Mechanism of As(III) S-adenosylmethionine methyltransferases and the consequences of human polymorphisms in hAS3MT

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ABSTRACT: Humans detoxify As(III) by methylation. Rapid methylation results in rapid clearance of arsenic from the body, leading to lower risk of arsenic-related diseases. Paradoxically, the methylated products, MAs(III) and DMAs(III) are more toxic and potentially more carcinogenic than inorganic arsenic. When methylation is slow, body clearance is slower, and the risk of arsenic-related diseases increases. We demonstrate that human single nucleotide polymorphisms in the AS3MT coding region lead to less active enzymes that slow the rate of methylation. We predict that these SNPs lead to increased risk of arsenic-related diseases. To understand the relationship between slower methylation and AS3MT activity, we conducted a structure-function analysis of an AS3MT ortholog and identified the rate limiting step in the catalytic cycle as slow reorientation of the methyl group of enzyme-bound MAs(III).

1 INTRODUCTION

Arsenic methylation, the primary biotransformation in the human body, is catalyzed by the enzyme As(III) S-adenosylmethionine (SAM) methyltransferases (hAS3MT). This process is thought to be protective from acute high-level arsenic exposure. However, with long term low-level exposure, hAS3MT produces intracellular methylarsenite (MAs(III)) and dimethylarsenite (DMAs(III)), which are considerably more toxic than inorganic As(III) and may contribute to arsenic-related diseases. Three previously identified exonic single nucleotide polymorphisms (SNPs) (R173W, M287T and T306I) may be deleterious. We identified five additional intragenic variants in hAS3MT (H51R, C61W, I136T, W203C and R251H) (Li *et al.*, 2017). We purified the eight polymorphic hAS3MT proteins and characterized their enzymatic properties. Each enzyme had low methylation activity through decreased affinity for substrate, lower overall rates of catalysis and/or lower stability. We propose that amino acid substitutions in hAS3MT with decreased catalytic activity lead to detrimental responses to environmental arsenic and may increase the risk of arsenic-related diseases.

Methylarsenite (MAs(III)) is both the product of the first methylation step and the substrate of the second methylation step. When the rate of the overall methylation reaction was determined with As(III) as substrate, the first methylation step was rapid, while the second methylation step was slow (Dheeman *et al.*, 2014). In contrast, when MAs(III) was used as substrate, the second methylation step was as fast as the first methylation step when As(III) was used substrate.

These results indicate that there is a slow conformational change between the first and second methylation steps. The structure of the AS3MT ortholog CmArsM was determined with bound MAs(III) at 2.27 Å resolution (Packianathan & Rosen, unpublished). The methyl group is facing solvent, as would be expected when MAs(III) is bound as substrate rather than facing the SAM binding site, as would be expected for MAs(III) as a product. We propose that the rate-limiting step in arsenic methylation is slow reorientation of the methyl group from the SAM binding site to solvent.

2 METHODS

2.1 Construction of hAS3MT variant

The eight mutations in the synthetic hAS3MT gene (Dheeman *et al.*, 2014) were introduced by site-directed mutagenesis. The variants were purified and assayed by previously reported methods (Dheeman *et al.*, 2014).

2.2 Crystallization of CmArsM with bound MAs(III)

Co-crystallization of CmArsM with MAs(III) was performed in hanging drops (Ajeev *et al.*, 2012). Data sets were collected at the Southeast Regional Collaborative Access Team (SER-CAT) facility at Advanced Photon Source (APS), Argonne National Laboratory. The structure was by molecular replacement with the unliganded crystal structure of CmArsM (PDB ID 4FS8) as a template.

