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A Novel *S*-Adenosyl-L-methionine:Arsenic(III) Methyltransferase from Rat Liver Cytosol*

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***S*-Adenosyl-L-methionine (AdoMet):arsenic(III) methyltransferase, purified from liver cytosol of adult male Fischer 344 rats, catalyzes transfer of a methyl group from AdoMet to trivalent arsenicals producing methylated and dimethylated arsenicals. The kinetics of production of methylated arsenicals in reaction mixtures containing enzyme, AdoMet, dithiothreitol, glutathione (GSH), and arsenite are consistent with a scheme in which monomethylated arsenical produced from arsenite is the substrate for a second methylation reaction that yields dimethylated arsenical. The mRNA for this protein predicts a 369-amino acid residue protein (molecular mass 41056) that contains common methyltransferase sequence motifs. Its sequence is similar to Cyt19, a putative methyltransferase, expressed in human and mouse tissues. Reverse transcription-polymerase chain reaction detects *S*-adenosyl-L-methionine:arsenic(III) methyltransferase mRNA in rat tissues and in HepG2 cells, a human cell line that methylates arsenite and methylarsonous acid. *S*-Adenosyl-L-methionine:arsenic(III) methyltransferase mRNA is not detected in UROtsa cells, an immortalized human urothelial cell line that does not methylate arsenite. Because methylation of arsenic is a critical feature of its metabolism, characterization of this enzyme will improve our understanding of this metalloid's metabolism and its actions as a toxin and a carcinogen.**

In many species, including humans, exposure to inorganic arsenic results in urinary excretion of methylated and dimethylated arsenicals (1–3). Cullen and co-workers (4) summarized the conversion of inorganic arsenic into these methylated products in a reaction scheme which incorporates oxidative methylation and the cycling of arsenic between the pentavalent (As^V)[1] and trivalent (As^{III}) oxidation states,

$$As^VO_4^{3-} + 2e \rightarrow As^{III}O_3^{3-} + CH_3^+ \rightarrow CH_3As^VO_3^{2-} + 2e \rightarrow CH_3As^{III}O_2^{2-}$$
$$+ CH_3^+(CH_3)_2As^VO_2^- + 2e \rightarrow (CH_3)_2As^{III}O^- + CH_3^+ \quad \text{(Eq. 1)}$$

Because reduction of arsenic to trivalency is a prerequisite for its oxidative methylation, pentavalent arsenicals are reduced by endogenous thiols such as glutathione (GSH) (5, 6) or by As^V reductases (7–9). A protein has been purified from rabbit liver cytosol that catalyzes the methylation of both arsenite and methylarsonous acid (10, 11); however, this protein has not been sequenced. These activities are designated arsenite methyltransferase (EC 2.1.1.137) and methylarsonite methyltransferase (EC 2.1.1.138), respectively. This protein (estimated molecular mass 60 kDa) uses *S*-adenosyl-L-methionine (AdoMet) as the methyl group donor. The methylation of arsenite by this protein is stimulated by a monothiol (GSH) and the methylation of methylarsonous acid is highly stimulated by a dithiol, dithiothreitol (DTT).

The methylation of arsenic has been commonly regarded as a mechanism for its detoxification (12). However, recent research has shown that methylated arsenicals that contain As^{III} are important intermediates in the metabolism of inorganic arsenic. Methylated arsenicals that contain As^{III} are found in the urine of individuals who chronically consume drinking water that contains inorganic arsenic and in cells cultured in the presence of arsenite (13–18). These As^{III}-containing metabolites are more cytotoxic and genotoxic than either arsenate or arsenite (19–21). Methylated arsenicals that contain As^{III} are also more potent than arsenite as inhibitors of the activities of various enzymes, including GSH reductase (22, 23), thioredoxin reductase (24, 25), and pyruvate dehydrogenase (26). These results suggest that the methylation process can be properly regarded as a means for the activation of inorganic arsenic to more reactive and toxic species. Consequently, characterization of enzymes that catalyze formation of methylated arsenicals is an important step in understanding the consequences of chronic exposure to inorganic arsenic.

Although there are extensive data on the kinetics of formation of methylated arsenicals in *in vitro* systems containing

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[1] The abbreviations used are: As^V, pentavalent arsenic; AdoMet, *S*-adenosyl-L-methionine; As^{III}, trivalent arsenic; GSH, glutathione; DTT, dithiothreitol; [^{73}As]iAs^{III}, arsenite; $MAs^{III}O$, methylarsine oxide; RT, reverse transcription.

TABLE I
Purification of S-adenosyl-L-methionine:arsenic(III) methyltransferase from rat liver cytosol

Fraction	Total protein (mg)	Specific activity[a]	Total activity[b]	Purification
Cytosol	1200	0.16	192	1
Acidified cytosol	678	0.48	325	3
Chromatofocusing	22	216	4752	1347
S-Adenosyl-L-homocysteine affinity chromatography	0.76	1490	1132	9312

[a] Specific activity expressed as pmols of methylated and dimethylated arsenic formed per mg of protein in a reaction mixture containing 0.1 μM arsenite that was incubated at 37 °C for 45 min.
[b] Total activity (picomols of methylated and dimethylated arsenic formed) is the product of the specific activity of a fraction and the total protein content of that fraction.

crude cytosolic preparations or purified enzyme, in cultured cells, and in intact animals, little is known about the properties of the enzymes that catalyze the methylation reactions. The work reported here describes the purification of a novel protein from rat liver cytosol that catalyzes the time-dependent formation of methylated and dimethylated arsenicals in reaction mixtures that contain the purified protein, arsenite, GSH, DTT, and AdoMet. Mass spectrometric sequencing of peptides generated from this protein indicates a high degree of homology with Cyt19, a putative methyltransferase of unknown function that is expressed in mouse and human tissues. Using degenerate oligonucleotide primers based on the amino acid sequence of peptides from the purified protein, the complete nucleotide sequence of the mRNA encoding the protein has been determined. This novel protein is designated *S*-adenosyl-L-methionine:arsenic(III) methyltransferase. The mRNA for the protein is expressed in a variety of rat tissues and in HepG2 cells, a human hepatoma cell line. However, it is not expressed in UROtsa cells, an SV40 large T antigen-immortalized human urothelial line (27), that do not methylate inorganic arsenic.

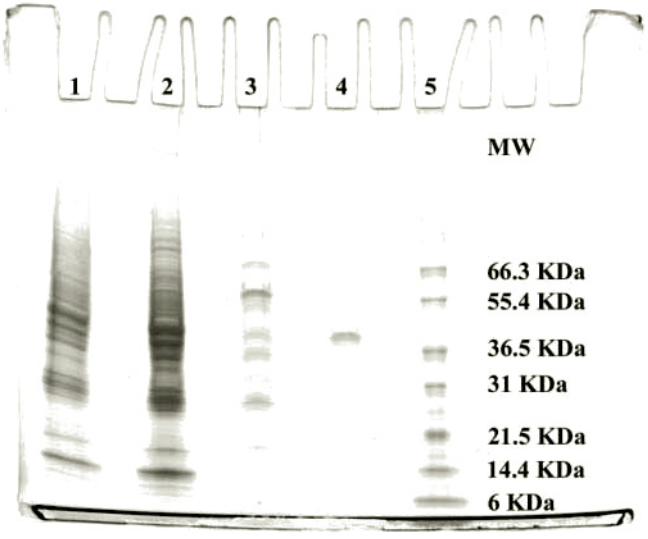

FIG. 1. **Purification of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase.** Fractions from purification scheme were electrophoresed on a 5–20% polyacrylamide gradient and gel stained with Coomassie Brilliant Blue. *Lane 1*, rat liver cytosol; *Lane 2*, acidified cytosol; *Lane 3*, pooled active fractions from chromatofocusing gel chromatography; *Lane 4*, pooled active fractions from *S*-adenosylhomocysteine-Sepharose gel chromatography; *Lane 5*, molecular weight markers.

