O-Phosphorylethanolamine Ammonia Lyase, A New Pyridoxal Phosphate-Dependent Enzyme[1]

H. Lee Fleshood[2] and Henry C. Pitot, McArdle Laboratory,
University of Wisconsin Medical School, Madison, Wisconsin 53706

Received May 23, 1969

SUMMARY

O-phosphorylethanolamine ammonia lyase, a new enzyme present in vertebrate liver, has been shown to catalyze the catabolism of O-phosphorylethanolamine to equimolar quantities of ammonia, acetaldehyde, and inorganic phosphate. The enzyme occurs in the soluble fraction of the cell and has been partially purified from rabbit liver. Using ^{14}C O-PE as substrate, the ^{14}C acetaldehyde formed enzymatically was characterized as the 2,4-dinitrophenylhydrazone by infrared spectra and chromatography. Ethanolamine is not a substrate for the enzyme nor does the cobamide coenzyme affect its rate. The Km for O-phosphoryl-ethanolamine was found to be 6.1×10^{-4}M and for pyridoxal phosphate 2.7×10^{-7}M.

MATERIALS AND METHODS

O-PE ^{14}C was synthesized using a modification of the Plimmer and Burch (7) procedure. One gm of ethanolamine and 10 gm of polyphosphoric acid (prepared from a ratio of 1 gm P_2O_5 and 1.3 gm 85% phosphoric acid), are heated in a closed test tube for 3 hours at 100°. Five volumes of H_2O were added and the mixture heated at 100° for 15 minutes. The solution

---

1 The work reported here was supported in part by grants from the National Cancer Institute (CA-07175) and the American Cancer Society (P-314).

2 Graduate Trainee of the National Cancer Institute (T01 CA-05002). The studies reported here have been incorporated into a thesis to be submitted by this author to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

was distilled under reduced pressure to dryness (or a thick syrup) at less than 30°, and triturated with ethanol to remove excess phosphoric acid. The amorphous residue was converted into a pure crystalline form by recrystallizations from small volumes of hot H_2O by the addition of ethanol or methanol giving a yield of 50 to 70%. The synthesis was carried out using redistilled reagent grade ethanolamine and ethanolamine-1,2-^{14}C obtained from New England Nuclear. The labelled compound had a specific activity of 4.5×10^{-3} μC/μM.

The enzyme was obtained by preparing a 25% homogenate of rabbit liver by means of a polytron homogenizer. All manipulations of the enzyme (unless otherwise specified) were carried out at 0° using buffer A containing 1.62×10^{-4} M pyridoxal-P. Buffer A consists of 0.1 M Tris-HCl, pH 7.8, 10^{-3} M dithiothreitol and 10^{-3} M EDTA. The 105,000 x g supernatant from the homogenate was brought to 35% saturation with $(NH_4)_2SO_4$ and the precipitate discarded. The protein precipitating between 35 and 50% saturation was collected. This pellet was extracted (50 ml solution/gm protein) once with 45%, twice with 40% and twice with 35% saturation ammonium sulfate. The salt must be removed by G-25 or dialysis before assaying. The enzyme extracted with 35% $(NH_4)_2SO_4$ had a specific activity 7 to 10-fold greater than that of the 105,000 x g supernatant. Protein was measured by the method of Lowry *et al.* (8). Table I shows the results of this procedure.

Acid and alkaline phosphatase catalyze the conversion of O-PE[3] in mammalian tissues (1,2) to ethanolamine and P_i. Horie and Shimazono (3) have also described the deamination of O-PE to glycolaldehyde phosphate in the presence of a liver homogenate and α-ketoglutarate. That ethanolamine itself is converted *in vivo* to glycine through a series of reactions involving glycolaldehyde and glycolic acid has been shown by Weissbach and Sprinson (4). In an Arthrobacter species Narrod and

[3] The abbreviations used in the text are as follows: O-PE, O-phosphoryl-ethanolamine; 2,4-DNP, 2,4-dinitrophenylhydrazine; DBC,α-(5,6-dimethyl-benzimidazolyl) cobamide coenzyme; TCA, trichloroacetic acid.

Table I

Partial Purification Procedure

[a]One unit of enzyme activity is defined as the release of 1 umole of acetaldehyde per min. mU = millunit.

