Serbian Biochemical Society

Proceedings

Editor: Ivan Spasojević
Technical support: Jovana Trbojević-Ivić, Milena Dimitrijević, Tijana Ćulafić
Cover design: Zoran Beloševac
Publisher: Faculty of Chemistry, Serbian Biochemical Society
Printed by: Colorgrafx, Belgrade
No of printed copies: 130

Serbian Biochemical Society
Twelfth Conference

International scientific meeting

September 21-23, 2023, Belgrade, Serbia

"***Biochemistry in Biotechnology"***

PROGRAMME

Day 1 – Thursday, September 21st

(Serbian Academy of Sciences and Arts: Ceremony Hall)

09:00–10:00	Participants registration
10:00–10:20	Opening ceremony Welcome messages by Marija Gavrović Jankulović - SBS and Vladimir Stevanović - SASA

Section 1

10:20–11:00	Mario Gabričević Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia **Protein-ligand interactions – Alpha-1-acid glycoprotein (Orosomucoid) with drugs: Multitechnic approach** Plenary / FEBS3+ lecture
11:00–11:30	Marija Stojadinović University of Belgrade – Faculty of Chemistry **Macrophage polarization and infectious diseases** Invited lecture
11:30–12:00	Coffee Break

Section 2

12:00–12:30 Jelena Bašić

University of Niš, Faculty of Medicine

Apolipoprotein E and matrix remodeling – a link to neurodegeneration in Alzheimer's disease

Invited lecture

12:30–13:00 Nevena Tomašević

University of Kragujevac, Faculty of Science

Histone deacetylase 4 (HDAC4), an epigenetic target for spinal muscular atrophy

Invited lecture

13:00–13:30 Jasmina Ivanišević

University of Belgrade – Faculty of Pharmacy

HDL-associated proteins in hypertensive disorders of pregnancy

Invited lecture

13:30–15:15 **Poster Session 1 & Lunch break**

(University of Belgrade – Faculty of Chemistry)

Section 3

15:30–16:00 Sophie Combet

Laboratoire Léon Brillouin, UMR12, CEA-CNRS, Université Paris-Saclay, France

Stability of food proteins at high pressure conditions

ANSO PRESSION Lecture

16:00–16:30	Annie Brûlet Laboratoire Léon Brillouin, UMR12, CEA-CNRS, Université Paris-Saclay, France **Effect of structure on digestion of plant protein gels** ANSO PRESSION Lecture
16:30–17:00	Ali Assifaoui PAM Unit, AgroSupDijon, University of Burgundy, France **Polysaccharide-based hydrogels: Structure and function** ANSO PRESSION Lecture
18:30–22:00	Social event 1 - guided tour and dinner

Day 2 – Friday, September 22nd

(University of Belgrade – Faculty of Chemistry: Ceremony Hall)

9:00–10:00	Participants registration and poster posting

Section 4

10:00–10:30	Zhao Minyan / Li Qian / Xu Shuwen Alliance of International Science Organizations **Presentation of the ANSO program** ANSO PRESSION Lecture
10:30–10:45	Ana Vesković University of Belgrade - Faculty of Physical Chemistry **EPR imaging of redox-responsive hydrogels** Oral presentation
10:45–11:00	Nikolina Sibinčić Innovative Centre ltd., University of Belgrade – Faculty of Chemistry **Expression of recombinant SARS-CoV-2 nucleocapsid protein in mammalian cells** Oral presentation
11:00–11:15	Jovana Stevanović University of Belgrade - Institute for the Application of Nuclear Energy **Evaluation of long noncoding RNAs *H19* and *MALAT1* as oxidative stress indicators in gestational diabetes** Oral presentation

11:15–11:45 Coffee Break

Section 5

11:45–12:15 Jelena Purać

University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology

The effect of low-dose spermidine supplementation on polyamine content and antioxidative defence mechanisms in honey bees

Invited lecture

12:15–12:45 Neda Aničić

University of Belgrade – Institute for Biological Research 'Siniša Stanković'

Insights into iridoid biosynthesis in *Nepeta* species (subfam. *Nepetoidae*, fam. *Lamiaceae*): Functional characterization of a key enzyme

Invited lecture

12:45–13:00 Jelena Spremo

Faculty of Sciences, Department of Biology and Ecology, University of Novi Sad

The impact of spermidine supplementation on genes involved in autophagy in honey bee (*Apis mellifera* L.)

Oral presentation

13:00–13:15 Antos Sachanka

Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus, Belarus

Design and property of the fusion enzyme of bovine DNA-exotransferase and DNA binding protein *Sso7d* from *S. solfataricus*

Oral presentation

13:15–13:30 Natalija Andrejević

Faculty of Chemistry, University of Belgrade

Amyloid fibrillation of egg-white proteins and its tendency to bind synthetic dye from water solutions

Oral presentation

13:30–15:00 **Poster Session 2 & Lunch break**

Section 6

15:00–15:30 Camille Loupiac

UMR PAM, Team PCAV, Institut Agro Dijon, Université de Bourgogne Franche Comté, France

Proteins under stresses

ANSO PRESSION Lecture

15:30–16:00 Andreja Rajković

Faculty of Bio-science Engineering, Department of Food Technology, Safety and Health, Ghent University, Belgium

Be serious about *B. cereus*: facts that do(not) age well

ANSO PRESSION Lecture

16:00–16:30 Aleksandra Martinović

Food Hub, University Donja Gorica, Montenegro

The significance of the contemporary tools of the microbial food safety risk assessment

ANSO PRESSION Lecture

18:30–22:00 Social event 2 - dinner / ANSO PRESSION organized event

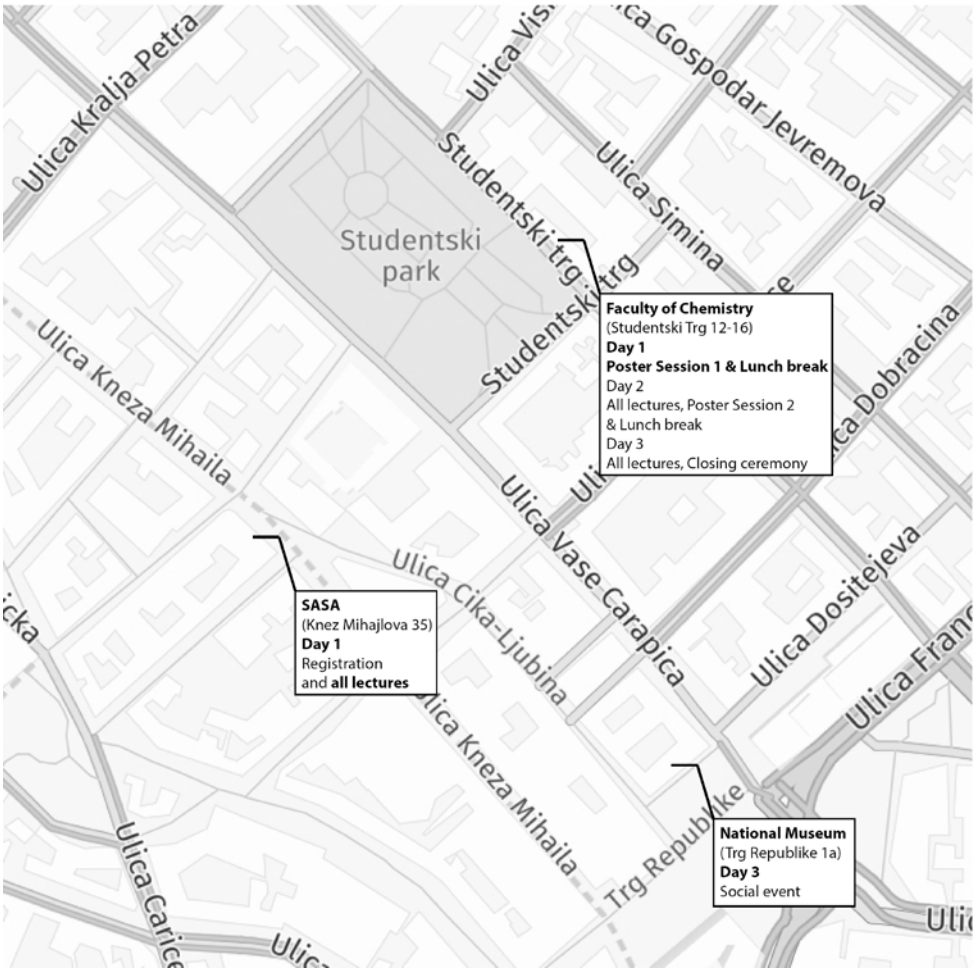
Day 3 – Saturday, September 23rd

(University of Belgrade – Faculty of Chemistry: Ceremony Hall)

Section 7

10:00–10:30	Jaroslav Katrlik Institute of Chemistry, Slovak Academy of Sciences, Slovakia **Study of biomolecular interactions by biosensors and biochips** ANSO PRESSION Lecture
10:30–11:00	Jelena Žakula University of Belgrade - Institute of Nuclear Sciences Vinča **Cancer cell death induced by ruthenium complexes** Invited lecture
11:00–11:30	Ivan Spasojević University of Belgrade - Institute for Multidisciplinary Research **Microalgae and transition metals - adaptation and opportunities** ANSO PRESSION Lecture
11:30–12:15	Coffee Break & Cocktail
12:15–12:30	Posters and speed talks awards announcement
12:30–13:00	Closing ceremony
14:30–17:00	Social event 3 - guided tour / visit to the National Museum

Map of events



Posters

(abstracts are enumerated for referencing purposes)

POSTER SESSION 1

P101
Ana D. Obradović
Antitumor potential of Tanacetum balsamita L. steams essential oil on human breast cancer cell lines

P102
Ana Vesković
EPR imaging of redox-responsive hydrogels

P103
Danijel Jakovljević
Long-term influence of specific antiepileptic drugs on redox and antioxidant parameters levels in human erythrocytes and plasma

P104
Danilo Četić
Simple two-step semi-preparative isolation and purification of transferrin from human serum

P105
Dušica J. Popović
Metformin synergized anticancer effect of other repurposed drugs in hamster fibrosarcoma

P106
Ekatarina Mihajlović
Anticancer potential of diiron thiocarbyne complexes

P107
Goran Miljuš
Proteomic profiling of anti-transferrin pull-down in patients with underlying oxidative stress

P108
Gorana Ilić
Effects of phenolic acids and their metabolites on oxidative stress and inflammation in U937 cells

P109
Isidora Protić-Rosić
Evaluation of the immunomodulatory potential of chimera Bv1a-BLwt and its mutants on the co-culture model system

P110
Jelena S. Katanić Stanković
Comparative *in vitro* analysis of the antioxidant, antigenotoxic, and anti-inflammatory properties of summer and winter savory (*Satureja* spp.)

P111
Jelica Milošević
Chaperone self-assemblies: Dissociation of DNAJb6 oligomers

P112
Jelica Simeunović
Soil cyanobacteria as producers of polysaccharides and fatty acids

P113
Jovana Jagodić
Circulatory levels of trace elements in pre-dialysis and hemodialysis patients

P114
Jovana Stevanović
Assessment of miR-27a, miR-222 and miR-340 as indicators of oxidative stress in gestational diabetes

P115
Jovana Stevanović
Evaluation of long noncoding RNAs *H19* and *MALAT1* as oxidative stress indicators in gestational diabetes

P116
Kosta J. Popović
ROS and NF-κB role in repurposed drugs treatment of hamster fibrosarcoma

P117
Ksenija Veličković
Diet- and age-dependent changes of intestinal injury in rats

P118
Ksenija Veličković
Glutamine deficiency suppresses adipogenic differentiation *in vitro*

P119
Luka Veličković
Exploring if *Porphyra* sp. extract functions as serum substitute in HT29 cell culture

P120
Marija Gvoić
Influence of bile acids and their derivatives on drug transport into central nervous system

P121
Maryia Kisel
The amino acid substitution N204H affects the interactions of CYP2C9 with ligands

P122
Milica Markelić
Proferroptotic response to nutrient deprivation in hepatocellular carcinoma cells is related to p53 status

P123
Milica R. Milenković
The effect of pH on coordination interactions between levofloxacin and Fe^{3+} in water

P124
Nevena Zelenović
Interactions of different urolithins with human serum albumin: Insights from fluorescence spectroscopy

P125
Nikolina Sibinčić
Expression of recombinant SARS-CoV-2 nucleocapsid protein in mammalian cells

P126
Olgica Martinis
Biochemical parameters according to the National Cholesterol Education Program (NCEP) for the prediction of metabolic syndrome in adolescents

P127
Sanja Erceg
Expression of miR-21-5p and glutathione peroxidase 1 (*GPX1*) in patients with steatosis and T2D

P128
Sanja Jelača
Antitumor potential of novel triphenyltin(IV) complexes with carboxylato N-functionalized 2-quinolone ligands

P129
Tamara Krajnović
Shikonin derivatives trigger phenotype reprogramming of B16 mouse melanoma cells

P130
Teodora Komazec
Cisplatin-naproxen conjugate free and loaded in SBA-15 indicate morphological changes and antitumor activity in vivo in mouse melanoma model

P131
Tino Šeba
Determination of Schiff base copper(II) complexes-HSA binding affinity using microscale thermophoresis

P132
Yaraslau U. Dzichenka
Molecular dynamics simulation of human sterol-7α-hydroxylase adsorption on the surface of anatase

P133
Zorana Lopandić
Analysis of newly designed H1sD2 glycoproteins by bioinformatic tools

POSTER SESSION 2

P201
Aleksa Savić
Evaluation of an in-house developed colorimetric and other assays for PET-degrading activity

P202
Aleksandar Ivanov
Examination of C-phycocyanin interactions with selected vitamins

P203
Ante Blajić
Genetic transformation of monoterpene biosynthesis and consequential modifications of the taste of citrus fruits and their defensive abilities against pathogens

P204
Antos Sachanka
Design and property of the fusion enzyme of bovine DNA-exotransferase and DNA binding protein *Sso7d* from *S. solfataricus*

P205
Dragana S. Josić
Temperature stability of *Streptomyces cyaneus* lacasse heterologously expressed within *S. cerevisiae* cell walls immobilized within tyramine-modified alginate beads using visible light photopolymerization

P206
Faiza Zafar
Optimization of the protocol for isolation of lipid transfer protein from lentil

P207
Filip Štrbac
Differences in chemical composition of the essential oils of peppermint (*Mentha piperita* L.) and spearmint (*Mentha spicata* L.) and their anthelmintic properties

P208
Georgios Sotiroudis
Antioxidant study of natural pigments and their incorporation into nanosystems

P209
Isidora Santrač
The accumulation of manganese by *Chlamydomonas acidophila* strains isolated from acid mine drainage

P210
Jelena Radović
Applying immobilised phycobiliproteins onto chitosan for efficient mercury removal

P211
Jelena Spremo
The impact of spermidine supplementation on genes involved in autophagy in honey bee (*Apis mellifera* L.)

P212
Jovana Trbojević-Ivić
Design and evaluation of *Candida rugosa* lipase hybrid nanoflowers

P213
Lidija Filipović
Nanobody-based immunoaffinity chromatography for the capture of urine-derived extracellular vesicles

P214
Ljiljana Milovanović
Biological activity of Serbian orange wines: polyphenolic content and inhibition of oxidative stress

P215
Ljubodrag Aleksić
Examining fatty acid interactions with *Arthrospira platensis*-derived C-phycocyanin

P216
M. Aydin Akbudak
Genome-wide identification of sucrose non-fermenting kinase-1 (SNRK1) genes in the maize genome

P217
Marija Marin
Antioxidant potential of the methanol extract of *Lavandula multifida* L.

P218
Marija Tanović
Magnetic, redox and structural properties of Mn-O-Ca cluster, synthesized by the green microalga *Chlorella sorokiniana*

P219
Michail A. Shapira
Unexpected regulators of cholesterol oxidase from *Pseudomonas aeruginosa*

P220
Milena Rašeta
Exploring the antioxidant potential, aldo-keto reductase inhibition, and estrogen receptor binding affinity of two autochtonous macrofungal species

P221
Mirjana Radomirović
Immuno-PCR for crustacean tropomyosin quantification

P222
Mirna Jovanović
Methanol extract of strawberry cultivar 'Aprika' increases glucose uptake in 3T3-F442A adipocytes

P223
Natalija S. Andrejević
Amyloid fibrillation of egg-white proteins and its tendency to bind synthetic dye from water solutions

P224
Nemanja Živanović
Chemical characterization and biological activities of six new genotypes of edible roses (*Rosa hybrida*)

P225
Nikola Radenković
Silk of Indian meal moth induces apoptosis of SW-480 colorectal cancer cells

P226
Nikola Srećković
The effect of carboxymethylcellulose and different natural ingredients on the stability of double emulsions (water-in-oil-in-water)

P227
Robert Kerep
An in vitro study on the binding affinity of Schiff base Rh(III) complexes to human serum α1-acid glycoprotein utilizing microscale thermophoresis

P228
Roko Vladušić
Colored proteins in biochemistry teaching

P229
Sanja Krstić
Phenolic profile, antioxidant and antidiabetic potential of traditionally prepared sage and peppermint juices

P230
Slađana Laketić
The impact of laser surface scanning on β-titanium alloy surface features and biocompatibility

P231
Suzana Jovanović-Šanta
Development of high-throughput elicitor screening (HiTES) system for usage in directed enzyme evolution

P232
Tafadzwa Kaseke
Binding of proteins in skimmed cow milk to polypropylene-microplastics

P233
Tamara Lujić
Impact of MPs on trypsin activity in simulated intestinal fluid

P234
Teodora Đukić
Mass spectrometry analysis reveals impact of peanut roasting on post-translational modifications of key allergens and their hinderence of trypsin digestion

P235
Vanja Tatić
Insect-derived active peptides: alternatives for combating multidrug-resistant bacteria

P236

Zorana Jovanović

Combined hydrogels of starch and β-lactoglobulin as matrices for the preservation of C-phycocyanin

Foreword

Dear colleagues

Welcome to the XII Conference of The Serbian Biochemical Society, entitled 'Biochemistry in Biotechnology'.

This year we have the richest program ever. In addition to our tradition to invite promising young researchers from four main university centers in Serbia to deliver lectures, we have eight guests from aboard that will participate through FEBS3+ program or within ANSO PRESSION project. This is a turning point in the organization of the conference which undergoes a transformation into scientific event with strong international character.

As always, we cherish the participation of PhD students and early career researchers. We are glad that many colleagues took the opportunity to show what they do and to find their place within the scientific ecosystem.

Organizing Committee

Plenary lecture

Protein-ligand interactions – Alpha-1-acid glycoprotein (Orosomucoid) with drugs: Multitechnic approach

Mario Gabričević*, Robert Kerep, Tino Šeba

Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

**e-mail: mgabricevic@pharma.hr*

Human serum alpha-1 acid glycoprotein (AAG) is an acute-phase plasma protein involved in the binding and transport of many drugs, especially basic and lipophilic substances.[1] It has been reported that the sialic acid groups that terminate the *N*–glycan chains of AGP change in response to certain health conditions and may have a major impact on drug binding to AGP. In pharmaceutical science, where future drugs are developed by targeting a selected protein, assessment of ligand affinity is important since binding of drugs to proteins affects their pharmacokinetics and pharmacodynamic action. In the blood, the drug is distributed in the body in free form and binded to plasma proteins to certain extend. The interaction between native and desialylated AAG with eight drugs (carvedilol, diltiazem, dipyridamole, imipramine, lidocaine, propranolol, vinblastine, warfarin) from different therapeutic areas was quantified using microscale thermophoresis, isotermal titration calorimetry and polarisation fluorescence. The results show that desialylation influences AAG-drug binding affinity. Also, we were able to detect both increase and decrease of AGP-drug equilibrium binding affinity caused by desialylation which can be linked with conformational change manifested by increased denaturation temperature of desialylated AAG. Therefore, different degree of sialylation may result in different binding affinities, and the clinical significance of changes in sialylation or glycosylation of AGP in general should not be neglected.

Acknowledgements

This study was supported by Croatian Science Foundation grants (IP-2016-06-3672 and UIP-2017-05-9537), European Structural and Investment Funds (KK.01.1.1.07.0055) and European Regional Development Fund FarmInova (KK.01.1.1.02.0021).

References

1. Israili ZH, Dayton PG. Human alpha-1-glycoprotein and its interactions with drugs. Drug Metab Rev 2001;33:161–235.

Invited lectures

HDL – associated proteins in hypertensive disorders of pregnancy

Jasmina Ivanišević[1*], Daniela Ardalić[2], Aleksandra Zeljković[1], Jelena Vekić[1], Tamara Gojković[1], Sandra Vladimirov[1], Tamara Antonić[1], Jelena Munjas[1], Željko Miković[2], Aleksandra Stefanović[1]

[1]Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Serbia
[2]Gynecology and Obstetrics Clinic „Narodni Front", Belgrade, Serbia

[*]e-mail: jasmina.ivanisevic@pharmacy.bg.ac.rs

Hypertensive disorders of pregnancy are associated with various complications and long-term health risks for both mother and child. Preeclampsia, a form of hypertensive disorder, is characterized by gestational hypertension and proteinuria. It is a serious condition that can lead to maternal morbidity and fetal mortality, as well as increased risk of cardiovascular disease later in life. The role of high-density lipoproteins (HDL) in hypertensive disorders of pregnancy has also been investigated. HDL contains many different proteins and is known for its anti-atherogenic, anti-inflammatory, and anti-oxidant properties. Because of their beneficial vascular effects, HDL proteins may prevent vascular damage in physiological pregnancy. However, dysfunctional HDL may be associated with vascular damage and hypertension in pregnancy. Further research is needed to elucidate the HDL proteome and its role in these conditions.

Acknowledgements

This work was supported by the scientific project HIgh-density lipoprotein MetabolOMe research to improve pregnancy outcome - HI-MOM, grant no. 7741659, under the auspices of Science Fund of the Republic of Serbia.

References

1. Rohatgi A, Westerterp M, Von Eckardstein A, Remaley A, Rye KA. HDL in the 21st century: A multifunctional roadmap for future HDL research. Circulation 2021;143:2293-309.
2. Roberts J, Gammill H. Preeclampsia: recent insights. Hypertension 2005;46:1243-9.

Apolipoprotein E and matrix remodeling – a link to neurodegeneration in Alzheimer's disease

Jelena Bašić[1*], Vuk Milošević[2,3], Branka Đorđević[1], Vladana Stojiljković[1], Milica Živanović[4], Tatjana Jevtović Stoimenov[1], Ivana Stojanović[1]

[1]*Department of Biochemistry, Faculty of Medicine, University of Niš, Serbia*
[2]*Clinic of Neurology, University Clinical Center Niš*
[3]*Faculty of Medicine, University of Niš*
[4]*Radiology Center, University Clinical Center Niš*

[*]*e-mail: jelena.basic@medfak.ni.ac.rs*

Apolipoprotein E (APOE) is a glycoprotein primarily produced by astrocytes and microglia. It plays a crucial role in complexing with amyloid β (Aβ) to accelerate its clearance. APOE genotyping holds great importance in determining whether an individual carries the APOE ε2/ε3/ε4 allele and the corresponding APOE2/E3/E4 protein isoform. Carrying the APOE ε4 allele has been associated with an increased risk of Aβ accumulation, amyloid plaque formation, and late-onset Alzheimer's disease (LOAD). The identification of novel biomarkers that indicate the earliest pathophysiological processes involved in Alzheimer's disease (AD) and the analysis of their diagnostic value in patients, especially through less invasive and cost-effective procedures that can visualize AD in a minimally invasive manner, are the primary focus of numerous researchers. Matrix metalloproteinases, their tissue inhibitors, and activators play a significant role in extracellular matrix remodeling, disruption of blood-brain barrier integrity, prolonged neuroinflammation, and Aβ clearance. These biomarkers are showing promise as potential blood-based diagnostic markers for patients with AD. In this context, we will discuss the possible mechanisms underlying the interrelation between APOE ε4 carrier status, matrix remodeling enzymes, and neurodegeneration in AD. Additionally, we will explore the diagnostic accuracy of these biomarkers in AD dementia patients based on the results obtained by our research group.

Acknowledgments

This study was supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia (Grant No. 451-03-47/2023-01/200113), and by the Faculty of Medicine University of Niš, Serbia (internal scientific project No. 44).

The effect of low-dose spermidine supplementation on polyamine content and antioxidative defence mechanisms in honey bees

Jelena Spremo[1], Elvira Vukašinović[1], Danijela Kojić[1], Marko Kebert[2], Tatjana Čelić[1], Srđana Đorđievski[1], Jelena Purać[1*]

[1]*Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia*
[2]*Institute of Lowland Forestry and Environment, University of Novi Sad*

[*]*e-mail: jelena.purac@dbe.uns.ac.rs*

The honey bee, a widespread pollinator, contributes to the conservation of biodiversity. In recent decades, a trend of declining colony numbers has emerged. The unsustainable exploitation of the environment may be the cause of this phenomenon. One protective strategy of organisms is to strengthen their antioxidant capacity. A class of positively charged molecules, polyamines, play important roles in various cellular processes. They exert a regulatory effect on gene expression, have antioxidative properties, and promote longevity in model organisms. The three main representatives are putrescine, spermidine, and spermine. The aim of this study was to determine whether supplementation of bees with low-dose spermidine leads to an increased level of the mentioned polyamines and whether this could strengthen the antioxidative defence systems. Two experimental groups were established: C group (control), fed with 50% (w/v) sucrose solution, and $S_{0.01}$ group, whose diet was supplemented with 0.01 mM spermidine. The experiment lasted for 10 and 17 days. A significant increase in putrescine, spermidine, and spermine content was noted in the supplemented group after 17 days, compared to its respective control. These results show a positive impact of spermidine supplementation on maintaining polyamine levels throughout aging. FRAP and MDA biochemical assays were used for the assessment of oxidative status. FRAP assay showed increased antioxidative capacity in the $S_{0.01}$ group. These results are in accordance with the results obtained from the MDA assay, which showed a decreased level of lipid peroxidation in the supplemented group in comparison to the control. The potential practical outcome of this study could be the use of spermidine in beekeeping practice to promote overall honey bee health.

Acknowledgements

This study was supported by the Science Fund of the Republic of Serbia (Program IDEAS, Grant No. 7721972, project title: Implications of dietary and endogenous polyamines for the health and longevity of honey bees B-HEALTH) and Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03- 47/2023-01/200125).

