Archives of Biochemistry and Biophysics 492 (2009) 82–92

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

An investigation of the catalytic mechanism of *S*-adenosylmethionine synthetase by QM/MM calculations

George D. Markham [a,*], Fusao Takusagawa [b], Anthony M. DiJulio [a], Charles W. Bock [a,c]

[a] *Institute for Cancer Research, Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111, USA*
[b] *Department of Molecular Biosciences, University of Kansas, 1200 Sunnyside Avenue, Lawrence, KS 66045-7534, USA*
[c] *Department of Chemistry and Biochemistry, School of Science and Health, Philadelphia University, School House Lane and Henry Avenue, Philadelphia, PA 19144, USA*

ARTICLE INFO

Article history:
Received 7 July 2009
Available online 20 August 2009

Keywords:
S-Adenosylmethionine
Methionine adenosyltransferase
Enzyme mechanism
Substrate-assisted-catalysis
QM/MM

ABSTRACT

Catalysis by *S*-adenosylmethionine synthetase has been investigated by quantum mechanical/molecular mechanical calculations, exploiting structures of the active crystalline enzyme. The transition state energy of +19.1 kcal/mol computed for a nucleophilic attack of the methionyl sulfur on carbon-5′ of the nucleotide was indistinguishable from the experimental (solution) value when the QM residues were an uncharged histidine that hydrogen bonds to the leaving oxygen-5′ and an aspartate that chelates a Mg^{2+} ion, and was similar (+18.8 kcal/mol) when the QM region also included the active site arginine and lysines. The computed energy difference between reactant and product was also consistent with their equimolar abundance in co-crystals. The calculated geometrical changes support catalysis of a S_N2 reaction through hydrogen bonding of the liberated oxygen-5′ to the histidine, charge neutralization by the two Mg^{2+} ions, and stabilization of the product sulfonium cation through a close, non-bonded, contact between the sulfur and the ribose oxygen-4′.

Introduction

S-Adenosylmethionine, AdoMet,[1] and its metabolites play a vast number of roles in cellular life [1]. AdoMet is one of the few sulfonium ions found in nature, and the cationic center endows it with a chemical versatility matched by few other biological entities, enabling it to act as an alkylating agent and free radical precursor, as well as a regulatory agent [2–4]. Methyl transfer from AdoMet is perhaps its most widely recognized role, participating in intermediary metabolism and in the modification of nucleic acids and proteins. DNA methylation forms a basis for the burgeoning field of epigenetics [5], while aberrant DNA methylation is common in cancers wherein errors are associated with alterations in DNA replication and transcription [6,7]. In a different family of pathways, decarboxylation of AdoMet followed by transfer of the propylamine moiety leads to the polyamines spermine and spermidine which are utilized in the regulation of cell proliferation [8,9]. In a distinct role, increasing numbers of AdoMet dependent enzymatic reactions are being recognized as having 5′-deoxyadenosyl free radicals as transient intermediates formed by reductive C5′—S bond cleavage [4,10].

The only known biosynthetic route to *S*-adenosylmethionine is catalyzed by *S*-adenosylmethionine synthetase, (ATP:L-methionine *S*-adenosyltransferase, often abbreviated as MAT) [11,12]. The two-step reaction catalyzed by MAT has a number of features that are unique in biology, as it encompasses displacement of the entire tripolyphosphate chain from ATP by the sulfur of methionine, followed by hydrolysis of the resultant tripolyphosphate (PPP_i) moiety to PP_i and P_i before product release; P_i originates from the γ-phosphoryl group of ATP and incorporates an oxygen atom from a water molecule [12,13]. Thus the enzyme has a bifunctional active site that catalyzes both AdoMet formation and PPP_i hydrolysis, the latter step being required to remove a kinetic and thermodynamic trap that arises due to the high affinity of PPP_i for the enzyme [12,14]. MAT sequences from eucarya and bacteria are highly conserved, with typically greater than 80% sequence homology, and the polar active site residues are retained in all of the hundreds of known sequences [15]. MATs exist as dimers or tetramers in nature, and vary substantially in kinetic behavior (e.g. k_{cat} and K_m values, cooperativity) [11,15]. MATs from archaea have distinct sequences that are highly conserved within that kingdom, and representatives of the archaeal class are found in a few bacteria [15,16].

