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Crystallization and Partial Characterization of Glutamate Dehydrogenase from Ox Liver Nuclei†

Guido di Prisco* and Felice Garofano

ABSTRACT: Glutamate dehydrogenase has been obtained in crystalline form from purified ox liver nuclear fractions. The enzyme appeared homogeneous, as judged by several electrophoretic techniques at two pH values. A comparative study with the widely known ox liver mitochondrial glutamate dehydrogenase revealed several common features, such as the allosteric effect of the nucleotides ADP and GTP, the activation at high concentrations of the cofactor NAD^+, and the existence of a concentration-dependent reversible monomer–polymer(s) equilibrium. However, the two enzymes differed in many other respects. Inorganic phosphate activated nuclear glutamate dehydrogenase to a much greater extent than the mitochondrial enzyme; the substrate NH_4^+ showed cooperative homotropic interactions only with nuclear glutamate dehydrogenase; kinetic differences were detected with most of the reaction substrates, as well as different rates of oxidative deamination of other L-amino acids; the nuclear enzyme had a higher anodic mobility and a different chromatographic behavior on anionic exchangers. The latter evidence indicates that the glutamate dehydrogenase activity in liver is associated with two proteins which are structurally different, thus confirming the results of a separate immunological study. Preliminary evidence suggests that the enzyme in nuclei is attached to the nuclear envelope, probably the inner membrane, from which it can be solubilized by the addition of salts.

In a previous report (di Prisco and Garofano, 1974) we have described the partial purification of glutamate dehydrogenase associated with purified ox liver nuclear fractions. Some of its properties, related to the response of activity to pH variations, in the absence and presence of some allosteric modifiers, have also been described. The enzyme has been obtained now in homogeneous and crystalline form. We wish to report its final purification as well as some of its characteristics.

The results of this study show that while some aspects of the mechanism of allosteric activation and inhibition, as well as of the molecular organization of the nuclear enzyme, resemble those of mitochondrial GDH,[1] some others notably differentiate the two enzymes.

Moreover, differences between the two enzymes were found when examining their electrophoretic and chromatographic behavior, suggesting a higher net negative charge of nuclear GDH. The two proteins appear, therefore, to have some structural differences, as indicated also by immunological studies (Casola et al., 1974; di Prisco and Casola, 1975).

Experimental Section

Preparation of Nuclear Fractions. Ox liver nuclei were isolated essentially according to the procedure of Pogo et al.

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[1] Abbreviation used is: GDH, glutamate dehydrogenase.

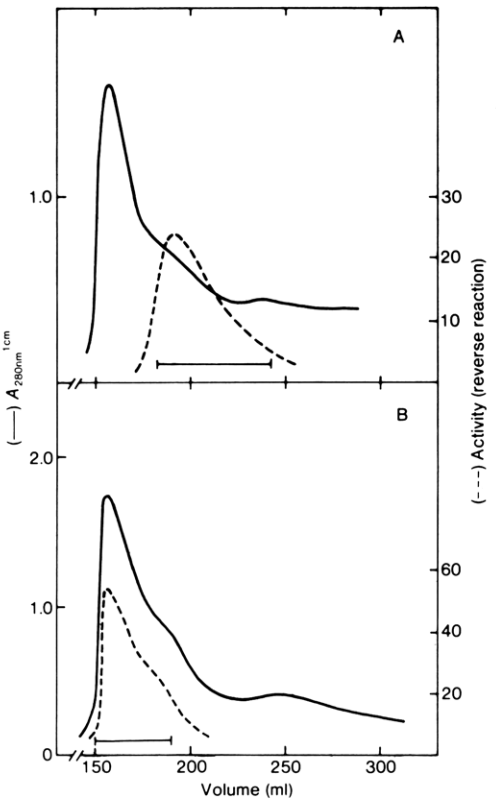

FIGURE 1: Elution profiles of nuclear GDH on a Sephadex G-200 column (2.5 × 100 cm). The fractions indicated by the solid bar (A) were pooled and, after concentration, run again on the same column (B). Following concentration of the fractions indicated by the solid bar, crystallization was carried out. See text for other experimental details.

