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PHOSPHOGLYCERATE KINASE FROM YOUNG AND OLD *TURBATRIX ACETI*

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Summary

Phosphoglycerate kinase (ATP:3-phospho-D-glycerate-1-phosphotransferase, EC 2.7.2.3) from young and old *Turbatrix aceti* has been purified to homogeneity. The "old" enzyme exhibits a marked reduction in specific activity both in crude homogenates and in pure form when compared to preparations from young nematodes. The specific activities for pure "young" and "old" enzymes are 650—750 and 300—400 units/mg, respectively. All other properties of "young" and "old" enzymes were nearly identical, including molecular weight (43 000), K_m, behavior on columns, thermal stability and mobility during gel electrophoresis at three pH values. The results are discussed in terms of the possible mechanism of formation of "altered" enzymes. In addition, certain properties of the nematode phosphoglycerate kinase are compared with those of the enzyme from yeast and rabbit muscle.

Introduction

Very little is known about changes at the molecular level which may occur in animals during aging. One clear example of such a change is the recently documented modification of enzymes which occurs in old animals. This area has recently been reviewed by Rothstein [1].

The age-related alteration of enzymes has been shown to result in a reduced catalytic ability of the molecules. The phenomenon has been demonstrated in several enzymes: isocitrate lyase [2], aldolase [3] and enolase [4] in the free-living nematode *Turbatrix aceti* and fructose 1,6-diphosphate aldolase in mouse

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Abbreviation: TEM, Tris/EDTA/mercaptoethanol buffer.

liver [5,6]. The "old" enzyme preparations have been shown to have a reduced specific activity either by demonstrating the presence of extra cross-reacting material or by direct comparison of pure enzyme from old and young organisms. Bolla and Brot [7] have recently reported an age-related decline in DNA polymerase activity in homogenates of *T. aceti*, and provided evidence for the progressive accumulation of inactive or partially active elongation factor 1.

In general, the only change observed in the properties of "old" enzymes, besides a lower specific activity, is in thermal stability [4,8—10]. The "old" preparations, in most cases, yield a biphasic curve, showing a rapidly denatured component, whereas "young" preparations show a constant rate of deterioration. The exception is nematode aldolase, which yields similar biphasic curves when measured in homogenates of both young and old *T. aceti* [3].

In our laboratory, study of isocitrate lyase from young and old *T. aceti* has provided the first comparative data of enzyme properties based upon work with pure enzyme preparations [2,9]. However, isocitrate lyase is unsuitable for further studies because of its large molecular weight (480 000) and complexity (4 subunits; 5 isozymes). On the other hand, phosphoglycerate kinase from yeast and rabbit muscle is known to have a molecular weight of 43 000 and to possess no subunits [11]. Therefore, studies of phosphoglycerate kinase from *T. aceti* were undertaken with the expectation that this would be a suitable enzyme for the study of the structure and mechanism of altered enzymes. This paper describes the purification of phosphoglycerate kinase (ATP:3-phospho-D-glycerate-1-phosphotransferase, EC 2.7.2.3) from young and old *T. aceti* and compares several properties of the "young" and "old" enzymes. In addition, the nematode enzyme is compared, in certain respects, with phosphoglycerate kinases from other sources.

Materials and Methods

D-3-Phosphoglyceric acid, sodium salt, ATP, NADH, glyceraldehyde-3-phosphate dehydrogenase (rabbit muscle) and phosphoglycerate kinase from yeast were obtained from Sigma Chemical Co. Standard proteins (ovalbumin, serum albumin, chymotrypsinogen, trypsin and myoglobin) were obtained from Boehringer-Mannheim. Sephadex G-100 and DEAE-Sephadex A-50 were products of Pharmacia Fine Chemicals. All other chemicals were of analytical grade.

Growth and aging conditions

T. aceti was grown axenically and aged in the presence of fluorodeoxyuridine as described earlier [2,4,12]. The worms were harvested at the desired ages by filtration onto sintered glass funnels, washed several times with TEM buffer (0.05 M Tris · HCl, pH 7.6, containing 1 mM EDTA and 1 mM mercaptoethanol) and suspended in the same buffer.

