Direct Analysis of Methylated Trivalent Arsenicals in Mouse Liver by Hydride Generation-Cryotrapping-Atomic Absorption Spectrometry

Jenna M. Currier,[†] Milan Svoboda, [§,‡,§] Diogo P. de Moraes[§,~], Tomáš Matoušek,[§] Jiří Dědina, [§] and Miroslav Stýblo[,‖]*

Department of Nutrition and Curriculum in Toxicology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

styblo@med.unc.edu

SUPPORTING INFORMATION

Experimental Methods

Arsenicals

Sodium arsenite (iAs^{III}) and sodium arsenate (iAs^{V}), (>99% pure) were purchased from Sigma-Aldrich (St. Louis, MO). A 1000 μg/L As AAS standard solution was purchased from Merck (Darmstadt, Germany). Because sodium arsenite is commonly contaminated with arsenate, arsenic trioxide (As2O3; >99.9% pure from Sigma-Aldrich) was used as the trivalent inorganic As specie for validation of the oxidation state specific analyses. Methylarsonic acid ($MAs^{V)}$, disodium salt ($CH_3As^{V}O(ONa)_2$) and dimethylarsinic acid ($DMAs^{V)}$ both better than 98% pure were purchased from Chem Service (West Chester, PA). The methylated trivalent arsenicals, oxomethylarsine ($MAs^{III}O$) and iododimethylarsine ($DMAs^{III}I$), were custom synthesized by Dr. William Cullen (University of British Columbia, Vancouver, Canada). In aqueous solutions, $MAs^{III}O$ and $DMAs^{III}I$ hydrolyze to form the methylarsonous (MAs^{III}) and dimethylarsinous ($DMAs^{III}$) anions, respectively.

Speciation Analysis of As

We used a semi-automated HG-CT-AAS system similar to that described in our previous reports *(1, 2)*. AAnalyst 800 spectrometer (Perkin-Elmer, Norwalk, CT, USA) equipped with a multiple microflame quartz tube atomizer (multiatomizer) and coupled to a cryotrap was controlled by FIAS 400 flow injection accessory (Perklin-Elmer). Using this system, iAs and the methylated arsenicals in samples are reduced to the corresponding arsines (arsine, methylarsine, and dimethylarsine) in a reaction mixture containing sodium borohydride ($NaBH_4$; Sigma-Aldrich, St. Louis, MO) and a 0.75M Tris(hydroxymethyl)aminomethane (TRIS)-HCl buffer (pH 6; Sigma-Aldrich). The arsines are cryotrapped and then separated by their boiling points prior to detection by the AA spectrometer. Here, two aliquots of each sample are analyzed to determine As^{III} species and As^{III+V} species. Trivalent arsenicals (iAs^{III}, MAs^{III} and $DMAs^{III}$) are analyzed directly without sample extraction or pretreatment. For analysis of total, tri- and pentavalent arsenicals (iAs^{III+V}, MAs^{III+V} and $DMAs^{III+V}$), the 2^{nd} sample aliquot is pretreated with 2% L-cysteine (biochemistry grade, EMD Chemicals, Inc., Gibbstown, NJ) for 1 hour at room temperature. L-cysteine reduces the pentavalent arsenicals to trivalency, allowing arsine generation from both As^{III} and As^{V} species. The concentrations of iAs^{V}, MAs^{V}, and $DMAs^{V}$ are then determined by subtracting the results of analysis in the 1^{st} sample aliquot from results of the analysis in the 2^{nd} aliquot.

Calibration curves for quantification of As species were generated using aqueous solutions of the pentavalent standards, iAs^{V}, MAs^{V}, and $DMAs^{V}$ treated with L-cysteine. To account for contamination, the As content in each of the standards was determined by graphite furnace-AAS *(1)*. We have shown that the slopes of these curves are identical with slopes of curves generated from solutions of the trivalent arsenicals in the absence of cysteine *(2)*. There are no standard reference materials available with certified levels of MAs^{III} and $DMAs^{III}$. We have routinely used custom synthesized MAs^{III} and $DMAs^{III}$ standards ($MAs^{III}O$ and $DMAs^{III}I$) for quality control, including calibration (when needed) or the standard addition techniques with relevant biological matrices.

Animals

12 week-old C57BL/6 mice were obtained from Jackson Laboratory (Bar Harbor, ME, USA) and housed in polycarbonate cages (2/cage) with corn cob bedding at the University of North Carolina Animal Facility (12 h light/dark cycle, 22 ± 1 °C and humidity of 50 ± 10%), accredited by the American Association for Accreditation of Laboratory Animal Care. Mice were allowed free access to food (Lab Diet 5058, Nutrition International, Brentwood, MO) and pure deionized water (DIW) or DIW containing iAs^{III} (50 mg As/l).

