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Detection of Arsenobetaine in Human Blood

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Arsenobetaine was detected and quantified unambiguously in human plasma, serum and red blood cells by the combination of HPLC with ICP MS. Three different column conditions, i.e. two ion-pair chromatographies for anionic (LC-1) and cationic (LC-2) compounds and gel-permeation chromatography (LC-3), were employed to confirm the assignment. Arsenobetaine was detected in every sample as a major component of the water-soluble arsenic compounds, with an increasing concentration in plasma < serum < blood cell fractions. It was the sole detectable arsenic compound in LC-1 and LC-2, while a broad peak corresponding to high-molecular-weight compounds was identified in addition to arsenobetaine in LC-3.

Keywords: HPLC–ICP MS, arsenic speciation, arsenobetaine, human blood, plasma, serum

INTRODUCTION

Arsenic is contained in marine organisms at the $\mu g\, g^{-1}$ or a higher level in wet tissue and much attention has been paid to its chemical form, its metabolic fate, and its cycling through the food web and the environment, mainly from a toxicological standpoint. Arsenic is notorious as a toxic element, but is also reported to be essential for some animals, and the mechanisms of its essentiality have attracted attention too. The analytical method for its speciation (i.e. the determination of its chemical form and quantity) of arsenic is essential for environmental and toxicological studies as well as studies on the possible essentiality of the element.

Among more than 20 arsenic compounds identified in the marine environment,[1–3] arsenobetaine $[(CH_3)_3As^+CH_2COO^-]$ has attracted much attention. Arsenobetaine is a ubiquitous and dominant compound in marine animals such as fish, crustaceans and molluscs, and is easily absorbed in the human body and excreted in urine, apparently without metabolic conversion.[4] The occurrence of arsenobetaine in blood, especially after ingestion of fish, shrimp etc., was expected from previous studies,[5–7] but, to our knowledge, an unambiguous indication of its presence in human blood has not yet been obtained. Here we report the identification and quantification of arsenobetaine in human plasma, serum and red blood cells by the HPLC–ICP MS method developed in our laboratory.[2,8,9]

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EXPERIMENTAL

Fifteen water-soluble arsenic compound standards were prepared as reported previously.[8] HPLC–ICP MS conditions used in the present experiment are summarized in Table 1.[8,9] Human plasma, serum and red blood cells lysate samples were prepared (according to the standard protocol) from blood sampled from the cubital vein of healthy male volunteers. An aliquot (typically 5–10 µl for LC-1 and LC-2, and 25–50 µl for LC-3) of the sample was injected into the HPLC–ICP MS and the signals at $m/z = 75$ (corresponding to $^{75}As^+$) were monitored. Interference from chloride (at $m/z = 75$ from the molecular ion $^{40}Ar^{35}Cl^+$) was assigned by the simultaneous appearance of a smaller (1/3) peak at $m/z = 77$ (corresponding to $^{40}Ar^{37}Cl^+$).[28] Quantification was performed by comparison of the peak area with that of a standard injection containing a known amount of dimethylarsinate ($100\ ng\ As\ cm^{-3}$).

RESULTS AND DISCUSSION

The chromatograms of a human serum sample for three different chromatographic conditions are shown in Fig. 1. A small peak at 4.1 min (desig-

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Table 1 HPLC–ICP MS conditions

HPLC system		
System	Model 410 Bio LC System (Perkin–Elmer)	
Column conditions		
LC-1	Inertsil ODS (4.6 mm × 250 mm; GL Science Co., Japan) 10 mM tetraethylammonium hydroxide–4 mM malonic acid–0.05% methanol (pH 6.8 adjusted by HNO_3)	
LC-2	Inertsil ODS 10 mM 1-butane sulphonic acid sodium salt–4 mM tetramethylammonium hydroxide–0.05% methanol (pH 3.0 adjusted by HNO_3)	
LC-3	Asahipak GS220 (7.6 mm × 500 mm; Showa Denko, Japan) 25 mM tetramethylammonium hydroxide–25 mM malonic acid (pH 6.8 adjusted by ammonium hydroxide)	
ICP MS		
System	PMS 2000 (Yokogawa Analytical Systems, Japan)	
Condition	Ar Nebulizer	0.8 L min^{-1}
	Aux.	1.0 L min^{-1}
	Plasma	14 L min^{-1}
	Forward power	1.2 kW

nated AB in the figure) in LC-1 showed the same retention time as the authentic arsenobetaine, and coinjection of the sample with arsenobetaine confirmed this assignment (data not shown). A peak corresponding to arsenobetaine was also detected in LC-2 and LC-3, though separation of

