Lactate dehydrogenase isoenzymes from human heart: separation by preparative gel electrophoresis; and a crystalline preparation of isoenzyme 1.

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A flat-bed apparatus for preparative gel electrophoresis with intermittent elution [1-3] permits the collection simultaneously of anode- and cathode-migrating components of a mixture. We employed this first for the separation of isoenzymes of lactate dehydrogenase (EC 1.1.1.27) in crude tissue extracts. Enzyme activity was determined by reaction rate observed at 340 nm in the presence of 0.14 mM NADH, 0.66 mM sodium pyruvate and 100 mM tris-acetate, pH 7.4. Units are according to Wroblewski and La Du [4]. Human hearts were obtained from the autopsy room within 24 h after death. Pieces of heart muscle (2-3 g) were chilled and washed twice in ice cold 0.15 M sodium chloride. The tissue was subsequently minced and homogenized using a Top-Drive homogenizer (M.S.E.) at top speed for 3-4 min. The resultant suspension was filtered through muslin, the filtrate centrifuged at 100,000 g for 20 min at 4°C and the supernatant was used for separation by preparative electrophoresis with results shown in Figure 1.

Heart muscle revealed three peaks of lactate dehydrogenase activity. The major activity was found in isoenzyme 1. Each of the separated zones gave a single band on analytical electrophoresis after staining for enzyme activity. Thus isoenzymes 1, 2, and 3 are easily and completely separated from each other in a single run.

Fig. 1. Separation of human heart-muscle lactate dehydrogenase isoenzymes by preparative polyacrylamide gel electrophoresis. In a similar experiment with liver, not illustrated, isoenzyme 5 emerged and was collected at the cathode end of the gel.

Purification of human heart muscle lactate dehydrogenase isoenzyme 1 was then conducted, in the first stages by conventional methods. Using a combination of ammonium sulphate fractionation, DEAE-cellulose chromatography, acetone precipitation and, again, ammonium sulphate precipitation, we found that the heart lactate dehydrogenase activity was only enriched 20-fold. The product obtained had a specific activity of 101,000 units/mg protein and was found to be contaminated with isoenzymes 2 and 3. In view of these results, we fractionated the above product by preparative polyacrylamide gel electrophoresis using the flat-bed apparatus described previously [1-3]. The recovery of enzyme activity from the gel was 85%. The active fractions were pooled and concentrated by ultrafiltration. The specific activity of the enzyme after this step was 1.1 x 10^6 units/mg protein.

This enzyme solution was crystallized by dialysis against 35% saturated ammonium sulphate solution also containing EDTA (1 mM) and β-mercaptoethanol (1 mM) for 40 h at 4°C. The insoluble material was removed by centrifugation and the supernatant was then transferred into a small cellophane bag tied on to a magnetic stirrer. The bag was then inserted into a beaker containing the same salt solution. The salt saturation in the beaker was brought to 65% by slow addition of a saturated ammonium sulphate solution also containing EDTA (1 mM) and β-mercaptoethanol (1 mM) with stirring. After 4-5 days the resulting crystalline precipitate was examined under the microscope, which revealed the presence of lactate dehydrogenase isoenzyme 1 crystals [Fig. 2B].

Fig. 2(A). Analytical polyacrylamide gel electrophoresis patterns of lactate dehydrogenase. From left to right, (a) crude heart extract, (b & c) crystalline isoenzyme 1.

2(B). Crystals of heart lactate dehydrogenase isoenzyme 1 (magnification x 100).