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Arsenite release on enzymic transformation of arsonomethyl substrate analogues: a potentially lethal synthesis by glycerol-3-phosphate dehydrogenase

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The isosteric arsenical analogue of glycerol 3-phosphate, 3,4-dihydroxybutylarsonic acid, is a good substrate for rabbit muscle glycerol-3-phosphate dehydrogenase. Its oxidation is accompanied by release of arsenite. This release seems to be due to a spontaneous elimination of arsenite by 3-oxoalkylarsonic acids, as it is also observed in (1) the oxidation of 3-hydroxypropylarsonic acid by yeast alcohol dehydrogenase, (2) treatment of 3,4-dihydroxybutylarsonic acid with periodate and (3) non-enzymic transamination of the glutamate analogue 2-amino-4-arsonobutyric acid. Enzymic formation of 3-oxoalkylarsonic acids in cells can therefore be lethal, as arsenite is poisonous to most organisms because of its high affinity for dithiols such as dihydrolipoyl groups.

INTRODUCTION

Enzymes that act on phosphates R-O-PO_3^{2-} often also act on their arsonomethyl analogues R-CH_2-AsO_3^{2-}. If the catalysed reaction alkylates, acylates or phosphorylates the arsono group, the product hydrolyses. This is presumably because arsenic is a larger atom than phosphorus, so that a fifth ligand (water) may more readily enter the co-ordination shell of a four-co-ordinate arsenic atom, and one of the original four ligands can leave. The presence of R-CH_2-AsO_3H_2 can therefore endow such enzymes with an artificial hydrolytic activity.

This activity can create futile cycles, e.g. the arsonomethyl analogue of diphosphate gives RNA polymerase an exonuclease activity [1], the arsonomethyl analogue of AMP gives adenylate kinase an ATPase activity [2] and 2-aminoethylarsonic acid, a non-isosteric analogue of phosphoethanolamine, gives ethanolamine–phosphate cytidylyltransferase the ability to hydrolyse CTP to CMP and diphosphate [3]. We now describe a different way in which enzyme reactions can be diverted from their usual course when the natural substrate R-O-PO_3^{2-} is replaced by R-CH_2-AsO_3^{2-}.

First we report that the isosteric arsonomethyl analogue of glycerol 3-phosphate, namely 3,4-dihydroxybutylarsonic acid, is a good substrate for rabbit muscle glycerol-3-phosphate dehydrogenase, with kinetic constants similar to those of the natural substrate. As the analogue was prepared as a racemate we planned to separate the enantiomers by oxidizing the (*S*)-isomer with the enzyme glycerol-3-phosphate dehydrogenase to 4-hydroxy-3-oxobutylarsonic acid, isolating it from the unoxidized enantiomer, and then reducing it back to (*S*)-3,4-dihydroxybutylarsonic acid using the same enzyme.

We failed, however, to isolate the expected oxidation product. This failure was explained when we found that arsenite was released during the enzyme-catalysed oxidation. We also found that three other reactions, all of which similarly generate a 3-oxoalkylarsonic acid, likewise release arsenite. These were (1) oxidation of 3-hydroxypropylarsonic acid by alcohol dehydrogenase, (2) treatment of 3,4-dihydroxybutylarsonic acid with periodate and (3) non-enzymic transamination of 2-amino-4-arsonobutyric acid, an arsenical analogue of glutamic acid. It therefore appears that the presence of a carbonyl group at C-3 in any alkylarsonic acid results in arsenite release. The arsenite will poison most cells because of its high affinity for dithiols such as dihydrolipoyl groups. Hence arsonomethyl analogues, each fairly harmless by itself, may become toxic when cellular enzymes produce the 3-oxo group. Such toxicity will be selective if cells differ in their abilities to take up these compounds; after such uptake the lethal conversion is likely to occur.

