SHORT COMMUNICATIONS

Microbial Metabolism of Amino Alcohols. Ethanolamine Catabolism Mediated by Coenzyme B₁₂-dependent Ethanolamine Ammonia-Lyase in *Escherichia coli* and *Klebsiella aerogenes*

By F. ANNE SCARLETT AND J. M. TURNER

Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX

(Received 29 December 1975)

INTRODUCTION

Ethanolamine catabolism by micro-organisms has been little studied although several different routes are known in bacteria. A pseudomonad grown on the amino alcohol as sole source of carbon and nitrogen possessed a narrowly specific ethanolamine oxidase and metabolized the glycolaldehyde formed (Narrod & Jakoby, 1958, 1964, 1966). The metabolism of ethanolamine to acetaldehyde was initially observed with strains of *Proteusmorganii* (Miyaki, Hayashi & Unemoto, 1959*a, b*), but the deamination of ethanolamine by an ammonia-lyase enzyme was first demonstrated with cell-free extracts of a *Clostridium* sp. by Bradbeer (1965*a*), who also found a cobamide coenzyme requirement (Bradbeer, 1965*b*). Considerable work has been done on the clostridial enzyme (see Barker, 1972) but little is known of the enzyme from other sources. The catabolism of ethanolamine to acetaldehyde by the sequential action of kinase and phospho-lyase (deaminating) enzymes has been found in *Erwinia* and other species (Jones, Faulkner & Turner, 1973; Jones & Turner, 1973; Faulkner & Turner, 1974*a*). In one strain of *Pseudomonas* ethanolamine was degraded by an ammonia-lyase whereas its homologue 1-aminopropan-2-ol was degraded by the kinase-phospho-lyase route (Faulkner & Turner, 1974*b*). We report here evidence for the vitamin B₁₂-dependent metabolism of ethanolamine by *Escherichia coli* and *Klebsiella aerogenes*, and some properties of ethanolamine ammonia-lyase enzymes in these bacteria.

METHODS

Organisms. *Escherichia coli* (NCIB8114) and *Klebsiella aerogenes* (NCIB8267) were obtained from the National Collection of Industrial Bacteria, Aberdeen, and were maintained at 30 °C on 2 % (w/v) nutrient agar slopes.

Media. Bacteria were cultivated in liquid media containing (g/l distilled water): glycerol, 5; K₂HPO₄, 7; KH₂PO₄, 3; Na₂SO₄, 1; MgSO₄·7H₂O, 0·1; ethanolamine, 1; vitamin B₁₂, 0·00004. The pH was adjusted to 7·0 before sterilization. Cultures were incubated at 30 °C in an orbital incubator (100 rev./min, radius of gyration 25 mm). The amount and nature of the nitrogen source was modified as indicated in the text.

Distribution of radioactivity from [¹⁴C]ethanolamine. [2-¹⁴C]Ethan-1-ol-2-amine was purchased from The Radiochemical Centre, Amersham, Buckinghamshire. Radioactivity in culture media and washed bacteria was measured as described previously (Jones & Turner, 1971, 1973).

Assay of ethanolamine ammonia-lyase activity. Bacteria were harvested in the exponential phase of growth ($E_{540}^{1.0}$, 0.8 to 1.0) by centrifuging at 5000 *g* for 20 min, and disrupted using an Aminco-French pressure cell. Cell-free extract was obtained by centrifuging at 100 000 *g* and 4 °C for 90 min. Assay mixtures contained (in a total vol. of 1 ml): Tris-HCl buffer, pH 8, 100 μ mol; ethanolamine, 2 μ mol; 5'-deoxyadenosyl cobalamin, 2 nmol; cell-free extract, 1 to 2 mg protein. Incubation was usually for 5 min at 37 °C. Reactions were started by the addition of extract, or by the addition of cofactor. The formation of acetaldehyde was measured colorimetrically with *N*-methyl-benzothiazolone hydrazone (Paz *et al.*, 1965) as described previously (Jones & Turner, 1973). Coenzyme-B₁₂ solutions were prepared immediately before use, treated to remove any hydroxocobalamin, and incubated with other components in subdued light (Joblin *et al.*, 1975).

RESULTS AND DISCUSSION

Studies on the biosynthetic utilization of [¹⁴C]ethanolamine, with a variety of bacteria, showed that vitamin B₁₂ was sometimes necessary for utilization of ethanolamine as a source of nitrogen for growth on glycerol plus mineral salts medium and for incorporation of radioactivity into cells (Clough, Shukla & Turner, 1975). *Escherichia coli* and *K. aerogenes* showed a similar response when half of the nitrogen was present as (NH₄)₂SO₄ and the rest as ethanolamine, the total amount (35 mg N/l) being growth-limiting (Fig. 1). When vitamin B₁₂ (40 μ g l⁻¹) was present initially, growth was paralleled by the incorporation of radioactivity into cells. In the absence of vitamin B₁₂, growth was incomplete and no radioactivity was assimilated. After adding the vitamin, a second phase of growth occurred and radioactivity was incorporated, although to a smaller extent. It appeared that vitamin B₁₂ was required for the deamination of ethanolamine. The presence of (NH₄)₂SO₄ did not prevent the metabolism of [¹⁴C]ethanolamine when vitamin B₁₂ was in the medium (Fig. 1*a, c*). Experiments with each nitrogen source over the range 0 to 60 mg N/l showed that ethanolamine promoted about 30 % more growth than the equivalent amount of (NH₄)₂SO₄. The reason for this is not known.

