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REVIEW

Enzyme-catalysed Transformations of Compounds Containing the $-CH_2-AsO_3H_2$ Group

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Enzymes that act on substrates $R-O-PO_3H_2$ often work on substrate analogues $R-O-AsO_3H_2$; such substrates are unstable, since esters of H_3AsO_4 hydrolyse easily. They also form easily, so that an enzyme that acts on $R-O-PO_3H_2$ often acts on a mixture of R–OH and arsenate via an ester that forms at the active site. Similarly coenzyme analogues may be formed; for example, a stable and active aspartate aminotransferase forms from the apoenzyme with free pyridoxal and arsenate.

Enzymes that convert $R-O-PO_3H_2$ into a diester often act on $R-CH_2-AsO_3H_2$, a stable substrate analogue; then the product is unstable and hydrolyses to re-form the analogue, giving a futile cycle. For example, RNA polymerase acquires exonuclease activity in the presence of $H_2O_3P-CH_2-AsO_3H_2$; adenylate kinase acquires ATPase activity in the presence of the arsonomethyl analogue of AMP.

A recent observation is that $HO-CH_2-CHOH-CH_2-CH_2-AsO_3H_2$ is a good substrate for glycerol-3-phosphate dehydrogenase. The product is unstable and eliminates arsenite, sharing this ability with other 3-oxoalkylarsonates. Thus this enzyme–catalysed oxidation is a lethal synthesis, in view of the toxicity of arsenite. Another unusual biochemical reaction of an arsonic acid is seen in the ability of a bacterium to use arsonoacetate as its sole source of carbon and energy.

In contrast with the elimination of arsenite by 3-oxoalylarsonic acids, 3-oxoalkylphosphonic acids, $R-CO-CH_2-CH_2-PO_3H_2$, are stable. 2-Oxoalkylphosphonic acids, $R-CO-CH_2-PO_3H_2$, however, are moderately unstable to hydrolysis, yielding phosphate and $R-CO-CH_3$. 2-Oxoalkylarsonic acids, $R-CO-CH_2-AsO_3H_2$, decompose in the same way, but much more readily, yielding arsenate.

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BIOCHEMICAL ACTIONS OF ARSENATE

Enzymes that act on phosphate normally act on arsenate too. Such enzymes normally alkylate, acylate or phosphorylate the phosphate. With arsenate, however, the products hydrolyse rapidly. They are less stable than phosphates because the arsenic atom is large, and can accommodate water to enter as a fifth ligand, so that one of the existing ligands can then leave. Typically esters of arsenate have half-lives in water at neutral pH of about 30 min;[1] if the arsenate is acylated or phosphorylated instead of being alkylated, i.e. the arsenic atom bears a better leaving group, the half-life falls to seconds.[2] This explains the long-known biochemical actions of arsenate: enzymes accept it in place of phosphate to incorporate into other compounds such as ATP, but the analogues formed hydrolyse forthwith.[3]

ESTERS OF ARSENATE AS ENZYME SUBSTRATES AND COENZYMES

Esters of arsenate also form easily, so that, as Lagunas and colleagues[4, 5] showed, an enzyme that acts on $R-O-PO_3H_2$ often acts on a mixture

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of R–OH and arsenate via an ester, R–O–AsO_3H_2, that forms at the active site. The conversion is slow, but a faster reaction can be obtained if the arsenate and R–OH form a coenzyme analogue rather than a substrate analogue, since then esterification of one molecule can lead to the formation of many molecules of product. Thus Ali and Dixon[6] found that a stable and active aspartate aminotransferase forms from the apoenzyme with free pyridoxal and arsenate.