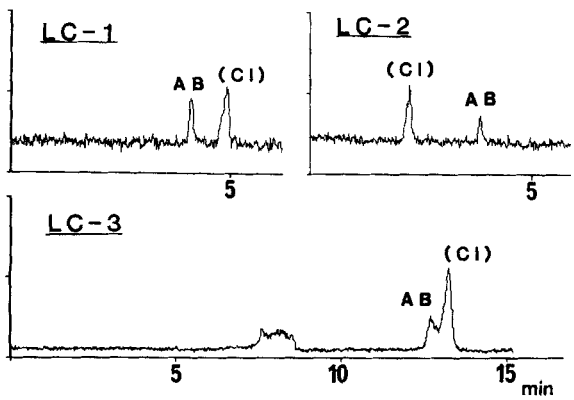

Figure 1 Chromatograms of a serum sample on three different column conditions.

Table 2 Contents of arsenobetaine in human blood (ng As cm^{-3})

Volunteer	1	2	3
Sex	Male	Male	Male
Age	29	31	39
AB in plasma	3.3	—[a]	0.9
AB in serum	4.6	1.6	1.7
AB in cell lysate	10.1	—[a]	5.7

Quantified based on the chromatograms on LC-1

[a] Not determined because of partial clotting during the pretreatment procedure.

the arsenobetaine peak from chloride interference (Cl) was not satisfactory under the latter conditions. The amount of arsenobetaine in each fraction for three male volunteers is calculated based on the chromatograms of LC-1, and is summarized in Table 2. The detection limit of arsenobetaine in LC-1 was calculated to be around 0.3 ng cm^{-3} based on the 3σ of the noise level (5 µl injection). No peak corresponding to other arsenic compounds including arsenate, arsenite, methanearsonate and dimethylarsinate was detected by in LC-1 or LC-2 in the present study. The detection limits of these inorganic and simple methylated arsenic compounds were similar to that of arsenobetaine, and the peaks corresponding to them were detected when a sample and the authentic standards were coinjected.

An interesting aspect of the distribution of arsenobetaine is inferred from the data in Table 2, i.e. that the plasma concentration of arsenobetaine is considerably lower than the concentration in red blood cells in every sample. In addition, the serum concentration is higher than the plasma concentration in two cases (the third one was discarded because of partial clotting), suggesting that a reasonable amount of arsenobetaine may be released from some components of the cells during the coagulation process. Vahter *et al.* reported the distribution of ^{73}As after i.v. administration of synthetic arsenobetaine to mice.[5] In their report, arsenic levels in both plasma and red blood cells were highest after one hour and then decreased, though the rate of decrease was slower in blood cells than in plasma. Yamauchi and Yamamura administered fish and synthetic arsenobetaine orally to human volunteers[6] and hamsters,[10] respectively, and analysed the resultant distribution in blood. They also reported a slower decrease for trimethylated arsenic (i.e.

compounds evolving trimethylarsine by reduction after alkaline digestion) levels in red blood cells compared with its plasma concentration. In the present experiment, all the three volunteers ate a fish meal about 12 h before sampling, and the present data may reflect the difference of the clearance rate of arsenobetaine in plasma and red blood cell fractions.

While no arsenic peak other than arsenobetaine was detected under LC-1 and LC-2 conditions, a broad peak was found in the chromatogram of each sample upon gel-permeation chromatography (LC-3) in a void volume fraction of the column. The chemical nature of the arsenic in this fraction is not clear at this stage. Inorganic and dimethylated arsenic up to the ng cm^{-3} level were reported in Japanese human blood,[7] and these levels, if present, can be detected in the present study. The characterization of this high-molecular-weight arsenic is now under way.

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