PURIFICATION AND SOME PROPERTIES OF GLUTAMATE DEHYDROGENASE FROM OX LIVER NUCLEI

Guido di Prisco and Felice Garofano

International Institute of Genetics and Biophysics,
Via Marconi 10, 80125 Naples, Italy

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SUMMARY

The existence of glutamate dehydrogenase, an enzyme believed of mitochondrial origin only, has been demonstrated in ox liver nuclei. The activity of the nuclear enzyme constitutes 10-20% of the total homogenate activity. The nuclear fractions are free from significant cytoplasmic contamination, as judged by phase microscopy examination, RNA/DNA ratios and absence of mitochondrial marker enzymes.

The nuclear enzyme has been purified 82-fold; the preparation appears very close to homogeneity. The pH optimum of the forward reaction differs from that of mitochondrial glutamate dehydrogenase; moreover, the reversal of effect, shown at low pH by the allosteric modifiers ADP and GTP on the mitochondrial enzyme, does not occur with nuclear glutamate dehydrogenase.

INTRODUCTION

The finding of glutamate dehydrogenase activity associated with purified nuclear fractions from rat liver (1,2,4) and Chang's liver cells (2,3) was the first indication that in animal tissues this enzyme is not located exclusively in the mitochondria. Recently, the existence of glutamate dehydrogenase associated with nuclei has been shown by other investigators in rat (5) and pig liver (6) and in single dorsal root ganglion cells of rabbit (7).

We have extended our investigation to ox liver, and detected the presence of glutamate dehydrogenase activity associated with highly purified nuclear preparations. We wish to report its purification as well as some of its properties, related to the response of the activity and of the effect of allosteric modifiers to pH variations. Preliminary reports of some of these findings have been presented (8,9).

EXPERIMENTAL

Preparation of nuclear fractions. Ox liver nuclei were isolated according to two procedures. One of these (10) involves low speed centrifugation of the sucrose homogenate in the presence of $MgCl_2$ and Triton X-100. Due to the presence of the detergent, mitochondria were dissolved and eliminated; however this procedure became unsuitable when large amounts of liver were processed, since many washings with several liters of medium were necessary in

order to finally obtain a clear supernatant, absolutely free from mitochondrial glutamate dehydrogenase activity. For larger scale preparations, therefore, we have resorted to the procedure of Pogo et al. (11), as modified by Cacace and Nucci (12). When such preparations were carried out, 1-3 kg of liver were homogenized in 4 parts of 0.32 M sucrose containing 3 mM $MgCl_2$. At the end of the procedure, the nuclear pellet was occasionally washed by resuspending in 0.32 M sucrose containing 1 mM $MgCl_2$ and 0.5% Triton X-100, and centrifuged at 700-800 x g. This step was however omitted in routine preparations, since it did not improve significantly the purity of the nuclei. These preparations yielded a very clean nuclear fraction, as judged by phase microscopy examination, by RNA/DNA ratios, which were very close to those reported previously (13), and by the almost total absence of NADH oxidase and succinate dehydrogenase activities.

Enzyme assay. Enzyme activities were measured at 23-25°, in cuvettes of 1-cm light path, by recording at 340 nm either the initial reduction of NAD^+ with glutamate added (forward reaction) or the initial oxidation of NADH with α-ketoglutarate and NH_4Cl added (reverse reaction); alternatively, the forward reaction was measured by recording the appearance of the fluorescence due to NADH. Activity is expressed as μmoles of cofactor reduced or oxidized per minute, using a molar extinction coefficient for NADH of 6.22×10^3 (14), one μmole representing one activity unit.

RESULTS AND DISCUSSION

Table 1 shows the distribution of glutamate dehydrogenase in ox liver. 10-20% of the activity was found associated with the purified nuclear fraction, a value comparable to that reported for other tissues and species (1,3,4,5,7).

The purified nuclei were resuspended in 0.32 M sucrose -1 mM $MgCl_2$ (approximately one tenth of the original homogenate volume), and were fragmented by 2-minute exposure to sonic oscillation (Branson Sonifier Cell Disruptor): the activity was then extracted by addition of potassium phosphate buffer, pH 7.6, at a final concentration of 0.1 M. This addition was necessary in order to solubilize the enzyme, as it had been demonstrated in other species (1,2,3). Following 1-hour centrifugation at 50,000 x g, the activity was totally recovered in the supernatant. This supernatant was fractionated with ammonium sulphate, by slow addition of the solid salt at 4°. The precipitate collected in the saturation range between 28 and 40% was dissolved in 50 mM potassium phosphate buffer, pH 7.6. The solution was heated at 55° for 3 minutes, immediately cooled at 0° and centrifuged; all of the activity was recovered in the supernatant. A second ammonium sulphate step was carried

TABLE 1

Distribution of glutamate dehydrogenase in ox liver

The assay conditions were 50 μM NADH, 1.25 mM α-ketoglutarate, 50 mM NH_4Cl, 50 mM potassium phosphate buffer, pH 7.6, in a final volume of 3 ml.

fraction*	μmoles NADH oxidized min^{-1} g^{-1}
homogenate	4.0
nuclei	0.76
mitochondria	2.8

*105,000 x g supernatant after sonic disruption and addition of 0.1 M potassium phosphate buffer, pH 7.6.

out under conditions similar to the first one (28-40% saturation). The precipitate, redissolved in a small volume of potassium phosphate buffer, was passed through a column of Sephadex G-200 (cm 2.5 x 100), equilibrated with the same buffer; the activity appeared soon after the void volume. The fractions with the highest specific activity were pooled; the final yield was 64%, with 82-fold purification of the initial extract. Table 2 outlines the purification procedure, from 3 kg of fresh ox liver. Polyacrylamide gel-SDS and starch gel electrophoresis indicated that the preparation was only slightly (5-10%) contaminated.