MATERIALS AND METHODS

Materials

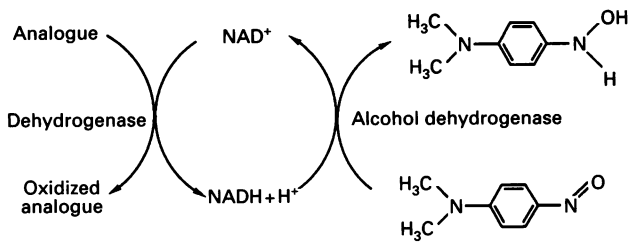
4-Bromobut-1-ene and *p*-nitroso-*N*,*N*-dimethylaniline were purchased from Aldrich Chemical Co. Arsenic(III) oxide and Amberlite CG-400 were purchased from BDH. Dowex 50W (X8) was from Aldrich Chemical Co. The enzymes *sn*-glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) from rabbit muscle and alcohol dehydrogenase (EC 1.1.1.1) from baker's yeast were purchased from Sigma Chemical Co. The coenzymes NAD^+ and NADH were purchased from Boehringer-Mannheim.

General methods

High-voltage paper electrophoresis was performed on Whatman 3MM paper cooled by immersion in white spirit. Samples and markers were spotted on paper and the paper wetted with buffer. The buffers used contained 10% (v/v) pyridine and 0.3% acetic acid for pH 6.5, 0.5% (v/v) pyridine and 5% (v/v) acetic acid for pH 3.5 and 2% (v/v) formic acid and 8% (v/v) acetic acid for pH 2.0. Runs were for 15–20 min at 5 kV (about 100 V/cm). The papers were dried and sprayed with a reagent containing $FeCl_3$ (10 g/l) and sulphosalicylic acid (10 g/l). Phosphates, phosphonates and arsonates were detected on paper by their ability to bind Fe^{3+} and thus prevent the formation of the purple complex with sulphosalicylic acid. These compounds therefore appear as white spots on a purple background [4].

Chemical syntheses

3,4-Dihydroxybutylarsonic acid, the arsonomethyl analogue of glycerol 3-phosphate, was synthesized [5, route B] by treating bis(diethylamino)chloroarsine, $(Et_2N)_2AsCl$, with but-3-enylmagnesium bromide, Br-Mg-CH_2-CH_2-CH=CH_2 [6] to obtain $(Et_2N)_2As$-CH_2-CH_2-CH=CH_2, which was oxidized to but-3-enylarsonic acid, H_2O_3As-CH_2-CH_2-CH=CH_2. This was hydroxylated with performic acid to give the 3,4-dihydroxybutylarsonic acid.



Scheme 1 Recycling of NAD$^+$ using *p*-nitroso-*N,N*-dimethylaniline

The NADH produced as the analogue is oxidized by the dehydrogenase in turn reduces *p*-nitroso-*N,N*-dimethylaniline, regenerating NAD$^+$. The reaction is followed by the fall in A_{440} as the *p*-nitroso-*N,N*-dimethylaniline is reduced [12].

2,3-Dihydroxypropylarsonic acid was synthesized [7] by the Meyer [8] reaction of alkaline arsenite on 3-chloropropan-1,2-diol.

3-Hydroxypropylarsonic acid was prepared by the method of Gough and King [9] with some modifications [10]. 3-Chloropropan-1-ol (95 g; 1 mol) was added to a solution of arsenic(III) oxide (As_4O_6; 100 g; 1 mol of As) and NaOH (120 g; 3 mol) in 300 ml of water. The mixture was heated to 75 °C with stirring until a homogeneous solution was obtained. The solution was diluted with an equal volume of water, acidified to pH 2 with conc. HCl and concentrated by rotary evaporation under reduced pressure. The arsonic acid was extracted twice with 150 ml portions of hot ethanol. The ethanol was removed by rotary evaporation. The syrupy solution of 3-hydroxypropylarsonic acid obtained was titrated to pH 10.5 with lithium hydroxide solution and dried down. The dilithium salt was crystallized from water on addition of acetone.

