The Distribution of Two Chromatographically Distinguishable Forms of Phosphofructokinase in the Tissues of the Rat

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The glycolytic sequence is required to subserve different functions in different tissues, so that, although the intermittent provision of utilizable energy may be regarded as its primary function in skeletal muscle, facilitation of the production of C_2 units from glucose is probably more important in the liver. Currently, control of glycolysis is usually explained in terms of the properties of three enzymes: hexokinase, phosphofructokinase and pyruvate kinase. Two of these, namely hexokinase and pyruvate kinase, are already known to have isoenzymes, and it is becoming clear that many of the distinguishing characteristics in the control of glycolysis in different tissues are achieved by variations in the proportions of these isoenzymes in the tissues. It would therefore be of interest to know whether the third enzyme, phosphofructokinase (ATP-D-fructose 6-phosphate 1-phosphotransferase, EC 2.7.1.11), also has isoenzymes and to determine to what extent the distribution and properties of these might influence the nature of glycolytic control from tissue to tissue. Layzer, Rowland & Bank (1969) have already reported that phosphofructokinases from human muscle and erythrocytes may be distinguished electrophoretically and chromatographically as well as in some kinetic properties. The work reported here is an investigation of two chromatographically distinguishable forms of phosphofructokinase in rat tissues, the method of separation being an elaboration of that described by Layzer *et al.* (1969).

Phosphofructokinase activity was assayed spectrophotometrically by the method of Underwood & Newsholme (1965), in which the reaction is coupled to those of aldolase and α -glycerophosphate dehydrogenase in the presence of triose phosphate isomerase. For all tissues except blood, extracts to be used for chromatography were prepared as follows: a 1-in-4 (w/v) homogenate made in 100 mM-tris-phosphate buffer, pH 8.0, containing 0.2 mM-EDTA and 1 mM-dithiothreitol (solution I) was centrifuged at 157 000g for 30 min. Blood was haemolysed by dilution with 6 vol. of 1 mM-dithiothreitol and centrifuged at 5000g for 15 min. The supernatant fluids from the centrifuged tissue

extracts were removed and used in chromatography without further treatment.

Two methods of DEAE-cellulose chromatography were employed. In method I, which was essentially similar to that of Layzer *et al.* (1969), the material was run on to a DEAE-cellulose column (10 cm long $\times$ 0.9 cm diam.) previously equilibrated with solution I, 25 ml of this same solution was run through the column and thereafter the enzyme was eluted on a linear gradient from solution I to 0.8 M-tris-phosphate buffer, pH 8.0, containing 0.2 mM-EDTA and 1 mM-dithiothreitol (solution II), 2 ml fractions being collected from the column. In method II a longer column was used (30 cm long $\times$ 0.9 cm diam.) and the enzyme was eluted in two stages. The material was run on to the column and treated with 25 ml of solution I as before and then eluted with 40 ml of 260 mM-tris-phosphate buffer, pH 8.0, containing 0.2 mM-EDTA and 1 mM-dithiothreitol (solution III), followed by a linear gradient from solution III to solution II. All procedures were carried out at 2-5°C.

Initially the phosphofructokinases of several tissues were tested by method I, elution by a simple linear gradient. It was found that the muscle enzyme gave a sharp elution peak with its maximum corresponding to 190 mM-tris-phosphate buffer. No other elution peak was ever found for the muscle enzyme in these experiments. The liver enzyme gave a much broader elution band, which overlapped the muscle-enzyme band to some extent but had a maximum at about 350 mM-tris-phosphate buffer. This method would not effect the separation of a mixture of the liver and muscle enzymes, but yielded a single broad elution band spanning the range of the elution peaks characteristic of the two tissues. A similar result was noted by Layzer *et al.* (1969) for mixtures of muscle and blood phosphofructokinases. Kidney phosphofructokinase gave a single peak with shoulders, the maximum value occurring at about 260 mM-tris-phosphate buffer. This pattern clearly suggests a mixture of approximately equal quantities of muscle-type and liver-type enzyme. The brain, adipose-tissue and heart enzymes all gave elution patterns with peaks similar to that of the muscle enzyme, but each showed a

marked tail at higher concentrations of buffer consistent with the view that in these tissues the muscle-type enzyme was mixed to various extents with liver-type enzyme. The blood enzyme had a broad peak rather similar to that of the liver enzyme.

A successful separation of the two forms was accomplished by method II, in which the muscle enzyme is eluted with 260 mM-tris-phosphate buffer before application of the gradient (Fig. 1). This result reinforces the view that the muscle contains only the one form of the enzyme (muscle-type), whereas the liver, although showing a preponderance of another type of phosphofructokinase (liver-type), also apparently contains about 5–10% of the muscle-type enzyme. This technique was used to carry out a preliminary screening of rat tissues to determine the distribution of the two forms of phosphofructokinase. It should be stressed that in such a treatment there is the tacit assumption that only two forms of the enzyme exist and also that fractions of phosphofructokinase from different tissues but exhibiting similar elution patterns are in fact identical. This may prove to be an oversimplification, and testing by other criteria will certainly be necessary. The relative amounts of the two types of enzyme were determined by

measuring the areas under the peaks in the elution diagrams. The results are conveniently expressed as the percentages of the total enzyme activity eluted by 260 mM-tris-phosphate buffer. The following results were obtained: muscle, 100%; liver, 5–10%; whole blood, 16%; brain, 80%; adipose tissue, 58%; kidney, 50%. These values are entirely consistent with the elution patterns found for these tissues with method I.

It will clearly be of importance to determine the nature of the phosphofructokinase in tumour tissue in view of the dramatic changes noted in the isoenzyme patterns of pyruvate kinase and hexokinase in the rapidly growing Morris hepatomas (Criss, 1969; Farina, Adelman, Lo, Morris & Weinhouse, 1968; Taylor, Morris & Weber, 1969). In preliminary experiments we found that the enzyme from ascites cells of the Novikoff hepatoma appeared to be still predominantly of the liver type, its elution pattern being qualitatively similar to that of the enzyme extracted from liver. Similarly, preliminary chromatographic analysis of the enzyme from the livers of alloxan-diabetic and starved rats gave no indication of gross changes in the proportions of the two forms of the enzyme.

These studies strongly suggest that phosphofructokinase of rat tissues exists in two forms,

Fig. 1. Elution of phosphofructokinases of liver and muscle from DEAE-cellulose. In each case the column was 30 cm long $\times$ 0.9 cm diam., and 2.0 ml fractions were collected. The enzyme was eluted with 260 mM-tris-phosphate buffer, pH 8.0, containing 0.2 mM-EDTA and 1 mM-dithiothreitol (solution II) applied to the column from tube 1 to tube 20. At tube 21 a linear gradient was applied to the column from solution II to 800 mM-tris-phosphate buffer, pH 8.0, containing 0.2 mM-EDTA and 1 mM-dithiothreitol. The enzyme activity unit is expressed as μ mol of substrate converted/min at 25°C. (a) Muscle enzyme; (b) liver enzyme; (c) muscle enzyme + liver enzyme.

designated here muscle-type and liver-type. Purification and detailed comparison of the properties of the two forms of phosphofructokinase will be necessary before any metabolic advantage in their particular distribution can be assessed.

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