The MAT from *Escherichia coli* (denoted cMAT) is the best characterized family member in terms of catalytic mechanism

* Corresponding author. Address: Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111, USA. Fax: +1 215 728 2412.
E-mail addresses: gd_markham@fccc.edu, m_estes@fccc.edu (G.D. Markham).

[1] *Abbreviations used:* AdoMet, *S*-adenosylmethionine; AMPPNP, adenylylimidodiphosphate; cMAT, *S*-adenosylmethionine synthetase from *Escherichia coli*; DFT, density functional theory; MAT, *S*-adenosylmethionine synthetase (methionine adenosyltransferase); PPNP, imidotriphosphate, $O_3P–NH–PO_2–O–PO_3$; QM/MM quantum mechanical/molecular mechanical; TS, transition state.

0003-9861/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2009.08.010

[13,14,17–22]. The crystal structure of cMAT provided the first insight into the architecture of a MAT, and structures have been reported for the ligand-free (apo) enzyme, as well as for several complexes [17,23,24]. cMAT is composed of 383 residue subunits and is typically found as a tetramer [13]. The four active sites are located in ~30 Å deep cavities between subunits and have contributions from residues from two subunits; cMAT does not show any cooperativity in kinetics [13]. Some mutants of cMAT are also active as dimers, supporting this state as the minimal functional unit (20). Additional crystal structures have shown that rat liver MAT and the human non-hepatic MAT have the same topology as cMAT [25,26], with an rmsd for the main chain carbons of less than 1.3 Å between any two of the structures. AdoMet formation is postulated to occur in a single chemical step *via* a direct S_N2 attack of the methionine on C5′ of ATP, based on the observed inversion of stereochemical configuration at C5′ during the reaction [27] and the magnitudes of primary and secondary kinetic isotope effects [19]. The free energy profile for the steps in the conversion of enzyme-bound substrates to products shows that in the active site AdoMet formation is energetically favorable while the subsequent PPP_i hydrolysis step has an equilibrium constant near unity [14,22]. MATs utilize two divalent cations (M^{2+}) per subunit for both catalytic activities, and certain monovalent cations stimulate these reaction rates by nearly three orders of magnitude [13]; *in vivo*, these cations are presumably Mg^{2+} and K^+. Crystallographic and spectroscopic data show that one of the divalent metal ions binds to all three phosphoryl groups while the second is ligated to the α- and γ-phosphoryl groups [18,24]. The monovalent cation activator also binds at the active site and appears to organize the active-site structure rather than directly partake in catalysis [24]. Coordination of two divalent metal ions to the α-phosphoryl group is anticipated to facilitate C5′—O5′ bond cleavage by polarizing the C5—O5′ bond in the reactant and neutralizing the negative charge that develops during AdoMet formation; however there are no data to support this notion. Furthermore the roles of the protein itself in the catalysis of AdoMet formation remain elusive, and the results of side-direct mutagenesis studies are largely ambiguous [20–22,28–30].

Crystal structures of cMAT have provided a foundation for understanding the means by which the surrounding protein facilitates the formation of AdoMet. Most notably are the structures of the catalytically active crystalline protein in complexes formed by incubation of the protein crystals with methionine, the alternate substrate adenylylimidodiphosphate (AMPPNP), and the activators Mg^{2+} and K^+ [24]. Utilization of AMPPNP stops the reaction sequence after formation of AdoMet and PPNP, the non-hydrolyzable analog of the normal tripolyphosphate intermediate [13]. These crystals contained approximately equal abundances of the reactant complex, E·2Mg^{2+}·AMPPNP·methionine·K^+ and the intermediate analog complex, E·2Mg^{2+}·AdoMet·PPNP·K^+ [24]. These crystal structures provided the starting points for our computational studies which were directed toward understanding the various molecular interactions involved in catalysis.