2-Amino-4-arsonobutyric acid, a glutamate analogue, was made [11] by treating dipropyl 2-chloroethylarsonate with the carbanion from diethyl acetamidomalonate and hydrolysing the product.

Enzyme assays

The reaction of glycerol-3-phosphate dehydrogenase was followed in 3 ml of 0.1 M glycine/NaOH buffer, pH 9.6, containing 0.1 M hydrazine, 0.33 mM NAD$^+$ and 1 μg of glycerol-3-phosphate dehydrogenase, with different concentrations of *sn*-glycerol 3-phosphate, 2,3-dihydroxypropylarsonic acid or 3,4-dihydroxybutylarsonic acid. The reaction of alcohol dehydrogenase was followed similarly, except that the hydrazine was replaced with 0.1 M semicarbazide, and ethanol or 3-hydroxypropylarsonic acid was used as substrate. Reactions were initiated by the addition of substrate. Initial rates of reaction were determined spectrophotometrically by measuring the rate of formation of NADH by its absorbance at 340 nm (in a Unicam SP 500 spectrophotometer with a Gilford 222-G attachment and with a Philips potentiometric recorder, PM8251A, for continuous monitoring).

Progress curves of substrate oxidation and arsenite release

The oxidation of the substrate could be followed and made to go to completion by allowing the NADH produced to reduce *p*-nitroso-*N,N*-dimethylaniline in the presence of alcohol dehydrogenase [12] and following the fall in absorbance ($\epsilon = 3.54 \times 10^4\ M^{-1} \cdot cm^{-1}$) at 440 nm (Scheme 1). The assay mixture contained 0.1 M glycine/NaOH buffer, pH 9.6, 0.5 mM 3,4-dihydroxybutylarsonic acid or 0.25 mM 3-hydroxypropylarsonic acid, 20 μg of enzyme, 0.5 mM *p*-nitroso-*N,N*-dimethylaniline and 0.33 mM NAD$^+$ in a total volume of 20 ml.

Arsenite was detected qualitatively in reaction mixtures by paper electrophoresis. It appeared as a spot of zero mobility, but treatment with H_2O_2 converted the spot into one with the same mobility as arsenate; both spots were revealed because they decolorized iron(III) sulphosalicylate. We first tried to determine the arsenite released by titrating it with iodine, but this not only oxidized the NADH formed (when this was allowed to accumulate), which could have been allowed for, but it also slowly reacted with other reaction products. We therefore determined the arsenite quantitatively after converting it into arsenate; samples in $NaHCO_3$ solution were titrated with 2.5 mM I_2 in 0.2 M KI until colour remained, and the arsenate was then measured [13] by the method of Ames [14] for phosphate, in which the samples were incubated in a mixture of ascorbic acid and acidic ammonium molybdate, and absorbance was read at 820 nm. A standard curve was constructed with known concentrations of arsenic(III) oxide.

Periodate treatment

3,4-Dihydroxybutylarsonic acid (free acid) was treated with $NaIO_4$ both in 1:1 and 1:2 ratios. For the 1:1 ratio, $NaIO_4$ (0.107 g; 0.5 mmol) in 2 ml of water was added dropwise to a solution of the analogue (0.113 g; 0.5 mmol) in 2 ml of water. For the 1:2 treatment, $NaIO_4$ (0.213 g; 1 mmol) in 2 ml of water was added dropwise to 0.113 g (0.5 mmol) of the analogue in 2 ml of water. In another experiment, the solution of the analogue (9 mg; 0.04 mmol) in 1 ml of water was added dropwise to a solution of $NaIO_4$ (17.2 mg; 0.08 mmol) in 1 ml of water so that there was an excess of periodate for most of the time. All the reactions were carried out at room temperature. Products were analysed by paper electrophoresis.