Cancer cell death induced by ruthenium complexes

Jelena Žakula[1*], Maja D. Nešić[2], Milica Matijević[2], Milutin Stepić[2], Marijana Petković[2], Lela Korićanac[1]

[1]Department of Molecular Biology and Endocrinology, Vinča Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade, Serbia
[2]Center for light-Based Research and Technologies COHERENCE, Department of Atomic Physics, Vinča Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade

[*]e-mail: pozegaj@vin.bg.ac.rs

Cancer is a complex and often fatal disease characterized by uncontrolled cell division. The most commonly used chemotherapeutics target rapidly dividing cancer cells but, at the same time, damage healthy dividing cells. New metal-based complexes, such as ruthenium complexes, that possess cytotoxic properties, have been developed to overcome these challenges[1,2]. Ruthenium complexes achieve their antitumor effect mainly by inducing apoptosis. However, its use is limited due to cancer cells' inherent and acquired resistance to apoptosis. Inducing ferroptosis in cancer cells is one way to overcome the problem of resistance. The dual role of autophagy in cancer cells is a major challenge for the application of metallocomplexes in cancer treatment, either as inducers or inhibitors of autophagy. Also, the effect of ruthenium complexes on other cellular processes such as cell cycle, cell migration, and adhesion are promising approaches in cancer treatment. Our results indicated a significant influence of Ru(II) complexes on these processes in melanoma, cervical and pancreatic cancer. The aim of this review is to summarize the latest data on the effect of ruthenium complexes on different types of cell death.

Acknowledgements

The research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (451-03-47/2023-01/200017).

References

1. Gałczyńska K, et al. Antitumor activity of Pt(II), Ru(III) and Cu(II) complexes. Molecules 2020;25:3492.
2. Sun Q, et al. Ruthenium complexes as promising candidates against lung cancer. Molecules 2021;26:4389.

Macrophage polarization and infectious diseases

Marija Stojadinović

Center of Excellence for Molecular Food Sciences, Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia

e-mail: mstojadinovic@chem.bg.ac.rs

Macrophages are a heterogeneous cell population present in most mammalian tissues with a wide range of functions. They are an essential component of optimal tissue homeostasis and an essential first line of defense against pathogens. Activated macrophages are typically divided into two phenotypes, M1 macrophages and M2 macrophages, which are influenced by microorganisms, the tissue microenvironment, and cytokine signals[1]. Primed M1 and M2 macrophages can be generated *in vitro* from human monocytes or murine bone marrow cells and subsequently activated to full phenotype with M1 or M2 stimuli[2]. In the long competitive history between pathogens and hosts, some pathogens have evolved various immune evasion strategies by utilizing and inter-converting macrophage phenotypes according to their needs[3]. Therefore the management of macrophage polarity is crucial for the prevention and treatment of infections and inflammatory disorders. We will evaluate the current state of knowledge regarding macrophage polarity and discuss how some pathogens exploit macrophage phenotypes for efficient replication and disease progression because studying the biology of these intriguing cells offers a great opportunity for future drug discovery.

Acknowledgements

This study was supported by Ministry of Education, Science and Technological Development of Republic of Serbia Contract number 451-03-68/2022-14/200168.

References

1. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008;8:958-69.
2. Yona S, Gordon S. From the reticuloendothelial to mononuclear phagocyte system - The unaccounted years. Front Immunol 2015;6:328.
3. Yu S, Ge H, Li S, Qiu H.-J. Modulation of macrophage polarization by viruses: Turning off/on host antiviral responses. Front Microbiol 2022;13.

Insights into iridoid biosynthesis in *Nepeta* species (subfam. *Nepetoidae*, fam. *Lamiaceae*): Functional characterization of a key enzyme

Neda Aničić[1*], Dragana Matekalo[1], Marijana Skorić[1], Jelena Božunović[1], Jasmina Nestorović Živković[1], Uroš Gašić[1], Milica Milutinović[1], Slavica Dmitrović[1], Luka Petrović[1], Biljana Filipović[1], Tijana Banjanac[1], Branislav Šiler[1], Boban Anđelković[2], Milena Dimitrijević[3], Danijela Mišić[1]

[1]*Department of Plant Physiology, Institute for Biological Research "Siniša Stanković"-National Institute of Republic of Serbia, University of Belgrade, Serbia*
[2]*Faculty of Chemistry, University of Belgrade*
[3]*Departmen of Life Sciences, Institute for Multidisciplinary Research, University of Belgrade*

[*]*e-mail: neda.anicic@ibiss.bg.ac.rs*

Nepeta L. holds a prominent position as the largest genus in the *Lamiaceae* family and serves as the exclusive representative of the iridoid-lacking *Nepetoidae* subfamily which produces iridoids. The genus showcases a rich chemodiversity, encompassing taxa both with and without iridoids. Our study investigates the genetic basis of iridoid diversity using omics-guided and functional genomics approaches. We identified functional iridoid synthases in iridoid-producing *N. rtanjensis* (NrIS2) and *N. sibirica* L. (NsIS), as well as in iridoid-lacking *N. nervosa* L. (NnIS). Remarkably, *N. nervosa* carries a dormant iridoid biosynthetic platform, suggesting a loss of iridoid production during its evolutionary history. Moreover, we explore regulatory mechanisms through comparative iridoid profiling and co-expression analysis of biosynthetic genes and transcription factors under various stress conditions (e.g., dehydration, UV − B radiation, pathogens) or elicitors (MeJA). These mechanisms influence plant productivity and the presence/absence of iridoids or their specific groups (iridoid aglycones and iridoid glycosides). Our research offers valuable insights into the molecular mechanisms driving iridoid biosynthesis and the chemical evolution of iridoids within *Nepeta*. This study enhances our understanding of the intricate relationship between genetics and the environment, providing comprehensive insights into iridoid metabolism, chemical evolution and ecology.

Acknowledgements

This work is financed by the Science Fund of the Republic of Serbia, Program IDEAS (project NEPETOME, No. 7749433), and is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/200007).

Histone deacetylase 4 (HDAC4), an epigenetic target for spinal muscular atrophy

Nevena Tomašević

Department of Chemistry, Kragujevac Center for Computational Biochemistry, Faculty of Science, University of Kragujevac, Serbia

e-mail: nevena.tomasevic@pmf.kg.ac.rs

Spinal muscular atrophy, a neurodegenerative recessive disease, is one of the leading genetic causes of death in early infancy and childhood worldwide, having an etiology in a mutation or deletion of the motor neuron 1 gene and deficient expression of the survival motor neuron protein[1, 2]. Being developed, spinal muscular atrophy is manifested in the denervation and consequent overexpression of histone deacetylase 4 in skeletal muscle, an epigenetic protein further having a role in the upregulation of two E3 ligases, atrogin-1 and MuRF1 via the myogenin-dependent pathway and leading to the structural and functional muscle protein breakdown through the ubiquitin-proteasome pathway [3]. Being not medically treated, spinal muscular atrophy can progress toward the loss of movement and even death, and, therefore, great efforts have been made so far to find an adequate therapy, with therapies already approved in Europe and the United States, yet of limited availability due to the high prices and severe side-effects. In that sense, there are continuous efforts among the scientific community worldwide to develop novel, cost-efficient approaches in therapy. The development of selective histone deacetylase 4 inhibitors and their epigenetic modifying capabilities has been of high interest in an attempt to find potential candidates for the effective treatment of spinal muscular atrophy. Nevertheless, none of the histone deacetylase 4 inhibitors has been repurposed for treating spinal muscular atrophy.

Acknowledgements

This study was supported by the Serbian Ministry of Science, Technological Development, and Innovation (Agreement Nos. 451-03-47/2023-01/200122 and 451-03-47/2023-01/200378)

References

1. Kolb SJ, Kissel JT. Spinal muscular atrophy. Neurol Clin 2015;33:831-46.
2. Lunke S, El-Osta A. 2009. The emerging role of epigenetic modifications and chromatin remodeling in spinal muscular atrophy. J Neurochem 2009;109:1557-69.
3. Bricceno KV, Sampognaro PJ, Van Meerbeke JP, Sumner CJ, Fischbeck KH, Burnett BG. Histone deacetylase inhibition suppresses myogenin-dependent atrogene activation in spinal muscular atrophy mice. Hum Mol Gen 2012;21:4448-59.

ANSO PRESSION lectures

The significance of the contemporary tools of the microbial food safety risk assessment

Aleksandra Martinović[1*], Amil Orahovac[1], Andrea Milačić[1], Nadja Raičević[1], Giuseppe Paderni[1], Werner Ruppitsch[2*]

[1]*Centre of Excellence for Digitalisation of Microbial Food Safety Risk Assessment and Quality Parameters for Accurate Food Authenticity Certification, University of Donja Gorica, Podgorica, Montenegro*
[2]*Institute of Medical Microbiology and Hygiene, Austrian Agency for Health and Food Safety, Vienna, Austria*

[*]*e-mail: aleksandra.martinovic@udg.edu.me; werner.ruppitsch@ages.at*

The ultimate priority of food production and processing is food safety. To fulfil strict food safety requirements accurate and reliable assessment models of food-borne risks (hazards) to human health are needed, along with an effective control system in place. The Codex Alimentarius defines a risk assessment process as intended to calculate or estimate the risk to a given target organism, system, or (sub) population, including the identification of attendant uncertainties, following exposure to a particular agent[1]. The process of risk assessment includes four steps: hazard identification, hazard characterization, exposure assessment, and risk characterization. Data gained through epidemiological surveys and food outbreak reports show that the foodborne disease burden is constantly growing. Microbiological risk assessment (MRA) minimises food-borne risks to the population, by applying tools for assessment of the hazards, their proper identification and quantification, and determination of the occurrence probability and prioritisation of microbiological risks based on country-specific uncertainties. MRA is a scientifically based analytical tool that encompasses the analysis of all the steps in the food production chain (from collection of raw materials, through processing, to the different food consumption patterns, retails, restaurants, and homes of people). Due to the high genetic diversity of microorganisms, it is still a challenge to encompass all the niches of their occurrence, transmission patterns along the food chain and impact to human health. MRA strategies applied in the EU, as well as the other developed countries worldwide, enable comprehensive evaluation of risks that influence the shaping of the food safety strategies globally. Aiming to ensure early detection of precise signals, new technologies, apart from traditional microbiological methods, are increasingly available. The application of new technological developments (known as omics) significantly improved general understating of microbial patterns, physiological triggers, and behaviors of microorganisms in different food matrices. Among these techniques certainly the most important ones are genome sequencing, protein

analyses and assessment of the metabolic profiles of microorganisms. These technologies are recently applied as powerful tools in MRA[2]. Metagenomics can be used to evaluate a microbial community, providing data on the hazard in specific foods or environments. Novel omics techniques in combination with machine learning algorithms, are becoming a big step forward, a promising area for collection and analysis of the vast data on pathogens, their characteristics, relation to the food matrixes and human health. These discoveries open a new era of fast, reliable, science-based identification and characterization of food borne pathogens, a paradigm shift will be required for the current risk assessment models to include genomic information[3].

References

1. Codex Alimentarius. Principles and guidelines for the conduct of microbiological risk assessment. CAC/GL-30(1999). Food and Agriculture Organisation Corporate Document Repository; 1999. Available at: http://www.fao.org/docrep/005/ y1579e/y1579e05.htm.
2. Wood, J. Predictive metagenomics profiling: why, what and how? Bioinform Rev 2016;2:1–4.
3. Franz E, Gras L.M, Dallman T. Significance of whole genome sequencing for surveillance, source attribution and microbial risk assessment of foodborne pathogens. Curr Opin Food Sci 2016;8:74–9.

Polysaccharide-based hydrogels: Structure and function

Ali Assifaoui[1*], Aline Maire du Poset[1,2,3], Andrea Zitolo[2], Adrien Lerbret[1], Fabrice Cousin[3]

[1]*UMR PAM Institut Agro Dijon - Université de Bourgogne, Dijon, France*
[2]*Synchrotron SOLEIL, L'Orme des Merisiers, Gif-sur-Yvette, France*
[3]*Laboratoire Léon Brillouin, CEA-Saclay, Gif-sur-Yvette, France*

[*]*e-mail: ali.assifaoui@u-bourgogne.fr*

Biopolymer-based hydrogels with a tuneable structure have drawn significant attention due to their wide range of applications in the food, pharmaceutical, and biomedical fields. This can be attributed to their remarkable features such as desirable mechanical properties, high water content, biodegradability and biocompatibility. At first order, hydrogels can be described as a network containing a crosslinked polymer and open spaces (meshes) between polymers chains enabling the diffusion of liquids and entrapped molecules such as proteins, peptides, and vitamins. In this work, we prepared polygalacturonate-based hydrogels by diffusing divalent cations X^{2+} (eg., Ca^{2+}, Zn^{2+}, Fe^{2+}) from an external reservoir, through a dialysis membrane, to the polygalacturonate (polyGalA) solution. These hydrogels exhibited various degrees of inhomogeneity and showed a significant gradient of both polyGalA and divalent cation concentrations, ranging from the part of the gel formed near the dialysis membrane (i.e., the initial stage of the gelation process) to the furthest part of the gel. We demonstrated that Fe^{2+} cations keep their oxidation sate in the entire gel, making them suitable for iron fortification purposes[1]. Furthermore, we observed that the mesh size (ξ) in the regions of the gel closer the dialysis membrane remained close to 7.5 nm, regarless of the divalent cation used (Ca^{2+}, Zn^{2+}, Fe^{2+})[2]. However, in the farthest parts of the gel, the mesoscopic structure depended on the nature of the cation, particularly on the different modes of interaction known to occur between the cation and the GalA unit, such as bidentate versus "egg-box" geometry.

Acknowledgements

Laboratoire Léon Brillouin, Synchrotron SOLEIL and Région Bourgogne Franche-Comté are acknowledged for their financial support.

References

1. Maire du Poset A, Lerbret A, Zitolo A, Cousin F, Assifaoui A, Design of polygalacturonate hydrogels using iron(II) as crosslinkers: A promising route to protect bioavailable iron againstoxidation. Carbohydr Polym 2018;188:276−83.

2. Maire du Poset A, Lerbret A, Boué F, Zitolo A, Assifaoui A, Cousin F, Tuning the structure of galacturonate hydrogels: External gelation by Ca, Zn, or Fe cationic cross-linkers. Biomacromolecules 2019;20:2864−72.

Be serious about *B. cereus*: facts that do(not) age well

Andreja Rajković, Jelena Jovanović

Research Unit Food Microbiology and Food Preservation, Department of Food Technology, Safety and Health, Faculty Bio-Science Engineering, Ghent University, Belgium

e-mail: andreja.rajkovic@ugent.be

Bacillus cereus has been seen as an established foodborne pathogen. Yet, emerging understanding of its taxonomy, pathogenic traits and foodborne related behavior change the way we go about it[1]. In our latest work we showed that unexpected properties, such as growth temperarures and toxigenic profiles, of *B. cereus* group members were found in unexpected different foods[2]. We have also seen that very low doses of *B. cereus* emetic toxin cereulide can cause sub-toxic responses in mammalian cells and their metabolic profiles. Important effects on ATP production were seen on all tested mammalian cells including intestinal cell lines, liver cell lines and viable spermatozoa[3]. How foodborne enterotoxigenic *B. cereus* (those strains producing HBL, NHE and cytK enterotoxins) rewires energy metabolism during intestinal tract infection is still not understood, but using Seahorse XFe analyzer we analyzed oxygen consumption and acidification rates to estimate bioenergetic changes in the intestinal Caco-2 cell line after exposure to the *B. cereus* enterotoxin-producing pathotypes. Infection of Caco-2 led to a more energetic phenotype due to increased flux through oxidative phosphorylation and glycolysis. Effects were strain dependent.

Acknowledgements

This study was supported by Special Research Fund of Ghent University for research grant BOFSTA2017004201 and GlobalMinds operational grant of Ghent University.

References

1. Jovanovic J, Ornelis VFM, Madder A, Rajkovic A. *Bacillus cereus* food intoxication and toxicoinfection. Compr Rev Food Sci Food Saf 2021;20:3719−61.
2. Jovanovic J, Tretiak S, Begyn K, Rajkovic A. Detection of enterotoxigenic psychrotrophic presumptive *Bacillus cereus* and cereulide producers in food products and ingredients. Toxins 2022;14:289.
3. Decleer M, et al. Oxygen consumption rate analysis of mitochondrial dysfunction caused by *Bacillus cereus* cereulide in Caco-2 and HepG2 cells. Toxins 2018;10:266.

Effect of structure on digestion of plant protein gels

Annie Brûlet[1*], Maja Napieraj[1], Evelyne Lutton[2,3], Javier Perez[4], François Boué[1]

[1]*Laboratoire Léon Brillouin, UMR12 CEA-CNRS, Université Paris-Saclay, CEA Saclay, Gif sur Yvette, France*
[2]*Mathématiques et Informatique Appliquée - Paris, UMR518 AgroParisTech-INRAE, Université Paris-Saclay, Palaiseau, France*
[3]*Institut des Systèmes Complexes, Paris, France*
[4]*SWING, Synchrotron SOLEIL, Saint-Aubin – BP 48, Gif sur Yvette, France*

[*]*e-mail: annie.brulet@cea.fr*

Although the biochemical processes of protein digestion are widely studied, the biophysical ones, like conformational changes, are more neglected, especially when resolving both time and space scales. We address the issue of structural changes of canola protein gel, as a solid food model, during in situ gastrointestinal digestion. We have used synchrotron Small-Angle X-ray Scattering (SAXS) with narrow beam, which provided structural information on macromolecules at the 0.5-nm up to 50nm scale, to study the in situ digestion of two gels, prepared by heating solutions at pH 8 and pH 11 in capillaries. Digestion was carried out by diffusion of enzymatic juices into the gel from the top of the capillary for several tens of hours. Kinetic measurements at different positions of the gel in the capillary showed trends consistently observed at longer digestion times on positions further from the digestive juice. Data analysis allowed us to extract parameters describing the proteins structures on a wide range of scales. Plotting the compactness as a function of their characteristic size measured at the protein scale reveals master curves in position and time, clearly showing the different digestion processes at the protein level. First, for the gel prepared at pH 11, with initially locally quite folded proteins, the digestive processes include back and forth evolutions of protein structure with unfolding (1st and 3rd steps), re-compaction – aggregation phenomena due to gastrointestinal pH conditions and enzymatic cleavage (2nd step), further unfolding – disaggregation once enzymatic reaction has acted sufficiently (3rd step), before the final protein cleavages (4th step) resulting in small peptides. Second, for the pH 8 gel, the 1st unfolding step is omitted because the proteins are already highly unfolded after the heat treatment of the gel preparation. It is replaced, due to an easier access of enzymes to unfolded proteins, by a weak re-compaction. The following mechanisms, in intestinal condition, are accelerated by the already unfolded then weak aggregated structures of the gel. At the scale of large aggregates, we mainly observe for both gels the decreasing of the size and/or number of these aggregates during digestion and the alteration of their interfaces, partly due to the loosening of the local protein network (protein unfolding and cutting). These studies are completed by imaging experiments,

allowing to explore micron scale, i.e. structure and sizes of protein aggregates (confocal and neutron imaging).

Proteins under stresses

Camille Loupiac

UMR Procédés Alimentaires et Microbiologiques, Equipe PhysicoChimie des Aliments et du Vin, Instiut Agro Dijon, Université de Bourgogne Franche Comté, Dijon, France

**e-mail:camille.loupiac@agrosupdijon.fr*

The current global context is marked by environmental degradation, an increase in population density and a scarcity of agricultural production areas and natural resources. These persistent stresses around proteins productions and being able to provide enough proteins to feed the world population has led governments and a number of food companies to seek new natural resources with both nutritional and energy interests. Despite the strong interest in alternative proteins from insects and microalgae, two natural resources rich in protein and whose production performance is high, there are still scientific and technological barriers to be lifted to consider the creation of new products and ingredients, on an industrial scale, economically competitive and sustainable. These include the cost, both energy and environmental, of extraction processes that generally rely on the use of solvents. Indeed, most conventional processes for extracting protein of agri-food interest are costly, denaturant, and not always compatible with food and sustainability constraints. We will present the results obtained in our laboratory[1-3], on the use of green extraction methods to extract and valorize proteins from microalgae, insects and hemp press cakes. We studied the impact of extraction processes on the structure and functionality of proteins from insects, hemp press cakes, and a red microalgae porphyridium cruentum. We have been able to establish a correlation between the quality of insects, hemp and microalgae proteins and the processes adopted for extraction, on their functional properties and formulation.

Acknowledgements

Results presented on hemp proteins have been obtained during the Valvigne project 2020-2022. This project has been supported by the Conseil Régional de Bourgogne Franche-Comté and the European Union through the PO- FEDER-FSE Bourgogne 2014-2020 programs.

References

1. Bou-Maroun E, et al. Impact of preparation process on the protein structure and on the volatile compounds in *Eisenia foetida* protein powders. Food Nutr Sci 2013;4:1175.
2. Tran T, et al. Effect of high hydrostatic pressure on the structure of the soluble protein fraction in *Porphyridium cruentum* extracts. Innov Food Sci Emerg 2019;58:102226
3. Martinez Sanchez M. et al. Nano-microstructure of extruded Spirulina/Starch foams in relation to their textural properties, Food Hydrocoll 2020;103:105697.

Microalgae and transition metals - adaptation and opportunities

Ivan Spasojević

Departmen of Life Sciences, Institute for Multidisciplinary Research, University of Belgrade, Serbia

e-mail: ivan@imsi.bg.ac.rs

The metabolism of metals in microalgae and adaptation to metal excess are of significant environmental importance. An excess of transition metals, such as copper, manganese or nickel, poses a serious threat to aquatic ecosystems and beyond. Microalgae use multi-step mechanisms and different strategies to inactivate metal ions. These include mucilage release, metal binding to the cell wall, transport across the membrane, temporary chelation with different oligo- and polymers, and storage of metals in the form of deposits or well-organized metal clusters[1]. The removal of metal pollutants by microalgae is considered a promising route in water remediation. More importantly, we may utilize adaptive traits of microalgae and employ them as potential vehicles for biosynthesis of catalytically active metal cluster compounds[2]. *De novo* synthesis of metal cluster compounds that mimic structural, redox and catalytic properties of biological clusters in metalloproteins represents an interdisciplinary challenge. Chemical synthetic pathways come with limitations and large environmental footprint, which calls for finding novel routes of green synthesis.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development and Innovation, contract number 451-03-47/2023-01/200053.

References

1. Vojvodić S, et al. Mechanisms of detoxification of high copper concentrations by the microalga *Chlorella sorokiniana*. Biochem J 2020;477:3729-41.
2. Vojvodić S, et al. A three-step process of manganese acquisition and storage in the microalga *Chlorella sorokiniana*. J Exp Bot 2023;74:1107-22.

Study of biomolecular interaction by biosensors and biochips

Jaroslav Katrlík

Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia

e-mail: katrlik@yahoo.com

Biosensors and biochips offer great advantages over conventional analytical techniques because they enable the direct, real-time and often also high-throughput detection of many biological and chemical substances. Their advantages include high specificity, sensitivity, and cost-effectiveness, and grow most in importance in the healthcare, biomedical, biopharmaceutical and food sectors. They can provide new analytical tools with reduced size as well as facilitate large-scale high-throughput sensitive analysis and screening of a very wide range of biomolecular interactions and samples for many different parameters and have been successfully applied in many fields such as medicine, pharmacy, food safety, environment, biotechnology, defence and security. One of the rapidly developing and increasingly desirable areas of bioanalysis is glycomics, where biosensors and biochips have a huge scope and potential for targeted applications. Determination of protein glycosylation may reveal changes in glycan composition occurring due to disease, ageing, lifestyle or other reasons. Altered glycosylation is one of the disease-related markers and information on glycosylation status can significantly increase the informative value of glycoprotein biomarkers. One such case is cancer where changes in the glycan composition of glycoprotein cancer biomarkers are markers for early diagnosis, prognosis, stratification and follow-up of patients. We have developed various lectin-based biosensing systems using analytical platforms such as protein microarrays, surface plasmon resonance and lateral flow assay. These biosensor and biochip analytical systems provide effective glycoprofiling of samples and screening/analysis of normal and aberrant glycosylation, and glycan biomarkers with attractive applications in biomedicine, biotechnology and biology. Although analytical assays based on lectin-glycan interactions do not allow the identification of glycan structures, in configuration with microarray platforms are suitable for rapid screening of glycosylation changes or abnormalities making them promising technologies for biomarker research and diagnostics. We have applied biosensors and microarray biochip platforms for the study of glycan changes in a number of various cases, *e.g.* cancer, gestational diabetes mellitus, kidney diseases, COVID-19, a congenital disorder of glycosylation, attention-deficit hyperactivity disorder, age-related glycosylation changes, and glycostructure of therapeutic proteins.

Acknowledgement

This work was supported by the grants ANSO-CR-PP-2021-01, APVV SK-SRB-21-0046 and VEGA 2/0120/22.

Stability of food proteins at high pressure conditions

Simeon Minić[1,2], Burkhard Annighöfer[1], Annie Brûlet[1], Sophie Combet[1*]

[1]*Laboratoire Léon-Brillouin (LLB), UMR12, CEA, CNRS, Université Paris-Saclay, Gif-sur-Yvette CEDEX, France*
[2]*Faculty of Chemistry, University of Belgrade, Serbia*

[*]*e-mail: sophie.combet@cea.fr*

High pressure (HP) is particularly suited to study protein folding/unfolding, revealing subtle structural rearrangements not accessible by other types of denaturation. HP also has many industrial-scale advantages over heat treatments, including "greener" processing and preservation of nutritional values, colors, and flavors of foods. We have combined *in situ* HP with small-angle (X-ray and neutron) scattering (SAS) and spectrophotometry to follow the structure in solution of proteins of interest for the food industry. SAS is an essential technique for obtaining structural, but low-resolution, information about proteins, when conventional high-resolution structural biology methods are not possible. I will illustrate this approach with two studies on proteins of food interest: (*i*) bovine β-lactoglobulin (BLG), a whey protein with a high propensity to bind to various bioactive molecules. We probed by SANS[1] and absorbance the effects on pressure stability and reversibility of BLG of the binding of retinol (vitamin A), resveratrol (polyphenol), and biliverdin (linear tetrapyrrole chromophore) to different sites on the protein[2, 3]. (*ii*) C-phycocyanin (CPC), a phycobiliprotein from cyanobacteria, to which tetrapyrrole chromophores are covalently attached and which can be used as a natural blue dye in the food industry. We studied by SAXS and absorbance HP-induced CPC unfolding and reversibility from two oligomeric states of the protein as a function of pH.

