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Methylation of arsenic by recombinant human wild-type arsenic (+3 oxidation state) methyltransferase and its methionine 287 threonine (M287T) polymorph: Role of glutathione

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ABSTRACT

Arsenic (+3 oxidation state) methyltransferase (AS3MT) is the key enzyme in the pathway for methylation of arsenicals. A common polymorphism in the *AS3MT* gene that replaces a threonyl residue in position 287 with a methionyl residue (AS3MT/M287T) occurs at a frequency of about 10% among populations worldwide. Here, we compared catalytic properties of recombinant human wild-type (wt) AS3MT and AS3MT/M287T in reaction mixtures containing *S*-adenosylmethionine, arsenite (iAs^{III}) or methylarsonous acid (MAs^{III}) as substrates and endogenous or synthetic reductants, including glutathione (GSH), a thioredoxin reductase (TR)/thioredoxin (Trx)/NADPH reducing system, or tris (2-carboxyethyl) phosphine hydrochloride (TCEP). With either TR/Trx/NADPH or TCEP, wtAS3MT or AS3MT/M287T catalyzed conversion of iAs^{III} to MAs^{III}, methylarsonic acid (MAs^{V}), dimethylarsinous acid ($DMAs^{III}$), and dimethylarsinic acid ($DMAs^{V}$); MAs^{III} was converted to $DMAs^{III}$ and $DMAs^{V}$. Although neither enzyme required GSH to support methylation of iAs^{III} or MAs^{III}, addition of 1 mM GSH decreased K_m and increased V_{max} estimates for either substrate in reaction mixtures containing TR/Trx/NADPH. Without GSH, V_{max} and K_m values were significantly lower for AS3MT/M287T than for wtAS3MT. In the presence of 1 mM GSH, significantly more $DMAs^{III}$ was produced from iAs^{III} in reactions catalyzed by the M287T variant than in wtAS3MT-catalyzed reactions. Thus, 1 mM GSH modulates AS3MT activity, increasing both methylation rates and yield of $DMAs^{III}$. AS3MT genotype exemplified by differences in regulation of wtAS3MT and AS3MT/M287T-catalyzed reactions by GSH may contribute to differences in the phenotype for arsenic methylation and, ultimately, to differences in the disease susceptibility in individuals chronically exposed to inorganic arsenic.

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Introduction

Enzymatically catalyzed methylation of inorganic arsenic (iAs) is the main pathway for the metabolism of iAs (Styblo et al., 1995; Vahter, 1999). Conversion of iAs to methylated metabolites affects the distribution and retention of arsenic (As) and also produces As species that mediate some of the toxic effects associated with iAs exposure (Chen et al., 2011; Hughes et al., 2010; Thomas et al., 2001). Enzymatically catalyzed methylation transfers methyl groups from *S*-adenosylmethionine (AdoMet) to As to produce monomethylarsenic (MAs), dimethylarsenic (DMAs), and trimethylarsenic (TMAs) metabolites that contain either trivalent As (As^{III}) or pentavalent As (As^{V}) (Challenger, 1951; Cullen et al., 1984). Strong evidence suggests that arsenic (+3 oxidation state) methyltransferase (As3mt, EC 2.1.1.137) is the key enzyme catalyzing reactions that form all known methylated oxyarsenical metabolites of iAs (Lin et al., 2002; Thomas et al., 2007). Two pathways have been proposed for As3mt-catalyzed methylation of iAs (Fig. 1). The oxidative methylation pathway proposed by Challenger involves oxidative addition of a methyl group to a trivalent arsenical to yield a methylated product containing pentavalent As (Challenger, 1951). The pentavalent arsenical is then reduced to trivalency allowing repeated cycles of oxidative methylation. Coupled rounds of oxidative methylation of trivalent As and reduction of

Abbreviations: AAS, atomic absorption spectrometry; AdoMet, *S*-adenosylmethionine; ANOVA, analysis of variance; As, arsenic; As^{III}, trivalent As; As^{V}, pentavalent As; AS3MT or As3mt, arsenic (+3 oxidation state) methyltransferase; iAs, inorganic arsenic; iAs^{III}, arsenite; iAs^{V}, arsenate; CT, cryotrapping; DMAs, dimethylarsenic; $DMAs^{III}$, dimethylarsinous acid; $DMAs^{V}$, dimethylarsinic acid; GSH, glutathione; hAS3MT, human AS3MT; HG, hydride generation; MAs, monomethylarsenic; MAs^{III}, methylarsonous acid; MAs^{V}, methylarsonic acid; rAs3mt, rat As3mt; TCEP, tris (2-carboxyethyl) phosphine hydrochloride; tDMAs, $DMAs^{III}$ + $DMAs^{V}$; tMAs, MAs^{III} + MAs^{V}; TMAs, trimethylarsenic; $TMAs^{III}$, trimethylarsine; $TMAs^{V}O$, trimethylarsine oxide; TR, thioredoxin reductase; Trx, thioredoxin; Wt, wild-type.

