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PURIFICATION AND CHARACTERIZATION OF RAT BRAIN GLYCEROL PHOSPHATE DEHYDROGENASE

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SUMMARY

Glycerol-3-phosphate dehydrogenase (*sn*-glycerol-3-phosphate: NAD^+ 2-oxidoreductase, EC 1.1.1.8) was isolated from rat brain tissue and its physical and kinetic properties investigated. The purification method resulted in the separation of fractions enriched for six other brain proteins and this procedure is presented.

The apparent Michaelis constant (K_m) for dihydroxyacetone phosphate is 0.17 mM whereas the K_m for L-glycerol-3-phosphate is 0.30 mM. Under the conditions of the assay, the pH optimum occurs at pH 7.15. The molecular weight determined by Sephadex G-100 chromatography is 72 000 whereas ultracentrifugation in a sucrose gradient yields a molecular weight of 75 000. Furthermore using an antiserum prepared against purified rat brain glycerol-3-phosphate dehydrogenase, it was shown that rat brain glycerol-3-phosphate dehydrogenase is lighter than rabbit muscle glycerol-3-phosphate dehydrogenase (M_r 78 000). The purified enzyme is extremely sensitive to *p*-mercuribenzoate, being demonstrably inhibited by 10 nM, although it is much less sensitive to *N*-ethylmaleimide and iodoacetate. The specific activity of the purified enzyme is 152 units/mg of protein, representing a 2800-fold increase in specific activity. Analytical acrylamide gel electrophoresis separates the purified enzyme into two bands of protein, each of which exhibits glycerol-3-phosphate dehydrogenase activity. The isozymes differ from each in charge and neither one represents an oligomeric form of the other.

INTRODUCTION

It has previously been demonstrated that the developmental appearance of brain glycerol-3-phosphate dehydrogenase (*sn*-glycerol-3-phosphate: NAD^+ 2-oxidoreductase, EC 1.1.1.8) in the rat is regulated by an X-ray-sensitive control mechanism [1]. In addition, the level of rat brain glycerol-3-phosphate dehydrogenase has been shown to be directly controlled by the presence of cortisol both in vivo [2, 3] and in tissue culture [3, 4]. To examine this brain specific hormonal regulation it was deemed necessary to isolate glycerol-3-phosphate dehydrogenase in a pure form and characterize its kinetic and physical parameters.

The cytosol glycerol-3-phosphate dehydrogenase has been purified from a number of sources including honey bee [5], bumble bee [6], rabbit muscle [7, 8] and liver [9], chicken muscle and liver [10] and from rat muscle [11], liver [12] and fat tissue [13]. Our study is the first report of the purification of glycerol-3-phosphate dehydrogenase from brain tissue.

METHODS

Enzyme assays

Glycerol-3-phosphate dehydrogenase activity was routinely measured at 30 °C in a 1-ml reaction volume containing a final concentration of the following reagents: 10 mM sodium phosphate buffer, pH 7.15, 1 mM EDTA, 5 mM mercaptoethanol, 100 μg/ml bovine serum albumin, 0.833 mM dihydroxyacetone phosphate and 0.167 mM NADH. The reaction was started by the addition of substrate and the change in absorbance was monitored at 340 nm in a Beckman, Kintrac VII recording spectrophotometer. One enzyme unit was defined as that amount of enzyme which would catalyze the oxidation of one μmole of NADH in 1 min under the conditions of the assay.

For the determination of the apparent K_m values of α-glycerol phosphate and NAD^+, the rate of appearance of NADH was measured as above. The reaction mixture contained 0.1 M glycine–NaOH and 0.02 M hydrazine sulfate, pH 8.5, in a final volume of 1.0 ml. When the K_m of α-glycerol phosphate was to be determined NAD^+ was present at 0.6 mM whereas α-glycerol phosphate was present at 5 mM for the determination of the K_m for NAD^+.

Lactate dehydrogenase was assayed as described previously [1]. Malate dehydrogenase was assayed as described by Ochoa [14] and alcohol dehydrogenase activity was determined by the method of Valley and Hock [15].

Protein was determined as described using crystallized bovine serum albumin as a standard [16]. Column eluants were monitored at 280 nm using an L.K.B. Uvicord II.

