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EVOLUTIONARY ANALYSIS OF LUCIFERASE [LUC GENE] STRUCTURAL AND BIOCHEMICAL PROPERTIES IN RENILLA.RENIFORMIS

Biotechnology

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ABSTRACT

Luciferase is the most common enzyme which is mostly present in aquatic species that helps in preventing from capturing by other predators. Naturally Luc gene signaling expressed level of the luciferase enzyme in the cell of various luminescent organisms. In some cases, they might be sessile or free swimming, and their structure a huge piece of the skimming tiny fish. Similar species are regularly discovered broadly isolated in time and also in space. A few animal groups may have spread over topographical periods surpassing 100 million years. In firefly luciferase is a type of second reporter which is used for high resolution wide analysis of promoter activities. Reporter system has full capacity to oxidize luciferin protein in presence of oxyluciferin + CO2 + H2O and light, its reaction contains an enzyme called luciferase then a luciferin-binding protein which helps in sequestering and later in releasing the luciferin, apart from that they contain GFP i.e., green fluorescent protein. Bioluminescence signaling is a powerful biological property that has been repurposed by the many scientists as a reporting pathway in animals and plants. Such as luciferin substrate (soluble in water) can be used for visualization of dynamic changes expression of gene in luminescent species.

KEYWORDS

Artificial light, bioluminescence, luminescent bodies, Single-celled planktons, reporter gene, oxyluciferin.

INTRODUCTION

Human beings have 5 sense of organs among which sight is one of them they use this sensing organ **to** see things and to navigate themselves, they also use the artificial light sources such as torches, lights etc. Also if a person is blind it becomes worse for them to get around without light. There are also animals and birds like owl use their big eyes to collect a lot of light and to navigate themselves accordingly. On the other hand there are certain bioluminescent life forms which use their own different approach by making and carrying their own light. It was in 1600s that researchers started to research on the topic that how basically these animals make their own light. But it was quite hard for them to get along this topic because it was hard for them to know that what exactly is the regulatory on-off system which is present in their luminescent bodies. And other hindrance was that many species start to degrade their luminescent property when got captured, because this capturing knock down their light producing organs. There are so many bioluminescent species which are present on our planet, Earth. For instance, jack-o'-lantern mushroom (in which only a part of the fungus emits light). This is the land species. But the majority of the bioluminescent species are found in the ocean especially at the twilight zone (also known as poorly lit zone) of the depths of ocean. Because this region is quite low in light, in general, the twilight zone extends from about 660 feet (201 meters) to about 3,300 feet (1006 meters) deep(1).

There is very interesting fact to know and learn that there are certain places where in night-time there is formation of blue light on the edges of the sea level i.e., the sea water appear to glow translucently. This occurs due to the presence of the dinoflagellates and the phenomenon is known as milky sea, which makes the ocean to glow. The cause of this phenomenon is the disturbance of these single-celled planktons. Sometimes, these dinoflagellates glow so brightly that it hinders the navigation of marine objects like boat or the ships.

How To Some Produce Light?

When we talk about the bioluminescence there is contribution of the 2 things which makes the light-producing reaction possible. The first one is the luciferin and the other one is luciferase. Now what are these? You must be thinking! Term of luciferase and luciferin protein both are derived from Latin word "Lucifer" which mean Light bringers or bioluminescent (Burbelo, Peter D.Kisailus, Adam E.Peck, Jeremy W).

According to the Oxford languages, Luciferin is an organic substance, present in luminescent organisms such as fireflies, that produces light when oxidized by the action of the enzyme luciferase.

And According to the Oxford languages, Luciferase is the enzyme that catalyses the oxidation of a luciferin, causing it to produce a visible glow(2).

When we talk about the bioluminescence there is requirement of certain things to make that happen such as ATP molecules and oxygen molecule (there is requirement of these charged ions basically to activate the reaction of bioluminescence) to make this light making process happen. As we know that it is the ATP which we called as energy currency of the cell helps in the storing energy as well as in transporting the energy in the living organisms such as human beings. In reaction there is also the formation of certain by products such as oxyluciferin and water.

There is also the production of this bioluminescence from the food they eat i.e, the production of this luciferin in organism depends on the food they use to feed upon. Also, there certain dinoflagellates whose luminescence depends on the photosynthesis i.e, they bright in great manner after very sunny days.

In this article, we'll see the diversification of few species of genera i.e., Metridian, Pleuromamma, Lucicutia, Danio, Renilla and Heterorhabdus. Also we will look into the evolutionary data of the species and how they are linked together. Also, we will check basic characters and principle behind luminescence pathways, also analysis how animals use luminescent abilities to their advantage. Apart from this we will put light on the biochemistry of the mechanism of the *renilla reinformis* (sea pansy).

Principle

In a bioluminescent response, there is decay to the electronic ground state and release of energy in the form of visible light, the prosthetic phospho-groups or phosphoprotein are response (intermediate) in chemical oxidative reaction to form complex pathway for emit light. The effectiveness of the by and large measure is portrayed by a quantum yield for bioluminescence (fBL). This is the result of the part of the atoms responding division of particles entering the bioluminescence pathway that become electronically energized fluorescence quantum yield of the energized state item. Most bioluminescent responses are generally inefficient (e.g., for the Renilla luciferin–luciferase response in vitro, fBL ¼ 0.05); nonetheless, the firefly response is interesting in having a quantum yield for bioluminescence near to unity (fBL ¼ 0.88). The power of light outflow is 42 - 108 photon $s^{1}cm^{2}$ in the Flashlight fish (Photoblepharon) and 42 -109 phophoton s^{1} cm^{2} in the dinoflagellate Gonyaulax. Investigations of bioluminescent spectra uncover that the maximum amount of light discharge of most remote ocean species is in the reach