A variety of ethanolamine analogues was used to examine the specificity of the vitamin B₁₂-dependent ethanolamine deamination system. 1,3-Diaminopropan-2-ol and 2-phenylethylamine each served as a nitrogen source for the growth of *E. coli* and *K. aerogenes* on glycerol medium, but vitamin B₁₂ was not required. Analogues incapable of serving as nitrogen sources were 2-aminobutan-1-ol, 1-amino-3-diethylaminopropan-2-ol, 4-amino-3-hydroxybutyrate, 2-aminoisobutyrate, 1-aminopropan-2-ol, 1-dimethylaminopropan-2-ol, 2-hydroxy-2-phenylethylamine and 2-methylaminoethan-1-ol.

The fate of radioactivity from [¹⁴C]ethanolamine used as a growth-limiting nitrogen source (35 mg N/l) was investigated. After 20 h of growth, bacteria had assimilated about 25 % of the radioactivity supplied; they were then fractionated by the sequential extraction procedure of Roberts *et al.* (1957). For both *E. coli* and *K. aerogenes*, the 'alcohol-soluble' fraction was the most radioactive, suggesting that radioactivity had been incorporated into lipids. When [¹⁴C]ethanolamine was growth-limiting (2.5 mM), the presence of 8 mM-acetate in the medium reduced the incorporation of radioactivity by *E. coli* and *K. aerogenes* by 63 and 45 % respectively. A strong odour of acetaldehyde was noted during growth of each species on glycerol plus ethanolamine medium. It seemed likely that the vitamin B₁₂-dependent utilization of ethanolamine involved its deamination to acetaldehyde, which was further metabolized via acetate or acetyl-CoA.

Cell-free extracts of both species contained coenzyme-B₁₂ (5'-deoxyadenosyl cobalamin)-

Fig. 1. Effect of vitamin B₁₂ on the growth and assimilation of [¹⁴C]ethanolamine by *Escherichia coli* (a, b) and *Klebsiella aerogenes* (c, d). The growth medium contained glycerol as the major source of carbon and growth-limiting amounts of (NH₄)₂SO₄ (17.5 mg N/l) and [¹⁴C]ethanolamine (17.5 mg N/l) as the sole sources of nitrogen (see Methods). Vitamin B₁₂ was either present initially (a, c) or added at the time indicated by the arrows (b, d). ○, Extinction of bacterial suspension at 540 nm; ●, radioactivity assimilated by the bacteria, measured by filtering through Millipore filters and end-window counting.

dependent ethanolamine ammonia-lyases. Omission of substrate, cofactor or extract from otherwise complete reaction mixtures, demonstrated their requirement. Both enzymes had optimum activity at pH 7.5 to 8.0, the apparent K_m values for ethanolamine and coenzyme-B₁₂ were about 0.2 mM and 0.1 μ M respectively (with coenzyme-B₁₂ or ethanolamine saturating at the concentrations given in Methods), and vitamin B₁₂ (cyanocobalamin) was a potent inhibitor. Under optimum conditions, enzyme activities of 100 to 230 nmol acetaldehyde formed/min per mg protein were measured for extracts of *E. coli* and *K. aerogenes* grown on glycerol plus ethanolamine medium. The enzyme activity in extracts of these aerobically grown bacteria is thus comparable in magnitude with that in extracts of the *Clostridium* sp. grown anaerobically on semi-synthetic medium containing ethanolamine (Kaplan & Stadtman, 1968). Extracts of bacteria grown on glycerol plus (NH₄)₂SO₄ (210 mg N/l) medium were devoid of ammonia-lyase activity but growth on medium containing both (NH₄)₂SO₄ and ethanolamine as nitrogen sources (each non-limiting at 210 mg N/l) gave fully active extracts. Although ethanolamine was not needed for growth on medium containing (NH₄)₂SO₄, it induced ammonia-lyase formation. This finding accounted for the assimilation of [¹⁴C]ethanolamine in the presence of (NH₄)₂SO₄ (Fig. 1).

During the course of this work, Chang & Chang (1975) presented nutritional evidence for vitamin B₁₂-dependent ethanolamine deamination by *Salmonella typhimurium* LT2, *E. coli*

K12 and *Enterobacter aerogenes* (ATCC1033), but did not assay enzyme activities. Ethanolamine ammonia-lyases appear to be more widespread than recognized hitherto.

