UDP-GLUCOSE DEHYDROGENASE FROM THE CHICK EMBRYO: TISSUE-SPECIFIC FORMS OF THE ENZYME[+]

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Tissues of the chick embryo contain two principal forms of UDP-glucose dehydrogenase (EC 1.1.1.22). These forms can be demonstrated by starch gel electrophoresis of partially purified samples of the enzyme. The more acidic form has been found only in the liver. The more basic form occurs in several other tissues, including the eye. The existence of the two forms has been confirmed by analyzing enzyme prepared from mixtures of eye and liver, by the technique of isoelectric focusing. The two forms also differ with respect to their inhibition by the feed-back inhibitor, UDP-xylose.

We have found two electrophoretically distinct forms of UDP-glucose dehydrogenase in chick embryo, one predominant in liver and the other in the eye. These two forms also differ in their response to inhibition by UDP-xylose, which has been shown by Neuffeld and Hall (1) to have the kinetic behavior of an allosteric feedback inhibitor. Such differences in the properties of the enzyme in the different tissues of the chick could reflect differentiation from one tissue to another with respect to the metabolic function of the reaction product, UDP-glucuronic acid.

EXPERIMENTAL: The chick embryos (15-day, white leghorn) were killed by decapitation. The gall bladder was removed from the liver, and the eyes were punctured and squeezed to drain off the clear fluids, which were discarded. Both tissues were frozen by

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immersion in liquid nitrogen and stored at -60° until used. Tissues could be stored for at least 3 months without detectable alteration of the properties of the enzymes.

Tissues were ground to a homogeneous paste with an equal weight of abrasive grain alumina in a mortar and pestle which was chilled in a salt-ice mixture. All subsequent operations were performed at 0-4° except for adjustments of pH which were made at room temperature. The paste was suspended in three times the weight of the tissue of 150 mM KCl, 10 mM 2-(N-morpholino) ethanesulfonate (MES), 1 mM EDTA, 0.5 mM dithiothreitol (DTT), pH 7.0, at 0°, and centrifuged for 50 minutes at 17,000 rpm in the SS-34 rotor of the Servall RC-2-B centrifuge. Details of all subsequent centrifugations were the same unless specified otherwise. The supernatant solution was brought to 0.35 saturation with solid $(NH_4)_2SO_4$ (20.9 g/100 ml), stirred for 30 minutes and centrifuged for 20 minutes. The supernatant solution was brought to 0.6 saturation with solid $(NH_4)_2SO_4$ (16.4 g/100 ml), and stirred and centrifuged as before. The resulting precipitate was taken up in a minimal volume (about 0.2 ml per g of eye and 0.7 ml per g of liver) of 50 mM potassium phosphate buffer, 1 mM EDTA, 0.5 mM DTT, pH 7.3, referred to as buffer I. The resulting solution was dialysed for 6 hours against 100 volumes of buffer I, in cellulose dialysis tubing 0.64 cm in diameter which had been washed in distilled water and presoaked in buffer I for 4 hours. The contents of the bag were stored overnight at 4° and then centrifuged in the number 30 rotor of the Spinco Model L Centrifuge at 27,000 rpm for 1 hour. Ten ml of the supernatant solution was then applied to a column of DEAE-cellulose, 1.5 cm x 22 cm, equilibrated with buffer I. The column was developed with buffer I at 0.5 ml/min, and 1 ml fractions were collected. The fractions were assayed (see the legend to Fig. 3 for the assay

method and definition of unit), and the most active fractions were combined, representing 90% of the total activity. Typical preparations from liver or eye had about 0.01 units/mg protein.

Salt was removed from the enzyme preparations prior to electrophoresis or isoelectric focusing by dialysis for 6 hr versus 1% glycine, 0.5 mM DTT, pH 6. The enzyme preparations were stored frozen in small portions at -60°.