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of 450–490 nm (blue), that that of coastal front species is in the reach of 490–520 nm (green), and some of the terrestrial and fresh-water species also emit light or in either yellow light at 550-580 and green light at 510-540 nm range(3). A typical component of bioluminescent responses is that they are oxidation responses. Just oxidation responses can give the energy which is typically needed for the delivery of high-energy states that will transmit photons when they decay to the ground state (e.g., the energy needed to create blue light at 450 nm is 265.4 kJ mol 1). The recent and advance development has been done for the elucidation of the molecular biology of bioluminescence and the cloning of a considerable lot of the qualities that encode luciferases and photoproteins. These qualities, for the study of gene regulation as well as expression especially the firefly quality (luc), have been utilized as in contrast to the chloramphenicol acetyl-transferase quality (CAT) as reporter genes. Additionally, sets of bioluminescent qualities (e.g., firefly and Renilla luciferase) have been utilized for two-shading observing of the expression of two distinct qualities. Qualities for bioluminescent proteins have been moved to and expressed in various microbes, yeast, mammalian cells, transgenic mice, and plants. There is also a thing in which there is expression which is done to produce a bioluminescent protein which is done by fusing these with the genes that encode other proteins and those are the fused gene which helps in their expression. The combined/fused proteins are being discovered to use as forms in immunoassay and have replaced conjugates prepared by conventional covalent coupling techniques.

Figure 1.*R. Reniformis Fluorescent Org.*

Renilla Reniformis (Sea Pansy)

Renilla belongs to the Order, Pennatulacea; Family, Renillidae It is local to warm mainland rack waters of the Western Hemisphere. It is every now and again discovered washed aground on North-east Florida sea-shores following northeasterly breezes or unpleasant surf conditions. It additionally can regularly be discovered living intertidally totally covered in the sand. Its hunter is the striped ocean slug, Armina tigrina]

A draft genome of *R. reniformis* was sequenced in 2018, delivering a haploid genome size of 172 megabases (Mb). This makes it one of the most small and minimal coral genomes found to date.

Range Of Wavelength For Bioluminescent

Table.1 This table shows the marine species with their luminescence range (in nm) and the colour which they produce in that range. We have tried to compare these species on the basis of their luminescent ranges. These are the approximate values that we have got through literature study**. In this we can analyse that mostly the species emit green or the blue light, the reason behind this is that there is presence of the GFP (Green Fluorescent Protein). If there is presence of this protein the colour emit is green otherwise there is emission of blue light. Rest the Danio rerio ranges from red to yellow to green colour that is because of the recombination reason in which the the gene for the luminiscence is inserted by genetic modification.

S. No.	Luciferase (Luc gene) containing Species	Wave-length Range(nm)	Colour Appearance (Bioluminescence)
1	Metridia longa	482nm	Blue light
2	Metridia curticauda	482nm	Blue light
3	Metridia pacifica	480nm	Blue light
4	Pleuromamma xiphias	492nm	Blue light
5	Pleuromamma abdominalis	485 nm	Green light
6	Geminin Danio rerio	600nm,570nm, 520nm	Red, Yellow, Green colour
7	Gaussia princeps	480nm	Blue light
8	Heterorhabdus tanneri	460nm-489nm	Blue light
9	Renilla reniformis	508nm	Green light
10	Renilla mulleri	508nm	Green light
11	Lucicutia ovaliformis	493nm	Green light

Diversity Evolutionary Tree

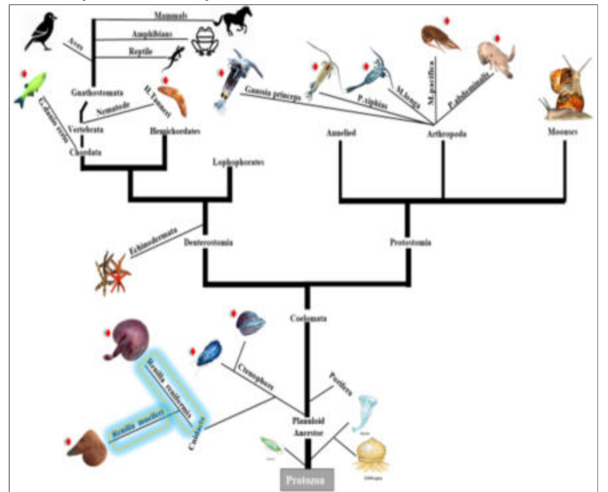


Fig.2 Diversity evolutionary tree of luciferase consisting species which are arises from different-different phylum of the same Animalia kingdom. Reference for Biology 11 - Component 5 (mrsoverholt. blogspot.com) Renilla muelleri and Renilla reniformis are evolved from cnidaria of Planuloid ancestor, and left of species are evolved from chordate, vertebrate (Nematode) of the Deuterostomia few of species (M.longa, P.xiphias, Gaussia princeps, M. pacifica and P.abdominalis are evolved from Protostomia (contain coelenterazine reaction substrate). These luciferase species are the ancestral species they are found in the deep marine where numbers of small micro-organism are attached to surface within algae components.

This picture depicts the diversity tree of all the species in which the luciferase is present. Protozoa are found in all those places where life exists. They are exceptionally versatile and effortlessly appropriated all around. They require dampness, regardless of whether they live in marine or freshwater living spaces, soil, rotting natural issue, or plants and creatures. They might be sessile or free swimming, and they structure a huge piece of the skimming tiny fish. Similar species are regularly discovered broadly isolated in time and also in space. A few animal groups may have spread over topographical periods surpassing 100 million years.

Protozoa are the most bountiful phagotrophs in the biosphere, yet no logical system has arisen that may permit exact meaning of the components of protozoan variety on a worldwide scale. We have begun this task by searching for the common ground between taxonomy and presence of an enzyme. We have used the methods such as taxonomic analysis software (http://cran.r-project.org/bin/windows/base/), and normal powerpoint presentation – to analyze the presence of Luciferase enzyme in all these species. Here we have distributed the classification in the following manner. We have started the diversity classification from eukaryote (as higher classification). It further leads to, planuloid ancestor. There has been a theory which told it is very similar to the planula larva of coelenterates.

