Detoxification system for inorganic arsenic: transformation of As_2O_3 into TMAO by vitamin B_{12} derivatives and conversion of TMAO into arsenobetaine†

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A new two-step synthetic pathway developed for the transformation of arsenic trioxide [iAs(III); As_2O_3] into arsenobetaine (AB; $Me_3As^+CH_2CO_2^-$) involves treatment of iAs(III) with native B_{12} or biomimetic B_{12} in the presence of glutathione (GSH) to give TMAO with a high selectivity and a high conversion rate; subsequent treatment of TMAO with iodoacetic acid in the presence of GSH gives arsenobetaine.

Inorganic arsenics (iAs) are widely used in non-ferrous metal refining, the glass industry, and in the production of arsenic agricultural chemicals, wood preservatives, defoliants, and arsenic semiconductors. However, chronic poisoning by arsenicals has occurred through large-scale environmental pollution,[1] and acute poisoning has occurred through the use of these compounds as a means of suicide or homicide.[2] Causal associations between the use of inorganic arsenics and occurrences of chronic poisoning, lung cancer, skin cancer, and bladder cancer have been demonstrated.[3] With regard to arsenic trichloride, a raw material for metallic arsenic, an ingredient of Group III/Group V compound semiconductors (GaAs), new research has been reported on the synthesis of chelate compounds through control of their oxidation states.[4] Although this research shares with our research a common goal of synthesizing new arsenic compounds, our research differs in that our principal goal is to synthesize arsenic compounds that are nontoxic or which have a level of toxicity that is as low as possible. The toxicity of arsenic compounds is markedly dependent on their chemical structure, and some methylated arsenic compounds have lower toxicities than those of inorganic arsenic compounds. In particular, arsenobetaine [AB; $Me_3As^+CH_2CO_2^-$; (trimethylarsonio)acetate] has a low toxicity (LD_{50}, 10 g kg^{-1}, mouse, oral)[5] and is found in high levels in fishery products.[5] The acute toxicity of AB, as determined from animal experiments, is approximately one three-hundredth of that of arsenic trioxide [iAs(III); arsenite; As_2O_3:LD_{50}, 0.03 g kg^{-1}, mouse, oral].[5] Moreover, as a result of studies on the metabolism and excretion of AB in humans and animals,[6] it is now widely accepted that AB is chemically stable, has a low tissue affinity, and is rapidly excreted from the human body. Although AB is produced by the action of marine organisms, there have been no successful attempts to synthesize AB artificially in a safe and cost-effective way.

The conversion of highly toxic inorganic arsenic compounds into nontoxic AB (in other words, the elimination of toxicity) is defined as "detoxification". The detoxification process should be safe and cost-effective. In marine ecosystems, inorganic arsenicals are biologically methylated, resulting in accumulation, through the food chain, of arsenic in the form of AB mainly in fish and shellfish.[7] It has been hypothesized that this biological methylation is promoted by alternating reduction of As(V) to As(III) and oxidative methylation.[8] These biochemical reactions involve a methyltransferase and a reductase, respectively.[9] Although many enzymes have been assumed to be involved in the conversion of inorganic arsenicals into AB, no enzyme has been isolated that is directly associated with the synthesis of AB.[10] Although chemical methods have been proposed for converting arsenic trioxide into trimethylated arsenic by treatment with alkylaluminium or Grignard reagents, these methods are both difficult and hazardous to apply.[11] Because the former is a reagent that can only be used under anhydrous conditions and the latter is inactivated by moisture, they can only be used for small-scale syntheses, so the current chemical syntheses of methylated arsenics may have no contribution to make in reducing future health problems from exposure to arsenicals or in establishing countermeasures to existing health problems. In addition, these reactions are unlikely to be useful in safe treatment of inorganic arsenicals in the industrial sphere.

Recently, a scientific technology called "biomimetics" has been developed that involves the application of various biochemical reactions that are highly efficient and occur without the production of undesirable by-products.[12] The biochemical methylation of arsenic involves a methyltransferase and a reductase. The establishment of a biomimetic system using this reaction could enable artificial conversion of arsenic trioxide into AB in a highly efficient and environmentally friendly manner.