Enzyme assay

Phosphoglycerate kinase activity was routinely measured in a reaction mixture containing 0.05 M Tris · HCl, pH 7.5; 10 mM $MgCl_2$; 10 mM D-3-phosphoglycerate; 5 mM ATP; 0.2 mM NADH; 20 μg (about 0.7 unit) of glyceralde-

hyde-3-phosphate dehydrogenase in a total volume of 1 ml. The enzyme preparation was added and the decrease in absorbance at 340 nm was recorded at 30°C on a Gilford recording spectrophotometer. The unit is defined as initial activity catalyzing the phosphorylation of 1 μmol of 3-phosphoglycerate per min at 30°C. Specific activity is defined as the number of enzyme units per mg of protein.

Gel electrophoresis

Polyacrylamide gel electrophoresis was used to monitor the purity of the enzyme. The enzyme sample (20—50 μg of protein), containing 2.5% glycerol, was placed on top of 7.5% acylamide gel. Electrophoresis was conducted for 4 h using a constant current of 4 mA/tube in Tris/glycine buffer, pH 8.3. Gel electrophoresis was also run in diethylbarbituric acid/Tris buffer, pH 7.0, and Tris · HCl, pH 9.3 [13]. The gels were stained for protein with naphthol blue black (0.2% dye in 7% acetic acid). Enzyme activity was determined by cutting the gel in 2 mm slices and eluting the enzyme with 0.05 M Tris · HCl, pH 7.5. The method of Weber and Osborn [14] was used for SDS-polyacrylamide disc gel electrophoresis at room temperature.

Purification of phosphoglycerate kinase

All procedures were carried out at 0—4°C. Protein was determined by the method of Lowry et al. [15].

(a) Preparation of crude homogenate. Young worms (7—11 days) employed for purification had a specific activity of 3.6—4.5 units/mg of crude homogenate while old worms (26—28 days) had a specific activity of 2.0—2.5 units/mg. Homogenates were prepared by treatment of a suspension of $2—6 \cdot 10^7$ worms in 60—70 ml of TEM buffer in a cold French pressure cell at 18 000 lb/inch^2. The homogenate was diluted with two volumes of TEM buffer and centrifuged at 25 000 × *g* for 15 min at 4°C. The supernatant fluid was recentrifuged at 105 000 × *g* for 60 min. The supernatant fluid was filtered through a double layer of cheese cloth, and is referred to as "crude homogenate".

(b) Ammonium sulfate precipitation. The crude homogenate was saturated to 55% by addition of 0.351 g/ml of $(NH_4)SO_4$. The precipitate was removed 30 min later by centrifugation at 10 500 × *g* for 10 min. The supernatant fluid was adjusted to 80% saturation by adding 0.179 g/ml of $(NH_4)SO_4$. The precipitate was collected 30 min later by centrifugation at 10 500 × *g* for 10 min. The precipitate thus obtained was dissolved in a minimum amount of TEM buffer and dialyzed against the same buffer for 5 h with a change of buffer.

(c) First Sephadex G-100 column chromatography with TEM buffer. The dialysed sample was applied to a column (2.5 × 100 cm) of Sephadex G-100 which had been equilibrated with TEM buffer. The column was developed with the same buffer and the peak showing enzyme activity (184—246 ml) was pooled and concentrated by use of an Amicon PM-10 membrane.

(d) Rechromatography on Sephadex G-100 column with phosphate buffer. The above concentrate was loaded onto another Sephadex G-100 column (2.5 × 100 cm), this time pre-equilibrated with phosphate buffer (0.1 M, pH 7.6) containing 1 mM EDTA. The column was eluted with the same buffer. The fractions showing phosphoglycerate kinase activity were pooled, concentrated

through a PM-10 membrane and then dialyzed against 10 mM Tris · HCl buffer, pH 8.0, containing 1 mM EDTA.

(e) DEAE-Sephadex A-50 chromatography. The dialyzed solution was applied to a DEAE-Sephadex A-50 column (1.5 × 50 cm) and was eluted with 120 ml of 10 mM Tris · HCl buffer (pH 8.0) containing 1 mM EDTA. The protein was eluted with a linear gradient of sodium chloride in Tris · HCl buffer (0—150 mM), total volume, 400 ml. Fractions of 2 ml were collected.

Phosphoglycerate kinase activity related to age

Crude homogenates were prepared, as described above, from worms of various ages by homogenizing a suspension of 0.5—1 · 10^6 worms in approximately 5 ml of TEM buffer. The phosphoglycerate kinase activity was determined as described above.

