BBA 66398

ELECTROPHORETIC AND CHROMATOGRAPHIC SEPARATION OF PHOSPHOENOLPYRUVATE CARBOXYKINASES

F. J. BALLARD
CSIRO, Division of Nutritional Biochemistry, Kintore Avenue, Adelaide, S.A. 5000 (Australia)

(Received February 23rd, 1971)

Electrophoresis of phosphoenolpyruvate carboxykinases on acrylamide gel gives marked separation between the cytosol enzyme from rat liver and the mitochondrial enzyme from chicken liver. These enzymes are also resolved on hydroxylapatite chromatography as are the mitochondrial and cytosol enzymes from sheep liver. The experiments indicate that the two activities found in livers of most mammalian species do indeed represent distinct enzymes.

INTRODUCTION

Unlike most enzymes, the intracellular distribution of phosphoenolpyruvate carboxykinase is not uniform but differs from one species to another. This enzyme is confined to the cytosol fraction of rat or mouse liver[1], the mitochondria of pigeons and chickens[2], and is in both hepatic cell compartments of most other species[1,3]. While a physiological explanation of these differences has not been found, it is established that the mitochondrial enzyme is constitutive and the cytosol enzyme adaptive to changes in gluconeogenesis[4,5]. Although differences have been found in the kinetic[6,7] and immunochemical[8] properties of phosphoenolpyruvate carboxykinases from cytosol and mitochondrial fractions of liver, no differences between physical properties of the enzymes have been reported.

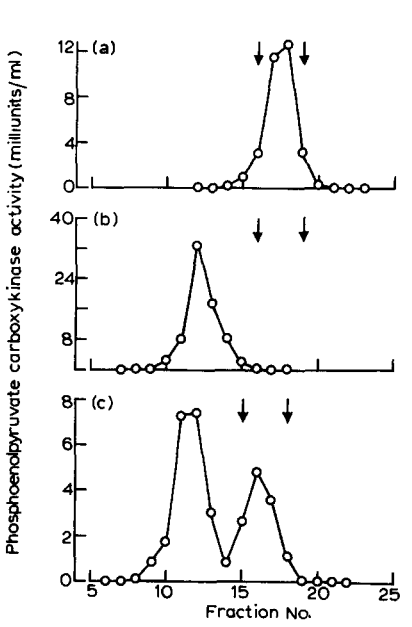
Phosphoenolpyruvate carboxykinase was assayed by the carboxylation method of CHANG AND LANE[9]. The enzyme samples used were purified from either the cytosol fraction of rat liver or washed mitochondria from chicken liver[8]. Both enzymes were homogenous on Sephadex G-100 chromatography, disc electrophoresis and hydroxylapatite chromatography. In each case the specific activity at 37° was 12 to 14 μmoles bicarbonate fixed per min per mg protein.

Electrophoresis of phosphoenolpyruvate carboxykinase was accomplished using the principle of disc electrophoresis with 3-mm-thick gels polymerised between two plates 70 mm × 70 mm. The methods of DAVIS[10] were followed with Tris–glycine (pH 8.3) as buffer. Portions of enzyme (20 μl) in 40% sucrose were layered beneath the buffer and on to the stacking gel with the aid of a 12-compartment spacer. In

this way several different samples of enzyme could be electrophoresed on a single gel slab .A buffer temperature of 6–8° was maintained by passing the buffer through a plastic bag immersed in an ice bath (Gradipore Apparatus, Townson and Mercer Pty. Ltd., Sydney, Australia). After electrophoresis for 4 h at 140 mV and 20–30 mA an internal haemoglobin marker had separated into two distinct bands and migrated 3–4 cm into the separating gel. For measurements of phosphoenolpyruvate carboxykinase activity the gels were cut longitudinally and then traversely into 1.5-mm sections. Each section was homogenised in 1.5 ml of 0.05 M imidazole–HCl (pH 7), centrifuged, and 200 μl of the supernatant used for enzyme assay.

