A HEAT-STABLE FACTOR WHICH AGGREGATES 3-PHOSPHOGLYCERATE KINASE FROM TURBATRIX ACETI

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SUMMARY: 3-Phosphoglycerate kinase from Turbatrix aceti, when partially pure, shows a molecular weight which varies from 43,000 to 160,000 on columns of Sephadex G-150 or G-100, depending upon the eluting buffer and its ionic strength. The pure enzyme has an unchanging molecular weight of 43,000 ± 2,000. Similar shifts of molecular weight have been observed in partially purified preparations of phosphoglycerate kinase from rat liver and from yeast. A low molecular weight factor, presumably a protein, appears to be associated with partially pure preparations of the nematode enzyme. This material is removed from the enzyme by ion-exchange chromatography and is responsible for the aggregation of phosphoglycerate kinase molecules.

3-Phosphoglycerate kinase (EC 2.7.2.3) (PGK) has been purified from several sources. It is reported to have a molecular weight of 43,000 to 50,000 and is composed of a single polypeptide chain (for review, see (1)). During the development of a procedure for the purification of PGK from the free-living nematode, Turbatrix aceti, we observed an unusual phenomenon: the enzyme, when partially pure, can be eluted from Sephadex G-150 or G-100 columns as a protein with a molecular weight ranging from 43,000 to 160,000 depending upon the nature of the buffer and its ionic strength.

In this report, we present evidence for the existence of a heat-stable, dialysable factor which causes the aggregation of PGK molecules when the enzyme is in a solution of low ionic strength.

PROCEDURES

T. aceti was grown axenically in a defined medium (2). Adult worms were harvested by filtration. Crude homogenates and ammonium sulfate fractions (55-80%) were prepared by the method of Sharma et al. (3). Details for purification of PGK from T. aceti will be reported elsewhere.** The procedure involves the following steps:

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First Sephadex G-100 Column: The 55-80% ammonium sulfate fraction (15-25 mg of protein/ml) was eluted from a column (2.5 x 110 cm) of Sephadex G-100 with TEM buffer (0.05M Tris-HCl containing 1 mM EDTA and 1 mM mercaptoethanol), pH 7.6. The fractions containing PGK were combined and concentrated using a PM-10 membrane.

Second Sephadex G-100 Column: The concentrated enzyme preparation (10-20 mg of protein/ml) was treated as above, except that equilibration and elution were carried out with 100 mM phosphate buffer containing 1 mM EDTA, pH 7.6. The PGK was concentrated as above and then dialyzed against 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA.

DEAE-Sephadex A-50 Chromatography: The concentrate from above (protein concentration, 1-2 mg/ml) was loaded onto a DEAE-Sephadex A-50 column (1.5 x 60 cm) and eluted with 120 ml 10 mM Tris-HCL buffer, pH 8.0, and then with a linear gradient of 0-0.15M NaCl in 10 mM Tris-HCl buffer, pH 8.0 (see Fig. 3). The fractions containing PGK activity were pooled.

Isolation of PGK "Factor": After emergence of the enzyme, the above column was treated with 1M NaCl in 10 mM Tris-HCl, pH 8.0. Peak F (Fig. 3) was pooled and placed in boiling water for 15 min. The precipitate was centrifuged and discarded. The supernatant consisted of the aggregation "factor".

Standardization of Sephadex G-100 and G-150 columns: The columns (2.5 x 110 cm) were calibrated using aldolase from rabbit muscle (158,000), enolase (82,000), bovine serum albumin (67,000), ovalbumin (45,000) and myoglobin (17,000) as standard proteins for determination of molecular weights of PGK under different conditions.

RESULTS

The procedure devised for purification of PGK from T. aceti involves elution of an ammonium sulfate fraction from Sephadex G-100 using 50 mM TEM buffer, pH 7.6. Under these conditions, the enzyme has an apparent molecular weight of 110-130,000 although the peak of activity is unsymmetrical (Fig. 1). The next step is similar but employs 100 mM phosphate buffer, pH 7.6. In this case, PGK appears from the column as a symmetrical peak with a molecular weight of 43,000 ± 2,000 (Fig. 2). When the original ammonium sulfate fraction (at the same protein concentration) was chromatographed on Sephadex G-100 directly with 100 mM phosphate buffer, PGK again appears as a protein of 42,000 to 48,000 molecular weight. If this low molecular weight product is dialyzed and rechromatographed in TEM buffer, the molecular weight returns to the 110-130,000 range.