Measurement of molecular weight

The molecular weight of the pure enzyme was determined by chromatography on Sephadex G-100 using bovine serum albumin (67 000), ovalbumin (43 000), chymotrypsinogen (25 000) and myoglobin (17 000) as standard proteins. SDS gel electrophoresis was also utilized to determine molecular weight, using ovalbumin (43 000), enolase (41 000), triosephosphate isomerase (26 500), trypsin (23 000) and myoglobin (17 000) as standard proteins.

Heat inactivation

Heat inactivation of phosphoglycerate kinase at 45 ± 0.2°C and 46 ± 0.2°C was carried out by taking an aliquot (5—10 μl) for assay from heated samples (0.1—0.5 ml, in various experiments) at given time intervals.

Results

Phosphoglycerate kinase obtained from Sephadex G-100 in TEM buffer (50 mM) shows a molecular weight of 110—130 000, although the peak is not symmetrical (Fig. 1). The elution pattern for the enzyme in phosphate buffer (100 mM) shows a molecular weight of 43 000 ± 2 000 (Fig. 2).

After final purification on DEAE-Sephadex A-50, the molecular weight was also found to be 43 000 ± 1000 (Fig. 3). The molecular weight as determined by SDS gel electrophoresis shows a single component of 43 000. Therefore, it can be concluded that nematode phosphoglycerate kinase is a monomer with a molecular weight of 43 000. A small molecular weight product which can be isolated from partially pure phosphoglycerate kinase preparations is responsible for aggregation of the enzyme in 50 mM TEM buffer [16].

As shown in Fig. 4, two peaks of phosphoglycerate kinase activity were obtained from DEAE-Sephadex A-50. Peak A is small and not homogeneous, containing only 5—10% of the total activity. Peak B contains 90—95% of the total activity, and this parallels the peak of the protein determined at 280 nm. Glycerol (2.5%) was added to the pooled major peak which was then concentrated using an Amicon PM-10 membrane. In some cases, a rerun on DEAE-Sephadex A-50 was required in order to obtain a homogeneous preparation.

Purification data from young and old *T. aceti* are summarized in Table I.

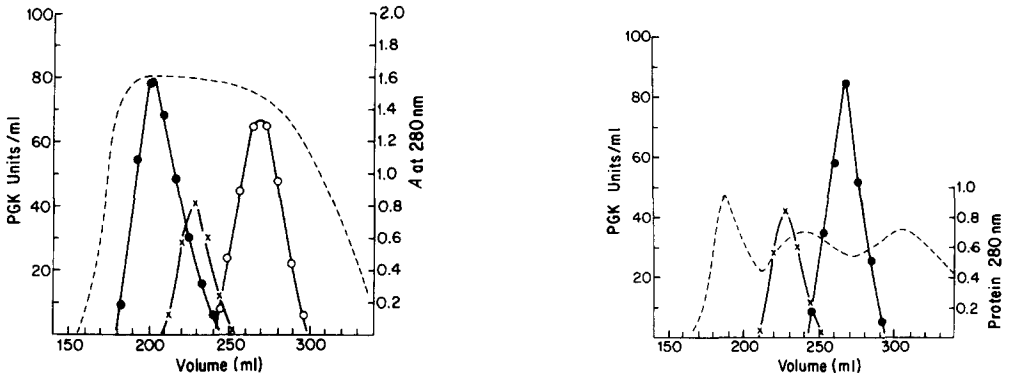

Fig. 1. Elution pattern of phosphoglycerate kinase (PGK) from the first Sephadex G-100 column (TEM buffer, 0.05 M). For details, see text. ------, protein (absorbance at 280 nm); ●———●, phosphoglycerate kinase activity; X———X, enolase activity (M_r = 82 000); ○———○, triosephosphate isomerase activity (M_r = 43 000).

Fig. 2. Elution pattern of phosphoglycerate kinase (PGK) from the second Sephadex G-100 column (phosphate buffer, 0.1 M). Details are given in the text. ------, protein (absorbance at 280 nm); ●———●, phosphoglycerate kinase activity (M_r = 43 000); X———X, enolase activity (M_r = 82 000).

