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PURIFICATION AND CHARACTERIZATION OF TWO ISOZYMES OF 3-PHOSPHOGLYCERATE KINASE FROM THE MOUSE

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Summary

Two isozymes of 3-phosphoglycerate kinase (ATP:3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3), designated PGK-A and PGK-B, were purified from separate extracts of muscle and testicular tissue of DBA/2J mice, respectively. A similar procedure was used to purify the corresponding isozymes from C57BL/6J mice in order to make inter-strain comparisons. The purification involved the use of affinity chromatography with an 8-(6-aminohexyl)amino-ATP-Sepharose column and DEAE-Sephadex chromatography. Lactate dehydrogenase isozyme LDH-X was also co-purified from extract of mouse testes by this two-step procedure. The same isozyme isolated from either mouse strain was found to be identical in physical and biochemical properties. Both isozymes are monomeric as determined by gel filtration chromatography and by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Furthermore, the isozymes have similar molecular weights, of 47 000 ± 2000 and exhibit similar K_m values for both coenzymes and substrate, as well as temperature dependence of enzyme activity. However, it was observed that the B isozyme is more labile than the A isozyme by denaturation at high temperature, urea and acidic pH.

Two of the glycolytic enzymes in sperm are known to exist in specific isozyme forms [1,3]. They are 3-phosphoglycerate kinase (ATP:3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3) [2,3] and lactate dehydrogenase [1]. The presence of such tissue-specific isozymes may be functionally and

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Abbreviations: LDH-X: Sperm-specific isozyme of lactate dehydrogenase; PGK-A: somatic isozyme of 3-phosphoglycerate kinase; PGK-B: sperm-specific isozyme of 3-phosphoglycerate kinase.

physiologically important to sperm. This has been suggested by the previous extensive studies with lactate dehydrogenase X from various mammalian species [5—10].

In the case of 3-phosphoglycerate kinase, the two isozymes extracted from somatic and germinal tissues are designated as PGK-A[3] and PGK-B, respectively [2]. They differ in electrophoretic mobility [2,3]. PGK-B is found only in mature testes and sperm of mammals, and represents as much as 50—70% of total phosphoglycerate kinase activity in the extract of testicular tissue. However, it is this phosphoglycerate kinase isozyme which is only found in sperm of many mammalian species [2,3].

In humans, the gene coding for PGK-A is associated with the X-chromosome [11], while in mice indirect evidence suggest that it may also be X-linked [12]. PGK-B, on the contrary, has been shown to be an autosomal enzyme in the mouse (refs. 2 and 3 and Eicher, E.M., personal communication). This raised some speculations regarding the expression of this particular isozyme on the physiological function of sperm [3,13].

In this paper, we report the use of general ligand affinity chromatography [14] as a tool to obtain pure PGK-B and lactate dehydrogenase X from extract of mouse testes, and PGK-A from muscle extract. The physical and biochemical properties of these two 3-phosphoglycerate kinase isozymes isolated from both DBA/2J and C57BL/6J mouse were mutually compared in detail. Through this study, we hope to understand more about the possible structural and functional differences of these two 3-phosphoglycerate kinase isozymes and their strain variations.

Materials and Methods

Chemicals

The chemicals used in this study were obtained from Sigma Chemical Company, Missouri. These include: NAD^+ (acid form), β-NADH (sodium salt), 5'-AMP, ADP, ATP, GTP, UTP, CTP (sodium salt), 3-phosphoglycerate (sodium salt), α-ketoglutaric acid, lactate (sodium salt), pyruvate (sodium salt), DL-glyceraldehyde 3-phosphate (hemiacetal) and glyceraldehyde-3-phosphate dehydrogenase.

Reduced NAD^+-pyruvate adduct employed in the elution of lactate dehydrogenase from affinity column was prepared by a direct mixing of equal amounts of NAD^+ and pyruvate (100 mg each) in 1 ml distilled water where pH was adjusted to and maintained at 11.5 for 10 min with 1 M NaOH. It was used immediately after preparation without any further purifications.