Treatment of 3,4-dihydroxybutylarsonic acid with an excess of periodate gave a product that co-ran with arsonopropionic acid on electrophoresis. This suggested that the aldehyde produced by periodate oxidation might be oxidized further to the acid in the presence of an excess of periodate. We therefore checked whether periodate was capable of oxidizing aldehydes under our conditions. First we checked that periodate oxidizes arsenite to arsenate; arsenic(III) oxide (0.197 g; 1 mmol) in 1 ml of water was treated with $NaIO_4$ (0.213 g; 1 mmol) in 1 ml of water at room temperature and electrophoresis showed formation of arsenate. To check whether periodate oxidizes aldehydes under the conditions of the oxidation of 3,4-dihydroxybutylarsonate, i.e. in the presence of arsenate, a stable aldehyde was used as a model. Dilithium 2-oxoethylphosphonate (70 mg; 0.5 mmol) was treated with equimolar amounts of both $NaIO_4$ and Na_2HAsO_4 at room temperature; electrophoresis at pH 6.5 showed formation of a spot that co-ran with phosphonoacetate.

Stability of the aldehyde (3-oxopropylarsonic acid)

The stability of the aldehyde formed by the periodate oxidation of 3,4-dihydroxybutylarsonic acid, i.e. 3-oxopropylarsonic acid, was determined by oxidizing it with $KMnO_4$, at various times after it was formed, to the stable 3-arsonopropionate, which could be identified by electrophoresis. 3,4-Dihydroxybutylarsonic acid was treated with $NaIO_4$ as described above (1:2); samples were removed at various times for electrophoresis after $KMnO_4$ solution had been added to each until the purple colour remained.

Transamination

2-Amino-4-arsonobutyric acid was transaminated by treating it with glyoxylate and pyridine acetate in the presence of copper(II) acetate as catalyst [15].

RESULTS AND DISCUSSION

Enzyme kinetics

2,3-Dihydroxypropylarsonic acid, a non-isosteric analogue of *sn*-glycerol 3-phosphate, did not show substrate activity with the enzyme glycerol-3-phosphate dehydrogenase, but the isosteric analogue, 3,4-dihydroxybutylarsonic acid, did. The K_m obtained was 0.55 mM and, under the same conditions, *sn*-glycerol 3-phosphate gave a K_m of 0.29 mM. The limiting velocity, V, for *sn*-glycerol 3-phosphate was 0.130 μmol·min^{-1} compared with 0.098 μmol·min^{-1} obtained for 3,4-dihydroxybutylarsonic acid (in each case with 1 μg of enzyme). These represent catalytic constants for the enzyme subunit of 84 s^{-1} and 63 s^{-1} respectively. The value for the natural substrate is lower than that given by the manufacturers, but we made no attempt to use exactly their conditions of assay; we therefore do not stress these absolute values, but the ratio between them should be correct. We note that the phosphonate analogue, 3,4-dihydroxybutylphosphonic acid, has a K_m slightly smaller than that for the natural substrate, and the limiting velocity diminished to about 80% [16,17].

In the determination of the kinetic parameters of the enzyme glycerol-3-phosphate dehydrogenase with the analogue 3,4-dihydroxybutylarsonic acid, the racemic mixture was used. Since the enzyme is specific for *sn*-glycerol 3-phosphate, and therefore probably bound only the (*S*)-isomer of 3,4-dihydroxybutylarsonic acid, it seems that both the natural substrate and the analogue have similar K_m and V values. Since the isosteric analogue is a good substrate, the fact that the non-isosteric analogue, 2,3-dihydroxypropylarsonic acid, was completely inert must mean that its molecular shape, with a shortened distance between its hydroxy and arsono groups, rather than absence of a phosphoester bond, prevents its correct binding to the enzyme.

Arsenite release

Repeated failures to isolate 4-hydroxy-3-oxobutylarsonic acid, the presumed product of the enzyme-catalysed oxidation of 3,4-dihydroxybutylarsonic acid, followed by repeated failures to prepare it by methods similar to those used for the corresponding phosphonate [18], led us to try another method of preparation, starting with the transamination of the glutamate analogue 2-amino-4-arsonobutyrate. Electrophoretic analysis of the reaction mixture showed that arsenite was eliminated, as it had been when Ali and Dixon [19] tried to transaminate the aspartate analogue 2-amino-3-arsonopropionate. Hence all the failures could be explained if 3-oxoalkylarsonic acids eliminated arsenite in aqueous solution.