In order to determine if the observed shifts in molecular weight are due to the ionic strength of the buffer or to the effect of phosphate ions, crude PGK (ammonium sulfate fraction) was chromatographed on Sephadex G-100 with 5 mM, 50 mM and 150 mM TEM buffer, respectively. As is evident from Fig. 1, with 5 mM

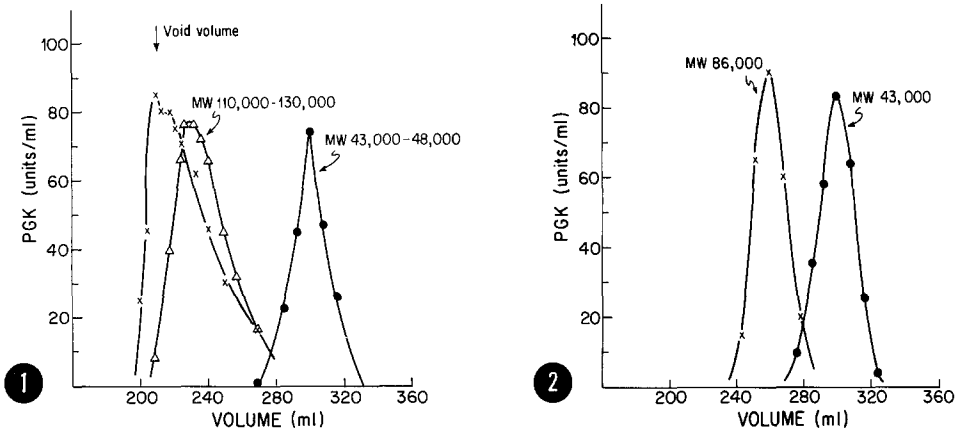


Figure 1: Elution pattern of PGK on Sephadex G-100. Samples of the 55-80% ammonium sulfate fraction were chromatographed and the PGK was eluted with: x——x, 5 mM TEM; Δ——Δ, 50 mM; ●——●, 150 mM TEM or 50 mM TEM + 100 mM NaCl. The same results were obtained if the enzyme preparation from the second Sephadex column (see Methods) was used.

Figure 2: Elution of PGK from Sephadex G-100 with phosphate buffer. x——x, 10 mM buffer; ●——●, 100 mM buffer.

TEM the enzyme appears in the void volume (chromatography on Sephadex G-150 showed a molecular weight of 140-160,000). With 50 mM TEM, the PGK appears in an unsymmetrical peak with a molecular weight of 110-130,000. With 150 mM TEM or 50 mM TEM containing 100 mM NaCl, the enzyme yielded a symmetrical peak with a molecular weight of 43,000 ± 2,000. Similarly, in 10 mM phosphate buffer, PGK appeared to have a molecular weight of approximately 80,000 whereas with 100 mM phosphate buffer, it is eluted with a molecular weight of 43,000 ± 2,000 (Fig. 2). When enzyme samples of greater purity were tested (50 to 60-fold purification from the second Sephadex G-100 column) similar results were obtained.

The final purification step for PGK involves use of DEAE-Sephadex A-50 (Fig. 3). The enzyme, after this treatment, had a molecular weight of 43,000 ± 2,000 on Sephadex G-100 irrespective of the ionic strength of the TEM or phosphate buffer. In other words, the enzyme had lost the ability to change its molecular weight once it had passed through the DEAE-Sephadex A-50. SDS gel electrophoresis of the pure enzyme showed a monomer with the same molecular weight as that obtained by the ion-exchange procedure (43,000).

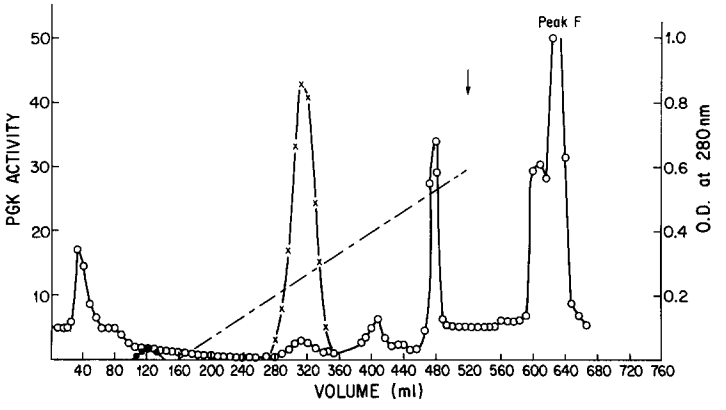
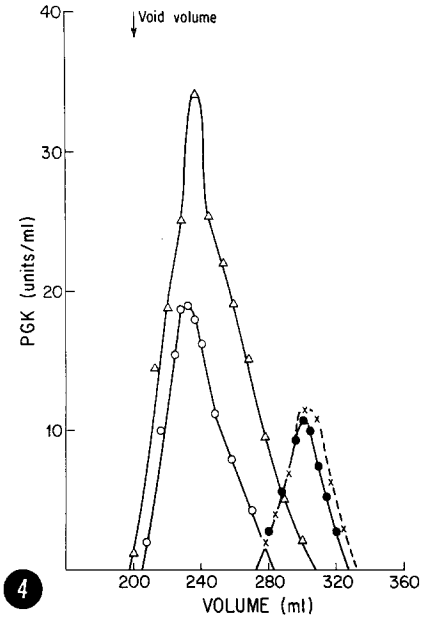


Figure 3: Purification of PGK on DEAE-Sephadex A-50. x——x, PGK activity; o——o, A_{280}; -----, 0-150 mM NaCl gradient. The arrow represents the point at which 1M NaCl in 10 mM Tris-HCl buffer (pH 8) was added. Peak F contains the aggregation factor.

