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The Similarity of the Glyceraldehyde 3-Phosphate Dehydrogenases Isolated from Rabbit Brain and Muscle*

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ABSTRACT: Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) has been isolated from rabbit brain. The crystalline enzyme appears homogeneous by chromatographic, electrophoretic, and ultracentrifugal criteria. The properties of the rabbit brain GAPDH are essentially the same as those of rabbit muscle GAPDH. The following characteristics of the brain enzyme were defined: (a) molecular weight, 147,000; (b) turnover number, 13,750 moles of nicotinamide–adenine dinucleotide (NAD) reduced/mole of enzyme per min; (c) 12.1 sulfhydryl groups/molecule; (d) four "active" sulfhydryl residues per mole; and (e) 2.5 moles of reducible NAD/mole of enzyme. No significant differences were detected in the peptide maps obtained after tryptic digestion or in the immunological properties of the two enzymes. It is concluded that the amino acid sequences of the brain and the muscle GAPDH are remarkably similar or identical.

Since the isolation and studies of D-glyceraldehyde 3-phosphate dehydrogenase from yeast by Warburg and Christian (1939), and from rabbit muscle by Baranowski (1939) and Bailey (1940), the molecular and catalytic properties of GAPDH[1] from these two sources have been studied extensively (Caputto and Dixon, 1945; Cori *et al.*, 1948). In recent years, comparative studies have been performed on GAPDH isolated from the muscle of a number of species including cat, dog, beef, pig, chicken, turkey, halibut, sturgeon, and lobster, as well as from *Escherichia coli* and *Bacillus stereothermophilus* (Elödi and Szörényi, 1956; Elödi, 1958; Allison and Kaplan, 1964; Amelunxen, 1966). From these studies, it seems that these proteins form a series of distinct but closely related and presumably homologous enzymes.

Multiple electrophoretically distinct forms of GAPDH have been found in yeast and, more recently, tissue-specific distribution patterns have been discovered in a number of species (Krebs, 1953; Lebherz and Rutter, 1967); however, no evidence of multiplicity of this enzyme was detected in various mammalian tissues tested (Lebherz and Rutter, 1967). In analogy with the tetrameric molecules, lactic dehydrogenase (Markert and Møller, 1959; Kaplan *et al.*, 1960) and aldolase (Penhoet *et al.*, 1966, 1967), five-membered sets of GAPDH isozymes are usually apparent in tissue extracts which exhibit GAPDH multiplicity (Lebherz and Rutter, 1967). The physiological significance, if any, of these GAPDH isozymes has not been defined. Moreover, the possible absence of multiple forms of this enzyme in various mammalian tissues is curious in view of the known multiplicity of several other glycolytic enzymes in these tissues. GAPDH has been isolated from a number of different tissues of the same species (human) including heart (Allison and Kaplan, 1964), skeletal muscle (Baranowski and Wolny, 1963), and red cells (Oguchi *et al.*, 1966), but no evidence concerning the possible identity of these molecules is available. Recently, Papadopolous and Velick (1967) have reported that crystalline rabbit liver GAPDH resembles the muscle enzyme in many molecular parameters but differs markedly in kinetic characteristics.

In the present investigation, we report the isolation of crystalline GAPDH from rabbit brain and compare its properties with those of rabbit muscle GAPDH.

Experimental Procedure

Substrate. DL-Glyceraldehyde 3-phosphate diethylacetal barium salt was purchased from Sigma Chemical

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: GAPDH, D-glyceraldehyde 3-phosphate dehydrogenase; TPCK, tosyl-2-amidophenylethyl chloromethyl ketone; PMB, *p*-hydroxymercuribenzoate.

