Density Functional Theory Investigation of As(III) S-Adenosylmethionine Methyltransferase

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ABSTRACT: Arsenic is one of the most pervasive environmental toxins. It enters our water and food supply through many different routes, including the burning of fossil fuels, the application of arsenic-based herbicides, and natural sources. Using a density functional theory (DFT) cluster approach, the mechanism of arsenic (III) S-adenosylmethionine methyltransferases and various selenium-containing analogues was investigated. Notably, the methylation of arsenic by arsenic (III) S-adenosylmethionine is proposed to be a way to remove arsenic from contaminated water or soil. From the DFT cluster results, it was found that the selective substitution of the active-site Cys44, Cys72, and Cys174 residues with selenocysteines had a marginal effect on the barrier for CH$_3$ transfer. Specifically, the average Gibbs activation energy was calculated to be only 4.2 kJ mol$^{-1}$ lower than the Gibbs activation energy of 107.4 kJ mol$^{-1}$ for the WT enzyme. However, importantly, it was found that with selective mutation, the methylation process becomes considerably more exergonic, where the methylation reaction can be made to be 26.4 kJ mol$^{-1}$ more exergonic than the reaction catalyzed by the WT enzyme. Therefore, we propose that the selective substitution of the active-site Cys44, Cys72 and Cys174 residues with selenocysteines could make the process of methylation and volatilization more advantageous for bioremediation.

INTRODUCTION

Arsenic is known to be carcinogenic and is believed to be involved in causing skin, lung, and bladder cancer. Moreover, arsenic is also speculated to be associated with diabetes and circulatory, respiratory, and cardiovascular diseases. Arsenic is one of the most pervasive environmental toxins because it enters the water and food supply through many different routes, including the burning of fossil fuels, the use of arsenic-based herbicides, and natural sources. Because of both mining and irrigation of groundwater contaminated with arsenic, widespread soil contamination with arsenic has occurred. High levels of arsenic have been found in drinking water in India, Bangladesh, and China. As a result, there has been an increase in arsenic accumulation in food crops.

The methylation of inorganic arsenic results in the formation of the monomethylated arsenic (MAs) species which can then be subsequently methylated to dimethylated (DMAs) and trimethylated (TMAs) species. It has been proposed that the methylation of arsenic is an essential part of the cycling of arsenic among terrestrial, aquatic, and atmospheric environments. The end product of this methylation is trimethylarsine (i.e., TMAs(III)), which is volatile and has been detected in the air above rice paddy soils. Importantly, arsenic methylation has been investigated as a means to remove arsenic from contaminated water or soil and thus can be advantageous for means of bioremediation. Arsenic can be methylated by many different organisms, including bacteria, plants, fungi, and animals.

Arsenic (III) S-adenosylmethionine methyltransferases (AsMTs) (ArsM in microbes and AS3MT in higher eukaryotes) are enzymes that methylate arsenic. These enzymes are ubiquitous in nature, with the majority found in prokaryotic and eukaryotic microbes. These enzymes utilize S-adenosyl methionine (SAM) as a methyl source, which is subsequently converted to S-adenosylhomocysteine (SAH).

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after transferring a methyl group forming MAs.²² In nearly
every ArsM/AS3MT analogue, there are four conserved
cysteine residues involved in the methylation of As.²³ In the
case of ArsM found in the red alga Cyanidioschyzon (PDB
code: 6CX6), these residues are Cys44, Cys72, Cys174, and
Cys224. The presently accepted mechanism is shown in
Scheme 1.²³
The first step in the methylation by AsMTs is the transfer of
the methyl group from SAM to As(III) via an SN₂-type
reaction, resulting in the formation of a MA(V) intermediate.²³
In the second step, the formation of a disul¬
fi¬de bond between Cys44 and Cys72 provides electrons to reduce MAs(V) to
MAs(III).²³ Not shown in Scheme 1 is the regeneration of the
active site by thioredoxin, which reduces the disul¬
fi¬de bond in
AsMTs to regenerate the cysteine residues for successive
methylation cycles to form the di- and trimethylated species.²³
In the present work, density functional theory (DFT) was used
to investigate the two-step mechanism (Scheme 1) for the
methylation of As(III) S-adenosylmethionine methyltransferas
to form MAs(III).

