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Binding free energy based analysis of arsenic (+3 oxidation state) methyltransferase with *S*-adenosylmethionine

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ABSTRACT

Human arsenic methyltransferase (hAS3MT) is identified as the enzyme catalyzing the methylation of arsenic, acting as a carcinogen. Discovery of *S*-adenosylmethionine (SAM) binding site is essential for understanding the methylation of arsenic. Here, the structure of human arsenic methyltransferase (hAS3MT) is proposed and SAM-hAS3MT complex is optimized using molecular dynamics. Furthermore, hydrogen bond network around SAM is studied for stable structure of hAS3MT and to have an insight into the binding affinity, free energy calculations are performed using Molecular Mechanics energies combined with Poisson-Boltzmann or Generalized-Born Surface Area continuum solvation (MM(PB/GB)SA) methods. The role of each residue contributing to the free energy is explored through energy decomposition analysis. To understand the significance of active site residues, eight mutants of hAS3MT are prepared, mutating the residues reported to have functional significance in experimental studies. Mutants are subjected to alanine scanning analysis to determine crucial residues for SAM binding. The findings may lead to the treatment and prevention of serious health threats caused by arsenic.

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1. Introduction

Arsenic (As) is an environmental toxin and carcinogen contributing to cancers of lung, bladder and skin. Due to its ubiquity, it is necessarily detoxified in every organism usually through methylation. Arsenic methylation is catalyzed by an enzyme called As(III) *S*-adenosylmethionine (SAM) methyltransferase, known as ArsM in microbes and hAS3MT in humans. hAS3MT methylates inorganic As into trivalent methylarsenite MAs(III) and dimethylarsenite DMAs(III), these are believed to be more carcinogenic and toxic than inorganic As [1]. Development of drugs for AS3MT is important to prevent diseases caused by arsenic toxicity. For this purpose, identification of inhibitors or activators is vital as a preliminary step [2]. It is primarily important to understand the mechanism of hAS3MT methylation and most essentially SAM binding site in hAS3MT for the identification of drug-like molecules [3].

SAM, a conjugate of nucleotide adenosine and amino acid methionine, is an essential metabolic intermediate in every organism having several SAM-dependent methyltransferases [4]. AS3MT utilize SAM as the methyl group donor, leading to *S*-adenosylhomocysteine (SAH). Mechanisms of arsenic methylation and transfer of methyl group from SAM to arsenic atom have remained the topic of debate since last few years [3,5–8]. The crystal structure of hAS3MT has not been resolved, but the crystal structures of eukaryotic red alga *Cyanidioschyzon merolae* CmArsM have been reported with or without bound SAM or As(III) (PDB ID: 4FS8, 4FR0, 4FSD) [9]. The protein CmArsM is used as a template for the development of hAS3MT homology model. Identification of SAM and arsenic binding positions is essential for having an insight into the methylation mechanism of arsenic by hAS3MT. SAM binding sites, deduced majorly from the AS3MT from diverse species through sequence alignment, have been elucidated in enough detail in the experimental studies [10–12]. Residues from hAS3MT that form a prospective network of hydrogen bonds around SAM are TYR59, GLY78, SER79, ASP102, MET103, GLN107, ILE136, GLU137 and CYS156. A stretch of amino acid residues from 74-ILDLGSGSG-82 has been recognized from the sequence alignment of several methyltransferases utilizing SAM as SAM-binding motif I. This includes GLY78 and SER79 from the putative hydrogen bonding network. The function and importance of these residues have been examined experimentally. Herein, the importance of these residues through computational study is highlighted.

A combination of homology modeling, molecular dynamic simulations, hydrogen bond analysis, and free energy calculations is used to investigate the binding of SAM with hAS3MT. Molecular Mechanics/Generalized Born Surface Area (MMGBSA) and Molecular Mechanics/Poisson-Boltzmann Surface Area (MMPBSA) are employed for free energy calculations. These methods use molecular mechanics force fields and implicit solvation models with additional benefits including fast sampling of conformational space and speed of computing [13,14]. MMGB/PBSA methods are combined with MD simulation studies in explicit water solvent models to give the best methods for calculation of absolute ligand binding affinities in terms of accuracy [15]. In our knowledge this is the first detailed in silico study on hAS3MT. We performed alanine scanning by mutating the residues reported to be of

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Table 1
Template and target information.

Target protein	Accession number	Length	Template ID	Template protein	Source	Query coverage	e-value	Maximum identity
Human arsenite methyltransferase	Q9HBK9	375	4FR0_A	ArsM arsenic(III) *S*-adenosylmethionine methyltransferase with SAM	Cyanidioschyzon sp.	83%	$2e^{-75}$	42%

functional importance into alanine, except for GLY78. Alanine scanning is a method primarily aimed to determine how a particular side chain pertaining to a particular amino acid effect the protein activity and contribute to the energy changes such as electrostatic and van der Waal (vdW) energies. Due to its smaller structure, mutation of glycine might lead to significant conformational changes resulting in high energy values [16]. In this study, free energy calculations are combined from per residue energy decomposition analysis and in silico alanine scanning mutagenesis methods. The contributions of different energy components including electrostatic, van der Waals and solvation energies have been thoroughly explored. Moreover, enzymatic interactions with its substrate, such as hydrogen bonds and salt bridge interactions are also investigated.

