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Arsenic (+3 oxidation state) methyltransferase and the inorganic arsenic methylation phenotype

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Abstract

Inorganic arsenic is enzymatically methylated; hence, its ingestion results in exposure to the parent compound and various methylated arsenicals. Both experimental and epidemiological evidences suggest that some of the adverse health effects associated with chronic exposure to inorganic arsenic may be mediated by these methylated metabolites. If iAs methylation is an activation process, then the phenotype for inorganic arsenic methylation may determine risk associated with exposure to this metalloid. We examined inorganic arsenic methylation phenotypes and arsenic (+3 oxidation state) methyltransferase genotypes in four species: three that methylate inorganic arsenic (human (*Homo sapiens*), rat (*Rattus norwegicus*), and mouse (*Mus musculus*)) and one that does not methylate inorganic arsenic (chimpanzee, *Pan troglodytes*). The predicted protein products from arsenic (+3 oxidation state) methyltransferase are similar in size for rat (369 amino acid residues), mouse (376 residues), and human (375 residues). By comparison, a 275-nucleotide deletion beginning at nucleotide 612 in the chimpanzee gene sequence causes a frameshift that leads to a nonsense mutation for a premature stop codon after amino acid 205. The null phenotype for inorganic arsenic methylation in the chimpanzee is likely due to the deletion in the gene for arsenic (+3 oxidation state) methyltransferase that yields an inactive truncated protein. This lineage-specific loss of function caused by the deletion event must have occurred in the *Pan* lineage after *Homo-Pan* divergence about 5 million years ago.

Keywords: Arsenic; Inorganic arsenic; Oxidation state

Introduction

Chronic exposure to inorganic arsenic (iAs) in occupational and environmental settings has been linked to increased prevalences of cancers (International Agency for Research on Cancer, 2004) and degenerative diseases (Abernathy et al., 2003; Tseng, 2004; Yoshida et al., 2004). In humans and many other species, iAs is converted to methylated metabolites. Thus, ingestion of iAs results in exposure to iAs and to methyl As (MAs) and dimethyl As (DMAs), its two major metabolites. The pathway for conversion of iAs into these metabolites can be summarized as $iAs^{III} \rightarrow MAs^{V} \rightarrow MAs^{III} \rightarrow DMAs^{V} \rightarrow DMAs^{III}$. Here, alternating steps of oxidative methylation and the reduction of As^{V} to As^{III} yield a series of intermediates and products (Cullen et al., 1984). Although methylation of iAs has sometimes been described as a detoxification process (Gebel, 2002), the intermediates and products of this pathway, particularly those that contain As^{III}, may be the putative mediators of some of the deleterious effects

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associated with iAs exposure. For example, methylated metabolites containing As^{III} exceed iAs in potency as cytotoxins or DNA-damaging agents and as enzyme inhibitors (Thomas et al., 2001); these metabolites also disrupt critical cell signaling processes (Drobná et al., 2003). Thus, iAs methylation can be considered an activation process. Recent evidence shows that a protein encoded by arsenic (+3 oxidation state) methyltransferase (*AS3MT*) catalyzes the multistep process that converts iAs to its methylated metabolites (Lin et al., 2002; Waters et al., 2004a). That is, this enzyme catalyses S-adenosylmethionine (AdoMet)-dependent methylation and the reduction of As^{V} to As^{III}. Like other arsenate reductases (Mukhopadhyay and Rosen, 2002), it uses thioredoxin or glutaredoxin to reduce As^{V} to As^{III}. Other work shows the critical role of *AS3MT* in the capacity to methylate iAs. Heterologous expression of rat *AS3MT* in human urothelial (UROtsa) cells which normally do not express AS3MT confers capacity to methylate iAs and alters the cytotoxicity of iAs^{III} and MAs^{III} (Drobná et al., submitted for publication). In cultured primary human hepatocytes, heterozygosity at the *AS3MT* locus has been associated with a high capacity to methylate iAs (Drobná et al., 2004). Polymorphisms of *AS3MT* may underlie a reported association between atypical patterns of methylated arsenicals in urine and the occurrence of As-induced skin lesions (Yu et al., 2000), although other factors could account for increased susceptibility.

