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PURIFICATION AND PROPERTIES OF LIVER TRIOSE PHOSPHATE ISOMERASE

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SUMMARY

Triose phosphate isomerase (D-glyceraldehyde-3-phosphate ketol-isomerase, EC 5.3.1.1) was prepared from horse and human livers with specific activities of 3183 and 2397 I.U./mg, respectively. The molecular weights of the human liver isomerase and the corresponding rabbit muscle enzyme were not significantly different. The rabbit muscle, horse liver, and human liver isomerases were resolved into five, three and three isozymes, respectively, by polyacrylamide gel electrophoresis. Kinetic studies demonstrated similar K_m's for glyceraldehyde-3-phosphate and dihydroxyacetone phosphate and similar equilibrium constants among the unresolved enzymes and the isolated isozymes.

INTRODUCTION

We previously described the separation of liver triose phosphate isomerase (D-glyceraldehyde-3-phosphate ketol-isomerase, EC 5.3.1.1) from liver alcohol dehydrogenase (alcohol:NAD oxidoreductase, EC 1.1.1.1), two activities which were thought to be a property of the same protein[1]. Further studies required a highly purified preparation of isomerase. Although the methods for its purification from calf muscle[2], rabbit muscle[3], and bovine lens[4] have been reported, no procedure was available for the purification of triose phosphate isomerase from liver when this work was begun. We, therefore, developed procedures for the isolation of the enzymes from horse and human liver. The electrophoretic properties, molecular weights and kinetics of these enzymes were also studied.

MATERIALS AND METHODS

Triose phosphate isomerase was coupled with L-glycerolphosphate dehydrogenase (EC 1.1.1.8, Boehringer Mannheim Co., New York, N.Y.) when glycerldehyde-3-phosphate was the substrate (25°). Cuvettes contained either 0.5 mM glyceraldehyde-3-phosphate for routine analysis or concentrations ranging between 0.1 mM and 3.8 mM in kinetic studies, an excess of L-glycerolphosphate dehydrogenase, 0.21 mM

NADH, 5.4 mM EDTA, and 0.02 M triethanolamine buffer, pH 7.9, in a total of 3 ml. When dihydroxyacetone phosphate was the substrate, isomerase was coupled to glyceraldehyde phosphate dehydrogenase (EC 1.2.1.9, Sigma Chemical Co., St. Louis, Mo.). The cuvettes contained between 0.25 mM and 2.57 mM dihydroxyacetone phosphate, an excess of glyceraldehyde phosphate dehydrogenase, 0.5 mM NAD, 6 mM arsenate, 5.4 mM EDTA, and 0.02 M triethanolamine buffer, pH 7.9, in a total of 3 ml.

In kinetic studies K_m and v_{max} were determined using LINEWEAVER–BURK plots[5]. When dihydroxyacetone phosphate was the substrate, DIXON plots[6] were used to correct for the inhibition of isomerase by arsenate and to evaluate K_i. K was calculated using the HALDANE[7] relationship.

Commercial preparations of rabbit muscle triose phosphate isomerase (Boehringer Mannheim Co., New York, N.Y.) were used for purposes of comparison.

Polyacrylamide gel electrophoresis was performed in a continuous buffer system[8] using an E-C Vertical Gel Electrophoresis cell (E-C Apparatus Corp., Philadelphia, Pa.). Protein was detected with amido black; alternatively, gels were sliced, the proteins eluted, and samples assayed for isomerase. The method of RAYMOND[10] was used for orthogonal acrylamide gel electrophoresis.

The molecular weights of rabbit muscle and human liver triose phosphate isomerases were determined by the gel filtration technique of WHITAKER[11] and by the equilibrium sedimentation technique of YPHANTIS[12].

Protein concentration was determined using the method of LOWRY *et al.*[13].

RESULTS

Ground horse liver was extracted with 2.5 parts of 0.05% EDTA (pH 6.8, 4 h, 25°) (Table I). Acetone was added to a final concentration of 35% (v/v) at 3°, the precipitate discarded and additional acetone added to a final concentration of 60%. The precipitate was taken up in 0.05% EDTA, dialyzed to remove acetone and heated (pH 6.8) successively at 40° for 15 min and 50° for 30 min. The resulting supernatant was applied to a Sephadex QAE A-50 column (2.3 cm × 28 cm, 0.007 M phosphate buffer, pH 7.8), eluted with 0.1 M NaCl in the same buffer and crystallized as rectangular needles in 3.5 M $(NH_4)_2SO_4$. The crystals, dissolved in 0.002 M phosphate buffer, pH 7.8, were filtered through a Sephadex G-75 (Superfine) column (2.5 cm × 40 cm) to yield a preparation exhibiting a specific activity of 3 183 I.U./mg. In the case of human liver triose phosphate isomerase, the fraction precipitating between 50% and 60% acetone was chromatographed as above and the first portion of isomerase eluted (specific activity, 2 397 I.U./mg) was used for further studies.

