Tissue-Characteristic Forms of Glyceraldehyde 3-Phosphate Dehydrogenase

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Glyceraldehyde 3-phosphate dehydrogenase exists in two different forms in various human tissue preparations. One of them is exhibited, after starch-gel electrophoresis, by a rapidly migrating or 'fast' band and the other by a 'slow' band. The proportion of the total activity in each of the two forms is characteristic of the type of tissue. A particulate fraction, obtained after centrifugation of homogenates, inhibits the enzyme activity and tends to convert the slow band into a fast one. The conversion is reversible. The fast band can also be converted into the slow one by addition of NAD^+ or ADP, or by dialysis against saturated sodium chloride solution. Conversions occur with the purified enzyme as well as with crude homogenates. The relevance of these findings to previous investigations and to glycolytic control mechanisms are discussed.

Glyceraldehyde 3-phosphate dehydrogenase is one of the enzymes that has been considered important in the control of glycolytic activity (Maitra, Ghosh, Schoener & Chance, 1964). Various reports indicate that changes in the conformation of the enzyme can be induced by substances such as NAD^+ (Durchslag, Puchwein, Kraty, Schuster & Kirschner, 1969; Kirschner, 1968) and that the enzyme may be bound (together with other glycolytic enzymes) to structural components of the cell (Arnold & Pette, 1968; Fahimi & Karnovsky, 1966; Green *et al.* 1965). The binding of the enzyme to particulate components of tissue preparations appears to be highly dependent on ionic strength.

The present report describes the use of high-resolution starch-gel electrophoresis to separate forms of glyceraldehyde 3-phosphate dehydrogenase. This method can be a sensitive means for the detection of different forms or conformations of the same enzyme molecule, since the rate of migration depends on molecular shape as well as on molecular size and mobility (Smithies, 1965).

EXPERIMENTAL

Purified enzymes. Suspensions of crystals of rabbit muscle glyceraldehyde 3-phosphate dehydrogenase in $(\text{NH}_4)_2\text{SO}_4$ (obtained from both Sigma Chemical Co., St Louis, Mo., U.S.A., and Schwartz BioResearch Inc., Mount Vernon, N.Y., U.S.A.) were used. Most preparations catalysed the reduction of 20–40 μmol of NAD^+ /min per mg of protein at 25°C.

Preparation of tissue homogenates and supernatants. Samples of human tissues were obtained at autopsies,

generally within one day after death. Portions of whole organs were used. The tissues were homogenized at 3°C with a glass homogenizer in 1.5 ml of water per g wet wt. The tissues could be stored frozen at –22°C for several days before homogenization but the homogenates had to be used on the same day that they were prepared to avoid loss of activity. Supernatant and particulate fractions were prepared by centrifuging the homogenate at 14000g for 15 min at 3°C. Water, purified by passage through a general-purpose exchange resin made by Crystal Research Laboratories Inc., Hartford, Conn., U.S.A., was used throughout.

Starch-gel electrophoresis. Electrophoresis was carried out horizontally for 18 h at 3°C (Ressler & Tuttle, 1963). The starch was prepared as described by Smithies (1965) and was obtained from the Connaught Laboratories, Toronto, Canada. The potential gradient was not critical and was generally about 4 V/cm. The buffer used for both the gel and electrode vessels contained 0.05 M-glycine and 0.03 M-LiCl, at pH 8.9. The glycine and LiCl were dissolved in slightly less than the final volume, adjusted to pH 8.9 with 4 M-NaOH, and then brought to volume. 0.05 M-Potassium phosphate buffer, pH 8.6, containing 3 mM-KCl, could also be used, but other buffers did not resolve the glyceraldehyde 3-phosphate dehydrogenase bands. One volume of each homogenate or supernatant was diluted with one volume of a solution containing 0.05 M-tris and 4 mM- β -mercaptoethanol, pH 7.3, before application. The dilution with this concentration of mercaptoethanol resulted in sharper bands. Applications were made by saturating a piece of Whatman no. 17 filter paper with each sample, and inserting it in the gel.