EXPERIMENTAL PROCEDURES

Materials—Unless otherwise noted, all reagents were obtained from Sigma. HepG2 cells were obtained from ATCC and UROtsa cells were obtained from Dr. N. Unimye, Department of Urology, School of Medicine, West Virginia University. Radiolabeled arsenite ([^{73}As]iAs^{III}) was prepared by the procedure of Reay and Asher (28) from radiolabeled arsenate [^{73}As]iAs^{V} obtained from the Los Alamos Meson Production Facility (Los Alamos, NM). The methylated arsenical, methylarsine oxide (MAs^{III}O), was synthesized and characterized as previously described (22).

Enzyme Purification—Male Fischer 344 rats (4 to 6 weeks old) from Charles River Laboratories were maintained in a 12-h light, 12-h dark photocycle at 21 ± 2 °C with free access to PMI 5001 Rodent Chow (PMI Nutrition International) and tap water. Rats were euthanized by cardiac exsanguination under nembutal anesthesia. All subsequent steps were performed at 4 °C. Livers were perfused *in situ* with 154 mM NaCl, removed, blotted, and weighed. Pooled livers (up to 150 g) were homogenized in a buffer containing 25 mM Tris, 5 mM GSH, 1 mM DTT, and 250 mM sucrose, pH 8.3, at a tissue-to-buffer ratio of 1:5 (w/v) using a glass-Teflon homogenizer. The homogenate was centrifuged at 110,000 × *g* for 45 min to prepare a cytosolic fraction. After removal of the lipid layer, the cytosolic fraction was adjusted to pH 5 by dropwise addition of 1 M acetic acid. The acidified cytosol was immediately centrifuged at 10,000 × *g* for 15 min; the supernatant was removed and adjusted to pH 8.3 by the dropwise addition of 1 M ammonium hydroxide. The treated supernatant could be stored overnight at 4 °C without loss of arsenic methyltransferase activity.

Treated supernatant was chromatofocused on a column of Polybuffer Exchanger PBE94 gel (Amersham Biosciences Inc.) that was eluted with Polybuffer 96 diluted 1:9 (v/v) in 25 mM Tris, 5 mM GSH, and 1 mM DTT, pH 6. The arsenic methylating activities of eluate fractions were assessed using a thin layer chromatography (TLC) method for the separation of ^{73}As-labeled products. Briefly, [^{73}As]iAs^{III} (final concentration 0.1 μM As^{III}) and 1 mM AdoMet were added to aliquots of eluate fractions and reaction mixtures were incubated at 37 °C for up to 120 min. Reactions were stopped by the addition of 0.2 M CuCl and heating to 100 °C for 5 min (29). Denatured proteins were removed by centrifugation and supernatants were oxidized with H_2O_2 (final concentration 10%). Aliquots of oxidized supernatants were analyzed by TLC as previously described (30). Fractions that contained the highest levels of the activity that converted arsenite to methylated and dimethylated products were pooled and applied to a column of an *S*-adenosyl-L-homocysteine-Sepharose affinity gel that was prepared by the method of Reeve and co-workers (31). The chromatographic gel was fully equilibrated with 50 mM Na phosphate buffer containing 5 mM GSH, 1 mM DTT, and 5% (v/v) glycerol, pH 7.4. After application of high activity fractions, the gel bed was washed with 5 column volumes of 50 mM sodium phosphate buffer containing 5 mM GSH, 1 mM DTT, and 5% (v/v) glycerol, pH 7.4. The arsenite methylating activity was eluted by addition of 1 mM AdoMet to the eluting buffer. Fractions containing the highest arsenic methylating activity were pooled and stored at −20 °C.

Anion Exchange Chromatography, Hydride Generation, Atomic Fluorescence Spectrometry—The enzyme-catalyzed conversion of methylarsine oxide to dimethylated arsenic was examined by the method of Zhang and associates (32). Aliquots of reaction mixtures were chromatographed on an anion-exchange column (Supelcosil LC-SAX, 250 mm × 4.6 mm, Supelco) using 30 mM potassium phosphate buffer, pH 4.5, as the mobile phase (1 ml/min). Arsines of the separated species were generated and detected using a Millennium Excalibur atomic fluorescence spectrometer (PS Analytical, Kent, England).

Peptide Sequence Analysis—SDS-PAGE of pooled active fractions from *S*-adenosyl-L-homocysteine-Sepharose affinity chromatography identified one Coomassie Blue-reactive band. This band was excised from the gel, transferred to a siliconized tube, and was washed and destained overnight in 200 μl of 50% methanol. Gel pieces were then dehydrated in acetonitrile and rehydrated in 30 μl of 10 mM DTT in 0.1

M ammonium bicarbonate. After reduction for 30 min at room temperature, the DTT-containing solution was removed and the sample was alkylated in 30 μl of 50 mM iodoacetamide in 0.1 M ammonium bicarbonate for 30 min at room temperature. The alkylating mixture was removed and the gel pieces were dehydrated in 100 μl of acetonitrile. After removal of the acetonitrile, the gel pieces were rehydrated in 100 μl of 0.1 M ammonium bicarbonate. Gel pieces were again dehydrated in 100 μl of acetonitrile. After removal of the acetonitrile, gel pieces were fully dried by vacuum centrifugation. Dried gel pieces were rehydrated on ice for 10 min in a solution containing 20 μg of trypsin/ml in 0.05 M ammonium bicarbonate. Excess trypsin-containing solution was removed and an additional 20 μl of 0.05 M ammonium bicarbonate was added. This sample was incubated overnight at 37 °C. Peptides were extracted from the polyacrylamide gel matrix with two 30-μl aliquots of 50% acetonitrile in 5% formic acid. Extracts were combined and evaporated to a volume of 25 μl for further analysis.

The liquid chromatography-mass spectrometry system consisted of a Finnigan LCQ ion trap mass spectrometer with a Protana nanospray ion source that was interfaced with a self-packed Phenomenex Jupiter 10 μM C_{18} reversed-phase capillary column (8 cm × 75 μm inner diameter). Samples of the extract (0.5 to 5 μl) were injected on the column that was eluted with an acetonitrile, 0.1 M acetic acid gradient at a flow rate of 0.25 μl/min. The nanospray ion source operated at 2.8 kV. The extracts were analyzed using the instrument's double play capability. Here, full scan mass spectra were acquired to determine the molecular weights of peptides and product ion spectra were acquired to determine the amino acid sequence in sequential scans. This mode of analysis yields about 400 CAD spectra of ions that range in abundance over several orders of magnitude. Data were analyzed by data base searching using the Sequest search algorithm. Peptides that were not matched by this algorithm were interpreted manually and searched in the EST data bases using the Sequest algorithm.