RESULTS AND DISCUSSION

Mechanism of the Wild Type. As noted in Compu-
tational Methods, there are four active cysteine residues in
CmArsM that are believed to be involved in the binding of As
(Scheme 1). These residues are Cys44, Cys72, Cys174, and
Cys224 (Figure 1).
For the reactive complex (RC), the calculated As(III)···CH₃
distance was 3.88 Å, whereas the S_SAM···C(H₃) distance was 1.83 Å. The Mulliken charge on the sulfur atom of SAM is +1.06e, whereas the total charge on the CH₃ moiety is −0.08e, indicating that at the present level of theory, the positive charge is localized on the S-atom of SAM, which is of course consistent with the standard picture of the formal charges present in SAM. The initial step of the mechanism is the SN₂
attack by the As, resulting in the transfer of the CH₃ moiety from SAM, forming the MA(V) intermediate (Figure 2 TS1). It is noted that such reactions are well-known for SAM enzymes.²⁷ For the transfer of the CH₃ from SAM to As(III), a transition state (TS) was obtained, where a frequency of 1401.8 cm⁻¹ was observed. Visual inspection of the imaginary
frequency confirmed that the TS corresponds to the transfer of CH₃ from SAM to As. The Gibbs activation energy for this step was calculated to be 107.4 kJ mol⁻¹ (Figure 2).
For TS1, the calculated value of r(As(III)···CH₃) was 2.45 Å, whereas the value of r(S_SAM···C(H₃)) was calculated to be 2.37 Å. Thus, the CH₃ is approximately centered between the sulfur atom of SAM and As. The calculated Mulliken charges indicate that the positive charge is still highly localized on the sulfur atom of SAM. Specifically, the calculated Mulliken charge on the S-atom was +0.71e. However, for the transferring CH₃, partial positive charge transfer does exist, where the summed Mulliken charge on the CH₃ is +0.12e. Regarding the As atom, it has not gained any positive character in the TS, where the Mulliken charge was calculated to be +0.06e. In the RC, the charge on the As was calculated to be +0.07e. Thus, charge transfer and CH₃ transfer do not occur concomitantly.

With complete transfer of the CH₃, the intermediate complex ICI is formed. For ICI, the As(III)···CH₃ distance is 1.93 Å, whereas the S_SAM···C(H₃) distance is 3.47 Å, indicating complete transfer of the CH₃ moiety from SAM to As(III)-forming SAH and MAs(V), respectively. The oxidation of As is evidenced by the considerable increase in the Mulliken charge on the As atom, which has increased from +0.07e in RC to +0.51e in ICI. In the case of the ligating sulfur atoms, the
change in the Mulliken charge was calculated to be on average +0.12e, indicating charge transfer from the ligating sulfurs to help stabilize the high oxidation state of As(V). For IC1, the As···S72 bonds have on average shortened by 0.05 Å (Table S1). This shortening is expected, given the oxidation of As(III) to As(V), where the tighter binding of the S atoms of the ligating Cys residues would help to stabilize the highly oxidized As center. From Figure 2, IC1 lies 32.8 kJ mol\(^{-1}\) higher in energy than RC.

The next step in the mechanism is formation of the disulfide between Cys72 and Cys44 with concomitant two-electron reduction of MAs(V) (Scheme 1). However, prior to this step, the attacking thiol of Cys72 was deprotonated such that the anionic S\(^{-}\) is able to nucleophilically attack the S\(^{···}\)As bond. As noted above, the proton was modeled to be lost to the bulk solvent, where a chemical potential of −1124.2 kJ mol\(^{-1}\) was used for a proton in a dilute aqueous environment. With the deprotonation of Cys72, the thiolate attacked Cys174 and not the proposed Cys44, resulting in the formation of the disulfide bond and reduction of the MAs(V) to MAs(III) without a TS. With the attack of Cys174 by Cys72, the reduction of MAs(V) is supported by the change in Gibbs reaction energy was found to be −6.750 Å apart, whereas the sulfur atoms of Cys174 and Cys72 are only 4.02 Å apart. For IC1, the sulfur atoms of Cys72 and Cys44 are 6.750 Å apart, whereas the sulfur atoms of Cys174 and Cys72 are 3.53 Å apart. Thus, from the experimentally obtained crystal structure and present QM model (i.e., IC1), Cys72 is in better position to attack Cys174 and not Cys44.