These findings suggest a critical role for *AS3MT* in the determination of the capacity to methylate iAs. In the work reported here, we have examined the relation between *AS3MT* genotype and the iAs methylation phenotype in four mammalian species. Of these, three (rat, mouse, human) are known to be efficient methylators of iAs. In contrast, the chimpanzee has been shown to lack the capacity to methylate iAs (Aposhian, 1997; Vahter et al., 1995). This examination of AS3MT at the genomic and proteomic levels suggests a molecular basis for this interspecies difference in iAs methylation phenotype.

Methods

Table 1 lists the sources and identifies the nucleotide sequence data used to extract *AS3MT* cDNA sequences for human (*Homo sapiens*), rat (*Rattus norwegicus*), mouse (*Mus musculus*), and chimpanzee (*Pan troglodytes*). Alignment of cDNA sequences for mouse, rat, human, and chimpanzee AS3MT used Ensembl (http://www.ensembl.org/) cDNA databases of mouse, human, or chimpanzee with rat *AS3MT* cDNA as query sequence using BLASTN program set to $E < 10^{-4}$ and other parameters as defaults. cDNA sequences and deduced protein sequences were aligned with CLUSTAL W (Thompson et al., 1994). The secondary and tertiary structures of portions of rat and chimpanzee AS3MT were modeled with 3D-JIGSAW Comparative Modeling Server using AdoMet-dependent methyltransferase (1VLM_A) with 219 amino acids from the database as template (Bates and Sternberg, 1999). The coordinates of 3D structures of rat or chimpanzee AS3MT were constructed and displayed with RasMol (Sayle and Milner-White, 1995).

Table 1
Sources and identities of nucleotide data used in construction of cDNA sequences

Species	NCBI nucleotide database ID	Ensembl cDNA database transcript ID
Human		ENST00000210555
Rat	NM_080890	
Mouse	BC013468	ENSMUST00000003655
Chimpanzee		ENSPTRT00000005526-ENSPTRT00000005527

The cloning of rat *AS3MT* and the expression and purification of the wildtype recombinant protein (AS3MT) have been described (Waters et al., 2004a). A mutant AS3MT in which Cys156 is replaced with a Ser residue was produced using a QuikChange Multi Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). The expression and purification of the recombinant mutant AS3MT (Cys156Ser) followed protocols used for wildtype AS3MT. The enzymatically catalyzed conversion of iAs^{III} to methylated arsenicals was monitored by measuring the conversion of [^{73}As]-iAs^{III} to methylated products using either wildtype AS3MT or Cys156Ser. Conditions for assay of methylation activity and for separation and quantitation of radiolabeled products have been described (Waters et al., 2004b). Briefly, reaction mixtures containing 10 g wildtype AS3MT or Cys156Ser with 1 mM AdoMet, 1 μM [^{73}As]-arsenite, and 1 mM tris (2-carboxylethyl) phosphine in 100 mM tris-100 mM phosphate, pH 7.4, were incubated at 37 °C for 20 or 60 min or overnight (18 h). Radiolabeled arsenicals were separated by thin layer chromatography for detection.

Results and discussion

We compared the *AS3MT* genotypes in human (*H. sapiens*), rat (*R. norwegicus*), and mouse (*M. musculus*) which methylate iAs with the *AS3MT* genotype of chimpanzee (*P. troglodytes*), a species that lacks the capacity to methylate iAs (Aposhian, 1997; Vahter et al., 1995). The *AS3MT* cDNA sequences (Fig. 1A) and the predicted protein sequences (Fig. 1B) for these species identify a striking interspecies difference. A 275 nucleotide deletion beginning at nucleotide 612 in the chimpanzee cDNA sequence causes a frameshift that leads to a nonsense mutation for a premature stop codon after amino acid 205. Notably, the deleted 275 nucleotides were located in three subfragments inserted about 103 Mb away from truncated chimpanzee *AS3MT* on chromosome 8. In contrast, a substantially larger AS3MT is predicted for rat (369 amino acids), mouse (376 amino acids), or human

