Rapid Equilibrium Kinetic Analysis of Arsenite Methylation Catalyzed by Recombinant Human Arsenic (+3 Oxidation State) Methyltransferase (hAS3MT) *‡

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Background: Oxidative methylation and successive methylation are two possible enzymatic mechanisms of arsenite methylation.

Results: Rapid equilibrium kinetic analysis established that hAS3MT-catalyzed arsenite methylation is a completely ordered reaction.

Conclusion: The methyl transfer step occurs on hAS3MT. Reductant reduces a disulfide bond and exposes the active site cysteine residues.

Significance: This work clearly elucidates the completely ordered mechanism of arsenite methylation by a rapid equilibrium kinetic model.

In the human body, arsenic is metabolized by methylation. Understanding this process is important and provides insight into the relationship between arsenic and its related diseases. We used the rapid equilibrium kinetic model to study the reaction sequence of arsenite methylation. The results suggest that the mechanism for arsenite methylation is a completely ordered mechanism that is also of general interest in reaction systems with different reductants, such as tris(2-carboxyethyl)phosphine hydrochloride, cysteine, and glutathione. In the reaction, cysteine residues of recombinant human arsenic (+3 oxidation state) methyltransferase (hAS3MT) coordinate with arsenicals and involve the methyl transfer step. S-Adenosyl-L-methionine (AdoMet) is the first-order reactant, which modulates the conformation of hAS3MT to a best matched state by hydrophobic interaction. As the second-order reactant, reductant reduces the disulfide bond, most likely between Cys-250 and another cysteine residue of hAS3MT, and exposes the active site cysteine residues for binding trivalent inorganic arsenic (iAs3+) to give monomethylarsonic dicysteine (MADC3+). In addition, the reaction can be extended to further methylate MADC3+ to dimethylarsonic cysteine (DMAC3+). In the methylation reaction, the β-pleated sheet content of hAS3MT is increased, and the hydrophobicity of the microenvironment around the active sites is decreased. Similarly, we confirm that both the high β-pleated sheet content of hAS3MT and the high dissociation ability of the enzyme-AdoMet-reductant improve the yield of dimethylated arsenicals.

Arsenic is one of the most significant hazards in the environment, affecting millions of people around the world. Exposure to arsenic is associated with cancers of the skin, lung, urinary bladder, kidney, and liver as well as several non-cancer diseases, such as diabetes mellitus, hypertension, and cerebrovascular and cardiovascular diseases (1–4). The relationship between arsenic and its related diseases is complicated by many aspects, such as dose-response relationships, oxidative stress, cellular signaling, cell cycle control, gene amplification, and chromosomal abnormalities (5–7). In many species, including humans, methylation is a major metabolic transformation of arsenic, producing mainly monomethylated arsenicals (MMAs)2 and dimethylated arsenicals (DMAs) (8–11), both of which have been detected in human urine (12). In recent years, it has become apparent that methylation is not necessarily a detoxification process of inorganic arsenic (iAs) (13). The methylated products and intermediates may be more reactive and toxic than inorganic arsenic. For example, Kligerman et al. (14) suggested that methylation of the trivalent forms of arsenic increased their genotoxicity and cytotoxicity. Compared with iAs3+, methylated trivalent arsenicals, such as MMA3+ and DMA3+, were found to be more potent in causing DNA strand breaks in human lymphocytes and to induce a greater extent of cytotoxic and genotoxic effects, such as micronucleus formation, chromosome aberrations, and sister chromatid exchange (15, 16). A better understanding of the biotransformation metabolism of arsenic should shed light on the relationship between arsenic and its related diseases (17, 18).

Arsenic (+3 oxidation state) methyltransferase (AS3MT) has been proposed as the authentic enzyme that catalyzes the...
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Preparation of hAS3MT—The cloning, heterologous expression, and purification of recombinant hAS3MT were carried out as described previously (20). Details are shown in the Supplemental Materials.

The Initial Velocity Assay of hAS3MT—The enzymatic methylation of arsenite was designed according to Myllylä et al. (27) and tested in a standard system (100 µl) containing hAS3MT (2.0 µM), AdoMet (1.0 mM), iAs³⁺ (1.0 µM), phosphate buffer (25 mM, pH 7.0), and each of the reductants (GSH, cysteine, and TCEP at 7, 10, and 0.7 mM, respectively). The initial velocity curve was obtained by varying the concentration of one of the three reactants (reductant, AdoMet, and arsenite) while the other two were fixed. All of the reactions were carried out in capped tubes at 37 °C for 30 min and then stopped by adding H₂O₂ to a final concentration of 3% (23). Arsenicals were analyzed by HPLC-inductively coupled plasma-MS (28). The methylation rates were calculated as mole equivalents of methyl groups transferred from AdoMet to arsenic (i.e. 1.0 pmol of CH₃/1.0 pmol of MMA or 2.0 pmol of CH₃/1.0 pmol of DMA) (29).