Computer simulations of enzymatic reactions are capable of providing tests of postulated reaction mechanisms, as well as insight into the catalytic contributions of individual residues and cofactors at a resolution currently unattainable by experimental methods [31–37]. This is an important convergence for MAT; despite decades of effort, cMAT crystals have had limiting resolution of 2.5 Å [17,23,24] while the crystal structures of rat liver MAT (for which some mechanistic data are also available) have been reported at resolutions in the range of 2.5–3.0 Å (cf. [15,26]). Recently, the 1.2 Å resolution structure of the non-hepatic human MAT, for which there have been no mechanistic studies, has been deposited by a structural genomics project (PDB file 2P02) [25]. Comparison of the highest resolution structures of each of these three MATs reveals an rmsd for α carbons of <1 Å, and <1.4 Å for all atoms in conserved residues, indicating that the cMAT structure provides a sound framework for further mechanistic studies despite the modest resolution of the structural model. Furthermore the initial steps in computational investigations include addition of the appropriate hydrogens (which are nearly X-ray transparent) and geometry optimization, which can mitigate some experimental uncertainty in atomic positions.

The combined quantum mechanics (QM)/molecular mechanics (MM) methodology (QM/MM) enables the rigor of QM methods to be applied to the reactive center while the remainder of the system is described by computationally more economical MM methods. The MM region exerts its influence *via* the combination of an anisotropic electrostatic environment and a framework within which the reaction site is constrained. The use of density functional theory (DFT) in the QM region allows some effects of electron correlation to be incorporated into the calculations while retaining sufficient computational efficiency to allow application to larger QM regions; the 229 atom QM region in our calculations of the Large_QM system is, to our knowledge, among the largest active site models that have been studied by DFT using these methods. The nearly identical crystal structures of the reactant and product complexes of cMAT show that large-scale protein conformational alterations need not be included in these calculations, reinforcing the suitability of a QM/MM approach utilizing a single protein conformation [24]. In the present study, QM/MM calculations of the MAT reaction in the architecture of the cMAT crystal structure have been used to investigate the molecular interactions that catalyze AdoMet formation and to evaluate whether an S_N2 reaction with the experimentally determined rate is consistent with the available crystal structures.

Computational methods

The protein structural model used as the starting point for the QM/MM calculations was derived from the experimental 2.5 Å resolution crystal structure reported in the PDB files 1P7L and 1RG9, which contain the reactants L-methionine and AMPPNP [24] or products AdoMet and PPNP. Extensive interactions between two subunits of the protein are evident in the crystal structures, consistent with a dimer (with the subunits denoted by A and B in the PDB file) being the minimal functional unit [20]. Thus a dimer was chosen as the protein framework for our studies.

The protein structure was prepared by removing all ligands, followed by the addition of hydrogens as appropriate for ionization states at neutral pH, positioning the polar side chains in tautomers and rotamers to maximize hydrogen-bonding and ion-pairing interactions. Computational software from Schrodinger L.L.C. (New York, NY) was used throughout this study. The protein was then subjected to a restrained MM minimization using the OPLS-AA force field (2001) in the program IMPACT; during this minimization all heavy atoms were constrained by a modified harmonic potential with an energy penalty $\Delta E = C * (r - r_0)^2$ for $(r - r_0) > 0.3$ Å (with $C = 25$ kcal/(mol Å^2)) and $\Delta E = 0$ for $(r - r_0) \leqslant 0.3$ Å so that there was no energy penalty for movement within 0.3 Å of the crystallographic positions, The tolerance of 0.3 Å was chosen based on an analysis of the experimental uncertainty in atomic coordinates according to Luzatti [38]. The final prepared structure included a total of 11,921 atoms and had an rmsd of 0.17 Å for all heavy atoms relative to the un-minimized protein structure, with a maximal deviation of 0.33 Å. The system was not "solvated" by the addition of explicit surface water molecules because the active site is buried deep within the protein structure and the surface atoms were frozen throughout the calculations. There is little quantitative information available in the lit-