Determination of mRNA Sequence—Total RNA was isolated from the livers of adult male Fischer 344 rat using Trizol reagent (Invitrogen). Total RNA was treated with DNase (Invitrogen) and cDNA was made using Promega Reverse Transcriptase System. A set of degenerate oligonucleotide primers for use in polymerase chain reactions (PCR) were designed on the basis of the translation of peptide sequences from *S*-adenosyl-L-methionine:arsenic(III) methyltransferase. With these primers, the cDNA was amplified using Hot Start PCR and Ampli *Taq* Gold enzyme (PerkinElmer Life Sciences). PCR products were purified using QIAquick PCR purification. A 900-base sequence was obtained and was sequenced at the UNC-CH Automated DNA Sequencing Facility on a Model 377 DNA Sequencer (Applied Biosystems) using the ABI Prism™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase. Using this sequence information, the sequences of the 5′ and 3′ ends were obtained using the GeneRacer system (Invitrogen). The resulting cDNAs were sequenced, providing the entire mRNA sequence.

Reverse Transcription (RT)-PCR Analysis of Gene Expression—Adult male Fischer 344 rats were euthanized by cardiac exsanguination under nembutal anesthesia. Tissues were quickly harvested, snap frozen in liquid nitrogen, and stored at −65 °C. RNA was also isolated from two human cell lines. HepG2 cells were grown in an amended Dulbecco's modified Eagle's medium (4.5 g of glucose and 110 mg of sodium pyruvate per liter) with 15% fetal bovine serum and UROtsa cells were grown in RPMI 1640 medium with 10% fetal bovine serum. RNA was isolated from both cell lines using Trizol reagent. RNA was treated with DNase (Promega) and further purified with an RNeasy Minikit (Qiagen). mRNA was reverse transcribed into cDNA which was used for PCR reactions for detection of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase and β-actin. PCR was performed on a PerkinElmer Geneamp 9700 thermal cycler and PCR products were separated on a 1% agarose gel and stained with ethidium bromide.

RESULTS

Protein Purification and Reaction Characteristics—A combination of acid treatment of cytosol, chromatofocusing, and affinity chromatography on an *S*-adenosyl-L-homocysteine-Sepharose gel column allowed 9300-fold enrichment of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase activity from rat liver cytosol (Table I). Notably, the total activity of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase recovered in this purification scheme increased as the specific activity of the fractions increased. The enhanced yield may reflect removal of an endogenous inhibitor of the enzyme's activity in

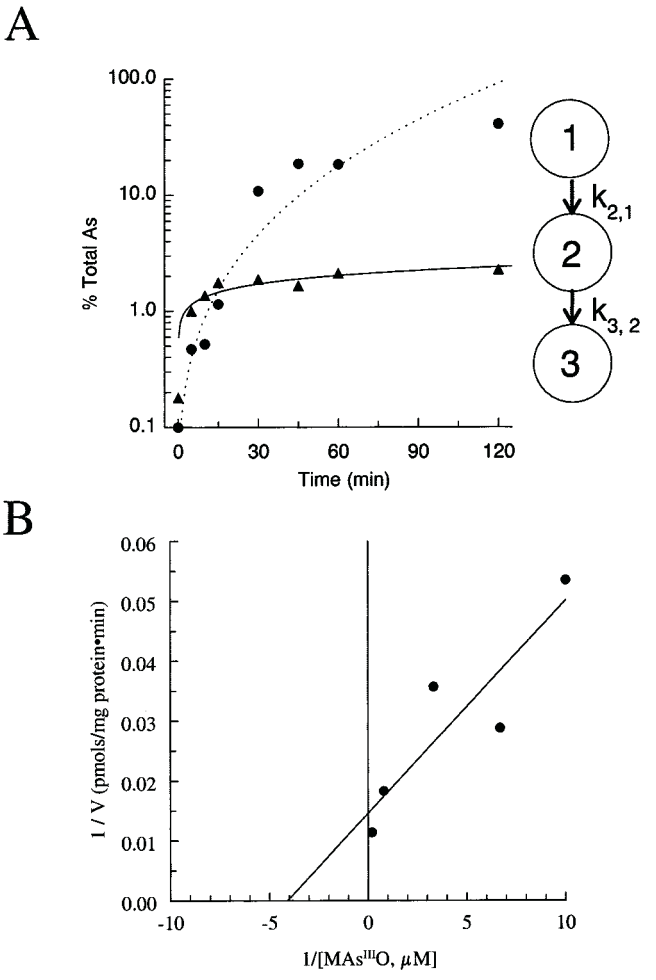

FIG. 2. **Production of methylated and dimethylated arsenic by *S*-adenosyl-L-methionine:arsenic(III) methyltransferase.** *A*, time course of methylated (▲) and dimethylated (●) arsenic in a reaction mixture that contained 25 μg of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase, 0.1 μM arsenite, 1 mM AdoMet, 5 mM GSH, and 1 mM DTT in 50 mM Na phosphate buffer, pH 7.5, with 5% (v/v) glycerol and was incubated at 37 °C for up to 120 min. Structure of the three-compartment model used to estimate the overall rates of conversion of arsenite to methyl arsenic ($k_{2,1}$) and of methyl arsenic to dimethyl arsenic ($k_{3,2}$) shown. *B*, double-reciprocal plot of the relation between the concentration of methylarsine oxide (μM) and the rate of conversion of methyl arsenic to dimethyl arsenic (picomoles converted per mg of protein per minute). The reaction mixture contained 25 μg of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase, 0 to 5 μM methylarsine oxide, 0.2 mM AdoMet, 5 mM GSH, and 1 mM DTT in 50 mM Na phosphate buffer, pH 7.5, with 5% (v/v) glycerol and was incubated at 37 °C for 15 min.

the purification scheme or an altered ratio of enzyme to DTT as purification progresses. A critical factor in the success of this purification scheme was inclusion of GSH and DTT in all buffers. Earlier work demonstrated that GSH was critical to the maintenance of arsenic methylating activity in an *in vitro* reaction system that contained rat liver cytosol (33, 34) and that DTT promoted the dimethylation reaction catalyzed by the purified rabbit liver protein (10). *S*-Adenosyl-L-homocysteine-Sepharose affinity chromatography yielded a single Coomassie Blue staining band on SDS-PAGE (Fig. 1). This material was used for studies of the characteristics of arsenic methylation and for preparation of peptides for amino acid sequence determination.