We therefore used the methods described above to measure the release of arsenite during enzyme-catalysed oxidation of the substrate. Figure 1 shows the time course of oxidation of 3,4-dihydroxybutylarsonic acid by glycerol-3-phosphate dehydrogenase and the consequent release of arsenite. The proposed mechanism of release of arsenite from the oxidized analogue is shown in Scheme 2. The concentration of arsenite increases as the concentration of oxidized analogue increases, but with a marked lag. The initial concentration of the racemic analogue was 500 μM and therefore only half of this, 250 μM, was expected to be oxidized. The concentration of analogue oxidized, as determined by the reduction of *p*-nitroso-*N*,*N*-dimethylaniline, was 277 μM, which is 11% more than expected. The amount of arsenite released was 204 μM and this is 18% below the 250 μM expected. Figure 2 shows a similar release of arsenite when 3-hydroxypropylarsonic acid was oxidized by alcohol dehydrogenase in the presence of NAD$^+$. The reaction mixture contained 250 μM 3-hydroxypropylarsonic acid and the concentration of substrate oxidized was 271 μM, 9% more than expected. Arsenite released was 201 μM, 19% down on the expected value of 250 μM.

Thus with both 3,4-dihydroxybutylarsonic acid and 3-hydroxypropylarsonic acid oxidations, the arsenite concentration obtained was less than expected, and the concentration of *p*-nitroso-*N*,*N*-dimethylaniline reduced was more than the concentration of oxidizable substrate. In each case the determinations were conducted at least twice.

To check that the assays based on the absorbance of *p*-nitroso-*N*,*N*-dimethylaniline were valid, a solution of 250 μM ethanol was oxidized. At the end of the reaction 244 μM ethanol had been oxidized as judged by absorbance at 440 nm. Hence the estimate of alcohol oxidized was 3% low. This implies that the unexpectedly high value for the amount of NADH produced was, if anything, an underestimate. We also checked that arsenite did not reduce the *p*-nitroso-*N*,*N*-dimethylaniline under the conditions of the assay.

To check the accuracy of the arsenate determination, on which the arsenite concentrations were based, solutions of arsenate were prepared in 0.4 M $NaHCO_3$ solution by (1) weighing out sodium arsenate, and (2) weighing out arsenic(III) oxide (As_4O_6) and oxidizing it by titration with I_2/KI solution. The arsenate estimation described above was then applied. Both gave the same standard curve to within 0.5%, and it was identical with that obtained earlier based on weighing sodium arsenite.

The overestimates of substrate oxidized therefore seem to be reproducible. Conceivably they are due to interference with the assay by the organic product of the elimination, which is expected to be a highly reactive vinyl ketone. It may (slowly) reduce some of the *p*-nitroso-*N*,*N*-dimethylaniline, and so give a falsely high indication of the quantity of substrate oxidized. The overestimate is higher than expected by approximately the same factor in each case, 11% for 3,4-dihydroxybutylarsonic acid and 9% for 3-hydroxypropylarsonic acid.

These over-reductions of NAD$^+$ and the deficit in the release of arsenite (18% for 3,4-dihydroxybutylarsonic acid and 19% for 3-hydroxypropylarsonic acid) could have the same cause if the product of substrate oxidation underwent competing reactions: (1) the arsenite elimination and (2) an enzyme-catalysed NADH-producing oxidation to a form that does not lose arsenite. This seems straightforward for the oxidation of 3-hydroxypropylarsonic acid that is catalysed by alcohol dehydrogenase, since the hydrated form of the product, 3-oxopropylarsonic acid, could well be a substrate for the enzyme. ^{13}C-NMR spectroscopy shows that the reaction mixture contains several substances, and we have failed to characterize the nature of any such minor competing reaction. If there is also such a competing reaction when 3,4-dihydroxybutylarsonic acid is the starting substance, it must be more complex. The presumed product of oxidation, 4-hydroxy-3-oxobutylarsonic acid, may reversibly form 3-hydroxy-4-oxobutylarsonic acid via enolization, and this might be a substrate for the alcohol dehydrogenase (although we admit that 3,4-dihydroxybutylarsonic acid is not), which would stabilize it against elimination by oxidizing it to 4-arsono-2-hydroxybutyrate. A model of a competing oxidation of the unstable 3-oxoalkylarsonic acid is provided by the periodate oxidation of 3-hydroxypropylarsonic acid (see below).