From planuloid ancestor, branches for Ctenophora and porifera got distinguished. From the Ctenophora, branch for the Cnidaria have led out for the Renilla species. Further, Coelomata have branched into two i.e, Deuterostomia and Protostomia. Deuterostomia is further divided into Chordata and hemichordates. In chordates we have the species G. danio rerio and further in vertebrata we have H. tanneri (nematode). On the other hand, in Protostomia we have species in Arthropoda i.e, Gaussia princeps, P. xiphias, M. longa, M. pacifica and P. abdominalis. All red dotes basically show bioluminescent species which has consist luciferase and coelenterazine substrate for oxidative reaction, which help in emitting light.

Phylogenetic Tree Of Active Luciferase Marine Fishes

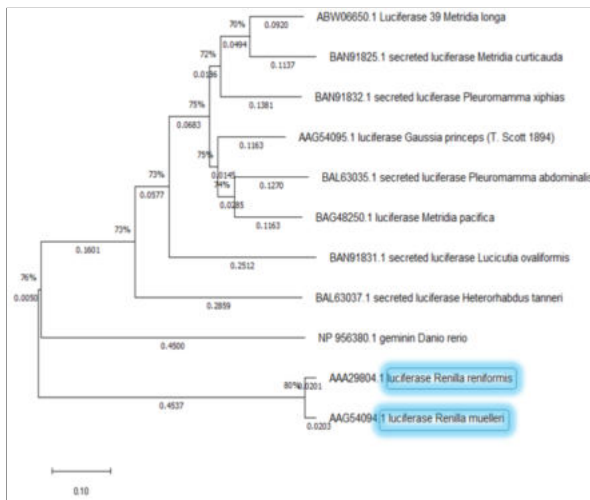


Fig.3 Test Neighbor joining phylogeny tree of related signaling luciferase species. The tree has been constructed with luminous species of different genera which is consists luciferin gene for the bioluminescence. In which all taxa species of this group clustered together in the bootstrap 100 replicates to show the next to the branch and showing the length of branches with the coverage data in the percentile manner which interconnected together by the nodes of the phylogeny tree. This evolutionary tree was conducted by the MEGA X (https://www.megasoftware.net/dload_win_gui*)* after alignment of the sequences.

This phylogenetic tree was constructed with mixed eleven luciferase consisting caudal species which is founds in the deep marine. All species of this tree are belonging from animalia kingdom but has different-different phylum some of from arthropda, molluscs and others. So many types of luminous species are founded on the earth (insect, fungi, molluscs, marine arthropods, caudal fish, and dinoflagellates etc). At the marine bank there are lot of small dinoflagellate move or locomotes in the evening and emits light by the bioluminescence. This unrooted tree was construct with Mertedia, Pleuromamma, Gaussia, Lucicutia, Heterorhabdus, geminin Danio and Renilla genera which are interconnected to each other based on molecular reaction and functionl analysis. Renilla reniformis and Renilla muelleri are outgroup species in the phylogeny luciferase tree which are connects to Geminin danio reno with 0.437 branch length by the group nodes. Metridia longa and Metridia curticauda show on top with 70% coverage data frequency and another species of Metridia (M.Pacific) is located with the Pleuromamma abdominalis with 0.1163 and 0.1270 branch length at 74% statistical data coverage frequency. Basically, luciferase coding protein is activated by the oxidative substrate of the species like coelenterazine was used as a substrate for the bioluminescent reaction in the luminous species. Metridia Curticauda luciferase is a hidden luciferase from the sea copepod and uses coelenterazine as a substrate to produce blue bioluminescence (λmax = 480 nm). The bioluminescent work of copepod M. Curticauda is supported by at least four pairs of mutant genes that emerged independently from the same parental gene after multiple duplication in the genome of the bright ancestor copepod. UM. This allows M. Curticauda retain light bioluminescence at various temperatures and can also serve as an example of cellular evolution in radical environmental changes. This luciferase has been used successfully as a bioluminescent reporter in mammals. Most of the calanoid species of the Lucicutia genus luminescence by the glandular cells of the bioluminous gland located at the caudal rami. One of the strongest evidence for the presence of luminescent character in the particular luminous organisms is the molecular and functional identification or functional analysis of luciferase. Homogenate species of the Lucicutia, Metridia, Heterorhabdus and Pleuromamma are earlier reported based on oxidative luciferase gene which is encoded at time of activation Coelenterazine substrate(4). The bright bioluminescence of copepod Metridia longa is enclosed in a small secret luciferase and copepod M. longa can maintain bioluminescence through the activity of four distinctly differentiated genes. which deviated outside the ancestralcopepod luciferase gene after several repetitions. Gaussiaprinceps luciferase (GLuc) produces a large burst of blue light when exposed to coelenterazine in the absence of ATP. Gaussiaprinceps luciferase (Gluc) is widely used as a reporter in eukaryotes as like another the ocean pansy is strikingly bioluminescent when disturbed in view of the interaction between a luciferase (Renilla-luciferin 2-monooxygenase) and green fluorescent protein (GFP). The two molecules have as of late become critical in biology. It likewise creates optional metabolites for synthetic protection that may make it an intriguing source of marine common products.