(1966), as modified by Cacace and Nucci (1973), but omitting tris(hydroxymethyl)aminomethane and keeping $MgCl_2$ at 0.5–1 m*M*. For large scale preparations (1–3 kg of liver) this method was preferred to that of Hymer and Kuff (1964), which involves low-speed centrifugation of the sucrose homogenate in the presence of $MgCl_2$ and Triton X-100. The detergent completely dissolves and eliminates contaminating mitochondria, but the procedure required too large a number of washings of the low-speed crude nuclear pellet with several liters of medium before eliminating every trace of solubilized mitochondrial activity. For small scale preparations, the procedure of Blobel and Potter (1966) was also used.

Fresh liver (1–3 kg) was processed each time as previously described (di Prisco and Garofano, 1974), with a final yield of 20–30 mg of crystalline enzyme. The purity of the nuclear preparations was examined each time by phase microscopy examination, determination of the RNA/DNA ratios (Ceriotti, 1952; Fleck and Munro, 1962), and determination of NADH oxidase and succinate dehydrogenase activities. Our results and those reported in the literature for similar procedures (Pogo et al., 1966; Kay et al., 1972) indicated that mitochondrial and cytoplasmic contamination did not exceed 0.5–1%, and could not possibly account for the levels of activity associated with the nuclear fractions.

Determination of Enzyme Activity. Rates were measured at 23–25°, in cuvettes of 1-cm light path, by recording at 340 nm either the initial reduction of $NAD(P)^+$ with glutamate added (forward reaction) or the initial oxidation of NAD(P)H with α-ketoglutarate and NH_4Cl added (reverse reaction). Alternatively, the forward reaction was measured by recording the fluorescence due to NAD(P)H (λ_{ex} 340 nm; λ_{em} 400 nm), in a Turner Model 111 fluorimeter, calibrated with standard solutions of reduced nucleotides.

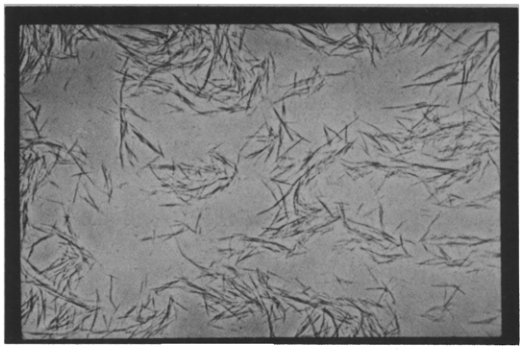

FIGURE 2: Crystals of GDH from ox liver nuclei, photographed with a Zeiss phase-contrast microscope; magnification, 80×.

Activity is expressed, unless otherwise indicated, as micromoles of cofactor reduced or oxidized per minute, using a molar extinction coefficient for NAD(P)H of 6.22×10^3 (Horecker and Kornberg, 1948), 1 μmol representing 1 activity unit.

Determination of Protein. Proteins were determined according to the method of Lowry et al. (1951), using crystalline serum albumin as standard.

Electrophoresis on Cellulose Acetate Strips and Cellogel. Electrophoresis was performed at room temperature, in 50 m*M* phosphate buffer (pH 7.6) or 40 m*M* Veronal buffer (pH 8.6), at 300 V for 25 min (Cervone et al., 1973). Strips or plates were stained for 3 min with 0.2% Ponceau S Red in 3% trichloroacetic acid. Destaining, in 3% acetic acid, was instantaneous.

Materials. Microgranular DE 52 was from Whatman; Sephadex G-200 from Pharmacia, Uppsala, Sweden; cellulose acetate strips from Gelman Instruments, Ann Arbor, Mich.; Cellogel (a ready-to-use cellulose acetate gel) plates from Chemetron, Milan, Italy; soluble hydrolyzed starch from Connaught, Medical Research Laboratories, Toronto, Canada. Highest grade coenzymes and nucleotides were supplied by Boehringer, Mannheim, Germany, as well as mitochondrial GDH. All other reagents were of the highest purity commercially available.

Results

Crystallization. The original procedure of partial purification (di Prisco and Garofano, 1974) has been further simplified. Following disruption of nuclei by sonic oscillation in 0.1 *M* phosphate buffer (pH 7.6), ammonium sulfate fractionation, and heating at 55°, as described, gel column chromatography was directly carried out, omitting the second ammonium sulfate step.