Acknowledgements

LLB, SOLEIL, and I2BC facilities are acknowledged for beamtime and proteomic expertise. This work was also supported by ANSO Project No. ANSOCR-PP-2021- 01.

References

1. Annighöfer B, et al. High pressure cell to investigate protein unfolding up to 600 MPa by small-angle neutron scattering. Rev Sci Instr 2019;90:025106.
2. Minic S, et al. Effect of ligands on HP-induced unfolding and oligomerization of β-lactoglobulin. Biophysical J 2020;119:2262-74.
3. Minic S, et al. Structure of proteins under pressure: covalent binding effects of biliverdin on beta-lactoglobulin. Biophysical J 2022;121:2514-25.

Posters / Session 1

Antitumor potential of *Tanacetum balsamita* L. steams essential oil on human breast cancer cell lines

Ana D. Obradović[1]*, Milena D. Vukić[2], Nenad L. Vuković[2], Milica Paunović[1], Branka Ognjanović[1], Miroslava Kačániová[3,4], Miloš M. Matić[1]

[1]*Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Serbia*
[2]*Department of Chemistry, Faculty of Science, University of Kragujevac*
[3]*Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture, Nitra, Slovakia*
[4]*Department of Bioenergy, Food Technology and Microbiology, Institute of Food Technology and Nutrition, University of Rzeszow, Rzeszow, Poland*

**e-mail: ana.obradovic@pmf.kg.ac.rs*

Breast cancer is the leading cause of death among female cancers. Excessive production of free radicals is associated with tumor development, but also with induction of apoptosis and inhibition of tumor cell progression. The aim of this study was to investigate the antitumor capacity of steams-derived *Tanacetum balsamita* L. essential oil (EO) by measuring cell viability, apoptosis rate, redox status parameters, migration capacity and Nrf-2 expression level on human breast cancer cell lines MBA-MB-468 and MDA-MB-231. The cells were treated with different concentrations of EO (from 1 μg/mL to 200 μg/mL) during 24 h and 72 h. The tested essential oil expressed antiproliferative activity, determined by MTT assay, proapoptotic effects, as well as dose- and time-dependent increase of nitrites and decreased of $O_2^{\cdot-}$ production. The investigated oil significantly decreased migration capacity and the expression levels of Nrf-2. The obtained data suggest that the tested oil exerts considerable antitumor activity by reducing cell viability rate, elevating apoptosis level and inhibiting the motility of tested breast cancer cell lines. The reduced levels of Nrf-2 expression indicate decreased antioxidative defence potential of breast tumor cells, which may also be one of antitumor mechanisms of action of the EO. Based on the results this essential oil is a good candidate for further investigation as a potential therapeutic agent against breast cancer.

Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-47/2023-01/200122).

EPR imaging of redox-responsive hydrogels

Ana Vesković*, Barbara Bekić, Marija Kratovac, Ana Popović Bijelić

EPR Laboratory, Faculty of Physical Chemistry, University of Belgrade, Serbia

**e-mail: ana.veskovic@ffh.bg.ac.rs*

Hydrogels have attracted notable attention for various biomedical applications, including controlled drug delivery, wound dressing and healing, and tissue engineering. The significance of the stimuli-responsive degradation for sustained and "on-demand" release of the active agent has been recently recognized. Redox-responsive disulfide-containing polymeric networks that undergo degradation when exposed to a thiol-rich environment have become particularly attractive as systems for controlled delivery[1]. Expanding on this idea, the focus of this study was shifted from the therapeutic, to the diagnostic potential of redox-responsive hydrogels. Instead of introducing cleavable redox-active junctions into the network, in the approach used here, the bovine serum albumin (BSA)-based matrix remains intact, and the stimuli-responsive indicator, 3-carbamoyl-PROXYL (3CP), is located within the protein hydrogel water pores. 3CP is an electron paramagnetic resonance (EPR) spin probe, particularly suitable as a redox environment marker, considering its sensitivity to both reduction and oxidation, observed through the probe EPR signal loss. Thermally-induced BSA hydrogel is not only a safe 3CP-indicator reservoir, in terms of biocompatibility and biodegradability, but owing to its antioxidative properties, BSA can serve as the first line of defense from reactive oxygen species, thus preserving the probe signal during the initial experiment phase. EPR imaging experiments showed that during the incubation of the 3CP-labeled BSA hydrogel with activated yeast cells, the decrease of the EPR signal intensity is dependent on the number of live cells, as well as the time of exposure. It is important to note that the observed signal loss was not attributed to 3CP diffusion out of the hydrogel, for yeast cell volumes up to 1 ml, as the spin-labeled hydrogel signal remained constant during several hours of its incubation in 1 ml of water or physiological saline. Ongoing experiments are likely to confirm the application of EPR imaging of spin-labeled hydrogels as a promising method for redox environment monitoring in human cell lines, and hopefully allow the translation of this methodology to *in vivo* studies.

References

1. Kilic Boz R, Aydin D, Kocak S, Golba B, Sanyal R, Sanyal A. Redox-responsive hydrogels for tunable and “on-demand” release of biomacromolecules. Bioconjug Chem 2022;33:839–47.

Long-term influence of specific antiepileptic drugs on redox and antioxidant parameters levels in human erythrocytes and plasma

Danijel Jakovljević[1*], Milan Nikolić[1], Vesna Jovanović[1], Aleksandra Nikolić-Kokić[2], Čedo Miljević[3], Maja Milovanović[3], Duško Blagojević[2]

[1]Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia
[2]Department of Physiology, Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade
[3]Institute of Mental Health, School of Medicine, University of Belgrade

[*]e-mail: jakovljevicdanijel96@gmail.com

Epilepsy is a chronic brain disease affecting over 50 million people worldwide. Literature data suggest the influence of oxidative stress on the development of epilepsy. However, little is known about the impact of selected antiepileptic drugs on redox homeostasis. This study aimed to investigate the effects of long-term use of antiepileptic drugs in monotherapy: oral antiepileptic drugs lamotrigine (LTG), carbamazepine (CBZ), and valproate (VPA) on the activities of antioxidant enzymes, haemoglobin and methaemoglobin levels in the erythrocytes of epileptic patients, as well as total protein and thiol concentration, $NO^{\cdot}$ (as nitrite), lipid peroxides and total glutathione (GSH) levels in plasma. All these parameters were compared to those of drug-naïve (control) patients. The results showed that CuZn superoxide dismutase activity was statistically reduced in the LTG patient group ($p<0.01$) and especially under CBZ ($p<0.001$). Catalase activity was significantly lower in the VPA group ($p<0.001$), despite lower activity in all groups. A statistically significant ($p<0.05$) lower concentration of total thiol groups in the LTG group was also found. The CBZ group had the highest concentration of $NO^{\cdot}$, statistically significantly higher than the controls ($p<0.05$). There was a statistically significant decrease in lipid peroxides concentration between LTG and the control group ($p<0.005$) and both CBZ and VPA groups ($p<0.001$). Correlation analysis showed that increased oxidative stress was not generally compensated by changes in the activity of complementary enzymes, with only two statistically significant (positive) correlations between GSH peroxidase and reductase ctivity ($p<0.05$) and GSH peroxidase and catalase activity ($p<0.05$), both in the LTG group. Our results indicate that long-term monotherapy with studied antiepileptics could modify the blood oxidants–antioxidants balance.

Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant Nos. 451-03-47/2023-01/200007 and 451-03-47/2023-01/200168).

Simple two-step semi-preparative isolation and purification of transferrin from human serum

Danilo Četić[1*], Goran Miljuš[1], Zorana Dobrijević[1], Nikola Gligorijević[1], Aleksandra Vilotić[2], Olgica Nedić[1], Ana Penezić[1]

[1]*Department for Metabolism, Institute for the Application of Nuclear Energy, University of Belgrade, Serbia*
[2]*Department for Biology of Reproduction, Institute for the Application of Nuclear Energy, University of Belgrade*

[*]*e-mail: danilo.cetic@inep.co.rs*

Human transferrin (Tf) is a bilobal 76 kDa iron-binding glycoprotein present in human serum. Each lobe has the ability to bind one ferric ion (Fe^{3+}) and a single synergistic bicarbonate anion. The main role of Tf is to transport Fe^{3+} ions through the circulation to cells, via interaction with transferrin receptor (TFR) on the cell surface. Previously described methods for Tf isolation and purification are either very time-consuming or provide Tf of lower final purity. Here we describe a fast and simple FPLC method for the isolation and purification of Tf from human serum. Serum samples were prepared by precipitation, while protein purification was performed on FPLC system, using an anion-exchange column. Several different buffers at the same pH were tested. Tf purified by this method was analyzed by Western blot, followed by immunodetection, as well as with silver staining after SDS PAGE. Its functionality was tested with respect to iron-binding capacity (ferozzine method) and its ability to interact with TFR by immunofluorescent staining. The conformation of purified Tf was analyzed by recording intrinsic fluorescent emmision spectra originating from Trp residues. The method itself is highly reproducible (intra- and interday), easy to perform (only two steps) and fast (under an hour), yielding 98% to 99% pure Tf with all buffers. Purified Tf was shown to have retained its iron-binding capacity, as well as the ability to interact with TFR. Purified Tf also retained its native three-dimensional structure. Described method for the isolation and purification of Tf is fast, simple and highly reproducible, yielding a functional Tf of high purity in its native state while offering the flexibility of using different buffer systems. All of these features make this protocol a method of choice for the isolation and purification of Tf on a semi-preparative scale.

Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-9/2022-14/200019).

Metformin synergized anticancer effect of other repurposed drugs in hamster fibrosarcoma

Dušica J. Popović[1*], Kosta J. Popović[2], Dejan Miljković[3], Dušan Lalošević[3], Zana Dolićanin[1], Mihalj Poša[2], Ivan Čapo[3], Jovan K. Popović[4]

[1]*Department of Biomedical Sciences, State University of Novi Pazar, Serbia*
[2]*Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Serbia*
[3]*Department of Histology and Embryology, Faculty of Medicine, University of Novi Sad*
[4]*Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad*

[*]*e-mail: jovan.popovic@mf.uns.ac.rs; jovapopmf@gmail.com*

The tumor growth rate positively correlates with glucose levels. The Warburg effect promotes the glucose uptake of cancer cells. The cancer glucose starvation is an effective way for suppressing tumor growth. Reduction of blood glucose can inhibit the proliferation of cancer cells. In addition, the combination of glucose inhibitors could be a promising direction for cancer therapy. Anticancer efficacy of the glucose lowering antidiabetic drug, metformin, in combination with 2-deoxy-*D*-glucose, deoxycholic acid, caffeine, itraconazole, nitroglycerin and disulfiram was tested on fibrosarcoma experimentally induced by BHK21/C13 cells in Syrian golden hamsters. Tumor biophysical characteristics, histology and immunohistochemistry were assessed. Blood samples were collected for hematological and biochemical analyses and the main organs were toxicologically analyzed. The Combination Index (CI) analysis was used to determine synergistic ($CI < 1$), additive ($CI = 1$) or antagonistic ($CI > 1$) metformin interaction with other investigated drugs. Two-drug combinations with metformin showed significant synergistic ($CI < 1$) antitumor effects on hamster fibrosarcoma compared to control, regarding all tested tumor parameters ($P < 0.05$) without toxicity. Combinations of metformin with other repurposed drugs might be an effective and safe approach in novel nontoxic adjuvant anticancer treatment.

Acknowledgements

This study was supported by the Republic of Serbia, Autonomous Province of Vojvodina, Provincial Secretariat for High Education and Scientific Research (Grants Nos. 142-451-3155/2022-02 (JM), 142-451-2626/2021 (DL)) and the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200114).

Anticancer potential of diiron thiocarbyne complexes

Ekatarina Mihajlović[1*], Sanja Jelača[1], Lorenzo Biancalana[2], Lorenzo Chiaverini[2] Biljana Dojčinović[3], Sanja Mijatović[1], Stefano Zacchini[4], Fabio Marchetti[2], Danijela Maksimović-Ivanić[1]

[1]Department of Immunology, Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade, Serbia
[2]Department of Chemistry and Industrial Chemistry, University of Pisa, Italy
[3]Institute of Chemistry, Technology and Metallurgy – Institute of National Importance for the Republic of Serbia, University of Belgrade
[4]Department of Industrial Chemistry "Toso Montanari", University of Bologna, Italy

[*]e-mail: ekatarina.mihajlovic@ibiss.bg.ac.rs

To improve safety and efficacy of conventional chemotherapeutics, it is important to target cancer cells more selectively. Potential strategies could arise from differences in iron metabolism between healthy and cancer cells, based on cancer cells high demands for iron. Their, so-called, "iron addiction" sets a foundation for new therapeutic approach. In this study, the cytotoxic effect of three diiron carbonyl complexes with a bridging thiocarbyne ligand was evaluated on different human cancer cell lines (HCT116 colorectal carcinoma, MCF-7 breast cancer and A2780 ovarian cancer), as well as on human embryonic lung fibroblasts (MRC-5), which were used for selectivity assessment. The most potent compound (FETPY) decreased viability of all cancer cell lines in dose-dependent manner, while A2780 cells emerged as the most sensitive. Therefore, they were selected for further investigation. On the other hand, the effect of FETPY on lung fibroblasts viability was remarkably less potent, showing its great selectivity towards malignant phenotype. Additionally, it was shown that intracellular iron concentration was much higher in A2780 than in MRC-5 cells after treatment with FETPY. Viability decrease of A2780 cells was a consequence of cell death – ferroptosis, caused by iron-dependent lipid peroxidation and membrane damage. Oxidative stress that caused ferroptosis evolved from intensive production of nitric oxide and superoxide anion. Controversially, it was followed with scavenging of hydrogen peroxide and peroxynitrite. Treatment with FETPY also caused significant decrease of A2780 cells division rate. Overall, these results indicate that the considered diiron derivatives show great potential for further investigation in cancer treatment.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200007).

Proteomic profiling of anti-transferrin pull-down in patients with underlying oxidative stress

Goran Miljuš[1*], Ana Penezić[1], Dragana Noe[2], Zorana Dobrijević[1], Marko Baralić[3], Dragana Robajac[1], Miloš Šunderić[1], Nikola Gligorijević[4], Ivan Dimitrijević[5], Goran Barišić[5], Olgica Nedić[1]

[1]Department for Metabolism, Institute for the Application of Nuclear Energy, University of Belgrade, Serbia
[2]Precision Biomarker Laboratories, Cedars-Sinai Medical Center, Los Angeles, USA
[3]Clinic of Nephrology, University Clinical Centre of Serbia, Faculty of Medicine, University of Belgrade
[4]Institute of Chemistry, Technology and Metallurgy - Institute of National Importance for the Republic of Serbia, University of Belgrade
[5]Digestive Surgery Clinic, University Clinical Centre of Serbia, Faculty of Chemistry, University of Belgrade

[*]e-mail: goranm@inep.ac.rs

Human serum tansferrin (hsTf) is a major circulatory protein crucial for the transport/metabolism of Fe^{3+} ions. By sequestering and delivering ferric ions to target tissues/cells hsTf maintains redox homeostasis. Oxidative stress (OS), one of the hallmarks of (patho)physiological conditions, alters protein structure and function. The main role of hsTf hinges on specific interaction with cellular Tf receptor (TfR) while other interactions contribute to diverse functions. The aim of this study was to profile interacting partners of hsTf in the samples of serum coming from patients diagnosed with a wide range of pathological conditions with underlying OS status. Anti-hsTf pull-down samples were analysed using mass spectrometry. Data went through analysis by appropriate bioinformatic tools. Results reveal differences in expression of hsTf interacting proteins in sample groups of patients suffering from kidney insufficiency subjected to dialysis treatment (peritoneal-PD or hemo-HD) also with patients with gestational diabetes compared to respective healthy sample groups. Colorectal cancer stage T3 versus T2 stage shows an inverse distribution of expression profiles in comparison to healthy samples. Most prominent differences are seen in hsTf interacting partners involved in the complement and coagulation cascades and cholesterol metabolic pathways, suggesting a multifaceted role of hsTf in these processes throughout the course of the disease.

Acknowledgements

This study was supported by the Science Fund of Republic of Serbia (Grant No. 6436701).

Effects of phenolic acids and their metabolites on oxidative stress and inflammation in U937 cells

Jelena Radević, Gorana Ilić*, Ljiljana Milovanović, Tatjana Majkić, Ivana Beara

[1]*Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Serbia*

**e-mail: gorana.ilic4@gmail.com*

Phenolic acids are secondary plant metabolites, which could exhibit different bioactivities, including antioxidant and anti-inflamatory[1]. Oxidative stress, an imbalance between production of reactive oxygen species and antioxidant defence, could lead to cardiovascular and neurodegenerative diseases, diabetes, cancer, etc. Overproduction of prostaglandin E_2 (PGE_2) and thromboxane A_2 (TXA_2) in cyclooxygenase pathway of arachidonic acid metabolism has important role in inflammation. The aim of this study was to determine the total antioxidant potential of thirteen selected phenolic acids and their metabolites (gallic acid; 3-O-methyl gallic acid; 4-hydroxybenzoic acid; 2,5-dihydroxybenzoic acid; 2,6-dihydroxybenzoic acid; 3,4-dihydroxybenzoic acid; 2-hydroxyphenylacetic acid; 3-hydroxyphenylacetic acid; 3,4-dihydroxyphenylacetic acid; *p*-coumaric acid; caffeic acid; ferulic acid; 3-(4-hydroxyphenyl)propionic acid), and their ability to inhibit production of PGE_2 and TXA_2 in *in vitro* cell-based model (human monocytes, U937 cell line). The oxidative stress was induced by AAPH, while DCFH-DA was used to monitor intracellular level of oxidative stress. Results showed significant antioxidant activity of several examined compounds (0.10–2.24 mg trolox equivalents/mmol of substance). The inflammation was induced by LPS and isolated products were quantified using the HPLC-MS/MS technique. It was shown that one compounds significantly exhibited inhibition of PGE_2 production (13.6%), while five of them significantly inhibited TXA_2 production (25– 37%). The results indicate that not all phenolic acids and their metabolites have considerable antioxidant and anti-inflammatory potential at physiological concentrations.

Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2023-14/200125).

References

1. Kiokias S, Proestos C, Oreopoulou V. Phenolic acids of plant origin - A review on their antioxidant activity in vitro (O/W emulsion systems) along with their in vivo health biochemical properties. Foods 2020;9:534-9.

Evaluation of the immunomodulatory potential of chimera Bv1a-BLwt and its mutants on the co-culture model system

Isidora Protić-Rosić[1*], Zorana Lopandić[2], Dragan Popović[3], Gordan Blagojević[4], Marija Gavrović-Jankulović[1]

[1]Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia
[2]Institute for Chemistry in Medicine, Faculty of Medicine, University of Belgrade
[3]Institute of Chemistry, Technology and Metallurgy – National Institute of the Republic of Serbia, University of Belgrade
[4]Allergy and Immunology Diagnostic Department, Institute of Virology, Vaccines and Sera "Torlak", Belgrade, Serbia

[*]e-mail: proticrosic@chem.bg.ac.rs

Allergen immunotherapy (AIT) is currently the only disease-modifying treatment for allergies. Pre-clinical models for the evaluation of novel therapeutics are crucial for ensuring their efficacy and safety. While cell culture models are cost-effective and efficient, they cannot fully replicate the cellular interactions *in vivo*. Therefore, it is essential to use more sophisticated model systems, such as co-cultures, to assess the potential of new therapeutics more accurately. Immunomodulatory protein banana lectin (BLwt) is an attractive candidate for adjuvant in AIT. Its mutant BL_{H84T} was developed to reduce its potential mitogenicity. The aim of this study was the development of the co-culture model system for testing the immunomodulatory effect of chimeras composed of the major birch pollen allergen (Bv1a) and BLwt (Bv1a-BLwt, Cwt), the hypoallergenic isoform of Bv1a (Bv1l) and BL_{H84T} (Bv1l-BL_{H84T}, C1 and BL_{H84T}-Bv1l, C2). Chimeric structures were designed *in silico*, fully minimized, and relaxed without van der Waals atomic clashes. Afterward, proteins were successfully expressed in *Escherichia coli* and purified by IMAC yielding around 0.4 mg per 1L of expression medium. The IgE binding capacity was assessed using ELISA inhibition with birch pollen allergic patients' sera. Caco-2 intestinal epithelial cells and THP-1 differentiated macrophages were used for the co-culture model system development. After protein application on the apical side of the co-culture, the integrity of the epithelial monolayer was not disturbed. The immunomodulatory potential of antigens was tested by measuring the gene expression levels for pro- and anti-inflammatory cytokines in both cell lines from co-culture. The obtained results indicate that the best anti-inflammatory response was favored after treatment with Cwt. Additionally, to further confirm the immunomodulatory effect of the recombinant chimeras, PBMCs obtained from individuals allergic to birch pollen were employed and treated with recombinant proteins. Only after treatment with Cwt, PBMCs secreted the anti-inflammatory cytokine IL-10. Obtained results suggest that Cwt chimera could have a therapeutic effect in AIT in birch pollen allergy.

Acknowledgments

This study was supported by Ministry of Science, Technological Development and Innovation of Republic of Serbia (Grant No. 451-03-47/2023-01/200168).

Comparative *in vitro* analysis of the antioxidant, antigenotoxic, and anti-inflammatory properties of summer and winter savory (*Satureja* spp.)

Jelena S. Katanić Stanković[1*], Vladimir Mihailović[2], Nikola Srećković[2], Sanja Matić[1], Sanja Krstić[3], Anna Nickl[3], Rudolf Bauer[3]

[1]*Department of Science, Institute for Information Technologies, University of Kragujevac, Serbia*
[2]*Department of Chemistry, Faculty of Science, University of Kragujevac*
[3]*Department of Pharmacognosy, Institute of Pharmaceutical Sciences, University of Graz, Austria*

[*]*e-mail: jkatanic@kg.ac.rs*

Satureja spp. (*Lamiaceae*) have been used in traditional medicine as muscle pain relievers, tonics, and carminative agents to treat stomach and intestinal disorders. *Satureja hortensis* L. (summer savory) is among the best-known of the savory genus. It is used as a spice and for digestion improvement. *S. montana* L. (winter savory) has a similar application and is widely used in Serbia as a tea. This study aimed to analyze and compare the biological activities of *S. hortensis* and *S. montana* methanolic extracts. Both extracts showed good antioxidant properties, higher for *S. hortensis* in comparison to *S. montana* extract. The *S. hortensis* and *S. montana* extracts were investigated *in vitro* for the ability to prevent the oxidative damage of DNA induced by hydroxyl and peroxyl radicals. According to the relative electrophoretic band densities at various concentrations (25, 50, 100, and 200 µg/mL), both extracts showed a significant reduction of DNA damage against oxidative changes caused by the free radicals ($HO^{\cdot}$ and $HOO^{\cdot}$). The anti-inflammatory activity was assessed based on the inhibition of cyclooxygenase-1 and -2 (COX-1 and COX-2) activities. The results for *S. hortensis* and *S. montana* methanolic extracts showed strong inhibition of COX-1 (90.87 and 87.03%, respectively) and COX-2 (66.98 and 59.56%, respectively) activities at 50 µg/mL. The obtained results suggest deeper investigations of pharmacological potential and mechanistic studies of both *Satureja* spp.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development and Innovation (Grant No. 451-03-68/2022-14/200378) and the bilateral project of scientific and technological cooperation between the Republic of Serbia and the Republic of Austria (Grant No. 337-00-577/2021-09/9).

Chaperone self-assemblies: Dissociation of DNAJb6 oligomers

Jelica Milošević[1,2*], Sara Linse[2]

[1]*Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia*
[2]*Department of Biochemistry and Structural Biology, Chemical Centre, University of Lund, Sweden*

[*]*e-mail: jelica@chem.bg.ac.rs*

DNJb6 is a chaperone protein expressed in various human tissues. Like other members of the DnaJ family, this protein serves as a co-chaperone for Hsp70 in ATP-dependant increase of amyloid protein solubility. Nevertheless, DNAJb6 also has an autonomous inhibitory effect on amyloid aggregation that is ATP-independent. It is known to inhibit the aggregation of polyQ peptides, amyloid-β, α-synuclein, IAPP, and TDP43. It is also known to be polydisperse and form micelle-like oligomers above roughly 100nM concentration[1]. A recent hypothesis explains the formation of DNAJb6 self-assemblies and its interactions with various clients prone to amyloid fibrillation as a consequence of its high chemical potential[2]. Testing this hypothesis and further understanding of the mechanism of chaperone activity requires a comprehensive study of the nature of both DNAJb6 self-aggregates and potential co-aggregates with amyloid peptides. With the aim to further understand the self-aggregation of DNAJb6, we investigated the kinetic and thermodynamic aspects of its oligomer dissociation using isothermal titration calorimetry (ITC) and Forster resonance energy transfer (FRET). Low enthalpy change upon dissociation of DNAJb6 oligomers indicates that its self-aggregation is highly entropy-driven. The dissociation monitored by FRET appears to be a slow process taking place in the timescale of hours when diluted to below 100nM. Oligomer dissociation is temperature-dependent with the ΔG of 36.3 kJ/mol.

Acknowledgements

This study was funded by grant 2015–00143 from the Swedish Research Council, 2022.0059 from the Knut & Alice Wallenberg Foundation, and 101097824 from the European Research Council.

References

1. Linse S, Thalberg K, Knowles T. The unhappy chaperone. QRB Discov 2021;2:1–10.
2. Carlsson A, Olsson U, Linse S. On the micelle formation of DNAJB6b. QRB Discov 2023;4:1–7.