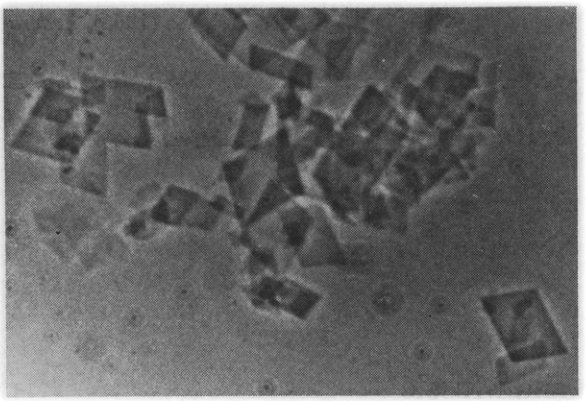

FIGURE 1: Photomicrograph of crystalline brain D-glyceraldehyde 3-phosphate dehydrogenase, ×1000.

Co. The compound was deionized on Dowex 50-X4 and the resulting solution was heated at 100° for 3 min to obtain the free aldehyde. The concentration of the D-glyceraldehyde 3-phosphate was determined spectrophotometrically in the presence of excess NAD and D-glyceraldehyde 3-phosphate dehydrogenase according to Velick (1955).

Activity Measurements. Enzyme activity was determined as described by Velick (1955). The reaction mixture contained 5×10^{-2} M pyrophosphate (pH 8.5), 2.5×10^{-4} M NAD, 2.5×10^{-4} M D-glyceraldehyde 3-phosphate, 5×10^{-3} M sodium arsenate, and 1×10^{-3} M sodium EDTA. The reaction was initiated by the addition of 0.01 ml of enzyme solution to make 3-ml final volume in a cuvet with a 1-cm light path. The absorbance change at 340 mμ between 15 and 45 sec was determined on a Gilford recording spectrophotometer at 25°. Prior to the assay, the enzyme was diluted in 10^{-3} M EDTA and 10^{-3} M β-mercaptoethanol so that the total change of absorbance during the 30-sec interval was not greater than 0.05. Under these conditions, one unit of specific activity is defined as 1 μmole of NAD reduced/min per mg of protein. The protein concentration was estimated spectrophotometrically assuming $E_{cm}^{1\%}$ 10 at 280 mμ.

Enzyme Purification. Rabbit brain GAPDH was isolated by a modification of the procedure developed by Allison and Kaplan (1964) for the muscle enzyme. β-Mercaptoethanol (0.01 M) was included in all the solutions employed in the procedure. A slightly modified ammonium sulfate fractionation procedure was used, and desalting was accomplished using a Sephadex G-25 column. DEAE Sephadex A-50 instead of DEAE-cellulose was employed for chromatography.

The specific activity of rabbit muscle GAPDH isolated by this procedure is twice the value reported by Allison and Kaplan (1964). It is similar to that obtained by the procedure of Cori (Velick, 1955) and approaches the value found by Amelunxen and Carr (1967) for the enzyme assayed in the absence of pyrophosphate. The specific activity of six GAPDH preparations obtained by this method varied within ±5%.

1. CRUDE EXTRACT. Frozen rabbit brains (8.5 kg) (Pel-Freeze Biologicals, Rogers, Ark.) were broken into small pieces and suspended in 17 l. of cold 0.01 M Tris-Cl, 0.001 M EDTA, and 0.01 M β-mercaptoethanol (pH 7.5). After thawing, the mixture was homogenized in a commercial blender for 30 sec and then centrifuged at 53,000*g* for 1 hr. The precipitate was discarded.

2. AMMONIUM SULFATE FRACTIONATION. The opalescent supernatant was brought to 60% saturation by the addition, over a 2-hr period, of 39 g of solid ammonium sulfate (enzyme grade)/100-ml solution. The pH was adjusted to 7.5 with 5 N ammonium hydroxide, the solution was allowed to stand 3 hr at 0° and was then centrifuged for 1 hr at 14,000*g*. The supernatant fluid was brought to 80% saturation by the addition of 14 g of ammonium sulfate/100-ml solution. The pH was adjusted to 8.2 with 5 N ammonium hydroxide, and the solution was allowed to stand overnight at 0°. The precipitate was collected by centrifugation at 14,000*g* for 1 hr.