Fig. 2*A* illustrates the time course of the production of methylated and dimethylated arsenicals in a system that contained 25 μg of enzyme, 0.1 μM [^{73}As]iAs^{III}, 1 mM AdoMet, 5 mM GSH, and 1 mM DTT in 50 mM sodium phosphate buffer with 5% (v/v)

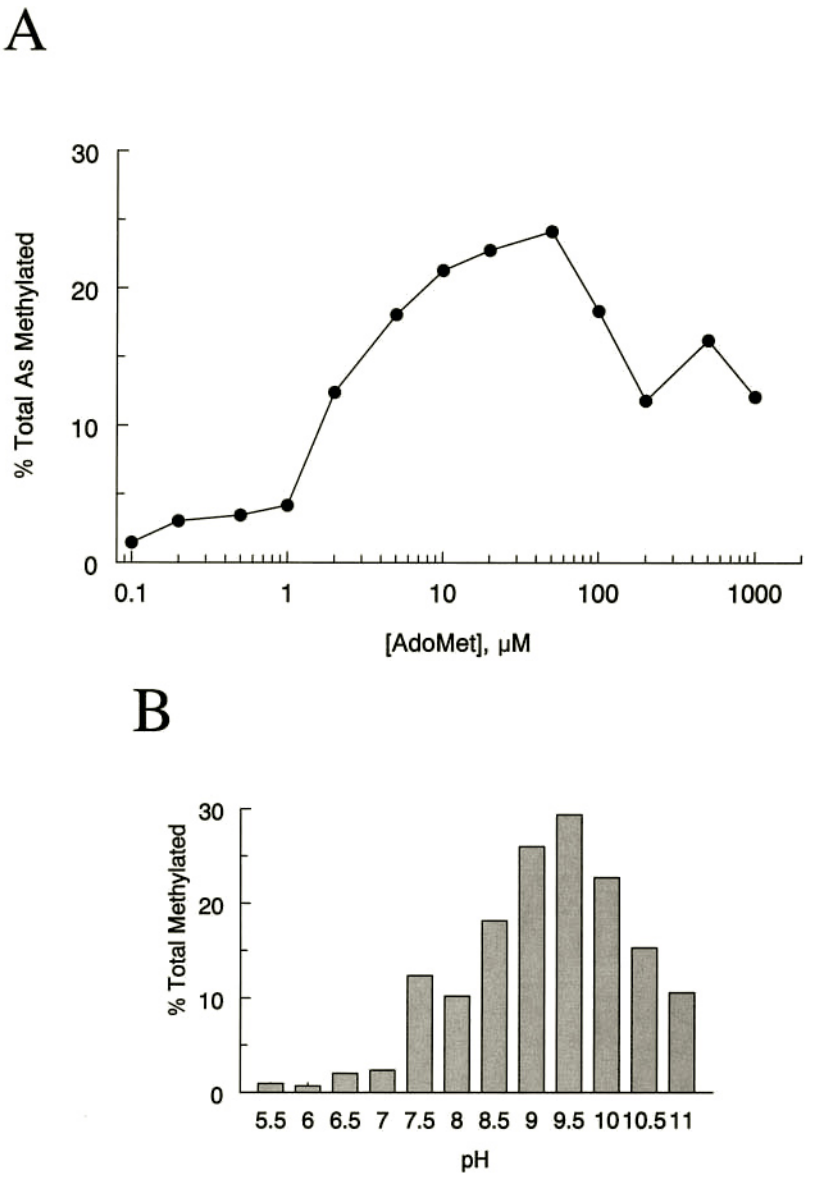

FIG. 3. **Effect of pH and AdoMet concentration on the activity of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase.** *A*, effect of AdoMet concentration on the activity of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase. The concentration of AdoMet was varied from 0 to 1000 μM in a reaction mixtures that contained 25 μg of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase, 0.1 μM arsenite, 5 mM GSH, and 1 mM DTT in 50 mM Na phosphate buffer, pH 7.5, with 5% (v/v) glycerol which were incubated at 37 °C for 12 min. *B*, effect of pH on the activity of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase. Reaction mixtures with final pH ranging from 5 to 11 that contained 2.5 μg of *S*-adenosyl-L-methionine: arsenic(III) methyltransferase, 0.1 μM arsenite, 50 μM AdoMet, 5 mM GSH, and 1 mM DTT in 50 mM Na phosphate buffer, pH 7.5, with 5% (v/v) glycerol were incubated at 37 °C for 15 min.

TABLE II
Amino acid sequences of peptides generated by the trypsin digestion of purified S-adenosyl-L-methionine:arsenic (III) methyltransferase as determined by liquid chromatography-mass spectrometry

Peptide	Measured molecular mass ($M+H^+$, Da)	Sequence
1	729.2 +2	DLAVIAK
2	827.8 +2	FVSATFR
3	833.3 +2	GVPEYIR
4	846.4 +2	IGFCPPR
5	878.2 +2	YXNEVXK
6	974.5 +2	ILDLGSGSGR
7	1088.5 +2	GHXTGIDM(O)TK
8	1242.7 +2	LVTANIITVGNK
9	1312.6 +2	DVQNYYGNVLK
10	1344.7 +2	ELIFDANFTFK
11	1409.7 +3	DCYVLSQLVGKK
12	1410.7 +3	SLQNVHEEVXSR
13	1608.7 +3	CKVVYDGGI—K
14	1731.2 +2	EGEAVEVDEETAAXLR
15	2232.2 +3	FAHDFLFTPVEASLXAA—

glycerol, pH 7.4. Here, incubation at 37 °C for intervals up to 120 min lead to the appearance of both methylated species. Notably, the appearance of methylated arsenic preceded the appearance of dimethylated arsenical. This pattern was consistent with the stepwise formation of ^{73}As-labeled methylated arsenic from [^{73}As]iAs^{III} and of ^{73}As-labeled dimethylated arsenic from ^{73}As-labeled methylated arsenic. A three-compartment kinetic model with the structure shown in Fig. 2*A* was constructed using SAAMII (version 1.1.1), a program for compartmental analysis (SAAM Institute, University of Washington). This model provided estimates of the overall rates for the appearance of methylated and dimethylated arsenic in this assay system. Based on the reduction in the residual sum of squares, $k_{2,1}$ was estimated as 0.00443% per minute and $k_{3,2}$ as 0.183% per minute. Under similar conditions, neither arsenate (As^V) nor methylarsonic (As^V) acid was methylated (data not shown). In other studies, the conversion of MAs^{III}O to dimethylated arsenic was assessed using anion exchange chromatography-hydride generation-atomic fluorescence spectrometry to quantify the substrate and the product. Here, reaction mixtures contained up to 5 μM MAs^{III}O, 25 μg of purified enzyme, 0.2 mM AdoMet, 5 mM GSH, and 1 mM DTT in 50 mM sodium phosphate, pH 7.4, with 5% (v/v) glycerol. Following incubation for 15 min at 37 °C, reactions were stopped by snap freezing on dry ice and stored at −20 °C. A double-reciprocal plot of the dependence of the reaction rate on the concentration of methylarsine oxide is shown in Fig. 2*B*. This analysis yielded an

TABLE III
Peptide sequences and oligonucleotide primers

Name	Description	Sequence
mtf1F	Peptide	QNYYGNVLK
	Oligonucleotide (5′ → 3′)	RAAYTAYGGNAAYGTNYTNAA[a]
mtf2F	Peptide	SLQNVHEE
	Oligonucleotide	WSNYTNCARAAYGTNCAYGARGAR
mtf3F	Peptide	ILDLGSGS
	Oligonucleotide	ATHYTNGAYYTNGGNWSNGGNWSN
mtf4F	Peptide	FCPPRLVT
	Oligonucleotide	TYTGYCCNCCNMGNYTNGTNACN
mtf5F	Oligonucleotide	AAGTGGATGAGGAGACGGCAG
mtf6F	Oligonucleotide	GGTGGCTAAAGGGCAGTCACAA
mtf7F	Oligonucleotide	TTCAGAGTTCTGGCGCCACCTA
mtf1R	Peptide	LSAEVPTF
	Oligonucleotide	ARNSWNGCYTCNACNGGNGTRAA
mtf2R	Peptide	AATEEDVE
	Oligonucleotide	NGCNGCNGTYTCYTCRTCNACYTC
mtf3R	Peptide	TVLRPPCF
	Oligonucleotide	NGTNACNARNCKNGGNGGRCARA
mtf-4R	Oligonucleotide	AGCCACTTCCACCTGGACCTTA
poly dT	Oligonucleotide	TTTTTTTTTTTTTTTTTTTTTTTTTT

[a] Degeneracies indicated as R = A + G, Y = C + T, M = A + C, K = G + T, S = G + C, W = A + T, H = A + T + C, N = A + C + G + T.

estimated K_m of 250 nM and a V_{max} of 68 pmol/mg of protein per min.

