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Nat Methods. Author manuscript; available in PMC 2009 August 1.

Published in final edited form as:
Nat Methods. 2008 October ; 5(10): 853–854. doi:10.1038/nmeth1008-853.

Large-scale evaluation of protein reductive methylation for improving protein crystallization

Youngchang Kim[1], **Pearl Quartey**[1], **Hui Li**[1], **Lour Volkart**[1], **Catherine Hatzos**[1], **Changsoo Chang**[1], **Boguslaw Nocek**[1], **Marianne Cuff**[1], **Jerzy Osipiuk**[1], **Kemin Tan**[1], **Yao Fan**[1], **Lance Bigelow**[2], **Natalia Maltseva**[1], **Ruiying Wu**[1], **Maria Borovilos**[2], **Erika Duggan**[2], **Min Zhou**[1], **T Andrew Binkowski**[1], **Rong-guang Zhang**[1], and **Andrzej Joachimiak**[1]

1*Midwest Center for Structural Genomics, Biosciences Division, Argonne National Laboratory, 9700 S. Cass Ave., Argonne, Illinois 60439, USA.*

2*Midwest Center for Structural Genomics, On Assignment, Labsupport Services, 9450 W. Bryn Mawr Avenue, Rosemont, Illinois 60018, USA.*

To the editor: Obtaining diffraction-quality crystals is a major bottleneck in protein X-ray crystallography. For example, the current success rate for protein structure solution at the Midwest Center for Structural Genomics (starting from purified protein) is ~10%. Protein crystallization is influenced by many factors, and many methods have been developed to enhance crystallization. In particular, reductive methylation of proteins has been successfully applied to obtain high-quality crystals[1-4]. Several studies[3,5,6] have indicated that methylating the solvent-exposed ε-amino group of lysines changes protein properties (pI, solubility and hydropathy)[7,8], which may promote crystallization via improving crystal packing. Reductive methylation of proteins is a simple, generic method; it is fast, specific and requires few steps under relatively mild buffer and chemical conditions and can be executed for several proteins in parallel. Native and methylated proteins have very similar structures, and, in most cases, methylated proteins maintain their biochemical function[2,5,9]. Some proteins can only be crystallized after methylation[3,10], and crystals of modified proteins often diffract to higher resolution[3,9]. The efficacy of the method has been previously tested on 10 proteins, with a 30% success rate[3].

Here we investigated the application of reductive methylation on a large scale. We applied a previously described reductive methylation protocol[2,11] (Supplementary Methods online) to 370 sequence-diverse proteins selected from protein families that had no structural homologs with >30% sequence identity. We expressed 370 recombinant proteins and purified them using standard methods[12] and screened them using standard crystal screening methods (Supplementary Methods). Of the 370 proteins, 269 proteins had not previously yielded crystals suitable for structure determination (crystals were too small, poorly ordered, twinned, highly mosaic or multiple), 85 proteins had previously failed to crystallize and 16 proteins were a reference set (not previously screened for crystallization; Table 1 and Supplementary Tables 1 and 2 online). After reductive methylation, we obtained diffraction-quality crystals for 40 of the 370 proteins, and so far we solved 26 crystal structures (Table 1). The crystallization success rate of methylated proteins did not correlate with the number of lysines, pI, hydropathy or molecular weight (Supplementary Table 1).

Correspondence to: Andrzej Joachimiak.

e-mail: E-mail: andrzejj@anl.gov.

Note: Supplementary information is available on the Nature Methods website.

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We also determined the structures of 4 proteins in their native as well as their methylated states (Supplementary Methods). By comparing these structures, we obtained insight into how methylation affects protein crystallization. We observed a decrease in the isotropic *B* factor (Fig. 1), which is likely a result of more ordered crystal packing and which leads to better diffraction limits. Indeed, the resolution of the methylated structures (average, 2.07 Å) was better than that of their native counterparts (average, 3.05 Å; Supplementary Table 1). The methylated lysines were engaged in various intra- and intermolecular interactions with protein and solvent (carboxylates and main chain carbonyls as reported earlier[2-4] as well as with histidine and arginine residues and water; Supplementary Figs. 1 and 2 online). Adding methyl groups effectively increases the lysine interaction radius by 1-1.2 Å, allowing a weak, long-distance ε-amine (>4.2 Å) interaction with oxygen or nitrogen to be replaced with stronger (3.3 Å) ε-amine-(*N*-methyl) oxygen or nitrogen interactions (Supplementary Fig. 3 online). Methylation of lysine may be considered as similar to replacing it with an arginine, which has a higher propensity for interactions and is found more often on protein-protein[13] and crystal packing interfaces[14-16] (Supplementary Figs. 1, 4 and 5 online). *Ab initio* quantum mechanical calculations using ethylamine and *N*,*N*-dimethylethyl amine model compounds (Supplementary Methods) provided an estimate of the binding energy between *N*-methyl groups. The computation results predicted the optimal methylated amine interaction distance with the water oxygen to be ~3.3 Å (Supplementary Fig. 3), consistent with observations in the crystal structures.