3. DESALTING. The 60–80% ammonium sulfate precipitate containing about 43 g of protein was dissolved in 500–700 ml of 0.005 M Tris-Cl (pH 7.5), 0.001 M sodium EDTA, and 0.01 M β-mercaptoethanol. The solution (approximately 700 ml) was immediately passed through a Sephadex G-25 coarse (Pharmacia, Uppsala) column (10 × 110 cm) previously equilibrated with 0.005 M Tris-Cl, 0.001 M EDTA, and 0.01 M β-mercaptoethanol (pH 7.5). The protein-containing effluent (usually diluted less than 25%) was collected within 3–4 hr.

4. CHROMATOGRAPHY ON DEAE SEPHADEX. The desalted solution was layered on the top of a DEAE Sephadex A-50 coarse column (3.7 × 110 cm) previously equilibrated with 0.005 M Tris-Cl (pH 7.5), 0.001 M EDTA, and 0.01 M β-mercaptoethanol. After passage of the sample into the column, elution was begun with the same buffer used for equilibration. GAPDH activity was highest in the earlier fractions and subsequently declined. A volume corresponding to that of the applied sample, usually containing about one-half of the original activity at a specific activity of 15–20, was selected for crystallization.

5. CRYSTALLIZATION. The combined DEAE Sephadex fractions containing approximately 4 mg of protein/ml (in some preparations, concentration in an Amico ultrafiltration unit was required) of specific activity 15–20 was brought to 70% saturation by the addition of 47.4 g of ammonium sulfate/100-ml solution. After standing at 0° for 4–5 hr, the mixture was centrifuged at 35,000*g* for 30 min and the precipitate was discarded. The supernatant was adjusted to pH 8.2 with 5 N ammonium hydroxide, and the solution became opalescent. After standing overnight at 0°, small crystals were apparent from microscopic examination of the solution. The suspension was then allowed to stand for 1 week to complete crystallization. The crystals were collected by centrifugation at 14,000*g* for 30 min and dissolved in 0.005 M Tris-Cl, 0.001 M EDTA, and 0.001 M β-mercaptoethanol (pH 7.5) to give a concentration of about 60 mg/ml. The specific activity of the preparation at this stage was at least 40.

6. RECHROMATOGRAPHY ON DEAE SEPHADEX. The solution of the first crystals was desalted on a Sephadex G-25 column (3 × 90 cm) and the resulting solution was

TABLE I: Summary of Procedure for Isolation of Rabbit Brain D-Glyceraldehyde 3-Phosphate Dehydrogenase.

Step	Procedure	Total Protein (g)	Total Act. (units)	Recov %	Protein Concn (mg/ml)	Sp Act.
1	Extract	161	132,000	100	11.5	0.82
2	60–80% $(NH_4)_2SO_4$ precipitate	42.8	83,000	63	62.7	1.93
3	Desalting (Sephadex G-25)	42.8	83,000	63	48.2	1.93
4	DEAE Sephadex A-50 chromatography	2.23	43,000	33	2.5	19.3
5	Crystallization	0.877	38,000	28	1.7	43.5
6	DEAE Sephadex A-50 rechromatography	0.670	37,000	28	27.0	55.6
7	Recrystallization	0.620	30,000	23	25.0	48.3

rechromatographed on DEAE Sephadex A-50 (2 × 90 cm column as outlined in step 4). Traces of hemoglobin were removed by this procedure.

7. RECRYSTALLIZATION. The fractions of specific activity greater than 55 were adjusted to approximately 27 mg of protein/ml, pH 8.0–8.2, and crystallized by the slow addition of solid ammonium sulfate. Figure 1 shows the typical crystals obtained by this procedure. The specific activity of GAPDH was not increased by further recrystallization nor by chromatography on carboxymethyl Sephadex (0.005 M phosphate, pH 6.0) with elution by a linear gradient of 0–1 M NaCl. The purification procedure is summarized in Table I.