Upon polyacrylamide gel electrophoresis, horse liver, human liver and commercial rabbit muscle isomerases were separated into three, three and five isozymes respectively (Fig. 1). Isomerase activity coincided with bands which stained for protein and was distributed among the isozymes as shown in Table II. No other proteins were observed and areas of gel containing no isomerase activity were found between the isozymes.

Orthogonal gel electrophoresis of the rabbit muscle and horse liver enzymes resulted in equal migration ratios for the major isozymes suggesting that they were equal in molecular size and shape, but had different surface charges (Table III).

TABLE I

PURIFICATION OF HORSE LIVER AND HUMAN LIVER TRIOSE PHOSPHATE ISOMERASES

Fraction	*Specific activity (I.U./mg)**		*Purification factor*		*Total activity (I.U.)*		*Recovery (%)*	
	Horse	*Human*	*Horse*	*Human*	*Horse*	*Human*	*Horse*	*Human*
Water extract	3.5	6.6	1.0	1.0	200 880	735 336	100.0	100.0
Acetone precipitate: Horse 35–60% Human 50–60%	15.8	28.3	4.5	4.8	216 992	397 143	58.3	54.0
Supernatant after heat treatment	23.1	181	6.6	27.3	105 000	145 541	52.3	19.8
QAE-Sephadex chromatography	367	2397	105	362	105 350	97 291	52.6	13.2
2.6 M $(NH_4)_2SO_4$ supernatant	1 238	—	354	—	85 656	—	42.6	—
2.6–3.5 M $(NH_4)_2SO_4$ precipitate	1 918	—	548	—	53 071	—	26.4	—
Sephadex G-75 gel filtration	3 183	—	909	—	39 420	—	19.6	—

* I.U. = 1 μmole of substrate converted per min.

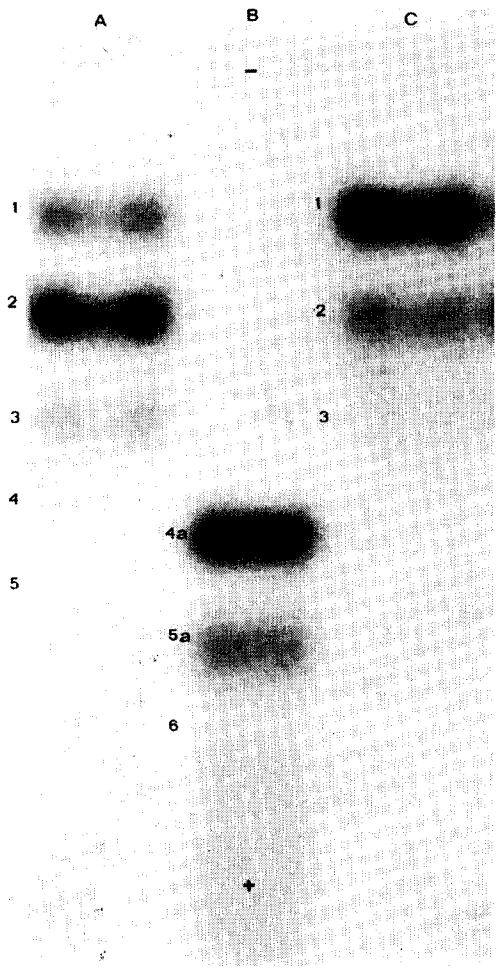

Fig. 1. Electrophoresis of rabbit muscle (A), human liver (B), and horse liver (C) triose phosphate isomerases on 5% polyacrylamide gel (14 h, 4°) visualized with amido black stain.

TABLE II

DISTRIBUTION OF TRIOSE PHOSPHATE ISOMERASE ACTIVITY AMONG ITS ISOZYMES

Isozymes	*Rabbit muscle*	*Horse liver (% of total activity)*	*Human liver*
1	22	50	—
2	44	38	—
3	24	12	—
4	6	—	—
4a	—	—	62
5	4	—	—
5a	—	—	26
6	—	—	11

TABLE III

ORTHOGONAL ACRYLAMIDE GEL ELECTROPHORESIS OF RABBIT MUSCLE AND HORSE LIVER TRIOSE PHOSPHATE ISOMERASES

Source of enzyme	*Isozyme*	*Distance migrated (cm)*		*Migration ratio (B/A)*
		A (5% gel)	*B (8% gel)*	
Rabbit Muscle	1	8.0	4.0	0.50
	2	8.9	4.5	0.51
	3	9.9	5.0	0.51
Horse Liver	1	8.9	5.1	0.57
	2	9.9	5.7	0.58

The molecular weights of rabbit muscle and human liver triose phosphate isomerases determined using gel filtration and equilibrium sedimentation were in good agreement (Table IV). The ultracentrifugation studies also indicated that the enzyme was extremely pure and homogeneous.

