Stereospecific DPN-independent lactic dehydrogenases of *Lactobacillus arabinosus* 17.5

A DPN-independent lactic dehydrogenase specific for L(+)lactic acid has been partially purified from cell-free extracts of *Lactobacillus arabinosus*[1]. Owing to the poor yields obtained a new method of purification has been developed using chromatography on cellulose ion-exchange resins[2], after an initial purification involving $(NH_4)_2SO_4$ fractionation, protamine sulphate treatment and batchwise elution from DEAE-cellulose.

Chromatography of the partially purified lactic dehydrogenase on a second DEAE-cellulose column resolved the activity in two peaks (Fig. 1). The lactic dehydrogenase was assayed as described previously[1] by measuring the reduction of 2,6-dichlorophenolindophenol in the presence of DL-lithium lactate. Re-examination of the activity of each peak using D(—)sodium lactate and L(+)sodium lactate in separate assays revealed that the first peak marked (1 in Fig. 1) contained predominantly D(—)lactic dehydrogenase while the second peak (2 in Fig. 1) contained predominantly L(+)lactic dehydrogenase activity.

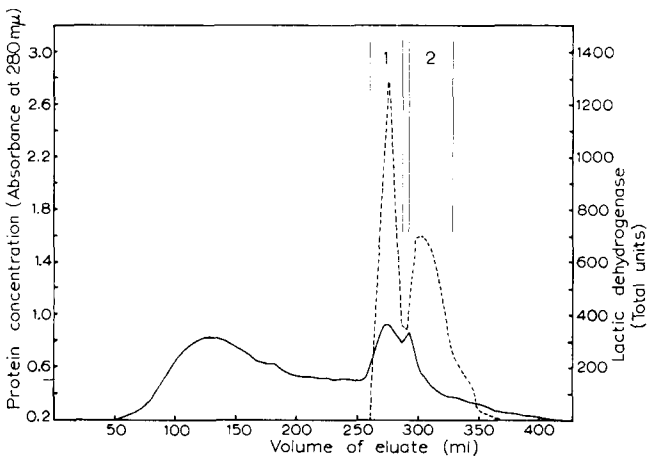

Fig. 1. Chromatography of purified lactic dehydrogenase on DEAE-cellulose. 40 mg protein applied to the column (20 × 2 cm) equilibrated in 0.005 *M* Tris-maleate (pH 7.5), 0.002 *M* sodium lactate. Elution with sodium lactate (pH 7.5) using a linear gradient to 0.3 *M*. Solid line, protein; dotted line, lactic dehydrogenase. A unit of lactic dehydrogenase was defined as described previously[1].

Rechromatography of the second peak on a CM-cellulose column completely separated the L(+)lactic dehydrogenase, which was eluted as a sharp symmetrical peak, from the small amount of contaminating D(+)lactic dehydrogenase (Fig. 2).

Rechromatography of the first peak on an identical CM-cellulose column resulted in the complete separation of the D(—)lactic dehydrogenase (eluted at 110 ml see Fig. 2) from the small amount of contaminating L(+)lactic dehydrogenase (eluted at approx. 15 ml, Fig. 2). However, quantitatively there was considerably less D(—)

Abbreviations: DPN, diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; TPNH, reduced triphosphopyridine nucleotide; Tris, tris(hydroxymethyl) aminomethane; DEAE-cellulose, diethylaminoethyl cellulose; CM-cellulose, carboxymethyl cellulose.

lactic dehydrogenase (as taken on a protein basis). This probably explains why only the L(+)lactic dehydrogenase had been detected previously[1].

The L(+)lactic dehydrogenase sedimented as a single sharp symmetrical peak (as observed by Schlieren optics, $S_{20,w} = 9.46$) in the Spinco Model E ultracentrifuge. This result, together with the elution patterns obtained with the two different types of cellulose columns, suggested that the L(+)lactic dehydrogenase was homogeneous.

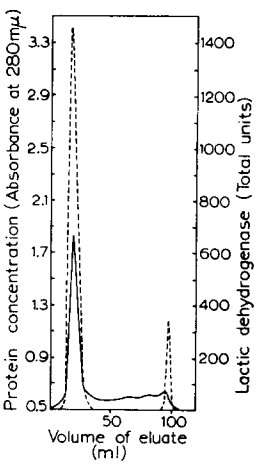

Fig. 2. Purification of L(+)lactic dehydrogenase on CM-cellulose. 10 mg protein from peak 2 of Fig. 1 applied to a CM-cellulose column (20 × 1 cm) equilibrated in 0.005 *M* Tris–maleate (pH 6.0), 0.002 *M* sodium lactate. Elution with Tris–maleate buffer using a linear gradient to 0.01 *M* and pH 7.5. Solid line, protein; dotted line, lactic dehydrogenase. The minor peak eluted at approx. 100 ml represents contaminating D(—)lactic dehydrogenase.

The two stereospecific lactic dehydrogenases showed different pH optima, K_m values and heat stabilities. Oxalate gave differing degrees of inhibition with the two enzymes, while *p*-chloromercurybenzoate only gave slight inhibition.

Neither enzyme reduced DPN (or TPN) in the presence of lactate nor oxidized DPNH (or TPNH) in the presence of pyruvate. Thus these two enzymes appear to be quite different from the stereospecific lactic dehydrogenases purified from *Lactobacillus plantarum* by DENNIS AND KAPLAN[3].

The L(+)lactic dehydrogenase showed a normal protein absorption spectrum and did not contain any bound DPN, TPN or lipoic acid. Also the enzyme did not contain any flavin as previously suggested[1] although it is capable of reducing a natural flavoprotein derived from the organism.

The fact that the lactic dehydrogenases described here are capable of reducing indophenol would suggest either (a) they contained some, as yet, undetected cofactor or that (b) due to some inherent property of the protein, hydrogen may be passed directly to indophenol without involving any intermediate carrier.

These aspects of the properties of the two enzymes are being investigated further and will be reported later in a more detailed publication.

The author is indebted to Dr. J. H. COATES of the Department of Agricultural Chemistry for the ultracentrifugal examination of the L(+)lactic dehydrogenase and to Dr. E. S. HOLDSWORTH of this department for the samples of DEAE and CM-cellulose and for helpful advice and discussion during this work.

This work was supported by a grant from the National Health and Medical Research Council.

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Received July 20th, 1959