Polyacrylamide Gel Electrophoresis. Electrophoresis was performed in 5% polyacrylamide gel according to the method of Ornstein (1964) and Davis (1964). Neutralized thioglycolate (1 μmole) was added to the protein sample (approximately 400 μg) to reduce any persulfate remaining in the gel after polymerization (Brewer, 1967). A constant current of 3 mA/gel column (0.5 × 5.1 cm) was applied, and electrophoresis was carried out at 8° for approximately 1 hr during which the bromophenol blue dye migrated the length of the column. The columns were then stained with 1% Amido Black 10B in 7% acetic acid and destained by electrophoresis in 7% acetic acid.

Cellulose Acetate Electrophoresis. Zone electrophoresis on cellulose polyacetate strips (Gelman Instrument Co.) was performed in 0.05 M barbital (pH 8.6) containing 0.01 M β-mercaptoethanol at 250 V for 180 min at 37°. The point of application of the enzyme was equidistant from the electrodes. GAPDH activity on the strips was revealed by the staining procedure described by Lebherz and Rutter (1967).

Determination of Sulfhydryl Groups. The number of sulfhydryl groups was determined by the spectrophotometric method of Boyer (1954). Titration with *p*-hydroxymercuribenzoate (PMB) was performed in 4 M urea–0.1 M phosphate buffer (pH 7.0).

Peptide Maps. GAPDH (10 mg/ml) in 8 M urea containing 0.1 M ammonium bicarbonate (pH 8.2) and 0.1 M β-mercaptoethanol was stirred for 4 hr at 45°. A fivefold molar excess of iodoacetamide over the β-mercaptoethanol was then added, and the pH was maintained at 8.2 according to Craven *et al.* (1965). After 15-min incubation at 25°, approximately tenfold molar excess β-mercaptoethanol over iodoacetamide was added. After extensive dialysis against 0.2 M ammonium bicarbonate buffer (pH 8.6), bovine trypsin (TPCK treated, Gallard Sleshinger Chemical Manufacturing Corp.) was added (GAPDH:trypsin ratio of 100:1, w/w). The solution was incubated for 4 hr at 25°, an equivalent amount of trypsin was added, and the incubation was continued for another 4-hr period. After freeze drying, approximately 3 mg of peptide mixture was dissolved in 20 μl of water and applied to No. 3MM Whatman filter paper. Chromatography was performed in butanol–acetic acid–water (4:1:5) for 16.5 hr. After drying for 24 hr, the sheet was rotated 90° and electrophoresis was carried out in pyridine–acetic acid–water (1:10:289) (pH 3.6) for 1 hr, 2600 V, as described by Katz *et al.* (1959).

Immunological Analysis. Antibodies to the rabbit brain and muscle GAPDH were prepared in mature roosters. Each bird received four injections each of 12.5 mg of protein in complete Freund's adjuvant (Difco). Ten days after the last injection, the animals were bled and the serum was prepared. The immunochemical studies were carried out without further purification of the sera. The double-diffusion analysis was carried out by a modified Ouchterlony technique as described by Campbell *et al.* (1963) in 0.7% agar in borate–saline buffer (pH 8.4) at room temperature. Immunoelectrophoresis in agar or agarose was carried out in barbital–acetate buffer (pH 8.6), ionic strength 0.0125. Electrophoresis was carried out at 6 V/cm for 60 min. The antiserum was then added and, after diffusion into the gel, three volumes of saturated NaCl were added for maximal precipitation.

Results

Catalytic Activity. The specific activity of brain GAPDH was 56 μmoles of NAD reduced/mg of protein per min under the conditions of our assay (25°, 0.001 M EDTA–0.05 M pyrophosphate buffer, pH 8.5). As expressed in terms of the second-order rate constant, *k*,

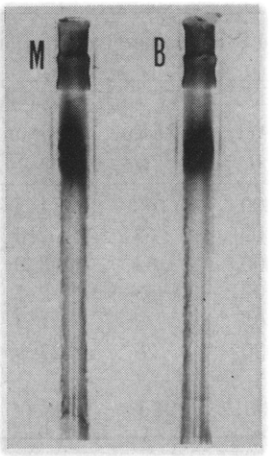

FIGURE 2: Electrophoresis of the muscle (M) and brain (B) GAPDH. Electrophoresis was performed in 5% polyacrylamide gel (pH 8.6). The gels were stained for proteins as described in Experimental Procedure.

