The rate of oxidation of dimethylarsinous acid to dimethylarsinic acid is pH dependent: implications for the analysis and toxicology of arsenic metabolites in urine

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A ^{1}H NMR spectroscopic study has shown the rate oxidation of dimethylarsinous acid to dimethylarsinic acid in buffered aqueous solutions to depend upon pH. Dimethylarsinous acid has been reported to be a highly toxic arsenical metabolite and component of the urine of persons exposed to inorganic arsenic, particularly through drinking water. As the pH of human urine can range from 4.5 to 8, the pH dependence of the oxidation rate of dimethylarsinous acid to dimethylarsinic acid has profound implications for the detection and analysis of these compounds in urine samples, and for the relevance of the experimental toxicology of dimethylarsinous acid.

Keywords*:* dimethylarsinous acid; oxidation; dimethylarsinic acid; ^{1}H NMR spectroscopy; kinetics

The effects of inorganic arsenic in drinking water represent one of the most serious current and continuing problems for human health on a world-wide basis.[1,2] Much effort has focused on the metabolites of inorganic arsenic in the urine of exposed persons both as biomarkers of exposure[3] and to provide possible toxicological insights.[4] In recent years several studies have reported that dimethylarsinous acid is a significant metabolite in the urine of persons exposed to inorganic arsenic through drinking water.[4-7] Indeed, in the study of Valenzuela *et al.*[7] dimethylarsinous acid was present in almost all samples and was the major arsenical metabolite constituting about 50% of the total arsenic. It has long been accepted that dimethylarsinic acid (the analogue of dimethylarsinous acid containing pentavalent arsenic) is a metabolite of inorganic arsenic that is found in the urine of exposed persons.[3] The reported presence of dimethylarsinous acid in urine has very much increased concerns about the safety of human exposure to inorganic arsenic because it has been shown that dimethylarsinous acid presents a far greater health hazard than does dimethylarsinic acid.[8,9] However, Francesconi and Kuehnelt[10] reviewing the above reports of the occurrence of dimethylarsinous acid in urine, found the results unconvincing. They suggested that confirmation with appropriate sample collection, storage and analytical preparation procedures was necessary. We report here that the rate of oxidation of dimethylarsinous acid to dimethylarsinic acid is pH dependent and the variation in rate is marked in the normal pH range of human urine. This finding has profound implications for the storage and analysis of urine samples and for the toxicology of dimethylarsinous acid.

For the reaction Me_2AsOH to $Me_2As(O)OH$ the rate constants were determined using the intergrated first-order rate law:

$$\ln[A]_t/[A]_0 = -kt$$

where $[A]_0$ is the initial concentration of dimethylarsinous acid, and $[A]_t$ is the concentration remaining after time t. Example plots of $\ln[A]_t$ versus time are shown in Figures 1a and b and confirmed that the reactions are first order; the gradients of the plots provided the rate constants (Table 1 and Figs 2 and 3). The half lives for the reaction, calculated from $t_{1/2} = 0.693/k$, at the range of pH values studied are given in Table 1 and Fig. 4. The values in Table 1 supply precise experimental values for rate constant and half life, whereas Figs 2 and 4 make it obvious that the rate constant is still increasingly as the pH approaches 3 (Fig. 2), and that the half life is still increasingly rapidly as the pH rises through 6 (Fig. 4).

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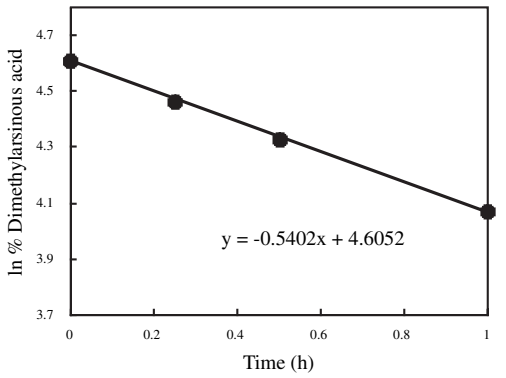