At this stage, the protein concentration of the solution was usually 5–10 mg/ml. It was chromatographed on a column of Sephadex G-200 (2.5 × 100 cm), equilibrated with 50 m*M* potassium phosphate buffer (pH 7.6). The activity peak emerged later than the void volume protein peak (Figure 1A). The fractions with the highest specific activity were pooled and solid ammonium sulfate was slowly added at 4° to 45% saturation. The centrifuged precipitate was redissolved in a small volume of buffer, achieving a protein concentration of 12 mg/ml or higher, and rechromatographed through the same column, or a shorter one (2.5 ×

Table I: Purification of GDH from Ox Liver Nuclei.[a]

Fraction	Units/ml	Vol (ml)	Protein (mg/ml)	Sp Act. (Units/mg)	Yield (%)	Times Purified
Supernatant after disruption of nuclei	4.8	370	11.2	0.4	100	
$(NH_4)_2SO_4$ fraction (28–40% saturation)	75.6	16	18.4	4.1	68	10.2
Supernatant after heating	75.0	15	9.2	8.2	63	20.5
First G-200 eluate (concentrated pooled fractions)	385.5	3	12.5	30.9	65	77.2
Second G-200 eluate (crystals)	525.0	2	15.0	35.0	59	87.8

[a] Assay conditions: 50 m*M* potassium phosphate buffer (pH 7.6), 50 μM NADH, 1.25 m*M* α-ketoglutarate, and 100 m*M* NH_4Cl, in a final volume of 1 ml.

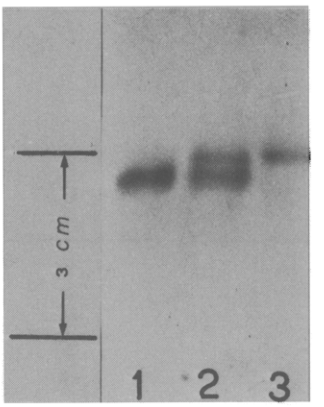

FIGURE 3: Cellulose acetate electrophoresis of: (1) crystalline mitochondrial GDH; (2) a mixture of mitochondrial and nuclear GDH; (3) crystalline nuclear GDH. Protein concentration, 3–5 mg/ml; 2 μl was applied to the strip. Experimental details are given in the text.

50 cm), using the same buffer as eluent. Under these conditions, the activity emerged with the void volume, in an asymmetrical peak (Figure 1B) of constant specific activity. The shape of the peak indicated that a concentration-dependent dissociation was occurring at the trailing edge (Winzor and Scheraga, 1963). The implications inherent to the gel filtration results will be discussed later.

The protein peak which emerged with the void volume consisted of pure nuclear GDH, as judged by the single band observed in polyacrylamide gel–sodium dodecyl sulfate electrophoresis, as well as starch gel, Cellogel, and cellulose acetate electrophoresis at pH 7.6 and 8.6. A lower molecular weight contaminant was eliminated following the second chromatography on G-200. The enzyme was precipitated at 45% saturation of ammonium sulfate, centrifuged, and dissolved in 0.1 *M* potassium phosphate buffer (pH 7.6). The protein concentration was adjusted to a minimum of 15 mg/ml, and the solution was clarified by a 15-min centrifugation at 50,000*g*. Solid ammonium sulfate was added until development of a faint cloudiness; crystallization occurred upon standing overnight at 0°, or almost immediately upon seeding with crystals of another preparation. Figure 2 represents nuclear GDH crystals, which appear as small needles. No significant increase in specific activity was noticed after crystallization, even when the step was repeated three times. Table I outlines the purification procedure, from 2 kg of fresh ox liver.

Polymerization. The behavior of nuclear GDH upon gel filtration indicated the existence of a concentration-dependent reversible monomer–polymer(s) equilibrium, similarly to mitochondrial GDH. This characteristic could be exploited to eliminate higher molecular weight contaminants in a first run, and lower molecular weight contaminants in a second one, simply by selecting appropriate enzyme concentrations. Judging from the specific activities at the various steps, GDH constituted not more than 20% of the total protein run through the first G-200 column, namely 1–2 mg/ml. This concentration, further decreased by the dilution occurring during the run, was such as to fully convert the enzyme to the dissociated form, and the activity peak thus emerged later than the void volume peak. In contrast, the second column was run at a GDH concentration of at least 10–11 mg/ml. Under these conditions, the enzyme was in the associated form, and was eluted with the void volume, the rapid association–dissociation equilibrium being evident at the trailing edge (Winzor and Scheraga, 1963), as shown in Figure 1B. Ultracentrifugation experiments (di Prisco et al., 1972) confirmed the existence of such an equilibrium between a monomer of mol wt ~300,000 and polymers that, in the case of mitochondrial GDH, are linear aggregates and may have a mol wt of up to 3.5×10^6 (Eisenberg, 1970).

