GLUTAMATE DEHYDROGENASE IN NUCLEAR AND MITOCHONDRIAL FRACTIONS OF RAT LIVER

G. di Prisco*, M. Banay-Schwartz and H.J. Strecker

Department of Biochemistry
Albert Einstein College of Medicine, Yeshiva University
New York, N.Y. 10461

Received October 11, 1968

Summary. Two glutamate dehydrogenases appear to be present in rat liver, one associated with nuclei, and the other with mitochondria. The two enzymes differ in regard to activation by inorganic phosphate, in the effects of pH on activity, in apparent Km values for some of the substrates and in the shape of curve obtained by a plot of NAD concentration versus activity.

Glutamate dehydrogenase, a key enzyme in metabolic pathways interconverting amino acids and carbohydrates, is distributed widely and has been extensively studied (1). It has been generally accepted that this enzyme is located in the mitochondria of animal cells, and indeed it has been sometimes used as a marker for these organelles. In fact, glutamate dehydrogenase activity found in nuclear fractions has been ascribed to contaminating mitochondria (2,3). However, more recent studies in our laboratory on enzymatic components of rat liver nuclei indicated that glutamate dehydrogenase, and several other enzymes, showed a bimodal distribution, in this case being found in nuclei as well as in mitochondria (4). This observation prompted an investigation of glutamate dehydrogenase activity in rat liver nuclei and in mitochondria, which revealed major differences between the two activities both from each other and from the well characterized crystalline enzyme from ox liver. These different

* Present address, International Laboratory of Genetics and Biophysics, Naples, Italy.

properties include the "tightness" of binding to the particulate structure, the response to inorganic phosphate, effect of pH on activity, and the apparent K_m values for the substrates, glutamate, α-ketoglutarate, NH_4^+ and NAD. Especially interesting is the observation that the activity in rat liver nuclei provides a sigmoid curve when rate is plotted versus concentration of NAD.

Methods. Rat liver nuclei were prepared essentially by the method of Hymer and Kuff (5). After the final washing, the sedimented nuclei were suspended in 0.1M potassium phosphate solution, pH 7.6 in a volume one-third of the original homogenate volume and sonicated for 2 minutes. After centrifugation at 105,000 x g for 60 minutes, all of the glutamate dehydrogenase activity was recovered in the supernate. If phosphate were omitted from the suspending medium, the activity was retained in the sedimented nuclear fragments. Rat liver mitochondria were isolated by conventional techniques (6). The particles were suspended in 0.25M sucrose solution and sonicated for 2 minutes. About 60-70 per cent of the glutamate dehydrogenase activity was recovered in the supernate obtained after centrifuging at 105,000 x g for 1 hr. Activity was measured at 23°-25° by recording either the initial reduction of NAD with glutamate added (forward reaction) or the initial oxidation of $NADH_2$ with α-ketoglutarate and NH_3 added (back reaction) at 340 mμ. The activity is expressed as μmoles per minute per ml of enzyme solution. The concentration of the nuclear preparation accounts for the greater activity shown in the figures. The extinction coefficient for $NADH_2$ of 6.22×10^6 (7) was used to calculate activity.

RESULTS

Isolation of nuclear and mitochondrial enzymes. A number of investigators have reported that although glutamate dehydrogenase is predominantly in the mitochondrial fraction of rat liver, appreciable amounts are found also in the nuclear fraction. Thus up to 18 per cent of the total activity in a homogenate has been reported present in washed nuclear fractions obtained

from homogenates prepared in 0.25M sucrose solutions (3). Although this activity in preparations of nuclei generally has been ascribed to mitochondrial contamination, we observed that nuclei prepared with the aid of Triton and Mg^{++} according to Hymer and Kuff (5) and retaining very little succinic dehydrogenase activity, still contained 25 per cent or more of the total glutamate dehydrogenase activity as assayed by oxidation of $NADH_2$. The total amount of enzyme activity associated with the nuclear fraction depended on the concentration of Mg^{++} in the homogenizing medium, as will be reported elsewhere. Magnesium ions did not affect the activity associated with the mitochondrial fraction and thus was not transferring enzyme from one compartment to the other.

Solubilization. The mitochondrial enzyme was brought readily into solution by fragmenting mitochondria suspended in 0.25M sucrose solution. Under otherwise identical conditions, the nuclear enzyme required the addition of 0.1M potassium phosphate (pH 7.6) for solubilization. In agreement with previous reports, disruption of the mitochondrial fraction caused an approximately 10-fold increase of glutamate dehydrogenase (2,3), whereas no increase was obtained with the nuclear fraction under the same conditions.

Effect of pH on activity. In the forward direction and under the specific assay conditions shown in Fig. 1, the pH-activity curve for the nuclear enzyme was considerably steeper, at each side of the optimum value, than the corresponding curve for the mitochondrial enzyme. The pH optima for both enzymes were however not too different at values of 8.0-8.5. The pH-activity curves in the reverse direction were rather similar for both enzymes with an optimum at about pH 7.6 (Figs. 1A and 1B).