TABLE II: Molecular Weights Determined for Brain and Muscle GAPDH.[a]

	Muscle Enzyme	Brain Enzyme
M_n	148×10^3	148×10^3
M_W	148×10^3	146×10^3
M_z	148×10^3	144×10^3
$(2M_n - M_w)_{c=0}$[b]	146×10^3	146×10^3

[a] Molecular weight determinations were performed in 0.1 M phosphate buffer (pH 7.0) for 14 hr at 15,000 rpm using the high-speed sedimentation equilibrium method of Yphantis (1964). [b] $(2M_n - M_w)_{c=0}$ denotes $2M_n - M_w$ extrapolated to $c = 0$ by least-squares procedures (Yphantis, 1964).

per milligram of protein (Velick, 1955), this corresponds to a value of about 1.4×10^6. If a molecular weight of 145,000 is assumed, this corresponds to a turnover number of approximately 13,750 moles of reduced NAD/mole of enzyme per min at initial concentrations of 4.8×10^{-4} M of D-glyceraldehyde 3-phosphate and NAD. These values are almost identical with those for the muscle GAPDH preparation reported by Velick (1955) (approximately 12,400 at pH 8.6, 27°) and close to that reported by Cori *et al.* (1948) (approximately 9700 under assay conditions similar to those employed here). These discrepancies do not necessarily reflect differences in the purity of the preparations, but rather in the catalytic activity of the isolated GAPDH (see Amelunxen and Carr, 1967). After the second recrystallization, the activity decreased approximately 15%.

Zone Electrophoresis. Electrophoresis of the muscle and brain GAPDH in polyacrylamide gel (pH 8.6) is shown in Figure 2. Single protein bands were obtained in each case. There were no significant differences in the electrophoretic mobilities of the two preparations.

Zone electrophoresis of the two enzymes was also performed on cellulose acetate strips (Figure 3). Single bands of activity corresponding to the protein stains were found in each preparation. A slight difference in mobility was detected after electrophoresis for 3 hr in barbital buffer (0.05 M, pH 8.6) at 37°. Coelectrophoresis of the two enzymes, however, resulted in a single discreet band.

Microheterogeneity was detected in both brain and muscle preparations using an isoelectric fractionation technique (Svensson, 1961; Vesterberg and Svensson, 1966; Vesterberg, 1967) with a remarkable resolving capacity. In both preparations, four and possibly five distinct peaks of protein and activity were detected with very similar isoelectric points in the range of pH 8.0–8.5. The chemical basis of this heterogeneity has yet to be elaborated.

Molecular Weight. In collaboration with Dr. David C. Teller (Department of Biochemistry, University of Washington), the molecular weights of muscle and brain GAPDH were determined using the high-speed sedimentation equilibrium method of Yphantis (1964). The rotor speed was 15,000 rpm with initial concentration of 0.75 mg/ml in 3-mm solution columns. The partial specific volume, $\bar{v}$, was assumed to be 0.739 (Taylor and Lowry, 1956), a value close to that calculated from the amino acid composition of muscle GAPDH (0.742). The strong similarity in the amino acid composition of the two enzymes as deduced from the peptide maps justifies the utilization of the same $\bar{v}$ value. The results of the determinations are presented in Table II. There is no significant difference in the molecular weight values obtained for these enzymes in the present studies. A constant value of M_n was found along the length of the cell indicating a high degree of homogeneity of the preparations. These values correspond to those recently reported for rabbit muscle GAPDH (146,000 ± 5000