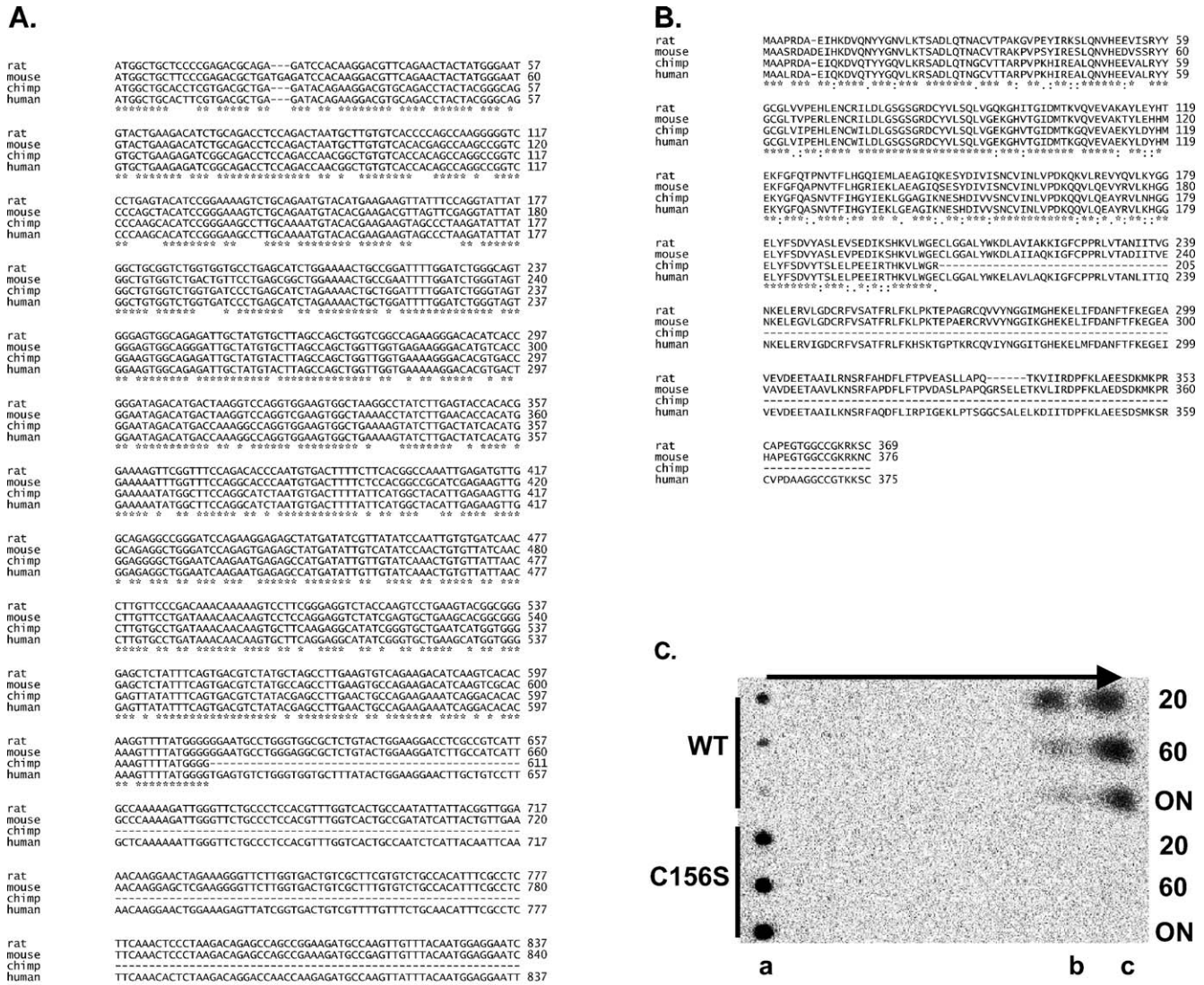

Fig. 1. AS3MT sequences, alignments, and activities. (A) Alignment of cDNA sequences for mouse, rat, human, and chimpanzee *AS3MT*. (B) Alignment of predicted protein sequences for mouse, rat, human, and chimpanzee AS3MT. Identical residues in predicted protein sequences indicated by asterisk (*). (C) Effect of Cys156Ser mutation on the catalytic activity of rat recombinant AS3MT. Reaction mixtures with wildtype (WT) or mutant (C156S) were incubated for 20 or 60 min or overnight (ON, 18 h). Positions indicated for migration of inorganic arsenic (a), dimethylarsenic (b), and trimethylarsenic (c), and direction of chromatography indicated by arrow.