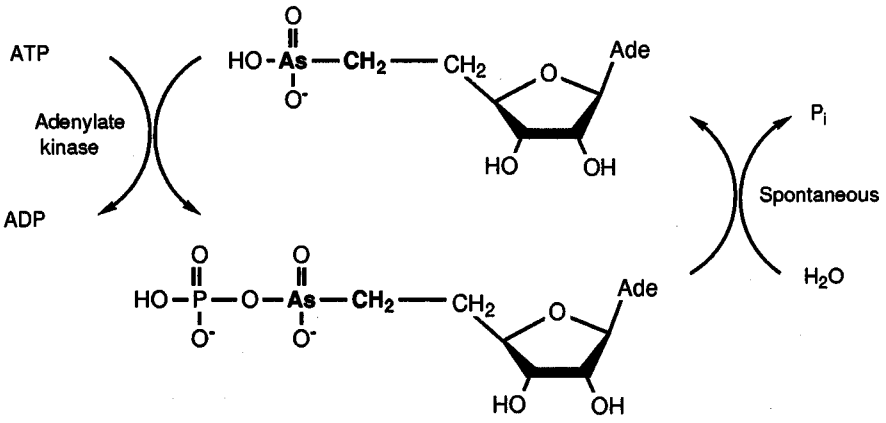
ARSONOMETHYL ANALOGUES OF NATURAL PHOSPHATES AND NEW FUTILE CYCLES

Enzymes that indirectly convert R–O–PO_3H_2 into a diester, R–O–P(O)(–O^-)–O–R′, often act on R–CH_2–AsO_3H_2, a stable substrate analogue; then the product, R–CH_2–As(O)(–O^-)–O–R′, is unstable and hydrolyses to re-form the analogue, giving a futile cycle in which the donor of the group R′ is used up. Thus, for example, RNA polymerase acquires[7] exonuclease activity in the presence of H_2O_3P–CH_2–AsO_3H_2. Similarly adenylate kinase acquires ATPase activity in the presence of the arsonomethyl analogue of AMP,[8] as shown in Scheme 1.

Synthesis of the analogue of AMP was achieved (Scheme 2) from adenosine in seven steps including protection and deprotection. The route thus provides a pathway for converting R–CH_2–OH into R–CH_2–CH_2–AsO_3H_2, i.e. an alcohol into the arsonomethyl analogue of its phosphate.

A somewhat different futile cycle is formed when CTP and 2-aminoethylarsonic acid are added to ethanolamine-phosphate cytidylyltransferase. This enzyme normally catalyses the nucleophilic attack of phosphoethanolamine (i.e. aminoethyl phosphate) on CTP to expel diphosphate (pyrophosphate) and yield CDP-ethanolamine. Unlike most enzymes that act on R–**O**–PO_3H_2, which accept R–**CH_2**–PO_3H_2 but not R–PO_3H_2, it acts on this last, so that in place of phosphoethanolamine (H_2N–CH_2–CH_2–**O**–PO_3H_2), aminoethylphosphonate (H_2N–CH_2–CH_2–PO_3H_2, ciliatine), which occurs in lower organisms, can be incorporated into phospholipids, even by mammalian enzymes.[9] Hence it was not surprising that the mammalian enzyme also acted on 2-aminoethylarsonic acid, and that the resulting CDP–ethanolamine analogue hydrolysed, so that the net reaction was the hydrolysis of CTP to CMP and diphosphate.[10] 2-Aminoethylarsonic acid is also a substrate for the bacterial transaminase responsible for the breakdown of aminoethylphosphonate,[11] but the 2-oxoethylarsonate formed (i.e. arsonoacetaldehyde) is not hydrolysed by the enzyme that hydrolyses the corresponding phosphonate in a major pathway for the biological breaking of the C–P bond.

Several other enzymes, e.g. phosphoglycerate kinase[12] and enolase,[13] also accept arsonomethyl analogues of their natural substrates although these analogues are usually poor substrates. Phophoenolpyruvate mutase, the main enzyme responsible for the natural synthesis of the C–P bond by interconverting phosphoenolpyruvate and 3-phosphonopyruvate, also uses 3-arsonopyruvate, which binds well to it but is a poor substrate.[14] The 3-arsonopyruvate was synthesized in four steps from glycine; it proves to be



Scheme 1. The action of adenylate kinase on the arsonomethyl analogue of AMP.