Detection of enzyme activity after electrophoresis. After electrophoresis, the gels were sliced and the bands containing glyceraldehyde 3-phosphate dehydrogenase activity were detected by a modification of the methods used by Williams (1964) and Lebherz & Rutter (1967).

Fructose 1,6-diphosphate (0.4g) and aldolase (0.8ml) were added to 8.0ml of 0.1 M-tris-HCl buffer, pH 7.5. The mixture was incubated at 37°C for 30 min. (The aldolase was a Sigma preparation, with 10mg of aldolase/ml. One mg converted 8.7 μ mol of fructose 1,6-diphosphate into dihydroxyacetone phosphate and glyceraldehyde 3-phosphate/min at pH 7.4 and 25°C.) Difco Noble agar (0.4g) was added to 100ml of 0.05 M-potassium phosphate buffer, pH 8.5. After dissolving the agar by heating to boiling and cooling to 50°C, enough of the solution was poured off to leave 70ml. The following were then added to it in the order given: the incubated aldolase mixture, 80mg of NAD⁺, 40mg of Nitro-Blue Tetrazolium and 0.8ml of aqueous phenazine methosulphate (2mg/ml). The solution was then poured over each starch-gel surface to a depth of about 1mm and the incubation was conducted in the dark at room temperature. After the bands developed, the gels were immersed overnight in ethanol-acetic acid-water (5:1:4, by vol.). The use of agar in the staining solution resulted in a better resolution by decreasing the rate of diffusion. When glyceraldehyde 3-phosphate was added directly, instead of having been generated by aldolase, the results were the same. If the substrate had been omitted, no bands were detected [except for the 'nothing dehydrogenase' in liver tissue, which could readily be identified (Shaw & Koen, 1965; Ressler & Stitzer, 1967)].

Assay of glyceraldehyde 3-phosphate dehydrogenase activity. Total glyceraldehyde 3-phosphate dehydrogenase activities were determined as described by Racker, Klybas & Schramm (1959) but with some modifications to make the assay conditions more comparable with those used for the detection of activity on the starch gels. Each

absorption cell contained 2.6ml of the glycine buffer used for electrophoresis, 0.1 ml of 42.7 mM-NAD⁺, 0.1 ml of 1 M-K₂HPO₄, 0.1 ml of the enzyme solution and 0.2 ml of the aldolase preparation described above (added last). The diluent for enzyme solutions was the glycine buffer, containing 2 mM- β -mercaptoethanol. Measurements were made within the range in which the initial slopes were proportional to the enzyme concentrations.

RESULTS

Fig. 1 shows the results obtained with various human tissues. Glyceraldehyde 3-phosphate dehydrogenase activity is essentially restricted to two bands, a rapidly migrating 'fast' band, and a 'slow' band. The mobility of each is constant regardless of the tissue or the amount of enzyme applied (if not overloaded).

In the supernatant fractions, the proportions of the total activity found in the fast and in the slow band were characteristic for the different tissues. In supernatants of heart, skeletal muscle and liver, essentially all of the activity was in the slow band. A trace of the fast band was observed in a few skeletal muscle or liver supernatants, but it never constituted more than a very small fraction of the activity in the slow band. The activity in supernatants of kidney tissues was approximately equally divided between the slow and the fast bands. In the other tissues studied, all or most of the activity was in the fast band. Several dozen

Fig. 1. Glyceraldehyde 3-phosphate dehydrogenase activity in human tissues. For each tissue, the homogenate is on the left, and the supernatant is on the right. The three samples of liver are from the same organ, homogenized and run on different days. All other samples are from separate organs. Migration towards the anode is upward.

samples were analysed over a period of about a year without any contradictions to these classifications.

Fig. 1 also shows that the total activity in the homogenate was usually much less than in the corresponding supernatants. Enzyme activities were therefore determined in the spleen supernatant fraction with and without the addition of the spleen particulate fraction at the same relative concentration as in the original tissue. It was found that in the presence of the particulate fraction, the activity was decreased by approx. 50%.