3 RESULTS AND DISCUSSION

3.1 Effect of SNPs on hAS3MT activity

In this study we searched repositories of human genomic data and identified eight nonsynonymous missense variants for further analysis. We introduced each mutation into a synthetic hAS3MT gene (Li *et al.*, 2017) and purified the resulting enzymes. We compared their enzymatic properties and stability with the most common form of hAS3MT using either As(III) or MAs(III) as substrate. The location of each substitution in the structure of hAS3MT was identified using a homology structural model of hAS3MT (Dheeman *et al.*, 2014), allowing correlation of structure with enzymatic properties. For example, a I136T substitution could be placed in the N-terminal domain that contains the SAM binding domain, and this single amino acid substitution resulted in lower affinity for SAM. A C61W substitution eliminates one of the four conserved cysteine residues (Cys32, Cys61, Cys156 and Cys206) involved in substrate binding and specificity (Fig. 1). As predicted, the C61W enzyme methylated MAs(III) but was unable to methylate As(III). Each of the other hAS3MT polymorphic enzymes has lower methylation capacity, with reduction in the V_{max} , K_{cat}/K_m and affinity for As(III), resulting in lower DMA:MAS ratios. Compared to wild type hAS3MT, those SNPs also show lower thermal stability. Thus, individuals with any of the eight variants would be predicted to have a longer total arsenic retention time in the body, leading to elimination of more iAs and MAs and less DMAs with a lower urinary DMAs:MAS ratio. These individuals could be at greater risk for arsenic-related diseases.

3.2 Reorientation of the methyl group of enzyme-bound MAs(III) is the rate-limiting step in the arsenic methylation pathway

We determined the rates of the first two steps of methylation, As(III) to MAs(III) and MAs(III) to DMAs. When As(III) was used as substrate, the first methylation step, (As(III)→MAs(III)), was rapid, while the second methylation step, MAs(III)→DMAs, was considerably slower. In contrast, when the second methylation step was assayed using MAs(III) as substrate, it was slightly faster than the first methylation step. Thus, the rate of the second methylation step, MAs(III)→DMAs, depends on whether the initial substrate is As(III) or MAs(III). Immediately following methylation of As(III) to MAs(III), the orientation of the methyl group is inferred to be facing the S-adenosylhomocysteine (SAH) product in the SAM binding site. In order to carry out the next methylation reaction, the methyl group must leave the SAM site to allow a new molecule of SAM to bind. We propose that, when MAs(III) is the product of As(III) methylation, the orientation of the methyl group adjacent to the SAM binding site prevents the SAH product from leaving and entrance of a second SAM molecule, creating a kinetic block between the first and second

Figure 1. Homology model of hAS3MT and polymorphisms. The structural model of the human AS3MT consists of three domain, the N-terminal SAM binding domain (light blue), the central As(III) binding domain (grey) and the C-terminal domain (green). The location of the eight residues altered by the SNPs are shown in stick form, and the predicted structure of the amino acid substitutions are shown on the boarder superimposed on the original residues. Four (H51R, C61W, I136T and R173W) are located in the large N-terminal domain that includes the SAM binding domain, two (W203C and R251H) are located in the As(III) binding domain, and two (M287T and T306I) are in the C-terminal domain.

Figure 2. Reorientation of the methyl group is the rate-limiting step in As(III) methylation.

methylation steps. To examine the structural basis for this slow step, we obtained a new crystal structure of MAs(III)-bound CmArsM. In this structure the orientation of the methyl group is facing toward solvent, the predicted conformation when MAs(III) is substrate. In this conformation an aqueous channel to the active site is open, allowing exchange of the product SAH for another SAM substrate. We propose that slow reorientation of the methyl group from the SAM binding site to solvent is rate-limiting in methylation of inorganic arsenic (Fig. 2).

4 CONCLUSIONS

Eight nonsynonymous missense variants in hAS3MT all had reduced catalytic activity, leading to high MAs:DMAs ratios. We propose that individuals with these SNPs may be at increased risk of arsenic-related diseases. To understand how the enzyme controls formation of MAs and DMAs, we identified the rate-limiting step in the overall reaction as a slow reorientation of the methyl group of enzyme-bound MAs(III).

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