Effect of Reductant on the Conformation of hAS3MT in the Methylation Reaction—The effect of the reductant on the hAS3MT disulfide bonds was determined by the improved Ellman’s test (28, 30). After incubation of hAS3MT (15 µM) with the different reductants (20 mM cysteine, 15 mM GSH, and 1.5 mM TCEP, respectively) in phosphate buffer at 37 °C for 30 min, the mixture was thoroughly dialyzed against phosphate buffer at 4 °C. The reduced hAS3MT was incubated with 0.1 mM 5,5’-dithiobis(2-nitrobenzonic acid) in Tris–HCl (20 mM, pH 7.0) at 25 °C for 90 min. The absorbance at 412 nm was then monitored to estimate the number of cysteine residues in hAS3MT. The effect of the reductant on the secondary structure was measured by CD spectroscopy after the reduced enzyme was thoroughly dialyzed against phosphate buffer at 4 °C. After the methylation reaction was catalyzed at 37 °C for 120 min, the secondary structure of hAS3MT was also determined by CD spectroscopy after being thoroughly dialyzed against phosphate buffer at 4 °C. The methylation reaction system was the same as that used in the velocity assay.

Mass Spectrometry—The methylation reaction system contained hAS3MT (4.0 µM), reductant (TCEP (0.7 mM), cysteine (10 mM), or GSH (7 mM)), AdoMet (1.0 mM), iAs³⁺ (1.0 µM), and phosphate buffer. After incubation at 37 °C for 60 min, the reaction mixture was immediately lyophilized and then separated by 12% non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The band corresponding to hAS3MT was excised and treated with 20 mM iodoacetamide in the dark at room temperature for 1.5 h followed by incubation with 5% trypsin overnight at 37 °C. Subsequently, 200 µl of 60% acetonitrile containing 0.1% (v/v) TFA was added to stop the reaction. Mass spectra were obtained on an AUTOFLEX II MALDI-TOF mass spectrometer (Bruker).

RESULTS

The Differences between hAS3MT-catalyzed Arsenite Methylation in Different Reductive Systems—Both MMA and DMA were detected in each of the reaction systems with different reductants. The differential distribution of the methylated...
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products is shown in Fig. 1. The characteristics of the reaction in the GSH and cysteine systems were similar. MMA was generated at the beginning of the reaction, DMA increased as the reaction proceeded, and the methylation efficiency was not high. Interestingly, the yields of MMA and DMA were nearly the same in the GSH and cysteine reductive systems when the reaction proceeded, and the methylation efficiency was not significantly different (Table 1). The values of $K_A$ in the cysteine, GSH, and TCEP reaction systems were 62.50, 90.91, and 98.95 mmol/liter, respectively. This finding suggests that AdoMet binds to hAS3MT more easily in the cysteine system than in the GSH or TCEP system. Additionally, the values of $K_{ABC}$ were 0.91 μmol/liter in the GSH system, 1.30 μmol/liter in the cysteine system, and 4.55 μmol/liter in the TCEP system, suggesting that the enzyme-AdoMet-TCEP iaAs$^{3+}$ complex dissociates most readily among the three reductant systems. In the TCEP system, the value of $K_{AB}$ was 1233.74 mmol/liter, which was much higher than in either the cysteine (36.92 mmol/liter) or GSH system (5.50 mmol/liter). With the increase of $K_{AB}$ from GSH to cysteine and then to TCEP, the $V_{\text{exp}}$ of the reactions also increased. Thus, the second step, which forms the enzyme-AdoMet-reductant complex, is the most likely rate-limiting step for arsenite methylation. The dissociation of the enzyme-AdoMet-reductant may be essential for iAs$^{3+}$ binding to hAS3MT in the third step.