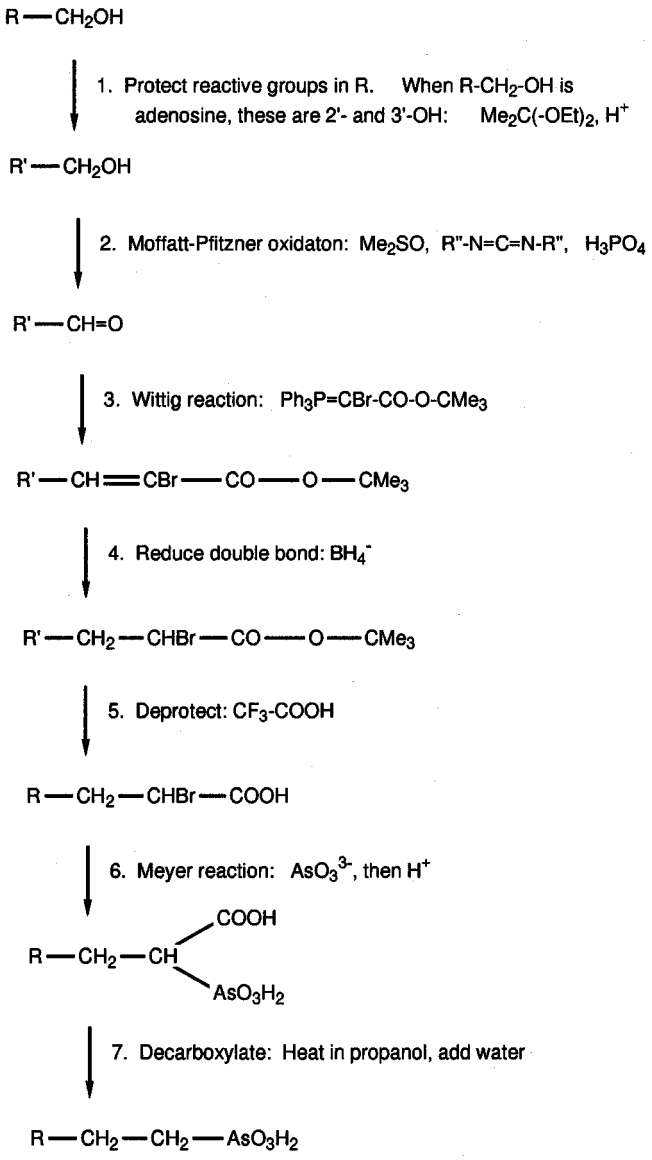
easily hydrolysed to pyruvate and arsenate, just as arsonoacetaldehyde is easily hydrolysed to acetaldehyde and arsenate.

THE ARSONO GROUP AS A CARBOXY ANALOGUE

Ali and Dixon[15] made 3-arsonoalanine, an analogue of aspartate, from arsonoacetaldehyde; the equivalent analogue of glutamate was made earlier.[12] These have not yet proved to be good substrates for enzymes that act on the natural amino acids, although they bind to some of them, and they transaminate the pyridoxal phosphate of glutamate decarboxylase.[16]

ENZYMIC PRODUCTION OF 3-OXOALKYLARSONIC ACIDS — A LETHAL SYNTHESIS

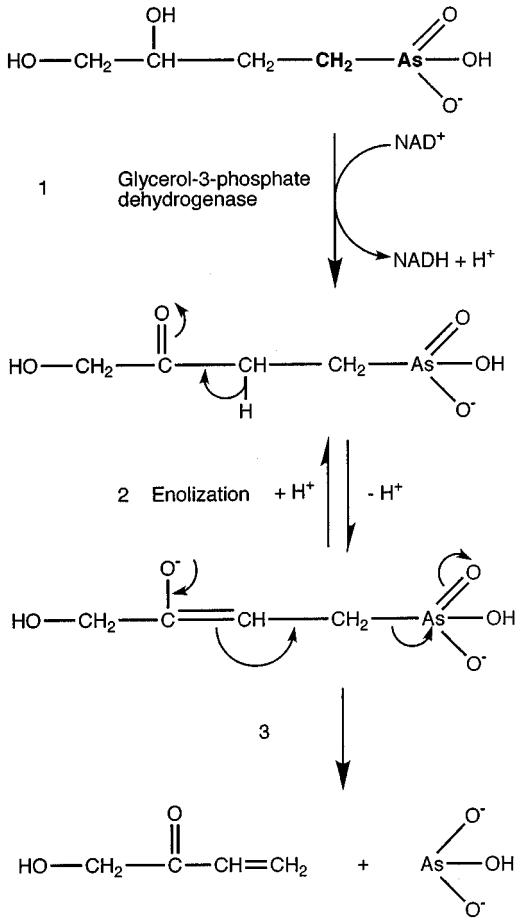
As expected for an arsonomethyl analogue of a phosphate, $HO–CH_2–CHOH–CH_2–CH_2–AsO_3H_2$



