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Possible key intermediates in arsenic biochemistry: Synthesis and identification by liquid chromatography electrospray ionization mass spectrometry and high resolution mass spectrometry

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ABSTRACT

Arsenic is a type 1 carcinogen and its toxicity is critically dependent on chemical speciation. However, after decades of research, the biogenesis of at least fifty naturally occurring arsenic species is still not well understood. Here, based on experimental work, it is proposed a set of pathways for the formation of multiple arsenic species that might help to clarify the present situation.

These are focused on the thiol protein arsenic bond and on its interaction with reactive metabolites. In fact, arsenic bound to glutathione interacting with sulfur adenosyl methionine (SAM), MethylCB$_{12}$ and AdoCB$_{12}$, forms a number of complexes that might be key intermediates in arsenic biochemistry. These include dimethylarsino glutathione (DMAG) *m/z* 412 $[M+H]^+$, synthesized non-enzymatically from glutathione and cacodylate. Trimethylarsonio glutathione (TMAG) *m/z* 426 $[M]^+$ synthesized from DMA, GSH and SAM, apparently by a classical Challenger methylcarbonium attack. Tetramethyl arsonium ion *m/z* 135 $[M]^+$ is formed in a third step, apparently by carbanion methylation. The presence of trimethylarsine oxide (TMAO) *m/z* 137 $[M+H]^+$ is attributed to the hydrolysis of TMAG or TMA, or to carbanion methylation of dimethylarsinoyl glutathione (*m/z* 428 $[M]^+$) formed from cacodylate and GSH. Cantoni type attacks of DMAG on SAM were unsuccessful, eventually due to competition of the trivalent S^+ atom of SAM for the As^{III} atom attack. The presence of dimethylarsonio diglutathione (DMADG *m/z* 717 $[M]^+$), is suggested to result from a GS^- attack on dimethylarsenoyl glutathione (*m/z* 428 $[M+H]^+$). The presence of dimethylarsenoyladenosine (*m/z* 372 $[M+H]^+$), trimethylarsenosugar adenine (*m/z* 370 $[M]^+$), and dimethylthioarsenosugar adenine (*m/z* 388 $[M+H]^+$), is explained by the synthesis of the pecursor dimethylarsonio-adenosine glutathione DMAAG (*m/z* 661 $[M]^+$), a likely source of oxo-and trimethylated arsenosugars, as well as of thio-arsenosugars by the cleavage of its S-C bond. The results gathered suggest that cell vacuoles might play a major role in arsenic metabolism, and that the dominance of oxo-As sugars, in algae extracts, may be supported by a mechanism of synthesis independent of DMAAG (*m/z* 661).

Abbreviations: AB, Arsenobetaine or trimethyl(carboxymethyl) arsonium *m/z* 179; AB2, Trimethylarsonio propionate (TMAP) or trimethyl(2-carboxyethyl) arsonium *m/z* 193; AB3, Trimethylarsonio butyrate or trimethyl(3-carboxypropyl) arsonium *m/z* 207; AC, Arsenocholine or trimethyl (2-hydroxyethyl)arsonium *m/z* 165; AdoCB$_{12}$, Adenosyl cobalamin *m/z* 1580; ATG, Tri(glutamyl–cysteinyl–glycinyl)trithio-arsenite *m/z* 994; Cyt19, S-adenosylmethionine:arsenic (III) methyltransferase; DMA, Dimethylarsinic acid or cacodylate *m/z* 139; DMAA, Dimethylarsinoyl acetic acid *m/z* 181; DMAE, Dimethylarsinoyl ethanol or dimethyloxarsyl ethanol *m/z* 167; DMAP, Dimethylarsinoyl propionate *m/z* 195; DMAAG, Dimethylarsonio–adenosine glutathione complex (*m/z* 661) or adenosine 5′(glutathione-kS)dimethylarsenic(1+) (complex 6); DMAG, Dimethylarsino glutathione or L-glutamyl–cysteinyl–glycinyl) dimethyl-thio-arsinite *m/z* 412 (complex 2); DMADG, Dimethylarsonio diglutathione complex (*m/z* 717) or bis(glutathione-kS)dimethylarsenic(1+) (complex 5); DMAOA, Dimethylarsinoyl adenosine *m/z* 372; DMAOG, Dimethyarsinoyl glutathione complex or (glutathione-kS)hydroxidodimethylarsenic(1+) (*m/z* 428), (complex 4); DMATG, Dimethylarsinothioyl glutathione *m/z* 444; DMARF, Dimethylarsinoyl ribofuranosone, or 5-dimethylarsinoyl-β-ribofuranosone m/z 239; DMTAA, Dimethylthioarsenosugar adenine or Dimethylarsinthioyl adenosine *m/z* 388; FTICR–ESI-MS, Fourier Transform Ion Cyclotron Resonance Electrospray Ionization Mass Spectrometry; GSH, Glutathione *m/z* 308; HPLC–ESI-MS^n, High Performance Liquid Chromatography Electrospray Ionization Multiple Mass Spectrometry; MADG, Di(glutamyl–cysteinyl–glycinyl) methyl-dithio-arsonite m/z; MetCB$_{12}$, Methyl-cobalamin *m/z* 1344; MMA, Monomethylarsonate *m/z* 141; PCn, Phytochelatin (n = 1 to 10); SAM, Sulfur-adenosyl-L-methionine, *m/z* 399; S-DMAE, Thio-Dimethylarsinoyl ethanol *m/z* 183; S-DMAA, Thio-Dimethylarsinoyl acetic acid *m/z* 197; S-DMAP, Thio-Dimethylarsinoyl propionate *m/z* 211; TMA, Tetramethylarsonium ion *m/z* 135; TMAG, Trimethylarsonio glutathione or Glutathione Trimethylarsonium cation complex *m/z* 426, (complex 3); TMAO, Trimethylarsine oxide *m/z* 137; TMAS, Trimethylarsine sulfide *m/z* 153; TMASA, Trimethylarsenosugar adenine *m/z* 370; TMAS-OH, Trimethylarsonio-ribofuranosol or 3-(5′-deoxy-5′-(trimethylarsonium)-β-ribofuranosol *m/z* 253.