As judged by disc gel electrophoresis at pH 7.0, 8.3 and 9.3, the major phosphoglycerate kinase peak (peak B; Fig. 4) shows a single activity and protein band in matching positions. There were no differences in behavior of "young" and "old" phosphoglycerate kinase during purification.

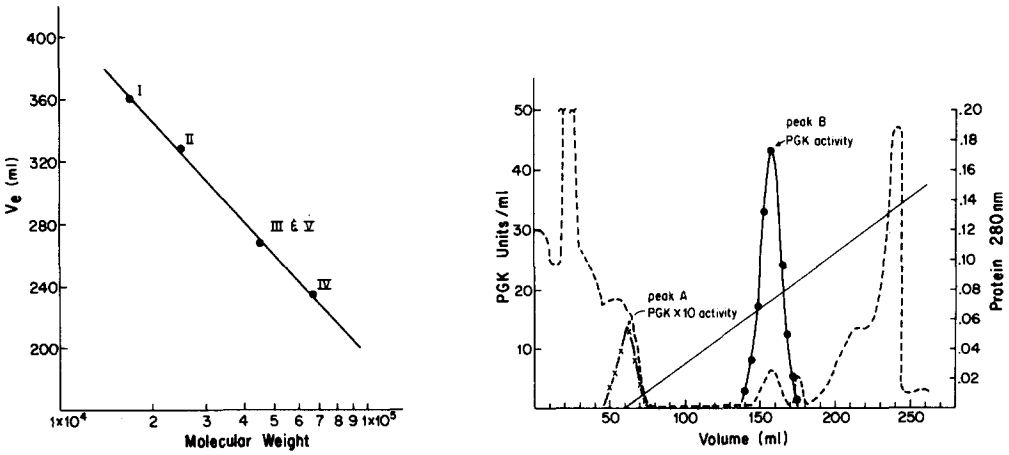

Fig. 3. Molecular weight determination of pure phosphoglycerate kinase on a Sephadex G-100 column. I = myoglobin (M_r = 17 000); II = chymotrypsinogen (M_r = 25 000); III = ovalbumin (M_r = 43 000); IV = bovine serum albumin (M_r = 67 000); V = phosphoglycerate kinase (M_r = 43 000).

Fig. 4. Elution pattern of phosphoglycerate kinase (PGK) from DEAE-Sephadex A-50. For details, see text. ———, 0—150 mM gradient of NaCl; ------, protein at 280 nm; X———X, phosphoglycerate kinase activity X 10 (peak A); ●———●, phosphoglycerate kinase activity (peak B).

TABLE I

PURIFICATION OF PHOSPHGLYCERATE KINASE FROM YOUNG AND OLD *T. ACETI*

Fraction	Total protein (mg)		Total activity (units)		Specific activity (units/mg)		Yield (%)		Purification (fold)	
	Young	Old	Young	Old	Young	Old	Young	Old	Young	Old
Crude homogenate	1517	518	5928	1140	3.9	2.2	100	100	—	
Ammonium sulfate (55—80%) fraction after dialysis	265	91	4207	799	15.9	8.7	71	70	4.0	3.9
First Sephadex G-100 after concentration	143	45	2955	660	20.7	14.7	50	50	5.3	6.1
Second Sephadex G-100 after dialysis	10	4.8	2197	389	219.7	81.1	37	34	56	37
DEAE-Sephadex A-50 after concentration (peak B) [a]	1.25	0.5	924	182	677.0	357.5	16	16	173	162

[a] In some cases, a rerun on DEAE-Sephadex A-50 was required in order to obtain homogeneous preparations.

Properties of phosphoglycerate kinase from young and old T. aceti

The relationship of phosphoglycerate kinase activity to the age of *T. aceti* is shown in Fig. 5. In general, the reduction in specific activity in crude homogenates follows the survival curve as was the case for isocitrate lyase [2], enolase [4] and triosephosphate isomerase [17]. The kinase in homogenates of old nematodes (26—28 days) has approximately a 50% lower specific activity compared to that from young (7—11 days) nematodes. The use of fluorodeoxyuridine for synchonizing the nematode cultures can safely be ignored as "altered" enolase was found in old nematodes not treated with this agent [4]. Moreover, "altered" superoxide dismutase has been reported in old rats (Dr. David Gershon, Israel Institute of Technology, private communication) and "altered" aldolase in old mice [5,6]. Needless to say, flurodeoxyuridine was not present in the diets of these animals.