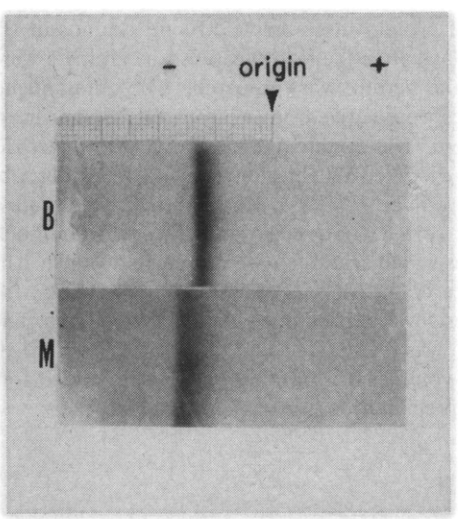

FIGURE 3: Cellulose acetate electrophoresis of the muscle (M) and brain (B) GAPDH. Electrophoresis was performed in 0.05 M barbital (pH 8.6) containing 0.01 M β-mercaptoethanol at 240 V for 180 min at 37°. The strips were stained for activity as described in Experimental Procedure.

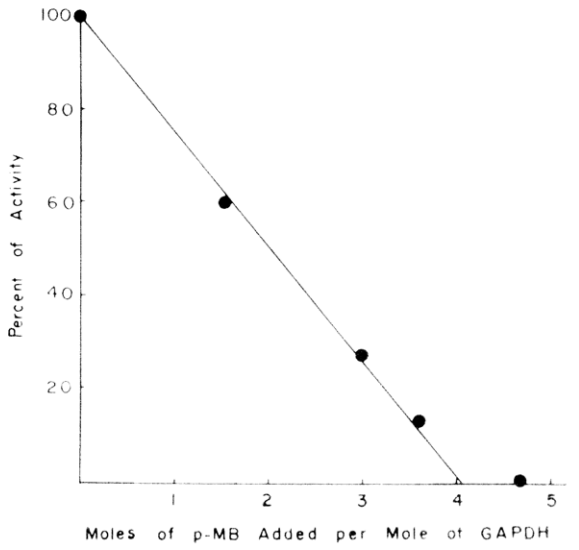

Figure 4: Titration of brain GAPDH with *p*-hydroxymercuribenzoate. Titration of enzyme (1 mg/ml) was performed in 0.3 M acetate buffer (pH 4.6). The loss of activity was measured within the first 30 sec of assay.

by Harris and Perham (1965), Harrington and Karr (1965), and Závodszky *et al.* (1966)).

Sulfhydryl Groups. Titration of brain and muscle GAPDH using the *p*-hydroxymercuribenzoate method developed by Boyer (1954) gave values of 12.1 and 13.6 sulfhydryl groups per mole for the brain and muscle enzymes, respectively. The value obtained for the brain enzyme is within the range of values reported for the muscle enzyme (Boyer, 1954; Velick and Furfine, 1963; Koeppe *et al.*, 1956; Segal and Boyer, 1953; Velick and Ronzoni, 1948; Benesch *et al.*, 1955). The variation probably is the result of different degrees of oxidation of enzyme preparations.

The number of "active" sulfhydryl residues for the brain GAPDH was also determined by *p*-hydroxymercuribenzoate titration. The enzyme, at a concentration of 1 mg/ml in 0.3 M acetate buffer (pH 4.6), was titrated with PMB. The loss of activity measured within the first 30 sec of assay was proportional to the amount of PMB added. As shown in Figure 4, titration of four sulfhydryl groups per mole of enzyme resulted in the loss of all catalytic activity. This value agrees with the value (3.5) of "active" sulfhydryl residues for muscle GAPDH obtained by Velick (1953) and Harris *et al.* (1963). It is consistent with the studies of Harris and Perham (1965) who have shown that the muscle GAPDH is composed of four identical chains, each of which contains one reactive cysteine residue.

Reducible NAD Bound to Brain GAPDH. The amount of bound NAD which can be reduced by the enzyme was determined by the increase in extinction at 340 mμ after reduction of bound NAD to NADH by addition of glyceraldehyde 3-phosphate according to Allison and Kaplan (1964). Values of 2.53 and 1.94 reduced NAD per mole of brain and muscle GAPDH, respectively, were obtained. Allison and Kaplan (1964) reported approximately 2 moles of NAD reduced/mole of rabbit muscle GAPDH.