The activity of the purified *S*-adenosyl-L-methionine:arsenic(III) methyltransferase was dependent on the concentration of AdoMet in the reaction mixture. Because buffer containing 1 mM AdoMet was used to elute the enzyme from *S*-adenosyl-L-homocysteine-Sepharose gel, the pooled active fraction in AdoMet-containing buffer was dialyzed against 50 mM Na phosphate buffer containing 5 mM GSH, 1 mM DTT, and 5% (v/v) glycerol, pH 7.4, using dialysis tubing with a nominal molecular weight cutoff of 12,000. The dialysate was used in a reaction mixture that contained 25 μg of enzyme, 0.1 μM [^{73}As]iAs^{III}, 5 mM GSH, 1 mM DTT, and up to 1000 μM AdoMet in 50 mM Na phosphate buffer with 5% (v/v) glycerol, pH 7.4. Following a 12-min incubation at 37 °C, reaction mixtures were processed for analysis of methylated arsenicals. Fig. 3*A* shows the influence of AdoMet concentration on the extent of methylation of arsenic. Maximal conversion of arsenic to methylated products occurred at 50 μM AdoMet; yield fell at higher AdoMet concentrations. In the absence of added AdoMet, methylated arsenicals were not detected in reaction mixtures (data not shown). The pH dependence of the arsenic methylating activity of the protein was examined over the pH range of 5 to 11 (Fig. 3*B*). Here, 2.5 μg of purified enzyme in 50 mM Na phosphate buffer containing 0.1 μM [^{73}As]iAs^{III}, 5 mM GSH, 1 mM DTT, and 5% (v/v) glycerol, pH 7.4, was diluted into 95 μl of buffer at the desired final pH. AdoMet concentration was 50 μM in the final reaction mixture. Substantial activity was found between pH 7.5 and 10 with peak activity at pH 9.5.

Peptide and cDNA Sequencing—Mass spectrometry of the purified protein provide 15 unique peptide sequences (Table II). BLASTP analysis found a high degree of identity between these peptides and the predicted sequences of two proteins (NCB accession number NP065602 and NP05733). These proteins of mouse and human origin, respectively, have been provisionally identified as methyltransferases and designated as Cyt19. Using peptide sequence data, a set of degenerate oligonucleotide primers was designed for PCR and subsequent sequencing of the products (Table III). Based on initial sequence results, additional oligonucleotide primers were synthesized to complete the sequencing of the full-length cDNA. The sequence of the full-length cDNA for *S*-adenosyl-L-methionine:arsenic(III) methyltransferase and its conceptual translation are shown in Table IV. The predicted protein contains 369 amino acids and has a calculated molecular mass of 41,056.

Expression of S-Adenosyl-L-methionine:Arsenic(III) Methyltransferase mRNA—RT-PCR analysis detected *S*-adenosyl-L-methionine:arsenic(III) methyltransferase mRNA in rat heart, adrenal, urinary bladder, brain, kidney, lung, and liver (Fig. 4*A*). In HepG2 cells, a human hepatoma cell line, this mRNA was detected after 35 or 45 cycles of amplification. However, in UROtsa cells, an immortalized cell line derived from normal human urothelium, *S*-adenosyl-L-methionine:arsenic(III) methyltransferase mRNA was not detected after 45 cycles of amplification (Fig. 4*B*). Notably, both sets of forward and reverse primers amplified mRNA in rat tissues and in HepG2 cells and failed to amplify mRNA in UROtsa cells. The results shown for rat tissues and HepG2 cells are for the primer sets that gave maximal amplification.

DISCUSSION

The methylation of arsenicals is a common feature of the metabolism of this metalloid in organisms ranging in complexity from microorganisms to humans (35–37). Although reservations have been expressed concerning the use of the rat in studies of the systemic disposition of arsenic (38), both rat liver cytosol and primary rat hepatocytes have been shown to be efficient methylators of arsenic (33, 34, 39). Hence, we used rat liver as the starting material for the purification of the enzyme that methylates arsenic. A novel enzyme has been purified from rat liver cytosol that catalyzes the formation of methylated arsenic and dimethylated arsenic using [^{73}As]iAs^{III} as the initial substrate. The high yield of activity in the purification scheme may be due to removal of endogenous inhibitors or alteration of the enzyme:DTT ratio. Others have noted the presence of endogenous inhibitors for arsenite and methylarsonous acid methyltransferases in cytosol prepared from the livers of rabbits or marmosets (10, 40). The pattern of appearance of the methylated arsenicals suggests that the initial reaction forms methylated arsenic from arsenite; a second reaction then forms dimethylated arsenic from methylated arsenic. Because the apparent first-order rate constant for the dimethylation reaction is much larger than that for methylation, dimethylated arsenic will be the predominant metabolite in the reaction mixture. An uncharacterized aspect of the reaction scheme catalyzed by *S*-adenosyl-L-methionine:arsenic(III) methyltransferase is the mode of reduction of pentavalent arsenic in methylarsonic acid, the product of the first oxidative methylation reaction, to trivalency. The formation of methylarsonous acid in the reaction mixture used in the present studies may depend on the presence of DTT. Zakharyan and associates

TABLE IV
Nucleotide sequence and conceptual translation of rat liver S-adenosyl-L-methionine:arsenic(III) methyltransferase

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                                                  Met Ala Ala Pro Arg Asp
 1  AGAGGGAATCCCTGGTTCTGGAAGTGGAGATCGTGAGTC ATG GCT GCT CCC CGA GAC

    Ala Glu Ile His Lys Asp Val Gln Asn Tyr Tyr Gly Asn Val Leu Lys
58  GCA GAG ATC CAC AAG GAC GTT CAG AAC TAC TAT GGG AAT GTA CTG AAG

    Thr Ser Ala Asp Leu Gln Thr Asn Ala Cys Val Thr Pro Ala Lys Gly
107 ACA TCT GCA GAC CTC CAG ACT AAT GCT TGT GTC ACC CCA GCC AAG GGG

    Val Pro Glu Tyr Ile Arg Lys Ser Leu Gln Asn Val His Glu Glu Val
156 GTC CCT GAG TAC ATC CGG AAA AGT CTG CAG AAT GTA CAT GAA GAA GTT

    Ile Ser Arg Tyr Tyr Gly Cys Gly Leu Val Val Pro Glu His Leu Glu
205 ATT TCC AGG TAT TAT GGC TGC GGT CTG GTG GTG CCT GAG CAT CTG GAA

    Asn Cys Arg Ile Leu Asp Leu Gly Ser Gly Ser Gly Arg Asp Cys Tyr
253 AAC TGC CGG ATT TTG GAT CTG GGC AGT GGG AGT GGC AGA GAT TGC TAT