Polyacrylamide-gel electrophoresis

Polyacrylamide-gel electrophoresis was performed by two different procedures. The first system [17, 18] concentrates the proteins at pH 8.3 and separates them at pH 9.5. For concentrating at pH 7.0 and separating at pH 8.0, the second system [19] was used. Protein was detected by staining the gels with a fresh solution containing 0.025% Coomassie Brilliant Blue and 10% trichloroacetic acid in acetic acid–methanol–water (14:40:160, by vol.) [20]. Destaining was done in acetic acid–methanol–water (14:40:160, by vol.). Staining for enzyme activity was performed in foil-wrapped, screw-cap tubes containing 0.156 M (DL)-α-glycerol phosphate, nitro blue tetrazolium salt (0.234 mg/ml), NAD^+ (1.56 mg/ml), and phenazine methosulfate (0.625 mg/ml) in 0.078 M glycine–NaOH, pH 9.0. The tubes were incubated at 37 °C in a circulating water bath for 15 min and the reaction terminated by replacing the staining solution with 10% acetic acid.

Rabbit anti-rat brain glycerol-3-phosphate dehydrogenase serum

The purified rat brain glycerol-3-phosphate dehydrogenase was subjected

to polyacrylamide-gel electrophoresis [17, 18] and then incubated in the enzyme staining solution for one min at 37 °C. The more concentrated band of activity was cut out and homogenized with an equal volume of 10 mM sodium phosphate buffer, pH 7.5, 5 mM mercaptoethanol and this was then homogenized with an equal volume of Freund's complete adjuvant. Each of two New Zealand White rabbits received a total of 900 μg of enzyme which was injected into the foot pads and intradermally along the back over a period of three weeks. They were bled on the fourth week. Despite the fact that only one of the two enzyme bands was injected, the antisera was capable of completely inactivating all of the enzyme present in the purified sample.

Determination of molecular weight by gel filtration

The column used for molecular weight determinations was 2.5 cm × 100 cm and was fitted with a plunger to provide even application of the sample. The bed (Sephadex G-100) occupied a total volume of 500 ml and was equilibrated with 0.1 M phosphate, pH 7.5, and 5 mM mercaptoethanol. The solution of proteins was composed of the following: ovalbumin (10 mg), lactate dehydrogenase (5.0 units), alcohol dehydrogenase (10 units), malate dehydrogenase (9.0 units), rabbit muscle glycerol-3-phosphate dehydrogenase (4.0 units) and rat brain glycerol phosphate dehydrogenase (4.0 units) in 5.0 ml of 0.1 M phosphate, pH 7.5, and 5 mM mercaptoethanol. The sample was put through a 0.45-μm millipore filter to remove any insoluble material; made 5% (w/v) with respect to glycerol, and 4.0 ml of this solution was applied to the column. The flow rate of the column was 0.4 ml/min and 3.0-ml fractions were collected. The location of ovalbumin was determined by monitoring the absorbance at 280 nm whereas the positions of all other proteins were determined by enzymatic assay.

For the glycerol-3-phosphate dehydrogenases the fractions were assayed once to determine their location and then aliquots from those fractions containing glycerol phosphate dehydrogenase were incubated at 37 °C for 30 min in the presence of rabbit anti-rat brain glycerol-3-phosphate dehydrogenase serum. The amount of antiserum was in excess of the amount required to inactivate all of the rat enzyme present. The activity remaining after incubation with antiserum (rabbit enzyme) was then subtracted from the amount present in the absence of antiserum (rabbit plus rat) to obtain the peak of the rat enzyme activity.

Sucrose gradient ultracentrifugation

The method used for sucrose gradient ultracentrifugation was essentially the same as that described previously [21] except that the samples were centrifuged in an SW27 swinging bucket rotor in a Beckman L2-65B ultracentrifuge. The sample (0.2 ml) was layered on top of 17.3 ml of a 5–20% (w/v) linear sucrose gradient in 0.1 M phosphate, pH 7.5. The sample contained 0.05 unit of alcohol dehydrogenase, 0.15 unit of rabbit muscle glycerol-3-phosphate dehydrogenase, 0.15 unit of rat brain glycerol-3-phosphate dehydrogenase and 2.0 units of malate dehydrogenase. The rabbit glycerol phosphate dehydrogenase, in this case, was used as a standard and assigned the molecular weight previously determined (78 000).