Phosphoglycerate kinase was purified to homogeneity, attaining 160—180 fold purification from six different young and old batches of *T. aceti.* The specific activity of pure phosphoglycerate kinase from young worms (peak B, Fig. 4) varied between 650—750 units/mg; the actual values being related to the initial activity found in the crude homogenates. Phosphoglycerate kinase from old *T. aceti* had a consistently lower specific activity. The values were 300—400 units/mg (Table II).

The specific activity of pure phosphoglycerate kinase from young *T. aceti* is comparable to values obtained from phosphoglycerate kinase from yeast [11], rabbit muscle [18], rat muscle [19], bovine liver [20] and human erythocytes [21]. The pure nematode enzyme preparations did not show any measurable activity when assayed for triosephosphate isomerase, glycerol-3-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase or pyruvate kinase.

Michaelis constant

K_m values were determined for D-3-phosphoglycerate and ATP under the standard assay conditions. K_m values for D-3-phosphoglycerate for "young" and "old" enzyme were essentially the same, 1.0 ± 0.2 mM (Table II), and these values are similar to the values obtained from yeast [11,22], rabbit muscle [22] and human erythocytes [21]. The K_m for ATP in "young" nematode

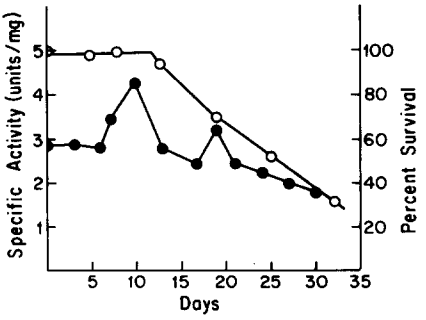

Fig. 5. Specific activity of phosphoglycerate kinase vs. age of *T. aceti.* ●———●, specific activity; ○———○, survival curve.

TABLE II

COMPARISON OF PHOSPHOGLYCERATE KINASE FROM "YOUNG" AND "OLD" *T. ACETI*

	Young (7—11 days)	Old (26—28 days)
Specific activity		
a. Crude homogenate	3.6—5.6 units/mg	2.0—2.5 units/mg
b. Pure enzyme	650—750 units/mg	300—400 units/mg
K_m for D-3-phosphoglycerate	1.0 ± 0.2 mM	0.9 ± 0.2 mM
K_m for ATP	0.26 ± 0.03 mM	0.52 ± 0.04 mM
Molecular weight	43 000 ± 1500	43 000 ± 1500
Subunits	None	None

enzyme is 0.26 mM ± 0.03, about half the figure for "old" enzyme, i.e., 0.52 ± 0.04. This difference was consistent. These values are similar to those found for the kinase from other sources [11,21,22].

Nucleotide specificity

Replacement of 2.5 mM ATP by an equimolar concentration of ITP or GTP yielded no reaction with "young" or "old" phosphoglycerate kinase from *T. aceti*. With rabbit muscle and yeast phosphoglycerate kinase, these nucleotides could substitute for ATP with approximately 70 and 55% activity, respectively (Table III).

Metal ion specificity

As with yeast phosphoglycerate kinase, Mn^{2+} and Ca^{2+} could be substituted for Mg^{2+} in the nematode enzyme. However, when Mn^{2+} was substituted at optimal concentration, only 70% of the Mg^{2+} activity was obtained with phosphoglycerate kinase from young or old *T. aceti*. The yeast phosphoglycerate kinase showed 90% efficiency. Ca^{2+} substitution gave similar results with all of the phosphoglycerate kinase enzymes tested (Table IV).

Specificity of the enzyme

2,3-Diphosphoglycerate, DL-α-, and β-glycerophosphate and 2-phosphoglycerate at a concentration of 10 mM were used to test their ability to func-

TABLE III

COMPARISON OF NUCLEOSIDE TRIPHOSPHATE SPECIFICITY OF *T. ACETI*, RABBIT MUSCLE AND YEAST PHOSPHOGLYCERATE KINASE

Nucleotide [a]	Phosphoglycerate kinase (% of activity)		
	T. aceti (young and old)	Rabbit muscle [b]	Yeast [b]
ATP	100	100	100
ITP	0	77	70
GTP	0	56	55

[a] The nucleotides were substituted in the reaction mixture for ATP in equal concentration (2.5 mM). Activity with ATP was taken as 100%.