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They also offer an explanation for the reason why arsenobetaine, and tetramethylarsonium are loosely bound to biotic tissues. Four arsenic species new to science, to the best of our knowledge, and a number of known arsenic compounds were synthesized in this work, identified by HPLC–ESI-MS^n and FTICR–ESI-MS, and suggestions regarding their mechanisms of synthesis were advanced. These results provide a framework for arsenic biochemistry which may explain the origin of a significant part of arsenic known metabolites.

1. Introduction

Inorganic arsenic in water supplies represents a serious risk of cancer [1,2], diabetes [3], heart failure [1] and endocrine disruption [4]. Light methylated arsenic species and thioarsenicals are carcinogenic to Man [5,6]. Gallium arsenide and arsenocholine have serious effects upon the hematopoietic, kidney and immunitary systems [7,8]. On the other hand some arsenicals show carcinostatic properties against promyelocytic leukemia, targeting genetic lesions [9], and inducing apoptosis [10]. This clearly shows that arsenic is a paradigmatic case of toxicity dependence on speciation.

Understanding arsenic metabolism is therefore not only an important scientific issue but also a matter of great public health concern. And arsenic speciation, following the pioneer identification of arsenobetaine in rock lobsters [11] became one of the most active fields in environmental analytical chemistry [12,13]. Since then, at least fifty different arsenic species have been identified in nature [11,22], their number increasing permanently.

Yet, despite the proposal of a varied number of concurrent mechanisms [14–22], we are still far from understanding the biogenesis of the majority of organoarsenicals [11,22]. Therefore, there is an obvious deficit of a comprehensive and unifying theoretical framework that investigated step by step could unravel the most complex aspects of the issue. The present work aims at giving a contribution to such a framework.

Recent research [22–30], has refocused on the key role of sulfur and of the thiol groups of proteins in arsenic metabolism. However, the S–As^{III} bonds of phytochelatins–As^{III} complexes, presumed to be induced in plants by arsenic stress [31], were found to be unstable, easily giving off arsenite during extraction or chromatographic separation.[23,25,31,32]. Such instability apparently explains their difficult detection in biotic tissues. The GSH analogs of phytochelatins–As^{III} complexes, ATG, MADG and DMAG were also found to be unstable [23,29,33].

The report that these complexes are labile, is somewhat puzzling as it is known the strong affinity of arsenic for thiol groups [14,34,35] , and that protein bound arsenic, can be rather difficult to extract [36,37].

On the other hand, that observation raises the possibility that most or all of the arsenite identified in plant extracts may originate from As^{III}–sulfur compounds identified *in panta* by XAS and XANES [38–40]. Moreover the lability of PC-As^{III} complexes is apparently enhanced by oxygenated and basic conditions, these complexes being better preserved in an acidic, cold and reduced environment [23,25]. At least part of the arsenite and arsenate that appear as end products or as side effects of sample processing and analysis, might not be present as such in the parent plant material, being rather an artifact of these processes [39].

