2-Aminoethylarsonic acid as an analogue of ethanolamine phosphate

Endowment of ethanolamine-phosphate cytidylyltransferase with CTP pyrophosphatase activity

Emma VISEDO-GONZALEZ* and Henry B. F. DIXON
Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

INTRODUCTION

Aminoethyl phosphate (O-phosphoethanolamine, ethanolamine phosphate, \(\text{HO}_3\text{PCH}_2\text{CH}_2\text{NH}_3\)) is incorporated into phospholipids via CDP-ethanolamine, which is synthesized by the transfer of a cytidyl group from CTP on to the phosphi group of the aminoethyl phosphate. Its analogue, aminoethylphosphonic acid, \(\text{HO}_3\text{POCH}_2\text{CH}_2\text{NH}_3\), is incorporated into the phospholipids of some lower organisms by a similar route of cytidyl transfer to form a CDP derivative [Liang & Rosenberg (1986); see also reviews by Florin-Christensen et al. (1986) and Kittredge & Roberts (1969)]. Further, the mammalian ethanolamine-phosphate cytidylyltransferase accepts the phosphonate as a substrate to catalyse this reaction (Tamari et al., 1973). This led us to think that it might also accept the corresponding arsonate, 2-aminoethylarsonic acid, \(\text{HO}_3\text{AsCH}_2\text{CH}_2\text{NH}_3\).

The interest in this would be that the transferase would be endowed by the analogue with a CTP pyrophosphatase activity, since enzymes and anhydrides of arsonic acids, should hydrolyze spontaneously with regeneration of the substrate analogue and CMP formation; such CMP production was observed. The limiting velocity with aminoethylarsonic acid is about 90% that with ethanolamine phosphate, and the Michaelis constant is below 20 mM.

2-Aminoethylarsonic acid was tested for its ability to act as a substrate for ethanolamine-phosphate cytidylyltransferase as a cytidyl acceptor in place of ethanolamine phosphate. The expected product, like all mixed anhydrides of arsonic acids, should hydrolyse spontaneously with regeneration of the substrate analogue and CMP formation; such CMP production was observed. The limiting velocity with aminoethylarsonic acid is about 90% that with ethanolamine phosphate, and the Michaelis constant is below 20 mM.

MATERIALS AND METHODS

2-Aminoethylarsonic acid

This was prepared as described in the preceding paper by Geoghegan & Dixon (1989).

Ethanolamine-phosphate cytidylyltransferase (EC 2.7.7.14)

The enzyme was prepared from rat liver by the method of Sundler (1975), involving homogenization, collection of the material precipitated by 25–50% saturation with \((\text{NH}_4)_2\text{SO}_4\) and chromatography on DEAE-cellulose. On chromatography in Sundler’s (1975) Tris/HCl buffer, pH 7.6, containing dithiothreitol, and a gradient from 0 to 0.3 M-NaCl, the active fractions were found to be those of 0.2–0.3 M-NaCl, although Sundler (1975) had found the enzyme to be eluted at slightly lower salt concentrations. The active fractions (30 ml from 15 g of rat liver) were pooled and stored at 0°C.

Separation of cytidine nucleotides

Samples were analysed for CMP and CDP-ethanolamine by chromatography on a column (1 cm x 10 cm) of the strongly basic resin Dowex 1 (X8; 200–400 mesh; formate form) equilibrated and developed with a solution of 40 mM-formic acid/20 mM-triethylamine, pH 3.7, run at 0.9 ml/min. The effluent was monitored at 270 nm. In this system 2 ml samples of 0.25 mM-nucleotide were applied: CDP-ethanolamine ran at 2.8 column volumes, CMP at 8 column volumes, and CTP did not emerge by 12 column volumes.

Enzyme assay

The ethanolamine-phosphate cytidylyltransferase was assayed by incubating 0.5 ml samples of enzyme (representing the enzyme from 0.25 g of liver) at 37°C with 0.5 ml of a solution containing 4 mM-CTP, 2 mM-
ethanolamine phosphate, 20 mM-MgCl₂, 10 mM-dithiothreitol and 40 mM-Tris that had been adjusted with conc. HCl to pH 7.8. Incubations were for 0.5, 1.0, 1.5 and 2.0 h. The reaction was stopped by adding 1 ml of the much more acid chromatographic buffer (40 mM-formic acid/20 mM-triethylamine). It was then heated at 100 °C for 20 min, and the precipitate was removed by centrifugation. A 1 ml sample of the supernatant was then subjected to chromatography, as above, and the amount of CDP-ethanolamine produced was assessed from the area of the peak determined by its absorbance at 280 nm. When aminohydrolyase acid was used in place of ethanolamine phosphate, the area of the peak of CDP was similarly assessed. The rate was measured from the slope of the progress curves, which remain constant for 2 h.

**RESULTS**

The active fraction of enzyme was incubated with 0.5 mM-ethanolamine phosphate and 1 mM-CTP. A rate of production of CDP-ethanolamine of $6.8 \times 10^{-4}$ mm/min was observed. Since the Michaelis constant of this enzyme is 65 μM for ethanolamine phosphate (Sundler, 1975), the limiting velocity was about $7.7 \times 10^{-4}$ mm/min.

When 2-aminohydrolyase acid (20 mM and 50 mM) was used, CMP was produced at rates of $5.85 \times 10^{-4}$ mm/min and $6.38 \times 10^{-4}$ mm/min respectively. This corresponds to a limiting velocity of about $6.8 \times 10^{-4}$ mm/min and a Michaelis constant of well under 20 mM (about 3 mM).

**DISCUSSION**

**Catalysed reaction**

Far the simplest interpretation of the production of CMP that we have demonstrated is that the 2-aminohydrolyase acid is a substrate for the cytidylyltransferase (Scheme 1). This analogue is isosteric with aminohydrolyase phosphonate, shown by Tamari et al. (1973) to be a substrate, so substrate action is not surprising. The presumed product must be unstable, so spontaneous hydrolysis completes the cycle shown in the lower half of Scheme 1. We have not, however, actually demonstrated that the mixed anhydride is an intermediate. Such an intermediate anhydride was trapped by Gresser (1981) for the mixed anhydride of ADP with arsenate. Similarly Teipel & Koshland (1970) obtained kinetic evidence for the finite lifetime of the 3-phosphoglyceroyl arsenate formed by glyceraldehyde-3-phosphate dehydrogenase.
when it oxidizes glyceraldehyde 3-phosphate in the presence of arsenate.

**Chromatographic properties**

The basic resin might have been expected to retard CDP-ethanolamine more than CMP, since it possesses $-\text{PO}_2\text{OPO}_2\text{CH}_2\text{CH}_2\text{NH}_3^+$ in place of $-\text{PO}_3\text{H}^-$, and the extra negative charge should have more effect than an extra positive charge, since it will be closer to the exchanging groups of the resin. The fact that the reverse was found may be explained by the ability of the $-\text{PO}_3\text{H}^-$ group of the CMP to dissociate to $-\text{PO}_2\text{O}_2^-$, which may be the predominant form bound even at a pH where $-\text{PO}_3\text{H}^-$ predominates in solution, and by the hydrophilic properties of the diphosphate grouping.

We thank many colleagues, especially Mr. M. J. Sparkes and Dr. J. Knudsen, for advice and discussion, and the Comissió Interdepartamental de Reserca i Innovació Tecnologica of Barcelona for support for E. V.-G.

**REFERENCES**

Braunstein, A. E. (1931) Biochem. Z. 240, 68–93

Received 2 February 1989; accepted 8 March 1989