Soil cyanobacteria as producers of polysaccharides and fatty acids

Jelica Simeunović[1*], Olivera Babić[1], Dajana Blagojević[1], Ivan Milovanović[2], Petar Davidović[1], Tamara Važić[1], Ivana Mihalj[1], Zorica Svirčev[1]

[1]*Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia*
[2]*Institute of Food Technology (FINS), University of Novi Sad*

[*]*e-mail: jelica.simeunovic@dbe.uns.ac.rs*

Cyanobacteria represent a rich source of biotechnologically important compounds. Many of them, such as polysaccharides and fatty acids exhibit a certain type of bioactivity, which is why they are used in the biomedical, pharmaceutical, food, and cosmetic industries as well as environmental protection and bioremediation. In this work, the metabolic potential of selected soil cyanobacterial strains was studied in terms of the production of polysaccharides and fatty acids. The modified colorimetric method adapted to work in microtiter plates, was used to determine the polysaccharide content. Using gas chromatography with a flame ionization detector, the composition of fatty acid methyl esters was determined. The obtained results showed the glucose-rich polysaccharides dominance in all tested strains, whereby the *Nostoc* M1 strain showed an exceptional production capacity. The results of the fatty acid composition analysis indicated the dominance of unsaturated over saturated fatty acids in all tested strains. The most abundant fatty acid was 18-carbon omega-3 α-linolenic acid with the highest content in the *Tolypothrix* K11 strain (43.15%). The fatty acids that also dominated were oleic acid and linoleic acid. The highest prevalence of oleic acid was found in the strain *Phormidium* M1 (22.46%), while the highest prevalence of linoleic acid was detected in the strain *Lyngbya* T7 (35.21%). γ-Linolenic acid was identified only in strains *Calothrix* M2 (25.34%) and *Calothrix* SP2 (49.28%), while eicosapentaenoic acid was found only in *Lyngbya* T7 (2.57%). The 16-carbon long-chain palmitic and palmitoleic acids were also present in most strains tested. The obtained results indicated that soil cyanobacteria can be a significant source of polysaccharides and essential fatty acids with high potential for biotechnological application.

Acknowledgements

This study was supported by the Science Fund of the Republic of Serbia (Grant No. 7726976), Integrated Strategy for Rehabilitation of Disturbed Land Surfaces and Control of Air Pollution–RECAP and by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200125).

Circulatory levels of trace elements in pre-dialysis and hemodialysis patients

Jovana Jagodić[1*], Dragan Manojlović[1], Aleksandar Stojsavljević[2]

[1]*Faculty of Chemistry, University of Belgrade, Serbia*
[2]*Innovative Centre Ltd., Faculty of Chemistry, University of Belgrade*

[*]*e-mail: jovanaj@chem.bg.ac.rs*

The homeostasis of trace elements is affected by the excretion of bile and the good function of kidneys[1]. When normal excretory capacity becomes insufficient as a result of a chronic kidney disease (CKD), haemodialysis is a therapy option that allows important functions to be preserved[2]. Vital substances can be lost if their amounts in the dialysate are lower than the amounts present in whole blood, or if those substances are not present in the dialysate. Taking that into consideration, the elemental profile of haemodialysis patients should be monitored on a regular basis. Therefore, this study aimed to examine and provide elemental status in serum samples of many trace elements (Al, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Cd, Pb, and U) in in haemodialysis patients (HD group) and pre-dialysis patients with stage 3 CKD (PD group) and compare their trace element levels with the healthy individuals. The results have shown that the levels of Cr, Zn, Rb, Cd, and U were lower, while Al, Mn, Co, Ni, Cu, As, Se, Sr, and Pb were higher in HD patients than in the healthy individuals. Lower levels of As, Sr, Zn, Rb, and U levels and higher of Al and Se, were significant in differentiating HD group from PD group. This study also demonstrated that the Cu/Zn ratio might be utilized as a marker for both early and late identification of renal failure. Significant variations in trace element levels in sera imply new pathophysiological processes in CKD, which could contribute to HD patients' pre-existing higher morbidity.

Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia Contract Number: 451–03-9/2021–14/200288 and by the Ministry of Defence of the Republic of Serbia, Project MFVMA/8/15–17.

References

1. Esmaeili M, Rakhshanizadeh F. Serum trace elements in children with end-stage renal disease. J Ren Nutr 2019;29:48–54.
2. Filler G, Felder S. Trace elements in dialysis. Pediatr Nephrol 2014;29:1329–35.

Assessment of miR-27a, miR-222 and miR-340 as indicators of oxidative stress in gestational diabetes

Ognjen Radojičić[1], Jovana Stevanović[2], Ana Penezić[2], Dragana Robajac[2], Miloš Šunderić[2], Goran Miljuš[2], Danilo Četić[2], Daniela Ardalić[1], Vesna Mandić Marković[1,3], Željko Miković[1,3], Olgica Nedić[2], Zorana Dobrijević[2*]

[1]*Gynecology and Obstetrics Clinic "Narodni front", Belgrade, Serbia*
[2]*Department for Metabolism, Institute for the Application of Nuclear Energy, University of Belgrade*
[3]*Faculty of Medicine, University of Belgrade*

[*]*e-mail: zorana.dobrijevic@inep.co.rs*

Gestational diabetes (GDM) is the most frequent pregnancy-related metabolic disorder. It represents a risk factor for both mother and a child, during pregnancy and after delivery, as it contributes to the development of a metabolic disease, obesity, type 2 diabetes mellitus and cardiovascular disease later during life. Accumulating evidence point out the importance of the impairment of the anti-oxidative system as the driving force of the devastating consequences in GDM, while microRNA molecules were associated with major disturbances related to the presence of (glyco)oxidative stress ((g)OS) and emerged as potential sensors and effectors of (g)OS-associated mechanisms. The aim of our study was to investigate the potential of OS-related microRNAs miR-27a, miR-222 and miR-340 from peripheral blood mononuclear cells (PBMCs) to serve as indicators of oxidative stress in GDM. Selected microRNAs were quantified by real-time polymerase chain reaction in samples of patients with GDM and normoglycaemic controls (n=40 each). The expression levels were tested for correlations with the level of *NRF2* mRNA, as well as with the activities of glutathione reductase (GR) and superoxide dismutase (SOD). Significant upregulation was determined for both miR-27a and *NRF2* in GDM, but their expression levels were not correlated. However, the expressions of all three tested microRNAs were found to positively correlate with the activity of GD in GDM samples, while miR-27a and miR-340 further displayed the negative correlation with the SOD activity. GR activity at pregnancy week 24-28 was higher in GDM associated with later pregnancy or neonatal complications, while the analysed microRNAs failed to demonstrate the prognostic significance. The presented results illustrate the potential of microRNAs to indicate the specific OS-related changes associated with GDM.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200019).

Evaluation of long noncoding RNAs *H19* and *MALAT1* as oxidative stress indicators in gestational diabetes

Jovana Stevanović[1], Ana Penezić[1], Ognjen Radojičić[2], Dragana Robajac[1], Miloš Šunderić[1], Goran Miljuš[1], Danilo Četić[1], Daniela Ardalić[2], Milica Mandić[2], Vesna Mandić Marković[2,3], Željko Miković[2,3], Olgica Nedić[1], Zorana Dobrijević[1*]

[1]*Department for Metabolism, Institute for the Application of Nuclear Energy, University of Belgrade, Serbia*
[2]*Gynecology and Obstetrics Clinic "Narodni front", Belgrade, Serbia*
[3]*Faculty of Medicine, University of Belgrade*

[*]*e-mail: zorana.dobrijevic@inep.co.rs*

Numerous lines of evidence point out the crucial involvement of the dysregulation of long non-coding RNAs (lncRNAs) in the molecular pathogenesis of human pathologies, including gestational diabetes mellitus (GDM). Among frequently analysed lncRNAs in terms of biomarkers properties and/or mechanistic roles in diabetic conditions are those involved in the regulation of oxidative stress (OS) response and inflammation. Since these interconnected processed are involved in the aetiology of GDM and severe obstetric and neonatal complications, the aim of this study was to evaluate the utility of OS-related *H19* and *MALAT1* as indicators of OS response in GDM. Relative quantification of selected lncRNAs in samples of patients with GDM and normoglycaemic controls (n=50 each) was performed by real-time polymerase chain reaction. The expression levels were tested for correlations with *NRF2* expression, as well as with the activities of glutathione reductase (GR) and superoxide dismutase (SOD). Significant downregulation was determined for *H19* (p=0.012), while *MALAT1* (p=0.009) and *NRF2* (p=0.006) were upregulated in GDM, compared to normoglycaemic pregnancy. Furthermore, expressions of *MALAT1* and *NRF2* demonstrated correlation in both GDM and controls (r=0.314 and r=0.390, respectively). However, the expressions of lncRNAs were not found to correlate with the activities of GD and SOD in GDM samples, while the level of *NRF2* mRNA displayed positive correlation with SOD activity. Even though the results did not show the association of *H19* and *MALAT1* with antioxidative enzymatic activities in GDM, correlation of *MALAT1* and *NRF2* expression illustrates the potential of this lncRNA to indicate OS-related changes associated with glucose intolerance in pregnancy.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200019).

ROS and Nf-κB role in repurposed drugs treatment of hamster fibrosarcoma

Kosta J. Popović[1*], Dušica J. Popović[2], Dejan Miljković[3], Dušan Lalošević[3], Ivan Čapo[3], Mihalj Poša[1], Zana Dolićanin[2], Jovan K. Popović[4]

[1]*Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Serbia*
[2]*Department of Biomedical Sciences, State University of Novi Pazar, Serbia*
[3]*Department of Histology and Embryology, Faculty of Medicine, University of Novi Sad*
[4]*Faculty of Medicine, University of Novi Sad*

[*]*e-mail: jovan.popovic@mf.uns.ac.rs; jovapopmf@gmail.com*

The NF-κB activity in cancer cells promotes tumor growth by stimulation of cell proliferation, neoangiogenesis and by inhibition of apoptosis and ROS activity[1]. Anticancer combinations of two repurposed drugs: metformin[2] with 2-deoxy-*D*-glucose, deoxycholic acid, caffeine, itraconazole or disulfiram (< 50% LD_{50}, equivalent to usual human dose) mechanism of action was investigated by rescuing treated BHK-21/C13 fibrosarcoma growth in hamsters with ROS inhibitor nitroglycerin and NF-κB stimulator mebendazole. 19 days after inoculation, anticancer effects were assessed by biophysical measurements of fibrosarcoma growth and immunohistochemical markers of tumor proliferation (Ki-67, PCNA), neoangiogenesis (CD34, CD31), glucose metabolism (GLUT1), NO metabolism (iNOS) and apoptosis (COX4, Cytochrome C). The combinations have shown significant antitumor effects ($P < 0.05$) which were inhibited partly by addition of ROS inhibitor nitroglycerin and completely by NF-κB stimulator mebendazole. Results indicate the key role of NF-κB in anticancer action of investigated drug combinations of non-oncological drugs and their potential to be used in oncology.

Acknowledgements

This study was supported by the Republic of Serbia, Autonomous Province of Vojvodina, Provincial Secretariat for High Education and Scientific Research (Grant Nos. 142-451-3155/2022-02 (JM), 142-451-2626/2021 (DL)) and the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/200114).

References

1. Yenmis G, et al. Anti-cancer effect of metformin on the metastasis and invasion of primary breast cancer cells through mediating NF-kB activity. Acta Histochem 2021;123:151709.
2. Buczyńska A, Sidorkiewicz I, Krętowski AJ, Zbucka-Krętowska M, Adamska A. Metformin intervention-A panacea for cancer treatment? Cancers (Basel) 2022;14:1336.

Diet- and age-dependent changes of intestinal injury in rats

Ksenija Veličković[1*], Milica Markelić[1], Ana Stančić[2] , Vesna Otašević[2], Anđelija Gudelj[2], Nevena Savić[2], Vesna Martinović[2], Ilijana Grigorov[2]

[1]Department of Cell and Tissue Biology, Faculty of Biology, University of Belgrade, Serbia
[2]Department of Molecular Biology, Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade

[*]e-mail: ksenija@bio.bg.ac.rs

A high-sugar diet is associated with an increased risk of chronic intestinal disease[1], but the capacity of the gut to adapt to dietary changes in young and adult rats is unknown. Therefore, the effects of an 8-week dextrose-enriched diet (20% or 60%) on intestinal histology, antioxidative defence status, and the expression pattern of high mobility group box-1 (HMGB1), a mediator of the inflammatory response[2], were investigated. Numerous signs of tissue damage were associated with decreases in villus height (Vh), crypt depth (Cd), villus surface area (VSA), and muscle thickness (Mt) in adult rats fed with 60% dextrose. While the decrease in Vh and Cd was affected by age, the decrease in VSA and Mt was interactively affected by age and treatment. Structural changes were associated with decreased activity of antioxidative defence enzymes, particularly catalase, which is affected by treatment, and CuZnSOD, which is interactively modulated by age and treatment. Moreover, massive translocation of HMGB1 from the nucleus to the cytoplasm was detected in epithelial cells in the same group (interactive effect of age and treatment). We concluded that supraphysiological dextrose concentrations induce changes in the oxidative state, possibly leading to redox modification of HMGB1 and consequent tissue damage. The intestine undergoes dynamic functional and morphological changes with age that are more pronounced under high dextrose concentrations.

Acknowledgements

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2022-14/ 200007).

References

1. Rippe JM, Angelopoulos TJ. Relationship between added sugars consumption and chronic disease risk factors: Current understanding. Nutrients 2016;8:697.
2. Chen R, et al. The mechanism of HMGB1 secretion and release. Exp Mol Med 2022;54:91–102.

Glutamine deficiency suppresses adipogenic differentiation *in vitro*

Ksenija Veličković[1,2*], Hilda Anaid Lugo Leija[1], Amal Surrati[1], Dong-Hyun Kim[1], Harold Sacks[3], Michael E. Symonds[4], Virginie Sottile[1,5]

[1]*School of Medicine, University of Nottingham, United Kingdom*
[2]*Faculty of Biology, University of Belgrade, Serbia*
[3]*VA Endocrinology and Diabetes Division, Department of Medicine, University of California, Los Angeles, USA*
[4]*The Early Life Research Unit, Division of Child Health, Obstetrics and Gynaecology, University of Nottingham*
[5]*Department of Molecular Medicine, University of Pavia, Italy*

[*]*e-mail: ksenija@bio.bg.ac.rs*

Glutamine (Gln) is the major source of energy in cells after glucose and has recently been shown to be an important carbon source for *de novo* lipogenesis[1]. To investigate whether Gln status affects adipocyte differentiation, mouse mesenchymal stem cells (MSCs) were subjected to Gln deprivation during differentiation and compared with MSCs differentiated in Gln-supplemented medium (5, 10, and 20 mM). Gln deprivation decreased adipogenic differentiation and lipid droplet formation, intracellular Gln concentration and gene expression for classic adipogenic markers including PPARγ. Also, glutamine restriction suppressed gene expression of isocitrate dehydrogenase 1 (IDH1), an enzyme that produces citrate for lipid synthesis. In contrast, Gln supplementation promoted adipogenic differentiation in a dose-dependent manner. These results suggest that the Gln pathway may have a previously overlooked role in adipogenesis. The underlying mechanism involving the Gln-IDH1 pathway may represent a potential therapeutic strategy to treat excessive lipid accumulation and, consequently, obesity.

Acknowledgements

This study was supported by the EU-CASCADE fellowship scheme funded by the EU's 7th FP PCOFUND-GA-2012-600181 (KV), by CONACyT Mexico (HALL), and received support from the Cardiometabolic Disease Research Foundation (Los Angeles, USA). Part of the methods utilised were developed through work that was supported by the BBSRC Doctoral Training Partnership (Grant No. BB/F017014/1).

References

1. Yoo H, Stephanopoulos G, Kelleher JK. Quantifying carbon sources for de novo lipogenesis in wild-type and IRS-1 knockout brown adipocytes. J Lipid Res 2004;45:1324-32.

Exploring if *Porphyra* sp. extract functions as serum substitute in HT29 cell culture

Luka Veličković[1], Nikolina Sibinčić[2], Marija Stojadinović[3], Nikola Gligorijević[4], Milan Nikolić[1], Tatjana Srdić[5], Simeon Minić[1]

[1]Department of Biochemistry, Centre of Excellence for Molecular Food Sciences, Faculty of Chemistry, University of Belgrade, Serbia
[2]Innovative Centre Ltd., Faculty of Chemistry, University of Belgrade
[3]Faculty of Chemistry, University of Belgrade
[4]Institute of Chemistry, Technology and Metallurgy - National Institute of Republic of Serbia, University of Belgrade
[5]Institute for Oncology and Radiology of Serbia, Belgrade, Serbia

[*]e-mail: velickovicluka12@gmail.com

This study investigates the impact of *Porphyra sp.* extracts on HT29 cell line growth and viability at reduced serum conditions. The concentration-dependent effects of phycobiliproteins (PBPs) on cell proliferation were examined over various time intervals. Lower concentrations of PBPs (20 μg/mL) demonstrated an increase in HT29 cell viability after 48 hours and 5 days of cultivation at reduced serum concentration (final serum concentration was in the range from 5 to 8%). This suggests a potential positive influence on cell proliferation, likely due to their antioxidant properties. Conversely, higher concentrations of PBPs exhibited inhibitory effects on cell growth, possibly due to cytotoxicity at elevated levels. Remarkably, when HT29 cells were cultured solely in algal extract without fetal calf serum (FCS), complete growth inhibition was observed after 72 hours. This finding underscores the insufficient nutrient and growth factor provision of PBPs alone for sustaining cell viability. Morphological differences observed in cells cultured with 70 μg/mL of PBPs indicated potential alterations in cellular morphology. Notably, 70 μg/mL of PBPs in RPMI medium with 5% FCS displayed growth inhibition compared to the control (5% FCS). Furthermore, we assessed HT29 cell adaptability to changes in FCS concentration and PBP supplementation. Cells incubated under varying FCS and PBP conditions were subcultured into RPMI medium with lower FCS concentration and PBPs from *Porphyra*. The viability of cells following subculturing indicated sustained adaptability to reduced FCS levels. Overall, this study provides valuable insights into the concentration-dependent effects of PBPs from *Porphyra* extracts on HT29 cell growth and viability. The findings underscore the potential benefits of PBPs at lower concentrations for cell proliferation at reduced serum conditions and reveal the adaptability of HT29 cells to changing culture conditions.

Acknowledgements

This study was supported by the Good Food Institute, Field Catalyst Grant Program 2022 (Alg2Meat project) and Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200168).

Influence of bile acids and their derivatives on drug transport into central nervous system

Marija Gvoić[1*], Saša Vukmirović[1], Bojan Stanimirov[2], Karmen Stankov[2]

[1]*Department of Pharmacology and Toxicology, Faculty of Medicine, University of Novi Sad, Serbia*
[2]*Department of Biochemistry, Faculty of Medicine, University of Novi Sad*

[*]*e-mail: 1109d16@mf.uns.ac.rs; m_skendzic@yahoo.com.*

Primary brain tumors as well as central nervous system (CNS) metastases are in focus of clinical and scientific interest because of the complexity of their treatment due to their location behind the blood-brain barrier (BBB). The main obstacle for efficacy of antitumor drugs in treatment of brain tumors is their low penetrance across the BBB and decreased drug delivery to malignant cells. Previously published results of our scientific group show that bile acids (BAs) and their derivatives have impact on improvement of the absorption, bioavailability and pharmacodynamic of several drugs[1,2]. In addition, current preliminary results of our group provides the evidence supporting the claim that antitumor drugs have the ability to cross the BBB when administered with BAs. That ability can be exploited by taking a part in novel drug carrier designs. BAs represent a drug carrier system as a part of a mixed micelle composition, bilosomes and conjugates with various drugs. Modern strategies addressed to this issue comprize intranasal drug administration, ligand conjugation, BBB disruption and use of BAs and nanomaterials for CNS drug delivery. Our present research discusses the current knowledge related to bile acid molecules as drug penetration modifying agents, with the focus on central nervous system antitumor drug delivery.

Acknowledgements

This study was supported by the grant from Ministry of Education, Science and Technological Development, Republic of Serbia III41012 (Grant No. 451-03-47/2023-01/200114), and Project of special interest for sustainable development in the Autonomous Province of Vojvodina (Grant No. 142-451-2532/2021-01).

References

1. Gvoic M, Vukmirovic S, Al-Salami H, Mooranian A, Mikov M, Stankov K. Bile acids as novel enhancers of CNS targeting antitumor drugs: A comprehensive review. Pharm Dev Technol 2021;26617-33.
2. Vasović V, et al. Influence of bile acid derivates on morphine analgesic effect in mice. Vojnosanitetski Pregled 2014;71:767-71.

The amino acid substitution N204H affects the interactions of CYP2C9 with ligands

Maryia Kisel*, Andrei Gilep

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Minsk, Belarus

**e-mail: marusen.kee@gmail.com*

From the fundamental and practical perspectives, it is fairly important to gain an accurate and deep understanding of the structural consequences of natural genetic polymorphism in the cytochrome P450 (CYP) superfamily enzymes, as any of them may lead to altered functional state of these proteins. The missense variant CYP2C9.57 exhibits a single amino acid mutation N204H (2C9_N204H). The mutated position is within the highly conserved substrate recognition site-2 (SRS-2) region. The native amino acid residue is substituted with a more hydrophobic and bulkier amino acid[1]. The present study is focused on determination of ligand-binding properties of 2C9_N204H in comparison with wild-type CYP2C9 by the spectrophotometric titration through estimation of spectral dissociation constants as well as spectral signatures for several CYP2C9 substrates and inhibitors.

In current work, difference spectra obtained in the presence of the azole-containing type II ligands miconazole and econazole showed conventional spectral response with 1.7-fold higher affinity for 2C9_N204H. While the proteins were titrated with type I ligands, diclofenac and tamoxifen, a non-typical, reverse type II response with λ_{max} = ~410 nm and λ_{min} = 430–437 nm was observed. Unexpectedly, in some cases, the early spectral change corresponding to reverse type II then switched to typical I (for diclofenac) or II (for fluconazole and cyproconazole). The 2C9_N204H led to the second binding mode appearance (for cyproconazole) or disappearance (for diclofenac) in comparison with the wild-type. Based on our results it may be concluded that the amino acid substitution N204H affects the spectral manner and binding affinity of CYP2C9 protein interactions with some of its substrates and inhibitors defined as the type I and type II ligands.

Acknowledgements

This work was supported by the National Academy of Sciences of Belarus (Grants Nos. 2021-27-170 and 2022-27-023).

References

1. Nahar R, et al. Implication of novel CYP2C9*57 (p.Asn204His) variant in coumarin hypersensitivity. Thromb Res 2013;131:535–9.

Proferroptotic response to nutrient deprivation in hepatocellular carcinoma cells is related to p53 status

Milica Markelić[1*], Vesna Otašević[2], Andjelija Gudelj[2], Tamara Saksida[2], Ana Stančić[2], Ksenija Veličković[1], Jelena Krstić[3]

[1]*Faculty of Biology, University of Belgrade, Serbia*
[2]*Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade*
[3] *"Gottfried Schatz" Research Center, Medical University of Graz, Austria*

[*]*e-mail: milica.markelic@bio.bg.ac.rs*

Recently, it has been suggested that nutrient deprivation (ND) may be effective as an adjuvant therapy to hepatocellular carcinoma (HCC) cell treatment with sorafenib (Sfb)[1]. These results suggest that ND-mediated priming of HCC cells to Sfb is positively correlated with the p53 status, suggesting the essential role of p53 in priming of HCC cells for regulated cell death (RCD). Preliminary data indicated morphological signs of ferroptotic RCD, so we aimed to determine whether ferroptosis plays a role in the removal of HCC cells *in vitro* with respect to their p53 status. To this end, p53 wild-type (p53WT) and p53 knockout (p53KO) HepG2 cells were grown in growth medium or in starvation medium and treated with Sfb or with ferroptosis inducer, Rsl-3, for 6 h. Morphological signs of RCD and nuclear translocation (i.e. activation) of Nrf2, (master regulator of ferroptosis-related signalling pathways), as well as protein levels of antioxidative defence (AD) enzymes (CAT, CuZnSOD, MnSOD) and ferroptosis-related proteins (GPX4, xCT) were analysed. The AD response to Rsl-3 treatment in p53WT cells was similar regardless of nutritional status, as the level of all analysed enzymes increased. The response to Sfb was enhanced by ND as CAT and CuZnSOD were elevated. p53KO cells responded quite differently, even when treated with Rsl-3, increasing only MnSOD. Starved Sfb-treated p53KO cells even decreased expression of AD enzymes. All signs of a proferroptotic response examined were present in starved p53WT cells (regardless of treatment): decreased nuclear translocation of Nrf2, GPX4, and xCT expression. Nrf2 activation and GPX4 expression were also decreased in starved p53KO cells (especially upon treatment with Sfb or Rsl-3), but accompanied by compensatory overexpressed xCT. These results may be indicative of enhanced AD in p53KO cells and may therefore explain, at least in part, their resistance to treatment with Sfb+ND which, as presented here, induces ferroptosis in p53WT HepG2 cells.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development and Innovations of Serbia - Project of bilateral cooperation with Austria (Grant No. 337-00-577/2021-09/29).

References

1. Krstic J, et al. Fasting improves therapeutic response in hepatocellular carcinoma through p53-dependent metabolic synergism. Sci Adv 2022;8:eabh2635.

The effect of pH on coordination interactions between levofloxacin and Fe^{3+} in water

Milica R. Milenković[1*], Jelena Korać Jačić[2], Ivan Spasojević[2]

[1]*Department of General and Inorganic Chemistry, Faculty of Chemistry, University of Belgrade, Serbia*
[2]*Department of Life Sciences, University of Belgrade - Institute for Multidisciplinary Research*

[*]*e-mail: mrm@chem.bg.ac.rs*

Interactions of drugs with redox-active biometals can affect their pharmacological activity by changing their solubility, bioavailability, and redox properties or leading to their decomposition by hydrolysis or redox processes[1,2]. The effect of pH on metal ion-oral drug interactions is of special importance considering the pH changes along the digestive tract (GIT). Iron can be present in significant concentration in GIT due to the intake of iron-rich foods and supplements or celiac disease[3]. Herein, we investigated the interactions of Fe^{3+} with levofloxacin (LEV) in water at different pH using UV-Vis spectroscopy. The coordination of Fe^{3+} with LEV is pH dependent showing pH 5 as optimum for complex formation. The determined stoichiometry of Fe^{3+}- LEV complex is 1:3 (Fe^{3+}:LEV). Cyclic voltammetry results showed a lower tendency of Fe^{3+} in the complex toward reduction into Fe^{2+}, while LEV coordinated with Fe^{3+} is slightly more susceptible to oxidation than free LEV.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development and Innovation of Republic of Serbia (Grant Nos. 451-03-47/2023-01/200053 and 451-03-47/2023-01/200168).

