The absence of the soluble cytochrome *c*, however, does not prevent the oxidation of formaldehyde, formate, succinate or malate.

It is concluded from the characteristics of mutant PCT-76 that the soluble cytochrome *c* of *Pseudomonas* AM1 is not involved in the oxidation of the majority of substrates, but that it is essential for the oxidation of methanol and ethanol.

We thank the Science Research Council for financial assistance.

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The Involvement of Glycollate in the Pathway for Ethanol Assimilation in *Pseudomonas* AM1

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In *Pseudomonas* AM1 C_1 compounds are assimilated by the serine pathway (Large, Peel & Quayle, 1961); none of the enzymes of this pathway is involved in known routes of ethanol metabolism. A mutant (PCT-48) of *Pseudomonas* AM1, unable to grow on C_1 compounds or ethanol, has been isolated. This suggests a novel pathway for ethanol metabolism that has one or more enzymes common to the pathway for the assimilation of C_1 compounds.

Mutant PCT-48 has all of the known enzymes required for C_1 -unit assimilation. Further, wild-type ethanol-grown organisms have very low activities of serine-glyoxylate aminotransferase, an inducible enzyme of the serine pathway, indicating that ethanol is not metabolized by this route. Malate synthetase is the only enzyme of the glyoxylate by-pass found in ethanol-grown *Pseudomonas* AM1. The trace amount of the key enzyme isocitrate lyase (specific activity less than $0.1 \mu\text{mol/h}$ per mg) is insufficient to account for the growth rate on ethanol (mean generation time 7h).

Acetate is likely to be an intermediate in ethanol assimilation; ethanol-grown organisms contain enzymes that oxidize ethanol to acetate as well as the enzymes of the tricarboxylic acid cycle. The results of short-term incubation of ethanol-grown *Pseudomonas* AM1 with $[U-^{14}\text{C}]$ acetate indicate that malate and glycollate are early intermediates in acetate metabolism. Very little label was present at early times in succinate, fumarate, citrate, glycine or serine.

Substrate amounts of glycollate allow growth of mutant PCT-48 on ethanol or acetate. This supports the suggestion that glycollate is an intermediate in ethanol and acetate assimilation. Further,

the hydroxypyruvate reductase mutant 20-BL (supplied by Professor J. R. Quayle) does not grow on ethanol even if glycollate is present. This mutant grows on ethanol plus glyoxylate indicating that the oxidation of glycollate to glyoxylate is involved in ethanol assimilation. Malate synthetase could provide a mechanism for malate formation from glyoxylate and acetate.

A possible sequence of intermediates in the assimilation of ethanol is as follows: ethanol, acetaldehyde, acetate (or derivative), glycollate, glyoxylate, malate.

Mutant PCT-48 has possibly lost an enzyme involved in the formation of glycollate from an acetate derivative. This enzyme may also be important for growth on C_1 compounds, because mutant PCT-48 can grow on methanol only in the presence of glycollate or glyoxylate but not acetate.

We thank the Science Research Council for a grant to P.M.D.

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In Search of Lactate Dehydrogenase from *Bacillus stearothermophilus*

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In the course of a multienzyme preparation from *Bacillus stearothermophilus* (Suzuki & Harris, 1971) by using fractionation with ammonium sulphate followed by column chromatography on DEAE-cellulose and hydroxyapatite, what appeared to be a pure protein possessing both lactate dehydrogenase and alcohol dehydrogenase activities was isolated. The elution profiles of the two activities from both columns coincided exactly and the purified enzyme also appeared to be homogeneous when examined by electrophoresis on gelatinized cellulose acetate (Cellologel) at different pH values, with and without 8M-urea, as well as on polyacrylamide gel in 0.1% sodium dodecyl sulphate. It also gave a single symmetrical peak in the analytical ultracentrifuge ($s_{20,w}$ 6.0S) and the molecular weight was estimated to be $135000 \pm 3\%$ by the sedimentation-equilibrium method. The molecular weight in 0.1% sodium dodecyl sulphate was estimated to be approx. 34000, and from these data it appeared that we had succeeded in isolating a tetrameric enzyme possessing both lactate dehydrogenase and alcohol dehydrogenase activities.