Fig 1a

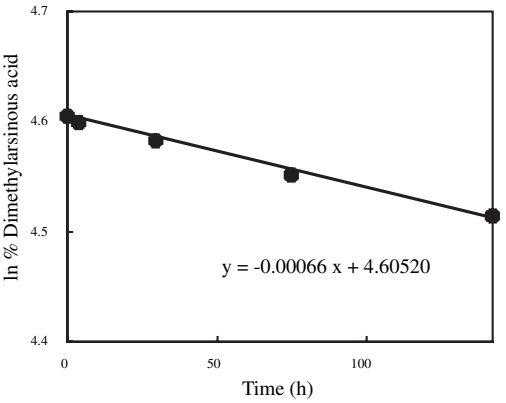

Fig 1b

Fig. 1 The oxidation of dimethylarsinous to dimethylarsinic acid. Natural logarithm of percentage dimethylarsinous acid remaining *versus* time at (a) pH 3.6, and (b) pH 6.0.

Table 1 Rate constants and half-lives for the oxidation of dimethylarsinous acid to dimethylarsinic acid at pH values from 3.2 to 6.0

pH	Rate constant k (h^{-1})	Half life (h)
3.2	0.98	0.71
3.6	0.54	1.28
4	0.25	2.77
4.4	0.078	8.88
4.8	0.013	53.3
5.2	0.0044	158
5.6	0.0021	330
6	0.00066	1,050

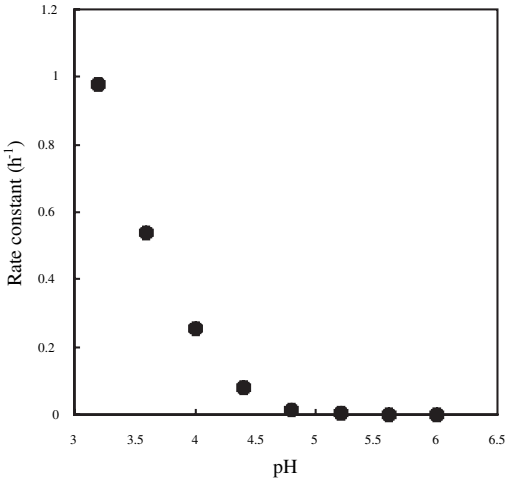

Fig. 2 The oxidation of dimethylarsinous to dimethylarsinic acid. Rate constant *k versus* pH.

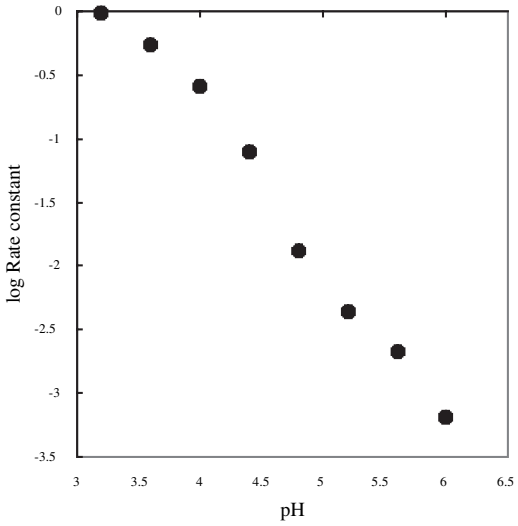

Fig. 3 The oxidation of dimethylarsinous to dimethylarsinic acid. Log 10 rate constant *k versus* pH.

At a pH of 3.2 and a temperature of 24.3 °C the half life of dimethylarsinous acid in aqueous solution is only 0.71 h. At pH 6.0 the half life is 1,050 h (44 days). Measurement of the rate of oxidation at higher pH values, *i.e.*, at slower rates of oxidation, was not practicable by the NMR spectroscopic method employed here. From pH 6.0 down to pH 3.2 the rate of oxidation rapidly increased (Fig. 4). The normal range of pH values for human urine is about 4.5 to 8.[13] At the lower end of that range (pH 4.5) the half life of dimethylarsinous acid in aqueous solution is only about 13 h. It is evident that results of analysis of dimethylarsinous acid in urine are likely to be unreliable if the pH is not controlled, and indeed the natural variation in urine pH would in any case make results difficult to interpret. The conditions of storage of urine samples prior to analysis would also have to be strictly controlled to yield reliable analytical results for dimethylarsinous acid; indeed consistently reliable measurements might not be possible. We suggest that previous studies that have apparently determined dimethylarsinous acid in urine samples that had been stored for many months,[1,3] or measured the stability of dimethylarsinous acid in urine without adequate control of pH,[14] should be critically re-examined in the light of work reported here.