Attempts to trap the vinyl ketone, the other product formed as

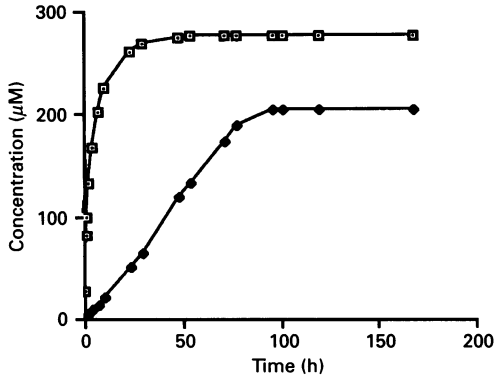


Figure 1 Time course of oxidation of 3,4-dihydroxybutylarsonic acid by glycerol-3-phosphate dehydrogenase

The oxidation of 3,4-dihydroxybutylarsonic acid was determined by monitoring the reduction of *p*-nitroso-*N,N*-dimethylaniline at 440 nm (Scheme 1). Arsenite released was oxidized to arsenate and determined by the method of Ames [14] for phosphate determination. The reaction mixture contained 0.1 M glycine/NaOH buffer, pH 9.6, 0.33 mM NAD^+, 0.5 mM *p*-nitroso-*N,N*-dimethylaniline, 0.5 mM racemic 3,4-dihydroxybutylarsonic acid, alcohol dehydrogenase and glycerol-3-phosphate dehydrogenase. ⊡, Analogue oxidized; ◆, arsenite released.

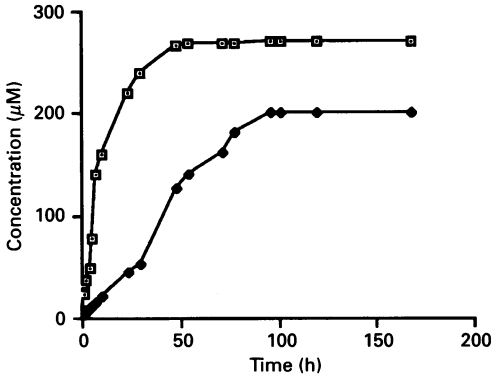


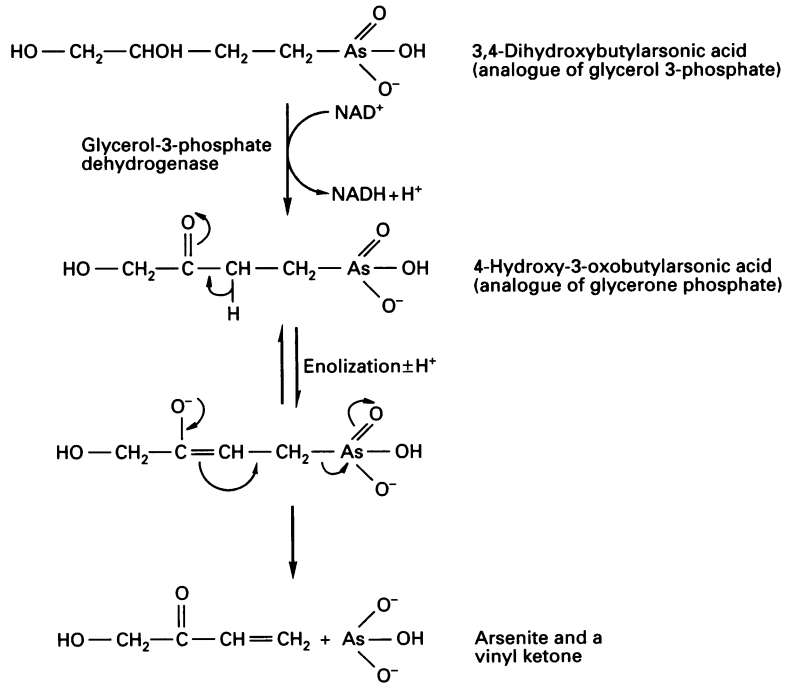
Figure 2 Time course of enzymic oxidation of 3-hydroxypropylarsonic acid by alcohol dehydrogenase