Enzyme purification

Source. The brains used were from Sprague–Dawley rats and were obtained

from Pel-Freeze Biologicals, Rogers, Ark. All operations were done at 4 °C and all buffers contained 1 mM EDTA and 5 mM 2-mercaptoethanol unless otherwise stated.

Homogenization. The frozen brains (1240 g) were allowed to thaw at 4 °C in 2 vol. of 0.1 M phosphate buffer, pH 7.5. They were homogenized in a commercial Waring blender at high speed (4 × 15 s) and stirred for 1 h. The homogenate was centrifuged in GSA heads in Sorvall centrifuges for 2 h at 11 000 rev./min. The precipitate was resuspended in one volume of buffer, stirred for 1 h, centrifuged as above and the supernatants combined.

$(NH_4)_2SO_4$ precipitation. The combined supernatants were treated over a period of 2 h with powdered $(NH_4)_2SO_4$ in two steps (40 and 60%) and each of the resulting precipitates was washed with the corresponding concentration of $(NH_4)_2SO_4$ in buffer. Following the addition of $(NH_4)_2SO_4$ each solution was allowed to stir overnight prior to centrifugation.

Desalting. The enzyme solutions were desalted, or the buffer exchanged, on Sephadex G-25 columns. The buffer used for this and all other columns was deaerated by heating at 80 °C for 2 h under line vacuum. Mercaptoethanol was added after the buffer had been cooled to 4 °C.

1st DEAE-cellulose column. DEAE-cellulose (Bio-Rad, Cellex-D, 0.9 mequiv/g) was equilibrated with 0.1 M phosphate, pH 7.5, and packed under pressure (2 lb/inch^2) in a column 5.0 cm × 40 cm. The precipitate from 60% $(NH_4)_2SO_4$ was dissolved in 0.1 M phosphate, pH 7.5, centrifuged at 21 000 rev./min for 1 h to remove insoluble material and desalted on a G-25 column. The sample (340 ml, 9.3 g protein) was then put onto the DEAE-cellulose column and washed through with the same buffer. Under these conditions, none of the glycerol-3-phosphate dehydrogenase is retained on the column although about 40% of the other proteins are bound.

1st G-100 column. The protein collected in the void volume of the DEAE-cellulose column was concentrated by $(NH_4)_2SO_4$ (60%) precipitation, redissolved in the same buffer and centrifuged at 21 000 rev./min for 1 h. The supernatant (120 ml, 5.2 g) was then loaded onto a Sephadex G-100 column (5 cm × 140 cm) equilibrated with 0.1 M phosphate, pH 7.5, 5% glycerol. The flow rate was 60 ml/h and the fractions (12.0 ml) containing enzyme activity were pooled and the protein precipitated by addition of $(NH_4)_2SO_4$ to 60%. Following centrifugation and resuspension in the above buffer, the sample (87.5 ml, 2.7 g) was put back through the same column (2nd Sephadex-G-100 column) and again the fractions containing enzyme activity were combined and precipitated with $(NH_4)_2SO_4$.

2nd DEAE-cellulose column. The above precipitate was resuspended in 0.01 M phosphate, pH 7.5, desalted on a Sephadex G-25 column, concentrated by ultrafiltration (Amicon PM-30 membrane) and applied (38.5 ml, 1.160 g) to a DEAE-cellulose column (2.5 cm × 40 cm) equilibrated with starting buffer, 0.01 M phosphate, pH 7.5. After collecting 300 ml of eluant (0.4 ml/min) the column was eluted with a linear gradient of 500 ml of starting buffer and 500 ml 0.3 M (DL)-α-glycerol phosphate in starting buffer.

3rd DEAE-cellulose column. The fractions from the above column containing enzyme activity were pooled and concentrated by ultrafiltration and the buffer exchanged on a Sephadex G-25 column equilibrated with 0.01 M Tris–HCl, pH 8.3. It (6.0 ml, 31 mg) was then applied to a DEAE-cellulose column (1.5 cm × 30 cm) equilibrated with the same buffer. After collecting 100 ml of eluant, glycerol-3-phos-

phate dehydrogenase was eluted with a linear gradient of 150 ml of starting buffer and 150 ml of starting buffer with 0.2 M (DL)-α-glycerol phosphate. The fractions containing the enzyme were combined, and stored in 60% $(NH_4)_2SO_4$.