Overall, the present QM cluster model appears to capture the details of the methyl transfer by CmArsM. The calculated barrier is within acceptable limits for an enzymatic reaction and the overall process is exergonic.

In the case of biochemical systems, sulfur can be readily oxidized to sulfoxides and sulfones,\(^{26,27}\) Se-containing catalysts on the other hand are known to have enhanced durability over their S-analogues. Specifically, selenium confers resistance to irreversible oxidation, generally seen in sulfur-containing systems.\(^{26,27}\) Thus, to determine the effects on the kinetics and thermodynamics of the methylation of As, we generated several models, where Cys44, Cys72, and Cys174 were mutated to their selenocysteine analogues.

**Effect of Mutating Select Active-Site Cysteine Residues.** Selenium lies directly under sulfur on the periodic table, thus shares many similar chemical and physical properties.\(^{28,29}\) However, there are also some key differences.\(^{27}\) For instance, selenium has a stronger reductive ability compared to sulfur.\(^{30}\) Moreover, thiol/disulfide exchange reactions are accelerated in solution with the replacement of S by Se because of selenolate being both a better nucleophile and a better leaving group than thiolate.\(^{31,32}\) In fact, it has been stated that almost all chemical reactions involving Se are faster than the analogous reactions involving S.\(^{27}\)

To investigate the effect of Se on the methylation mechanism by AsMT, five active-site models were created with different key active-site cysteine residues modified to selenocysteine residues. The single-mutant models investigated were Cys44Sec, Cys72Sec, and Cys174Sec. The double mutants investigated were Cys44Sec/Cys174Sec and Cys72Sec/Cys174Sec. The Cys174Sec mutant was investigated because as discussed above, Cys72 was found to attack Cys174 and not Cys44 as proposed.\(^{25}\)

For purposes of brevity, only key distances will be discussed herein; however, the geometries of all complexes investigated are provided in the Supporting Information (Table S1). Regarding key bond lengths, the mutation of the active-site Cys residues had marginal effects on the As···CH\(_3\) distances for the five RC mutant systems investigated. Specifically, the average As···CH\(_3\) distance for the five mutant models was calculated to be 3.79 Å, which is marginally shorter than the 3.88 Å distance seen in the wild-type (WT) model.

Regarding the TSs for the five mutants, the average values of \(r(\text{As(III)}···\text{CH}_3)\) and \(r(S_{\text{SAM}}···\text{C(H}_3))\) were calculated to be...
2.45, and 2.36 Å, respectively. Therefore, like the WT QM cluster model, the CH₃ is approximately centered between the S and As for the five mutant models. Moreover, like the WT QM cluster model, the average calculated Mulliken charge on the S-atom of SAM of 0.72e indicates that the positive charge is still highly localized on the sulfur atom of SAM for the five mutant models.

The Gibbs reaction and activation energies for the five QM models are provided in Table 1. From Table 1, the mutation of the active-site cysteine residues generally resulted in a marginal reduction in the Gibbs activation energy for the CH₃ transfer. Specifically, for the five mutant models, an average reduction of 4.2 kJ mol⁻¹ was observed in ΔGᵢ. Thus, the mutation of key active-site Cys residues has a marginal effect on the rate of methyl transfer. The exception being the Cys44Sec mutant which had a slightly greater Gibbs activation energy than the WT TS. In a stopped-flow spectrophotometry experimental study, the kinetics of the nucleophilic attack by a Cys or Sec residue to disulfides, diselenides, and mixed sulfide selenides were studied. It was found that reaction rates of selenium as a nucleophile or as an electrophile are 2–3 orders of magnitude faster than the analogous reaction involving cysteine.

