THE MOLECULAR WEIGHT AND SOME KINETIC PROPERTIES OF CRYSTALLINE RAT LIVER GLUTAMATE DEHYDROGENASE*

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Glutamate dehydrogenases have been isolated and studied from a large number of sources. In general, the enzyme isolated from non-animal sources is not affected by purine nucleotides and is specific either for DPNH or TPNH, while enzyme from animal sources is markedly affected by purine nucleotides like GTP and ADP, and can utilize either TPNH or DPNH almost equally well as coenzyme (Frieden, 1965). Furthermore, in all cases examined, except for the dogfish liver enzyme (Corman et al., 1967), the enzyme from animal sources undergoes a reversible association-dissociation reaction. Enzymes found to readily associate have been crystallized from such diverse sources as frog liver, chicken liver and beef liver.

The present communication deals with the purification and crystallization of the rat liver enzyme. The enzyme appears to be quite different from all other mammalian glutamate dehydrogenases which have been tested in that it

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does not appear to undergo an association reaction and it responds differently to purine nucleotides. Antigenic differences between the rat and beef liver enzyme have been noted previously (Talal and Tomkins, 1964).

In view of the fact that rat liver mitochondria are frequently the preferred system for studying metabolic pathways (e.g. glutamate metabolism) and that the glutamate dehydrogenase is an important enzyme in such studies, we report here some of the characteristics of this enzyme and indicate some of its differences from the commercially available beef liver glutamate dehydrogenase.

Enzyme Preparation: After several unsuccessful attempts were made to apply the procedures used in the purification of glutamate dehydrogenase from other sources, the enzyme was purified from rat liver acetone powder as follows: Acetone powder was extracted for 1 hour with 10 volumes of cold 0.05 M potassium phosphate buffer (pH 7.4). The supernatant fluid was treated as described by Olson and Anfinsen (1952) up to and including the step in which the acid-ethanol precipitate is taken up in a minimal volume of 0.05 M potassium phosphate buffer (pH 7.4). This solution was brought to 60% saturation with cold, saturated ammonium sulfate, and the resultant precipitate was collected by centrifugation at 10,000 rpm and 0°C and dissolved in a minimal volume of the phosphate buffer. The enzyme was precipitated in a 20-60% saturated ammonium sulfate cut and was subsequently taken up in a minimal volume of 0.1 M Tris-HCl buffer (pH 7.0) containing 10^{-4} M EDTA. Residual ammonium sulfate was removed by dialysis in the cold for 24 hours against 0.1 M Tris-HCl buffer (pH 7.0) containing 10^{-4} M EDTA and 10^{-3} M ADP. The dialyzed enzyme solution was then diluted to a protein concentration of about 50 mg/ml with the same buffer and incubated at 46°C for 30 minutes. Denatured protein was removed and the supernatant fluid was passed through a DEAE-Sephadex column equilibrated with 0.02 M potassium phosphate buffer (pH 7.0). The enzyme was eluted with a 0-0.5 M KCl gradient and was precipitated from the column effluent at 60% saturation with ammonium sulfate. The precipitate was

dissolved in a minimal volume of 0.02 M potassium phosphate buffer (pH 7.0), and the enzyme crystallized after the solution was brought to slight turbidity with ammonium sulfate and allowed to stand for several days. After 4 or 5 recrystallizations, a 200-fold purification over the extract had been attained.

RESULTS

Physical properties of the enzyme: In 0.02 M potassium phosphate buffer (pH 7.0) the enzyme sedimented as a single, sharp, symmetrical peak in the ultracentrifuge with a sedimentation coefficient of 12.9 S. At an enzyme concentration of 2.5 mg/ml there was no evidence for any association to higher molecular weight forms.

The enzyme was then examined in 0.1 M Tris-HCl buffer (pH 8), 10^{-4} M EDTA, under conditions which force either association or dissociation of the beef liver glutamate dehydrogenase, i.e. in the presence of 10^{-4} M DPNH with either 10^{-3} M ADP or 10^{-3} M GTP, respectively. The sedimentation coefficient in the presence of DPNH and ADP was 11.7 S and identical to that in the presence of DPNH and GTP (11.9 S). No evidence for heterogeneity was observed in either case, indicating that under these conditions there is no tendency of the enzyme to undergo a reversible association-dissociation reaction.

The molecular weight of the enzyme was determined in 0.02 M potassium phosphate buffer (pH 7.0) by low speed sedimentation equilibrium using the scanner attachment to the Spinco Model E ultracentrifuge. Plots of log O.D. vs x^2 were invariably linear and gave an average molecular weight, after several determinations, of 350,000 ± 20,000.