[b] The data were taken from Krietsch and Bücher [11].

TABLE IV

METAL ION SPECIFICITY OF *T. ACETI* PHOSPHOGLYCERATE KINASE

In all experiments, the ATP concentration was 2.5 mM.

Metal ion	Concn. required for max. activity [a]	Phosphoglycerate kinase (% of activity)	
		T. aceti young and old	Yeast [b]
Mg^{2+}	5—10 mM	100	100
Mn^{2+}	2 mM	70	90
Ca^{2+}	2—5 mM	55	60

[a] These data were obtained from Larsson-Raznikiewicz [26].
[b] The enzyme is inhibited by a higher than optimal concentration of Mg^{2+}, Mn^{2+} or Ca^{2+}.

tion as substrates. None of these reagents shows activity with *T. aceti* enzyme under the standard reaction conditions.

pH optimum

pH optimum was determined using sodium acetate buffer (pH 4.0 to 6.0), Tris/maleate (pH 6.0 to 8.0), Tris · HCl (pH 7.0 to 9.0) and sodium carbonate-bicarbonate (pH 8.0 to 10.5) in the reaction mixture. The maximal activity was found in the range of pH 7.3 to 10.0. All buffers were at a concentration of 0.05 M.

Heat lability

As can be seen from Fig. 6, heat lability of "young" and "old" phosphoglycerate kinase is identical at both temperatures tested. Both enzymes show biphasic patterns. Approximately 20% of each enzyme at 45°C is heat labile. At temperatures higher than 46°C, the enzyme depreciates rapidly.

Electrophoretic mobility

The electrophoretic mobilities of "young" and "old" phosphoglycerate kinase were found to be essentially the same at all three pH values tested. Fig. 7

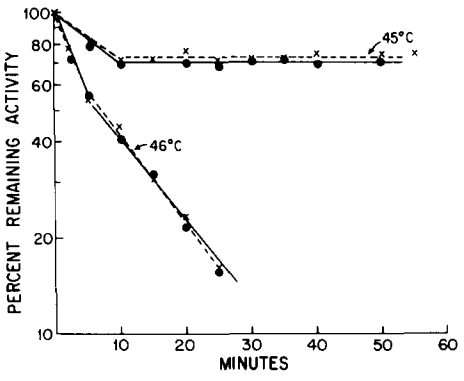

Fig. 6. Heat inactivation at 45 ± 0.2°C and 46 ± 0.2°C of pure phosphoglycerate kinase from "young" and "old" *T. aceti*. For details, see text. X———X, "young" enzyme; ●———●, "old" enzyme.

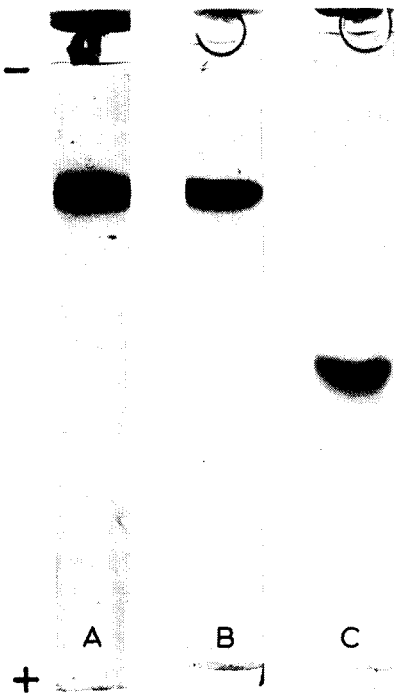

Fig. 7. Comparison of electrophoretic mobility of phosphoglycerate kinase from different sources at pH 8.3. A = "young" phosphoglycerate kinase (*T. aceti*); B = "old" phosphoglycerate kinase (*T. aceti*); C = yeast phosphoglycerate kinase.

shows the electrophoretic mobility of the enzyme in Tris/glycine buffer (pH 8.3) compared to that of yeast phosphoglycerate kinase.