Recently, Suzuki *et al.*[15] and Hansen *et al.*[16] have raised the possibility of confusion between dimethylarsinous acid and sulfur-containing arsenical metabolites in human urine.

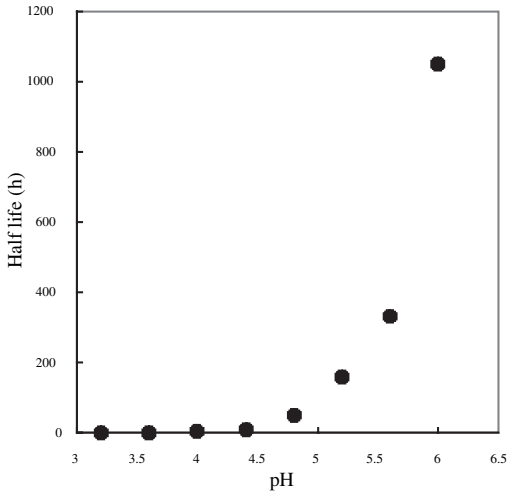

Fig. 4 The oxidation of dimethylarsinous to dimethylarsinic acid. Half-life for the reaction *versus* pH.

Our study reinforces the need for confirmation of the reported widespread presence of dimethylarsinous acid in urine samples and, until such confirmation is forthcoming, the relevance of toxicological studies[8,9] that have demonstrated the adverse effects of dimethylasinous acid should be questioned.

Experimental

Preparation of buffer solutions

Citrate/phosphate buffer solutions were made according to the formulas listed by Gomori.[11] Acetone-d_6 was obtained from Merck, Darmstadt, Germany. All other chemicals, including D_2O, were supplied by Wako Pure Chemicals, Osaka, Japan. The same quantities of reagents (weights of chemicals, volumes of solvent) were used for making buffers in D_2O as if they were being made in water. No corrections were made for isotope effects. Buffers solutions (0.1 M) in D_2O with nominal pH values of 3.2, 3.6, 4.0, 4.4, 4.8, 5.2, 5.6 and 6.0 were prepared.

Preparation of dimethylarsinous acid.

Dimethylarsinous acid was generated *in situ* in the buffer solutions by the hydrolysis of iododimethylarsine, which was prepared by the method of Burrows and Turner.[12]

NMR Spectrocopy

^{1}H NMR spectra were recorded in D_2O with a JEOL ECA 800 NMR spectrometer operating at 800 MHz. Chemical shifts were recorded as δ values relative to external Me_4Si.

Experimental procedures

A portion of iododimethylarsine (10 µl) was dissolved in acetone-d_6 (0.75 ml) to make a stock solution, which was was kept in a desiccator at 4 °C. It was used to make all dimethylarsinous solutions and was completely stable for the duration of the experiment (2 months) as shown by NMR spectroscopy.

An aliquot of the stock solution (5 µl) was added to a portion of buffer solution (1 ml) to make a solution 0.63 mM in dimethylarsinous acid. This was done for all buffers (pH values 3.2 to 6.0). The hydrolysis of iododimethylarsine in aqueous solution that produces dimethylarsinous acid also, of course, yields hydroiodic acid, which will lower the pH of the solution if it is not adequately buffered. Maintenance of the pH values used in the study would have required higher concentrations of buffer than were suitable for NMR measurement if we had used a higher concentration of dimethylarsinous acid. However, a much lower concentration would have resulted in very long times to accumulate the NMR spectra. Nevertheless, the concentration used in our study was very much higher than those found in contaminated urine (by a factor of approximately 1,000). This will, however, have made no difference to the significance of the results as the rate of oxidation was not dependent upon the concentration of dimethylarsinous acid in the total range that we were considering. Oxygen gas was bubbled through the solution in the NMR tube and the space in the tube above the solution