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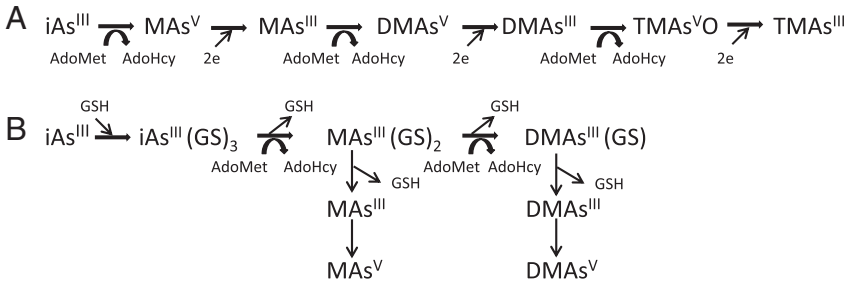

Fig. 1. Two postulated pathways for methylation of inorganic arsenic by AS3MT: A. Oxidative methylation pathway. B. Reductive methylation pathway; iAs^{III}, arsenite; MAs^{V}, methylarsonic acid; MAs^{III}, methylarsonous acid, $DMAs^{V}$, dimethylarsinic acid; $DMAs^{III}$, dimethylarsinous acid; $TMAs^{V}$, trimethylarsine oxide; $TMAs^{III}$, trimethylarsine; AdoMet, *S*-adenosylmethionine; AdoHcy, *S*-adenosylhomocysteine; GSH, glutathione; e, electron.

pentavalent As convert arsenite (iAs^{III}) to methylarsonic acid (MAs^{V}), methylarsonous acid (MAs^{III}), dimethylarsinic acid ($DMAs^{V}$), dimethylarsinous acid ($DMAs^{III}$), trimethylarsine oxide ($TMAs^{V}O$), and finally, trimethylarsine ($TMAs^{III}$). Hirano and associates have proposed a reductive methylation pathway in which trivalent arsenicals bound to glutathione (GSH) or a protein thiol are substrates for repeated rounds of enzymatically catalyzed methylation (Hayakawa et al., 2005). In this pathway, methylated trivalent arsenicals remain bound to thiols throughout the cycle and pentavalent methylated arsenicals are produced by oxidation of As^{III}-thiol complexes. Consistent with either mechanism, both trivalent and pentavalent inorganic and methylated arsenicals have been detected in urine of individuals chronically exposed to iAs, in cells and medium following *in vitro* exposure to arsenicals, and in reaction systems that contain recombinant As3mt (Del Razo et al., 2001, 2011; Devesa et al., 2004; Hernández-Zavala et al., 2008; Valenzuela et al., 2005).

Previous studies of kinetic aspects and cofactor requirements for methylation reactions catalyzed by recombinant rat As3mt (rAs3mt) found that catalysis was supported by endogenous dithiol reductants, thioredoxin (Trx), glutaredoxin, or lipoic acid (Waters et al., 2004a,b). Thus, incorporation of coupled systems consisting of thioredoxin reductase (TR), thioredoxin (Trx), and NADPH, or glutaredoxin/GSH/GSH reductase/NADPH, or lipoic acid/TR/NADPH into reaction mixtures supported rAs3mt-catalyzed methylation of iAs^{III} to yield mono-, di- and trimethylated arsenicals, including MAs^{V}, MAs^{III}, $DMAs^{V}$, $DMAs^{III}$, $TMAs^{V}O$ and $TMAs^{III}$. Synthetic reductants, dithiothreitol (DTT) and tris(2-carboxylethyl)-phosphine hydrochloride (TCEP), also supported rAs3mt-catalyzed methylation. Although the monothiol reductant GSH did not support rAs3mt-catalyzed methylation of iAs^{III} and inhibited conversion of DMAs to $TMAs^{V}O$ and trimethylarsine, its addition to reaction mixtures that also contained endogenous dithiol reductants or synthetic reductants increased methylation rates and DMAs yield. Studies with recombinant human AS3MT (hAS3MT) have also examined kinetic aspects of As methylation and the role of reductants in catalysis (Hayakawa et al., 2005; Song et al., 2009, 2010; Wood et al., 2006). Reductants evaluated with hAS3MT include monothiols (mercaptoethanol, cysteine, GSH) and a dithiol DTT. Reactions catalyzed by hAS3MT in the presence of DTT yielded high DMAs/MAs ratios that were similar to the ratios of these metabolites found in urine of humans ingesting iAs or in primary cultures of human hepatocytes exposed to iAs. In contrast, reaction mixtures containing hAS3MT and GSH as the sole reductant displayed low methylation rates, high MAs levels, and low DMAs/MAs ratios. Thus, hAS3MT and rAs3mt are absolutely dependent on a dithiol reductant for optimal activity. For both rAs3mt and hAS3MT, it is likely that GSH acts as a modulator, not a primary determinant, of catalytic activity.