2. Methods

2.1. Structure modeling

The crystal structure of CmArsM was used as template to build the homology model of hAS3MT using Modeller 9v12 [17]. The criteria for selection of template was query coverage, sequence identity and e-value among them. The detailed information and parameter values are given in Table 1. The first 35 residues of hAS3MT do not have the relative coordinates in CmArsM structure and hence could not be assigned to a structure. These residues were not reported in the CmArsM structure to be present in the binding domains. The sequences of hAS3MT and CmArsM were aligned using the align2d program within Modeller. The 3D structure of the hAS3MT model was generated and the conformation with lowest value of the Modeller objective function was selected and used as the starting point for further optimization. Homology modeling was also performed using four different web-based tools/servers for determining the best model: (i) SWISSMODEL [http://swissmodel.expasy.org/] (ii) I-TASSER [http://zhanglab.ccmb.med.umich.edu/I-TASSER/] (iii) Mod-web [http://modbase.compbio.ucsf.edu/ModWeb20-html/modweb.html] (iv) EsyPRED [http://www.fundp.ac.be/sciences/biologie/urbm/bioinfo/esypred/]. The criterion of selecting the model over other web-based servers was primarily based

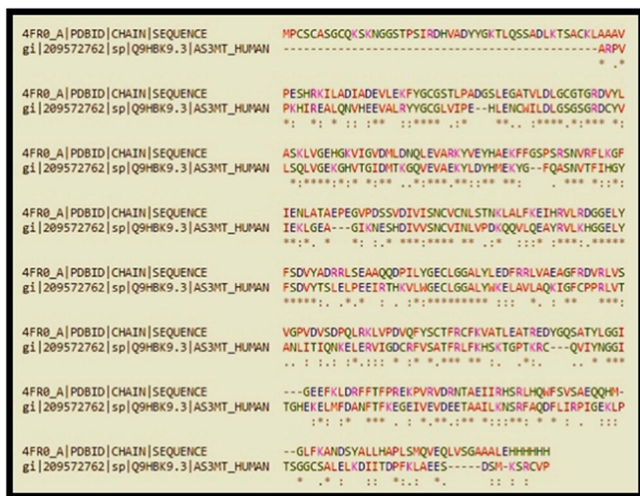

Fig. 1. Sequence alignment of target and template sequences.

upon the results of Ramachandran plots depicting the complete coverage of amino acids present in favorable regions and further confirmed through other validation tools.

The modeled structure was energy minimized using UCSF Chimera [18] for 750 steps of each conjugate gradient and steepest descent algorithms. The minimized structure was then subjected to PROCHECK analysis for the assessment of protein structure through Ramachandran plot [19]. Other tools used to assess the quality of homology model are Verify3D [20], ERRAT [21] and ProSA [22].

The bound structure of AS3MT is obtained through docking with SAM using GOLD5.2 software [23]. Default parameters in GOLD for genetic algorithm are selected with 10 number of runs. GoldScore Fitness function is used for docking which is based on:

$$\text{GoldScore Fitness} = S_{hb-ext} + S_{vdW-ext} + S_{hb-int} + S_{int} \tag{1}$$

where S_{hb-ext} and $S_{vdW-ext}$ are the hydrogen bond and van der Waals scores for enzyme-substrate complex, respectively. S_{hb-int} and S_{int} are the contributions to the GoldScore Fitness function due to intramolecular hydrogen bonds and intramolecular strain in the substrate, respectively. All other parameters were set as default. SAM-binding site was mapped from sequence alignment between CmArsM and hAS3MT sequences (Fig. 1).

2.2. Molecular dynamics simulation protocol

MD simulations of homology model and SAM bound AS3MT were performed for the purpose of structural investigation. The set of simulation studies were carried out using the AMBER software [24]. Preprocessing of SAM involved the application of antechamber program. The general AMBER force field (GAFF) [25] was chosen for SAM and for enzyme the ff14SB force field [26]. To record the topology of the enzyme and inhibitors, the LEaP module was employed. To bring electrostatic neutrality to the system, 8 and 9 Na + ions were added to SAM bound hAS3MT and homology model hAS3MT respectively. The system was placed in TIP3P box of water molecules. Such cubic box in simulations is used because of its geometrical simplicity [27]. Energy minimization was performed by executing 1500 steps of the steepest descent method followed by 1000 steps of the conjugate gradient method. The cutoff for non-bonded interaction was set as 8 Å. Heating for 10 ps of the system was performed at constant temperature of 300 K and constant volume (canonical ensemble). Next the system is equilibrated for 100 ps, periodic boundary conditions with constant pressure and Langevin thermostat is used.

The production runs for 10 ns for both systems were performed with explicit solvent models in the isothermal-isobaric ensemble (T = 300 K; P = 1 atm). Long-range electrostatic effects were modeled using periodic boundary conditions (PBC) and particle-mesh-Ewald method (PME) [28], with a weak coupling algorithm used to couple the temperature to an external bath [29]. The bond lengths involving hydrogen bonds were constrained by the use of SHAKE algorithm [30]. The temperature was kept constant through employing Langevin coupling integration algorithm. The time step equal to 2 fs was chosen to solve the Newton's equations and the trajectory files were collected every 1 ps for the succeeding exploration. Ptraj module in the AmberTools 14 was used for all MD trajectory analyses and visual examination is performed using VMD software [31].

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