Part of this work was carried out with the aid of a grant from the Medical Research Council. The interest and support of Professor T. W. Goodwin, F.R.S., is gratefully acknowledged.

REFERENCES

- BARKER, H. A. (1972). Corrinoid-dependent enzymic reactions. *Annual Review of Biochemistry* **41**, 55–90.
- BRADBEER, C. (1965*a*). The clostridial fermentations of choline and ethanolamine. I. Preparation and properties of cell-free extracts. *Journal of Biological Chemistry* **240**, 4669–4674.
- BRADBEER, C. (1965*b*). The clostridial fermentations of choline and ethanolamine. II. Requirement for a cobamide coenzyme by an ethanolamine deaminase. *Journal of Biological Chemistry* **240**, 4675–4681.
- CHANG, G. W. & CHANG, J. T. (1975). Evidence for the B₁₂-dependent enzyme ethanolamine deaminase in *Salmonella*. *Nature, London* **254**, 150–151.
- CLOUGH, H. B., SHUKLA, S. D. & TURNER, J. M. (1975). Biosynthetic utilization of ethanolamine by *Erwinia carotovora*. *Biochemical Society Transactions* **3**, 769–772.
- FAULKNER, A. & TURNER, J. M. (1974*a*). Phosphorylation of ethanolamine in catabolism: biodegradative adenosine triphosphate-ethanolamine phosphotransferase and related enzymes in bacteria. *Biochemical Society Transactions* **2**, 133–136.
- FAULKNER, A. & TURNER, J. M. (1974*b*). Microbial metabolism of amino alcohols. Aminoacetone metabolism via 1-aminopropan-2-ol in *Pseudomonas* sp. NCIB8858. *Biochemical Journal* **138**, 263–276.
- JOBLIN, K. N., JOHNSON, A. W., LAPPERT, M. F., HOLLOWAY, M. R. & WHITE, H. A. (1975). Coenzyme-B₁₂-dependent enzyme reactions: a spectrophotometric rapid kinetic study of ethanolamine ammonia-lyase. *FEBS Letters* **53**, 193–198.
- JONES, A. & TURNER, J. M. (1971). Microbial metabolism of amino alcohols via aldehydes. *Journal of General Microbiology* **67**, 379–381.
- JONES, A. & TURNER, J. M. (1973). Microbial metabolism of amino alcohols. 1-Aminopropan-2-ol and ethanolamine metabolism via propionaldehyde and acetaldehyde in a species of *Pseudomonas*. *Biochemical Journal* **134**, 167–182.
- JONES, A., FAULKNER, A. & TURNER, J. M. (1973). Microbial metabolism of amino alcohols. Metabolism of ethanolamine and 1-aminopropan-2-ol in species of *Erwinia* and the roles of amino alcohol kinase and amino alcohol O-phosphate phospho-lyase in aldehyde formation. *Biochemical Journal* **134**, 959–968.
- KAPLAN, B. H. & STADTMAN, E. R. (1968). Ethanolamine deaminase, a cobamide coenzyme-dependent enzyme. I. Purification, assay and properties of the enzyme. *Journal of Biological Chemistry* **243**, 1787–1793.
- MIYAKI, K., HAYASHI, M. & UNEMOTO, T. (1959*a*). Degradation pathway of ethanolamine in *Proteus morganii*. *Chemical and Pharmaceutical Bulletin (Tokyo)* **7**, 118–122.
- MIYAKI, K., HAYASHI, M. & UNEMOTO, T. (1959*b*). Ethanolamine deaminase in *Proteus morganii*. *Chemical and Pharmaceutical Bulletin (Tokyo)* **7**, 132–133.
- NARROD, S. A. & JAKOBY, W. B. (1958). Enzymatic conversion of ethanolamine to glycolaldehyde. *Federation Proceedings* **17**, 281.
- NARROD, S. A. & JAKOBY, W. B. (1964). Metabolism of ethanolamine. An ethanolamine oxidase. *Journal of Biological Chemistry* **239**, 2189–2193.
- NARROD, S. A. & JAKOBY, W. B. (1966). Ethanolamine oxidase. In *Methods in Enzymology*, vol 9, pp. 354–357. Edited by S. P. Colowick and N. O. Kaplan. New York: Academic.
- PAZ, M. A., BLUMENFELD, O. O., ROJKIND, M., HENSON, E., FURFINE, C. & GALLOP, P. M. (1965). Determination of carbonyl compounds with N-methyl benzothiazolone hydrazone. *Archives of Biochemistry and Biophysics* **109**, 548–559.
- ROBERTS, R. B., ABELSON, P. H., COWIE, D. B., BOLTON, E. T. & BRITTEN, R. J. (1957). Studies of biosynthesis in *Escherichia coli*. *Carnegie Institute of Washington Publication* **607**, 13–14.