$$\lambda_1 = \left(1 + \frac{K_A}{[A]} \right)^{-1}$$  \hspace{1cm} (Eq. 4)

$$\lambda_2 = \frac{K_{AB}}{[B]}$$  \hspace{1cm} (Eq. 5)

$$\lambda_3 = \left(1 + \frac{K_{ABC}}{[C]} \right)^{-1}$$  \hspace{1cm} (Eq. 6)

$$\lambda_4 = \frac{K_{AB}K_{ABC}}{[C]V_{\text{exp}}}$$  \hspace{1cm} (Eq. 7)
Effect of Reductant on Disulfide Bonds and the Conformation of hAS3MT in the Reaction

—Trivalent arsenicals easily bind to the sulfhydryl sites of proteins (33) and have been reported to coordinate to cysteine residues of hAS3MT to catalyze the methylation reaction (8, 11, 34). Our previous work suggested that GSH reduced the disulfide bond between the cysteine residues of hAS3MT that was formed after the release of the methylated products from the enzyme (21). From our current results, reductants are involved in the second step of the reaction. Thus, we propose that the reductant might reduce the disulfide bonds of hAS3MT and change the conformation of hAS3MT to expose the active site cysteine residues for iAs^{3+} binding. To confirm this hypothesis, we analyzed the number of sulfhydryl groups of the reductant-treated hAS3MT using the improved Ellman’s test. The numbers of cysteine residues was 6.31, 6.56, and 8.75 in cysteine-, GSH-, and TCEP-reduced hAS3MT, respectively, whereas 4.66 cysteine residues were detected on the surface of the control hAS3MT. This result agrees well with our earlier report that 4.42 thiol groups were detected on hAS3MT (28). We further analyzed the number of

| TABLE 1 |
| Values of the kinetic parameters KA, KAB, KABC, and Vfexp for the methylation reaction |
| Parameter | GSH | Cysteine | TCEP |
| --- | --- | --- | --- |
| KA (µmol/liter) | 90.91 ± 3.23 | 62.50 ± 2.12 | 98.85 ± 3.46 |
| KAB (mmol/liter) | 5.50 ± 0.67 | 36.92 ± 1.23 | 1233.74 ± 22.11 |
| KABC (µmol/liter) | 0.91 ± 0.03 | 1.30 ± 0.02 | 4.55 ± 0.06 |
| Vfexp (pmol CH₃H₃/(h·mg)) \times 10⁻⁴ | 4.55 ± 0.21 | 10.00 ± 0.62 | 18.52 ± 0.63 |

Effect of Reductant on Disulfide Bonds and the Conformation of hAS3MT in the Reaction—Trivalent arsenicals easily bind to the sulfhydryl sites of proteins (33) and have been reported to coordinate to cysteine residues of hAS3MT to catalyze the methylation reaction (8, 11, 34). Our previous work suggested that GSH reduced the disulfide bond between the cysteine residues of hAS3MT that was formed after the release of the methylated products from the enzyme (21). From our current results, reductants are involved in the second step of the reaction. Thus, we propose that the reductant might reduce the disulfide bonds of hAS3MT and change the conformation of hAS3MT to expose the active site cysteine residues for iAs^{3+} binding. To confirm this hypothesis, we analyzed the number of sulfhydryl groups of the reductant-treated hAS3MT using the improved Ellman’s test. The numbers of cysteine residues was 6.31, 6.56, and 8.75 in cysteine-, GSH-, and TCEP-reduced hAS3MT, respectively, whereas 4.66 cysteine residues were detected on the surface of the control hAS3MT. This result agrees well with our earlier report that 4.42 thiol groups were detected on hAS3MT (28). We further analyzed the number of
cysteine residues of hAS3MT by MALDI-TOF mass spectrometry. Cysteine residues of the samples were labeled by iodoacetamide before trypsin digestion and mass spectrometry analysis. As shown in Fig. 3, seven iodoacetamide-modified cysteine-corresponding peptides were detected in native hAS3MT. In the hAS3MT that underwent reaction, we detected additional peptides corresponding to two additional cysteine residues at sites 32 and 250. These results suggest that Cys-32 and Cys-250 are exposed in the reaction, although a direct disulfide bond between these two residues has yet to be confirmed.

CD spectroscopy was used to evaluate the effect of reductant on the conformation of hAS3MT. Compared with native hAS3MT, the α-helix content decreased, but the β-pleated sheet content increased in the presence of reductant (Fig. 4, A and B). The secondary structure of hAS3MT changed similarly in the methylation reaction. In particular, the β-pleated sheet content increased significantly in the TCEP reaction system (Fig. 4, C and D). Regardless of whether the structural alteration of hAS3MT occurred in the reductive systems or in the methylation reaction systems, the β-pleated sheet content of hAS3MT increased. This outcome proved that the increase in β-pleated sheet content of hAS3MT was induced by the reductant.