Scheme 2. A method for converting an alcohol, $R–CH_2–OH$, into the arsonomethyl analogue, $R–CH_2–\mathbf{CH_2–As}O_3H_2$, of its phosphate, $R–CH_2–\mathbf{O–P}O_3H_2$.

is a good substrate for glycerol-3-phosphate dehydrogenase. We failed to isolate the oxidized product and this led us to realize that it is unstable and eliminates arsenite.[17] This elimination seems to be a general property of 3-oxoalkylarsonic acids, since 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) attempted non-enzymic transamination of the glutamate analogue 2-amino-4-arsonobutyric acid. It is probably a consequence of enolization, so that the overall reaction with the analogue of glycerol phosphate may be represented as shown in Scheme 3.

Enzymic formation of 3-oxoalkylarsonic acids in cells can therefore be lethal, since arsenite is poisonous to most organisms because of its high affinity for dithiols such as dihydrolipoyl groups; it therefore inactivates enzymes such as pyruvate dehydrogenase and oxoglutarate dehydrogenase, which are central to oxidative metabolism.



Scheme 3. The action of glycerol-3-phosphate dehydrogenase on the arsonomethyl analogue of its natural substrate.

AN UNUSUAL NATURAL BREAKAGE OF THE C–As BOND

Quinn and McMullan[18] have found a bacterium that can live on arsonoacetate as its only source of energy and carbon. It excretes arsenate quantitatively. It can also oxidize arsenite to arsenate, so conceivably the degradation of the arsonoacetate is reductive, giving arsenite as the first product. It similarly breaks down racemic arsonochloroacetate quantitatively to arsenate, so must act on both enantiomers. It cannot use this as its source of energy and carbon, which may not be surprising, since it cannot use chloroacetate either.

CHEMICAL PROPERTIES OF OXO ARSONIC ACIDS

Unlike 3-oxoalkylarsonic acids, 3-oxoalkylphosphonic acids are stable; for example, the phosphonomethyl analogue of glycerone phosphate is substrate for aldolase.[19] The elimination of arsenite from 3-oxoalkylarsonic acids; but not of phosphite from 3-oxoalkylphosphonic acids, may reflect the fact that arsenite is relatively stable compared with arsenate, whereas phosphite is unstable compared with phosphate. 2-Oxoalkylarsonic acids, exemplified by arsonoacetaldehyde[11, 15] and 3-arsonopyruvate[14] (see above), are only moderately stable; they release arsenate by hydrolysis, rather than arsenite by elimination. 2-Oxoalkylphosphonic acids can be hydrolysed in this way,[20] as in the enzyme-catalysed hydrolysis of phosphonoacetaldehyde,[21–24] but with much more difficulty. The lability of 2-oxoalkylarsonic acids presumably reflects the ease with which water can more easily enter the coordination shell of arsenic than that of phosphorus, because of the larger size of the atom, i.e. the same feature expressed in the lability of esters and anhydrides of arsenate.

CONCLUSIONS

As reviewed here, and also in more detail elsewhere,[25] the arsonic acids $R–CH_2–AsO_3H_2$

enter many enzyme-catalysed reactions of phosphates $R–O–PO_3H_2$. When these reactions involve transfer of a group onto $–AsO_3H_2$, so that its esters or anhydrides are formed, these products hydrolyse rapidly, because of the easy displacement by water of oxygen ligands on arsenic. This gives a 'futile cycle', which uses up the donor of the group transferred. A different consequence of an enzyme-catalysed reaction of $R–CH_2–AsO_3H_2$ is the formation of toxic arsenite when a 3-oxoalkylarsonic acid is produced.

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