References

1. Božić B, et al. Coordination and redox interactions of β-lactam antibiotics with Cu2+ in physiological settings and the impact on antibacterial activity. Free Radical Biol Med 2018;129:279–85.
2. Chen J, Wang Y, Qian Y, Huang T. Fe(III)-promoted transformation of β-lactam antibiotics: Hydrolysis vs oxidation. J Hazard Mater 2017;335:117–24.
3. Talarico V, Giancotti L, Mazza GA, Miniero R, Bertini M. Iron deficiency anemia in celiac disease. Nutrients 2021;13:1695.

Interactions of different urolithins with human serum albumin: Insights from fluorescence spectroscopy

Nevena Zelenović[1*], Lidija Filipović[2], Milica Kojadinović[3], Milica Popović[4]

[1]Center for Chemistry, Institute for Chemistry, Technology and Metallurgy - National Institute of Republic of Serbia, University of Belgrade, Serbia
[2]Innovative Centre Ltd., Faculty of Chemistry, University of Belgrade
[3]Department for Nutritional Biochemistry and Dietology, Centre of Research Excellence in Nutrition and Metabolism, Institute for Medical Research - National Institute of Republic of Serbia, University of Belgrade
[4]Department of Biochemistry, Faculty of Chemistry, University of Belgrade

[*]e-mail: nevenazelenovic@gmail.rs; zelenovic@ihtm.bg.ac.rs

Urolithins (UROs), metabolites of ellagic acid (EA), are present in ellagitannins (ETs)-containing food such as fruits, nuts, and oak-aged wines[1]. In the presence of gut microbiota, ETs are hydrolyzed to EA, which is poorly absorbed in the intestines and therefore undergoes the reaction of decarboxylation of the one lactone ring and successive removal of hydroxyl groups, forming several URO isomers[2]. Free and conjugated UROs are found in the systemic circulation after ingestion. In the circulation, UROs interact with serum proteins and are transported to tissues where they perform their biological role (anti-inflammatory, anticarcinogenic, antiglycative, antioxidant, and antimicrobial)[1,2]. The interactions of UROs with HSA as carrier can be examined using fluorescence spectroscopy, where UROs act as molecular quenchers. The fluorescence emission spectra were performed at different temperatures (298 K and 310 K). The fluorescence intensity of the HSA decreased with increasing UROs concentration, but did not lead to a shift in the maximum emission λem[1-3]. The obtained constants decreased with temperature, indicating a static mechanism of binding (UroC Ksv 11.236×10^4 M^{-1} and UroD Ksv 4.028×10^4 M^{-1}; UroC 0.9007×10^4 M^{-1} and UroD Ksv 0.129×10^4 M^{-1} at 310K). The binding affinity of UROs to HSA is dependent on their hydrophobicity. Corresponding aglicons have higher binding contants in comparison with conjugated (UroB Ksv 5476×10^4 M^{-1} and URO BG 130×10^4 M^{-1} at 298K; UroB Ksv 6.301×10^4 M^{-1} and URO BG 2.46×10^4 M^{-1} at 310K).

Acknowledgements

This work was funded by the Ministry of Education, Science, and Technological Development of Republic of Serbia (Grant Nos: 451-03-9/2021-14/200288, 451-03-68/2022-14/200288, and 451-03-68/2022-14/200015).

References

1. Zelenovic N, Kojadinovic M, Filipovic L, Vucic V, Milcic M, Arsic A, Popovic M. Interactions of different urolithins with bovine serum albumin. Nat Prod Commun 2023;18:1-11.
2. Rashtbari S, et al. Interaction of bovine serum albumin with ellagic acid and urolithins A and B: Insights from surface plasmon resonance, fluorescence, and molecular docking techniques. Food Chem Toxicol 2022;162:112913.
3. Jing Z, Xuan G, Jinyang H, and Honghui W. Probing the interaction between human serum albumin and 9-Hydroxyphenanthrene: A spectroscopic and molecular docking study. ACS Omega 2020;5:16833-40.

Expression of recombinant SARS-CoV-2 nucleocapsid protein in mammalian cells

Nikolina Sibinčić[1*], Maja Krstić Ristivojević[2], Marijana Stojanović[3], Maja Mladenović Stokanić[2], Tamara Vasović[2], Tanja Ćirković Veličković[2,4], Marija Stojadinovic[2]

[1]Innovative Centre Ltd., Faculty of Chemistry, University of Belgrade, Serbia
[2]Department of Biochemistry, Center of Excellence for Molecular Food Sciences, Faculty of Chemistry, University of Belgrade
[3]Institute for Biological Research "Siniša Stanković" - National Institute of the Republic of Serbia, University of Belgrade
[4]Serbian Academy of Sciences and Arts, Belgrade

[*]e-mail:nsibincic@chem.bg.ac.rs

The SARS-CoV-2 nucleocapsid (N) protein plays a significant role in the coronavirus life cycle and participates in a variety of critical events following viral invasion[1]. In infected patients, high titers of immunoglobulin G (IgG) targeting N protein were detected and correlated with the clinical course of the disease[2]. Therefore, N protein and anti-N protein IgGs were recognized as important diagnostic indicators of COVID-19 infection in serological and quick antigen tests[3]. In this study, we optimized the expression of the recombinant form of SARS-CoV-2 N protein in a mammalian cell line HEK293T by comparing the transfection efficiency between Polyethylenimine (PEI) and Calcium Phosphate (CaP) DNA-complexing agents. Transfection potency was tested at different cell confluence and passage number, in several cell culture media, pre-transfection and post-transfection media change and in conditions of reduced serum. Chloroquine and glycerol treatments were included to enhance transfection efficiency as they might inhibit DNA degradation in lysosomes or increase membrane permeability. Protein expression was monitored in cell supernatants up to 7 days post-transfection in dot-bot and Western blot using anti-N protein antibodies. Both transfection methods have shown moderate to relatively high transfection efficiency dependent on the applied conditions, making them affordable and easy to use techniques for recombinant N protein production on a small-scale in adherent mammalian systems. PEI acts as a good delivery system regardless of the presence of the fetal bovine serum (FBS), while CaP transfection is more dependent on the presence of FBS which in turn favors N protein degradation. However, we have optimized both methods to achieve optimal expression of unfragmented N-protein in serum-free conditions. Apart from setting up a cost-effective platform for expression of N protein in mammalian cells, we plan on investigating the mechanisms behind the PEI and CaP non-viral gene delivery systems as there are still some uncertainties in the scientific community.

Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia (Grant Nos. 451-03-68/2022-14/200168 and 451-03-68/2022-14/200288). The authors have used antibodies obtained through the project COVID-19-CAPSIDO supported by the Science Fund of the Republic of Serbia under the Grant No. 7542203.

References

1. Wu W, et al. The SARS-CoV-2 nucleocapsid protein: Its role in the viral life cycle, structure and functions, and use as a potential target in the development of vaccines and diagnostics. Virol J 2023;20:1-16.
2. Di D, et al. Recombinant SARS-CoV-2 nucleocapsid protein: Expression, purification, and its biochemical characterization and utility in serological assay development to assess immunological responses to SARS-CoV-2 infection. Pathogens 2021;10:1039.
3. Garcia-Cordero J, et al. Recombinant protein expression and purification of N, S1, and RBD of SARS-CoV-2 from mammalian cells and their potential applications. Diagnostics 2021;11:1808.

Biochemical parameters according to the National Cholesterol Education Program (NCEP) for the prediction of metabolic syndrome in adolescents

Olgica Martinis*

Education and Teaching Training Agency, Zagreb, Croatia

**e-mail: olgica.martinis69@gmail.com*

Research on eating habits and prediction for metabolic syndrome (MetS) of adolescents aged 14 to 17 years was conducted in three regions of Croatia due to predisposition to different types of diet - Mediterranean diet (respondents from Hvar), continental diet - urban population of Central Croatia (Zagreb City, Pregrada, Varaždin) and Eastern Croatia (Beli Manastir). This study aimed to determine the nutritional status and prediction of MetS in adolescents according to the National Cholesterol Education Program (NCEP). Values of parameters for predicting MetS according to NCEP include anthropometric criterion (waist circumference), blood pressure measurement and biochemical parameters - glucose concentration (GUC), HDL cholesterol and triglycerides (TG)[1]. GUC concentration values in the examined adolescents confirm that one subject with MetS prediction has elevated GUC values before education and after education 2 subjects (≥ 5.6 mmol/L) according to NCEP criteria. HDL cholesterol concentrations were lowered in all subjects predicted for MetS according to the NCEP criteria before and after education[2]. In contrast, the differences in the representation of subjects concerning TG values were not significant either before or after education. Based on the results of this research, the importance of individual biochemical parameters, GUC, HDL cholesterol and TG concentration for determining the prediction of MetS in adolescents can be concluded.

References

1. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). J Am Med Assoc 2001;285:2486-97.
2. Weiss R, et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med 2004;350:2362-74.

Expression of miR-21-5p and glutathione peroxidase 1 (GPX1) in patients with steatosis and T2D

Sanja Erceg[1*], Miloš Mitrović [2], Ratko Tomašević[3], Jelena Munjas[1], Miron Sopić[1], Boško Misita[1], Milica Mamić[3], Aleksandra Klisić[4], Omar Ben Mariem[5], Ana Ninić[1]

[1]Faculty of Pharmacy, University of Belgrade, Serbia
[2]Clinical Hospital Center "Zvezdara", Belgrade, Serbia
[3]Clinical Hospital Center "Zemun", Belgrade
[4]Faculty of Medicine, University of Montenegro, Podgorica, Montenegro
[5]Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy

[*]e-mail: sanja.erceg@pharmacy.bg.ac.rs

Non-alcoholic fatty liver disease is a metabolic disorder caused by disturbances in glucose and lipid metabolism and is closely associated with type 2 diabetes (T2D) and oxidative stress[1]. In this study, we investigated whether microRNA-21-5p (miR-21-5p) and the antioxidant enzyme glutathione peroxidase-1 (GPX1) are associated with steatosis and T2D. The study included 203 participants who underwent ultrasonography and were divided into three groups: 66 patients with steatosis and T2D (group 1), 86 patients with steatosis (group 2), and 51 apparently healthy controls (CG). Both miR-21-5p and GPX1 messenger ribonucleic acid (mRNA) expression were determined by real-time polymerase chain reaction. The miR-21-5p expression was statistically significantly lower in group 1 (P=0.045), but higher in group 2 (P<0.001) than in CG. The expression of miR-21-5p was also statistically significantly lower in group 1 compared with group 2 (P<0.001). GPX1 mRNA expression was statistically significantly lower in both patient groups compared to CG (P=0.004). Spearman correlation analysis showed a significant positive correlation between miR-21-5p and GPX1 mRNA expression in all participants (P<0.001). Our results showed altered expression of miR-21-5p and GPX1 mRNA in participants with steatosis and T2D and participants with steatosis compared to CG.

Acknowledgements

This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia (Grant No: 451-03-68/2022-14/200161).

References

1. Kumar V, Xin X, Ma J, Tan C, Osna N, Mahato RI. Therapeutic targets, novel drugs, and delivery systems for diabetes associated NAFLD and liver fibrosis. Adv Drug Deliv Rev 2021;176:113888.

Antitumor potential of novel triphenyltin(IV) complexes with carboxylato *N*-functionalized 2-quinolone ligands

Sanja Jelača[1*], Marijana P. Kasalović[2,3], Nebojša Đ. Pantelić[4], Goran N. Kaluđerović[2], Sanja Mijatović[1], Danijela Maksimović-Ivanić[1]

[1]Department of Immunology, Institute for Biological Research "Siniša Stanković" - National Institute of the Republic of Serbia, University of Belgrade, Serbia
[2]Department of Engineering and Natural Sciences, University of Applied Sciences, Merseburg, Germany
[3]Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia
[4]Department of Chemistry and Biochemistry, Faculty of Agriculture, University of Belgrade

[*]e-mail: sanja.jelaca@ibiss.bg.ac.rs

Cancer is responsible for millions of deaths worldwide each year and, although great advances have been made in the treatment options, there are still many issues that must be addressed in order to improve cancer therapy. In the present work, anticancer effect of three novel Ph_3SnL complexes ($L1^-$,3-(4-methyl-2-oxoquinolinyl-1(2H)-yl)propanoato; $L2^-$,2-(4-methyl-2-oxoquinolin-1(2H)-yl)ethanoato; $L3^-$,2-(4-hydroxy-2-oxoquinolin-1(2H)-yl)ethanoato), was evaluated against several cancer cell lines (MCA-7, A375, HCT116, 4T1, B16 and CT26). The applied treatment decreased cell viability of all cell lines after 72 h in a dose-dependent manner with IC_{50} values in the low micromolar range. Flow cytometric assessment revealed apoptotic cell death in A375 but not B16 culture, exposed to tested drug. Morphological signs of apoptosis such as shrunk nuclei and condensed chromatin were further confirmed by fluorescent microscopy. Same treatment in B16 lead to cell division block coupled with two-fold increase in the amount of melanin and tyrosinase activity, indicating the differentiation of B16 cells towards melanocytes. In the background of different response of two melanoma cell lines lies dissimilar redox response to the treatment. While in A375 cultures, ROS/RNS production is inhibited in comparison to control, in B16 cells compound Ph_3SnL1 provokes ROS/RNS generation. Finally, when applied in therapeutic regiment, Ph_3SnL1 significantly reduced tumor volume in C57BL/6 mice.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (Grant Nos. 451-03-47/2023-01/200007 and 451-03-47/2023-01/200116).

Shikonin derivatives trigger phenotype reprogramming of B16 mouse melanoma cells

Tamara Krajnović[1*], Dijana Bovan[1], Nenad L. Vuković[2], Milena D. Vukić[2], Sanja Mijatović[1], Nikola Tanić[1], Nebojša Arsenijević[3] , Danijela Maksimović-Ivanić[1]

[1]Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade, Serbia
[2]Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia
[3]Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac

[*]e-mail:tamara.krajnovic@ibiss.bg.ac.rs

Shikonin is a naphthoquinone found in the roots of plants of the *Boraginaceae* family and is widely known for its numerous biological activities, including anticancer. In this study, the antitumor mode of action of shikonin derivatives isolated from the roots of *Onosma visianii* was investigated in mouse melanoma cell line B16. MTT and CV assays showed that six examined shikonins decreased B16 cell viability in a dose-dependent manner, with compounds 5 and 6 exhibiting the highest cytotoxic activity. This effect correlated with caspase-mediated apoptosis, which was detected by flow cytometry and fluorescence microscopy. In addition, CFSE staining revealed a strong blockage of cell division in response to treatment, with a more profound effect of compound 6. The altered cell morphology together with the loss of dividing potential upon exposure to both shikonins implied reprogramming of the B16 cell phenotype. The absence of melanogenesis enhancement coupled with an elevated level of myelin basic protein in response to treatment with both tested agents suggested that the cells transdifferentiated into a Schwann-like phenotype, with possible involvement of the autophagic process in this conversion. Differentiation of malignant cells has become favourable in cancer treatment, bearing in mind the phenomenon of apoptosis-induced proliferation. Hence, the specific antitumor mode of action of shikonin derivatives on melanoma *in vitro* shown here provides a good platform for new investigations of these promising natural compounds.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200007) and joint funding of Development and Research Projects of the Republic of Serbia and the People's Republic of China (Grant No. 06/2018).

Cisplatin-naproxen conjugate free and loaded in SBA-15 indicate morphological changes and antitumor activity *in vivo* in mouse melanoma model

Teodora Komazec[1*], Ekatarina Mihajlović[1], Dijana Bovan[1], Sanja Mijatović[1], Ivana Predarska[2,3], Goran N. Kaluđerović[2], Evamarie Hey-Hawkins[3], Danijela Maksimović-Ivanić[1]

[1]Department of Immunology, Institute for Biological Research "Siniša Stanković" - National Institute of the Republic of Serbia, University of Belgrade, Serbia
[2]Department of Engineering and Natural Sciences, University of Applied Sciences, Merseburg, Germany
[3] Institute of Inorganic Chemistry, Faculty of Chemistry and Mineralogy, Leipzig University, Germany

**e-mail: teodora.komazec@ibiss.bg.ac.rs*

Overexpression of cyclooxygenase (COX) and thus, prostaglandin E2 in numerous cancers justified COX inhibitors testing in cancer prevention or treatment[1]. Conjugate molecules of COX inhibitors and common chemotherapeutic drugs, as well as their immobilization in nanoparticles that increases drug delivery and accumulation in tumor tissue, can potentially improve approaches in cancer therapy. Cisplatin-naproxen conjugate and corresponding SBA-15 counterpart decreased the viability of B16 cells. Enlarged and elongated cells with distinctly granular cytoplasm and the increased presence of lipid droplets were noticed after haematoxylin–eosin and Oil Red O staining of treated cultures. In addition, enormous nuclei and markedly heterochromatin foci were confirmed by PI staining indicating establishment of senescent state upon the treatment. Alongside, differentiation of melanoma cells toward melanocytes was demonstrated by elevated tyrosinase activity and presence of melanin, thus leading to reduced tumorigenic potential *in vivo*. In addition, cisplatin-naproxen conjugate and corresponding SBA-15 counterpart significantly reduced melanoma growth in C57BL/6 mice, with lesser signs of toxicity compared to cisplatin as a positive control. Strong antitumor potential of both, free and immobilized conjugates on mouse melanoma cells opens numerous possibilities for further research.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200007).

References

1. Kolawole OR, Kashfi K. NSAIDs and cancer resolution: New paradigms beyond cyclooxygenase. Int J Mol Sci 2022;23:1432.

Determination of Schiff base copper(II) complexes–HSA binding affinity using microscale thermophoresis

Tino Šeba[1*], Aleksandar Mijatović[2*], Rada Baošić[3], Mario Gabričević[1]

[1]*Department of General and Inorganic Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia*
[2]*Department of Chemistry, Faculty of Mining and Geology, University of Belgrade, Serbia*
[3]*Department of Analytical Chemistry, Faculty of Chemistry, University of Belgrade*

**e-mail: tino.seba@gmail.com*

Metal-based anticancer drugs, such as cisplatin, have a significant role in cancer chemotherapy, but their clinical use is limited due to severe toxicity and drug resistance. To overcome these challenges, there has been a rapid expansion in the research and development of more effective and safer metal-based anticancer agents. Copper(II) complexes with Schiff bases as ligands exhibit various biological activities, such as anticancer, antimicrobial, antiviral, or anti-inflammatory. Copper(II) complexes are considered safer alternatives to platinum complexes, as copper is an endogenous metal[1]. The interactions between metal-based drugs with human serum albumin (HSA) play crucial roles in their distribution, metabolism, and activity. To that end, we determined the binding affinities (K_a) of six previously reported Schiff base copper(II) complexes 1–6 to HSA using microscale thermophoresis (MST). MST is a novel technique for quantifying biomolecular interactions based on thermophoresis, the directed movement of molecules in a temperature gradient, depending on various molecular properties such as size, charge, hydration shell, or conformation[2]. K_a, calculated from the MST data, fell within the range of $1.27 \cdot 10^4$–$1.13 \cdot 10^5$ M^{-1}, which indicates a moderately strong binding. Complexes 1–4 display a single transition throughout the titration, indicating a 1:1 binding relationship, while complexes 5 and 6 exhibit two transitions, indicating a 1:2 binding interaction.

Acknowledgments

This study was supported by funding from the Croatian Science Foundation grants (IP-2016-06-3672 and UIP-2017-05-9537), European Structural and Investment Funds (KK.01.1.1.07.0055), European Regional Development Fund FarmInova (KK.01.1.1.02.0021).

References

1. Krasnovskaya O, et al. Copper coordination compounds as biologically active agents. Int J Mol Sci 2020;21:3965.
2. Jerabek-Willemsen M, et al. Microscale thermophoresis: Interaction analysis and beyond. J Mol Struct 2014;1077:101-13.

Molecular dynamics simulation of human sterol-7α-hydroxylase adsorption on the surface of anatase

Yaraslau U. Dzichenka[1*], Natallia E. Boboriko[2]

[1]*Institute of Bioorganic Chemistry of National Academy of Sciences, Minsk, Belarus*
[2]*Chemistry Faculty, Belarusian State University, Minsk*

[*]*e-mail: dichenko@iboch.by*

At present time TiO_2 has gained great attention as a promising photocatalyst due to its excellent optical and electronic properties, high chemical stability, low cost, non-toxicity, and eco-friendliness. Usually it is used for the photodegradation of organic pollutants, but it is also possible to use inorganic material as a component of a conjugated "metal oxide/enzyme" system. Such systems are of great interest for enzymatic synthesis of different compounds in organic chemistry. In present work classical molecular dynamics simulations of TiO_2 with human enzymes, sterol-7α-hydroxylases (CYP7), containing different peptide tags, were performed. Force field parameters (charges on atoms of Ti and O, parameters of vdw interaction) were identified using systematic optimization approach based on the experimentally derived lattice parameters for anatase TiO_2 modification. Molecular dynamics simulation of human CYP7 interaction with the inorganic surface was performed in explicit solvent with periodic boundary conditions. It was found that protein structure remains stable in general and interacts with the surface mainly due to contacts that are formed between O atoms of inorganic material and atoms of positively charged amino acids (lysine, arginine) localized in peptide tags. CYP7 enzymes without added artificial tags form bonds between charged amino acids on the protein surface and O atoms of TiO_2 thus allowing the protein to adsorb differently on the inorganic surface. It was also confirmed during the simulation that water molecules form a layer on the TiO_2 surface, which plays significant role for protein binding to inorganic material. The data obtained will be used for the development of optimal immobilization protocol of human CYP7 enzyme on the surface of TiO_2 nanoparticles.

Acknowledgements

This study was supported by the project "Design of the effective approach for the immobilization of human cytochromes P450 on the surface of nanosize TiO_2 for the creation of photocontrolled catalytic systems" (Project No. X22Y-009).

Analysis of newly designed H1sD2 glycoproteins by bioinformatic tools

Zorana Lopandić[1*], Marija Gavrović-Jankulović[2]

[1]*Institute of Medical Chemistry, Faculty of Medicine, University of Belgrade, Serbia*
[2]*Department of Biochemistry, Faculty of Chemistry, University of Belgrade*

[*]*e-mail: lopandic93@gmail.com*

Allergen-specific immunotherapy (AIT) can induce allergen long-term tolerance after discontinuation and is the only curative approach for allergy treatment[1]. However, because this therapy is not always effective, novel approaches to increase its efficacy, such as the use of virus-like particles[2], are urgently needed. Furthermore, because *N*-glycans influence the innate immune response via pattern recognition receptors, hemagglutinin (HA), a surface glycoprotein and the main antigen of Influenza virus, is a promising adjuvant candidate. House dust mite allergy affects 65-130 million people worldwide[3], with Der p 2 being the most common allergen. To assess the effect of *N*-glycans on innate immunity modulation during AIT, we created *in silico* H1sD2 glycoproteins, which are made up of the receptor-binding domains of HA (H1s) and Der p 2 (D2). The NCBI database was used to obtain protein sequences. Clustal Omega multiple sequence alignment and the BepiPred 2.0 server were used for *in silico* analysis of linear B-cell epitopes. Using the PDB70 database, three-dimensional models of H1sD2 glycoproteins were predicted in ColabFold v1.5.2: AlphaFold 2 with an average RMSD of 0.28. Recombinant glycoproteins were produced in the *Pichia pastoris* expression system and purified using an IMAC/IEX combination. *In silico* analyses revealed that B-cell epitopes differ between chimeras.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development and Innovation of Republic of Serbia (Grant No. 451-03-47/2023-01/200168).

References

1. Pavón-Romero GF, Parra-Vargas MI, Ramírez-Jiménez F, Melgoza-Ruiz E, Serrano-Pérez NH, Teran LM. Allergen immunotherapy: Current and future trends. Cells 2022;11:212.
2. Kucuksezer UC, Ozdemir C, Cevhertas L, Ogulur I, Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy and allergen tolerance. Allergol Int 2020;69:549–60.
3. Huang HJ, Sarzsinszky E, Vrtala S. House dust mite allergy: The importance of house dust mite allergens for diagnosis and immunotherapy. Mol Immunol 2023;158:54–67.

Posters / Session 2

Evaluation of an in-house developed colorimetric and other assays for PET-degrading activity

Aleksa Savić[1], Nenad Drulović[2], Jelena Radosavljević[2*]

[1]*Innovative Centre of the Faculty of Chemistry Ltd., Serbia*
[2]*Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia*

[*]*e-mail: radosavljevic@chem.bg.ac.rs*

Plastic materials have become indispensable in the modern world, with their extensive use resulting in their environmental accumulation. A promising solution for overcoming this ecological threat may be found in recombinantly produced plastic-degrading enzymes. Due to the complexity of the heterogeneous catalysis occuring during enzymatic PET hydrolysis, quantifying and comparing activities of such enzymes is rendered difficult. Here, we have assessed various assays documented in existing literature, employing different preparations of the purified recombinant *Ideonella sakaiensis* PETase mutant W159H/S238F (expressed from commercial plasmid Addgene #112203). The investigated methods were as follows: p-nitrophenyl acetate (pNA) hydrolysis assay, bis-(2-hydrohxyethyl)-terephthalate (BHET) agar and PET agar diffusion assays, and UV absorbance monitoring after PET particle and PET bottle cut-out hydrolysis. Additionally, we introduced an indirect colorimetric assay using the indicator pyrocatechol violet (PCV). Our work reveals many advantages and problems for each of the tested methods. The pNA hydrolysis assay is the quickest, but many substances which are usually present in enzyme buffers and solutions tend to hydrolyse this compound (e.g. imidazole). It is also unspecific due to hydrolysis by other esterase enzymes. The BHET diffusion assay offers a great tool for activity comparison and estimation, with greater enzyme specificity. However, it is slow and accurate activity quantification is difficult. PET hydrolysis was conducted on in-house prepared PET particles with UV spectrophotometric measurement or by a diffusion assay. Due to the measuring wavelength (240 nm), the importance of proper blanking is critical, but accurate results can still be obtained. The sensitivity of the diffusion assay is much lower in comparison to the similar BHET assay. We also report on a modification of the phenol red indirect colorimetric assay using PCV as the indicator and PET particles as the substrate, which has not been previously described in existing literature.

Acknowledgements

This work was supported by the Ministry of Science, Technological Development and Innovation Contracts No: 451-03-47/2023-01/200168 and 451-03-47/2023-01/200288.