    Val Leu Ser Gln Leu Val Gly Gln Cys Gly His Ile Thr Gly Ile Asp
302 GTG CTT AGC CAG CTG GTC GGC CAG AAG GGA CAC ATC ACC GGG ATA GAC

    Met Thr Lys Val Gln Val Glu Val Ala Lys Ala Tyr Leu Glu Tyr His
351 ATG ACT AAG GTC CAG GTG GAA GTG GCT AAG GCC TAT CTT GAG TAC CAC

    Thr Glu Lys Phe Gly Phe Gln Thr Pro Asn Val Thr Phe Leu His Gly
399 ACG GAA AAG TTC GGT TTC CAG ACA CCC AAT GTG ACT TTT CTT CAC GGC

    Gln Ile Glu Met Leu Ala Glu Ala Gly Ile Glr Lys Glu Ser Tyr Asp
448 CAA ATT GAG ATG TTG GCA GAG GCC GGG ATC CAG AAG GAG AGC TAT GAT

    Ile Val Ile Ser Asn Cys Val Ile Asn Leu Val Pro Asp Lys Gln Lys
497 ATC GTT ATA TCC AAT TGT GTG ATC AAC CTT GTT CCC GAC AAA CAA AAA

    Val Leu Arg Glu Val Tyr Gln Val Leu Lys Tyr Gly Gly Glu Leu Tyr
546 GTC CTT CGG GAG GTC TAC CAA GTC CTG AAG TAC GGC GGG GAG CTC TAT

    Phe Ser Asp Val Tyr Ala Ser Leu Glu Val Ser Glu Asp Ile Lys Ser
595 TTC AGT GAC GTC TAT GCT AGC CTT GAA GTG TCA GAA GAC ATC AAG TCA

    His Lys Val Leu Trp Gly Glu Cys Leu Gly Gly Ala Leu Tyr Trp Lys
644 CAC AAG GTT TTA TGG GGG GAA TGC CTG GGT GGC GCT CTG TAC TGG AAG

    Asp Leu Ala Val Ile Ala Lys Lys Ile Gly Phe Cys Pro Pro Arg Leu
693 GAC CTC GCC GTC ATT GCC AAA AAG ATT GGG TTC TGC CCT CCA CGT TTG

    Val Thr Ala Asn Ile Ile Thr Val Gly Asn Lys Glu Leu Glu Arg Val
741 GTC ACT GCC AAT ATT ATT ACG GTT GGA AAC AAG GAA CTA GAA AGG GTT

    Leu Gly Asp Cys Arg Phe Val Ser Ala Thr Phe Arg Leu Phe Lys Leu
790 CTT GGT GAC TGT CGC TTC GTG TCT GCC ACA TTT CGC CTC TTC AAA CTC

    Pro Lys Thr Glu Pro Ala Gly Arg Cys Gln Val Val Tyr Asn Gly Gly
839 CCT AAG ACA GAG CCA GCC GGA AGA TGC CAA GTT GTT TAC AAT GGA GGA

    Ile Met Gly His Glu Lys Glu Leu Ile Phe Asp Ala Asn Phe Thr Phe
888 ATC ATG GGG CAC GAA AAG GAA CTA ATT TTC GAT GCA AAT TTT ACA TTC

    Lys Glu Gly Glu Ala Val Glu Val Asp Glu Glu Thr Ala Ala Ile Leu
937 AAG GAA GGT GAA GCT GTT GAA GTG GAT GAG GAG ACG GCA GCC ATC TTG

    Arg Asn Ser Arg Phe Ala his Asp Phe Leu Phe Thr Pro Val Glu Ala
986 AGG AAC TCT CGG TTT GCT CAC GAT TTT CTC TTC ACA CCT GTT GAG GCC

     Ser Leu Leu Ala Pro Gln Thr Lys Val Ile Ile Arg Asp Pro Phe Lys
1035 TCC CTG TTG GCT CCC CAA ACA AAG GTT ATA ATC AGA GAT CCA TTC AAG

     Leu Ala Glu Glu Ser Asp Lys Met Lys Pro Arg Cys Ala Pro Glu Gly
1084 CTT GCA GAG GAA TCT GAC AAG ATG AAG CCG AGA TGT GCA CCA GAA GGC

     Thr Gly Gly Cys Cys Gly Gly Arg Lys Ser Cys Ter
1151 ACT GGA GGC TGC TGT GGC AAA AGG AAA AGC TGC TAG ACCTAGGGCCAGCGT