Kinetic properties: Kinetic studies were initially carried out in 0.01 M Tris-acetate buffer, pH 8.0, 10^{-5} M EDTA, 100 μM DPNH (or TPNH), 5 mM α-ketoglutarate, and 20 mM NH_4Cl. The decrease in optical density at 340 mμ was measured spectrophotometrically and the initial velocities were recorded using a 0.1 optical density full scale. However, such assays were extremely

non-linear and this non-linearity was not corrected by the addition of 10^{-3} M dithiothreitol or 10^{-3} M phosphate, or by changing the pH of the buffer. Assays were linear in the presence of purine nucleotides and 0.1 mg/ml albumin, but both of these substances were considered undesirable as standard assay components. The addition of 3% glycerol to the assay mixture was finally used to stabilize the enzyme and achieve nearly linear assays without affecting the actual kinetic properties of the enzyme of either the rat or the beef liver.

The response of rat liver glutamate dehydrogenase to various purine nucleotides was examined with either DPNH or TPNH as coenzyme. The kinetic results are summarized in Table I and are compared with those previously obtained with the beef liver enzyme (Frieden, 1963; 1965). From the data obtained with DPNH or TPNH as the coenzyme, it can be seen that the binding constants for the various nucleotides are similar for the rat and beef liver glutamate dehydrogenases. However, the two enzymes differ quite markedly in the extent to which their initial velocities are affected by these nucleotides. The inhibition in the presence of GDP and GTP is so nearly complete that any differences between the two enzymes are not distinguishable. It was also found that increasing the concentration of DPNH causes a decrease in the binding constant for ATP and a change in the effect of this nucleotide upon the enzyme from activation to inhibition. However, lowering the concentration of DPNH has the opposite effect. The apparent dissociation constant for ATP is increased and the ability of the nucleotide to activate the enzyme is also increased. It must be mentioned here that the rates obtained with both DPNH and TPNH were still slightly non-linear at the lower concentrations of purine nucleotides even in the presence of 3% glycerol. However, at higher nucleotide levels, the non-linearity disappeared (DPNH) or was greatly decreased (TPNH). This stabilization probably accounts for some of the activation of the rat liver enzyme by ADP and ATP, but by no means eliminates the differences in response to these

TABLE I

Experiments were performed in 0.01 M Tris-acetate buffer, pH 8, 10^{-5} M EDTA, 3% glycerol, at 25°C. The DPNH or TPNH concentration was 100 μM and α-ketoglutarate and NH_4^+ ion were present at saturating levels. v'/v is the ratio of initial velocities in the presence and absence of saturating concentrations of the purine nucleotide being studied.

		DPNH		TPNH	
		Rat	Beef	Rat	Beef
ADP	Ka (μM)	29	30	26	15
	v'/v	9	2	18	3.5
ATP	Ka (μM)	--*	25	125	40
	v'/v	3.5	0.6	9	1
GDP	Ki (μM)	1.8	2	15	5
	v'/v	≤ 0.02	≤ 0.05	0.15	0.1
GTP	Ki (μM)	0.08	0.1	0.5	0.4
	v'/v	≤ 0.04	≤ 0.04	0.04	0.05

* Not determined

nucleotides of the rat liver and beef liver enzymes.

Initial studies using 10 mM glutamate and 1 mM DPN or TPN in 0.01 M Tris-acetate buffer (pH 8) with 10^{-5} M EDTA and 3% glycerol were performed. At high levels of the various purine nucleotides, the degree of activation or inhibition observed was quite similar to that previously reported for the beef liver enzyme, except that ATP caused slight inhibition of the DPN reaction, whereas with the beef liver glutamate dehydrogenase a low level of activation had been found (Frieden, 1963).

DISCUSSION

It has been shown that the various molecular weight forms of beef liver glutamate dehydrogenase bind purine nucleotides preferentially (Frieden and Colman, 1967). Thus GTP binds better to monomer (of 400,000 molecular weight) than to the polymer, while ADP binds better to the polymer than to the monomer. That this type of preferential binding may be important in control of the activity by purine nucleotides has been previously discussed in terms of a rather general mechanism (Frieden, 1967). It is of considerable interest, therefore, that this mechanism must be lacking in the rat liver enzyme. Perhaps some of the differences in purine nucleotide effects from the beef liver enzyme are reflected in the inability of the rat liver enzyme to undergo association. This conclusion is somewhat strengthened by the observation of Corman et al. that the dogfish liver enzyme, which also does not undergo association, is somewhat different kinetically (Corman et al., 1967). However, the dogfish is considerably lower on the phylogenetic scale than is the rat or for that matter the chicken, the glutamate dehydrogenase of which does undergo an association-dissociation reaction.

Studies are underway to define more closely the differences between the rat and beef liver enzymes.

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