T-Coffee Sequence Alignment Of Luciferase Genera

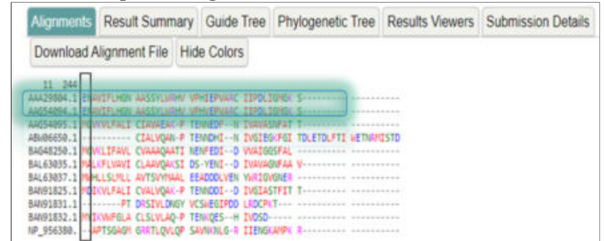


Fig.4 Sequence of luciferase Metridia, Pleuromamma, Gaussia, Lucicutia, Heterorhabdus, Geminin and Renilla genera are multiple sequence aligned by the Tree based Consistency (align bases length) Objective Function for Alignment Evaluation (T-Coffee) is used for generating pairs wise sequence alignment. Luciferase gene composed mainly seven exon which is non-coding part and revealed three TATA boxes and consist non-polar amino acid as side chain. T-Coffee (Results < T-Coffee < Multiple Sequence Alignment < EMBL-EBI) is measuring the sequence based on consistency which species of polar or non-polar amino acids interact with luciferase gene consistency.

Luciferase is an oxidative enzyme which has non-polar side chains of amino acids (valine, alanine, Proline, Methionine and Leucine or isoleucine) there is distinguish from the photoprotein because cause oxidative reaction and required ATPase for to emitting light or bioluminescent. Protein protein interaction basically occurs in all biological pathways process which is performed a particular function against the target substance. Like in signal transduction pathway in which interactions transmit signals that are regulates morphology of cell, cell apoptosis and cell regulation by transcription. There are variety of interaction database are present on online mode like on EMBL, BHL, IMEx, MINT and IDB consists in the interact form. Ranilla luciferase is a soluble protein in fusion that is exhibits high Renilla luciferase activity due to N terminal. *Renilla luciferase* consist negatively charged glutamic amino acid at C terminal of peptide sequence it can be used in the binding assay with immobilized protein. And Renilla luciferase and other genera (Geminin, Gaussia Metridia, Pleuromamma, Lucicutia, Heterorhabdus) fusion protein emit light from the coelenterazine substrate which is indicates the interaction between the two proteins that interact by the active transient protein protein interaction. This interaction is occurring between Cdc42 GTPase and its protein. *Renilla* and other *Pleuromamma, Lucicutia, Heterorhabdus luciferase* protein have different side amino acids molecules that are generates a particular stimulation towards the signaling proteins which is expresses by the coelenterazine substrate, coelenterazine founds in almost dinoflagellates species organisms mostly located at bank side of the deep marine. protein-protein interactions usually are unable to detect directly or provide molecular-level information about the activated signaling intermediates that are generated via conformation-induced steric changes. The Renilla reniformis luciferase gene expresses well in mammalian cells and is commonly used as a control co-reporter with firefly luciferase constructs Furthermore, Renilla luciferase and firefly luciferase have been used to study protein-protein interactions in more of references articles.

Crystal Structure Analysis Of *Renilla Reniformis* Fluorescent *Luciferase*

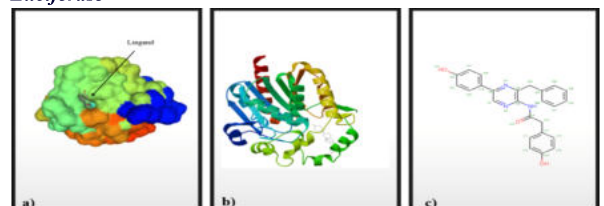


Fig.5 Crystal structure of Renilla reniformis luciferase with the ligand-

N-[3-BENZYL-5-(4-HYDROXYPHENYL)PYRAZIN-2-YL]-2-(4-HYDROXYPHENYL)ACETAMIDE which interact with coelenterazine substrate to emit light, it consists Alpha/Beta hydrolase fold, and catalytic domains help in protein ligand interaction. Coelenterazine substrate bind with ligand polar molecules or with hydrophobic interactions (W:156, D:162, L:165, F:180, K:189) with Hydrogen bonds E:144, D:162 and H:285.

a. This shows the surface structure of the Renilla-luciferin 2-monooxygenase, showing the presence of ligand.
b. This shows the ribbon structure of the protein with binding to its active site. The structure contains A and B chain having 2 binding sites at positions 162 and 285 respectively. The structure is been shown at the resolution of 1.80 Armstrong.
c. This picture shows the structure of ligand, N-[3-BENZYL-5-(4-HYDROXYPHENYL)PYRAZIN-2-YL]-2-(4-HYDROXYPHENYL)ACETAMIDE having molecular formula of $C_{25}H_{21}N_3O_3$. The molecular weight of the ligand is 411.45.

Renilla-luciferin 2-monooxygenase

Table.2 Sequential structure component analysis of Renilla luciferin 2-monoxygenase, consists number of Alpha helix and Beta strands with their binding site which are responsible for interaction between the ligand (Binding site CEI) and sequence of luciferin peptides and it is also consist a CIS peptide from base 258 to 259 which is responsible for stabilizing the helix by the disrupting the hydrogen bonds networking.

Feature	Position	Site	Peptide seq. no./Terminal
Alpha Helix	10-14	GLN A 10 MET A 14	5
Alpha Helix	16-23	THR A 16 ARG A 23	8
Alpha Helix	56-61	SER A 56 ARG A 61	6
Alpha Helix	63-67	VAL A 63 ILE A 67	5
Alpha Helix	93-106	ARG A 93 GLU A 106	14
Alpha Helix	121-133	TRP A 121 HIS A 133	13
Alpha Helix	158-168	ASP A 158 SER A 168	11
Alpha Helix	168-176	SER A 168 LEU A 176	9
Alpha Helix	179-184	ASN A 179 THR A 184	6
Alpha Helix	184-189	THR A 184 LYS A 189	6
Alpha Helix	195-204	GLU A 195 GLU A 204	10
Alpha Helix	205-207	PRO A 205 LYS A 207	3
Alpha Helix	210-212	GLY A 210 VAL A 212	3
Alpha Helix	213-222	ARG A 213 GLU A 222	10
Alpha Helix	230-246	LYS A 230 ALA A 246	17
Alpha Helix	263-271	SER A 263 LYS A 271	9
Alpha Helix	268-290	PHE A 286 ASP A 290	5
Alpha Helix	291-308	ALA A 291 LYS A 308	18
Beta Strand	25-29	LYS A 25 VAL A 29	5
Beta Strand	32-38	SER A 32 ASP A 38	7
Beta Strand	46-50	ALA A 46 LEU A 50	5
Beta Strand	72-76	ARG A 72 PRO A 76	5
Beta Strand	114-120	ILE A 114 ASP A 120	7
Beta Strand	137-145	ILE A 137 SER A 145	9
Beta Strand	252-259	LYS A 252 PRO A 259	8
Beta Strand	276-283	THR A 276 GLY A 283	8
Sequence Binding site	162	ASP A 162	N
Sequence Binding site	285	HIS B 285	C
Binding site CEI	156	TRP B 156	C
Binding site CEI	162	ASP B 162	C
Binding site CEI	174	MET B 174	N
Binding site CEI	180	PHE B 180	C
Binding site CEI	285	HIS B 285	N

