Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Procedure for reductive methylation of protein to improve crystallizability

Neil Shaw
National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China
Chongyun Cheng
National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China
Zhi-Jie Liu
National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

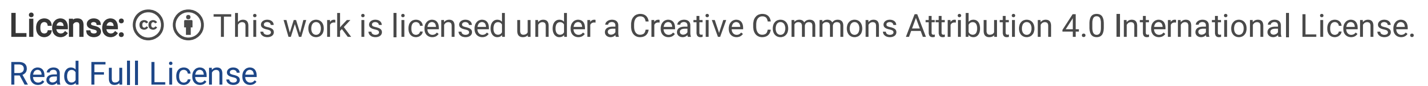
---

Method Article

Keywords: methylation of lysine

Posted Date: July 20th, 2007

DOI: https://doi.org/10.1038/nprot.2007.287



License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Introduction

Post genomics era has seen a number of new challenges emerge while moving towards the goal of covering the protein fold space such that eventually a protein could be modeled from its primary sequence using known protein structures. While expression is still a major challenge for a number of medically important proteins, a soluble expression may not always guarantee a 3D structure. Many soluble proteins purified to homogeneity, resist crystallization because of the intrinsic flexible nature of the surface residues. Lysine and glutamic acid residues found on the surface of the proteins, have long side chains that are mobile. These side chains need to be localized in space in order to promote homogenous inter molecular interactions necessary for formation of a crystal lattice. Addition of methyl groups to the side chain amine of lysines1-9 has been shown to promote crystallization presumably by immobilizing these side chains. The protocol outlined here describes the process of reductively methylating a protein for improving its crystallizability.

Reagents

1. Dimethylamine borane complex \(DMAB) \(Sigma, cat. no. – 15584) 2. 36 % solution of formaldehyde \(Sigma, cat. no. – F8775) Stock Solutions 1M DMAB Prepare 1ml of 1M stock solution of DMAB by dissolving 59mg of DMAB in 1ml of deionized water taken in a 1.5ml eppendorf tube. Cover the eppendorf tube with aluminum foil to prevent exposure of solution to light. 1M Formaldehyde Prepare 1ml of 1M stock solution of formaldehyde by dissolving 84µl of formaldehyde \(36 % solution) in 1ml of deionized water taken in a 1.5ml eppendorf tube.

Equipment

1. Gel shaker 2. Acta Purifier^{TM} \(Amersham Biosciences, USA)

Procedure

1. Pipette out 1ml of 10mg ml^{-1} protein sample \(critical in PBS and pure and homogeneous) each in two 1.5ml eppendorf tubes. 2. Cover the tubes with aluminum foil. Label one tube as “control”. Do not add any chemicals to this tube. Label “methylated” on the other tube. 3. Add 20µl of 1M DMAB solution followed by 40µL of 1M formaldehyde solution to the “methylated” tube. 4. Shake both the tubes \(“control” and “methylated”) at 4 °C in dark on a gel shaker maintained at 100 rpm for 2 h. 5. Repeat steps 3 and 4 twice. 6. Add 10µL of DMAB and incubate for 12 h at 4 °C in the dark while shaking. 7. Run size exclusion chromatography on “control” and “methylated” protein using 20mM Tris, 150mM NaCl, pH8.0 for buffer exchange. 8. Concentrate “control” and “methylated” protein to ~ 10-15 mg ml^{-1} 9. Analyze proteins using SDS PAGE and mass spectroscopy. 10. Screen control and methylated protein for crystallization under identical conditions.

Timing

18h

Critical Steps

Step 1: Protein should be pure, homogenous and in a buffer without primary amines. Phosphate buffered saline could be a good choice for this step. Step 6: Size exclusion chromatography using Tris-based buffer helps quench and remove excess chemicals.

Troubleshooting

Step 9: If there is no increase in molecular weight prepare fresh stock of reagents and repeat the procedure.

Anticipated Results

The above procedure results in dimethylation of free amine groups. An increase in molecular weight by 28 daltons for each free amine group \(lysines and the N-terminal amine) is expected. Usually a qualitative shift in protein band on SDS PAGE and size exclusion elution profiles is observed for the methylated protein when compared to the control. Mass spectroscopy is used to quantitatively confirm the success of the procedure. The methylated protein is expected to behave different than control during crystallization. Therefore, when methylation is used to improve crystal quality, the methylated protein may not crystallize under the same conditions where the control protein crystallized previously. In such cases, the methylated protein needs to be screened again for crystallization.

References

1. Liu, Z.-J. _et. al_.: The high-throughput protein-to-structure pipeline at SECSG. _Acta Crystallogr. D_ **61**, 679-84 \(2005). 2. Rayment, I. Reductive alkylation of lysine residues to alter crystallization properties of proteins. _Methods in Enzymology_ **276**, 171-179 \(1997). 3. Geoghegan, K., Cabacungan, J., Dixon, H. & Feeney, R. Alternative reducing agents for reductive methylation of amino groups in proteins. _Int. J. Pept. Protein Res_. **17**, 345-352 \(1981). 4. Gerken, T., Jentoft, J., Jentoft, N. & Dearborn, D. Intramolecular interactions of amino groups in ^{13}C reductively methylated hen egg-white lysozyme. _J. Biol. Chem_. **257**, 2894-2900 \(1982). 5. Means, G. & Feeney, R. Reductive alkylation of amino groups in proteins. _Biochemistry_ **7**, 2192-2201 \(1968). 6. Rypniewski, W., Holden, H. & Rayment, I. Structural consequences of reductive methylation of lysine residues in hen egg white lysozyme: An X-ray analysis at 1.8Ǻ resolution. _Biochemistry_ **32**, 9851-9858 \(1993). 7. Schubot, F. & Waugh, D. A pivotal role for reductive methylation in the de novo crystallization of a ternary complex composed of Yersinia pestis virulence factors YopN, SycN and YscB. _Acta Cryst. D_ **60**, 1981-1986 \(2004). 8. Kobayashi, M., Kubota, M. & Matsuura, Y. Crystallization and improvement of crystal quality for

X-ray diffraction of maltooligosyl trehalose synthase by reductive methylation of lysine residues. _Acta Cryst. D_ **55**, 931-933 \(1999). 9. Walter, T., Meier, C., Assenberg, R., Au, K.-F., Ren, J., Verma, A., Nettleship, J., Owens, R., Stuart, D. & Grimes J. Lysine methylation as a routine rescue strategy for protein crystallization. _Structure_ **14**, 1617-1622 \(2006)

Acknowledgements

This work was funded by the 863 \(Grant 2006AA02A316) and 973 \(Grant 2006CB910901) Project of the Ministry of Science and Technology of China，the National Natural Science Foundation of China \(30670427) and the Institute of Biophysics, CAS, China.