RESULTS AND DISCUSSION: Starch gel electrophoresis of enzyme prepared from eye and enzyme prepared from liver gave the patterns shown in Fig. 1. Control incubations without UDP-glucose, or with glucose and UMP were negative. It can be seen that the identity

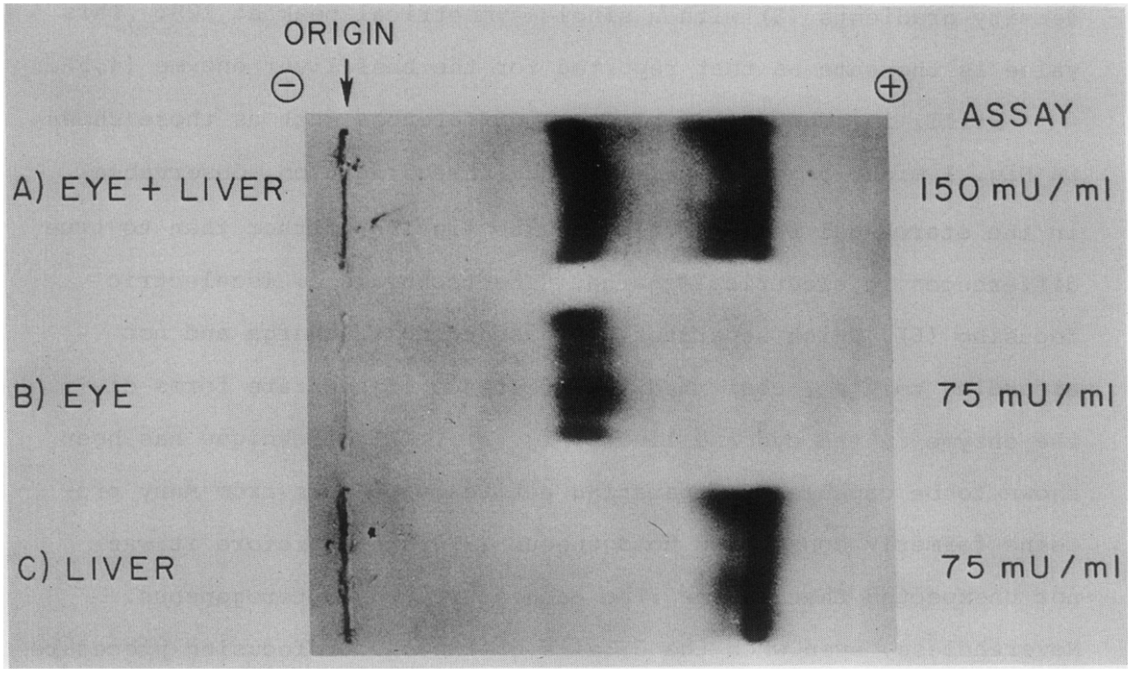

Fig. 1. Starch gel electrophoresis of UDP-glucose dehydrogenase from chick embryo. Equal volumes of enzyme solutions, assayed as in Fig. 3 and containing 0.5 mM DTT, were placed in each slot. Electrophoresis was performed at 4° at 400 V for 3 hr, using the discontinuous buffer system (pH 8.4) and apparatus described by Scopes (2). The gel was stained with a freshly prepared solution containing 1 ml 0.01 M UDP-glucose, 1 ml 0.01 M NAD^+, 1 ml 0.1 M NaCl, 1 ml 5 mM $MgCl_2$, 2.5 ml 0.5 M potassium phosphate buffer, pH 7.4, 2.5 ml nitro blue tetrazolium (1 mg/ml), and 0.25 ml phenazine methosulfate (1 mg/ml). The gel was incubated at 37° for 1 hr to develop the stain. Similar gels had 15 to 20 bands when stained with Amido Black.

of the bands and the relative intensity of staining was maintained when a mixture of the two enzyme preparations was run. Although we have observed, in other experiments, that the banding patterns in starch gel also depended on the nature and the amount of sulfhydryl compound present in the enzyme solution (0.5 mM DTT in Fig. 1), the two principal forms of the enzyme seen in Fig. 1 could always be distinguished. Other tissues of the 15-day chick embryo, including bone, heart, intestine, muscle, and brain, gave patterns like that of the eye.

Enzyme from eye did not appear to differ from enzyme from liver with respect to molecular weight, since each sedimented in sucrose density gradients (3) with a single symmetrical peak at 12S. This value is the same as that reported for the beef liver enzyme (4,5).