**Effect of AdoMet and iAs³⁺ on the Conformation of hAS3MT**—Tryptophan, tyrosine, and phenylalanine residues all contribute to protein fluorescence. However, phenylalanine has a very low quantum yield, and the fluorescence of tyrosine is easily quenched when it is ionized or close to an amino group, a carboxyl group, or a tryptophan (35). In hAS3MT, there are three tryptophan residues (Trp-73, Trp-203, and Trp-213) close to the cysteine residues (Cys-156, Cys-206, Cys-72, and Cys-250) that are important to the enzymatic activity (20, 26). Changes in intrinsic fluorescence intensity of hAS3MT reflect the perturbation of the active site.

The effect of AdoMet on hAS3MT fluorescence was static quenching. This effect was proved by the Stern-Volmer curves and the Lineweaver-Burk curves (36) (Fig. 5, A and B) and could be further supported by Förster’s energy transfer theory because the distance we calculated between AdoMet and the tryptophan residue in hAS3MT was less than 7 nm (37). The thermodynamic parameters were calculated according to the equation proposed by Bi et al. (38) (Fig. 6). The three parameters, \( \Delta G < 0, \Delta H > 0, \) and \( \Delta S > 0, \) suggested a hydrophobic interaction between AdoMet and hAS3MT (36). The value of \( \Delta G \) showed that when hAS3MT was in the active state at 37 °C it bound AdoMet more readily in the cysteine system than in the GSH system, and hAS3MT more readily bound AdoMet in the GSH system than in the TCEP system (Fig. 7A). The values of \( \Delta H \) and \( \Delta S \) showed the same trend, which confirmed that the AdoMet binding reaction was an entropy-driven reaction (Fig. 7B). These findings led to the conclusion, as also suggested by \( K_A \), that AdoMet bound to hAS3MT more readily in the cysteine system than in GSH or TCEP system.

**FIGURE 3. MALDI-TOF spectra of trypsin-digested hAS3MT.** The hAS3MT was separated by 12% non-reducing SDS-PAGE and alkylated by 20 mM iodoacetamide after having catalyzed the arsenite methylation at 37 °C for 60 min. The reaction system contained hAS3MT (4.0 μM), AdoMet (1.0 mM), iAs³⁺ (1.0 μM), phosphate buffer (25 mM, pH 7.0), and different reductant. The reductants were TCEP (B), cysteine (C), and GSH (D) at 0.7, 10, and 7 mM, respectively. The protein under natural condition was used as a control (A). Results are the average of three determinations.
The synchronous fluorescence spectra showed that the maximum emission wavelength of hAS3MT was slightly red-shifted in each reductive system (Table 2). Interestingly, red shifts were also observed with the titrated concentrations of AdoMet, demonstrating that the microenvironment around the active site was disturbed and that the hydrophobicity decreased in the presence of AdoMet (37). The fluorescence quenching efficiency of AdoMet on the enzyme calculated using \( \frac{F_0 - F}{F_0} \) (%) was 28.91% in the cysteine system, 26.52% in the GSH system, and 19.46% in the TCEP system (Table 2), confirming that AdoMet bound to hAS3MT more readily in the cysteine reductive condition than in the GSH or TCEP reductive condition.

The three-dimensional fluorescence spectra of hAS3MT were notably different among the different reductive systems (data not shown). In our test range, peak 1 was mainly dominated by the microenvironments of the tryptophan and tyrosine residues, and peak 2 mainly exhibited the fluorescence character of polypeptide backbone structures (37). Peak 2 of hAS3MT disappeared in both the GSH system and the cysteine system (Table 3). After AdoMet titration, the maximum emission wavelength of peak 1 showed a slight red shift, and the fluorescence intensity decreased by 22.77 and 17.33% in the cysteine system and the GSH system, respectively. After AdoMet titration in the TCEP system, the maximum emission wavelength of peak 1 was slightly red-shifted in each reductive system (Table 2). Interestingly, red shifts were also observed with the titrated concentrations of AdoMet, demonstrating that the microenvironment around the active site was disturbed and that the hydrophobicity decreased in the presence of AdoMet (37). The fluorescence quenching efficiency of AdoMet on the enzyme calculated using \( \frac{F_0 - F}{F_0} \) (%) was 28.91% in the cysteine system, 26.52% in the GSH system, and 19.46% in the TCEP system (Table 2), confirming that AdoMet bound to hAS3MT more readily in the cysteine reductive condition than in the GSH or TCEP reductive condition.
wavelengths of peak 1 and peak 2 had red shifts, and the fluorescence intensity decreased by 16.50 and 49.58%, respectively. The effect of AdoMet on peak 2 was more significant than that on peak 1, demonstrating a stronger impact of AdoMet on the polypeptide backbone structure of the enzyme.