Similarly, a past computational study of small thiol- and selenol-containing models found that nucleophilic attack by thiols at selenium is both kinetically faster and thermodynamically more favorable than at sulfur. Considering the average reduction in ΔGᵢ of 4.2 kJ mol⁻¹ seen for the present models, this corresponds to an acceleration of ~5× for methyl transfer relative to the WT.

With the transfer of the methyl group, all five mutant IC1 complexes lie higher in energy than their respective RCs (Table 1). However, as cysteine residues are mutated to selenocysteine residues, the endergonicity of the IC1 intermediate generally decreases. Of the five mutant models investigated, the double mutant Cys72Sec/Cys174Sec was the least endergonic and only lies 23.8 kJ mol⁻¹ higher in energy than RC. In the case of the single-mutant models, Cys174Sec only lies 25.8 kJ mol⁻¹ higher in energy than its respective RC. The reduction in endergonicity of IC1 is likely due to the greater polarizability of Se versus S, which helps to stabilize the greater positive charge on the As atom, resulting from CH₃ transfer. Indeed, the Mulliken charge on the As for IC1 was calculated to be +0.41e and +0.40e, for Cys174Sec and Cys44Sec, respectively. For WT IC1, the Mulliken charge was calculated to be +0.51e on the As atom.

The next step in the mechanism is formation of the disulfide with concomitant reduction of MAs(V) to MAs(III). Like the WT model, the residue Cys72 (or Sec72) was deprotonated for each of the five mutant models, allowing the anionic S (or Se) to attack the S_Cys72–As bond. With the removal of the proton on Cys72 (or Sec72), the formation of the disulfide bond between Cys72 (or Sec72) and Cys174 (or Sec174) occurred without a TS, which is analogous to that seen for the WT model.

For the five mutant models, the resulting product complexes are all considerably exergonic relative to their respective RCs. From the energies in Table 1, the mutation of Cys72 to Sec72 has the greatest effect on the overall Gibbs reaction energies. Specifically, relative to RC, ΔG_RC is −38.1 kJ mol⁻¹ for the WT, whereas for Cys72Sec, ΔG_RC is −62.7 kJ mol⁻¹, which corresponds to a difference in Gibbs reaction energies (ΔΔG_RC) of −24.6 kJ mol⁻¹ (similar results are found for the Cys44Sec/Cys72Sec and Cys72Sec/Cys174/Sec models). It is well-known that Sec is more acidic than Cys; thus, the difference in ΔΔG_RC is likely due to the difference in pKₐ values between the residues. Indeed, the commonly accepted pKₐ values of 8.3 and 5.3 for Cys and Sec, respectively, correspond to a difference of −17.0 kJ mol⁻¹ in the Gibbs reaction energy for deprotonation of Sec in an aqueous environment relative to the Gibbs reaction energy for deprotonation of Cys in an aqueous environment. Therefore, although the substitution of Cys with Sec does not affect the barrier for methyl transfer, the greater acidity of Sec results in greater exergonicity for the methylation of As.

With the experimentally observed enhancement in the durability of Se-containing catalysts relative to analogous S-containing catalysts and the considerable increase exergonicity with marginal reduction in barrier for methyl transfer discussed herein, we propose that the substitution of the active-site Cys72 residue could make the process of methylation and volatilization more advantageous for bioconversion by arsenic (III) S-adenosymethionine methyltransferases.

**CONCLUSIONS**

Given the toxicity of arsenic and its known carcinogenic properties, we have investigated the mechanism by which the ubiquitous enzyme arsenic (III) S-adenosymethionine methyltransferases methylates arsenic. Notably, the methylation of arsenic is an essential part of the cycling of arsenic among terrestrial, aquatic, and atmospheric environments and has been investigated as a means to remove arsenic from contaminated water or soil. Arsenic (III) S-adenosymethionine methyltransferases are ubiquitous enzymes in nature that use S-adenosyl methionine as a methyl source to methylate active-site-bound arsenic.