Stability of the enzyme

Both "young" and "old" pure enzymes at a concentration of 1 mg/ml (Tris · HCl; pH 8.0) lose approximately 50% of their activity after storage for 24 h at 4°C. Samples containing 2.5% glycerol (final concentration) stored at −20°C remained stable for a week. Freezing and thawing inactivated the enzyme. Attempts to stabilize the enzyme at 4°C by addition of 2.5% glycerol, 10 mM D-3-phosphoglyceric acid, 5 mM ATP or 5—10 mM $MgCl_2$ were unsuccessful. The enzyme is completely stable at 4°C for at least 48 h in crude homogenate and for long periods at the phosphate-Sephadex G-100 stage of purification.

Discussion

As is the case of isocitrate lyase [2], aldolase [3], enolase [4] and triosephosphate isomerase [17], PGK activity decreases with the age of *T. aceti* as measured in crude homogenates (Fig. 5). Studies with pure isocitrate lyase and enolase showed that the lowered specific activity was due to a reduced catalytic ability of the enzymes [2,4]. However, for triosephosphate isomerase, the

lower specific activity in the homogenate was a result of less enzyme being present [17]. Phosphoglycerate kinase purified from "old" *T. aceti* has a consistently lower specific activity (300—400 units/mg) than "young" enzyme (650—750 units/mg). These data prove that the reduction in specific activity observed in crude homogenates represents a loss of catalytic ability.

Properties of "young" and "old" enzymes such as molecular weight, position on gels and inhibitor effects fail to show significant changes in all previous enzymes studied and phosphoglycerate kinase falls within this general pattern. For example, both "young" and "old" preparations have the same electrophoretic mobility (Fig. 7) and show the same elution pattern from Sephadex and ion-exchange columns. Moreover, molecular weight and K_m for the substrate are unaltered, as is behavior with Mn^{2+} and Ca^{2+} as substitutes for Mg^{2+}. One noticeable change is the fact that the "young" enzyme does show half the value of "old" K_m for ATP (Table II). Though K_m values for some "old" enzymes have been reported as unchanged, others show a small increase. For example, the K_m of nematode aldolase increases with age until double the "young" value is reached [3]. Enolase also shows a 2-fold increase of K_m for old nematodes [4]. It is difficult at this time to determine the significance, if any, of these small but seemingly consistent changes. They could be due to the presence of altered enzyme molecules. If inhibitors are causing the alterations observed in old enzymes (isocitrate lyase, enolase, aldolase, phosphoglycerate kinase), they must remain tightly bound to the pure proteins. Mixing crude homogenates from young and old organisms failed to provide evidence for such materials [3,5,8]. It seems unlikely that there is a specific inhibitor for each "old" enzyme, especially since such enzymes may prove to be numerous. However, the possibility is not excluded.

The heat-lability pattern of isocitrate lyase [9] and enolase [4] showed that "young" enzyme lost activity monotonically (first order rate kinetics) while "old" enzyme showed a biphasic curve, indicating the presence of a heat-labile component. Taking these results into account, Reiss and Rothstein [9] have proposed the following sequence of reactions to explain the formation of "altered" enzymes in old organisms:

original enzyme → conformationally altered, partially active, metastable (heat-sensitive) enzyme → inactive enzyme → eventual proteolysis

The "metastable" enzyme or "inactive" enzyme would accumulate by kinetic or enzyme action because of the dramatically increased dwell time of proteins in the cells. (The half-life of proteins increases with the age of *T. aceti* from 33 h at 6 days of age to more than 250 h at 28 days of age [Muller, R. and Lane, R., Biochemistry Department, State University of New York at Buffalo, unpublished data]). Zeelon et al. [3] obtained similar results for the half-life of nematode aldolase.

In contrast to enolase and isocitrate lyase, both "young'. and "old" phosphoglycerate kinase have identical heat inactivation curves (Fig. 6). A similar result was reported for nematode aldolase [3]. These findings strongly suggest that, for the latter two enzymes, "old" preparations consist of active and inactive molecules as first proposed by Gershon and Gershon [8]. Based upon the

above scheme, phosphoglycerate kinase (and aldolase) in *T. aceti* would be converted to inactive enzyme without accumulating molecules in the metastable (but active) state. For "old" isocitrate lyase and enolase, a metastable form clearly exists. The respective proteins possess enzyme activity which is rapidly lost on heating.