If the pure PGK is mixed with the crude enzyme (ammonium sulfate fraction), the molecular weight increases from 43,000 to 110-130,000 (Fig. 4). Addition of albumin (10 mg/ml) did not affect the molecular weight of pure PGK (monomeric form) on G-100 columns.

It seemed clear that during the DEAE-Sephadex A-50 step, a "factor" which was responsible for association of PGK molecules was being removed from the enzyme. Several groups of fractions obtained from the DEAE-Sephadex A-50 column were pooled and mixed with pure enzyme. Only peak F (Fig. 3) caused an increase in the molecular weight of PGK as determined on Sephadex G-100 in 0.05M TEM buffer (Fig. 5). In some cases, a molecular weight of 60-70,000 was obtained which would suggest a monomer-dimer equilibrium. However, where more of the "factor" was added, the peak shifted to a molecular weight of 80-90,000 (Fig. 5).

The "factor" is stable to heating in boiling water for at least an hour. At the present stage of purification, it has an $A_{260/280}$ of 1.6-1.9, and it reacts with ninhydrin. Dialysis for 18-24 hours against 50 mM TEM buffer results in loss of the material. These properties, along with its retention on Sephadex G-25, suggest that the factor has a molecular weight of 3,000 or less.



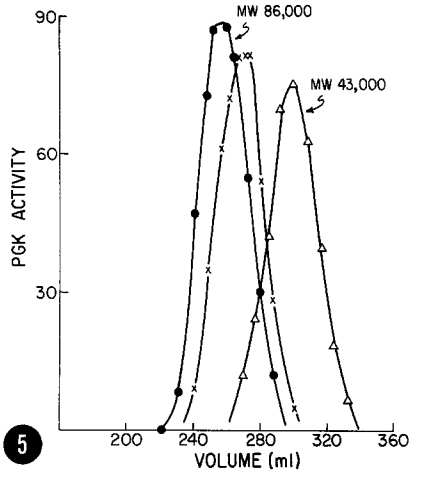


Figure 4: Effect of adding the ammonium sulfate fraction to pure PGK. Δ——Δ, 150 units of pure PGK + ammonium sulfate fraction containing 500 units of PGK; o——o, ammonium sulfate fraction alone; ●——●, pure PGK, 150 units; x----x, pure PGK (150 units) + 10 mg albumin/ml.

Figure 5: Effect of the aggregation factor isolated from Peak F on the molecular weight of PGK. Δ——Δ, 150 units of pure PGK; x——x, 150 units of pure PGK + 0.5 ml of "factor" solution (A_{280} = 5.0); ●——●, 150 units of pure PGK + 3 ml of the same "factor" preparation.

DISCUSSION

Pure PGK from T. aceti, in common with that from rabbit muscle, liver, yeast and human erythocytes, has a molecular weight of about 43,000 and consists of a single polypeptide chain (1). However, the partially pure nematode enzyme shows different molecular weights (43,000 to 160,000) depending upon the nature of the buffer (Figs. 1 and 2). It appears that in low ionic strength buffer (5 mM TEM), the enzyme exists as a tetramer-trimer; at 50 mM TEM, it appears as a trimer-dimer; at 150 mM TEM or 50 mM TEM containing 100 mM NaCl, a monomer results. Similarly, with 10 mM and 100 mM phosphate buffer, PGK appears as a dimer and monomer, respectively. It is reasonable to conclude that the ionic strength of the buffer plays a role in the association of PGK molecules in

partially pure preparations, though phosphate ions even at low ionic strength still favor the dimer form rather than the tetramer-trimer form as in the case of TEM buffer.

Once PGK has been passed through the ion-exchange resin, DEAE-Sephadex A-50, the enzyme always exhibits a molecular weight of 43,000 ± 2,000 when placed back on Sephadex G-100 irrespective of the nature and ionic strength of the buffer. In other words, the observed aggregation of PGK molecules in partially pure preparations cannot simply be due to the low ionic strength of TEM or phosphate buffer. A factor must exist which remains associated with the enzyme in partially pure preparations, but which is removed during the ion-exchange step.

Evidence for the existence of such a factor is provided by the experiment which shows that the addition of the ammonium sulfate fraction shifted the molecular weight of pure enzyme from 43,000 to 110-130,000 suggesting a trimer-dimer mixture. The shift of PGK to high molecular weight forms is not due to non-specific protein-protein interactions. Except for peak F, no fractions from the DEAE-Sephadex A-50 column had any effect, nor did albumin at the same protein concentration as the added ammonium sulfate fraction (Fig. 4).

Indeed, an aggregation factor can be isolated from the ion-exchange column. It is removed by addition of 1M NaCl to the column after elution of the enzyme. When mixed with homogenous enzyme, the factor causes association of PGK molecules, depending upon its concentration (Fig. 5).

We have observed that PGK from yeast and rat liver exists in similar aggregated forms before being treated on the ion-exchange column. Interestingly, the reported methods of purification for these preparations start with ion-exchange chromatography (4,5), so that the higher molecular weight forms would not be observed.

A number of recent papers,(6,7,8) suggest that small protein factors may play an important role in various aspects of enzyme regulation. This paper adds further evidence that such factors may be widespread.

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