Protein-Ligand Interaction

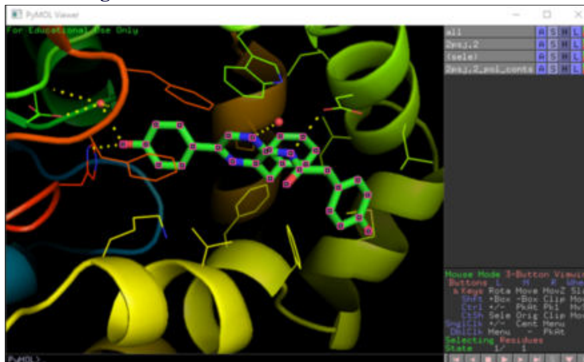
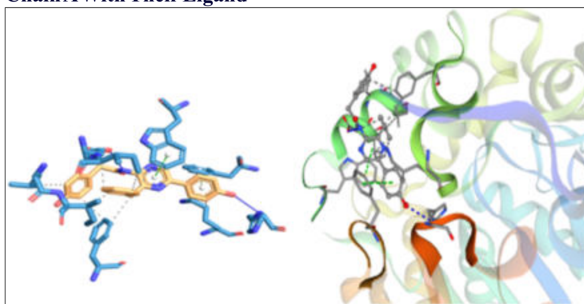


Fig.6 We have studied this Protein Ligand interaction via, the Pymol software, using the PDB ID: 2PSJ (DOI: 10.2210/pdb2PSJ/pdb).

In the protein Ligand interaction shown here in the picture they shows the 13 interactions, having Hydrophobic interactions: A:W.156, A:W.156, A:D.162, A:I.163, A:L.165, A:I.166, A:F.180, A:F.180, A:K.189 (B). But in comparison there is 14 interaction in the 2 part of the structure having Hydrophobic interactions at A:W.156, A:W.156, A:D.162, A:L.165, A:I.166, A:F.180, A:F.180, A:F.181, A:K.189 (D). Also, it shows the Hydrogen bondings between A:D.162 and A:H.285 (C) and in the 2 part it shows the Hydrogen bondings between A:E.144, A:D.162, A:H.285 (E). Here the coelenterazine (which is a substate of the Renilla luciferase). It consists of the aromatic imidazopyrazinone (at central position). Here RLUC, catalyzes an oxidative decarboxylation using the oxygen molecule present in the substrate. Due to this catalyzation, the imidazole ring got opened and there is release of CO_2. And hence, there is emission of photon due to the Relaxation of the electronically excited coelenteramide reaction (A).

Chain A With Their Ligand



Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	156A	TRP	3.68	5039	1251
2	156A	TRP	3.63	5028	1250
3	162A	ASP	3.67	5025	1298
4	163A	ILE	3.99	5027	1309
5	165A	LEU	3.76	5017	1322
6	166A	ILE	3.92	5026	1327
7	180A	PHE	3.56	5014	1444
8	180A	PHE	3.57	5022	1443
9	189A	LYS	3.91	5035	1513

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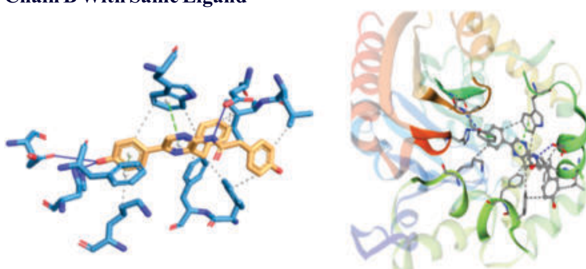
Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Sidechain	Donor Atom	Acceptor Atom
1	162A	ASP	2.25	3.08	141.99	✘	✔	5019 [Nam]	1301 [O3]
2	285A	HIS	2.44	3.02	120.02	✘	✔	5037 [O3]	2291 [N2]

Fig.7 Overview of the crystal structure of chain A ligand or Renilla luciferin 2-monooxygenase, which show the hydrophobic and binding site of the ligand (C25H21N3O3) at 156, 162, 174, 180 and 285 position. Also check bond angle and bond lengths by the following residue of Z score value which means the bond length depends on base pair or atoms of the chain (interrupt with ligand of the sequence). **Like,** this chain consists a Leucine molecule at 287 residue (atom is CA-CB-CG) which has Z score value is .07 that means there is no any chirality and planarity at this residue.