Electrophoretic Mobility and Chromatographic Behavior. In comparison with the mitochondrial enzyme, nuclear GDH showed a higher anodic mobility on starch gel electrophoresis at pH 7.6, and on Cellogel and cellulose acetate electrophoresis at pH 7.6 and 8.6. On cellulose acetate, a mixture of the two enzymes was resolved into two bands (Figure 3) after 25 min at 300 V in 40 m*M* Veronal buffer (pH 8.6), according to the method of Cervone et al. (1973). This seems to indicate that the molecules of the two enzymes are differently charged, nuclear GDH being more negative. A further indication of a difference in the net molecular charge was obtained by the elution profiles upon chromatography at pH 6.2 on a cellulose anionic exchanger, DE 52 (Figure 4). Under suitable conditions, nuclear GDH was not adsorbed, whereas mitochondrial GDH was strongly bound and successively eluted by a linear phosphate buffer gradient; an artificial mixture of the two enzymes could easily be separated, as shown in Figure 4C. The identity of each peak was ascertained by electrophoresis on cellulose acetate.

Allosteric Modifiers and Effect of pH. At the pH values commonly used in the enzymatic assay, mitochondrial GDH undergoes allosteric activation by ADP and inhibition by GTP. However, when the activity and allosteric effects were measured at pH 6.0–7.0, a reversal of effect was shown to occur, namely ADP became a strong inhibitor while GTP lost its inhibitory effect and slightly activated the reaction. This phenomenon, originally shown for ADP (Bitensky et al., 1965), has been recently studied in some detail, especially with regard to ionic strength influence (di Prisco, 1975). We have extended this investigation to nuclear GDH, and found that this enzyme, unlike the mitochondrial, was constantly activated by ADP and inhibited by GTP. The lack of reversed effect on nuclear GDH is illustrated in Table II, where the results obtained at pH 6.5 with the forward reaction are reported.

Effect of Phosphate. Inorganic phosphate and other elec-

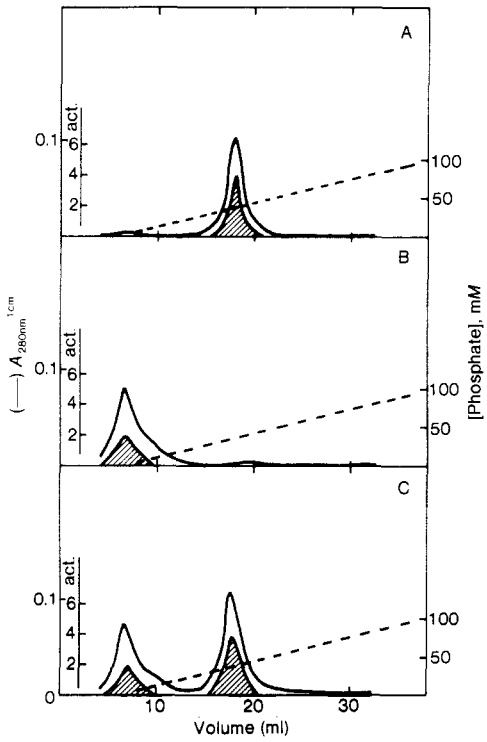

FIGURE 4: Ion-exchange chromatography of mitochondrial (A), nuclear (B) and a mixture of mitochondrial and nuclear GDH (C), on a column of DE 52 (0.8 × 7 cm). Each enzyme (1 mg) in 0.2–0.3 ml, alone or in the mixture, was dialyzed overnight against 5 m*M* potassium phosphate buffer (pH 6.2). The column was equilibrated with the same buffer. The elution was carried out with a linear gradient (5–100 m*M*) of the buffer; total volume of the gradient, 30 ml. The hatched areas represent activities (reverse reaction).

Table II: Effect of the Addition of ADP and GTP at pH 6.5.[a]

Addition	Activity (×10³)	
	Nuclear GDH	Mitochondrial GDH
None	0.018	0.025
0.5 m*M* ADP	0.032	0.006
50 μ*M* GTP	0.007	0.029

[a] The conditions of the fluorometric assay were 50 m*M* potassium phosphate buffer (pH 6.5), 10 m*M* sodium glutamate, 0.1 m*M* NAD^+, and 0.2 μg of enzyme, in a final volume of 1.2 ml.