was filled with dioxygen before the tube was capped. ^{1}H NMR spectra were then recorded for all samples at time intervals appropriate to the rate of reaction. Between recording spectra, dioxygen was bubbled through each solution and the tube was again filled with dioxygen. In this way we ensured that the dioxygen concentration was always in large excess and did not, in any case, limit the rate of oxidation. If an aqueous solution of dimethylarsinous acid in an NMR tube was purged with dinitrogen no oxidation occurred, but oxidation resumed if dioxygen was readmitted to the tube. For each spectrum, the integration of the resonance of the protons of the methyl groups of dimethylarsinous acid (δ=1.33) and that of the protons of the methyl groups of dimethylarsinic acid, the product of the oxidation of dimethylarsinous acid, were recorded. The ratio of these two peaks was a measure of the extent of the oxidation at the time of recording the spectrum. The chemical shift of the the dimethylarsinic acid methyl protons varied with pH (δ=2.00 at pH 3.2 to δ 1.81 at pH 6.0) whereas that of dimethylarsinous acid remained unchanged at δ 1.33. Presumably this reflects the fact that dimethylarsinous acid remains undissociated at pH values of 6 or less. Solutions were maintained at all times at 24.3 $\pm$ 0.1 °C.

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References

1 K. Breslin, *Environ. Health Perspect*., 2000, **108**, A224.
2 M.N. Mead, *Environ. Health Perspect*., 2005, **113**, A378.
3 Environmental Health Criteria 224. *Arsenic and Arsenic compounds*, 2nd edn; World Health Organization: Geneva; 2001.
4 X.C. Le, X. Lu, M. Ma, W.R. Cullen, H.V. Aposhian, H.V. and B. Zheng, *Analyt. Chem*., 2000**, 72**, 5172.
5 L.M. Del Razo, M. Styblo, W.R. Cullen and D.J. Thomas, *Appl. Pharmacol.,* 2001, **174**, 282.
6 B.K. Mandal, Y. Ogra and K.T. Suzuki, *Chem. Res. Toxicol*., 2001**, 14**, 371.
7 O.L. Valenzuela, V.H. Borja-Aburto, G.G. Garcia-Vargas, M.B. Cruz-Gonzalez, E.A. Garcia-Montalvo, E.S. Calderon-Aranda and L.M. Del Razo, *Environ. Health. Perspect*., 2005, **113**, 250.
8 M.J. Mass, A. Tennant, B.C. Roop, W.R. Cullen, M. Styblo, D.J. Thomas and A.D. Kligerman, *Chem. Res. Toxicol*., 2001, **14**, 355.
9 S. Nesnow, B.C. Roop, G. Lambert, M. Kadiiska, R.P. Mason, W.R. Cullen and M.J. Mass, *Chem. Res. Toxicol*., 2002, **15**, 1627.
10 K.A. Francesconi and D. Kuehnelt, *Analyst,* 2004, **129**, 373.
11 G. Gomori, *Meth. Enzymol.,* 1955, **1**, 138.
12 G.J. Burrows and E.E. Turner, *J. Chem. Soc*., 1920, **117**, 1373.
13 A. White, P Handler and E.L. Smith, *Principles of Biochemistry*, 5th edn; McGraw-Hill, Inc.: New York; 1973.
14 Z. Gong, X. Lu, W.R. Cullen, and C Le, *J. Anal. At. Spectrom.,* 2001, **16**, 1409.
15 K.T. Suzuki, B.K. Mandal, A. Katagiri, Y. Sakuma, A. Kawakami, Y. Ogra, K. Yamaguchi, Y. Sei, K. Yamanaka, K. Anzai, M. Ohmichi, H. Takayama and N. Aimi, *Chem. Res. Toxicol*., 2004, **17**, 914.
16 H.R. Hansen, A. Raab, M. Jaspars, B.F. Milne and J. Feldmann, *Chem. Res. Toxicol*., 2004, **17**, 1086.