[b]When this step was performed in the absence of pyridoxal-P the enzyme could be activated 20 to 30% by adding pyridoxal-P (1.62×10^{-4}M) to the assay system.

Fraction	Volume (ml)	Total mU[a]	Total Protein (mg)	S.A. mμ/ mg	% Yield
105,000 x g supernatant	333	894	1,130	0.79	100%
Ammonium Sulfate 35-50%	22	640	245	2.6	71.7
[b]35% Ammonium Sulfate Extract	6	530	81.5	6.5	59.3

Jakoby (5) demonstrated that ethanolamine was oxidized to glycolaldehyde but that O-PE was not a substrate for the enzyme. Bradbeer (6) has shown that a choline-fermenting clostridium catabolizes ethanolamine to acetaldehyde and ammonia by means of ethanolamine deaminase which requires DBC as a cofactor.

This paper describes a hitherto unreported enzyme, O-PE ammonia lyase, which is found in the soluble fraction of mammalian liver and is capable of catalyzing the reaction seen in equation 1:

$$H_3N^+CH_2CH_2OPO_3H^- \xrightarrow{\text{pyridoxal-P}} NH_4^+ + CH_3CHO + Pi \quad (1)$$

RESULTS

Proof that acetaldehyde is a product of the reaction was shown by comparing the 2,4-DNP derivative isolated from the reaction to the known purified derivative whose infrared spectra are shown in Figure 1. Thin

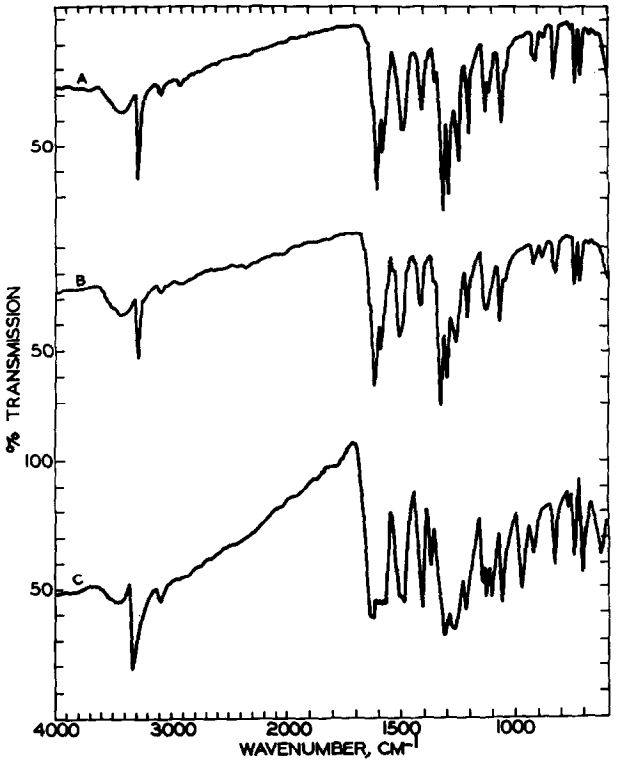

Figure 1. Infrared spectra from 1 mg of compound in a KBr pellet, (A) acetaldehyde 2,4-dinitrophenylhydrazone prepared as a standard; (B) same as A but isolated from the enzyme reaction; (C) 2,4-DNP. The derivative isolated from the enzyme reaction was obtained by trapping the acetaldehyde in the center well of a Conway diffusion chamber containing 40 mg of 2,4-DNP in 2 ml of 5N H_2SO_4. The outside well contained 100 µmoles of ^{14}C O-PE and enzyme solution giving 0.3 µmoles/min. Enzyme and substrate solutions were prepared using buffer A + 1.62 X 10^{-4} M pyridoxal-P (40 mg/l). The reaction was run 2 hours at 37° before addition of 1 ml of 10% TCA. Diffusion was allowed to proceed for 6 hours after addition of the TCA.

---

layer chromatography on Whatman MN polygram silica gel sheets in benzene:petroleum ether, 3:1 and benzene:ethyl acetate, 20:1, followed by scanning the chromatogram for radioactivity and staining with iodine vapor showed that this was the only DNP derivative formed.