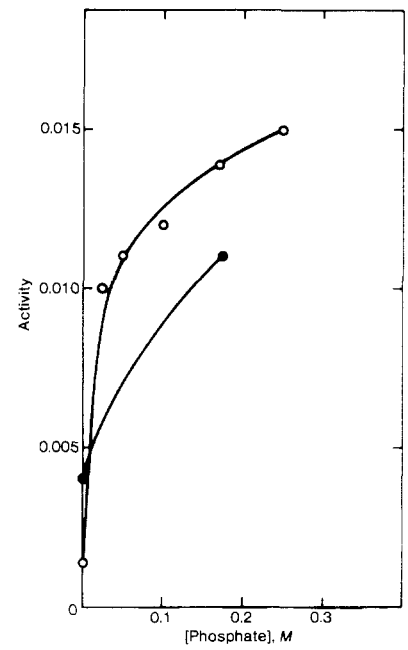

FIGURE 5: Effect of inorganic phosphate on the activity of GDH at pH 9.0. Assay conditions: phosphate concentrations as indicated, 25 m*M* sodium glutamate, 50 μ*M* NAD^+, 10 μg of nuclear (○) or mitochondrial (●) GDH, in a final volume of 1 ml.

Table III: Apparent Michaelis Constants of the Reaction Substrates and Cofactors.[a]

Substrate or Cofactor	Apparent K_m (m*M*)	
	Nuclear GDH	Mitochondrial GDH
Glutamate	2.5	4.5
α-Ketoglutarate	0.19	0.22
NADH	0.008	0.02
NAD^+	0.12	0.07
NH_4Cl	40.0[b]	20.0

[a] When used at fixed concentrations, glutamate was 25 m*M*; α-ketoglutarate, 1.25 m*M*; NH_4Cl, 100 m*M*; NAD^+ and NADH, 50 μ*M*. The forward reaction was carried out at pH 9.0, in the absence of buffer; the reverse reaction at pH 7.6, in 50 m*M* potassium phosphate buffer. [b] Half-saturation point.

trolytes have been shown to have an effect on the stability and the catalytic and allosteric properties of GDH from various sources (di Prisco and Strecker, 1966, 1969, 1970; di Prisco et al., 1968; Fourcade and Venard, 1971; Godinot and Gautheron, 1971, 1972). In rat liver and Chang's cells, the nuclear enzyme was strongly activated by phosphate at pH 9.0, whereas mitochondrial GDH was partially inhibited (di Prisco et al., 1968; di Prisco and Strecker, 1970); ox liver mitochondrial GDH, at the same pH, was activated threefold (di Prisco and Strecker, 1969). Figure 5 shows that the nuclear enzyme from ox liver is also activated by phosphate, and that this effect, 10- to 12-fold, is much more dramatic than that observed on mitochondrial GDH.

Like the mitochondrial enzyme, nuclear GDH showed activation by high concentrations of NAD^+. This activation may be the result of NAD^+ binding to a second site (Frieden, 1959; Pantaloni and Iwatsubo, 1967) or of negative cooperative interactions (Dalziel and Egan, 1972). This effect has been shown to be abolished by phosphate at pH 9.0 (di Prisco and Strecker, 1969); phosphate had a similar action on nuclear GDH, causing the abnormal kinetics obtained as a function of NAD^+ concentration to revert to normal Michaelis and Menten type.

Cooperative Homotropic Effect by NH_4^+. The plot of initial velocity as a function of NH_4^+ concentration had a sigmoid shape, similar to that observed with allosteric enzymes characterized by positive cooperative effects (Monod et al., 1965). Figure 6 illustrates this phenomenon, together with the heterotropic effect of the allosteric modifiers ADP and GTP. ADP (0.5 m*M*), the allosteric activator, abolished the cooperative effect and increased the affinity of the NH_4^+ sites for this ligand, lowering the half-saturation point from 40 to 11 m*M*, whereas the allosteric inhibitor GTP (8 μ*M*) increased the cooperativity by further decreasing the affinity of the sites; the half-saturation point by NH_4^+ was raised to 95 m*M*.