Transmethylation is among the enzymatic reactions in which vitamin B_{12} is involved. In this study, a system was

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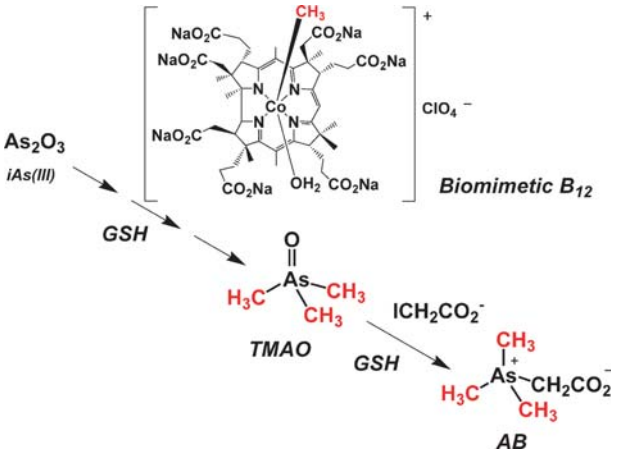

Scheme 1 Detoxification system for inorganic arsenic.

constructed that consists of "biomimetic vitamin B_{12}" [sodium (methyl)(aquo)cobyrinate perchlorate],‡ which mimics naturally occurring methylated vitamin B_{12} (methylcobalamin), together with a B_{12} coenzyme; these serve as a model for methyltransferase (Scheme 1), whereas the reduced form of glutathione (GSH) serves as a reductase model. We found that this system effected a highly efficient conversion of arsenic trioxide into trimethylarsine oxide (TMAO; $Me_3As{=}O$) (Table 1).

When naturally occurring coenzyme B_{12} was used as a source of methyl groups, the absolute yields of the products were as follows: inorganic arsenics (iAs) 0%, methylarsonic acid [MMA; $MeAs(O)(OH)_2$] 0%, dimethylarsonic acid [DMA; $Me_2As(O)(OH)$] 0%, TMAO 97%, tetramethylarsonium (TeMA; Me_4As^+) 1% (Table 1). When the biomimetic coenzyme B_{12} was used as the source of methyl groups, the absolute yields of the products were as follows: iAs 0%, MMA 0%, DMA 0%, TMAO 99%, and TeMA 1% (Table 1). Therefore, the biomimetic coenzyme system produced the same level of selectivity in conversion as that of the naturally occurring coenzyme. There have been no previous reports on the selective conversion of arsenic trioxide into TMAO by a naturally occurring coenzyme with an efficiency as high as 97%, and no reports of any such conversion by using a biomimetic coenzyme.

We also found that AB was quantitatively generated from TMAO and haloacetic acid in the presence of GSH in a neutral aqueous solution under mild conditions (Table 2).

Table 1 Arsenic methylation mediated by methyl B_{12} derivatives[a]

		Absolute yield (%)[b]				
Entry	Methyl donor	iAs[c]	MMA	DMA	TMAO	TeMA
1	Native B_{12}	0	0	0	97	1
2	Biomimetic B_{12}	0	0	0	99	1

[a] Methylation reactions were carried out in Tris-HCl buffer (pH 8) at 100 °C for 2 h. Initial concentrations were as follows. Native B_{12} (methylcobalamin) or biomimetic B_{12}, sodium (methyl)(aquo)cobyrinate perchlorate, $[(CH_3)(H_2O)Cob(III)(CO_2Na)_7]^+ClO_4^-$: 7.4×10^{-2} M; GSH: 1.3 M; iAs: 5.4×10^{-5} M. [b] Products were assayed by HPLC-ICP-MS. [c] iAs includes arsenite [iAs(III)] and arsenate [iAs(V)].

Table 2 Arsenic carboxymethylation of trimethylarsine oxide into arsenobetaine[a]

	Absolute yield (%)[b]				
Carboxymethyl donor	iAs	MMA	DMA	TMAO	AB
ICH_2CO_2H	0	0	0	0	99

[a] Carboxymethylation reaction were carried out in 100 mM phosphoric acid–citric acid buffer (pH 5) at 37 °C for 2 h. Initial concentrations were as follows. TMAO: 2.0×10^{-7} M; GSH: 5.0×10^{-3} M; iodoacetic acid: 5.0×10^{-3} M. [b] Products were assayed by HPLC-ICP-MS.

TMAO (0.2 μM) was also quantitatively converted into AB (99%) in the presence of GSH (5 mM) and iodoacetic acid (5 mM) in 100 mM phosphoric acid–citric acid buffer (pH 5, 37 °C) (Table 2).

For "biomimetic B_{12}" (methylcobyric acid), there is a previous report of a synthesis of methylcobyric acid from monocyano monoaquo cobyrinate,[13] but no details of the experimental procedures are given. We first synthesized hydrophobic B_{12} [(methyl)(aquo)cobyrinate heptamethyl ester perchlorate] from naturally occurring vitamin B_{12} (cyanocobalamin). Seven of the methyl ester groups located near the corrin ring of the hydrophobic B_{12} were then hydrolyzed with sodium hydroxide to give "biomimetic B_{12}".‡ This is a new synthetic method. In addition, the previous report[13] states that the authors synthesized methylcobyric acid with the aim of using it as a standard substance for the identification of methylated corrinoids biosynthesized by microbial cells. They did not assess the reactivity of methylcobyric acid as a methyl donor. Our study is the first, therefore, to report on the reactivity of methylcobyric acid in a transmethylation reaction. It follows, therefore, that this is the first study to report on the methylation of arsenic by "biomimetic B_{12}".