Maximum velocities (v_{max}), Michaelis constants (K_m), and equilibrium constants (K) are shown in Table V. The data for the unresolved enzymes are expressed as I.U./mg protein. The activity of the isozymes was not expressed on a protein basis because compounds which interfered with protein determinations were eluted from the gel with the isozymes. Accordingly, v_{max} values expressed as I.U./ml were used to calculate K for the isozymes. The apparent K_m for dihydroxyacetone phosphate was corrected for inhibition of isomerase by arsenate using a DIXON plot[6] ($K_{i,\ arsenate}$ = 5.5 mM).

TABLE IV

THE MOLECULAR WEIGHTS OF RABBIT MUSCLE AND HUMAN LIVER TRIOSE PHOSPHATE ISOMERASES

Source	*By gel filtration**	*By analytical ultracentrifugation***
Rabbit muscle	44 000	49 100
Human liver	48 750	45 740

* A Sephadex G-100 column was used with myoglobin, chymotrypsinogen A, ovalbumin, bovine serum albumin and horse liver alcohol dehydrogenase as the marker proteins. Blue Dextran 2000 was used to determine the void volume.

** Equilibrium sedimentation was performed using interference optics. The protein concentration was 0.5 mg/ml.

DISCUSSION

Recently, KRIETSCH *et al.*[14] and NORTON *et al.*[15] described the purification of yeast, rabbit liver and rabbit muscle isomerases 250-, 1 066- and 458-fold, respectively, using procedures similar to those employed in this laboratory to purify the horse and human liver enzymes 909- and 362-fold, respectively. Although the specific activity of the enzymes derived in this laboratory was somewhat lower than those prepared by KRIETSCH *et al.*[14] and NORTON *et al.*[15], it should be emphasized that the

TABLE V

MAXIMUM VELOCITIES, MICHAELIS CONSTANTS AND EQUILIBRIUM CONSTANTS OF UNRESOLVED TRIOSE PHOSPHATE ISOMERASE FROM HORSE LIVER, HUMAN LIVER AND RABBIT MUSCLE AND THEIR ISOZYMES

Source	*Glyceraldehyde-3-phosphate to Dihydroxyacetone phosphate*		*Dihydroacetone phosphate to glyceraldehyde-3-phosphate*		*K*
	v_{max}*	K_m (*mM*)	v_{max}*	K_m (*mM*)	
I. Horse Liver					
A. Unresolved	7935 ± 456**	0.42 ± 0.06	753 ± 125	0.59 ± 0.01	14.8 ± 2.5
B. Isozymes					
1	10.1	0.47	0.63 ± 0.08	0.57 ± 0.03	19.9
2	8.3	0.43	0.62 ± 0.05	0.60 ± 0.02	18.9
II. Human Liver					
A. Unresolved	2302 ± 119	0.40 ± 0.03	163 ± 5	0.59 ± 0.01	20.9 ± 3.1
B. Isozymes					
4a	6.0	0.40	0.39 ± 0.05	0.57 ± 0.03	22.8
5a	6.1	0.46	0.43 ± 0.10	0.58 ± 0.02	20.5
III. Rabbit Muscle					
A. Unresolved	7231	0.40	542	0.58	19.3
B. Isozymes					
1	5.8 ± 0.1	0.44 ± 0.03	0.41 ± 0.02	0.59 ± 0.01	19.2 ± 1.0
2	7.9 ± 0.1	0.48 ± 0.01	0.61 ± 0.05	0.60 ± 0.01	16.0 ± 0.8
3	7.6 ± 0.6	0.49 ± 0.02	0.51 ± 0.06	0.63 ± 0.03	16.2 ± 1.1

* v_{max} for unresolved enzymes is expressed as I. U./mg protein; v_{max} for isozymes expressed as I.U./ml of enzyme solution (see text).

** Variability is expressed as the standard deviation and was calculated when the experiment had been repeated in some cases three times but in most cases four times. When no standard deviation is reported the experiment was performed twice and the mean value is given.

enzymes were isolated from different species. The high purity of our preparations is supported by observations made during gel filtration, electrophoresis and ultracentrifugation.

The kinetics of rabbit muscle[16], calf muscle[17], human erythrocyte[18], bovine lens[4], yeast[15], rabbit liver[15], horse liver and human liver isomerases have now been studied. The K_m values reported for glyceraldehyde-3-phosphate range between 0.32 mM and 0.5 mM, except for yeast (1.27 mM). The K_m for dihydroxyacetone phosphate has been reported for rabbit muscle[16], rabbit liver[14], yeast[14], human liver and horse liver, and the values appear to center around 0.6 mM. When the K_m's of the individual isozymes were measured they were similar to those of the unresolved enzymes. Most of the values reported for K range between 18 and 22. Thus, the kinetic constants for the triose phosphate isomerase isolated from mammalian tissues and their isozymes do not appear to differ widely despite the differences in electrophoretic mobilities.

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