Examination of C-phycocyanin interactions with selected vitamins

Aleksandar Ivanov[1*], Luka Veličković[1], Zorana Jovanović[1], Nikola Gligorijević[2], Simeon Minić[1], Milan Nikolić[1]

[1]Department of Biochemistry & Centre of Excellence for Molecular Food Sciences, Faculty of Chemistry, University of Belgrade, Serbia
[2]Center for Chemistry, Institute of Chemistry, Technology and Metallurgy, National institute of Republic of Serbia, University of Belgrade

[*]e-mail: aleksandar_ivanov2001@hotmail.com

C-phycocyanin (C-PC) is a photosynthetic protein from *Arthrospira platensis* (cyanobacteria). Due to its intense blue colour, which is very rare in nature, C-PC has industrial applications as a food colourant as a substitute for synthetic food colourants. Disadvantages of C-PC as a food colourant are its poor stability at high temperatures (during thermal treatment of the food) and its sensibility to change pH value. The binding of food-derived small molecules, such as vitamins, could stabilize the structure of C-PC at high temperatures and wide pH ranges. In this study, we characterized the binding of selected vitamins to C-PC, purified from the commercial powder of *Arthrospira platensis*. We used hydrophilic vitamins (B_1, B_2, B_7, B_9, B_{12}), lipophilic vitamins (A, D_3) and provitamin (β-carotene). Fluorescent spectroscopy showed a decrease in fluorescence of C-PC in the presence of vitamin A, vitamin D3 and β-carotene (lipophilic molecules) compared to the control. In contrast, the fluorescence of C-PC in the presence of hydrophilic vitamins showed minimal change. The protein fluorescence quenching approach demonstrated hydrophobic (pro)vitamins binding affinities ranging from 0.02 to 5.9 x 10^5 M^-1, with the ability of hydrophobic (pro)vitamins to bind at the different sites on C-PC. UV-VIS spectrophotometry showed that the binding of hydrophobic (pro)vitamins does not affect the protein colour, while CD spectroscopy revealed that the binding of chosen molecules does not significantly influence the secondary structure of C-PC. Overall, this study demonstrated C-PC's significant potential in binding hydrophobic (pro)vitamins, while further research is required to test if these ligands could improve C-PC stability.

Acknowledgements

This work was supported by ANSO Project No. ANSO CR-PP-2021- 01 and Ministry of Science, Technological Development and Innovation of Republic of Serbia, contract number: 451-03-47/2023-01/200168.

Genetic transformation of monoterpene biosynthesis and consequential modifications of the taste of citrus fruits and their defensive abilities against pathogens

Ante Blajić

Department of Biology, Faculty of Science, University of Split, Croatia

e-mail: blajicante@gmail.com

Citrus fruits, including important crops such as oranges, lemons, grapefruits, pomelos, and limes, represent an essential food product worldwide. The variety of their aromas and flavors is characterized by mixtures of volatile terpenes that refine their peel. Limonene (1-methyl-4-(1-methylethenyl)-cyclohexene), a colorless liquid cyclic monoterpene, is the most abundant volatile component present in the oil of all commercially grown citrus fruits. Various genetic manipulation techniques are used to influence the expression of the gene encoding for limonene synthase, a key enzyme in monoterpenoid biosynthesis, in the antisense and sense orientation of the integrated sequence in the plasmid. Obtained transgenic citrus fruits have been shown to have changed organoleptic properties and have a different impact on the ecological balance in the environment in which they grow. This study provides an overview of the influence of transgenic lines of citrus fruits that either have reduced or elevated concentrations of limonene on the human sense of smell and the interaction of the plant with insect herbivores and pathogens.

Design and property of the fusion enzyme of *bovine* DNA-exotransferase and DNA binding protein *Sso7d* from *S. solfataricus*

Antos Sachanka*, Veronika Shchur, Sergei Usanov, Aleksei Yantsevich

Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus, Minsk, Belarus

**e-mail:antosuk@yandex.ru*

DNA-exotransferase (*TdT*) – is a unique enzyme, useful for template-independent DNA synthesis by nucleotides addition to the 3′-terminus of DNA. *TdT* is currently concerned as a single way to realization of enzymatic *de novo* synthesis of DNA[1]. Gene fusion techniques offer multiple opportunities to combine the desired properties of different proteins in a single polypeptide, for example high thermostability and DNA-binding properties of *Sso7d* and unique enzyme activity of *TdT*. DNA binding protein from *S. solfataricus* (*Sso7d*) has been successfully used before to improve the processivity of thermostable DNA polymerases[2]. Here, we report a novel strategy to enhance the thermostability and functional properties of *TdT* by fusion with *Sso7d*. The fusion gene (TdT_GGS_SSO7d) was obtained by OE PCR technique (*Sso7d* fused to amino terminus of *TdT*) from plasmids containing *TdT* and *Sso7d* genes. The TdT_GGS_SSO7d gene was ligated into the pCWori plasmid and transformed into DH5α *E. coli* competent cells. Enzyme was purified to homogeneity by immobilized metal affinity chromatography. The amount of isolated fusion protein is comparable to that of native *TdT* when expressed and purified under identical conditions. The thermostability of proteins was measured by thermal shift assay with DLS and fluorescent detection. Different principles of methods allowed to get a deep insight into structure and functional properties of designed enzyme. The fusion protein's thermal denaturation begins at a temperature that is 30% higher than that of the native *TdT*, as determined by fluorescence and DLS measurements. The denaturation midpoint was shifted from 45 to 49°C as detected by DLS and from 46 to 70°C as detected by fluorescence measurements. Therefore, thermal shift assay observed by independent measurements revealed significant growth in fusion protein thermostability. Functional properties of novel fusion enzyme are under study.

Acknowledgments

This research was financially supported by the National Academy of Sciences of Belarus, project grant agreement no. 2023-27-021 and BRFFI grant No X21M-056.

References

1. Tang L, Navarro LA Jr, Chilkoti A, Zauscher S. High-molecular-weight polynucleotides by transferase-catalyzed living chain-growth polycondensation. Angew Chem Int Ed Engl 2017;64:6778–82.
2. Wang Y, Prosen DE, Mei L, Sullivan JC, Finney M, Vander Horn PB. A novel strategy to engineer DNA polymerases for enhanced processivity and improved performance in vitro, Nucleic Acids Res 2004;32:1197–207.

Temperature stability of *Streptomyces cyaneus* lacasse heterologously expressed within *S. cerevisiae* cell walls immobilized within tyramine-modified alginate beads using visible light photopolymerization

Dragana S. Josić[1*], Nikolina Popović Kokar[2], Anja Stošić[2], Milica Crnoglavac Popović[2], Olivera Prodanović[3], Goran T. Vladisavljević[4], Radivoje Prodanović[2]

[1]*Innovation center of the Faculty of Chemistry, University of Belgrade, Serbia*
[2]*Faculty of Chemistry, University of Belgrade*
[3]*Institute for Multidisciplinary Research, University of Belgrade*
[4]*Department of Chemical Engineering, Loughborough University, UK*

[*]*e-mail: draganajosic@chem.bg.ac.rs*

Photo-crosslinked modified alginate hydrogels are useful in various biomedical applications, including tissue engineering, wound healing, regenerative medicine, controlled drug delivery, and 3D bioprinting[1]. Traditional UV-crosslinkable hydrogels, often used for injectable soft tissue regeneration, have faced hurdles in clinical translation due to concerns about UV light's potential harm[2]. The use of visible light and safer photoinitiators during hydrogel synthesis significantly boosts their efficacy in immobilizing biocatalysts and advancing tissue engineering applications[3]. In this study the synthesis of tyramine modified alginate were optimized and spectral characterization of the modified alginate by ultraviolet-visible spectroscopy, infrared spectroscopy and proton nuclear magnetic resonance. Laccase from *Streptomyces cyaneus* was expressed on the surface of yeast cell walls, followed by cell lysis and immobilization of obtained cell walls withinin calcium-tyramine-alginate beads. Additional cross-linking of the obtained immobilizers by photopolymerization with visible light, in the presence of the riboflavin photoinitiator, in order to obtain a biocatalyst with improved characteristics and the ability to be used multiple times. The temperature stability of both photopolymerized and non-photopolymerized biocatalysts was determined. At 60°C, the cell wall-immobilized laccase entrapped in photopolymerized alginate-tyramine beads exhibited a 30% increase in enzymatic activity compared to the non-photopolymerized tyramine-alginate biocatalyst and 2.5 times higher activity compared to the enzyme immobilized in native alginate beads.

Acknowledgments

This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia Grant No. 451-03-47/2023-01/200168 (University of Belgrade-Faculty of Chemistry), Grant no. 451-03-47/2023-14/200288 (Innovation Center of the Faculty of Chemistry)

(RS-200288), Grant No. 451-03-47/2023-01/200053 (University of Belgrade-Institute for Multidisciplinary Research).

References

1. Chiulan I, Heggset EB, Voicu ŞI, Chinga-Carrasco G. Photopolymerization of bio-based polymers in a biomedical engineering perspective. Biomacromolecules 2021;22:1795-814.
2. Kong X, et al. Comparative analysis of different laser systems to study cellular responses to DNA damage in mammalian cells. Nucleic Acids Res 2009;37:e68.
3. Donnelly PE, Chen T, Finch A, Brial C, Maher SA, Torzilli PA. Photocrosslinked tyramine-substituted hyaluronate hydrogels with tunable mechanical properties improve immediate tissue-hydrogel interfacial strength in articular cartilage. J Biomater Sci Polym Ed 2017;28:582-600.

Optimization of the protocol for isolation of lipid transfer protein from lentil

Faiza Zafar[1*], Zorana Lopandić[2], Isidora Protić-Rosić[1], Milena Zlatanova,[1] Marija Gavrović-Jankulović[1]

[1]Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia
[2]Institute for Chemistry in Medicine, Faculty of Medicine, University of Belgrade

[*]e-mail: faiza@chem.bg.ac.rs

A large group of lipid transfer proteins (LTPs) transfer lipids using hydrophobic cavities that stabilize lipid molecules outside of the membrane[1]. Nonspecific LTPs present in plants have "alpha –helices" fold and are involved in the transport of phospholipids, fatty acids, and prostaglandin B2. Plant LTPs, on the other hand, are frequently identified as IgE reactive molecules capable of traversing the epithelial barrier in the first line of defence[2]. The purpose of this study was to develop a protocol for isolating non-specific LTP from lentil (Len c 3) for use in epithelial transport studies. The lentil protein extraction was performed in citrate buffer pH 3.5 and purified using the QAE-Sephadex chromatography after dialysis against citrate buffer pH 7.0 followed by SP-Sepharose chromatography in Tris-HCl pH 7.0. The purification process was monitored using tricine SDS-PAGE. The identity of three isoforms indicated as nLTP2, nLTP4, and nLTP5 (UniProtKB A0AT29, A0AT33, and A0AT31, respectively) was confirmed by a mass fingerprint. Because epithelial barrier studies require highly purified allergens, Len c 3 purification must be further optimized.

Acknowledgements

The ALLPreT project that has received funding from the European Union's research and innovation programme under the Marie Sklodowska-Curie grant agreement No 101072377.

References

1. Wong LH, Gatta AT, Levine TP. Lipid transfer proteins: the lipid commute via shuttles, bridges and tubes. Nat Rev Mol Cell Biol 2019;20:85-101.
2. Egger M, Hauser M, Mari A, Ferreira F, Gadermaier G. The role of lipid transfer proteins in allergic diseases. Curr Allergy Asthma Rep 2010;10:326-35.

Differences in chemical composition of the essential oils of peppermint (*Mentha x piperita* L.) and spearmint (*Mentha spicata* L.) and their anthelmintic properties

Filip Štrbac[1*], Nataša Simin[2], Dejan Orčić[2], Slobodan Krnjajić[1], Antonio Bosco[3], Dragica Stojanović[4], Radomir Ratajac[5], Giuseppe Cringoli[3], Laura Rinaldi[3]

[1]*Department of Life Sciences, Institute for Multidisciplinary Research, University of Belgrade, Serbia*
[2]*Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Serbia*
[3]*Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Italy*
[4]*Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad*
[5]*Department of Drug Testing and Toxicology, Scientific Veterinary Institute Novi Sad*

[*]*e-mail: filip.strbac@imsi.bg.ac.rs*

Plants of the genus *Mentha* are well-known for their various medicinal properties including anti-inflammatory, antiemetic, antispasmodic, analgesic and antiparasitic effects, which are used for the treatment of various gastrointestinal and respiratory diseases. The aim of this study was to determine the chemical composition of the essential oils (EOs) of two *Mentha* species, peppermint (*M. piperita*) and spearmint (*M. spicata*), and to evaluate their anthelmintic activity against gastrointestinal nematodes, parasites that have a significantly negative impact on modern sheep farming. The main compounds of peppermint EO, determined by GC-MS analyses, were menthol (32.6%), menthone (22.0%) and isomenthone (9.39%), and those of spearmint were carvone (64.4%), trans-4-caranone (8.67%) and limonene (4.37%). Their anthelmintic effects, assesed using the egg hatch test conducted at eight different concentrations (50, 12.5, 3.125, 0.781, 0.195, 0.049, 0.025 and 0.0125 mg/ml), were 20.0-90.3% and 13.0-93.7%, respectively. Although both tested samples showed high and dose-dependent (R^2 = 0.93 and 0.96, respectively) anthelmintic potential, their effect was significantly different at five concentrations ($p<0.05$). The obtained results suggest the high influence of differences in chemical composition of EOs on their pharmacological properties, although the samples were extracted from similar plant species. These should not be neglected during the preparation of formulation, which is important for finding alternatives to combat resistance in nematode.

Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Rescript no. 451-03-1183/2021-14)

Antioxidant study of natural pigments and their incorporation into nanosystems

Konstantina Matskou, Irini Karandrea, Georgios Sotiroudis, Maria Zoumpanioti*

Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece

**e-mail: mariaz@eie.gr*

The administration and delivery of bioactive compounds is restricted by a variety of obstacles that result in limited biocompatibility and bioavailability. Efforts to maintain or even improve their bioactive properties have often employed their incorporation in hydrogels that are based on natural polymers. In this context, hydrogels are particularly widespread as biomolecule encapsulation and release systems. In the present work, chitosan and a cellulose derivative (HPMC) were used to develop hydrogels for the encapsulation of pigments from natural sources that also present antioxidant activity, namely, phycocyanin, phycoerythrin and curcumin. Firstly, phycocyanin and phycoerythrin were successfully extracted and purified. Moreover, the connection of the hydrophobic polyphenol curcumin to the hydrophilic protein carrier BSA was accomplished, allowing curcumin to be dissolved in aqueous solutions[1]. The extracted phycobiliproteins and curcumin-BSA complex were dissolved in an aqueous solution, and their antioxidant activity was studied using the ABTS and DPPH assays. Phycoerythrin had a higher antioxidant capacity than phycocyanin. When phycocyanin and curcumin-BSA were incorporated into natural hydrogels made of chitosan and HPMC, the inhibition of the DPPH radical was significantly enhanced due to the antioxidant activity of chitosan.

Acknowledgements

This study was supported by the PRESSION ANSO project (No: ANSO-CR-PP-2021-01). The implementation of the doctoral thesis of K. Matskou was co-financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the Act "Enhancing Human Resources Research Potential by undertaking a Doctoral Research" Sub-action 2: IKY Scholarship Programme for PhD candidates in the Greek Universities.

References

1. Matskou K, Kisaoglan B, Mavroidi B, Pelecanou M, Zoumpanioti M, Matis I, Xenakis A. Inducing the formation of a colloidal albumin carrier of curcumin. J Colloid Interf Sci Open 2022;6:100051.

The accumulation of manganese by *Chlamydomonas acidophila* strains isolated from acid mine drainage

Isidora Santrač[1*], Milena Dimitrijević[1], Marina Stanić[1], Marija Tanović[1], Valentina Ćurić[2], Snežana Kovačević[1], Ivan Spasojević[1]

[1]*Department of Life Science, Institute for Multidisciplinary Research, University of Belgrade, Serbia*
[2]*Faculty of Biology, University of Belgrade*

[*]*e-mail: isantrac@imsi.bg.ac.rs*

Acid mine drainage ponds represents a specific artificial ecosystem that gives advantage to extemophilic microalgae. The mechanisms of adaptaton of such strains to excess metal concentrations that are common for their habitat, are poorly understood. Herein, we analzyed two strains of the green microalga *Chlamydomonas acidophila* - 137 and PM01, which have been isolated from different mining sites, for their interactions with Mn ions. The effects of different concentrations of Mn^{2+} were investigated in the late exponential/early stationary phase of culture growth (15 days). Viability was determined by Evans blue assay. No toxic effects were observed at concentrations as high as 2 mM Mn^{2+}. The the time dynamics of Mn accumulation in the biomass was determined using ICP. It was shown that the maximum accumulation of Mn in strain 137 (3.79 ± 0.68 µg/mg) was reached at 24h, while for PM01 the highest uptake (3.23 ± 0.17 µg/mg) was observed at 72 h. Next we analyzed redox settings by measuring the levels of reduced thiols unsing *in vivo* EPR spin probing. The treatment with 2 mM Mn^{2+} induced a rapid and irreversible decrease in the level of thiols which indicates that Mn^{2+} activated prooxidtaive processes. The maximum drop of thios levels were: from 4.80 ± 0.05 to 2.70 ± 0.05nmol/mg fresh weight after 1h for 137; and from 4.70 ± 0.05 to 3.40 ± 0.05 nmol/mg fresh weiht after 30 min for PM01. Further, the level of reactive oxygen species was evaluated using fluorescent probe DCFH-DA assay. The changes were in agreement with thiol levels. Both tested algal strains show resistance to Mn and are therefore good candidates for application in water bioremediation.

Acknowledgements

This study was supported by Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. 451-03-47/2023-01/20053.

Applying immobilised phycobiliproteins onto chitosan for efficient mercury removal

Jelena Radović[1], Dragana Popović[2], Tatjana Ćurčić[2], Milan Nikolić[1,3], Simeon Minić[1,3], Nikola Gligorijević[4*]

[1]*Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia*
[2]*Institute for Biocides and Medical Ecology, Belgrade, Serbia*
[3]*Center of Excellence for Molecular Food Sciences, Faculty of Chemistry, University of Belgrade*
[4]*Department of Chemistry, Institute of Chemistry, Technology, and Metallurgy, National Institute of the Republic of Serbia, University of Belgrade*

[*]*e-mail: nikola.gligorijevic@ihtm.bg.ac.rs*

This study aimed to improve chitosan polymer's capabilities to absorb mercury by immobilising phycobiliproteins (PBPs) onto the surface of chitosan beads (chitosan–PBPs). Phycobiliproteins, light-harvesting proteins from algae and cyanobacteria, have several industrially essential applications. These proteins can bind heavy metals with high affinities. Protein extracts obtained from both *Arthrospira platensis*, with C-phycocyanin as the dominant phycobiliprotein and *Neoporphyra haitanensis*, with R-phycocyanin and R-phycoerythrin as the dominant PBPs, were covalently immobilised onto chitosan beads. Binding analysis showed that, on average, 54 µg of PBPs were immobilised per bead. Immobilised proteins were still in their native state, with no visible colour change after immobilisation. Chitosan–PBPs and chitosan alone were tested for mercury adsorption at pH 4 and pH 7 by atomic absorption spectroscopy. The tested concentration range of mercury was from 1 to 70 ppm. Affinity, calculated using Henry's binding isotherm, of chitosan–PBPs for mercury was higher at both pH values than chitosan alone. Furthermore, chitosan–PBPs beads were able to absorb significantly more mercury than chitosan alone. These results show that the covalent immobilisation of PBPs onto chitosan improves its mercury adsorption characteristics and creates a more efficient eco-friendly adsorbent for removing mercury ions in the tested concentration range.

Acknowledgements

This study was financially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract numbers: 451-03-47/2023-01/200168 and 451-03-47/2023-01/200026.

The impact of spermidine supplementation on genes involved in autophagy in honey bee (*Apis mellifera* L.)

Jelena Spremo*, Elvira Vukašinović, Tatjana Čelić, Srđana Đorđievski, Jelena Purać, Danijela Kojić

Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia

**e-mail: jelena.spremo@dbe.uns.ac.rs*

Spermidine (Spd) is a polyamine (PA) with various cellular functions. Like other PAs, Spd content declines with age. Exogenous Spd can replenish cellular PAs content with beneficial effects on lifespan and health of model organisms and humans. Autophagy is believed to be the key mechanism of anti-aging activity of Spd. In our previous study, we found that Spd added at mM concentrations to food extended lifespan and improved oxidative status in honey bees[1]. To see if Sdp induces autophagy in honey bees, we tested for indicators of autophagy induction in bees supplemented with Spd. We measured the relative gene expression of autophagy-related proteins (Atg3, Atg5, Atg9 and Atg13)[2], as well as gene of enzymes involved in epigenetic modifications related to autophagy, such as DNA methyltransferase (DNMT1A/B and DNMT3), histone deacetylases (HDAC1, HDCA3 and Sirt1), and histone acetyltransferases (KAT2A, KAT6B and p300) in abdomen and head of honey bees supplemented with Spd at 0.1 and 1 mM concentrations for 17 days. Results showed that both Spd concentrations elevated gene expression of most analyzed genes, especially in abdomen, but 0.1 mM was more effective. This pattern of gene expression probably triggers other geroprotective mechanisms as well, but autophagy could be crucial in honey bee life extension.

Acknowledgements

This study was supported by the Science Fund of the Republic of Serbia (Program IDEAS, Grant No. 7721972, project title: Implication of dietary and endogenous polyamines for the health and longevity of honey bees B-HEALTH) and Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200125).

References

1. Đorđievski S, Vukašinović E , Čelić T, Pihler I, Kebert M, Kojić D, Purać J. Spermidine dietary supplementation and polyamines level in reference to survival and lifespan of honey bees. Sci Rep 2023;13:4329.
2. Lee IH, Finkel T. Regulation of Autophagy by the p300 Acetyltransferase. J Biol Chem 2009;284:6322-28.

Design and evaluation of *Candida rugosa* lipase hybrid nanoflowers

Jelena Grujičić[1], Marija Gavrović-Jankulović[1], Jovana Trbojević-Ivić[2*]

[1]*Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia*
[2]*Innovative Centre, Faculty of Chemistry, University of Belgrade*

[*]*e-mail: jivic@chem.bg.ac.rs*

Nanoflowers are unique nanobiocatalysts with an attractive morphology, enhanced loading capacity and high specific surface area, resulting in remarkable catalytic activity and stability of the immobilized enzymes[1]. *Candida rugosa* lipase (CRL, EC 3.1.1.3) is a renowned biocatalyst with a great industrial potential in fats and oils processing and production of ester-based nutraceuticals, food additives and biofuels[2,3]. To tackle the issue of the low stability of CRL, we have devised a cost-effective and environmentally friendly method for production of CRL nanoflowers with calcium as an inorganic component (CRL-NF). Preservation of catalytic activity of CRL-NF was confirmed in hydrolytic zymograms with *p*-nitrophenyl palmitate (*p*NPP) and olive oil. Most importantly, immobilization has resulted in significant stabilization of CRL at temperatures $\geq 55^{o}$C and pH ≥ 8. Obtained CRL-NF could be effectively re-used in 4 consecutive cycles of *p*NPP hydrolysis. Accordingly, our preliminary data indicate the promising applicative potential of CRL-NF in fats and oils processing and production of functional food.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract numbers: 451-03-47/2023-01/200168 and 451-03-47/2023-01/200288).

References

1. Xu K, Appiah B, Zhang BW, Yang ZH, Quan C. Recent advances in enzyme immobilization based on nanoflowers. J Catal 2023;418:31-9.
2. Trbojević Ivić J, et al. Design of biocompatible immobilized Candida rugosa lipase with potential application in food industry. J Sci Food Agr 2016;96:4281-7.
3. Trbojević Ivić J, et al. Synthesis of medium-chain length capsinoids from coconut oil catalyzed by Candida rugosa lipases. Food Chem 2017;218:505-8.

Nanobody-based immunoaffinity chromatography for the capture of urine-derived extracellular vesicles

Lidija Filipović[1*], Milica Spasojević[2], Ario de Marco[2], Milica Popović[1]

[1]*Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia*
[2]*Innovation Centre of the Faculty of Chemistry, University of Belgrade*
[3]*Laboratory for Environmental and Life Sciences, University of Nova Gorica, Slovenia*

[*]*e-mail: lfilipovic@chem.bg.ac.rs*

Extracellular vesicles (EVs) are a heterogeneous group of lipid bilayer-closed structures derived from almost all types of cells. Urine is a highly preferred biofluid in disease detection because it's non-invasive, easy, and fast sampling that can be carried out by patients themselves. In this work, we tried to implement the previously developed immune-affinity procedure for the purification of EVs to capture vesicles from urine.[1] Nanobodies used in this work were isolated from a naïve pre-immune (heavy-chain only-VHH) library by direct panning against EVs. The method was successfully adapted to urine, allowing rapid isolation of EVs in single-step chromatography. For determining morphological and biochemical features of the isolated EVs we used different biochemical and instrumental methods (colorimetric sulfophosphovanilin- SPV assay, Bradford assay, Flow cytometry, Dynamic light scattering- DLS, Nanoparticle Tracking Analyzer NTA, Scanning electron Microscopy- SEM). The combined analyses indicated that the recovered EVs were exosomes by confirming diameter and biomarkers such as CD9, CD63, and CD81. This inexpensive, relatively fast and easy-to-perform method has excellent potential for isolating different classes of EVs from various biological sources.

Acknowledgements

This work was funded by the Ministry of Science, Technological Development and Innovation of Republic of Serbia Contract number: 451-03-47/2023-01/20288.

References

1. Filipović L, et al. Affinity-based isolation of extracellular vesicles using single-domain antibodies bound to macroporous methacrylate-based copolymer. N Biotechnol 2022;69:36-48.