The effect of iAs$_{3}^{+}$ on the secondary structure of hAS$_{3}$MT is very limited and irregular. A further analysis showed that the values of ($\alpha + \beta$)% and ($\beta/(\alpha + \beta)$)% increase slightly with increasing iAs$_{3}^{+}$ at 37 °C, whereas they were nearly unchanged at 29 °C (data not shown) (39).

**DISCUSSION**

It has been reported previously that the nonenzymatic methylation of arsenite by methylcobalamin proceeds via nucleophilic attack of the As-GSH complex on cobalt (40) and that the methylation of arsenite catalyzed by arsenic methyltransferase also proceeds via the formation of As-GSH complexes (23). These reports suggested that arsenicals are capable of forming a thiol-arsenic complex with cysteine in the reaction system. However, TCEP is a non-thiol reductant, which cannot form thiol-arsenic complexes with arsenicals in the reaction. Therefore, our results suggested that the thiol-arsenic complex formed by arsenicals and exogenous reductant might only exist in a thiol reductive reaction system and is not necessarily a substrate for the hAS$_{3}$MT-catalyzed methylation reaction.

Based on our results, we propose that the methyl group is transferred from AdoMet to iAs$_{3}^{+}$ on the enzyme. In the reaction, AdoMet first modulates the peptide backbone of hAS$_{3}$MT to a best matched state, and then the reductant reduces the

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**TABLE 2**

| Parameter          | Phosphate buffer (25.00 mM) | GSH (7.00 mM) | Cysteine (10.00 mM) | TCEP (0.70 mM) |
|--------------------|-----------------------------|---------------|---------------------|----------------|
| $F_0$              | 797.98                      | 680.60        | 680.54              | 869.86         |
| $F$                | 624.85                      | 500.08        | 483.80              | 700.56         |
| $\lambda_0$ (nm)   | 277.0                       | 279.0         | 279.5               | 278.0          |
| $\lambda$ (nm)     | 281.5                       | 282.0         | 282.5               | 281.5          |
| $(F_0 - F)/F_0$ (%) | 21.70                       | 26.52         | 28.91               | 19.46          |
| $\Delta \lambda$ (nm) | 4.5                         | 3.0           | 3.0                 | 3.5             |

**TABLE 3**

| Peaks          | Peak position $\AA_{ex}/\AA_{em}$ | Intensity, $F_0$ | Peak position $\AA_{ex}/\AA_{em}$ | Intensity, $F_{AdoMet}$ | $(F_{AdoMet} - F_0)/F_0$ |
|----------------|------------------------------------|------------------|-----------------------------------|-------------------------|-------------------------|
| hAS$_{3}$MT    |                                    |                  |                                    |                         |                         |
| hAS$_{3}$MT + AdoMet |                        |                  |                                    |                         |                         |
| Peak 1/cysteine| 277.0/349.5                       | 547.78           | 277.0/350.0                       | 423.04                  | −22.77                  |
| Peak 1/GSH     | 277.0/350.0                       | 600.67           | 277.0/350.5                       | 496.58                  | −17.32                  |
| Peak 1/TCEP    | 277.0/350.0                       | 718.44           | 277.0/351.0                       | 599.88                  | −16.50                  |
| Peak 2/TCEP    | 228.0/350.5                       | 453.41           | 228.0/353.0                       | 228.61                  | −49.58                  |
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![Diagram](https://example.com/diagram.png)

The disulfide bond of hAS3MT to expose cysteine residues in the active site for binding iAs^{3+} to form arsenic tricysteine (ATC^{3+}). An ion pair of ATC^{3+} attacks the cationic sulfur of AdoMet, and a methyl group of AdoMet is transferred to ATC^{3+}, resulting in monomethylarsonic dicysteine (MADC^{3+}) and S-adenosylhomocysteine. The extra reductant may function in cleaving S-adenosylhomocysteine from the enzyme. A portion of MADC^{3+} also dissociates from the enzyme to form MMA^{3+} in the presence of reductant (11, 28). The remaining MADC^{3+} is further methylated into dimethylarsonic cysteine (DAMC^{3+}) on hAS3MT in the presence of AdoMet and reductant and then dissociated into DMA^{3-}. MMA^{3-} and DMA^{3-} can be further oxidized by environmental oxygen into MMA^{5-} and DMA^{5-}, respectively (21) (Fig. 8, A and B).