Table II shows the stoichiometry of the reaction carried out in a Conway (9) diffusion chamber which was modified according to Lin and Greenburg (10). The acetaldehyde trapping solution was prepared using 0.4 gm of semicarbazide in 250 ml of 0.1 M Tris pH 7.3 at 25°. Ammonia

Table II

Stoichiometry of the Reaction Using Conway Diffusion Chambers[a]

[a]The initial reaction mixture contained 10 µmoles of O-PE and 0.5 ml of the partially purified enzyme with an activity of 0.047 µmoles/minute prepared in buffer A + 1.62×10^{-4} M pyridoxal-P. The reaction was run for 90 minutes at 37°. Reaction flasks were compared to zero time controls using 2 ml of 50% TCA or 2 ml of Na Borate pH 11.5 to stop the reaction. The values are given as µmoles ± standard deviation of quadruplicate determinations.

[b]The acetaldehyde produced is the total dpm trapped as acetaldehyde semicarbazone in the center well.

[c]The ^{14}C utilized is the total dpm remaining after 2 ml of 50% TCA was added and acetaldehyde was allowed to diffuse for 6 hours.

[d]In the case of phosphate produced, the value obtained is the total Pi in the outside well from the acetaldehyde and the ammonia determination, a total of eight separate determinations.

[e]Ammonia produced is the total gas trapped on glass rods containing 5N H_2SO_4.

[f]The O-PE utilized was determined using the ninhydrin reaction and subtracing the amount of O-PE in the reaction flasks from the values in the control flasks. O-PE tends to adhere to glass, especially in non-polar solvents such as those used in liquid scintillation. This problem can be avoided by adding 0.1 ml of 1.0N HCl to the counting vial.

Acetaldehyde[b]	^{14}C utilized[c]	Phosphate[d]	Ammonia[e]	O-PE utilized[f]
3.89 ± 0.03	3.96 ± 0.05	3.90 ± 0.09	3.90 ± 0.9	3.84 ± 0.31

was determined by the method of Cedrangolo et al. (11) except that the glass rod was placed inside the Conway diffusion flask (10). Inorganic phosphate was determined by the method of Baginski et al. (12). The ninhydrin reaction was that described in reference (13). It is apparent from the data that for each micromole of O-PE which disappears with the presence of the enzyme an equimolar amount of acetaldehyde, P_i and ammonia are formed.

It was found that pyridoxal phosphate was bound very tightly to the enzyme. Dialysis or repeated ammoniun sulfate precipitation would yield an enzyme which was only 30 to 40% dependent on the addition of pyridoxal-P

Table III

Activity of O-phosphorylethanolamine Ammonia Lyase After Treatment with Hydroxylamine[a]

[a]The native enzyme was passed through a G-25 fine column (1 X 12 cm) equilibrated with 10^{-3} M hydroxylamine in buffer A; an aliquot of this was then passed through another column equilibrated in buffer A to remove the hydroxylamine. The enzyme was now 90% dependent on pyridoxal-P for maximal activity. All assays were run at 37° at 340 mμ. The total volume of each reaction mixture is 1.0 ml containing: buffer A, 10 mM O-PE (except where ethanolamine is used), 0.282 mM NADH, 14 units alcohol dehydrogenase, 0.2 ml O-PE ammonia lyase (0.01 - 0.03 μmoles/minute).

Addition to Reaction Mix	% Activity
None	10
Pyridoxal-P (10^{-5}M)	100
Hydroxylamine (10^{-4}M) + Pyridoxal-P (10^{-5}M)	0
Ethanolamine (No O-PE present) (10^{-2}M)	0
Ethanolamine (No O-PE present) (10^{-2}M) + Pyridoxal-P (10^{-5}M)	0

for maximum activity. However, if one passes the enzyme through a G-25 sephadex column equilibrated with 10^{-3} M hydroxylamine which of itself inhibits the enzyme and then removes the hydroxylamine with another G-25 column, an enzyme preparation is obtained which is 90% dependent on the addition of pyridoxal phosphate for maximum activity (Table III). Ethanolamine itself is not a substrate for the enzyme. O-PE ammonia lyase does not utilize DBC or Vitamin B_{12} in place of or in addition to pyridoxal phosphate. p-Chloromercuribenzoate inhibits this enzyme, the inhibition being partly reversed by dithiothreitol.