1203 AGAGCCCACC GAGCATGAGG GGGTGGCTAA AGGGCAGTCA CAAAGTCTTC TGAGCCTGCT
1264 CTTCACCAGA GCACAGACTA TGGGAAGATG GCAAAGCCAC TGCTAAGAAA AAGTGATTTT
1325 TAGAGGGTGT TAATTTAAGG TTCACAGCAA ACTTTTTCTA TATTTCAGAG TTCTGGCGCC
1386 ACCTAGTGGT CAGAAGTAGA ACTTGTACGT CTAAGGTTTA CAAATATCTT CCTTCTGGCC
1447 TACCACAGGA CACCTCTGGG TTTTTCTCTG TGGTTATTCA GGAAGCACAG TACTTACTGA
1508 ATTTATGCTG ACTATGACAA AAGGTTGCCA ACTCAAATTT GGTAGGAGTA CTCTTTAGGT
1569 CGCTGTCTTC AAACTTTTTT CCCTGTAAAT GGAAATAAAT GAAAACAAAT GAAAAATAAA
1629 TTTGACTTCA TCCTGATAAA AAAAAAAAAA AAAAAAAAAA A
```

(11) reported that DTT strongly stimulated the methylation of methylarsonous acid by the enzyme purified from rabbit liver. Omission of DTT from reaction mixtures that contain rat liver *S*-adenosyl-L-methionine:arsenic(III) methyltransferase sharply reduces the conversion of arsenite to methylated products.[2] In cells, this reduction may depend on pentavalent arsenic reductase (7, 8) or on other endogenous reductants. Kinetic analysis indicated a high affinity of the enzyme for $MAs^{III}O$ ($K_m \sim 250$ nM). Concentrations of $MAs^{III}O$ greater than 5 μM inhibited the production of dimethylated arsenic. This finding is consistent with earlier results showing that high concentrations of $MAs^{III}O$ inhibited the formation of dimethyl arsenic in cultured cells (20). AdoMet served as the methyl group donor for both reactions. These results are consistent with evidence that trivalent arsenicals are the preferred substrate for this enzyme and that the pentavalent arsenicals must be reduced enzymatically or nonenzymatically before methylation (7, 11, 41). Based on estimated molecular weights, the protein purified from rat liver cytosol (~42.000) is probably not identical with the enzyme purified from rabbit liver cytosol (~60,000) that catalyzes the methylation of arsenite and methylarsonous acid (10, 11). *S*-Adenosyl-L-methionine:arsenic(III) methyltransferase from rat liver differs kinetically from arsenic methyltransferases purified from rabbit or hamster liver cytosol. For those enzymes, estimated K_m values for the conversion of MAs^{III} are orders of magnitude higher than the K_m determined for the rat liver enzyme. Further comparison of the arsenic methyltransferases awaits the availability of data on the sequence of the protein purified from rabbit or hamster liver and on additional kinetic characterization of the enzymes. Unique arsenic methyltransferases may be expressed in the tissues of different species or multiple enzymes capable of catalyzing this reaction may be expressed in tissues. *S*-Adenosyl-L-methionine:arsenic(III) methyltransferase is probably not identical with previously described proteins that catalyze methylation of selenium. Among these proteins, the best characterized is thioether *S*-methyltransferase (EC 2.1.1.96) which catalyzes the AdoMet-dependent formation of trimethylselenonium ion from dimethyl selenide (42). However, the smaller molecular mass of this enzyme (29.5 kDa) and its dissimilar amino acid sequence (43) indicate a lack of identity with *S*-adenosyl-L-methionine:arsenic(III) methyltransferase. Hsieh and Ganther (44) described two activities from rat liver and kidney that catalyze the synthesis of dimethyl selenide, a protein from the cytosolic fraction with a molecular mass of about 30 kDa, and an activity in the microsomal fraction that catalyzes this reaction. The protein purified in the present work is not likely to be identical with these proteins.

S-Adenosyl-L-methionine:arsenic(III) methyltransferase mRNA was detected in many rat tissues. Studies in *in vitro* systems containing cytosolic fractions prepared from rat liver or kidney have shown that arsenite is methylated in both tissues (33, 34, 45). Arsenic methylating activity has also been detected in cytosolic fractions prepared from mouse liver, kidney, lung, and testes (46). Both methylated and dimethylated arsenicals have been detected in livers and kidneys of arsenite-treated rats (47). Hence, the occurrence of *S*-adenosyl-L-methionine:arsenic (III) methyltransferase mRNA in rat tissues is consistent with evidence for the methylation of arsenic in these tissues. HepG2 cells expressed fairly high levels of *S*-adenosyl-L-methionine: arsenic(III) methyltransferase mRNA. These cells are efficient methylators of arsenite, containing both methylated and dimethylated products (18). In contrast, UROtsa cells which do not express this mRNA do not methylate arsenic (20). Thus, in this immortalized human cell line, absence of expression of *S*-

[2] S. Lin and D. J. Thomas, unpublished observations.

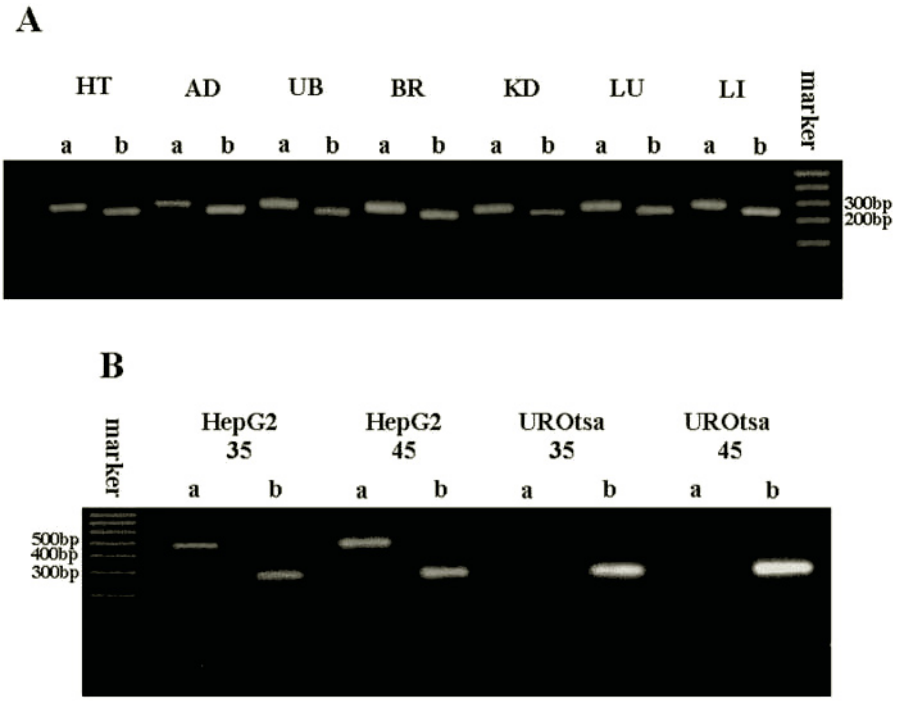

FIG. 4. **Detection of *S*-adenosylmethionine:arsenic(III) methyltransferase mRNA in tissues and cells by RT-PCR.** *A*, expression of *S*-adenosylmethionine:arsenic(III) methyltransferase mRNA in rat tissues. For *S*-adenosyl-L-methionine:arsenic(III) methyltransferase mRNA, the primers were 5′-TCTGCCCTCCACGTTTGGTCACT-3′ (mtf4F/rat, forward) and 5′-CTGCCGTCTCCTCATCCACTT-3′ (mtf2R/rat, reverse); the predicted product size was 250 bp. The primers for β-actin mRNA were 5′-TCATGAAGTGTGACGTTGACATCCGT-3′ (forward) and 5′-CCTAGAAGCATTTGCGGTGCACGATG-3′ (reverse). The predicted product size was 288 bp. PCR conditions were denaturation (30 s at 95 °C), annealing (30 s at 60 °C), and extension (30 s at 72 °C) for 35 cycles. For each tissue the PCR product for β-actin is shown in *Lane a* and the PCR product for *S*-adenosyl-L-methionine:arsenic(III) methyltransferase is shown in *Lane b*. *HT*, heart; *AD*, adrenal gland; *UB*, urinary bladder; *BR*, brain; *KD*, kidney; *LU*, lungs; and *LI*, liver. ϕX174/*Hae*III DNA size markers are shown in the *last lane*. *B*, expression of mRNA for *S*-adenosylmethionine:arsenic(III) methyltransferase in human cell lines. For *S*-adenosyl-L-methionine:arsenic(III) methyltransferase in HepG2 cells and UROtsa cells, the primers were 5′-ATTTTGGATCTGGGCAGTGGGAGT-3′ (mtf3F/rat, forward) and 5′-AGTGACCAAACGTGGAGGGCAGA-3′ (mtf3R/rat, reverse); the predicted product size was 477 bp. The primers for β-actin mRNA and predicted product size as in *A*. For the amplification of mRNA from HepG2 cells or UROtsa cells, a touchdown PCR protocol with either 35 or 45 cycles of amplification was used. Conditions for denaturation (30 s at 95 °C) and extension (30 s at 72 °C) were constant for either number of cycles. For 35 or 45 cycles of amplification, the annealing time was always 30 s; however, the annealing temperature of 65 °C decreased 0.5 °C per cycle for the first 20 cycles. The annealing temperature was then held at 55 °C for the final 15 or 25 cycles of amplification. The RT-PCR product for *S*-adenosyl-L-methionine:arsenic(III) methyltransferase is shown in *Lane a* and the RT-PCR product for β-actin is shown in *Lane b*. ϕX174/*Hae*III DNA size markers are shown in the *first lane*.

adenosyl-L-methionine:arsenic(III) methyltransferase mRNA is associated with a failure to produce methylated arsenicals. The factors that underlie *S*-adenosyl-L-methionine:arsenic(III) methyltransferase mRNA gene silencing in UROtsa cells have not been determined.

Alignment of the mRNA sequences of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase and human Cyt19 suggested a close relation between these gene products (Table V). The most striking difference in amino acid sequences of human Cyt19 (calculated molecular mass of 37,969 Da) and of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase (calculated molecular mass of 41,056 Da) was the N-terminal 31-amino acid sequence that was found only in the rat protein. This sequence contained 4 cysteine residues that may be critically involved in the binding of As^{III}. The overlapping amino acid sequences of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase and of human Cyt19 contained several motifs that have been found in other methyltransferases (48). Motif I with a consensus sequence of (V/I/L)(L/V)(D/E)(V/I)G(G/C)G(T/P)G most closely matched residues 74 to 82 (ILDLGSGSG) in predicted sequences of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase and human Cyt19. These sequences conform to the general consensus for motif I (hh(D/E)hG*X*G*X*G), where h is a hydrophobic amino acid, *X* is any amino acid, and the position of glycine residues is strongly conserved (49). Motif I has been associated with the binding of AdoMet by the enzyme and mutation of the conserved glycine residues in motif I in guanidinoacetate methyltransferase abolished its catalytic activity (50). An acidic residue which contributes to the binding of AdoMet has been found between 17 and 19 residues C-terminal to the end of motif I (48, 50). In *S*-adenosyl-L-methionine:arsenic(III) methyltransferase and human Cyt19, aspartate was found 20 residues C-terminal to the end of motif I. As in other methyltransferases, this aspartate residue was preceded by a hydrophobic amino acid, isoleucine. Motif II with the consensus sequence (P/G)(Q/T)(F/Y/A)DA(I/V/Y)(F/I)(C/V/L) has been found in other methyltransferases. A sequence containing FDA was found at residues 286 to 293 (LIFDANFT) in *S*-adenosyl-L-methionine:arsenic(III) methyltransferase; in human Cyt19, the cognate sequence was LMFDANFT. As in motif II sequences from other methyltransferases, these sequences in *S*-adenosyl-L-methionine:arsenic(III) methyltransferase and in human Cyt19 included aromatic amino acid residues in close proximity to an invariant aspartate residue. Notably, motif II in *S*-adenosyl-L-methionine:arsenic(III) methyltransferase and in human Cyt19 was 203 residues C-terminal to motif I. In other methyltransferases, the start of motif II is 36 to 90 residues C-terminal to motif I. The consensus sequence for motif III (LL(R/K)PGG(R/I/L)(L/I)(L/F/I/V)(I/L)) was identified in neither *S*-adenosyl-L-methionine:arsenic(III) methyltransferase nor in human Cyt19. Human Cyt19 could be an *S*-adenosyl-L-methionine-dependent arsenic(III) methyltransferase.

Genetically determined differences among individuals in the

TABLE V

Alignment of amino acid sequences of rat liver S-adenosyl-L-methionine:arsenic(III) methyltransferase and human Cyt19

Positions for common methyltransferase motif I (I) and motif II (II) and for invariant aspartate residue indicated by horizontal line. The protein sequence for human Cyt19 is from *Homo sapiens* Cyt19 (NCBI accession number AF226730).