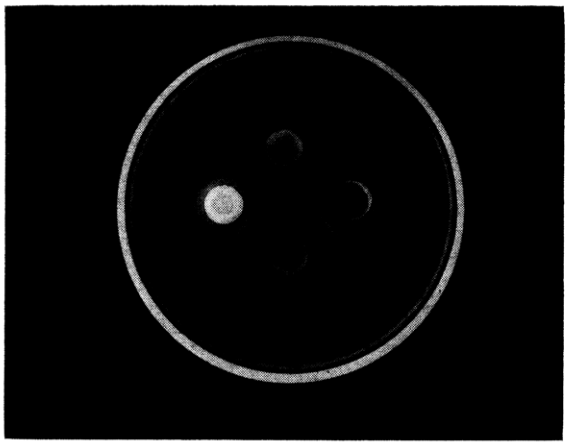

FIGURE 5: Ouchterlony diffusion patterns for brain and muscle GAPDH. Top well: brain enzyme; bottom well: muscle enzyme; left well: antiserum to brain enzyme; right well: antiserum to muscle enzyme.

These values are not indicative of the maximum number of NAD molecules which can be bound to the enzyme, but rather the bound NAD which is reducible under the experimental conditions. The somewhat larger value obtained here for the brain GAPDH probably indicates a higher degree of saturation of binding sites by NAD in the brain enzyme.

Immunochemical Studies. The results of a double-diffusion analysis of brain and muscle GAPDH are presented in Figure 5. The two enzymes behaved identically in these experiments. Antibodies prepared against either enzyme cross-reacted similarly with both of the enzymes. No spurs were detected in the patterns.

Immunoelectrophoresis of the enzymes was carried out in collaboration with Dr. William Sievert and Mr. Richard Findlay of Abbott Laboratories (North Chicago). As shown in Figure 6, the major components of both the muscle and brain enzymes appeared identical, but minor differences were apparent. The immunochemical studies taken collectively support the contention that the major fractions of brain and muscle GAPDH preparations are immunochemically identical;

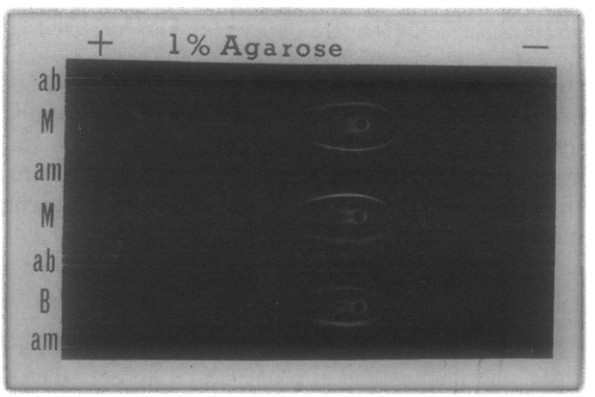

FIGURE 6: Immunoelectrophoretic pattern for brain and muscle GAPDH. M = muscle enzyme; B = brain enzyme; am = antiserum to muscle enzyme; ab = antiserum to brain enzyme. Immunoelectrophoresis was performed as described in Experimental Procedure.