(375 amino acids). Mouse AS3MT differs by unique insertion of Asp after Ala7; an additional six amino acids are inserted after Gln333 in mouse and Gly332 in human sequences. In the N-terminal portion of AS3MT common to all species, overall identity is 74.1%. Because Cys residues have been reported to be involved in reduction reaction of arsenate to arsenite in bacteria (Mukhopadhyay and Rosen, 2002), we examined the potential roles of Cys

residues in mammalian AS3MTs. Notably five Cys residues are fully conserved in the common N-terminal portion of AS3MT. In the C-terminal portion common to rat, mouse, and human, there are seven additional conserved Cys, including a CysCys pair near the C-terminus. Mouse lacks a Cys shared by rat (residue 354) and human (residue 360); human also has a unique Cys at residue 334. Site-directed mutagenesis has examined the role of conserved Cys (residues 32, 61, 85, and 156 in rat AS3MT) in the common N-terminal region of AS3MT. Among tested mutants of rat AS3MT, only Cys156Ser is completely inactive in iAs methylation, suggesting this residue to be critical for enzyme function (Fig. 1C). Molecular modeling of the common UbiE methylase-like domains of rat and chimpanzee AS3MT shows that the local environment of Cys156 may contribute to its catalytic function (Fig. 2). In rat AS3MT, Cys156 located in a well-defined cavity structure may be situated to participate in the binding of substrates which contain As^{III}. The predicted structure of chimpanzee AS3MT lacks this well-defined cavity for Cys156. This change could contribute to the loss of catalytic activity by chimpanzee AS3MT. The loss of function in chimpanzee AS3MT might also be due to the global loss of the C-terminal portion of the protein. The C-terminal half of AS3MT shares little sequence homology with those of other non-nucleic acid methyltransferases but may be required for some catalytic function unique to this enzyme (e.g., reduction of As^{V}-containing intermediates). By comparison, the C-terminal region in *Thermotoga maritima* isoaspartyl methyltransferase also shows little sequence homology with other methyltransferases; however, this region is required for protein stabilization and enzymatic activity (Ichikawa and Clarke, 1998).

The loss of AS3MT activity in the chimpanzee may be an example of a lineage-specific loss of function associated