Biological activity of Serbian orange wines: polyphenolic content and inhibition of oxidative stress

Ljiljana Milovanović[1*], Tatjana Majkić[1], Ljilja Torović[2], Emilija Svirčev[1], Ivana Beara[1]

[1]*Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Serbia*
[2]*Department of Pharmacy, Faculty of Medicine, University of Novi Sad*

[*]*e-mail: ljiljana.milovanovic@dh.uns.ac.rs*

In the past ten years orange wines have significantly attracted the attention of wine producers, someliers and consumers. Orange wine is essentially white wine that is produced similary to red wines, with long skin-contact fermentation, without any chemical or specific yeast. The phenolic profile and biological activity of orange wines have been poorly investigated[1]. The aim of this study was to compare the polyphenolic profile (HPLC analysis), content of total polyphenols, flavonoids and tannins (spectrophotometric methods) in 24 orange wines from Serbia and to evaluate their potential to inhibit oxidative stress in *in vitro* cell-based model (human monocytes, U937 cell line). The oxidative stress in U937 cells was induced by 2,2′-azobis(2-amidinopropane)dihydrochloride, while dichlorofluorescein diacetate was used to monitor intracellular level of oxidative stress. Among the examined polyphenolics, catechin (0–76.7 mg/L), followed by gallic (0–49.5 mg/L) and caffeic acid (0–22.2 mg/L) were dominant polyphenols in examined wines, while sporadic occurrence of some anthocyanins was noticed. All wines had significant amounts of total polyphenols (227.0–1318 μg gallic acid equivalent/mL of wine), flavonoids (33.55–104.9 μg quercetin equivalent/mL of wine) and tannins (145.8–1254 μg catechin equivalent/mL of wine). Regarding inhibition of oxidative stress, the activity range varied from 138.0 to 1063 μg trolox equivalent/mL of wine, implicating that examined wine samples are effective inhibitors of oxidative stress.

Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 451-03-68/2023-14/200125).

References

1. Bene Z, Kallay M. Polyphenol contents of skin-contact fermented white wines. Acta Alimentaria 2019;48:515–24.

Examining fatty acid interactions with *Arthrospira platensis*-derived C-phycocyanin

Ljubodrag Aleksić[1*], Luka Veličković[1], Nikola Gligorijević[2], Miloš Šunderić[3], Marija Takić[4], Milan Nikolić[1], Simeon Minić[1]

[1]*Department of Biochemistry & Center of Excellence for Molecular Food Sciences, Faculty of Chemistry, University of Belgrade, Serbia*
[2]*Center for Chemistry, Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, University of Belgrade*
[3]*Department for Metabolism, Institute for the Application of Nuclear Energy, University of Belgrade*
[4]*Group for Nutrition and Metabolism, Institute for Medical Research, National Institute of the Republic of Serbia, University of Belgrade*

[*]*e-mail: ljubodragal@protonmail.com*

Cultured meat requires less land and water and is less polluting, but still costly. The critical challenge in cultivated meat science is identifying and developing bovine serum albumin alternatives as the key component in cell media. Phycobiliproteins (PBPs) from micro- and macroalgae are promising candidates for albumin replacement due to their high abundance and well-known excellent antioxidative and metal-binding activities of covalently attached tetrapyrrole chromophores. Considering the importance of fatty acids (FA) binding by albumin for cell cultivation, the additional prerequisites for developing PBPs as albumin replacement components is their validation for the ability to bind FA. This study aims to examine the ability of C-phycocyanin (C-PC), the major PBP of microalgae *Arthrospira platensis*, to bind seven fatty acids (stearic, palmitic, oleic, elaidic, linoleic, linolenic and docosahexaenoic acid). For this purpose, we employed various optical spectroscopy techniques (fluorescence, CD, and VIS absorption spectroscopy). The protein fluorescence quenching approach demonstrated FA binding affinities ranging from 0.42 to 2.4 x 10^5 M^{-1}, with the ability of FA to bind at different sites on C-PC. Fatty acid binding induces substantial changes in the VIS absorption spectra of C-PC, indicating the FA are attached in the vicinity of C-PC chromophores. On the other hand, CD spectroscopy did not show significant effects of FA binding on C-PC secondary structure content. Overall, this study revealed C-PC's significant potential in binding FA, the critical prerequisite to replacing albumin for developing animal-free cell media for meat cultivation.

Acknowledgements

This study was supported by the Good Food Institute, Field Catalyst Grant Program 2022 (Alg2Meat project) and the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract number: 451-03-47/2023-01/200168.

Genome-wide identification of sucrose non-fermenting kinase-1 (*SNRK1*) genes in the maize genome

M. Aydin Akbudak[1*], Ertugrul Filiz[2]

[1]*Department of Agricultural Biotechnology, Akdeniz University, Antalya, Turkiye*
[2]*Cilimli Vocational School, Duzce University, Turkiye*

[*]*e-mail: akbudak@akdeniz.edu.tr*

SnRK1 (Sucrose Non-Fermenting Kinase-1) is an evolutionarily conserved protein kinase that plays a vital role as a regulatory enzyme with numerous functions in living organisms. In plants, it exerts control over energy metabolism, growth and development, stress responses, sugar homeostasis, seed germination, flowering time, abiotic and biotic stress tolerance, as well as plant adaptation. By coordinating plant metabolism, growth, and stress responses, SnRK1 ensures plant survival and adaptation under adverse conditions. Although SnRK1 is generally accepted as a defense regulator, its effects on plant growth and performance are still not fully understood. In the present study, we employed bioinformatics methods to identify the SnRK1 gene family throughout the maize genome, enabling us to determine the transcriptional changes of the identified genes under various stresses that hinder maize production, such as drought, cold, salinity, and aphid damage. Consequently, this study represents the first comprehensive exploration of the *Zea mays SnRK1* (*ZmSnRK1*) genes on a genome-wide scale, facilitating their characterization and the identification of their regulatory mechanisms under both biotic and abiotic stress conditions. Elucidating the functions of the *ZmSnRK1* gene family in these contexts holds promise for the development of maize varieties with enhanced yield, quality, and environmental stress tolerance. Overall, our findings provide valuable insights that can contribute to the breeding of maize cultivars capable of thriving under challenging biotic and abiotic conditions.

Acknowledgement

This study was funded by Akdeniz University Scientific Research Projects Coordination Unit. Grant number: FBA- 2023-6290.

Antioxidant potential of the methanol extract of *Lavandula multifida* L.

Marija Marin[1]*, Radmila Glišić[2], Snežana Branković[2]

[1]*Faculty of Biology, University of Belgrade, Serbia*
[2]*Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Serbia*

[*]*e-mail: majamarin@bio.bg.ac.rs*

The family of *Lamiaceae* includes a variety of fragrant and medicinal plants. In the Mediterranean region, numerous wild and cultivated lavender species are grown, which are used in folk medicine, cosmetics and pharmaceuticals[1]. In traditional medicine they are used to treat insomnia and anxiety. They have anti-inflammatory, antioxidant, antispasmodic, sedative, insecticidal, antibacterial and antifungal properties[2]. It is known that the composition of chemical components of plants is related to their response to environmental conditions. In addition, essential oil and different extracts of the same species have been shown to have different biological activities. The medicinal plant *Lavandula multifida* L., which grows in arid regions, plays a significant role in protecting against erosion. Many studies have proven that the essential oil and ethanol extracted from *L. multifida* have significant antimicrobial, anti-inflammatory and anti-septic activity[3]. In this study, the potential antioxidant properties of the methanol extract from the leaves of *L. multifida* L. were investigated. The 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay was used in the present study to evaluate the potential antioxidant activity (or radical scavenging activity) of methanol extract of *L. multifida*. The EC_{50} value of the tested extract was 0.0224 μg/ml (the amount of antioxidant required to scavenge 50% of DPPH radicals). Based on the obtained results, we can conclude that the methanol extract of *L. multifida* has a possible biological activity due to its antioxidant potential.

Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development (Republic of Serbia), grant number 451-03- 47/2023-01/200178

References

1. Hosseini SM, et al. The effect of aqueous extract of *Lavandula officinalis* to reduce cholesterol, triglyceride and other lipid metabolites on female BALB/c mice. Clin Investig Arterioscler 2020;32:111-16.
2. Batiha GS, et al. A review of the bioactive components and pharmacological properties of Lavandula species. Naunyn Schmiedebergs Arch Pharmacol 2023;396:877–900.
3. Tofah M, et al. A new lavender (*Lavandula multifida* L.) ecotype from arid Tunisia, with differential essential oil composition and higher antimicrobial potential. Life 2023;13:103.

Magnetic, redox and structural properties of Mn-O-Ca cluster, synthesized by the green microalga *Chlorella sorokiniana*

Marija Tanović[1*], Milena Dimitrijević[1], Milica Milenković[2], Marina Stanić[1], Zvonko Jagličić[3], Wilfred Hagen[4], Ivan Spasojević[1]

[1]*Life Sciences Department, Institute for Multidisciplinary Research, University of Belgrade, Serbia*
[2]*Faculty of Chemistry, University of Belgrade*
[3]*Faculty of Civil and Geodetic Engineering, University of Ljubljana, Slovenia*
[4]*Department of Biotechnology, Delft University of Technology, The Netherlands*

[*]*e-mail: marijatanovic98@gmail.com*

Metabolism of metals in microalgae, as well as their adaptation to metal excess, are of significant environmental importance. We have found previously that the green microalga *Chlorella sorokiniana* accumulates environmental Mn excess in the form of a multivalent Mn-O-Ca cluster with structure that is very similar of oxygen-evolving complex (OEC) in photosystem II[1]. The application of microalgae in the 'green' synthesis of catalytic metal clusters is very important, since the use of toxic chemicals for traditional synthesis could be avoided. The aim of this study was to investigate the magnetic, redox and structural properties of this cluster. The magnetic properties of the cluster, were tested using Low-T-EPR spectroscopy and SQUID magnetometry. Based on the analysis of the EPR spectra, it was concluded that the spin of Mn in the cluster is >1/2. In addition, the spin of Mn is not an integer since parallel mode EPR did not deliver any detectable spectrum. The paramagnetic nature of the Mn-O-Ca cluster was confirmed by the SQUID instrument. Effective magnetic moment calculated per ion Mn was ~5 BM (Mn ratio in biomass was 12,1%). For the investigation of redox and structural properties, an extract of cluster from microalga was used. Cluster extraction involved the application of a series of solvents - phenol in Tris-Cl buffer, chloroform, methanol, 0,17% sodium hypochlorite[2]. Cyclic and differential voltagrams were recorded in the range from -1,5 to 2 mV[3]. The analysis revealed the presence of different oxidation states of Mn (+2, +3, +4). The peak potentials resembled the potentials in OEC model compounds[2].The redox similarities between the Mn-O-Ca cluster in microalgae and OEC imply that cluster may be have a similar catalytic activity. More detailed analysis of activity and structure of Mn-O-Ca cluster is warranted.

Acknowledgements

This study was supported by the Ministry of Science and Technological Development of Serbia

References

1. Vojvodić S, et al. Mechanisms of detoxification of high copper concentrations by the microalga *Chlorella sorokiniana*. Biochem J 2020;477:3729-41.
2. Villalobos M, Toner B, Bargar J, Sposito G. Characterization of the manganese oxide produced by *Pseudomonas putida* strain MnB1. Geochim Cosmochim Acta 2003;67:2649-62.
3. Zhang C, Chen C, Dong H, Ren Shen J, Dau H, Zhao J. A synthetic Mn4Ca-cluster mimicking the oxygen-evolving center of photosynthesis. Science 2015; 348: 691-3.

Unexpected regulators of cholesterol oxidase from *Pseudomonas aeruginosa*

Michail A. Shapira[1*], Yaraslau U. Dzichenka[1], Anastasia I. Liaudanskaya[2], Aliaksey Yantsevich[1]

[1]*Institute of Bioorganic Chemistry of National Academy of Sciences, Minsk, Belarus*
[2]*Department of Genetics, Belarusian State University, Minsk*

[*]*e-mail: mshapira2016@gmail.com*

Pseudomonas aeruginosa is an opportunistic pathogen known for causing infections, particularly in immunocompromised individuals. Its resistance to multiple antibiotics poses challenges for effective treatment. In addition to the well-known virulence factors like adhesins, exotoxins, alginate, and quorum sensing[1], *P. aeruginosa* possesses two distinct factors that contribute to its pathogenesis. One is cholesterol oxidase (ChOx)[2], which oxidizes cholesterol in host cell membranes, rendering them susceptible to toxin injections via the Type III Secretion System. The other factor is a group of secondary metabolites called "phenazines" [3], which promote biofilm formation and affect host immune responses. While previous research did not establish a connection between these two features, our current study reveals the ability of phenazine metabolites as well as NADH to selectively regulate ChOx activity. Thus, we demonstrated that self-sufficient ChOx can be regulated by the NADH. Moreover, carboxyphenazine and 2-hydroxyphenazine were identified as potent enhancers of ChOx activity, surpassing the effects of NADH. Conversely, pyocyanine, another phenazine metabolite, that are a predominant in *P. aeruginosa* phenazines production, does not affect ChOx activity. The features found help to more fully understand the pathogenesis of the diseases that are caused by the *P. aeruginosa*.

References

1. Liao C, Huang X, Wang Q, Yao D, Lu W. Virulence factors of *Pseudomonas aeruginosa* and antivirulence strategies to combat its drug resistance. Front Cell Infect Microbiol 2022;12:1–17.
2. Doukyu N, Nihei S. Cholesterol oxidase with high catalytic activity from Pseudomonas aeruginosa: Screening, molecular genetic analysis, expression and characterization. J Biosci Bioeng 2015;120:24–30.
3. Shapira MA, et al. Novel approach of phenazine derivatives isolation from Pseudomonas culture medium. Process Biochem 2021;111:325–31.

Exploring the antioxidant potential, aldo-keto reductase inhibition, and estrogen receptor binding affinity of two autochtonous macrofungal species

Milena Rašeta[1*], Sofija Bekić[1], Yusufjon Gafforov[2], Nataša Simin[1], Anđelka Ćelić[3], Edward Petri[3], Dilfuza Berdieva[4]

[1]*Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Serbia*
[2]*Mycology Laboratory, Institute of Botany, Academy of Sciences of Republic of Uzbekistan, Tashkent, Uzbekistan*
[3]*Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia*
[4]*Department Faculty and Hospital Therapy, Tashkent Medical Academy, Uzbekistan*

[*]*e-mail: milena.raseta@dh.uns.ac.rs*

Exploration of the biochemical potential of autochtonous macrofungal strains for production of specific bioactive agents is an essential part of the continuous search for novel strains with improved biological activities. Fungi are a reliable source of bioactive compounds with significant antioxidant capacity, and can be used in the treatment of many diseases, including cancers. The aim of this study was to evaluate antioxidant potential, aldo-keto reductase inhibition, and estrogen receptor binding affinity of four types of extracts of two autochtonous species, *Lentinus tigrinus* and *Sanghuangporus lonicerinus* from Uzbekistan. Determination of antioxidant activity of the tested fungal extracts was done using standard in vitro assays: ABTS, DPPH and FRAP. The potential of fungal extracts to inhibit human aldo-keto reductases, AKR1C3 and AKR1C4, was measured by fluorescence spectroscopy, by monitoring consumption of NADPH cofactor. The relative binding affinities of fungal extracts for the ligand-binding domains (LBDs) of estrogen receptor α (ERα) and estrogen receptor β (ERβ) were evaluated using a fluorescent screen in yeast. The highest scavenging activity and reducing power was observed for *Sanghuangporus lonicerinus* ethanol extracts. Furthermore, all tested extracts showed high inhibition potential against AKR1C3, while *Sanghuangporus lonicerinus* ethanol extracts were the strongest inhibitors. In contrast, AKR1C4 inbition was weak. Finally, of the tested fungal extracts, *Lentinus tigrinus* H_2O extracts showed the highest binding affinity for ERα-LBD, similar to estrone, a natural ligand. Our results suggest that the extracts tested in the present study could serve as a valuable starting point for isolation of new compounds for design of anticancer drug candidates.

Acknowledgements

The research was financed by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200125) and CAS PIFI (Grant no. 2022VBA0021).

Immuno-PCR for crustacean tropomyosin quantification

Mirjana Radomirović[1*], Nikola Gligorijević[2], Dragana Stanić-Vučinić[1], Andreja Rajković[3,4], Tanja Ćirković Veličković[1,3,4,5]

[1]Center of Excellence for Molecular Food Sciences and Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia
[2]Center for Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade
[3]Ghent University Global Campus, Yeonsu-gu, Incheon, South Korea
[4]Faculty of Bioscience Engineering, Ghent University, Belgium
[5]Serbian Academy of Sciences and Arts, Belgrade, Serbia

[*]e-mail: radomirovicmirjana@chem.bg.ac.rs

Tropomyosin has been recognized as one of the most common allergens among shellfish allergens. Sensitive and specific quantification of traces of allergens present in food samples is of critical importance for people with food allergies. This study thus aimed to develop a highly sensitive immuno-PCR method for detecting crustacean tropomyosin in foods. Method couples conventional sandwich ELISA assay with real-time PCR amplification of marker DNA. Monoclonal mouse anti-tropomyosin antibody was used as a capture antibody, while polyclonal rabbit anti-tropomyosin antibody served as a detection antibody in sandwich ELISA. A double-stranded amino-DNA molecule of 77 base pairs was covalently conjugated to a secondary goat anti-rabbit antibody and subsequently amplified and quantified by real-time PCR. Tropomyosin was quantified using highly purified natural shrimp tropomyosin as standard. The sensitivity of immuno-PCR for quantification of tropomyosin was increased by up to 20-fold compared to ELISA, demonstrating accuracy as low as 19.8 pg/mL. Recovery of tropomyosin in vegetable soup as a food matrix was in the 87.7–115.6% range, with relative standard deviations in the 5–24.5% range. Tropomyosin was also quantified in the commercially available food products. Developed immuno-PCR technique thus shows the potential to be a method of choice for specific and ultrasensitive detection of tropomyosin in food samples, with the final aim of reducing risks of accidental food contamination.

Acknowledgements

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract number: 451-03-47/2023-01/200168], the Science Fund of the Republic of Serbia, Program DIASPORA, #6504499, ShellPCR, and IMPTOX European Union's Horizon 2020 research and innovation program (grant number 965173).

Methanol extract of strawberry cultivar 'Aprika' increases glucose uptake in 3T3-F442A adipocytes

Mirna Jovanović[1*], Dragica Milosavljević[2], Jelena Dragišić Maksimović[2], Vuk Maksimović[2], Jasminka Milivojević[3], Ana Djordjević[1], Jelena Brkljačić[1]

[1]*Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia, University of Belgrade, Serbia*
[2]*Institute for Multidisciplinary Research, University of Belgrade*
[3]*Faculty of Agriculture, University of Belgrade*

[*]*e-mail: mirna.jovanovic@ibiss.bg.ac.rs*

Insulin resistance is a state where a normal amount of insulin can't provoke an appropriate metabolic response. Insulin promotes membrane trafficking of the glucose transporter GLUT4 from the storage vesicles to the plasma membrane in white adipose tissue. Adipocytes use glucose for lipogenesis and store the energy as lipid droplets. If adipocytes are unable to uptake glucose, a chronic state of hyperglycemia is developed, with severe health consequences. Polyphenols are natural anti-inflammatory and antioxidant agents. Food rich in polyphenols has been suggested to exert an ameliorative effect on restoring insulin sensitivity, with the main identified target being AMPK[1,2], one of the key sensors of intracellular energy. Here, we tested the effect of methanol extracts from three newly introduced strawberry (*Fragaria x ananassa*, Duch.) cultivars – 'Aprika', 'Sandra' and 'Quicky' on glucose metabolism in 3T3-F442A adipocytes. It was determined that 'Aprika' has the highest total phenolic content, relative to the other two cultivars. After 72-h exposure, none of the strawberry cultivars affected adipocyte cell growth significantly. Protein expression analysis of the differentiated adipocytes suggested 'Aprika', but not the other two cultivars, significantly increased the AMPK expression, as well as GLUT4, thus increasing glucose uptake. Strawberry extracts did not significantly affect the differentiation of adipocytes (SIRT1 and PPARγ), nor the fatty acid synthesis (ACC). Conclusively, the 'Aprika' methanol extract with high phenolic content exerts an ameliorative effect on glucose uptake, presumably through activation of the AMPK-dependent mechanism of GLUT4 trafficking. The systemic effects of the 'Aprika' cultivar should be further investigated. Implications of the research are decreased hyperglycemia in obese and diabetic patients, by the introduction of the 'Aprika' strawberry cultivar into everyday diet.

Acknowledgements

This study was supported by The Ministry of Science, Technological Development and Inovation of the Republic of Serbia, grant numbers 451-03-47/2023-01/200007, 451-03-47/2023-01/200053 and 451-03-47/2023-01/200116.

References

1. Zhao L, Zou T, Gomez NA, Wang B, Zhu MJ, Du M. Raspberry alleviates obesity-induced inflammation and insulin resistance in skeletal muscle through activation of AMP-activated protein kinase (AMPK) α1. Nutr Diabetes 2018;8:39.
2. Xu W, Luo Y, Yin J, Huang M, Luo F. Targeting AMPK signaling by polyphenols: A novel strategy for tackling aging. Food Funct 2023;14:56-73.

Amyloid fibrillation of egg-white proteins and its tendency to bind synthetic dye from water solutions

Natalija S. Andrejević[1*], Filip Stevanović[2], Natalija Đ. Polović[1]

[1]*Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia*
[2]*Public Health Insitute of Belgrade, Serbia*

[*]*e-mail: natalija@chem.bg.ac.rs*

Water pollution represents one of the global leading factors for illness and death[1]. Synthetic dye compounds are frequently discharged as environmental waters originating from textile, paper and leather industries. It has been proven that synthetic dyes may have harmful and toxic effects on the environment, even in low concentrations[2]. Therefore, it is of great significance to investigate and produce novel materials for water purification. In this research, we have made amyloid fibrils[3], directly from the egg-white powder, without the need of purification. Then, we have investigated the optimal manner for amyloid precipitation and quantified dye-binding capacities of several amyloid preparations. Along side, we have examined the effect of acidic/alkali conditions on the amyloid dye-binding capacity. It is shown that pH level higly affected the dye-binding capacity due to electrostatic repulsions. We have put the foundations of novel method for the purification of water contaminated by synthetic dyes, emerging on its simplicity, low-costness and eco-friendliness.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development and Innovation of Republic of Serbia, Contract number: 451-03-47/2023-01/200168.

References

1. Bolisetty S, Mezzenga R. Amyloid–carbon hybrid membranes for universal water purification. Nature Nanotech 2016;11:365–71.
2. Ardila-Leal LD, Poutou-Piñales RA, Pedroza-Rodríguez AM, Quevedo-Hidalgo BE. A brief history of colour, the environmental impact of synthetic dyes and removal by using laccases. Molecules 2021;26,3813.
3. Milošević J, Petrić J, Jovčić B, Janković B, Polović N. Exploring the potential of infrared spectroscopy in qualitative and quantitative monitoring of ovalbumin amyloid fibrillation. Spectrochim. Acta A Mol Biomol 2020;229:1386-425.

Chemical characterization and biological activities of six new genotypes of edible roses (*Rosa hybrida*)

Nemanja Živanović[1*], Nataša Simin[1], Biljana Božanić Tanjga[2], Mirjana Ljubojević[2], Marija Lesjak[1]

[1]*Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Serbia*
[2]*Department of Fruit Growing, Viticulture, Horticulture and Landscape Architecture, Faculty of Agriculture, University of Novi Sad*

[*]*e-mail: nemanja.zivanovic@dh.uns.ac.rs*

Due to their specific aroma, rose petals have been used for centuries as food in the production of jams, teas, wine, cakes, flavor extracts and candies. They also can be rich in biologically active compounds that contribute to human health. In this study, flowers of 6 new genotypes of *Rosa hybrida*, classified as edible roses due to their specific aroma, were harvested from the experimental fields of Pheno Geno Roses company near Temerin, Vojvodina. Petals were macerated with 70% MeOH and extracts were chemically characterized by determining total phenolic (TPC), flavonoid (TFC) and monomeric anthocyanin contents (TAC) as well as LC-MS/MS quantitative analysis. Biological activity was evaluated by DPPH, FRAP and acetylcholine esterase inhibition assays. Examined extracts are rich in phenolic compounds (TPC was in the range of 91-217 mg gallic acid eq./g d.e., TFC – 17-56 mg quercetin eq./g d.e., and TAC – 0.2-6.7 mg cyanidin-3-O-Glc eq/g d.e.). LC-MS/MS analysis revealed that the dominant phenolics are quercetin and kaemferol glycosides. The extracts expressed great antioxidant potential. Total antioxidant power in the FRAP assay was 74-223 mg ascorbic acid eq./g d.e., while EC50 values in the DPPH assay ranged from 7.7 µg/mL to 45 µg/mL. The extracts also showed moderated neuroprotective activity determined by acetylcholine esterase inhibition assay, with results in the range of 16-68 ng of eserine eq./g d.e. Based on the obtained results, the new genotypes of edible roses investigated in this study can be considered as functional food due to the high level of bioactive compounds with health-promoting properties.

Acknowledgments

This study was supported by the Secretary for Higher Education and Scientific Research, Autonomous Province of Vojvodina (Grant No.142-451-2658/2021-01/1).

Silk of Indian meal moth induces apoptosis of SW-480 colorectal cancer cells

Nikola Radenković[1*], Milena Milutinović[1], Danijela Nikodijević[1], Jovana Jovankić[1], Stefan Blagojević[1], Vladimir Jurišić[2], Dragana Predojević[1], Filip Vukajlović[1], Snežana Pešić[1]

[1]*Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Serbia*
[2]*Faculty of Medical Science, University of Kragujevac*

[*]*e-mail: 269-2013@pmf.kg.ac.rs*

Several ongoing studies have indicated that various animal products may contain biologically active substances with the potential to be used in medicine[1]. The Indian meal moth (*Plodia interpunctella* Hbn.) silk imprimis exists to protect the larvae, particularly during pupation. Since the silk is produced by larval glandular apparatus, that silk contains, except for the dominant fibroin and sericin, a certain amount of mandibular enzymes which can potentially possess antitumor activity. Therefore, this study aimed to evaluate the antitumor potential of the DMSO-extracted silk (five days produced by the final larval stage of *P. interpunctella*) on the SW-480 human colorectal adenocarcinoma cells. The MTT test of cell viability showed a strong cytotoxic activity of silk on SW-480 cells after 72 h. After the same time of incubation, the selectivity was also shown due to non-cytotoxicity on normal human keratinocytes, HaCaT cells. The pro-apoptotic activity was examined 72 h after treatment by fluorescent staining to detect morphological changes on SW-480 carcinoma cells, as well as by flow cytometry. Also, the expression of apoptosis-related genes was determined by the qPCR method. The results show proapoptotic activity of silk extract and increased expression of *Caspase 8* and *9* genes compared to control, suggesting the caspase-induced apoptosis. Based on the observed results on cytotoxicity, selectivity, and proapoptotic activity of *P. interpunctella* silk, our results undoubtedly indicate the presence of antitumor substances in the tested silk, as well as a high level of biocompatibility with healthy human cells.