Because the catalytic activity of hAS3MT in presence of endogenous dithiol reductants has not been systemically examined, we characterized the function of two commonly occurring forms of hAS3MT. These are wild-type form (wtAS3MT) which contains a methionyl residue in position 287 and a variant form AS3MT/M287T in which this methionyl residue is replaced by a threonyl residue. This change arises from a single nucleotide C14458T polymorphism (rs11191439) in the coding region of *AS3MT* (NM_020682.3: c.860T>C) and occurs in ~10% of Caucasian, African American and Latino populations (Del Razo, et al., 2011; Wood et al., 2006). Individuals with wtAS3MT and AS3MT/M287T genotypes display different urinary profiles of methylated metabolites of iAs, including changes in the DMAs/MAs ratio (Agusa et al., 2009; Chung et al., 2009; Engström et al., 2007, 2011; Fujihara et al., 2009; Hernández et al., 2008a,b; Hwang et al., 2010; Valenzuela et al., 2009), suggesting that AS3MT genotype and As methylation phenotype can be linked. Therefore, we have compared the kinetics of As methylation in reactions catalyzed by recombinant wtAS3MT and AS3MT/M287T using physiological and synthetic reductants and examined the role of GSH as a modulator of catalytic activities of wtAS3MT and AS3MT/M287T. Differences in kinetic behavior

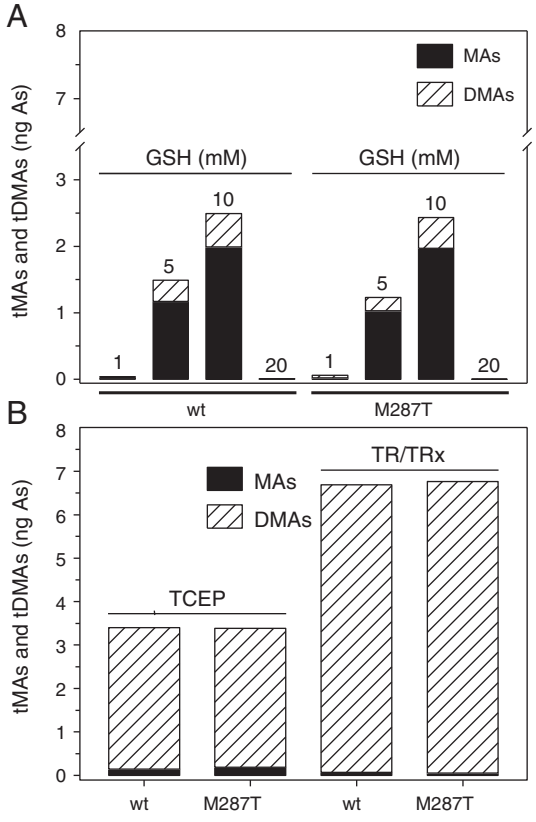

Fig. 2. Effects of reductants on methylation of arsenite (iAs^{III}) by wtAS3MT and AS3MT/M287T. Reaction mixtures contained 100 mM Tris–HCl buffer (pH 7.4), recombinant enzyme (5 μg), 1 mM AdoMet, 1 μM iAs^{III} and one of the following reductants or reducing systems: A. GSH (1, 5, 10 or 20 mM); B. 1 mM TCEP or a coupled enzymatic system consisting of 0.2 μM TR, 10 μM Trx and 300 μM NADPH. Reaction mixtures were incubated at 37 °C for 2 h. Cumulative yields of tMAs ($MAs^{III}+MAs^{V}$) and tDMAs ($DMAs^{III}+DMAs^{V}$) shown (mean, n=3).

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