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Bruton & B. F. C. Clark, unpublished work).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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