Still, it seemed possible that differences such as those shown in Fig. 1 might be due to differences in aggregation, observable in the starch gel because of molecular sieving, rather than to true differences in electrical charge. The technique of isoelectric focusing (6), which separates only according to charge and not according to size, confirmed the existence of separate forms of the enzyme in the eye and liver (Fig. 2). This technique has been shown to be capable of separating active components from many proteins formerly considered homogeneous (7), and therefore it was not unexpected that enzyme from each source was heterogeneous. Nevertheless, even when the details of the electrofocusing procedure were varied slightly, or a mixture of both enzymes was present, enzyme from one source was always clearly distinguishable from enzyme from the other source. Enzyme from eye gave a single principal peak near pH 7.0, with additional minor components, whereas enzyme from liver gave a double peak at around pH 6.5.

In order to show that the observed differences did not arise

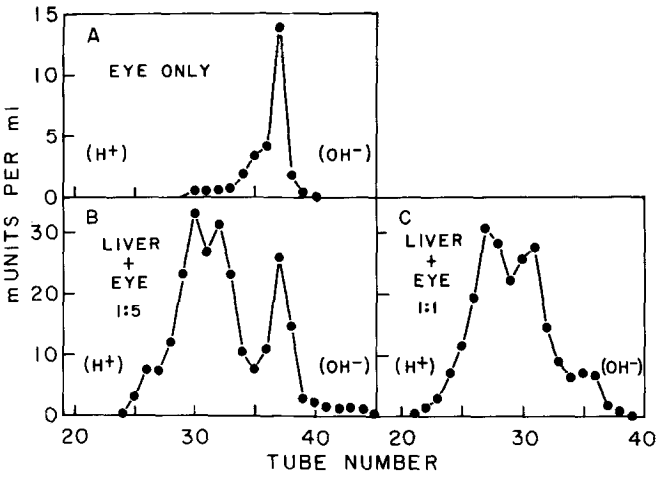

Fig. 2. Electrofocusing of UDP-glucose dehydrogenase prepared from mixed tissues of chick embryo. Runs were performed according to the instructions supplied with the equipment (8101 column) and chemicals provided by LKB Instruments, Rockville, Md.. All runs were done at 4°, 1% ampholyte, 690 V, with the anode at the bottom of the column, and assayed for enzymatic activity as in Fig. 3. A: 0.32 units (0.01 units/mg protein) of enzyme purified from eye, run for 70 hr.; pH of peak = 7.1; ampholyte range, pH 5-7; 0.083 units recovered; 3 ml fractions. B: 0.50 units (0.009 units/mg protein) of enzyme purified from a mixture of 5 g liver and 25 g eye, run for 45 hr.; pH of peaks = 6.5, 6.5, 6.8; ampholyte range, pH 5-8; 0.44 units recovered; 2 ml fractions. C: 0.51 units (0.009 units/mg protein) of enzyme purified from a mixture of 12 g liver and 12 g eye, run for 45 hr.; pH of peaks = 6.4, 6.6, 6.9; ampholyte range, pH 5-7; 0.33 units recovered; 3 ml fractions.

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during the preparation of the enzymes, for example by deamination or limited proteolysis, preparations were made from mixtures of the two tissues. The patterns shown in Fig. 2B and 2C come from such preparations, made with a weight ratio of liver to eye of 1:5 and 1:1, respectively. Since liver contained much more activity than eye, in agreement with our findings with individual tissues, the areas of the liver peaks in 2B and 2C are relatively constant. The eye peak in 2B was several times the size of the eye peak in 2C, reflecting the relative amount of tissue originally present. These data suggest that neither enzyme is modified during the preparative procedure.