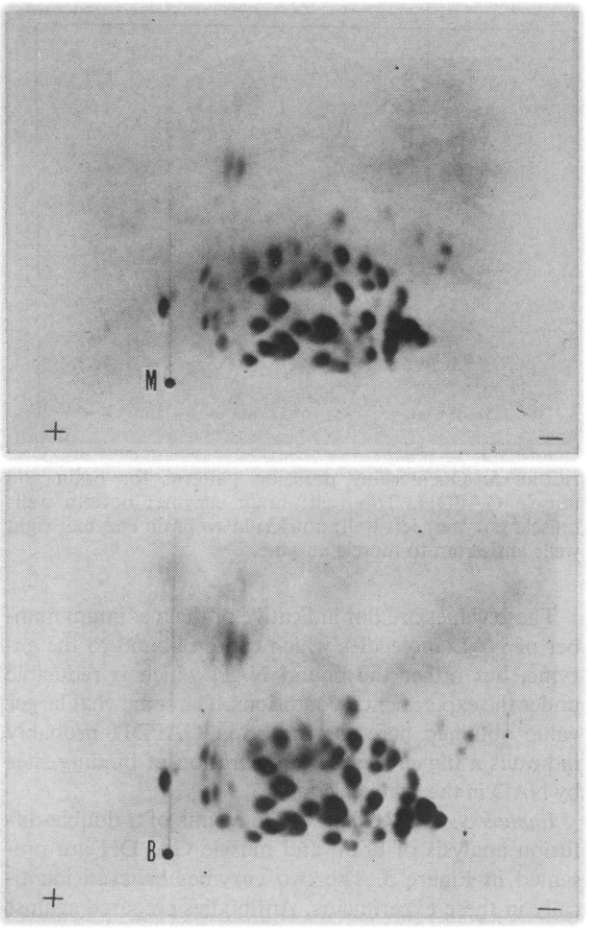

FIGURE 7: Peptide map patterns obtained from tryptic digestion of muscle (M) and brain (B) GAPDH. Enzymes were treated with iodoacetamide prior to digestion. All procedures are described in Experimental Procedure.

however, a minor degree of heterogeneity is apparent in both samples. This may represent partial degradation of the protein molecule or may be the result of microheterogeneity of the native enzyme.

A similar immunoelectrophoretic analysis of crystalline liver GAPDH (kindly provided by Dr. S. Velick) was performed using antibodies prepared against brain and muscle GAPDH. No differences were detected in the liver, brain, or muscle enzymes by this procedure.

Peptide Maps. Peptide maps of brain and muscle GAPDH are presented in Figure 7. There were no significant differences in the patterns observed from the two enzymes. A slight quantitative difference in one or two spots was observed (see arrows), but no evidence of qualitative differences in the peptide patterns was found.

Discussion

From electrophoretic mobilities, catalytic characteristics, and immunological properties, it appears that a number of glycolytic enzymes have a broad tissue distribution. This supposition has been rarely tested by a direct analysis of primary structure. In one study, Stewart and Margoliash (1965) found that cytochrome *c* preparations isolated from a number of tissues have similar fingerprint patterns and have concluded that the molecules are identical. In the present studies, no convincing differences between muscle and brain GAPDH have been observed by a number of parameters reflecting the primary structure. Of course, these experiments cannot prove the identity of the two molecules, but the close correspondence of the peptide maps, the immunological, chemical, and catalytic properties of these enzymes indicate the molecules must be very similar, if not identical. The small differences in the various parameters reported here appear more likely to be a result of subtle differences resulting from the isolation procedures or from the different originating cellular environments. However, slight differences in the primary structure of these preparations cannot be rigorously excluded by the present experiments. A more extensive comparison of the primary structure, especially employing less cumbersome methodology (Edman and Begg, 1967), would appear warranted to rigorously establish this point.

The probable identity of brain and muscle GAPDH and the close similarities with the GAPDH from various tissues (Lebherz and Rutter, 1967) contrasts with the easily distinguishable variants of other glycolytic enzymes (aldolase (Penhoet *et al.*, 1967; Rutter *et al.*, 1968), lactic dehydrogenase (Markert and Moller, 1959; Kaplan *et al.*, 1960), hexokinase (Sharma *et al.*, 1963; Walker, 1963; Ballard and Oliver, 1964; González *et al.*, 1964; Katzen and Schimke, 1965; Grossbard and Schimke, 1966), pyruvate kinase (Tanaka *et al.*, 1967; Susor and Rutter, 1968), and phosphorylase (Davis *et al.*, 1966)) which have distinctive tissue distributions and probably distinctive physiological functions. The possibility of a distinct GAPDH variant in liver tissue is raised by the recent studies of Papadopoulos and Velick (1967) which show that the GAPDH isolated from liver has different kinetic properties from those of the muscle GAPDH. We have not performed a similar kinetic analysis of the brain enzyme, however we have detected no differences in the electrophoretic or immunoelectrophoretic properties of all three enzymes. The great resolving capacity of the isoelectric fractionation procedure mentioned in the Results may be useful in determining the yet unknown structural basis of these kinetic differences.

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