The oxidation of 3-hydroxypropylarsonic acid was determined by monitoring the reduction of *p*-nitroso-*N,N*-dimethylaniline at 440 nm (Scheme 1). Arsenite released was oxidized to arsenate and determined by the method of Ames [14] for phosphate determination. The reaction mixture contained 0.1 M glycine/NaOH buffer, pH 9.6, 0.33 mM NAD^+, 0.5 mM *p*-nitroso-*N,N*-dimethylaniline, 0.25 mM 3-hydroxypropylarsonic acid and alcohol dehydrogenase. ⊡, Analogue oxidized; ◆, arsenite released.

a result of arsenite release, by addition of cysteine to the reaction mixture were unsuccessful.

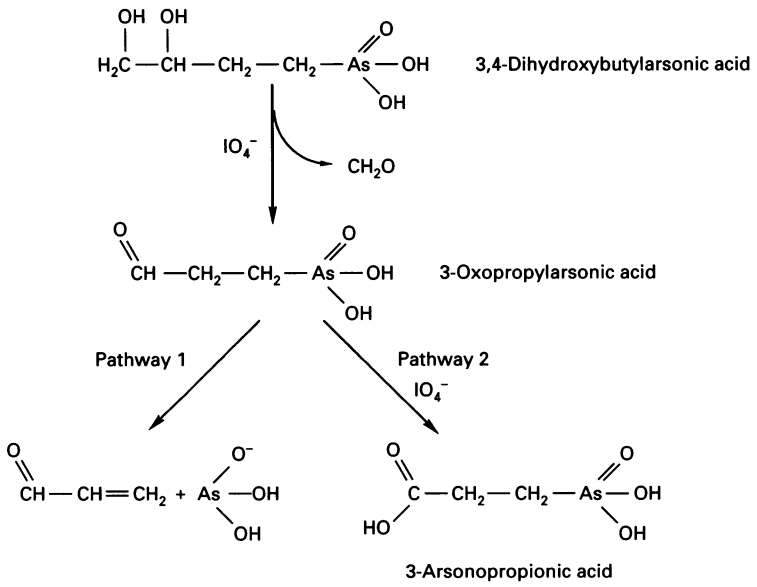
Periodate treatment

Since periodate oxidizes arsenite to arsenate, we here describe as arsenite release the appearance of either arsenite or arsenate (usually a mixture) on electrophoresis. Such release was observed when 3,4-dihydroxybutylarsonic acid was treated with periodate both in a 1:1 and 1:2 ratio. When the periodate was added dropwise to the analogue in a 1:1 ratio, only arsenite/arsenate and a trace of unchanged analogue were observed to give Fe^{3+}-binding spots after electrophoresis. No trace of unchanged analogue was observed in the 1:2 treatment. Addition of



Scheme 2 Proposed mechanism of arsenite release from 3,4-dihydroxybutylarsonic acid during oxidation by glycerol-3-phosphate dehydrogenase

The ketone produced by the dehydrogenase may enolize, and so lose arsenite by elimination. The vinyl ketone formed is likely to be unstable. This scheme will also apply to the action of alcohol dehydrogenase on 3-hydroxypropylarsonic acid when H- replaces $HO-CH_2-$.