We showed in this large-scale study that reductive methylation of proteins provides a generally useful, simple, inexpensive and efficient method to alter protein-surface properties that can improve the crystallizability of proteins. We achieved an overall success rate, from purified protein to structure, of 7.0% for proteins that did not yield a structure in initial attempts, thus demonstrating that reductive methylation can be an effective method for increasing the chances of obtaining a structure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We thank all members of the Structural Biology Center and Midwest Center for Structural Genomics at Argonne National Laboratory for their help in conducting these experiments, S. Li, C.B. Lindberg and C. Giometti for running mass spectrometry experiments, A. Edwards for reading and commenting on the drafts, and L. Butler for preparing the manuscript. This work was supported by US National Institutes of Health (GM62414, GM074942) and by the US Department of Energy, Office of Biological and Environmental Research, under contract DE-AC02-06CH11357.

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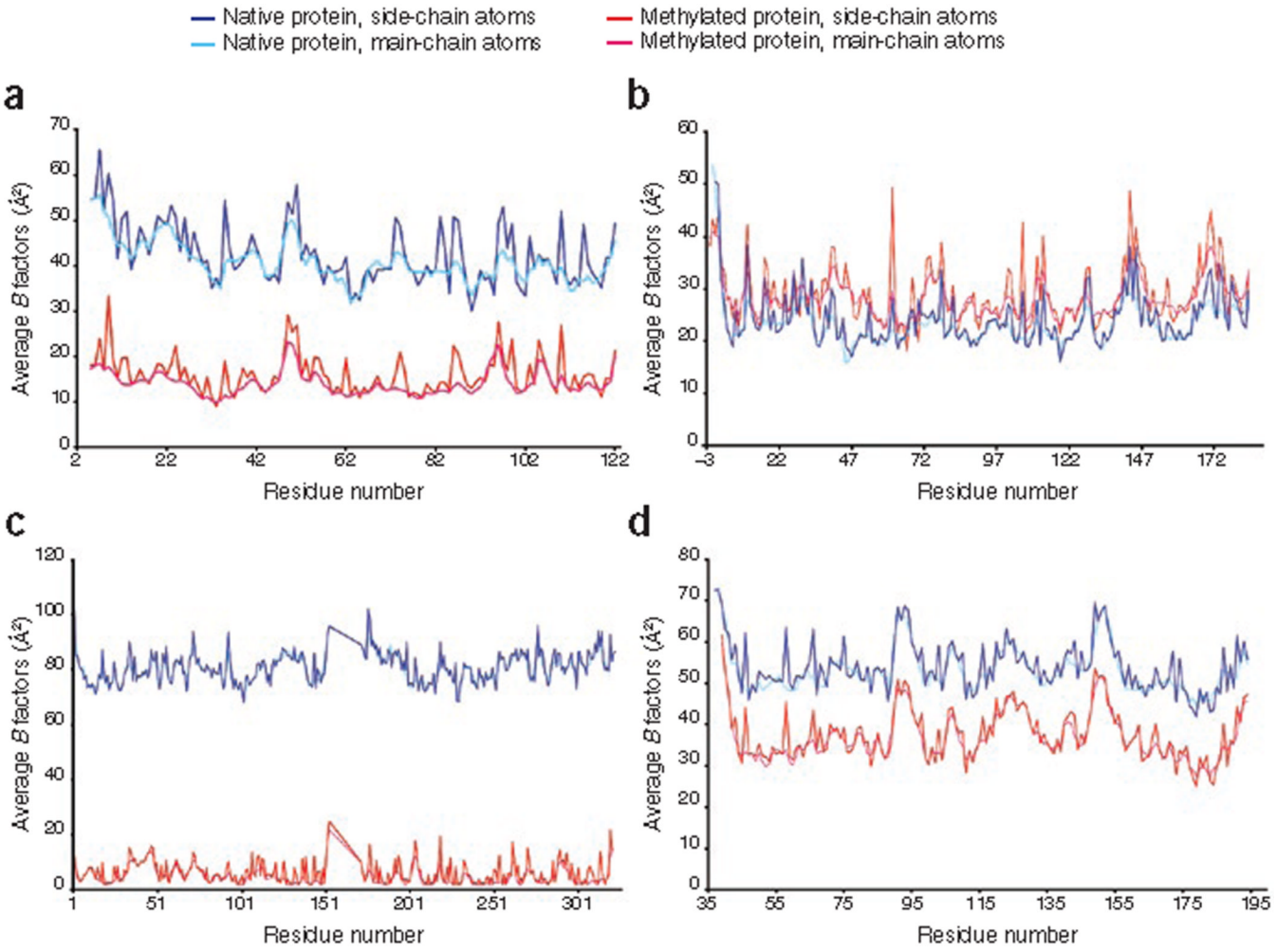

Figure 1.

Plots of isotropic *B* factors calculated for protein structures solved in both the native and methylated states. (**a**) Putative HopJ protein VP0580 (Protein Data Bank (PDB) identifiers 2QHQ (methylated) and 2QM2 (native)). (**b**) Flavodoxin FldA (PDB identifier 2ARK (methylated)). (**c**) Gfo/ Idh/MocA family oxidoreductase SP1482 (PDB identifier 2HO3 (methylated) and 2H05 (native)). (**d**) Extracellular domain of arabinofuranosyltransferase DIP0159 (PDB identifier 2IDL (methylated)).

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Table 1

Summary of methylation results for protein sets used in this study

Description of protein set	Number of proteins in the set	Macroscopic crystals after methylation	Diffraction-quality crystals after methylation	Structures solved	Overall success rate (percentage of structures solved)
Crystallized previously but crystals unsuitable for structure determination	269	59	33	20	7.4
Screened previously but no crystals obtained	85	8	5	5	5.9
Proteins not previously screened	16	5	2	1	6.3
Total	370	72	40	26	7.0