Tissues

Because of the distinct differences in genetic background, two strains of mice, DBA/2J and C57BL/6J were selected for enzyme purification and characterization. They were obtained from Jackson Laboratory at the age of 8—12 weeks. They were killed by cervical dislocation. Both abdominal muscle and testes were removed and stored at -60° until use for enzyme purification.

Enzyme assays

Lactate dehydrogenase was assayed according to a published procedure [15]

except that 0.1 M Tris · HCl, pH 8.0, was used as the assay buffer. LDH-X, in a mixture of lactate dehydrogenase isozymes, was estimated according to the procedure of Schatz and Segal [7]. 3-Phosphoglycerate kinase was routinely assayed by the formation of 1,3-diphosphoglycerate [16] (backward reaction) unless otherwise specified. In a final volume of 1 ml, the concentrations of the reaction mixtures and buffer were: 100 mM Tris · HCl, pH 8.0, 10 mM $MgCl_2$, 0.15 mM NADH, 2 mM ATP, 6 mM 3-phosphoglycerate and 10 μl glyceraldehyde-3-phosphate dehydrogenase (10 mg/ml in 1.2 M ammonium sulfate). The reaction was measured at 340 nm and 26 ± 1°C on a Beckman Model 25 spectrophotometer. Activity was expressed in terms of international units (μmol of 1,3-diphosphoglycerate formed per min).

The forward reaction of this enzyme (formation of 3-phosphoglycerate) was assayed according to the method of Krietsch and Bücher [16].

Protein determination

The protein concentration was routinely determined by the fluorescamine assay of Böhlen et al. [17]. Bovine serum albumin was employed as a standard for the protein determination.

Electrophoresis

Lactate dehydrogenase isozymes were routinely analyzed by disc acrylamide gel electrophoresis, using 7.5% acrylamide gel in 0.2 M Tris · HCl buffer, pH 8.5, and 0.04 M Tris/glycine buffer, pH 8.3, as the well buffer. Activity and protein staining of the gel followed a published procedure [18]. The purity of 3-phosphoglycerate kinase preparations was routinely analyzed by SDS acrylamide gel electrophoresis using the same buffer system except that 0.1% SDS was included in the well buffer and 1% SDS in the gel buffer. Isozymes of 3-phosphoglycerate kinase were analyzed by starch gel electrophoresis according to the procedure of Beutler [19].

Molecular weight determination

The molecular weight of native 3-phosphoglycerate kinase was determined by Sephadex G-150 chromatography where malate dehydrogenase (molecular weight 67 000), adenylate kinase (21 000) and cytochrome *c* (12 500) were employed as standards. The molecular weight of denatured 3-phosphoglycerate kinase was determined by SDS gel electrophoresis where phosphorylase *a* (subunit molecular weight 91 000), bovine serum albumin (68 500), enolase (41 000) and α-glycerophosphate dehydrogenase (34 000) were used as standards.

Preparation of affinity columns

An 8-(6-aminohexyl)amino-ATP-Sepharose column was employed as an affinity column for the purification of lactate dehydrogenase and 3-phosphoglycerate kinase. This affinity gel was prepared according to the procedure of Lee et al. [20]. This affinity gel can be regenerated by washing with a solution of 6 M urea and 2 M NaCl immediately after use. It can be used repeatedly at least 10 times without a significant loss in its capacity to separate these two enzymes.

Results

Purification of lactate dehydrogenase-X and 3-phosphoglycerate kinase isozymes

An identical purification procedure was performed for the purification of PGK-A from muscle as well as PGK-B and LDH-X from testes of either DBA/2J or C57BL/6J mice.

By starch gel electrophoresis, it was shown that only PGK-A is present in muscle extracts, whereas both PGK-A and PGK-B are present in testicular extracts [3]. With this purification procedure, a mixture of both PGK-A and PGK-B was first purified from mouse testes by an 8-(6-aminohexyl)amino-ATP-Sepharose column. They were separated in the subsequent step by DEAE-Sephadex ion exchange chromatography. Purification of LDH-X from mouse testes using this procedure was based on similar principles.