Chain B With Same Ligand



Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	156B	TRP	3.78	5059	3754
2	156B	TRP	3.88	5070	3755
3	162B	ASP	3.74	5056	3802
4	165B	LEU	3.72	5048	3826
5	166B	ILE	3.92	5057	3831
6	180B	PHE	3.63	5045	3948
7	180B	PHE	3.72	5053	3947
8	181B	PHE	3.88	5059	3957
9	189B	LYS	3.90	5066	4017

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Sidechain	Donor Atom	Acceptor Atom
1	144B	GLU	3.53	3.82	100.79	✔	✔	3674 [O3]	5068 [O3]
2	162B	ASP	2.18	3.01	141.90	✘	✔	5050 [Nam]	3805 [O2]
3	285B	HIS	2.37	2.89	114.13	✘	✔	5068 [O3]	4795 [N2]

Fig.8 Crystal structure of chain B and it's Ligand which interrupt with coelenterazine substrate, help in emitting light by the oxidative reaction. The prosthetic group of coelenterazine binds with the aqua or apoaequorin (hydrophobic group) to form active luciferin complex by the oxidative reactive reaction. Hydrophobic interacted residue are react with ligand atoms (174 and 285 residue). Chain B consists Non-aromatic side chain at 240 (Tyr) and 287 (Leu) position.

Mechanism

Renilla reniformis consist a R luciferase (RLuc) gene which is responses in oxidative reaction or also involve in catalytic degradation of coelenterazine substrate by releasing dioxetanone molecule intermediate. RLuc gene is present inside the intracellular membrane in the special light emitting cells that are response only when biological bioluminescence (green fluorescent) reaction is undergoes. After binding luciferin binding protein with coelenterazine luciferin in presence of Ca^{+2} ions (Ca^{+2} ions basically activate the luciferin binding protein) it forms Luciferin binding protein + coelenterazine luciferin complex pathway(6). All types of coelenterazine luciferin or substrate are interact with sequence chain protein of luciferase containing species, like all copepods contain coelenterazine independent substrate which at time of oxidative reaction by the releasing of di-oxygen molecules with carbon and left of photon help in emitting of light. When Coelenterazine oxyluciferin are interact with green fluorescent protein in the cytosol then it releases photon of blue light at 480 nm range (according to ward and seliger- photoprotein discover at protein bounding level) and also RLuc gene interact with fluorophore by via passing resonance energy of green photon at 505 nm which are emit green bioluminescence.

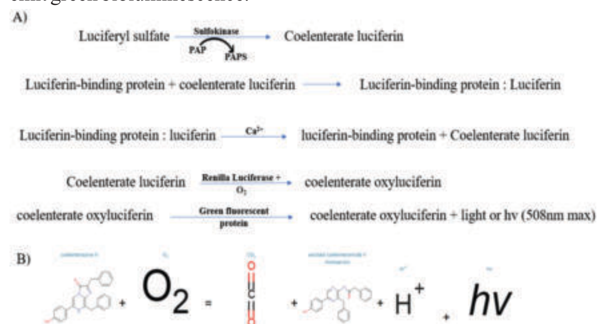


Fig.9 Basically, Luciferin protein undergoes oxidative enzyme catalyzed reaction with O_2 coelenterazine luciferin react with Renilla luciferase with oxygen and form and form coelenterazine oxyluciferin product, the result is splitting off a molecular fragment and intermediate between them that's help in emitting light upon decaying to its ground state. Basically this product react or undergoes a particular process of nonradiative energy which is transfer to an accessory protein (luciferin of Renilla), a green fluorescent protein (GFP), which results in green bioluminescence. In vitro, in the absence of GFP, the product emits blue light.

Catalytic Activity

Common name of the *Renilla reniformis* is sea pansy which in its reaction contains an enzyme called luciferase then a luciferin-binding protein which helps in sequestering and later in releasing the luciferin, apart from that they contain GFP i.e., green fluorescent protein. It apart from all these in the sequence of reaction involves a transfer of the charge between the GFP and the excited-state coelenterate oxyluciferin. There has been cloning done with the gene for *Renilla luciferase* and also has used for preparing a recombinant luciferase. Also, the GFP has been used in many gene expression which is being isolated from the Renilla species.

Ramachandran Plot Of *Renilla Reniformis*

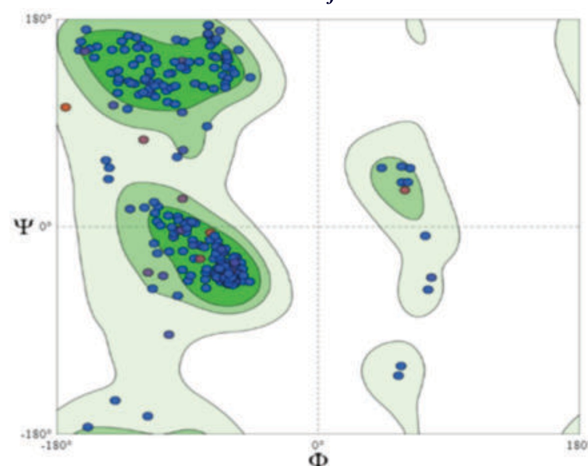


Fig.10 The figure shows the Ramachandran plot of the RLuc protein. The plot is made on the basis of Phi-Psi torsion angles of the protein backbone. Using this plot we are able to analyze the conformation of the protein as the Phi and Psi angles helps us to know that what the exact positions of the amino acids are.

The blue dots in the plot signify the amino acids present in the protein. The colored region shows the permissible configurations of the amino acid and the white region are unfavorable. The X axis denotes for the Phi angle (positive for the clockwise rotation and the negative for the anti-clockwise rotation of the bond), and the Y-axis denotes the Psi angle (the positive for the clockwise and the negative for the anti clockwise)(8).The upper half part of the plot tells us about the steric clash between carboxyl oxygen and side chain and on the other hand the lower half tells us about the steric clash between amide nitrogen and side chain The left upper corner told us about the beta sheets (parallel or the anti-parallel). The left below corner shows the right

handed alpha helix and the upper right upper shows the presence of the left handed alpha helix. the rotation of the bonds depends upon the R group of the amino acid but not all combinations of the Phi and Psi are possible due to physical clashes of the atoms in 3D space (if it is bulkier then because of the presence of the bulky group there comes the hindrance in it's rotation. And case is opposite when we talk about the light side chain or the R group. If it is light then there is no hindrance while rotating). To plot this Ramachandran plot by hand we need to collect the data regarding the atomic coordinates from the Protein Data Bank and then using structure we will estimate the Phi and Psi angles for the individual amino acids. Below we have generated the table by analyzing the Glycine and Proline side chain of the protein noting their Phi and Psi angles.