Effect of phosphate on activity. A striking effect of phosphate solutions was observed on the nuclear enzyme. At pH 9.0, addition of increasing concentrations of potassium phosphate increased the rate of the forward reaction up to 12-fold at an optimum phosphate concentration of 0.2M. Under the same conditions the mitochondrial enzyme was slightly inhibited (Fig. 2A). At this pH, the reverse reaction was not affected by phosphate with either en-

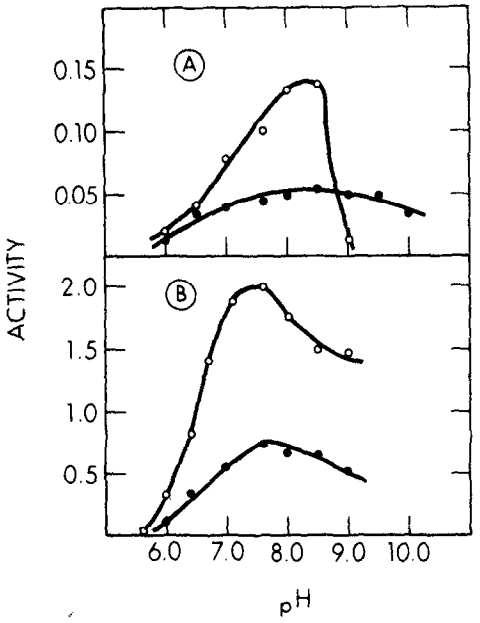

Fig. 1. Effect of pH on activities of rat liver nuclear (o—o) and mitochondrial (•—•) glutamate dehydrogenases. Assays were carried out in 50 mM potassium phosphate buffer; 50 mM Tris-HCl was substituted for determinations above pH 9.0. The other components of the reaction mixture were 8.3 mM potassium glutamate, 50 μM NAD (A, forward reaction); 1.25 mM α-ketoglutarate, 50 μM $NADH_2$, 50 mM NH_4Cl (B, back reaction).

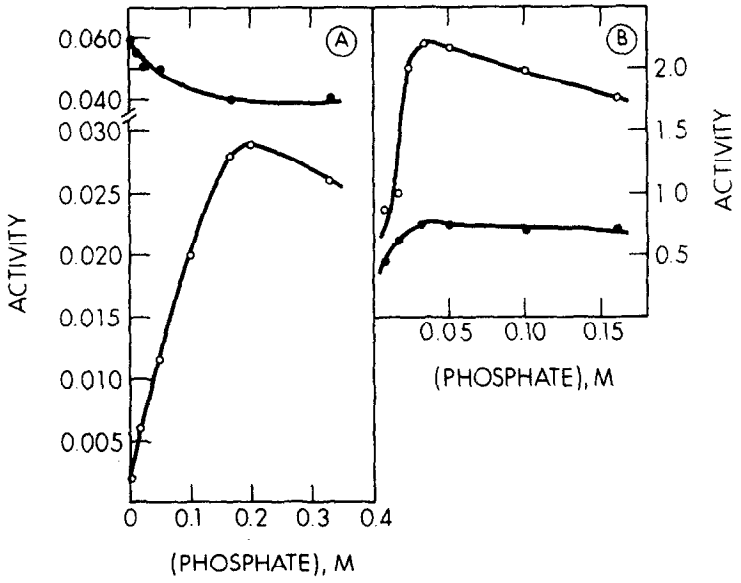

Fig. 2. Effect of phosphate on activities of rat liver nuclear (o—o) and mitochondrial (•—•) glutamate dehydrogenases. Assays were carried out at A, pH 9.0 with 25 mM potassium glutamate, 50 μM NAD; B, pH 7.6 with 1.25 mM α-ketoglutarate, 50 μM $NADH_2$, 50 mM NH_4Cl.

zyme. In contrast, at pH 7.6, the forward reaction catalyzed by either enzyme was relatively unaffected by phosphate, whereas the back reaction catalyzed by the nuclear enzyme was strongly enhanced by 0.05M phosphate (Fig. 2B).

Comparison of the data for the velocities for the forward and back reactions under different conditions of pH and phosphate concentrations underlined a significant differentiation of the two enzymes. The ratio of rates of the back and forward reactions at the conditions for optimum velocities, was about the same for both nuclear and mitochondrial enzymes being 14 and 12 respectively. However, at pH 9.0 and in the absence of phosphate, the nuclear glutamate dehydrogenase is so poorly active in the forward direction, that the ratio of the back to the forward reaction approaches 1100. Under these conditions the corresponding ratio for the mitochondrial enzyme is still about 12.

Kinetics. Plotting NAD concentration versus activity for the mitochondrial enzyme produced a modified hyperbolic curve, which as a double reciprocal plot yielded a biphasic curve characteristic also for the crystalline beef liver enzyme (8,9). However, the direct plot of velocity as a function of NAD concentration for the nuclear enzyme yielded a sigmoid curve similar to that described for certain allosteric enzymes (10). This sigmoid curve was markedly altered by addition of potassium phosphate to the incubation mixture in the direction of the same type of modified hyperbolic curve characteristic of the rat mitochondrial and crystalline beef liver enzymes (Fig.3).