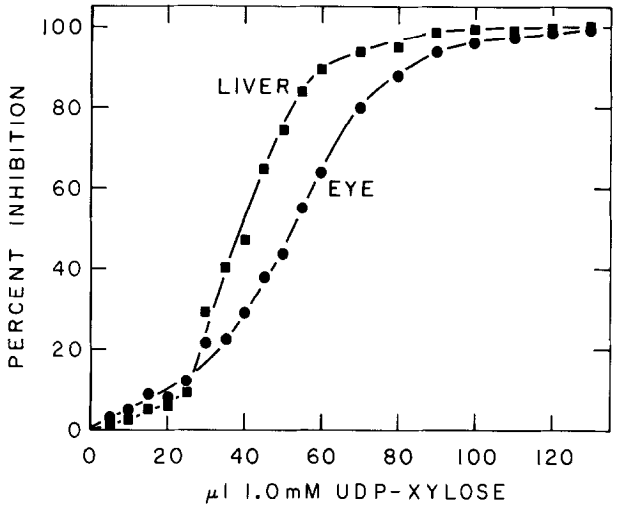

Fig. 3. Inhibition of UDP-glucose dehydrogenase from chick embryo by UDP-xylose. Enzyme from eye, (●); enzyme from liver, (■). Reactions were run at 25° in a cell having a 1-cm light path with a total volume of 0.62 ml, containing the stated amounts of UDP-xylose, plus 100 μmoles of sodium glycylglycinate buffer, pH 8.7, 50 μmoles of NAD^+, 50 μmoles of UDP-glucose, 50 μmoles of DTT, and 0.5 mg of crude bovine serum albumin. The reactions were started by the addition of 2.3×10^{-3} units of eye enzyme, or 3.2×10^{-3} units of liver enzyme, and the reaction monitored by the change in light absorption at 340 nm. One unit of enzyme (forming one μmole of NADH per minute when saturated with substrate) gives a change of optical density of 10 per minute in this reaction mixture.

The difference between enzyme from eye and from liver with respect to inhibition by UDP-xylose, shown in Fig. 3, suggests a functional difference between the two proteins. The possibility of isozymes having differing sensitivity to UDP-xylose was suggested by Neuffeld and Hall in their original description of inhibition of the enzyme by UDP-xylose (1).

The data in Fig. 3, when plotted using a form of the equation proposed by Hill for the binding of oxygen by hemoglobin (8), give a coefficient, n, of approximately 4 for the eye and 5 for the liver enzyme. These values for the Hill coefficient, which may reflect both the number of interacting sites and the degree of interaction between them (9), are considerably higher than those found by

Bdolah and Feingold (10) in enzyme from mature hen oviduct, where $n = 1.25$. Such a discrepancy could reflect a difference in cooperativity between enzyme from embryonic and adult tissue. A number of sources of UDP-glucose dehydrogenase have been examined by Neuffeld and Hall (1), and by Feingold and his colleagues (10,11,12), who found values for n from 1.0 (no cooperativity) to 2.3, for inhibition by UDP-xylose.

The observations reported here raise two general questions. 1) What is the structural basis for the distinction between the two forms of the enzyme? 2) Is there a functional differentiation, *in vivo*, between the two forms, as suggested by the difference in sensitivity to UDP-xylose?

References

1. Neufeld, E. F., and Hall, C. W., Biochem. Biophys. Res. Commun., 19, 456 (1965).
2. Scopes, R. K., Biochem. J., 107, 139 (1968).
3. Martin, R. G., and Ames, B. N., J. Biol. Chem., 236, 1372 (1961).
4. Wilson, D., Anal. Biochem., 10, 472 (1965).
5. Zalitis, J., and Feingold, D. S., Arch. Biochem. Biophys., 132, 457 (1969).
6. Vesterberg, O., and Svensson, H., Acta Chem. Scand., 20, 820 (1966).
7. Susor, W. A., Kockman, M., and Rutter, W. J., Science, 165, 1260 (1969).
8. Hill, A. V., Biochem. J., 7, 471 (1913).
9. Atkinson, D. E., Hathaway, J. A., and Smith, E. C., J. Biol. Chem., 240, 2682 (1965).
10. Bdolah, A., and Feingold, D. S., Biochim. Biophys. Acta, 159, 176 (1968).
11. Ankel, H., Ankel, E., and Feingold, D. S., Biochemistry, 5, 1864 (1966).
12. Bdolah, A., and Feingold, D. S., J. Bact., 96, 1144 (1968).