Similar to glutamate dehydrogenase from other mammalian sources, of both nuclear and mitochondrial origin, the ox liver nuclear enzyme utilized either of the two nicotinamide adenine nucleotides as cofactors. Under the standard assay conditions of these experiments, the rate of the reverse reaction (glutamate synthesis) was several-fold higher than that of the forward reaction.

Fig. 1 shows the response of the activity to the pH of the assay mixture. In comparison with mitochondrial glutamate dehydrogenase, the pH activity curve of the reverse reaction (B) obtained with the nuclear enzyme had a similar shape, with a maximum around pH 8.0. However, a marked difference was noted in the forward reaction (A): the curve obtained with the mitochondrial enzyme had a maximum at pH 7.6 - 8.0, whereas that of nuclear glutamate

TABLE 2

Purification of glutamate dehydrogenase from ox liver nuclei

Assay conditions as in Table 1.

fraction	units/ml	volume (ml)	protein* (mg/ml)	specific activity (units/mg)	yield %	times purified
Supernatant after nuclei disruption	0.15	1500	2.55	0.059	100	--
1st $(NH_4)_2SO_4$ fraction	1.6	100	5.6	0.286	72	4.8
Supernatant after heating	1.6	90	3.1	0.497	65	8.5
2nd $(NH_4)SO_4$ fraction	13.2	10	9.0	1.475	60	25
G-200 eluate (pooled fractions)	0.24	600	0.05	4.8	64	82

*determined by the method of Lowry et al. (15).

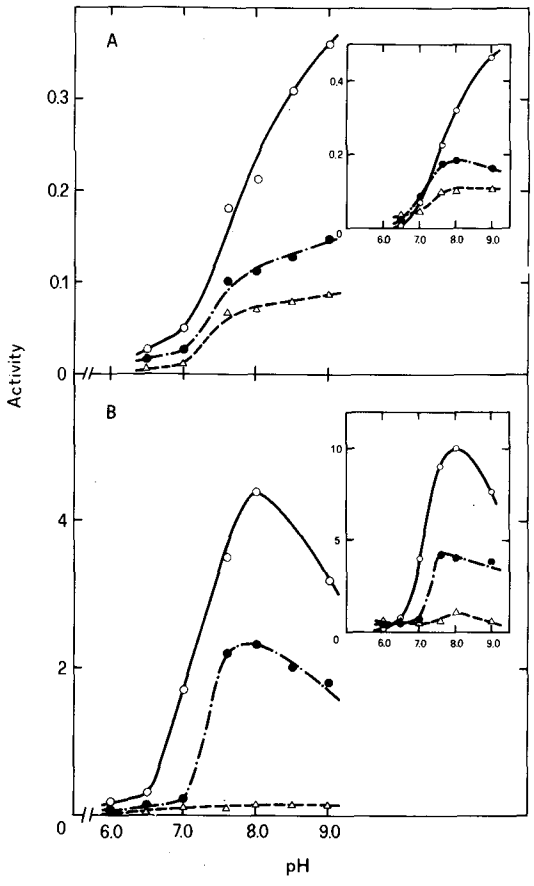

Fig. 1 - Effect of pH on activity of ox liver nuclear glutamate dehydrogenase, in the absence of effectors (●——·——●), in the presence of 0.5 mM ADP (O——O) and 50 μM GTP (Δ -- Δ). A, forward reaction, fluorometric assay (10 mM sodium glutamate, 0.1 mM NAD^+, 50 mM potassium phosphate buffer, 0.2 μg enzyme, in a final volume of 1.2 ml); B, reverse reaction (1.25 mM α-ketoglutarate, 50 μM NADH, 50 mM NH_4Cl, 50 mM potassium phosphate buffer, 1.2 μg enzyme, in a final volume of 3 ml. In the two inserts, the curves with mitochondrial glutamate dehydrogenase are shown for comparison.

dehydrogenase had not yet reached its maximal value at pH 9.0.

The effect of the allosteric effectors ADP and GTP as a function of pH is also illustrated in Fig. 1. It has been shown that lowering the pH produced a reversal of allosteric effect on mitochondrial glutamate dehydrogenase, namely ADP became a strong inhibitor (16,17,18) and GTP lost its inhibitory effect and slightly activated the reaction (17,18). The curves reported in Fig. 1 confirm once again these findings with the mitochondrial enzyme (see the inserts); however, nuclear glutamate dehydrogenase clearly differs in this respect, since GTP constantly inhibited, and ADP constantly

activated the reaction measured in both directions, even in the pH range 6.0 - 7.0, where the reversal of effect takes place with mitochondrial glutamate dehydrogenase. It seems therefore that the regulatory mechanism of nuclear glutamate dehydrogenase lacks at least part of the complexity characterizing that of the mitochondrial enzyme.

The availability of nuclear glutamate dehydrogenase in purified form permits now a more detailed study of the relationship between structure and function of two proteins with similar activities. Results on their structural, kinetic and immunological differences will be reported elsewhere.

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