The apparent Km of O-PE in the presence of saturating concentrations of pyridoxal-P was found to be 6.1×10^{-4}M. The apparent Km for pyridoxal-P was determined using the enzyme treated as in Table 2 and it was shown to be 2.7×10^{-7}M. Inorganic phosphate is a competitive inhibitor of the enzyme reaction with an apparent Km of 1.3×10^{-3}M.

DISCUSSION

Recently LaNauze and Rosenberg (14) demonstrated that 2-aminoethylphosphonate is metabolized by a transaminase found in a strain of Bacillus cereus to acetaldehyde and Pi in the presence of pyridoxal-P and pyruvate. The fact that pyridoxal-P is a cofactor for this enzyme in bacteria and for O-PE ammonia lyase in vertebrates is unique because a phosphate group is involved in the reaction instead of the classical carboxyl group. Longenecker and Snell (15) have shown the nonenzymatic deamination and dephosphorylation of a number of compounds to occur in the presence of a metal ion and pyridoxal-P. The phosphate esters of serine and threonine were reactive but O-PE was not. That O-PE ammonia lyase may function in a significant way in ethanolamine metabolism in vivo is evidenced by the finding of Sprinson and Coulon (16) that in contrast to glycine and serine, the carbon atoms of

ethanolamine preferentially label fatty acids. The conversion of O-PE to acetaldehyde by O-PE ammonia lyase may be the key step in this pathway.

A recent study by Blaschko et al. (17) demonstrated that the urine of rats on a pyridoxine deficient diet contains elevated levels of O-PE. Since O-PE ammonia lyase is a pyridoxal-P dependent enzyme, its low activity in these rats could account for these authors' findings. By a similar reasoning, the high levels of O-PE seen in patients with the familial disease, hypophosphatasia, may be the result of a lack or deficiency in O-PE ammonia lyase. This is especially plausible because of the recent findings of Danovitch et al. (18) who showed intestinal alkaline phosphatase levels in such patients to be normal. Earlier work had suggested that the disease resulted from a lack of alkaline phosphatase in the organism. Studies to confirm these hypotheses are presently under investigation in this laboratory.

ACKNOWLEDGEMENT

The authors express their thanks to Miss Ute Illie for her technical assistance.

REFERENCES

1. Smith, L. C. and Rossi, F. M., Proc. Soc. Exp. Biol. Med., 99, 754(1958)
2. Matsushima, T., Inouye, M., Higashi, K. and Oikawa, A., J. Biochem., 54, 123(1963)
3. Horie, S., and Shimazono, N., J. Biochem., 49, 768(1961)
4. Weissbach, A. and Sprinson, D. B., J. Biol. Chem., 203, 1031(1953)
5. Narrod, S. A. and Jakoby, W. B., J. Biol. Chem., 239, 2189(1964)
6. Bradbeer, C., J. Biol. Chem., 240, 4669(1965)
7. Plimmer, R. H. A. and Burch, W. J. N., Biochem. J., 31, 398(1937)
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., J. Biol. Chem., 193, 265(1951)
9. Conway, E. J., Microdiffusion Analysis and Volumetric Error, 2nd ed., Crosby Lockwood and Son, London, 1947, p.7
10. Lin, C. and Greenberg, D. M., J. Gen. Physiol., 38, 181(1954)
11. Cedrangolo, F., Salvatore, F., Cimino, F., Zappia, V., Enzymologia, 29, 143(1965)
12. Baginski, E. S., Foa, P. P., and Zak, B., Clinical Chemistry, 13, 326(1967)

13. Spies, J. R., in S. P. Colowick and N. O. Kaplan (Editors), Methods in Enzymology, Vol. III, Academic Press, New York, p. 468
14. LaNauze, J. M. and Rosenburg, H., Biochim. Biophys. Acta, 165, 438(1968)
15. Langenecker, J. B. and Snell, E. E., J. Biol. Chem., 225, 409(1957)
16. Sprinson, D. B. and Coulon, A., J. Biol. Chem., 207, 585(1954)
17. Blaschko, H., Datta, S. P., and Harris, H., Brit. J. Nutr., 7, 364(1953)
18. Danovitch, S. H., Baer, P. N., and Laster, L., N. Eng. J. Med., 278, 1253(1968)