The purification of PGK-B and LDH-X from testes and PGK-A from muscle of DBA/2J mouse are given as examples.

A. PGK-B and LDH-X from testes

Step 1. 20 g of frozen testes were placed in 100 ml of 10 mM phosphate buffer, pH 6.5, and homogenized with a Virtis homogenizer. After centrifugation at 27 000 × *g* for 20 min, the supernatant was passed through a double-layer cheese cloth to remove suspended lipids. It was then passed through an 8-(6-aminohexyl)amino-ATP-Sepharose column (30 ml, 2 × 10 cm) at 4°C. Lactate dehydrogenase was quantitatively adsorbed on the affinity column, whereas leakage of 3-phosphoglycerate kinase was observed at the end of the loading. About 30% of input 3-phosphoglycerate kinase activity was found in the eluent during the wash of the column with 150 ml of 10 mM phosphate buffer, pH 6.0. The remaining 3-phosphoglycerate kinase was sharply eluted with 2 mM ATP in the wash buffer. Lactate dehydrogenase was subsequently eluted with 0.2 mM reduced NAD-pyruvate adduct (2 absorbance units/ml at 340 nm) in the same buffer. The elution profile is shown in Fig. 1. The eluent containing 3-phosphoglycerate kinase which leaked during loading and subsequent washing of the column was rechromatographed on another 8-(6-aminohexyl)amino-ATP-Sepharose column of the same size. Quantitative adsorption of 3-phosphoglycerate kinase was observed. A biospecific elution with 2 mM ATP gave quantitative recovery of the readsorbed 3-phosphoglycerate kinase from the affinity column. Fractions obtained from these two passages were combined. The specific activity of pooled fractions is about 35 units/mg and overall recovery for this step is about 80%. The specific activity of eluted lactate dehydrogenase is about 280 units/mg, and the recovery is about 70%.

Step 2. The pH of the pooled 3-phosphoglycerate kinase fractions (40 ml) was adjusted to 8.0 by a slow dropwise addition of 1 M NH_4OH. The sample was then passed through a DEAE-Sephadex column (30 ml, 2 × 10 cm) which had been equilibrated with 10 mM phosphate buffer, pH 8.0. After the loading, about 20% of the input 3-phosphoglycerate linease activity was eluted as a broad peak with the same buffer. The remaining 3-phosphoglycerate kinase activity was eluted with a 0—0.1 M NaCl gradient (200 × 200 ml) in the wash buffer. The elution profile is shown in Fig. 2. By means of starch gel electro-

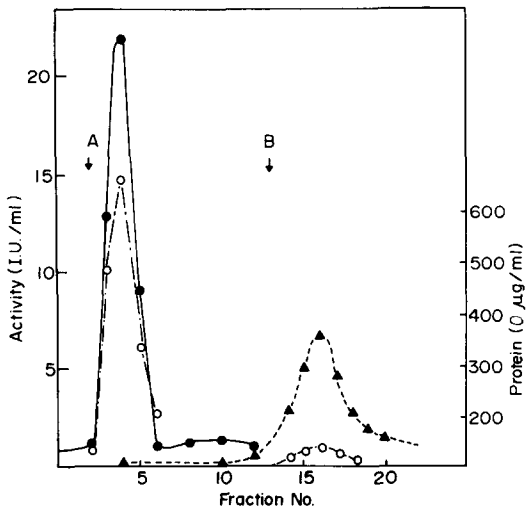

Fig. 1. Elution of 3-phosphoglycerate kinase (●) and lactate dehydrogenase (▲) from DBA/2J mouse testes from an 8-(6-aminohexyl)-amino-ATP Sepharose column (2 × 10 cm). A and B denote the elution by 2 mM ATP and 0.2 mM NAD^+-pyruvate adduct in 10 mM phosphate buffer, pH 6.5, respectively. (○) denotes the protein concentration. Fractions of 5 ml were collected for 3-phosphoglycerate kinase and 10 ml for lactate dehydrogenase.