Comparison of Michaelis Constants. Table III reports the values of the apparent Michaelis constants of the reaction substrates for the two enzymes, with small but signifi-

Table IV: Oxidative Deamination of L-Amino Acids.[a]

L-Amino Acid	Relative Rate (%)		Nuclear GDH/ Mitoch. GDH
	Nuclear GDH	Mitochondrial GDH	
Glutamate	100	100	1.00
Norleucine	15.19	18.00	0.85
Norvaline	6.92	6.94	0.99
Leucine	3.69	5.48	0.67
Valine	2.18	2.78	0.78
Isoleucine	1.50	2.25	0.67
Glutamine	1.45	1.94	0.75
Methionine	1.39	3.33	0.42
α-Aminobutyrate	1.09	1.67	0.65
Aspartate	0.44	0.55	0.80
Alanine	0.28	0.64	0.44
Asparagine	0.025	0.036	0.69
Lysine	0.024	0.036	0.67
γ-Aminobutyrate	0.020	0.030	0.67

[a] The conditions of the fluorometric assay were 200 m*M* potassium phosphate buffer (pH 8.8), 25 m*M* amino acid, 1 m*M* NAD^+, 0.03–24 μg of enzyme, in a final volume of 1.2 ml. Specific activities with glutamate were 5.76 (nuclear GDH) and 7.56 (mitochondrial GDH).

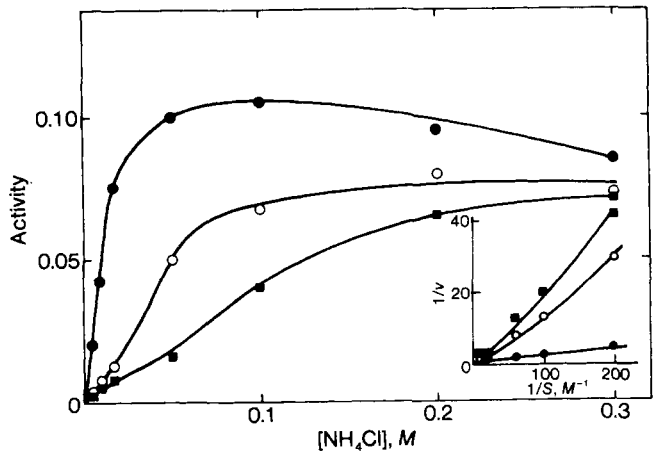

FIGURE 6: Reaction velocity as a function of NH_4Cl concentration, at pH 7.6, 50 m*M* potassium phosphate buffer, 50 μ*M* NADH, 1.25 m*M* α-ketoglutarate, 2 μg of nuclear GDH, in a final volume of 1 ml: (○) control without effectors; (●) 0.5 m*M* ADP; (■) 8 μ*M* GTP. The insert shows these results, replotted in double reciprocal form.

cant differences with all substrates, except α-ketoglutarate. Nuclear GDH was much more susceptible to inhibition by high concentrations of NADH and α-ketoglutarate than the mitochondrial enzyme.

Substrate Specificity. GDH from various sources is known to catalyze the oxidative deamination of a wide range of L-amino acids, at a generally much slower rate than the natural substrate L-glutamate (Struck and Sizer, 1960; Wiggert and Cohen, 1965, 1966; Tomkins et al., 1965). Other L-amino acids were also substrates of nuclear GDH (Table IV). The comparison of the substrate specificity of the two enzymes shows that nuclear GDH oxidatively deaminates all other amino acids at a slower relative rate in comparison with the mitochondrial enzyme. Two amino acids, alanine and methionine, were oxidatively deaminated at less than half, and six others at two-thirds of the rate recorded with mitochondrial GDH.

Stability of Association to the Nuclear Structure. A recent report (King and Frieden, 1970) questioned the existence of GDH activity associated with very pure nuclear fractions obtained from rat liver (di Prisco et al., 1968). King and Frieden employed the method of Blobel and Potter (1966) and found no activity in the nuclear pellet. Our evidence indicates that the bulk of the activity may be solubilized from nuclear preparations in the presence of salts, even under conditions in which the nuclear structure is not destroyed; since the method of Blobel and Potter involves exposure of nuclei (in hypertonic sucrose) to a somewhat concentrated ionic environment, containing 50 m*M* Tris-HCl, 25 m*M* KCl and 5 m*M* $MgCl_2$, experiments were carried out with small scale homogenates of both ox and rat liver, comparing the recovery of activity associated with the nuclear pellet, prepared in this complete medium, with that obtained when the salts (except 1 m*M* $MgCl_2$) were omitted. The nuclei of both preparations appeared morphologically very similar. The yields were also identical, somewhat lower than those reported (Blobel and Potter, 1966), as judged by quantitative determinations of DNA (Ceriotti, 1952). The results of these experiments showed that the activity found in the nuclei prepared in the salt-lacking medium was at least fivefold higher than that measured in the preparation performed with the complete medium.