RESULTS

Purification

Since each preparation involved 900–1000 rat brains we felt that others might be able to use our discarded fractions. We therefore consulted with a number of investigators engaged in brain research and determined which proteins were of interest to them and in which fractions the proteins could be found. An outline of the purification procedure and the protein of interest in the fractions given to various investigators is given below.

Following centrifugation of the crude homogenate, monoamine oxidase and other membrane and mitochondrial proteins are found in the precipitate. The first $(NH_4)_2SO_4$ cut results in the precipitation of about 55% of the protein and includes tryptophan hydroxylase. The 60% $(NH_4)_2SO_4$ cut precipitates glycerol-3-phosphate dehydrogenase and leaves the brain-specific S-100 protein [22] in the supernatant. Passage of the resuspended precipitate through the first DEAE-cellulose column separates 40% of the protein from glycerol-3-phosphate dehydrogenase and among the proteins retained on the column are the brain specific proteins 14.3.2 and 14.3.3 [23]. Because of the relatively small amount of enzyme present, it was necessary to combine a large number of the fractions separated on the 1st Sephadex G-100 column, concentrate them, and run them again on the same column. One of the proteins which easily separates from glycerol-3-phosphate dehydrogenase under these conditions is lactate dehydrogenase.

Although the purification scheme was specifically oriented toward the isolation of glycerol-3-phosphate dehydrogenase, it was possible to provide other investigators with fractions enriched for six different proteins. This type of approach is not limited to brain proteins and should be undertaken, whenever possible, during the purification of proteins from any source.

Table I summarizes the results of each of the steps in the purification pro-

TABLE I

PURIFICATION OF GLYCEROL-3-PHOSPHATE DEHYDROGENASE FROM RAT BRAIN
Abbreviation: GPDH, glycerol-3-phosphate dehydrogenase.

Isolation step	Total GPDH units	% yield	Total protein (g)	GPDH units per mg protein
Crude homogenate	6100	100	95.8	0.055
Centrifugation	6147	100	38.1	0.161
40% $(NH_4)_2SO_4$ supernatant	5840	95	16.4	0.356
60% $(NH_4)_2SO_4$ precipitate	5415	88	9.3	0.582
DEAE-cellulose, 0.1 M phosphate, pH 7.5	5247	87	5.2	1.00
Sephadex G-100 filtration No. 1	3741	61	2.7	1.40
Sephadex G-100 filtration No. 2	3100	50	1.16	2.7
DEAE-cellulose, 0.01 M phosphate, pH 7.5	886	15	0.031	28.6
DEAE-cellulose, 0.01 M Tris–HCl, pH 8.3	401	6.5	0.00263	152.4

cedure. The presence of mercaptoethanol was necessary throughout the purification. When glycerol-3-phosphate dehydrogenase was eluted with a linear salt gradient ($0 \rightarrow 0.3$ M) from DEAE-cellulose the recovery was variable and the activity was eluted in two relatively dilute peaks. However, when an α-glycerol phosphate gradient was used, the activity was recovered in one concentrated peak. This gradient, combining both affinity elution [24] and simple ionic elution resulted in higher recovery of glycerol-3-phosphate dehydrogenase from the column although the consistent loss of enzyme activity on DEAE-cellulose was not prevented. The specific activity of the final product was 152 units/mg of protein, representing an increase in purity of about 2800-fold over that found in the crude homogenate.

The homogeneity of the purified enzyme was examined by polyacrylamide-gel electrophoresis at two different pH values (Fig. 1). In both cases two very close, yet separate, bands of protein corresponding to two bands of enzyme activity were found. Gels stained for enzyme activity in the absence of substrate did not show any bands.

Fig. 1. Analytical acrylamide-gel electrophoresis of purified brain glycerol-3-phosphate dehydrogenase. The gels labeled 1 and 2 were run at high pH [17, 18] whereas the gels labeled 3 and 4 were run at low pH [19]. Gels 2 and 3 were stained for protein and gels 1 and 4 were stained for enzymatic activity as described in Methods. Each gel contained approximately 12 μg of protein.