GLYCINE		
Amino acid	Phi	Psi
a.a:85	-62.44	176.25
a.a:229	-65.80	160.16
a.a:118	-127.12	140.78
a.a:88	-57.04	-25.71
a.a:122	-65.48	-31.42
a.a:171	-60.43	-32.64
a.a:269	-68.73	-38.06
a.a:296	-60.94	-40.57
a.a:17	-48.71	-56.38
a.a:52	-117.79	-142.34
a.a:283	162.20	171.91
a.a:90	76.14	6.77
a.a:80	82.00	-5.85
a.a:228	93.44	-5.73
a.a:82	50.19	-128.20
a.a:210	-96.91	-150.53
a.a:260	71.63	-175.62
PROLINE		
Amino acid	Phi	Psi
a.a:111	-64.46	171.29
a.a:274	-68.67	161.19
a.a:76	-85.04	152.01
a.a:251	-66.92	136.30
a.a:224	-91.31	59.03
a.a:259	-81.96	-7.14
a.a:69	-70.96	-12.30
a.a:187	-72.56	-20.69
a.a:8	-65.96	-20.60
a.a:220	-69.33	-25.94
a.a:231	-66.06	-30.57
a.a:205	-60.60	-24.58
a.a:65	-54.90	-29.14
a.a:215	-56.54	-32.80
a.a:292	-62.79	-38.58
a.a:157	-68.92	-38.80
a.a:18	-49.09	-44.95
a.a:196	-48.15	-45.90

ROLE OF LUCIFERASE IN BIOLUMINESCENCE

Bioluminescence is characterized by a variety of marine organisms, from bacteria to large squid and fish. Light is released when flavin pigment, luciferin, is oxidized in the presence of luciferase, an enzyme also produced by the body. (The chemical system is like that of flies.) The light produced is usually blue green, which has an electrical spectrum near the point of high ocean water transfer and is most noticeable in many deep-sea creatures. chemical reaction (chemiluminescence) in which the conversion of chemical energy into radiation is direct and 100% effective; that is, it reduces the temperature slightly in the process. For that reason, emissions are called cold light or luminescence(9).

The main function of the copepod bioluminescence is perhaps a counter-intuitive response or defensive behavior, although at the moment this is just speculation. This suggests that copepods save their bioluminescence from what appears to be the most powerful threat by the predator(3).

In addition to the protective function of bioluminescence on copepods, it can also be used for communication as a warning signal between individuals. Luminescence, adenosine triphosphate (ATP) begins to react with firefly luciferase, ionic magnesium, and firefly luciferin to form complex (luciferase-luciferyl-adenylate) and pyrophosphate. That is complex and responds with cellular oxygen to emit light. Sufficient energy is released in the final step to transform the electronic suspension of the luciferase-luciferyl-adenylate complex from a low-energy ground to a high-energy happiness. The high-energy building then depletes it by releasing a photon of visible light and returning it to the ground surface. Prey-predator linkage between bioluminescent copepod families and their predators (cephalopods, myctophids, gonostomatids and cnidarians.

Fig.11 Prey-predator linkage between bioluminescent copepod families and their predators (cephalopods, myctophids, gonostomatids and cnidarians **https://doi.org/10.1002/pro.3433**. Luciferase species has luciferin gene which interact with predator coelenterazine substrate in the presence of oxygen, Coelenteramine endowed with chain breaking properties.

Role Of Bioluminescence In Metabolism

The light production shows that it is associated with the protection and survival of the species. This is evident in the variety of squid, which includes a bright cloud to confuse the enemy and escape, and to the many marine fish that hang light traps to attract prey or show easy prey to hide their enemies, scare off food, or simply light a path in the dark ocean waters. The light of light-reflecting bacteria is extinguished when oxygen is released, it has been suggested that bioluminescent reactions were originally used to remove toxic oxygen. The body's response, including oxygen and luciferin, releases enough energy to stimulate the molecule in the body to emit visible rays. Many ancient luminaries later developed oxygen systems but retained luminescent energy as part of the body's interconnected pathways. or for a certain amount of survival luminescence that I can give the body.

Applications

- Green Fluorescent Protein – from jelly Aequorea was first found and was extracated. It is utilized as a biomarker from multiple ways today, since its DNA can be cloned and even a small amount of it could be combined into cells of numerous different creatures. GFP and other same fluorescent proteins can initiate glowing in microbes, protozoa, plants, nematodes, feathered creatures, warm blooded animals, fish and a lot more living being. Other fluorescent proteins, for example, Yellow Fluorescent Protein (YFP) have likewise been created. Osamu Shimamura, Martin Chalfie and Roger Tsien shared a Nobel Prize for their work here. One of the life forms into which GFP has been embedded is the zebrafish, Danio rerio, a normally used model creature. The "Glofish" accessible as pets are a maybe shocking consequence of this exploration(6).
- Tourism- Bioluminescence in the seas has been noticed for quite a long time (see Darwin's record of smooth oceans in the History tab). Today, sightseers rush to territories, for example, Puerto Rico's Vieques Island to observe the enchantment. In 2005, a gleaming territory generally the size of Connecticut was first seen from space in the Indian Ocean (PNAS 102:14181-14184; article by Miller, Haddock, Elvidge, and Lee).
- Bioluminescent imaging – or BLI – takes into account noninvasive imaging of organic cycles in living creatures. Among different utilizations, this makes it conceivable to consider the cycles of different sicknesses and of therapies for those illnesses. It can likewise be utilized to find tumors. Bioluminescent Resonance Energy Transfer, or BRET, is utilized to plan neuronal circuits to comprehend cerebrum work(3)(5).
- Quorum sensing -Studies on bioluminescence in microbes (specifically, bacteria) in ocean water prompted the revelation of what is known as quorum sensing. J. W. Hastings and E. P.