Subsequently, however, while attempting to purify phosphofructokinase from the same cell extracts it was found that this activity was also associated with the lactate dehydrogenase and

alcohol dehydrogenase activities throughout the entire purification scheme. However, by repeated rechromatography under different conditions it eventually proved possible to discern a slight separation between the lactate dehydrogenase + alcohol dehydrogenase and phosphofructokinase activity peaks within the same protein peak eluted from hydroxyapatite, showing that the latter activity was associated with a different protein. It was then established that our earlier preparation of apparently 'pure' enzyme possessing both lactate dehydrogenase and alcohol dehydrogenase activities also contained an appreciable amount of phosphofructokinase.

The separation of lactate dehydrogenase + alcohol dehydrogenase from phosphofructokinase was eventually achieved by preparative isoelectric focusing (Vesterberg & Svensson, 1966). By this procedure the two enzymes were found to possess isoelectric points at pH 5.5 and 6.1 respectively. Moreover, as the former is more active towards ethanol than lactate, we conclude that our search for lactate dehydrogenase has led us instead to purify alcohol dehydrogenase and phosphofructokinase. The properties of the two enzymes are being studied and will be reported.

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Triose Phosphate Isomerase from *Bacillus stearothermophilus*

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Triose phosphate isomerase has been isolated in pure crystalline form from several sources including rabbit (Czok & Bücher, 1960) and chicken (Trentham, McMurray & Pogson, 1969) muscle, rabbit liver and yeast (Krietsch, Pentcher, Klingenberg, Hafstätter & Bücher, 1970). These enzymes possess molecular weights in the range 50 000–60 000 and have been shown to consist of two subunits. We now report the isolation and properties of triose phosphate isomerase from *Bacillus stearothermophilus*. Cell extracts were prepared as described by Suzuki & Harris (1971) and pure triose phosphate isomerase was obtained from the fraction that was precipitated with ammonium sulphate (55–70% saturation) by successive chromatography on DEAE-cellulose and hydroxyapatite. Alternatively the crude cell extract was adsorbed directly on DEAE-cellulose and the fraction eluted between 0.3M- and 1.0M-sodium chloride was treated with ethanol (2.0 vol.) at 4°C to precipitate tRNA (C. J.

Bruton & B. F. C. Clark, unpublished work). Pure triose phosphate isomerase was isolated (in a yield of approx. 65 mg/kg of cells) from the 66%-ethanol supernatant by chromatography on hydroxyapatite. Both procedures yielded pure enzyme with a specific activity of 4500 units/mg at 25°C. It gave a single band when examined by polyacrylamide-gel electrophoresis with and without 0.1% sodium dodecyl sulphate and a single symmetrical peak ($s_{20,w}$ 3.9S) in the analytical ultracentrifuge. In the presence of sodium dodecyl sulphate the molecular weight was estimated to be 27 000, indicating that the enzyme from *B. stearothermophilus*, like its counterparts from muscle (Norton, Pfuderer, Stringer & Hartman, 1970; Coulson, Knowles & Offord, 1970; Miller & Waley, 1971) and yeast (Krietsch *et al.* 1970), is composed of two subunits.

Carboxymethylation with iodo[2-¹⁴C]acetate, as well as titration with 5,5'-dithiobis-(2-nitrobenzoic acid) in 5M-guanidine hydrochloride, indicate the presence of four cysteine residues/subunit, and the results of amino acid analysis show that the subunit consists of a single polypeptide chain containing about 250 amino acid residues (including two tryptophan residues), giving a molecular weight of 27 600. Moreover a preliminary examination of tryptic peptides shows that the two subunits are of similar and probably identical sequence.

B. stearothermophilus triose phosphate isomerase appears to be stable indefinitely at room temperature and neutral pH, but is rapidly inactivated below pH 5. At 60°C (i.e. the growth temperature of the organism) in 50 mM-tris-HCl buffer, pH 8.1, it has a half-life of 4 h (as compared with a half-life of 9 min for the rabbit muscle enzyme under similar conditions).

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