We have also tried to establish whether nuclear GDH is attached to the nuclear membrane. The difficulty of avoiding enzyme solubilization is again encountered, since many methods commonly used to isolate nuclear membranes, including the procedure we have used (Kay et al., 1972), involve the use of salts. The omission of salts brings about very low activity yields, since GDH, once the nuclear structure is destroyed, is very unstable in the absence of stabilizing ions, as was found with the mitochondrial enzyme (di Prisco and Strecker, 1966). However, preliminary experiments indicate that the enzyme is associated with the inner membrane. (The outer membrane can be removed by washing with Triton X-100, without solubilizing the activity.) The attachment of nuclear GDH to the nuclear envelope confirms previous indications obtained with other tissues (Zbarsky et al., 1968).

Discussion

Since the existence of GDH associated with rat liver nuclei was first reported (di Prisco et al., 1968), a number of papers have appeared, confirming this finding in various tissues and organisms. Thus, nuclear activity has been found in rat liver (Zbarsky et al., 1968; Herzfeld et al., 1973), Chang's liver cells (di Prisco and Strecker, 1970; di Prisco et al., 1970), pig liver (Franke et al., 1970), single dorsal root ganglion cells of rabbit (Kato and Lowry, 1972), and yeast (Camardella et al., 1975).

The nuclear enzyme of ox liver has been obtained in crystalline and homogeneous form. The examination of some of its characteristics, in comparison with mitochondrial GDH, revealed many similarities, as could be expected in two proteins fulfilling the same biological function, but also substantial differences. Thus, both enzymes utilize the two nicotinamide adenine nucleotides as cofactors and oxidatively deaminate other amino acids besides L-glutamate (the relative rate with other amino acids is invariably lower with nuclear GDH, in some cases less than half that with the mitochondrial enzyme). Like mitochondrial GDH, the nuclear enzyme undergoes a concentration-dependent reversible polymerization. Other properties in common are the regulatory effects shown by ADP, GTP, and NAD^+.

However, differences have been detected in a number of other properties. In addition to the described different response of the activity to pH variations (di Prisco and Garofano, 1974), nuclear GDH is activated by ADP and inhibited by GTP throughout the pH range 6.0–9.0, in the presence and absence of high salt concentrations. At lower pH, mitochondrial GDH is, on the contrary, inhibited by ADP and activated slightly by GTP (di Prisco, 1975). The absence of a reversed effect may indicate that the regulatory mechanism of nuclear GDH lacks at least part of the complexity characterizing that of the mitochondrial enzyme.

Similar to previous findings with the two enzymes extracted from rat liver and Chang's liver cells (di Prisco et al., 1968; di Prisco and Strecker, 1970), the activity of ox liver nuclear GDH undergoes activation by phosphate to a much higher extent than the mitochondrial enzyme. Another property of nuclear GDH is the shape of the substrate saturation curve obtained with NH_4^+, which can be interpreted on the basis of a cooperative interaction of multiple sites. GTP enhances this interaction, and ADP suppresses it as demonstrated by the first-order kinetics observed in its presence. With mitochondrial GDH, normal Michaelis and Menten kinetics are obtained.

Finally, the higher anodic electrophoretic mobility of the nuclear enzyme constitutes a first indication of a structural difference, since, as confirmed also by the behavior on ion-exchange chromatography, the two proteins probably differ in their net charge. An immunological study has revealed that nuclear GDH has antigenic determinants in common with the mitochondrial enzyme, but also has specific determinants (Casola et al., 1974), which strengthens the evidence that the two activities are associated with proteins having structural differences, probably reflected in the respective amino acid sequences. Unlike nuclear GDH, the mitochondrial enzyme only possesses antigenic determinants common to the nuclear antigen (di Prisco and Casola, 1975).