Acknowledgements

This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Agreement no. 451-03-57/2023-03/2936; 451-03-47/2023-01/200122 and 451-03-47/2023-01/200111).

References

1. Milutinović M, et al. The silk of Plodia interpunctella as a potential biomaterial and its cytotoxic effect on cancer cells. Anim Biotechnol 2020;31:195–202.

The effect of carboxymethylcellulose and different natural ingredients on the stability of double emulsions (water-in-oil-in-water)

Amira Aguenarous[1*], Nikola Srećković[2*], Vladimir Mihailović[2]

[1]*Department of Hydraulics, University of Science and Technology Mohamed Boudiaf USTO.MB, Oran, Algeria*
[2]*Department of Chemistry, Faculty of Science, University of Kragujevac, Serbia*

[*]*e-mail: amiraaguenarous@outlook.com; nikola.sreckovic@pmf.kg.ac.rs*

Double emulsions (W/O/W) have a specific structure with small water droplets incorporated in oil drops dispersed in a continuous aqueous phase. This characteristic consistency enables their application for the encapsulation of specific molecules in pharmaceuticals, preparation of cosmetic products, agricultural or food products, and fat reduction in food. In this study, the stability and physicochemical properties of W/O/W emulsions prepared using different emulsifiers and stabilizers were compared. The oil phase of the W/O/W emulsions consisted of paraffin oil or *Calendula officinalis* L. infused oil and polyglycerol polyricinoleate as a lipophilic emulsifier. These lipid components were dispersed in water to obtain inner W/O emulsions that further, in the second step, homogenized with the outer aqueous phase with a hydrophilic emulsifier and stabilizer to obtain W/O/W emulsion. Different concentrations of carboxymethylcellulose or hyaluronic acid were used as stabilizers, while Tween 80 (polyoxyethylene (80) sorbitan monooleate), CreamMaker ANIO (glyceryl oleate citrate, caprylic/capric triglyceride), *Rosmarinus officinalis* L. water extract, and/or hydrolyzed wheat proteins were used as hydrophilic emulsifiers. The stability of the W/O/W emulsions was analyzed over a one-month storage period using physicochemical measurements. An excellent structure and stability of W/O/W emulsion were observed using paraffin oil and 1% of carboxymethylcellulose and 1% of Tween 80 in the outer aqueous phase. The formation of W/O/W emulsion using *C. officinalis* infused oil was possible using two combinations of emulsifiers and stabilizers from natural origin, 1% of CreamMaker ANIO and hyaluronic acid, as well as carboxymethyl cellulose and hydrolyzed wheat protein in the outer aqueous phase. These emulsions with all ingredients from natural origin showed lower stability with higher average particle size and creaming indexes than those obtained using paraffin oil and synthetic surfactant.

Acknowledgments

This study was supported by the Erasmus+ scholarship.

An *in vitro* study on the binding affinity of Schiff base Rh(III) complexes to human serum α1-acid glycoprotein utilizing microscale thermophoresis

Tino Šeba[1], Aleksandar Mijatović[2], Rada Baošić[3], Mario Gabričević[1], Robert Kerep[1*]

[1]*Department of General and Inorganic Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia*
[2]*Chair of Chemistry, Faculty of Mining and Geology, University of Belgrade, Serbia*
[3]*Department of Analytical Chemistry, Faculty of Chemistry, University of Belgrade*

[*]*e-mail: robert.kerep1@gmail.com*

Studying metal-protein interactions is essential for understanding the fate of metallodrugs in biological systems. Lately, alternative metal-based cancer therapies have received widespread attention since cisplatin was developed as an anticancer drug[1]. The purpose is to find drugs that have lower toxicity but more potency than Pt-based agents. Schiff base Rh(III) complexes are considered as one alternative to Pt drugs, which exhibit low toxicity to normal cells, as well as easily absorbed by tumor tissue and rapidly excreted from the body. AGP exerts a substantial impact on transporting and disposing of numerous drugs present in blood. It has been shown that the drug–AGP interactions in the blood can influence the drug stability, toxicity and distribution during the chemotherapeutic process. The nature and magnitude of drug–AGP interactions significantly influence the pharmacokinetics of drugs, and the binding parameters are useful in studying metal–protein binding[2]. Therefore, the binding affinity of two newly synthesized Rh(III) complexes H[Rh(acac$_2$en)Cl$_2$] (RhAA) and H[Rh(phacac$_2$en)Cl$_2$] (RhPP) with AGP have been studied by MST as a powerful technique in quantitation of binding events based on the movement of molecules in microscopic temperature gradient, depending on various molecular properties such as size, charge, hydration shell or conformation[3]. The results show that the binding affinity of RhPP was measurable (K_A = 7.69 mM^{-1}) than that of RhAA when bound to AGP, where RhAA did not show any significant binding indicating that RhAA might be more bioavailable and potent drug.

Acknowledgments

This study was supported by funding from the Croatian Science Foundation grants (IP-2016-06-3672 and UIP-2017-05-9537), European Structural and Investment Funds (KK.01.1.1.07.0055), European Regional Development Fund FarmInova (KK.01.1.1.02.0021).

References

1. Bruijnincx PC and Sadler PJ. New trends for metal complexes with anticancer activity. Curr Opin Chem Biol 2008;12:197–206.
2. Kerep R, et al. Potential clinically relevant effects of sialylation on human serum AAG-drug interactions assessed by isothermal titration calorimetry: Insight into pharmacoglycomics? Int J Mol Sci 2023;10.8472.
3. Jerabek-Willemsen M, et al. Microscale thermophoresis: interaction analysis and beyond. J Mol Struct 2014;1077:101–13.

Colored proteins in biochemistry teaching

Milan Nikolić[1], Roko Vladušić[2*], Dragica Trivić[3]

[1]*Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Serbia*
[2]*Department of Chemistry, Faculty of Science, University of Split, Croatia*
[3]*Department of Chemical Education, Faculty of Chemistry, University of Belgrade*

[*]*e-mail: vladusic@pmfst.hr*

Of all biomolecules, proteins have the most significant roles in living systems. It is not an exaggeration to say that proteins sustain life. Hence the entirely appropriate name for this group of molecules of life was coined by the famous Swedish chemist and inventor Berzelius: in Greek, the word *prōteios* means the first, the most important. The most famous colored protein is certainly hemoglobin, red due to the presence of the heme group, thus making the blood red-colored. Although hemoglobin is a textbook model system in protein biochemistry, for studying its quaternary structure, the relationship between structure/activity/function, and allostery, as a critical feature of protein regulation in all living cells, safety, and related ethical issues limit its use in the student laboratories. Cyanobacteria (such as the blue-green algae *Spirulina*) and red algae (such as the genus *Porphyra*) are the richest natural source of (complete) proteins, which have always been used in human nutrition. Their most abundant proteins (phycobiliproteins), as part of the complex photosynthetic apparatus, are all deep-colored, thanks to different, covalently bound tetrapyrrole chromophores (phycobilins). Given that the starting material for experimental work on phycobiliproteins is cheap and available and that they are relatively easy to obtain, purified enough in the required quantity, they could enter the curricula of all courses that study proteins. This presentation will briefly present our previous teaching experiences working with three colored algae proteins: blue C-phycocyanin, purple R-phycocyanin, and reddish-pink R-phycoerythrin. More specifically, how they can be used to consider the relationship between the structure and physicochemical properties of such macromolecules in the student laboratory. For example, for easier experimental understanding (on a visible, therefore macroscopic level) of protein structural stability in aqueous solutions and related conformational transitions, a particular challenge in general biochemical education.

Acknowledgments

The authors thank the members of the research group for Protein biochemistry at the Faculty of Chemistry in Belgrade (https://www.chem.bg.ac.rs/grupe/GPB/index-l.html) for their help in work with phycobiliproteins.

Phenolic profile, antioxidant and antidiabetic potential of traditionally prepared sage and peppermint juices

Sanja Krstić[1*], Saša Vukmirović[3], Sanja Berežni[2], Nebojša Stilinović[3], Nebojša Pavlović[4]

[1]Institute of Pharmaceutical Sciences, University of Graz, Austria
[2]Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Serbia
[3]Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Novi Sad
[4]Department of Pharmacy, Faculty of Medicine, University of Novi Sad

[*]e-mail sanja.krstic@uni-graz.at

Sage and peppermint have a number of biological properties that have been demonstrated to be helpful for the treatment of a range of diseases. Determination of phenolic profile and the investigation of the biological activity of traditionally made juices obtained from sage and peppermint leaves origin from one village in Republic of Montenegro was the main goal of the present study. Phenolic compounds have been quantified using LC-MS/MS technique. The most abundant phenolics in the sample of traditional sage juice were quinic acid (2571.86 ±1.15 μg/g) and apigenin-7-O-glucoside (324.36± 1.15 μg/g). Only two phenolic acids (caffeic and quinic) have been quantified, in significantly lower amount, in peppermint juice. In experimental mice (Mus musculus, NMRI Haan strain), the anti-diabetic (oral glucose tolerance and streptozotocin induced diabetes test) and antioxidant activity (measuring oxidative stress-related enzymes including superoxide-dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase and level of lipid peroxidation in liver homogenates) were examined. In both healthy and diabetic animals, a ten-day treatment with the tested juices caused the blood glucose level to decrease. Furthermore, the juices demonstrated notable antioxidant activity, which has boosted the activity of antioxidant enzymes and decreased the level of lipid peroxidation. The obtained results support traditional use of sage and peppermint juice as food with health and nutritional benefits.

The impact of laser surface scanning on β-titanium alloy surface features and biocompatibility

Slađana Laketić[1,*], Marko Rakin[2], Miloš Momčilović[1], Jovan Ciganović[1], Đorđe Veljović[2], Vesna Kojić[3], Ivana Cvijović-Alagić[1]

[1]Vinča Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Serbia
[2]Faculty of Technology and Metallurgy, University of Belgrade
[3]Oncology Institute of Vojvodina, Faculty of Medicine, University of Novi Sad, Sremska Kamenica, Serbia

[*]e-mail address: sladjana.laketic@vin.bg.ac.rs

Laser surface scanning was applied in the present work to achieve the improvement of biointegration properties of the titanium alloy with high niobium content as potential implant material. The use of laser surface scanning enabled modification of the surface chemistry, morphology, and roughness and in that way influenced the obtainment of alloy's excellent biocompatibility. Scanning electron microscopy analysis showed that the interaction of laser irradiation with β-titanium alloy surface resulted in the formation of specific scanned pattern lines, as well as distinctive surface damage features in the form of microcracks, periodic wave-like structures, and ripples. An increase in the laser pulse energy led to the appearance of more pronounced hydrodynamic effects. Moreover, the optical profilometric analysis showed that the laser surface scanning of the investigated alloy with higher laser pulse energy caused an increase in surface roughness. *In vitro* examination of the cell culture assays in contact with the investigated alloy indicated that there were no cytotoxic effects of the alloy under given examination conditions, while the cell morphology analysis demonstrated that the cell attachment and proliferation were enhanced after the laser beam irradiation. Taking into account the obtained results, it can be concluded that the laser surface modification of the β-titanium alloy, which resulted in the appearance of pronounced surface features, shows a high impact on the material properties and enhances its performance in the biological environment.

Acknowledgments

This work was financially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia through Contract No. 451-03-47/2023-01/200017 and the PhD fellowship of Slađana Laketić.

Development of high-throughput elicitor screening (HiTES) system for usage in directed enzyme evolution

Yaraslau U. Dzichenka[1], Michail A. Shapira[1], Antos B. Sachanka[1], Sergey A. Usanov[1], Suzana Jovanović-Šanta[2*]

[1]Institute of Bioorganic Chemistry of National Academy of Sciences, Minsk, Belarus
[2]Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Serbia

[*]e-mail: suzana.jovanovic-santa@dh.uns.ac.rs

Directed enzyme evolution approach is widely used for the creation of novel biocatalysts, producing molecules of interest: drugs, intermediate compounds for organic synthesis etc. A successful directed evolution experiment depends on the genetic diversity and high throughput selection approach. Usage of bacterial cells allows to analyze a large number of polymorphic variants of the protein ($>10^{11}$) with simple experimental procedures. Cytochromes P450 (CYPs) are enzymes, considered as the most versatile biocatalysts in nature because of the variety of substrate structures and the types of reactions they catalyze. They are extremely perspective for the usage in production of pharmaceuticals and other chemicals. Here we present preliminary results of the development of the bacterial high-throughput elicitor screening (HiTES) system for usage in directed evolution of CYPs. Elementary selection system was created on the basis of inducible *lac* operon. Gene coding aminoglycoside-3'-phosphotransferase was cloned into pACYC184 vector (restriction sites NdeI and SacI) in *lac* operon cassette and corresponding plasmid was transformed into *E. coli* cells (BL21 strain). Cells cultivation on gradient LB agar with different concentrations of kanamycin (Km) and IPTG (inducer of LacI protein) allowed to identify optimal concentrations of bioregulators (170 μg/ml Km and 0.1 mM IPTG) for the selection of producents. In the next step, bacterial library of mutant forms of LacI was constructed using a set of degenerate mutagenic primers, which was designed on the basis of the results of pharmacophore modelling. The results obtained will be used for the further screening of bacteria, containing mutant forms of LacI protein with high affinity to the molecules of interest – products of enzymatic reactions, mediated by different CYPs.

Acknowledgements

This study was supported by bilateral Belarus-Serbia project "A new screening method for using in the directed evolution of human steroid-metabolizing enzymes" (project No. X22SRBG-009 / 337-00-00230/2022-09/09)

Binding of proteins in skimmed cow milk to polypropylene-microplastics

Tafadzwa Kaseke, Vesna Jovanović, Tanja Ćirković Veličković*

Center of Excellence for Molecular Food Sciences, Department of Biochemistry, University of Belgrade, Serbia

**e-mail: tcirkov@chem.bg.ac.rs*

Microplastics (MPs) continue to be detected in various foods, including milk products[1]. There is increasing evidence from the literature on the potential negative effects of MPs on human health. Milk as a source of protein is one of the most commonly consumed food products. Polypropylene is a major polymer used to manufacture milk products packaging and has been identified as a contaminant of milk products[2]. However, its effect on the digestion of milk proteins is unknown. Therefore, the study described herein evaluated the interaction of polypropylene microplastics (PP-MPs) with proteins in skimmed cow milk (31.36 mg/mL proteins and 0.7 % fat) to understand the binding efficacy of proteins on the MPs. Different amounts (5, 10, 20, and 30 mg per 250 mL) of PP-MPs (63–180 mg in size) were incubated in simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) for 60 min with continual mixing. After the incubation process, proteins binding to the PP-MPs were extracted from the soft corona and hard corona using different extraction methods (once, twice, and thrice with 1X buffer solution). The protein profiles of the samples were analyzed using SDS-PAGE. In addition, quantification of proteins bound in the hard corona was done for the SGF incubated skimmed cow milk and PP-MPs. Binding of proteins such as α-casein, β-casein, k-casein, β-lactoglobulin, and α-lactoglobulin both in the soft and hard corona was observed in all the simulated digestive fluids, regardless of the amount of PP-MPs used. However, more proteins were bound to the PP-MPs in skimmed cow milk incubated with SGF compared to SSF and SIF, suggesting that proteins from skimmed cow milk have different capacities to bind to the PP-MPs in different digestive systems. Increasing the number of extractions and the amount of PP-MPs enhanced the protein content extracted from the hard corona. In addition, 20 mg/250 mL was determined as the optimum PP-MPs concentration for increasing the extraction of proteins from the hard corona; beyond that concentration, no significant increase in protein content was observed. This study lays the foundation for the *in vitro* digestion of skimmed cow milk proteins in the presence of PP-MPs.

Acknowledgments

This study was supported by the European Union's Horizon Europe program, Microprot project, Grant Agreement number 101090313.

References

1. Kaseke T, Lujic T, Cirkovic Velickovic T. Nano-and microplastics migration from plastic Ffood packaging into dairy products: Impact on nutrient digestion, absorption, and metabolism. Foods 2023;12:3043.
2. Da Costa Filho PA, et al. Detection and characterization of small-sized microplastics (≥ 5 μm) in milk products. Sci Rep 2023;11:24046.

Impact of MPs on trypsin activity in simulated intestinal fluid

Tamara Lujić[1], Nikola Gligorijević[2], Dragana Stanic-Vučinić[1], Maša Bićanin[1], Tanja Ćirković Veličković[1,3,4,5*]

[1]Faculty of Chemistry, University of Belgrade, Serbia
[2]Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, University of Belgrade
[3]Serbian Academy of Sciences and Arts, Belgrade, Serbia
[4]Faculty of Bioscience Engineering, Ghent University, Belgium
[5]Ghent University Global Campus, Incheon, Korea

[*]e-mail: tcirkov@chem.bg.ac.rs

Mircoplastics (MPs) are an abundant contaminant in the environment with ingestion being the most common way of exposure for humans. Binding of protein to MPs is proposed to be multilayered with the formation of a soft and hard corona[1]. It has been proven that MPs interact with enzymes present in the digestive system and impact their activity[2]. The aim of this study is to investigate the impact of MPs on the activity of trypsin in simulated intestinal fluid (SIF). For this purpose, two sizes of polypropylene (large – 180-500 μm, small – 63-180 μm) and one size of polyethylene terephthalate (<80 μm) have been studied. Activity in bulk and soft corona was determined in SIF at 405 nm with Nα-Benzoyl-DL-arginine 4-nitroanilide hydrochloride after different times of incubation. Activity in hard corona was determined after 1 h of incubation with the MPs. Although specific activity in the control decreases through time, there is a tendency for all MPs to preserve activity in bulk and soft corona trypsin after 4 h of incubation. Trypsin remains active in the hard corona, with the activity being an order of magnitude lower than in the control, possibly due to significant changes in structure.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 965173.

References

1. Monopoli MP, Åberg C, Salvati A, Dawson, KA. Biomolecular coronas provide the biological identity of nanosized materials. Nat Nanotechnol 2012;7:779-86.
2. de Guzman MK, et al. Small polystyrene microplastics interfere with the breakdown of milk proteins during static in vitro simulated human gastric digestion. Environ Pollut 2023;335:122282.

Mass spectrometry analysis reveals impact of peanut roasting on post-translational modifications of key allergens and their hinderence of trypsin digestion

Teodora Đukić[1], Katarina Smiljanić[2], Ivana Prodić[3], Tanja Ćirković Veličković[2,4]

[1] *Institute of Medical Chemistry, Faculty of Medicine, University of Belgrade, Serbia*
[2]*Center of Excellence for Molecular Food Sciences, Department of Biochemistry, Faculty of Chemistry, University of Belgrade*
[3] *Institute of Molecular Genetic Engineering, University of Belgrade*
[4]*Serbian Academy of Sciences and Arts, Belgrade*

[*]*e-mail: teodora.djukic@med.bg.ac.rs; teodora.djukic994@gmail.com*

A bottom-up proteomic study, using high-resolution tandem mass spectrometry (HRMS) and, PEAKS Studio X+ was performed to investigate the impact of peanut roasting on readily soluble allergens and their post-translational modification (PTM) profiles. Among four major peanut allergen groups, we found that Ara h 3 prevails in raw peanut extract (PE), similar to Ara h 1, while the opposite is true for Ara h 6, which is enriched in roasted PE; Ara h 2 bands are near the same intensity. HRMS detected more than 40 different types of modification in raw and roasted samples. Distinct variations in the types and occurrence of specific amino acid PTMs were identified between allergens present in raw and roasted samples. Roasting affected the most frequent modifications by enrichment of OxM, HyP, carbamoylation (KR), and deamidation. The PTMs could also be mapped to the regions of IgE-binding epitopes of Ara h 1–3 and Ara h 6. As porcine trypsin is used for HRMS sample preparation and is also a digestive protease, hindrance effects to trypsin efficacy regarding PTMs was assessed. Roasting caused dihydroxylation and formylation PTMs with hindrance effects to trypsin efficacy, while methylation on several K/R showed opposite effects. In the case of methylated R342, results suggested facilitation of tryptic performance at modified residue compared to unmodified counterpart, while in the rest of seven genuine sequences containing modified residues, trend was opposite. Further exploration of how different PTMs could affect digestion efficiencies of major gastric and intestinal peptidases is currently in works and a much-needed assessment to better understand the role of PTMs.

Acknowledgements

This research was funded by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia [Contract No: 451-03-9/2023-14/200168]; Belgian Special Research Fund

BOF [StG No: 01N01718]; Serbian Academy of Sciences and Arts [GA No. F-26]; and the European Commission, under the Horizon2020 FoodEnTwin project [GA No: 810752].

Insect-derived active peptides: alternatives for combating multidrug-resistant bacteria

Vanja Tatić[1], Fabiana Giglio[2], Ana Volarić[1], Dragan Radnović[1], Patrizia Falabella[2], Željko D. Popović[1*]

[1]*Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia*
[2]*Department of Sciences, University of Basilicata, Potenza, Italy*

[*]*e-mail: zeljko.popovic@dbe.uns.ac.rs*

One of the greatest advances in biochemistry and medicine was the discovery of antibiotics, but the same compounds that have saved many lives are now beginning to lose their efficacy. The rise of multi- and ultraresistant bacterial strains is one of the most pressing global problems. Active peptides with antibacterial properties, such as those obtained from insects, can offer alternative solutions. Insects, which have evolved an outstanding innate immune system that allows them to adapt to diverse environments, are still an untapped resource of biotechnological potential, notably with regard to food and human health. The chosen insect species, *Tenebrio molitor* (yellow mealworm, YMW), *Hermetia illucens* (black solider fly, BSF), and *Rhynchophorus ferrugineus* (red palm weevil, RPW), are recognized as model species in the field of biotechnology, with YMW and BSF and their products also being used as key sources of human food and animal feed. Gram-positive (*Micrococcus flavus*, Mf, and *Staphylococcus aureus*, Sa) and Gram-negative bacteria (*Escherichia coli*, Ec, and *Pseudomonas aeruginosa*, Pa) were used to infect larval stages, after which their haemolymph was extracted and target peptides were isolated via organic solvent precipitation. Infection of RPW and BSF was done via injection with Ec or Mf, while YMW was infected via food with Pa or Sa. Small peptides (below 30 kDa), similar in size to the majority of antimicrobial peptides (AMPs), were present in the precipitate. The activity of AMPs was tested using an agar diffusion assay, which showed that selected bacteria are susceptible to the active peptides from haemolymph, with Pa having greater zones of inhibition overall. More precisely, Pa was sensitive to YMW haemolymph, while Sa showed intermediate sensitivity due to the growth of bacteria back into the inhibition zone after 24 h. RPW haemolymph had a similar effect on both bacterial plates but with slightly wider initial inhibition zones. Both bacterial strains seem to be resistant to peptides from BSF haemolymph. Further testing of activity after 8 and 12 hours will be performed. Results show great potential for the use of insect-derived peptides as potential alternatives to antibiotics.

Acknowledgement

This study was funded by the project of Basilicata ERDF Regional Operational Programme, AAA – (SAFE SOS) grant no. C39J20001750002 and the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant no. 451-03-47/2023-01/200125.

Combined hydrogels of starch and β-lactoglobulin as matrices for the preservation of C-phycocyanin

Zorana Jovanović[1,2*], Burkhard Annighöfer[2], Daniel Dudzinski[2], Luka Veličković[1], Nikola Gligorijević[3], Milan Nikolić[1], Annie Brûlet[2], Ali Assifaoui[4], Sophie Combet[2], Simeon Minić[1]

[1] Faculty of Chemistry, University of Belgrade, Serbia
[2] Laboratoire Léon Brillouin (LLB) UMR12 CEA-CNRS, Université Paris-Saclay, Gif-sur-Yvette CEDEX, France
[3] Institute of Chemistry, Technology and Metallurgy, University of Belgrade
[4] PAM Unit, team Physical chemistry applied to Food and Wine – AgroSupDijon, University of Burgundy, Dijon, France

[*] e-mail: zorana.gr@gmail.com

The color of food products is an important aspect in food industry, and its preservation remains a big challenge. We aim to preserve the natural blue dye of C-phycocyanin (C-PC) phycobiliprotein from Spirulina microalgae. For this purpose, we incorporated C-PC in combined starch and β-lactoglobulin (BLG) hydrogels by using a high-pressure (HP) process. Indeed, in thermal treatment, the color derived from C-PC is entirely lost. We characterized the obtained HP gels by both rheology and small-angle X-ray scattering (SAXS). Various formulations of binary (BLG/C-PC) and ternary (starch/BLG/C-PC) systems were tested under HP up to 4,500 bar. A good preservation of the C-PC pigment was established by mixing BLG and starch with C-PC at pH 7, with concentrations of 180, 5, and 10 mg/mL, respectively. Identical component concentrations were maintained in the binary systems. Structure of gels was characterized by SAXS providing insight of C-PC interactions with BLG and starch after HP process which leads to the formation of solid gels with larger mesh compared to two-component systems. This results in enhanced mechanical properties, which were determined by amplitude and frequency sweep measurements using a rheometer with applied plane/plane geometry. Therefore, adding starch, even at small concentration, significantly improves gel visual appearance and mechanical properties. Our study reveals that preservation through HP treatment is more effective than high temperature treatment, as visually observed through the sustained color integrity of C-PC blue dye.

Acknowledgements

This work was supported by ANSO Project No. ANSOCR-PP-2021- 01.

Supported by

Република Србија

МИНИСТАРСТВО НАУКЕ,
ТЕХНОЛОШКОГ РАЗВОЈА И
ИНОВАЦИЈА

САНУ

СРПСКА АКАДЕМИЈА
НАУКА И УМЕТНОСТИ

Универзитет у Београду
ИНСТИТУТ ЗА МУЛТИДИСЦИПЛИНАРНА ИСТРАЖИВАЊА

University of Belgrade
INSTITUTE FOR MULTIDISCIPLINARY RESEARCH

Addendum

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

577.1(048)

SERBIAN Biochemical Society. International scientific meeting (12 ; 2023 ; Beograd)
"Biochemistry in Biotechnology" : [proceedings] / Serbian Biochemical Society, Twelfth Conference, International scientific meeting, September 21-23, 2023, Belgrade, Serbia ; [editor Ivan Spasojević]. - Belgrade : Faculty of Chemistry : Serbian Biochemical Society, 2023 (Belgrade : Colorgrafx). - 156 str. ; 23 cm

Tiraž 130. - Bibliografija uz većinu apstrakata.

ISBN 978-86-7220-140-6 (FOC)

a) Биохемија -- Апстракти

COBISS.SR-ID 124201993