phoresis, these two 3-phopshoglycerate kinase fractions were shown to be PGK-A and PGK-B, respectively [2]. In peak I (Fig. 2) the specific activity of PGK-A was determined to be 100 units/mg and was only 25% pure by analysis of the protein bands seen by SDS gel electrophoresis. In peak II, PGK-B was a homogeneous enzyme, with a specific activity of 450 units/mg in fractions 17—20. This represents about a 1000-fold purification over the crude extract. In fractions 14—16 the specific activity was approx. 100—260 units/mg. SDS gel electrophoresis revealed a high molecular weight protein which was

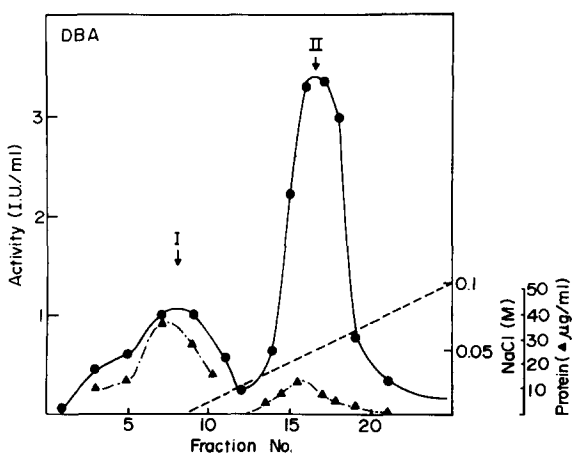

Fig. 2. Elution of 3-phosphoglycerate kinase isozymes from a DEAE-Sephadex A-50 column. This purification step was on enzyme fractions recovered from affinity columns. The dashed line (- - - - -) denotes a 0—0.1 M NaCl gradient in 10 mM phosphate buffer, pH 8.0. Fractions of 10 ml were collected and I and II denote the peaks of PGK-A and PGK-B, respectively.

co-eluted with PGK-B in the earlier fractions. To increase the overall yield of PGK-B, these fractions with low specific activity were pooled and concentrated to 1 ml by a pressure concentrator and chromatographed on a Sephadex G-75 column (1.5 × 75 cm) which had been equilibrated with 0.1 M phosphate buffer, pH 7.5, containing 10 mM β-mercaptoethanol. The single high molecular weight protein contaminant was removed by this step, and the resultant PGK-B was obtained with an overall yield of about 30%. Further purification of PGK-A from mouse testes was not pursued in this study.

Lactate dehydrogenase eluted from the affinity column was found to be a mixture of at least five different isozymes, based on the analysis by acrylamide gel electrophoresis [1,4]. Therefore, after affinity chromatography, all the lactate dehydrogenase fractions were combined and chromatographed on a DEAE-Sephadex column according to the same procedure as that described for the separation of 3-phosphoglycerate kinase isozymes. The lactate dehydrogenase eluted from the column in the second fraction was shown to be LDH-X. By acrylamide gel electrophoresis it was demonstrated to be homogeneous, free from other lactate dehydrogenase isozymes and proteins. The specific activity of LDH-X is 80 units/mg under the described assay conditions [15]. The overall yield is about 50% with respect to the original LDH-X activity in the extract. A general outline for the purification of LDH-X and PGK-B from testes is shown in Table I.

B. PGK-A from muscle

Purification of muscle PGK-A followed essentially the same procedure as that described for PGK-B from testes. The muscle extract was prepared from 5 g of abdominal muscle in 20 ml of 10 mM phosphate buffer, pH 6.5, containing 10 mM β-mercaptoethanol. After passing through an 8-hexyl-ATP-Sepharose column (47 ml, 2 × 15 cm) the adsorbed 3-phosphoglycerate kinase was

TABLE I

PURIFICATION OF LDH-X AND PHOSPHOGLYCERATE KINASE FROM TESTIS AND MUSCLE OF C57BL/6J

20 g of frozen testes and 5 g of frozen abdominal muscle were used in this experiment. PGK, 3-phosphoglycerate kinase; LDH, lactate dehydrogenase.