Our literature searches have failed to find any reports of studies on the conversion of arsenic trioxide into TMAO in the presence of methylcobalamin. One paper[14] reports the conversion of arsenic trioxide into MMA in the presence of methylcobalamin; however, this does not give a description of TMAO synthesis. Another study[15] reports that both MMA as the main product and DMA as a by-product were obtained in the presence of methylcobalamin and a reducing agent; however, this does not give a description of the synthesis of TMAO. Likewise, a third study[16] reports that both MMA as the main product and DMA as a by-product were obtained; however, that does not report a confirmation of the synthesis of TMAO. On the basis of the above information, it could be deduced that methylation of arsenic trioxide in the presence of methylcobalamin proceeds only as far as the synthesis of MMA and DMA. Our report is the first to describe the conversion of arsenic trioxide into TMAO in the presence of methylcobalamin, with an absolute yield of 97%.

TMAO is an arsenic compound of low toxicity [LD_{50}, 10 600 mg kg^{-1}],[17] and it is known, from an oral and intraperitoneal administration study in hamsters, that under *in vivo* conditions TMAO is reduced to trimethylarsine (TMA), part of which is excreted in exhaled breath. TMA is also an arsenic compound of low toxicity (LD_{50}, 7870 mg kg^{-1}), but a study

in animals has shown that it may cause mild transient hemolysis.[18–19] On the other hand, it is known that AB is stable *in vivo*, has low affinity for body tissues, and is rapidly excreted.[6] Thus, it can be inferred that desirable detoxification of arsenic trioxide should proceed from conversion of arsenic trioxide into TMAO with subsequent conversion into AB.

There is a report on a method for synthesizing AB from TMA with halogenated acetate ester in an organic solvent to form an AB ester that is subsequently hydrolyzed to produce AB.[11] However, there is no report describing the synthesis of AB from TMAO in an aqueous solution under mild conditions; our report is the first to describe such a synthesis. The reaction does not require an organic solvent and it proceeds in aqueous solution under mild conditions, and it can therefore be regarded as an economical and eco-friendly process.

In summary, [i] we propose a new treatment system for toxicants, *i.e.* "detoxification of arsenic" in which arsenic trioxide is transformed into AB. [ii] To accomplish this detoxification (iAs(III) → AB), we designed a two-step synthesis process [iAs(III) → TMAO → AB] involving reactions under mild aqueous conditions. [iii] The first step in this two-step process is the trimethylation of arsenic trioxide, which is very difficult to accomplish selectively. In other words, the following six types of arsenic compound may be produced during methylation of arsenic trioxide: unchanged iAs(III), iAs(V), MMA, DMA, TMAO, and TeMA. We succeeded, for the first time, in selectively synthesizing TMAO from arsenic trioxide by using naturally occurring vitamin B_{12} (methylcobalamin). [iv] To reproduce, in our biomimetic system, the high selectivity shown by naturally occurring vitamin B_{12}, we successfully synthesized "biomimetic B_{12}" by using a new synthesis method. [v] By using this "biomimetic B_{12}", we succeeded, in selectively synthesizing TMAO exclusively from arsenic trioxide in a similar manner to the reaction of naturally occurring vitamin B_{12}; this is the first report of such a synthesis. [vi] The second reaction (TMAO → AB) in the two-step synthetic pathway is the first reported case in which TMAO has been directly converted into AB in an aqueous solution under mild conditions without the use of organic solvents or the use of highly volatile TMA as reactant. As described above, we propose a detoxification system for converting a toxicant (arsenic trioxide) into a nontoxic compound (AB) without the production of toxic by-products, and our experiments have demonstrated the feasibility of this process.

The vitamin B_{12} derivatives and the GSH that are used in the detoxification method developed in this study are safe and environmentally friendly materials. Therefore, our detoxification process is also safe and environmentally friendly. Our method for detoxification of inorganic arsenics could be applied in the prevention of poisoning by inorganic arsenical compounds that are present in well water, which has been a widespread cause of chronic poisoning by arsenic. In addition, the method could be used to detoxify surplus inorganic arsenicals in industry, which currently present considerable difficulties in terms of their safe disposal. Finally, a more-specific application would be in the detoxification of inorganic arsenicals generated in the process of producing chemical agents.[20]

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Notes and references

‡ Biomimetic B_{12} was prepared as follows. First, 4 M aqueous NaOH (20 μL) was added to a solution of hydrophobic vitamin B_{12} (5 mg) in methanol (10 μL).[21] The mixture was stirred in a screw-capped polypropylene tube and incubated in a thermostated bath at 30 °C for 20 h. The solution was then neutralized with 6 M aqueous HCl and diluted with ultra-pure water (10 μL).

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