

COMPUTATIONAL STUDIES OF THE EFFECTS OF REDUCTIVE METHYLATION ON PROTEIN MOLECULES

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INTRODUCTION

X-ray crystallography is one of the most widely used methods for determining the structures of biomacromolecules, accounting for over 80% of the data deposited in the Protein Data Bank (PDB). In the crystallographic studies, protein crystals play a central role. Due to the limited understanding on the protein crystallization process, however, obtaining high quality protein crystals remains the rate-limiting step in the entire pipeline (1). Various approaches have been proposed to improve the success rate of protein crystallization, and the reductive methylation method, which selectively modifies the lysine residue in the protein, garners the most interest. In the Midwest Center for Structural Genomics (MCSG), it is part of the routine protocol, and has been shown to improve crystal quality and facilitate the crystallization of proteins that would otherwise not crystallize. Despite its success in practice, little is known about the underlying mechanism. In the present study, we employed quantum mechanical (QM) calculations and molecular dynamics (MD) simulations to investigate the atomistic details of reductive methylation and its effects on the protein molecules.

EXPERIMENTAL

The interaction of lysine residue sidechain (natural and methylated) with water molecule was calculated with GAMESS (2) at the HF/6-31G(d) level, using ethyl ammonium and dimethyl ethyl ammonium as the model compound, respectively. An interaction profile was obtained by plotting the intermolecule interaction energy values against the relative position of the water molecule to the nitrogen atom in the ammonium cation. Also, the molecular mechanics force field parameters of di-methylate lysine residue were optimized using the QM calculation results as a reference.

The atomic structures of both natural and methylated protein molecules were determined at the MCSG before being deposited to PDB. Hydrogen atoms were added to the PDB structures, assuming the environmental pH as 7.0, and both the natural and methylated lysine residues were modeled as protonated. The protein molecule was then soaked in

a cubic water box, which constitutes pre-equilibrated water molecules. Appropriate numbers of counter ions (sodium and/or chlorine) were randomly placed inside the water box to counter the charges from the protein molecule. The whole system first undergoes a brief energy minimization to eliminate bad contacts, then, with harmonic restraints on the heavy atoms of the protein molecule, was heated to 300K in 200ps. The harmonic restraints were gradually released during the heating process, and when the system reached 300K, an additional 250ps of equilibration was carried out without any restraints. Data collection began after equilibrium was reached, as judged from the changes in temperature and energy values. A total length of over 10ns MD trajectory was collected for each protein system. All aforementioned MD simulations were conducted using the NAMD package (3).

RESULTS

The interaction energy profile indicates that just like ethyl ammonium, which forms a strong hydrogen bond with the surrounding water molecule, dimethyl ethyl ammonium also interacts favorably with water molecules, albeit with a lower strength. The distance at which the diethyl methyl ammonium interacts most favorably with water is around 3.4Å. Dimethylated lysine residues are therefore believed to maintain this optimal distance when interacting with nearby molecules, which is exactly the case in the crystal structures.

One interesting finding from the MD simulations is that the methylated lysine residue is a medley of two seemingly contradictory properties, being heavily involved in hydrogen-bond like interactions while exhibiting considerable hydrophobicity. This unique combination leads to the restructuring of the local environment, which in turn propagates to other regions of the protein molecule, eventually altering the global motion patterns. The changed global movements are likely the origin of the different behaviors in crystallization.

REFERENCES

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