	Step	Enzyme (tissue)	Total activity (units)	Specific activity (units/mg)	Condition of elution
1.	Crude extract	LDH (testis)	428	0.5	—
		PGK (testis)	371	0.43	
		PGK (muscle)	300	3.0	
2.	Affinity column	LDH (testis)	310	280	0.2 mM NAD pyruvate adduct
	8-(6-aminohexyl)-amino-ATP-Sepharose	PGK (testis)	300	35	2 mM ATP
		PGK (muscle)	240	80	2 mM ATP
3.	DEAE-Sephadex	LDH-X	80	80	10 mM phosphate buffer, pH 8.0 and salt gradient
		PGK-B (testis)	133	260—450	
		PGK-A (muscle)	200	500	Negative adsorption

eluted biospecifically with 2 mM ATP in the same buffer. The specific activity of the eluted enzyme could be as high as 80—100 units/mg. Fractions containing 3-phosphoglycerate kinase activity were pooled and concentrated to 1 ml by a pressure concentrator. After adjusting the pH to 8.0 with 1 M NH_4OH, the sample was adsorbed on a DEAE-Sephadex column (30 ml) equilibrated with 10 mM phosphate buffer, pH 8.0, containing 10 mM β-mercaptoethanol. Only one peak of 3-phosphoglycerate kinase activity was eluted with the same buffer in 10 fractions (5 ml per fraction). By starch gel electrophoresis it was shown to be PGK-A, and in fractions 3—8 it was shown to be homogeneous by acrylamide gel electrophoresis. The specific activity of PGK-A in these fractions is about 500 units/mg. The overall recovery of this preparation was about 30%.

An identical procedure was applied to the purification of 3-phosphoglycerate kinase isozymes from the corresponding tissues of C57BL/6J mouse. Within experimental error, 3-phosphoglycerate kinase isozymes purified from either strain of mouse were shown to have similar specific activity after the same purification steps.

Comparative studies

1. Molecular weight determination. Based on the results of SDS polyacrylamide gel electrophoresis, the molecular weight of denatured PGK-B from DBA/2J was shown to be in the range of 47 000 ± 2000. The molecular weight of native PGK-B was found to be 45 000 ± 3000 as determined by Sephadex G-150 chromatography. Within the experimental error, the molecular weights of PGK-A and PGK-B from either DBA/2J and C57BL/6J mouse were found to be identical under the same conditions.

2. pH dependence of activity. The backward reaction catalyzed by PGK-A and PGK-B from DBA/2J mouse was assayed at different pH values in 0.1 M Tris/maleate buffer. It was found to decrease slightly with increasing pH from 6.5 to 9.0 with a peak activity around pH 6.5. At pH 8.0, identical activity was obtained when assayed in 0.1 M phosphate buffer instead of 0.1 M Tris · HCl buffer. Identical pH dependence of enzyme activity was observed when the same isozyme, isolated from DBA/2J and C57BL/6J mouse, was compared.

3. Thermal stability study. Relative thermal stability of PGK-A and PGK-B was studied by incubation at 50°C at three different pH values (6.0, 7.0 and 8.0) in 0.1 M phosphate buffer. It was generally observed that PGK-B has a lower thermal stability as compared to PGK-A. At pH 6.0 more than 80% of PGK-A activity remained after 10 min of incubation at 50°C, whereas only 20% of PGK-B activity remained under the same experimental conditions. The thermal stability of PGK-A does not vary significantly with pH changes. This is contrary to PGK-B for which low thermal stability was observed at low pH. No strain difference between DBA/2J and C57BL/6J mice was observed regarding the relative thermal stability of each isozyme. For each isozyme, the relative thermal stability was compared between the purified sample and crude muscle homogenate. No apparent difference was observed. The result of this study at pH 6.0 is given in Fig. 3.