Greenberg revealed in the Journal of Bacteriology in May 1999 that there was no luciferase gene transcription at low cell densities, however radiant qualities do enact at high cell densities when the light produced is sufficiently splendid to fill a need. It is currently acknowledged that cell-cell correspondence in bacteria's is normal.

- Tools- Devices have been built up that utilizes bioluminescence from various perspectives. Since adenosine triphosphate (ATP), an energy putting away particle found in every single living cell. ATP is needed for outflow of light in such a manner that the measure of light is directly relative to the measure of ATP. Estimations of ATP can in this way distinguish contamination definitely more rapidly and precisely than conventional refined. An illustration of a device that does this is the BioScan made by GE. This little instrument estimates microbes in water just and very quickly(2).

MATERIALS AND METHODS

In this article, we have used many databases and softwares. With the help of only these databases we were able to completely understand the concept.

List of the tools that we have used is as follows:

(The databases and software that we have used are all open source.)

1. PYMOL: Using this software we were able to analyze the 3-D structure of the protein. Mostly structures in this assignment have been analyzed using this software only. Binding site were also analyzed using this software.
2. SWISS- model: Using this database, we took the structure of the protein of interest. We also assessed the structure. Apart from that the Ramachandran plot study is also being done using this database.

The Protein Ligand interaction, along with the Ligand study was being done using this database.

3. Uniprot: This database helped to get the code for the protein of interest. Because the Swiss model only takes the code from this database. Also, the structural analysis was done using this database.

RESULTS AND DISCUSSION

After completing this article, we have learned and analyzed some points about the bioluminescence species containing luciferin protein. These points are as follows:

1. We have first generated the phylogenetic tree and diversity tree of eleven marine species using the MEGA X and in that we have compared the coverage data frequency level of all the species. And we have saw that there is a different branch that has formed for two species which are Renilla Reniformis and Renilla Muelleri having a 80% coverage data value with 100 bootstraps.

2. Second thing that we have discussed in this article is the T-Coffee sequence alignment in which we have shown the side chain of the amino acid which interacts via covalent or the non-covalent bond to the protein. We have highlighted the two Renilla species which have side chain containing the Glutamic amino acid. It also consists of alpha amino acid and negatively charged carboxylic group. There is this non-covalent interaction occurring in between the ligand and protein which helps glutamic amino acid to neutralize their negative charges. On the other hand, the rest of the 9 species consists of the Methionine group as a side chain molecule.

3. The third result that we have analyzed by our own understanding while studying the Protein-Ligand interaction in Renilla reniformis is the presence of the 5 interaction or the binding site of the ligand (TRP 156, ASP 162, MET 174, PHE 180 and HIS 285), these groups are interacting with the Protein via non-covalent bond, and the reason behind these 5 sites is the breaking of the sulfate bonds of substrate because of presence of weak interaction bonding. We also saw that the presence of the CIS peptide at position 258-259 helps in stabilizing the helix by maintaining the hydrogen bonding.

CONCLUSION

In Conclusion, to this paper we have learned about the Bioluminescence protein i.e, RLuc (Luciferase/Luciferin) in Renilla reniformis. T Coffee sequence Alignment was done for the various marine species. It was used for generating pairs wise sequence alignment of species, Metridia, Pleuromamma, Gaussia, Lucicutia, Heterorhabdus, Geminin and Renilla genera. Later in the article, there has been study of the crystal structure of Renilla reniformis fluorescent luciferase. It's the Ligand and protein interaction which helps in the production of light in the species. There has been ribbon structure and the ligand study done in the paper using the Pymol software. The Crystal structure of Renilla reniformis luciferase with the ligand-N-[3-BENZYL-5-(4-HYDROXYPHENYL)PYRAZIN-2-YL]-2-(4-HYDROXYPHENYL)ACETAMIDE which interact with coelenterazine substrate to emit light, it consists Alpha/Beta hydrolase fold, and catalytic domains help in protein ligand interaction. Coelenterazine substrate bind with ligand polar molecules or with hydrophobic interactions (W:156, D:162, L:165, F:180, K:189) with Hydrogen bonds E:144, D:162 and H:285. There has been a Sequential structure component analysis of Renilla luciferin 2-monoxygenase, consists number of Alpha helix from position 10- 14, 16-23, 56-61, 63-67, 93-106, 121-133, 158-166, 168-176, 179-184, 184-189, 195-204, 205-207, 210-212, 213-222, 230-246, 263-271, 268-290 and 291-308 and Beta strands from position 25-29, 32-38, 46-50, 72-76, 114-120, 137-145, 252-259 and 276-283 with their binding site which are responsible for interaction between the ligand (Binding site CEI) and sequence of luciferin peptides. There have been a lot of functions of this bioluminescence in the organisms such as for defense, for beauty, communication, warning signal. There is also a great role of metabolism in the bioluminescence. The application includes many certain points from Tourism, Bioluminescent imaging, Quorum sensing and as tools.

Abbreviations

fBL: Fibrillarin
MEGA X: Molecular Evolutionary Genetics Analysis X
CAT: Chloramphenicol Acetyl Transferase
R. Reniformis: Renilla Reniformis
M.Pacific: Metridia Pacific
EMBL: European Molecular Biology Laboratory
BHL: Biodiversity Heritage Library
IMEx: The International Molecular Exchange Consortium
MINT: Molecular INTeraction Database
IDB: Index DataBase
RLuc: Renilla Luciferase
GFP: Green Fluorescent Protein
YFP: Yellow Fluorescent Protein
BRET: Bioluminescent Resonance Energy Transfer
BLI: Bioluminescent Imaging

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