A picture is beginning to emerge of the range of properties to be expected of "altered" enzymes. One may speculate that they consist of active plus partially active molecules in the case of isocitrate lyase [2], entirely of partially active molecules in enolase [4] and active plus inactive molecules in nematode aldolase [3] and phosphoglycerate kinase. It is of interest to note that Rotman [23] has compared single molecules of stored and freshly prepared β-galactosidase from *E. coli.* He found that, in the former case, the individual enzyme molecules were heterogeneous and showed losses of activity which varied by a factor of up to 4. Fresh enzyme showed variations of 10% above random error. Indeed, some of the "aged" molecules had as little as 5% of the activity of freshly prepared enzyme. The behavior of these molecules is analogous to that of the "altered" enzymes from old animals: specific activity is reduced; K_m, molecular weight and electrophoretic mobility are unchanged. Moreover, dissociation and reassociation did not lead to an increased activity of the stored molecules.

The theory proposed by Reiss and Rothstein [9] can satisfactorily explain all of the data so far reported for "altered" enzymes without invoking major sequence changes as would be required by the "error catastrophe" theory of Orgel [24]. A more complete discussion of the relation of current research to this theory may be found in a recent review by Rothstein [1].

It is of interest to note that in the nematode phosphoglycerate kinase, ATP could not be replaced by ITP and GTP as is the case in yeast and rabbit muscle enzyme (Table III). Replacement of Mg^{2+} and Mn^{2+} in phosphoglycerate kinase from *T. aceti* results in 70% maximum activity as compared to the yeast enzyme where 90% activity was attained (Table IV). Yeast phosphoglycerate kinase has more mobility towards the anode as compared to "young" and "old" phosphoglycerate kinase from *T. aceti* (Fig. 7). Yeast phosphoglycerate kinase has been reported to yield different electrophoretic forms [25], although these had the same specific activity.

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References

1 Rothstein, M. (1975) Mech. Age. Dev. 4, 325–338
2 Reiss, U. and Rothstein, M. (1975) J. Biol. Chem. 250, 826–830
3 Zeelon, P., Gershon, H. and Gershon, D. (1973) Biochemistry 12, 1743–1750
4 Sharma, H.K., Gupta, S.K. and Rothstein, M. (1976) Arch. Biochem. Biophys. 174, 324–332

5 Gershon, H. and Gershon, D. (1973) Proc. Natl. Acad. Sci. U.S. 70, 909—913
6 Tan, C. and Makinodan, T. (1975) Fed. Proc. 34, 278
7 Bolla, R. and Brot, N. (1975) Arch. Biochem. Biophys. 169, 227—236
8 Gershon, H. and Gershon, D. (1973) Nature 227, 1214—1217
9 Reiss, U. and Rothstein, M. (1974) Biochem. Biophys. Res. Commun. 61, 1012—1016
10 Holliday, R. and Tarrant, G.M. (1972) Nature 238, 26—30
11 Krietsch, W.K.G. and Bücher, T. (1970) Eur. J. Biochem. 17, 568—580
12 Hieb, W.F. and Rothstein, M. (1975) Exp. Gerontol. 10, 145—153
13 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404—427
14 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406—4412
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
16 Gupta, S.K. and Rothstein, M. (1976) Biochem. Biophys. Res. Commun. 69, 48—54
17 Gupta, S.K. and Rothstein, M. (1976) Arch. Biochem. Biophys. 174, 333—338
18 Scopes, R.K. (1969) Biochem. J. 113, 551—554
19 Hass, L.F., Caban, C.E., Kappel, W.K., Ottinger, W.E., White, E.L., Kranz, K.R. and Fritz, P.J. (1974) Comp. Biochem. Physiol. 47B, 879—893
20 Bojanovski, M., Kulbe, K.D. and Lamprecht, W. (1974) Eur. J. Biochem. 45, 321—331
21 Yoshida, A. and Watanabe, S. (1972) J. Biol. Chem. 247, 440—445
22 Rao, D.R. and Oesper, P. (1961) Biochem. J. 81, 405—411
23 Rotman, M.B. (1970) in Lactose Operon (Beckwith, J.R. and Zisper, D., eds.), pp. 279—289, Cold Spring Harbor Laboratory, N.Y.
24 Orgel, L.E. (1963) Proc. Natl. Acad. Sci. U.S. 49, 517—521
25 Larsson-Raznikiewicz, M. (1970) Eur. J. Biochem. 15, 574—580
26 Larsson-Raznikiewicz, M. (1970) Eur. J. Biochem. 17, 183—192