Scheme 3 Arsenite release from 3,4-dihydroxybutylarsonic acid by periodate oxidation

Gradual addition of periodate (1 mol/mol) to a solution of 3,4-dihydroxybutylarsonic acid gave release of arsenite (left-hand pathway). In another treatment the analogue was added slowly to 2 mol/mol of periodate solution, so that the periodate was in excess during the addition, and arsonopropionic acid was also produced (right-hand pathway). This may mean that the aldehyde formed in the first periodate oxidation is further oxidized to arsonopropionate before it has the opportunity to release arsenite.

permanganate to the 1:2 reaction mixture also gave a spot with the mobility of 3-arsonopropionate, but only if the permanganate was added within 15 min of the reaction. When the analogue was added dropwise to 2 mol/mol of periodate, so that periodate was in excess during the reaction, the spot that co-ran with 3-arsonopropionate was also observed on electrophoresis, in addition to arsenite/arsenate. It is therefore likely that the aldehyde produced by the periodate treatment (Scheme 3) was oxidized to arsonopropionic acid by the excess of periodate (since periodate oxidized 2-oxoethylphosphonate to phosphonoacetate). From this it again appears that the release of arsenite from the 3-oxoethylarsonate is not instantaneous, as periodate oxidation can compete with it.

Mechanism and consequences

Arsenite elimination appears to be a characteristic of 3-oxoalkylarsonic acids, $R-CO-CH_2-CH_2-AsO_3H_2$, and may be due to enolization as shown in Scheme 2. Here 3,4-dihydroxybutylarsonic acid is oxidized to 4-hydroxy-3-oxobutylarsonic acid by the enzyme glycerol-3-phosphate dehydrogenase in the presence of NAD^+. Enolization may be followed by elimination of arsenite, as electrons flow back from O-3 of the enolate as the keto isomer is formed (Scheme 2).

Although the carbon–arsenic bond in alkylarsonic acids is formed from arsenite in the Meyer [8] reaction, breakage of this bond may occur at lower pH when arsenite becomes a better leaving group. The release of arsenite from benzylarsonic acid in HCl is known [20,21], as well as its release (see above) on attempted transamination of arsonoalanine [19].

A consequence of this pathway of arsenite release *via* enzymic generation of 3-oxoalkylarsonic acids is that a compound such as 3,4-dihydroxybutylarsonic acid, $HO-CH_2-CHOH-CH_2-CH_2-AsO_3H_2$, may be selectively toxic to cells that can take it up. The compound itself is fairly harmless. Delivery of such compounds into cells and their consequent oxidation inside the cell will result in release of arsenite. The arsenite will kill the cell because of its affinity for dithiol groups. The other product formed when arsenite is released, a vinyl ketone, is a strong electrophile, and hence also likely to be toxic.

By contrast with 3-oxoalkylarsonic acids, 3-oxoalkylphosphonic acids are stable; for example, the phosphonomethyl analogue of glycerone phosphate, namely 4-hydroxy-3-oxobutylphosphonic acid [18], is a substrate for aldolase [22]. The elimination of arsenite from 3-oxoalkylarsonic acids, but not of phosphite from 3-oxoalkylphosphonic acids, may be explained by the high stability of arsenite compared with arsenate, in contrast with the low stability of phosphite compared with phosphate. 2-Oxoalkylarsonic acids, exemplified by arsonoacetaldehyde [7,19] and 3-arsonopyruvate [23], are only moderately stable, but they release arsenate by hydrolysis, rather than arsenite by elimination. 2-Oxoalkylphosphonic acids can be hydrolysed in this way [24], as in the enzyme-catalysed hydrolysis of phosphonoacetaldehyde [25–27], but with much more difficulty. The lability of 2-oxoalkylarsonic acids presumably reflects the ease with which water can more easily enter the co-ordination shell of arsenic than that of phosphorus, because of the larger size of the atom. This is the same feature that is probably responsible for the lability of all esters and anhydrides of arsenate.

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