Since the homogenates generally showed a greater proportion of the total activity in the fast band than did the respective supernatants, attempts were made to see whether the particulate fraction would convert the slow band into the fast one. When 1g of a liver particulate fraction (with no detectable activity by itself) was added to 1ml of a heart supernatant fraction (with activity only in the slow band), the activity of the mixture was almost entirely converted into that of the fast band. Additions of particulate fractions to purified rabbit muscle glyceraldehyde 3-phosphate dehydrogenase also converted most of the original, slow band into the fast one. Similar conversions were produced by the addition of 1mg of thymus RNA (Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.) to 1ml of either various tissue supernatant fractions or purified enzyme. (All mixtures were incubated for 2–2½ h at 23°C before application to the gel. The

purified glyceraldehyde 3-phosphate dehydrogenase was at a concentration of 0.24mg/ml.)

Dialysis of various tissue supernatants at 3°C for 3h against 0.1M-potassium phosphate buffer, pH 7.3, containing 2mM-β-mercaptoethanol and saturated with sodium chloride, converted all of the fast bands into slow ones. Similar conversions occurred when samples of purified glyceraldehyde 3-phosphate dehydrogenase, in which fast bands had been produced by the addition of a particulate fraction, were dialysed in the same way. When the dialysis against saturated sodium chloride was followed by a second one for 1½ h against the same buffer, but without sodium chloride, the original fast bands were reproduced.

Additions of ADP or NAD⁺ also converted the fast band into a slow one (Fig. 2). NAD⁺ was more effective when added to the gel rather than to the sample (Fig. 2c).

DISCUSSION

The findings reported in this study show that two different forms of glyceraldehyde 3-phosphate dehydrogenase exist and that the proportion of the total activity in each form varies in different tissues. Conversion of one form into the other indicates that the two forms do not differ in their primary structure and the reversibility of the conversions suggests that they are not caused by selective destruction of enzyme activity.

A number of investigators have proposed that glycolytic activity is affected by the binding of a complex of glycolytic enzymes to structural components of the cell. Such binding appears to be decreased in tissues capable of high glycolytic activity, or when a high ionic strength is used for the extraction of the enzyme (Arnold & Pette, 1968; Margreth, Catani & Schiaffino, 1967; Poon & Wood, 1968; Starlinger, 1967).

In the present study it is shown that cell particulate fractions can convert a 'slow band' form of glyceraldehyde 3-phosphate dehydrogenase into a 'fast band' form, and that dialysis at high ionic strengths, which would be expected to disrupt any association of a cell particulate component with the enzyme, caused conversion back into the 'slow band' form. Further, the 'slow band' form, which may be assumed to correspond to enzyme unassociated with the cell particulate components, predominates in the more active tissues such as liver, skeletal muscle and heart. These results raise the possibility that the existence of two forms of the enzyme may have functional significance in the mammalian cell, as has already been suggested may be the case in *Chromatium* (Hudock, Mellin & Fuller, 1965). The findings that conversion of the 'slow band' form into the 'fast band' form by the cell particulate

Fig. 2. Conversion of glyceraldehyde 3-phosphate dehydrogenase bands in supernatants by ADP or NAD⁺. ADP was added to a concentration of 30mM, and NAD to a concentration of 1.3mM. In (c) NAD⁺ (0.7mM) was added to the starch gel before electrophoresis. The heart supernatant is included in (b) and (c) for comparison.

fraction occurs with a decrease in total activity, and that NAD^+ and ATP can bring about conversion in the reverse direction, are also consistent with this possibility. Reference has already been made to the facts that the conformation of the enzyme is altered by NAD^+ and that ADP is an activator of phosphofructokinase and other kinases.

These results, in conjunction with those of other studies, make it intriguing to consider the possibility that substrates and activators liberate certain groups of a glycolytic enzyme complex from associations with structural components, which permits coordinated conformational changes into active forms. An exhaustion of the supply of substrates and activators might then result in the reverse changes and inactivation.

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