These isozymes differ in charge but not in molecular weight. This conclusion was reached from the following observations and experiments. Two peaks of glycerol-3-phosphate dehydrogenase could be obtained from DEAE-cellulose when a salt gradient was used as the eluant suggesting that the peaks represented enzymes differing in charge. Molecular sieving and ultracentrifugation in a sucrose gradient yielded only one peak of activity suggesting the presence of only one molecular size species.

Finally, the most definitive evidence was obtained by electrophoresing the purified enzyme in several concentrations of acrylamide [25]. A plot of the log of the relative mobility against gel concentration ("Ferguson Plot", Fig. 2) demonstrated that the enzyme bands have parallel slopes and therefore identical retardation coefficients (K_R). Since K_R is a function of molecular size, these results indicate that the glycerol-3-phosphate dehydrogenase isozymes differ in charge and not in molecular weight.

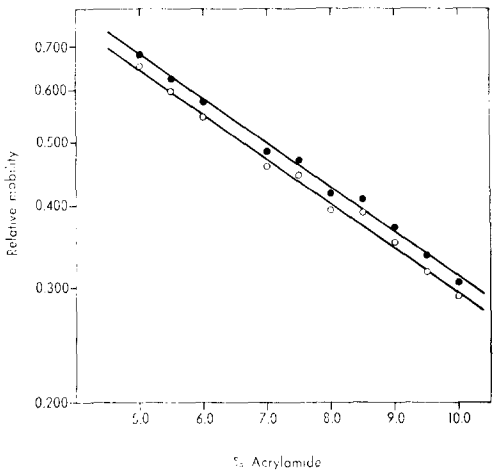

Fig. 2. Polyacrylamide-gel electrophoresis of glycerol-3-phosphate dehydrogenase at different gel concentrations. Each gel contained 30 munits of enzyme and was electrophoresed according to the method of Davis [18]. Following electrophoresis, the gels were stained for enzyme activity as described in Methods and the relative mobility of the bands was determined as described by Rodbard and Crambach [25].

Stability of glycerol-3-phosphate dehydrogenase

Repeated freezing and thawing results in the rapid loss of activity although some of the activity can be restored by incubation at room temperature for 30 min. Complete recovery was never obtained and there was a progressive loss of activity corresponding not only to the length of time for which samples were frozen but also to the number of times frozen and thawed. Lyophylization resulted in irreversible inactivation.

The enzyme is generally more stable at higher than at lower pH values. Its stability in various buffers was determined by incubating equal amounts of enzyme for 20 min at 30 °C in various buffers at pH 7.5 at a concentration of 10 mM. Glycerol-3-phosphate dehydrogenase is most stable in phosphate and least stable in Tris–glycine.

The enzyme is stable for at least 5 min at 45 °C, but loses activity at higher temperatures and is completely inactivated after 5 min at 60 °C. Rapid denaturation at 50 °C can be prevented with bovine serum albumin (100 μg/ml), $(NH_4)_2SO_4$ ($> 20\%$) or 0.1 M phosphate buffer, pH 7.5.

The effect of sulfhydryl reagents

The enzyme is extremely sensitive to sulfhydryl reagents being demonstrably

inhibited by 10 nM *p*-chloromercuribenzoate. At 1 μM, it inhibits more than 90% of the enzyme activity whereas 5 mM of *N*-ethylmaleimide was required to produce a similar inhibition. Iodoacetate was much less effective and even at 10 mM only inhibited 50% of the activity. The inhibition by any of these reagents was completely reversible with dithiothreitol.

The effect of pH on enzyme activity

The results shown in Fig. 3 indicate that the activity of glycerol-3-phosphate dehydrogenase is extremely dependent on the pH at which the reaction is run. The pH optimum for the purified enzyme is at 7.15 and the decline in activity is more rapid at higher pH values even though the enzyme is more stable at the higher values. The pH profile obtained using a crude homogenate is broader and the optimum is shifted upwards to between 7.3 and 7.5.