Using a DFT cluster model, the mechanism of the WT enzyme and various selenium-containing analogues was investigated to better understand the process and energetics for the methylation of As. At the IEFPPCM-B3LYP-GD3BJ/6-311+G(2df,2p)//IEFPPCM-B3LYP/6-31G(d) level of theory, the Gibbs activation energy for the methyl transfer in the WT model was found to be 107.4 kJ mol⁻¹, which is well within the range of typically accepted values for enzymatic reactions. Overall, the process of methylation was found to be exergonic −38.1 kJ mol⁻¹, thus indicating that the methylation of As by the WT arsenic (III) S-adenosymethionine methyltransferases is spontaneous under standard conditions.

Regarding the various selenocysteine mutants, it was found that the mutation of key active-site residues only marginally lowered the reaction barrier by an average value of 4.2 kJ mol⁻¹. This reduction in ΔG is an acceleration of ~5× for the methyl transfer relative to the WT. However, although the substitution of the active-site Cys44, Cys72, and Cys174 residues with selenocysteine...
residues had a marginal effect on the barrier for CH$_3$ transfer, the methylation process does become considerably more exergonic with the Cys72Sec mutation. Specifically, the reaction becomes on average 29 kJ mol$^{-1}$ more exergonic with mutation of the nucleophilic Cys72 to selenocysteine. Thus, as a means to make the process of methylation and therefore volatilization of As by arsenic (III) S-adenosylmethionine methyltransferases more advantageous, we propose that the substitution of the active-site Cys72 residue be further investigated.

## COMPUTATIONAL METHODS

The Gaussian 09 software suite was used for all calculations. Stationary points were obtained at the B3LYP/6-31G(d) level of theory. Harmonic frequencies were calculated at the same level of theory to confirm the nature of the optimized stationary points and to obtain the Gibbs energy corrections. Single-point electronic energies were obtained at the IEFPCM-B3LYP-GD3BJ/6-311+G(2df,2pd)/IEFPCM-B3LYP/6-31G(d) level of theory. The addition of the D3 and D3BJ corrections was used to investigate the effect of dispersion interactions on the reaction energies. The D3 corrections represent the D3 version of Grimme’s dispersion corrections. D3BJ combines the D3 version of Grimme’s dispersion with Becke-Johnson damping. The IEFPCM approach was used to model the presence of a nonpolar active-site environment on the reaction with diethylether chosen, given its dielectric constant of 4.33, which lies in the typical range of dielectric constants used to model a protein environment (i.e., 4.0–10). The single-point energies were then corrected to Gibbs energies by adding the Gibbs energy corrections. Mulliken charges discussed herein were obtained at the IEFPCM-B3LYP-GD3BJ/6-311+G(2df,2pd)/IEFPCM-B3LYP/6-31G(d) level of theory.

The DFT cluster model used in the present study was taken from the crystal structure of As(III) S-adenosylmethionine methyltransferase (CmArsM) found in the red alga Cyanidioschyzon (PDB code: 6CX6). The enzyme was cocrystallized with As(III) and S-adenosyl-L-homocysteine (SAH). Although the crystal structure is dimeric, CmArsM is a monomer in the crystal structure is dimeric, CmArsM is a monomer in the last step, where the truncation occurred were fixed to their crystallographic positions. The restrained carbon atoms are indicated in Figure 1 with asterisks.

In the reactive complex, Cys72 was modeled as protonated. However, for the formation of the disulfide in the last step, Cys72 must be deprotonated. The Gibbs reaction energy ($\Delta G^\ddagger$) of this deprotonation step was calculated, where the standard chemical potential of a proton in a dilute aqueous environment ($\mu^\ddagger_{298K}(H^+)$) was chosen to be $-1124.2$ kJ mol$^{-1}$. The dielectric constants used to model a protein environment (i.e., constant of 4.33, which lies in the typical range of dielectric constants used to model a protein environment (i.e., 4.0–10).

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