Columns of hydroxylapatite were prepared by suspending 4 g of dry resin (Biorad HPT) in 10 mM potassium phosphate (pH 7.0) and pouring into a column with 1 cm diameter. Enzyme fractions that had been equilibrated with 10 mM potassium phosphate (pH 7.0) by passage through a Sephadex G-25 column were applied to the hydroxylapatite and eluted with 120 ml of a linear gradient from 10 to 250 mM potassium phosphate (pH 7.0).

The separation of the mitochondrial phosphoenolpyruvate carboxykinase from chicken liver and the cytosol enzyme from rat liver has been achieved by disc electrophoresis (Fig. 1). In this system the cytosol enzyme migrates towards the anode at approximately the same mobility as haemoglobin and a considerably greater



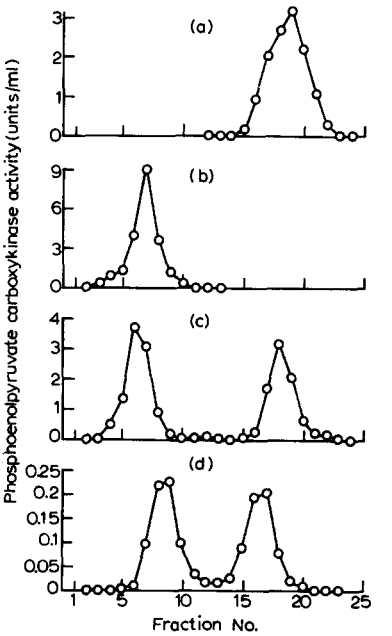


Fig. 1. Separation of phosphoenolpyruvate carboxykinase activities on disc electrophoresis. Fractions are numbered from the beginning of the separating gel. (a) Purified cytosol enzyme from rat liver, (b) Purified mitochondrial enzyme from chicken liver. (c) Mixture of both enzymes. Arrows indicate the migration of added sheep haemoglobin.

Fig. 2. Chromatography of phosphoenolpyruvate carboxykinases on hydroxylapatite columns. (a) Purified cytosol enzyme from rat liver. (b) Purified mitochondrial enzyme from chicken liver. (c) Mixture of both purified enzymes. (d) Crude extract of sheep liver prepared by mixing a cytosol fraction with an extract of freeze-dried mitochondria.

mobility than the mitochondrial enzyme. Similar results have been reported for malate dehydrogenase[11], aspartate aminotransferase[12] and isocitrate dehydrogenase (NADP)[13] where in all cases the cytosol enzyme migrates more rapidly towards the the anode than the respective mitochondrial enzyme. It is not known whether the consistency of this effect has any general importance.

A difficulty with the electrophoretic separation of phosphoenolpyruvate carboxykinases is that the recovery of enzyme is not usually greater than 30% and is much lower when crude enzyme preparations are applied to the gel. Attempts to overcome this recovery problem by performing the electrophoresis at lower pH resulted in poor separation of the enzymes.

A second successful method for the separation of the two phosphoenolpyruvate carboxykinase activities is chromatography on hydroxylapatite columns. With homogenous phosphoenolpyruvate carboxykinases the mitochondrial enzyme was loosely bound to the column and eluted with low concentrations of phosphate at pH 7, while the cytosol enzyme required approximately 0.15 M phosphate for elution. The two activities were completely separated and as long as the chromatography was carried out at 2° and at a flow rate sufficient to complete the procedure in 3 h, the recovery of each enzyme was quantitative. Furthermore, this separation method was applicable to large volumes of dilute enzyme as both activities were adsorbed on to the hydroxylapatite, and could also be used with impure enzyme preparations. An example is shown in Fig. 2d. Here cytosol and mitochondrial extracts from sheep liver have been combined and passed through a hydroxylapatite column. The separation of these two phosphoenolpyruvate carboxykinases is evident.

When considered with the previous kinetic[7] and immunochemical[8] data, these experiments offer strong evidence that the two phosphoenolpyruvate carboxykinases found in liver do indeed represent distinct enzymes with different physical properties.

ACKNOWLEDGEMENTS

I am grateful to the Queen Elizabeth II Fellowship Committee for financial assistance and to Miss J. M. Robinson for performing the electrophoretic separations.

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