4. Temperature dependence of activity. The temperature dependence of the backward and forward reaction catalyzed by these two isozymes was compared in 0.1 M Tris · HCl buffer, pH 8.0. The assay was performed at various temper-

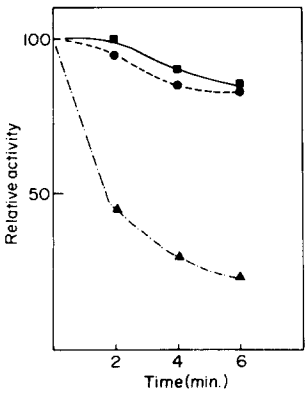

Fig. 3. Comparisons of relative heat stability of 3-phosphoglycerate kinase isozymes from DBA/2J mouse in 0.1 M phosphate buffer at pH 6.0, where (■) represents PGK-A in crude muscle extract, (●) and (▲) are the purified PGK-A and PGK-B isozymes, respectively. The protein concentration for the muscle extract is 250 μg/ml, whereas it is 3 μg/ml for the purified enzyme.

atures after 5 min of incubation of the assay mixtures. It was observed that the activity of both 3-phosphoglycerate kinase isozymes in either direction increased steadily with increasing temperature from 25 to 40°C. No significant difference for the temperature dependence of activity was observed between these two isozymes. Under the same experimental conditions virtually no strain difference was observed regarding the temperature dependence of activity of each 3-phosphoglycerate kinase isozyme.

5. Steady-state kinetic studies. K_m values for coenzymes and substrate were measured at two different temperatures. As shown in Table II, both isozymes exhibit quite similar K_m values for ATP, ADP and 3-phosphoglycerate. K_m

TABLE II

COMPARISONS OF K_m FOR COENZYMES AND SUBSTRATE OF PHOSPHOGLYCERATE KINASE ISOZYMES AT TWO TEMPERATURES

The apparent K_m values were determined by varying the substrate or coenzyme concentrations and keeping the rest of reaction mixtures identical with those of standard assays given in Materials and Methods. 3-PG, 3-phosphoglycerate.

Isozyme	Mouse strain	Coenzyme or substrate	$K_m \times 10^{-4}$ (M)	
			27°C	37°C
PGK-A	DBA/2J	ATP	2.2	2.6
		ADP	2.3	2.3
		3-PG	9.6	17.7
PGK-B	DBA/2J	ATP	3.5	3.2
		ADP	1.6	1.6
		3-PG	11.7	16.8
PGK-A	C57BL/6J	ATP	2.1	2.1
		ADP	2.3	2.3
		3-PG	7.1	11.6
PGK-A	C57BL/6J	ATP	3.0	3.2
		ADP	1.6	1.6
		3-PG	10.5	14.1

values for coenzymes are about one order of magnitude lower than those for the substrate. K_m values for coenzymes do not vary significantly from 27 to 37°C, whereas those for 3-phosphoglycerate increase approx. 50—100%. When K_m values were compared for each isozyme from these two strains of mouse, no differences were found.

6. Inactivation by urea and iodoacetate. Both PGK-A and PGK-B from DBA/2J and C57BL/6J mouse were inactivated with time in the presence of 3 M urea in the assay buffer at 0°C. In the case of PGK-A, about 75 percent of activity remained after 40 min of incubation, whereas more than 80% of PKG-B activity was lost under the same conditions. In the presence of 10 mM iodoacetate, both isozymes were inactivated to almost the same extent. For either isozyme, more than 50% of enzyme activity was lost after the first 10 min of incubation followed by a slow decrease in activity with time.

7. Coenzyme specificity. Under the standard assay conditions, other nucleotide triphosphates were substituted for ATP as a coenzyme. GTP exhibits about 60% of the enzymatic activity as compared to ATP, whereas UTP and CTP show only 1% of the activity. No strain or isozyme difference was observed regarding their specificity to these nucleoside triphosphates. However, 2′-deoxyATP exhibits 3 and 12% of the ATP activity for PGK-A and PGK-B, respectively.