Early studies showed that, in the GSH reaction system, arsenite and GSH form ATG^{2+}, which easily binds to hAS3MT (11, 23), and further form ATC^{3+} by exchanging the GSH with cysteine residues of the enzyme. GSH can also competitively coordinate with the arsenic of MADC^{3+}. This coordination leads to the dissociation of MADC^{3+} from the enzyme into MADG^{3+}, which can then be transformed into MMA^{3+}. Therefore, the transformation from iAs^{3+} to MMA^{3+} is the major methylation step in the initiation of the reaction, and MMA is the major methylated product at the beginning of the GSH reaction system. However, arsenite did not exist in thiol-arsenic form in the TCEP reaction system. The high reductive potential of TCEP leads to rapid dissociation of S-adenosylhomocysteine from hAS3MT, which favors the further methylation of MADC^{3+} into DAMC^{3+} and accelerates the velocity of the methyl transfer reaction. A large amount of DMA is thus generated at the beginning of the reaction with TCEP.

The completely ordered mechanism of AdoMet + reductant + iAs^{3+} → products and the role of each reactant in the reaction are detailed in Fig. 8B. As the first-order reactant, AdoMet affects the peptide backbone of hAS3MT and decreases the hydrophobicity of the microenvironment around the active site. The conformational change of the enzyme increases the exposure of the originally buried hydrophobic regions (41). Fomenko et al. (42) analyzed the structure model of mouse AS3MT and confirmed that the cysteine residues in the active site are surface-exposed on the β-pleated sheet of the enzyme. In our research, the β-pleated sheet content increased, and the hydrophobicity of the microenvironment around the active site decreased when reductant was added into the enzymatic system. Therefore, the reductant alters the conformation of hAS3MT to an active state before the methylation reaction. The effect of iAs^{3+} on the conformation of hAS3MT is insignificant.

The enzyme was thoroughly inactive in the GSH reaction system when any of the cysteine residues at positions 72, 156, 206, and 250 were mutated into serine residues (20, 26). Cys-156 and Cys-206 of hAS3MT were proved to be the cysteine residues in the active site that bind to iAs^{3+} (20). The results of our MALDI-TOF mass spectrometry experiment suggest that the disulfide bond reduced by the reductant is associated with Cys-250. Therefore, we concluded that the third active-site cysteine residue, which bound iAs^{3+} in the reaction, might be Cys-250, although a possible involvement of Cys-72 cannot be ruled out. The enzyme had no catalytic activity until the disulfide bond between Cys-250 and another cysteine residue was cleaved by reductant.

In conclusion, the methylation of arsenite by hAS3MT is a completely ordered mechanism that is of general interest in different reductive systems. The methyl transfer process occurs on hAS3MT, which agrees well with the views of Marapakala et al. (11) and Naranmandura et al. (34). In this process, arsenicals form thiol-arsenic complexes with the cysteine residues of hAS3MT, and the valence state of arsenic does not change. Although this mechanism is a successive methylation, arsenicals do not necessarily coordinate with GSH in the form of As-GSH complexes to facilitate the methylation reaction (23). The reductant cleaves the disulfide bonds of hAS3MT and exposes the active site cysteine residues for iAs^{3+} binding. Similarly, we further confirmed that the high β-pleated content increases the exposure of the active site on the surface of hAS3MT. The high dissociation ability of the enzyme-AdoMet-
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redundant intermediate might be beneficial to the binding of iAs\(^{3+}\) to the active site. This work used a rapid equilibrium kinetic model to analyze the reaction sequence of a real enzyme-catalyzed reaction and provided new insight into the mechanism of arsenite methylation catalyzed by hAS3MT. A deep understanding of the mechanism in vitro will guide further studies on the metabolism of arsenic in vivo in which GSH is a predominant endogenous redundant and assist in the endeavor to seek the antidotal pathway of arsenic poisoning.

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