On the other hand, the electronic structure of PC_2As^{III} and GS_n–As^{III} complexes still permits the methylation of the thiol bound arsenic atom. The fact is that $(PC_2)As^{III}$–CH_3 and MADG complexes have been identified in plant and animal materials [32,41], and shall therefore be naturally formed in one way or another.

1.1. The theory on trial

The set of hypothesis under investigation here stems from the assumption that the electronic structure of monomethylated proteinthiol-As^{III} complexes permits a second methyl transfer *in vivo*, leading to dimethylation of the arsenic atom and the formation of a key dimethylated arsenic complex (protein–$As(CH_3)_2$) (Fig. 1).

It is also assumed that further methylation of this intermediate dimethylated arsenic complex is possible up to a 3rd and 4th methyl group (assumption 2).

A third assumption is that the As^{III} atom of key dimethylated intermediate arsenic complexes (e.g. GS-$As(CH_3)_2$), can trigger nucleophilic attacks on the methylene carbon atoms of sulfur-adenosyl-L-methionine (SAM).

Further it is also assumed that intermediate dimethylated arsenic complex may react with adenosyl-cobalamin resulting in the transfer of the adenosine moiety to the arsenic atom (adenylation), forming a protein–dimethylarsonio-adenosine conjugate (assumption 4).

A fifth assumption is that a fraction of these protein–dimethylarsonio-adenosine conjugates, might be, under proper conditions, severed from the protein substrate (PCn, GSH or other) by the cleavage of the sulfur – carbon bond induced by a strong electron donor.

If the mechanisms proposed to occur in this set of assumptions are shown to be viable, that would open a new field of possibilities in the area of arsenic biochemistry. In this work it is presented what, to our best knowledge, is the first experimental evidence supporting the

Table 1
Synthesis, mass-to-charge ratio, formula, ESI-MS^2 main fragments and retention time of compounds and complexes.

Compounds and complexes	Synt	*m/z*	Formula	ESI-MS^2 main fragments	RT (min)
Glutathione (GSH)		308	$C_{10}H_{16}N_3O_6S$	MS^2, 290, 272, 233, 179,162, 144, and 116	8
Sulfur adenosyl methionine (SAM)	A, B, andC	399	$C_{15}H_{23}N_6O_5S$	MS^2 298, 264, 250, 163 and 136	3.5
Tetramethylarsonium ion (TMA)	C	135	$C_4H_{12}As$	MS^2……120, 117, and 105	4–5
Trimethylarsine oxide (TMAO)	C	137	$C_3H_{10}OAs$	MS^2, 122, 119, 117, 107, 105, 103, and 91	5
Dimethylarsino glutathione (DMAG)	A	412	$C_{12}H_{23}N_3O_6SAs$	MS^2 394,337, 283, 266, and 155	42
Trimethylarsonio glutathione (TMAG)	B	426	$C_{13}H_{25}N_3O_6SAs$	MS^2 408, 337, 297, and 280	41
^{34}TMAG	B	428	$C_{13}H_{25}N_3O_6^{34}SAs$	MS^2, 410, 339,299, and 282	41
Dimethylarsinoyl glutathione (DMAOG)	B	428	$C_{12}H_{23}$ N_3O_7S As	MS^2, 382, 353, 299, 285, 268, and 155	36
Dimethylarsonio diglutathione (DMADG)	A	717	$C_{22}H_{38}N_6O_{12}S_2As$	MS^2 699, 642, 588, 459, 412, 361, and 355	38
Dimethylarsinoyl adenosine (DMAOA)	D	372	$C_{12}H_{19}N_5O_4As^+$	MS^2, 250,237, 178,177, 160, and 145	5
Dimethylthioarsenosugar adenine (DMTAA)	D	388	$C_{12}H_{19}N_5O_3SAs^+$	MS^2 253, 250, 235, and 136	21
Trimethylarsenosugar adenine(TMASA)	E	370	$C_{13}H_{21}N_5O_3As^+$	MS^2 324, 302, 280, 250, 235, 175, and 120	5
Dimethylarsonio-adenosine glutathione (DMAAG)	D,E	661	$C_{22}H_{34}N_8O_9SAs^+$	MS^2 634,526, 456, 388, 356,306, 253, and 250	7–8
Dimethylarsinothioyl glutathione (DMATG)	A	444	$C_{12}H_{23}N_3O_6S_2As^+$	MS^2 346, 315, 306, 231, 212, and 177	30