```
                        1                                                  50
Methyltransferase - rat MAAPRDAEIH KDVQNYYGNV LKTSADLQTN ACVTPAKGVP EYIRKSLQNV
          Cyt19 - human MAALRDAEIQ KDVQTYYGQV LKRSADLQTN GCVTTARPVP KHIREALQNV

                        51                      I                          100
Methyltransferase - rat HEEVISRYYG CGLVVPEHLE NCRILDLGSG SGRDCYVLSQ LVGQKGHITG
          Cyt19 - human HEEVALRYYG CGLVIPEHLE NCWILDLGSG SGRDCYVLSQ LVGEKGHVTG

                        101                                                150
Methyltransferase - rat IDMTKVQVEV AKAYLEYHTE KFGFQTPNVT FLHGQIEMLA EAGIQKESYD
          Cyt19 - human IDMTKGQVEV AEKYLDYHME KYGFQASNVT FFHGNIEKLA EAGIKNESHD

                        151                                                200
Methyltransferase - rat IVISNCVINL VPDKQKVLRE VYQVLKYGGE LYFSDVYASL EVSEDIKSHK
          Cyt19 - human IVVSNCVINL VPDKQQVLQE AYRVLKHGGE LYFSDVYTSL ELPEEIRTHK

                        201                                                250
Methyltransferase - rat VLWGECLGGA LYWKDLAVIA KKIGFCPPRL VTANIITVGN KELERVLGDC
          Cyt19 - human VLWGECLGGA LYWKELAVLA QKIGFCPPRL VTANLITIQN KELERVIGDC

                        251                                   II           300
Methyltransferase - rat RFVSATFRLF KLPKTEPAGR CQVVYNGGIM GHEKELIFDA NFTFKEGEAV
          Cyt19 - human RFVSATFRLF KHSKTGPTKR CQVIYNGGIT GHEKELMFDA NFTFKEGEIV
                        301                                                350
Methyltransferase - rat EVDEETAAIL RNSRFAHDFL FTPVEASLLA PQTKVIIRDP FKLAEESDKM
           Cyt19 - human EVDEETAAIL KNSRFAQDFL IRPIGEKLPT SGAVLLWS 338

                        351
Methyltransferase - rat KPRCAPEGTG  GCCGKRKSC 369
```

capacity to methylate inorganic arsenic to methylated and dimethylated products could be important determinants of the risk associated with exposure to this metalloid. Epidemiological studies suggest an association between the pattern of arsenic-containing metabolites in urine and the occurrence of arsenic-induced skin lesions (51–53). In particular, a higher percentage of methylated arsenic in urine has been found in individuals with arsenic-induced skin lesions, including cancer, than in individuals without arsenic-induced lesions. Considerable interindividual variation has been found in the amounts of methylated and dimethylated arsenic excreted in urine (54). This variation could reflect polymorphisms in *S*-adenosyl-L-methionine:arsenic(III) methyltransferase that affect the rate of conversion of methylated arsenic to dimethylated arsenic. However, differences in the urinary excretion of these metabolites may also reflect differences among individuals in the systemic retention of methylated arsenicals. Studies of the rate and extent of methylation of arsenite in cultured primary human hepatocytes have also found severalfold variation among donors in the fraction of inorganic arsenic that is methylated (39). These results are consistent with a polymorphism in the rate of arsenic methylation in cultured human cells. Vahter (55) has suggested that genotypic differences in the activities of the enzyme that methylates arsenic or in enzymes involved in the recycling of homocysteine used in the synthesis of AdoMet could be the sources of variability among individuals in the urinary output of methylated arsenicals. To this list could be added genotypic differences in the activity of the reductases that convert arsenate and methylarsonic acid to arsenite and methylarsonous acid (7, 8). Additional studies of polymorphisms of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase will be required to determine the contribution of this factor to differences among individuals in metabolism, retention, and clearance of arsenic. By comparison, genetic polymorphisms of other methyltransferases have been linked to specific phenotypic changes. Single nucleotide polymorphisms in the open reading frame of the gene encoding thiopurine methyltransferase or polymorphic variable tandem repeat number in the 5′-flanking region of this gene have been linked to altered enzyme activity and differences in response to chemotherapeutics (56). An analogous polymorphism of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase in humans could affect the rate of formation and disappearance of methylated arsenicals in tissues. Given the unique toxicities of methylated arsenicals, especially those that contain As^{III} (57), genotypic variation in the activity of *S*-adenosyl-L-methionine:arsenic(III) methyltransferase might affect the consequences of chronic exposure to inorganic arsenic.

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