Table I presents the apparent Michaelis constants for the several substrates with the two enzymes. The apparent K_m values were lower for glutamate, α-ketoglutarate and NH_4Cl with the nuclear enzyme, and higher for NAD. The apparent K_m for $NADH_2$ appeared to be the same for both enzymes.

Other characteristics which appear to be qualitatively similar for the two enzymes of rat liver were inhibition by higher concentrations of α-ketoglutarate, NH_4Cl or $NADH_2$, stimulation by ADP and inhibition by GTP. Higher concentrations of glutamate inhibited only the nuclear enzyme. Inhibition

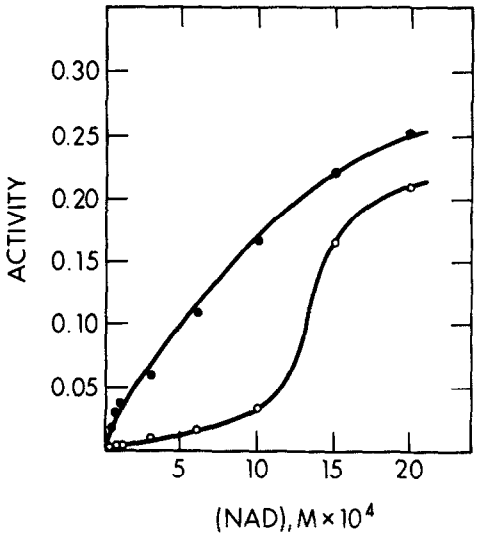

Fig. 3. Reaction velocity as a function of NAD concentration, at pH 9.0, in the absence (o—o) and presence (•—•) of 0.166 M potassium phosphate buffer. The other components were 25 mM potassium glutamate and NAD at the concentrations indicated.

Table I. Apparent Michaelis constants of the reaction substrates for rat liver nuclear and mitochondrial glutamate dehydrogenase

	Apparent K_m values (mM)	
	nuclear enzyme	mitochondrial enzyme
Glutamate	0.91	4.35
α-Ketoglutarate	0.133	0.45
NH_4Cl	11.0	30.0
NAD	0.058	0.022
$NADH_2$	0.023	0.023

When used at fixed concentration, glutamate was 25 mM; α-ketoglutarate 1.25 mM; NH_4Cl 50 mM; NAD 50 μM; $NADH_2$ 50 μM. The forward reaction was carried out at pH 9.0 in 50 mM potassium phosphate buffer; the reverse reaction at pH 7.6, in 50 mM potassium phosphate buffer.

by $NADH_2$ or GTP, and activation by ADP, are characteristic also of the crystalline beef liver enzyme.

Discussion. The demonstration of the presence of glutamate dehydrogenase in the nuclei of rat liver raises the question of a possible difference in function of the two enzymes in the two sub-cellular organelles. The striking decreases in rate of the nuclear enzyme in the forward direction at pH values on either side of the optimum of 8.5-8.6 raises the possibility that a cellular environment could exist in which the rate of the back reaction was much greater than that of the forward reaction. This suggestion is supported by the lower apparent K_m values for α-ketoglutarate and NH_4Cl. The sigmoid shape of the curve for velocity versus NAD concentration, also indicates that at low NAD concentrations the forward reaction would proceed very poorly. This sigmoid curve which is typical of certain allosteric enzymes, has not been described with other widely studied glutamate dehydrogenases from a variety of tissues, although in the latter cases NAD is known to be an allosteric modifier and at higher concentrations to bind to a regulatory site (9). Purification of the nuclear enzyme should permit a more detailed study of the relationship between structures and functions of the two presumably different proteins with similar activities.

Acknowledgements. One of us (H.J.S.) is a holder of a Career Award, 1-K6-GM-2487 from the U.S. Public Health Service. This work was supported by grants from the U.S. Public Health Service (GM 13012) and the American Cancer Society (P-431).

REFERENCES

1. Frieden, C., The Enzymes (Boyer, P.D., Lardy, H.A. and Myrbäck, K. ed.), Vol. 7, p. 3 (1963).
2. Hogeboom, G.H. and Schneider, W.C., J. Biol. Chem., 204, 233 (1953).
3. Beaufay, H., Bendall, D.S., Baudhuin, P. and de Duve, C., Biochem. J., 73, 623 (1959).
4. Banay-Schwartz, M., Benziman, M. and Strecker, H.J., J. Cell Biol., 35, 9A (1967).
5. Hymer, W.C. and Kuff, E.L., J. Histochem. Cytochem., 12, 359 (1964).
6. Schneider, W.C. and Hogeboom, G., J. Biol. Chem., 183, 123 (1950).
7. Horecker, B.L. and Kornberg, A., J. Biol. Chem., 175, 385 (1948).
8. Olson, J.A. and Anfinsen, C.B., J. Biol. Chem., 202, 841 (1953).
9. Frieden, C., J. Biol. Chem., 234, 809 (1959).
10. Changeux, L., Cold Spring Harbor Symp.Quant. Biol., 26, 313 (1961).