Tissues

Freshly dissected mouse livers were collected from unexposed (control) mice and from mice exposed to iAs^{III} in drinking water for 9 days. Liver homogenates were prepared in ice cold DIW (10% w/v). Liver homogenates from control mice were used for calibration and to prepare homogenates spiked with As standards. Liver homogenates from mice exposed to iAs^{III} were used for the method validation. Here, the As^{III} species were analyzed directly in freshly prepared homogenates; As^{III+V} species were analyzed in homogenates pretreated with 2% cysteine. To determine % recovery of As during these analyses, aliquots of the homogenates were digested using a MARS Microwave Reaction System equipped with MarsXpress Temperature Control (CAM, Matthews, NC). Briefly, 100 μL of 10% homogenate was digested in 3 mL of 2 M ultrapure phosphoric acid (EMD Chemicals, Inc.) for 10 hours at 90 ± 4°C. After digestion, 1 mL of each digestate was neutralized with 0.25 mL of 10 N NaOH to a final pH between 6 and 7. Since this digestion oxidizes all trivalent arsenicals to their pentavalent counterparts, the neutralized digestates were treated with 2% L-cysteine by the addition of 20% L-cysteine and incubated at room temperature for 1 hour prior to analysis.

Statistical Analyses

All statistical analyses were performed using GraphPad Instat software package (GraphPad Software Inc., San Diego, CA). Linear regression and correlation analyses were employed to determine characteristic of calibration curves of As^V species in liver homogenates and DIW. ANOVA followed by Bonferroni's multiple comparison posttest was employed to determine differences in As recovery between DIW and homogenate solutions spiked with As standards. Statistical significance was considered at the level of $p < 0.05$.

TABLES

Table S1. Characteristics of the calibration curves for As^{V} standards spiked into DIW or 10% liver homogenate (a)

Matrices	As-Standard	Linear regression (b)	Correlation coefficient
DIW	iAs^{V}	0.821x - 0.019	0.999
	MAs^{V}	0.825x - 0.024	0.999
	$DMAs^{V}$	0.835x - 0.024	0.999
homogenate	iAs^{V}	0.873x + 0.00004	0.999
	MAs^{V}	0.880x - 0.005	0.998
	$DMAs^{V}$	0.941x - 0.027	0.997

(a) Arsines and methyl substituted arsines were generated after 1-hour pretreatment with 2% L-cysteine.

(b) The linear regression for each As^{V} standard was determined over the range of 0.125 to 4 ng As/mL.

Table S2. The instrumental and tissue detection limits for analysis of As^{III} and As^{III+V} species in mouse liver homogenate.

Detection Limit	iAs^{III}	MAs^{III}	$DMAs^{III}$	iAs^{III+V}	MAs^{III+V}	$DMAs^{III+V}$
Instrumental (pg As) (a)	14	13	9	10	10	12
Tissue (ng As/g tissue) (b)	6	5	4	4	4	5

(a) The instrumental detection limits were calculated from the AAS spectra generated for blanks (control liver homogenates, n=8) as 3(SD/slope) for the absorbance areas with the retention times corresponding to arsine, methylarsine, and dimethylarsine signals.

(b) Tissue detection limits were calculated from the instrumental detection limits and reflect the concentration and dilution of the liver homogenates used for the analysis.

References

(1) Matousek, T., Hernandez-Zavala, A., Svoboda, M., Langrova, L., Adair, B. M., Drobna, Z., Thomas, D. J., Styblo, M., and Dedina, J. (2008) Oxidation State Specific Generation of Arsines from Methylated Arsenicals Based on L- Cysteine Treatment in Buffered Media for Speciation Analysis by Hydride Generation - Automated Cryotrapping - Gas Chromatography-Atomic Absorption Spectrometry with the Multiatomizer. *Spectrochim. Acta Part B At. Spectrosc. 63,* 396-406.

(2) Hernandez-Zavala, A., Matousek, T., Drobna, Z., Paul, D. S., Walton, F., Adair, B. M., Dedina, J., Thomas, D. J., and Styblo, M. (2008) Speciation Analysis of Arsenic in Biological Matrices by Automated Hydride Generation-Cryotrapping-Atomic Absorption Spectrometry with Multiple Microflame Quartz Tube Atomizer (Multiatomizer). *J. Anal. At. Spectrom. 23,* 342-351.