Kinetic constants

The values of the apparent Michaelis constants (K_m) were obtained from Lineweaver–Burke plots of the results of experiments in which the concentration of one substrate was varied while the other was present at a constant saturating level. In the direction of dihydroxyacetone phosphate reduction, the assay was done at 30 °C in 0.01 M phosphate, pH 7.15, 5 mM mercaptoethanol and 100 μg/ml bovine serum albumin. Under these conditions and NADH at 0.167 mM, the K_m for dihydroxyacetone phosphate is 0.17 mM whereas at constant dihydroxyacetone phosphate (0.833 mM) the K_m for NADH was less than 0.01 mM. For the oxidation of α-glycerol phosphate to dihydroxyacetone phosphate the reaction was conducted in glycine–NaOH (0.1 M) and hydrazine sulfate (0.02 M) buffer, pH 8.5, at 30 °C. With NAD^+ present at 0.6 mM, the K_m for α-glycerol phosphate was 0.3 mM whereas with 5 mM α-glycerol phosphate the K_m for NAD^+ was 0.03 mM.

Determination of molecular weight

The purified rat brain enzyme was mixed with the indicated proteins, chromatographed on Sephadex G-100 and its elution volume determined (see Methods). The average of the results of three such runs is plotted in Fig. 4 and indicates that rat brain glycerol-3-phosphate dehydrogenase has a molecular weight of 72 000. Brain

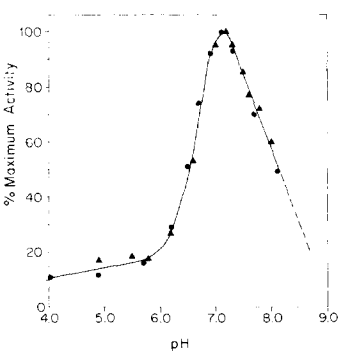

Fig. 3. Effect of pH on rat brain glycerol-3-phosphate dehydrogenase activity. Each point represents the average of three determinations and the circles and triangles represent the results of two different experiments. The pH was measured after the reaction.

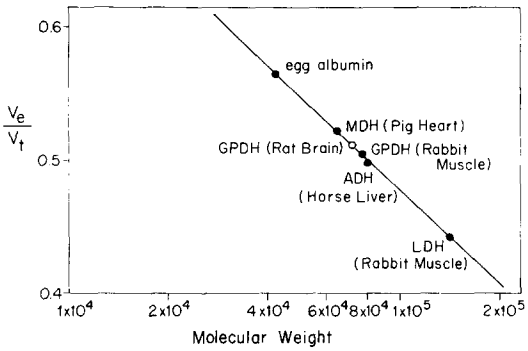

Fig. 4. Chromatographic determination of the molecular weight of rat brain glycerol-3-phosphate dehydrogenase (GPDH) by Sephadex G-100. A complete description of the procedure is in Methods. The ratio of the elution volume (V_e) of each of the proteins to the total column volume (V_t) is plotted against the log of the molecular weight. MDH, malate dehydrogenase; LDH, lactate dehydrogenase; ADH, alcohol dehydrogenase.

glycerol-3-phosphate dehydrogenase, either crude or purified, consistently exhibited one peak on Sephadex G-100 columns and on ultracentrifugation in sucrose gradients.

The literature values for the molecular weight of rabbit muscle glycerol-3-phosphate dehydrogenase range from 60 000 [8] to 78 600 [25]. Because of this variation and the availability of rabbit-antisera directed against the rat brain enzyme (see Methods), the rabbit muscle enzyme was run as an experimental protein and its molecular weight determined. In all experiments the peak activity of the rabbit muscle glycerol-3-phosphate dehydrogenase eluted before the peak of rat brain glycerol-3-phosphate dehydrogenase and when plotted against the standards, its molecular weight was 78 000.