8. pH stability. Both isozymes were incubated at 0°C in 0.1 M Tris, pH 9.6, for 90 min or 0.1 M acetate buffer, pH 4.7, for 45 min. It was observed that both PGK-A and PGK-B were stable at high pH for at least 30 min. However, under the acidic conditions, PGK-B was inactivated more rapidly than PGK-A and lost about 70% of its activity after 45 min of incubation. PGK-A retained 90% of its original activity under the same conditions.

Discussion

In this study, we have employed a generalized two-step procedure to obtain highly purified 3-phosphoglycerate kinase isozymes from mouse testes and muscle. Affinity chromatography using an 8-(6-aminohexyl)amino-ATP-Sepharose column was employed [20—22] as a major step for enzyme purification. In view of the bifunctional affinity properties of this affinity column, LDH-X was co-purified from mouse testicle extract [22]. This unique procedure was found to be very useful in multi-enzyme purifications where the source of the enzymes is limited [23]. The capacity of the 8-(6-aminohexyl)amino-ATP-Sepharose column to 3-phosphoglycerate kinase isozymes is about 50 μg/ml of affinity gel. This is considerably lower than that of other kinases such as creatine kinase and pyruvate kinase [20]. Leakage of 3-phosphoglycerate kinase activity could occur if the affinity column is overloaded with the crude extract; readsorption of the unbound enzyme to a second column often results in better enzyme purification, since the highly competitive enzymes in the extract have been effectively removed in the first passage. Several examples of this saturation-readsorption technique in the purification of dehydrogenases and kinases from crude homogenates [20—22] have illustrated the effectiveness of this method for the purification of weakly bound enzymes by general ligand affinity column.

In order to understand the nature of two 3-phosphoglycerate kinase isozymes purified from mouse testes and muscle, comparative biochemical studies were performed under many experimental conditions. These comparisons were made between two 3-phosphoglycerate kinase isozymes in a given strain of mouse. The strain variations of the same 3-phosphoglycerate kinase isozymes were also investigated. Enzymes from DBA/2J and C57BL/6J mice were compared because of marked differences in their genetic background. Comparisons of molecular weights of native and denatured 3-phosphoglycerate kinase isozymes showed that both are monomeric and have similar molecular size. The characteristics of the structural stability of each 3-phosphoglycerate kinase isozyme do not vary with the presence of other endogenous proteins. Without exceptions, it was generally observed that PGK-A from either strain of mice is more stable than PGK-B under the described conditions. No strain differences in the relative stability of either isozyme was found. The same degree of inactivation by iodoacetate is indicative of the presence of essential thiol group(s) in both isozymes. From the steady-state kinetic studies, when the apparent K_m values of these two isozymes were compared at 27 and 37°C, no dramatic difference was observed. When the temperature dependence of V was compared between these two isozymes, no significant difference was observed. Although PGK-A and PGK-B exhibit quite different structural stability, they do have similar molecular size, coenzyme specificity and catalytic properties.

The specific expression of PGK-B isozyme in sperm and meiotic cells could be attributed to some unique molecular properties of this isozyme in comparison to the somatic enzyme, PGK-A. However, besides the difference in relative structure stability, both isozymes exhibit almost identical biochemical properties. This is in contrast to the unique biochemical properties observed for LDH-X which is also a sperm-specific enzyme [8]. Although these two sperm-specific isozymes are key enzymes in the glycolytic pathways of sperm, our experimental observations do not draw any apparent functional correlations regarding their specific expressions. Perhaps the specific expression of PGK-B isozyme in sperm is the result of a series of responses to gene regulation by hormones during the meiotic processes of testicular cells [24]. Moreover, since there is an evidence of X-chromosome inactivation during spermatogenesis [25], the expression of an autosomal gene coding for PGK-B instead of X-linked PGK-A might be an essential consequence during meiosis [26]. Further studies on this sperm-specific 3-phosphoglycerate kinase isozyme, including the immunological approaches, detailed structural analysis, and its possible role in the physiological functions of sperm and meiotic cells are now in progress.

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