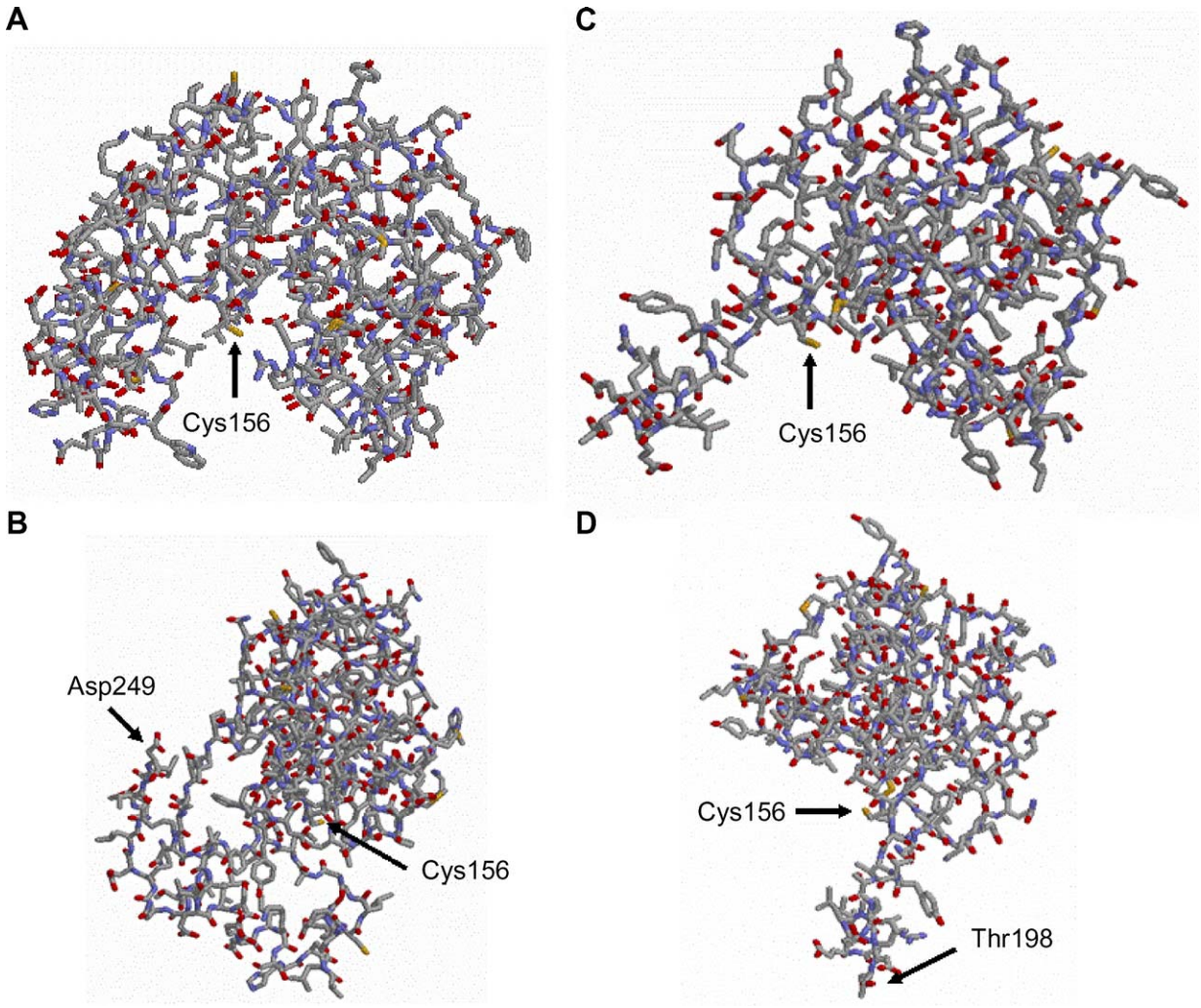

Fig. 2. Molecular modeling of rat and chimpanzee AS3MT. (A and B) Predicted structure of rat AS3MT (residues 70–249). (C and D) Predicted structure of chimpanzee AS3MT (residues 63–198). Both are shown in two complementary orientations. Asp249 and Thr198 are the last residues in the regional 3D structures (B and D), respectively.

with a deletion event in chimpanzee *AS3MT* leading to production of an inactive enzyme (Hacia, 2001). An analogous deletion in human CMP-sialic acid hydroxylase gene eliminates this enzyme activity and accounts for a human-chimpanzee difference in patterns of modification of cell-surface glycoproteins. Insertion/deletion events are thought to be a major source of divergence between the human and chimpanzee genomes (Britten, 2002). The human and chimpanzee discordance in AS3MT activity suggests that loss of function occurred in the *Pan* lineage after the *Homo-Pan* divergence about 4.6–6.2 million years ago (Chen and Li, 2001). Two New World monkeys, the common marmoset (*Callithrix jacchus*) and the tamarin (*Saguinus oedipus*), do not methylate iAs (Vahter and Marafante, 1985; Vahter et al., 1982; Zakharyan et al., 1996). In contrast, the Rhesus monkey (*Macaca mulatta*) which originated in the Old World methylates iAs (Zakharyan et al., 1996). Recent studies with cultured primary hepatocytes from a Rhesus monkey confirm that this species rapidly converts iAs^{III} to MAs and DMAs (M. Styblo et al., unpublished results). It has been speculated that the loss of the iAs methylation phenotype in New World monkeys and in chimpanzees confers resistance to trypanosomal diseases which are endemic to the regions in which these species arose (Aposhian, 1997; Zakharyan et al., 1996). In this model, chemotherapeutic concentrations of iAs in blood are sustained in the absence of methyltransferase activity. This is postulated to result in a selective advantage to non-methylating species. The lack of iAs methylation capacity in the marmoset and other New World monkeys could have arisen from a loss of function mutation in these species after their divergence from a common primate ancestor. Alternatively, the lack of activity could reflect the presence of an endogenous inhibitor of the activity of As methyltransferase. The toxicological implications of the lack of functional AS3MT in the chimpanzee have not been fully evaluated. Notably, the cytotoxicity of iAs^{III} in CRL-1609 cells, an SV40 virus-transformed chimpanzee skin fibroblast cell line, is similar to that seen in other cell lines known to methylate iAs^{III} (Sakurai et al., 2004), suggesting that the absence of methylation does not potentiate the actions of this metalloid.

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