To confirm the molecular weight determined by gel chromatography, purified rat brain glycerol-3-phosphate dehydrogenase was subjected to ultracentrifugation in a sucrose gradient [20]. In this case the rabbit muscle enzyme was used as a standard and assigned the molecular weight determined by gel chromatography (78 000). The average of two such runs is plotted in Fig. 5 and indicates that the rat brain enzyme

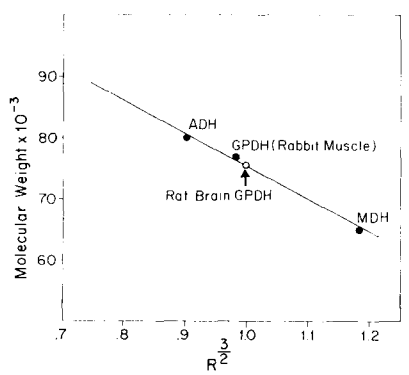

Fig. 5. Molecular weight determination by sucrose gradient ultracentrifugation. See Methods for a description of the procedure used. The molecular weights are plotted against $R^{3/2}$ where R is the ratio of the migration distance of the standard to the migration distance of rat brain glycerol-3-phosphate dehydrogenase. Abbreviations as in legend to Fig. 4.

has a molecular weight of 75 000. In all runs the peak of rat brain glycerol-3-phosphate dehydrogenase trailed the peak of rabbit muscle glycerol-3-phosphate dehydrogenase, confirming the results found with gel chromatography that the rabbit enzyme is heavier than the rat enzyme.

DISCUSSION

Although not original or novel, providing other investigators with fractions enriched for particular proteins does not appear to be done unless the investigators are part of the same laboratory or research groups. It should be obvious from the outline presented, that such provisions do not involve any extra work and can result in a substantial savings of time, money and effort as well as promoting cooperation and interaction among laboratories.

The existence of two or more soluble glycerol-3-phosphate dehydrogenase isozymes has been reported for chicken liver and muscle[10], rat liver [27], rat muscle [28], for mouse brain [29] and for bumblebees [6]. The salt elution of two glycerol-3-phosphate dehydrogenase peaks from DEAE-cellulose; the single peak from ultracentrifugation and Sephadex G-100 chromatography, and the results of the Ferguson plot of the electrophoresis data indicate that the brain isozymes differ in charge and that neither represents an oligomeric form of the other. The two isozymes were present in the crude homogenates in all of our preparations and were therefore not generated by the isolation procedure per se. However, using fresh rat brains we were unable to consistently demonstrate the presence of both isozymes. This is similar to the results obtained for the rat muscle isozymes [28] where two separable glycerol-3-phosphate dehydrogenases were usually found when muscle from older rats was used as the source of the enzyme. They also demonstrated that the more acidic form could be generated from the other upon standing at 4 °C for several days. Whether the presence of the two brain isozymes is dependent on the age of the rat and/or influenced by the condition of storage is currently being investigated.

The shape of the curve obtained when the pH of the reaction was varied, is similar to that found for the rabbit muscle enzyme [30] but the pH optimum of rat brain glycerol-3-phosphate dehydrogenase is 7.15 whereas the rabbit muscle enzyme optimum occurred at 7.6. Both of these sharp pH profiles are in marked contrast to the very broad pH optimum (around pH 6.6) exhibited by the honey bee enzyme [31].

The requirement for the continued presence of mercaptoethanol has also been demonstrated for the rat muscle enzyme [11] and is indicative of the similarity between the properties of the brain and muscle enzymes. The inhibition by low concentrations of *p*-chloromercuribenzoate is indicative of the importance of cysteine residues to the functional integrity, and probably the structural integrity of glycerol-3-phosphate dehydrogenase. In comparison, the relative insensitivity of brain glycerol-3-phosphate dehydrogenase to iodoacetate may reflect the presence of unreactive "buried" essential cysteine residues, analogous to the essential and non-essential cysteine's in lactate dehydrogenase [32].

The molecular weight of the brain enzyme (72 000–75 000) is about 10% heavier than the molecular weights published for the rat liver enzyme (63 000 $\pm$ 6000 [12]) and the rat muscle enzyme (58 000–63 000 [11]). The antisera against rat glycerol phosphate dehydrogenase allowed the direct comparison of the molecular

weight of the rabbit muscle and rat brain enzymes. These experiments indicate that the rabbit muscle glycerol-3-phosphate dehydrogenase is heavier than the rat brain glycerol-3-phosphate dehydrogenase and has a molecular weight of 78 000. This compares favorably with the reported values of 78 600 [30]; of 74 000–79 300 [33], and of 77 000 [9]. It is 10–15% higher than the values reported by White [34], 68 000 and that of Fondy et al. [8], 69 000.

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