A recent report (King and Frieden, 1970) questioned the existence of nuclear GDH in rat liver. These authors employed a method commonly used to prepare nuclei on a small scale (Blobel and Potter, 1966) and found no activity in their nuclear fractions. However, the fact that the activity can be easily solubilized, without disrupting the nuclei, in the presence of low salt concentrations, was overlooked. In fact, our experiments demonstrate that the salts present in the above-mentioned method are sufficient to wash out most of the nuclear activity, thus leaving an inactive pellet. The conclusion of King and Frieden (1970) was that the rat liver nuclear activity reported by us (di Prisco et al., 1968) was due to cytoplasmic contaminations and, thus, since the cytoplasmic activity is mitochondrial, to michondria. They questioned the effectiveness of the method of Hymer and Kuff (1964) for preparing nuclear fractions, and also our finding of a differential effect of phosphate on the two activities of rat liver, explaining this effect (activation of nuclear and inhibition of mitochondrial GDH), in the case of the mitochondrial activity, with a hypothetical inhibitor, present in the mitochondria. This explanation is rather unconvincing. If indeed the nuclear activity were due to mitochondrial contamination, why was it not then inhibited, in the presence of phosphate, by the same hypothetical contaminating inhibitor? It appears to us that the number of characteristics that differentiate nuclear from mitochondrial GDH (including the phosphate effect, which is difficult to ascribe to a factor, both enzymes being crystalline), together with the supporting evidence provided by other groups, leaves indeed little doubt as to the existence of a GDH of nuclear localization. In particular, Kato and Lowry (1972) extracted the nucleus from single cells and also "trimmed" it to eliminate any trace of cytoplasm. After these manipulations, they still found activity (at 50% of the cytoplasmic level) associated with the "trimmed" nucleus, hence localized *within* the organelle. Herzfeld et al. (1973) reported that fetal and neonatal rat liver contained nuclear and mitochondrial GDH activities in approximately the same amounts; then nuclear GDH decreased to about 15% of the total activity in adult liver.

The amount of GDH in ox liver nuclei is not small. Our nuclear preparations contain at least 10% of the total homogenate activity (di Prisco and Garofano, 1974), a level comparable to that reported in other tissues. GDH constitutes roughly 1% of the total soluble nuclear protein. Its biological significance, however, remains unclarified. Since there is no pathway as yet reported for reoxidation of NADH in the nucleus, it is tempting to speculate that GDH would perhaps fulfill this role. This, and other hypotheses, however, need experimental support, and work is in progress to this aim.

Acknowledgments

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Detection of Structural Differences between Nuclear and Mitochondrial Glutamate Dehydrogenases by the Use of Immunoadsorbents†

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ABSTRACT: Structural differences between crystalline mitochondrial and nuclear glutamate dehydrogenases from ox liver have been detected by immunological techniques. Antisera prepared against each enzyme precipitate both glutamate dehydrogenases; upon immunodiffusion, the antiserum against the nuclear enzyme gives a line of incomplete identity with the two antigens, whereas the antiserum against the mitochondrial enzyme gives a line of complete identity. Fractionation of the antibodies contained in each antiserum by means of an immunoadsorbent, to which the nuclear or the mitochondrial enzyme has been covalently linked, shows that nuclear glutamate dehydrogenase (GDH) contains specific antigenic determinants as well as determinants common to the mitochondrial enzyme, whereas the latter appears to have no antigenic portions which are not present in the nuclear antigen, in accord with the results of immunodiffusion. The antibodies against determinants common to both enzymes precipitate and inhibit them, whereas the specific anti-nuclear GDH antibodies precipitate but do not inhibit the nuclear antigen.

Nuclear fractions from different tissues have been shown to contain glutamate dehydrogenase (di Prisco et al., 1968, 1970; Zbarsky et al., 1968; di Prisco and Strecker, 1970; Franke et al., 1970; Kato and Lowry, 1972; Herzfeld et al., 1973; Camardella et al., 1975). This enzyme was formerly believed to be of mitochondrial localization exclusively.

GDH[1] from ox liver nuclei has been extracted, purified, and crystallized, and the study of its characteristics has revealed a number of kinetic and structural differences in comparison with the mitochondrial enzyme (di Prisco et al., 1972; di Prisco and Garofano, 1974, 1975).

Recently, an investigation of the immunological properties of the two dehydrogenases has been undertaken. Our initial results (Casola et al., 1974) indicated that nuclear GDH contains specific antigenic determinants as well as determinants common to the mitochondrial enzyme. Such conclusions were attained by performing an affinity chromatography of the anti-nuclear GDH antiserum on a mitochondrial GDH immunoadsorbent column. This work, however, did not yet provide an answer to the question whether

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A preliminary account of these findings has been presented (di Prisco et al., 1974).
[1] Abbreviation used is: GDH, glutamate dehydrogenase.