YEAST PHOSPHOGLYCERATE KINASE PURIFIED BY AFFINITY ELUTION HAS TIGHTLY BOUND 3-PHOSPHOGLYCERATE

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1. Introduction

Phosphoglycerate kinase (PGK, ATP-3-phospho-D-glycerate-1-phospho-transferase, EC 2.7.2.3) is the glycolytic enzyme that catalyses the following reaction: 1,3-diphosphoglycerate + ADP $\overset{Mg^{2+}}{\rightleftharpoons}$ 3-phosphoglycerate + ATP [1]. The enzyme is active as a monomer with a relative molecular mass M_r of 44 000 [2,3]. Treatment of PGK with the arginine-modifying reagent, phenylglyoxal leads to loss of activity [4–6]; the substrate, 3-phosphoglycerate can protect against inactivation.

We report here that PGK purified by affinity elution is not inactivated by phenylglyoxal, and it is shown that this is due to the presence of tightly bound 3-phosphoglycerate.

2. Materials and methods

2.1. *Purification and characterization of PGK*

PGK was purified from dried baker's yeast (*Saccharomyces cerevisiae*), obtained from the Distillers Co., by the method in [7–9]. The procedure involves extraction, ammonium sulphate precipitation and affinity elution from CM-cellulose by 3-phosphoglycerate. An additional gel filtration step using Sephadex G-200 was sometimes required. The PGK was characterized by enzymic assay, SDS–polyacrylamide gel electrophoresis, and amino acid analysis, and was shown to have spec. act. $\geqslant$650 U/mg and to be of high purity.

2.2. *Assay of enzymic activity*

The activity of PGK was assayed by coupling the reaction to that catalysed by glyceraldehyde-3-phosphate dehydrogenase, and monitoring the oxidation of NADH [8]. Protein was estimated as in [10], and spectrophotometrically, using $A^{1.0\%}_{1\ cm} = 5.0$ at 280 nm [11].

2.3. *Modification of arginine residues*

Reactive arginine residues were modified by the arginine-specific reagent phenylglyoxal according to [4]. PGK (2 mg/ml, 45 μM) was incubated with 2.0 mM phenylglyoxal, the conditions shown [4,6] to lead to 95% inactivation in the absence of 3-phosphoglycerate, but only 5% inactivation in the presence of 20 mM 3-phosphoglycerate.

3. Results and discussion

PGK purified by affinity elution was resistant to inactivation by 2.0 mM phenylglyoxal (fig.1). Gel filtration of PGK on Sephadex G-200 and/or exhaustive dialysis did not alter its resistance to phenylglyoxal inactivation. It seemed possible that the resistance to inactivation might result from tightly-bound 3-phosphoglycerate, and three procedures were used to examine this possibility.

(i) Guanidinium chloride: The PGK was treated with 2.0 M guanidinium chloride followed by dialysis against water at 4°C, conditions known to cause the reversible unfolding of PGK [12]. This procedure was repeated 3 times.

(ii) Sodium sulphate: The PGK was suspended in 0.5 M sodium sulphate, dialysed against 0.5 M sodium sulphate and then dialysed against water. The procedure was repeated 3 times.

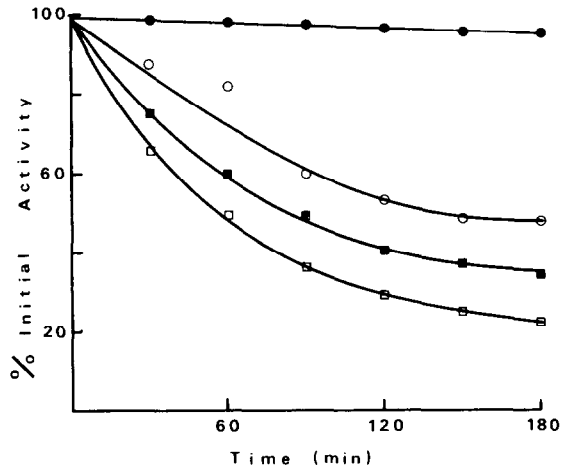

Fig.1. Phenylglyoxal inactivation of PGK. The susceptibility of different PGK preparations to inactivation by 2.0 mM phenylglyoxal was measured. (•) PGK purified by affinity elution; PGK after the following treatments; (○) 2.0 M guanidinium chloride; (▪) 0.5 M sodium sulphate; (□) glyceraldehyde-3-phosphate dehydrogenase, ATP and NADH. Details of these treatments are given in section 2.

(iii) Glyceraldehyde-3-phosphate dehydrogenase [GAPDH (Sigma) from yeast, crystalline suspension in 3.0 M ammonium sulphate solution]: The PGK (10 nmol) was incubated with a 5-fold molar excess of NADH, ATP (50 nmol) and 0.36 pmol GAPDH for 45 min at 37°C, and then dialysed against water. (The GAPDH may subsequently be removed by gel filtration if desired.)

The susceptibility to phenylglyoxal inactivation of PGK treated by these 3 procedures is shown in fig.1. It is apparent that all 3 procedures have rendered PGK sensitive to phenylglyoxal treatment, and that the GAPDH procedure allowed the most extensive phenylglyoxal modification.

As a control, the effect of the 3 treatments on the specific activity of PGK was measured (table 1). The GAPDH treatment caused virtually no loss of specific activity, whereas the repeated denaturation of PGK in guanidinium chloride led to ~20% inactivation.

The extent of modification of arginine residues by phenylglyoxal was correlated with loss of activity by measuring the loss of arginine detected by amino acid analysis (fig.2). It can be seen that modification of between 2 and 3 arginine residues occurs with complete inactivation.

These results are consistent with purified PGK

Table 1
Recovery of PGK activity after treatment

Treatment	Initial spec. act. (units/mg)	Spec. act. after treatment (units/mg)	% Decrease spec. act.
(i) Guanidinium chloride	660	540	18
(ii) Sodium sulphate	660	640	3
(iii) GAPDH	660	650	2

Details of the treatment procedures are given in section 2

containing tightly bound 3-phosphoglycerate even after gel filtration or dialysis. The removal of the 3-phosphoglycerate by GAPDH treatment was examined in more detail to quantify the amount of bound 3-phosphoglycerate. The rate and extent of NADH oxidation due to the 3-phosphoglycerate bound to PGK is shown in fig.3. The conditions of assay using an excess of GAPDH were chosen to ensure that the GAPDH reaction was not rate-limiting. A semi-log plot of these data gives a straight line, indicating that the reaction is first order with respect to 3-phosphoglycerate concentration. The amount of 3-phosphoglycerate bound to PGK was calculated to be 1.1 mol/mol PGK (fig.3).

PGK is the object of much interest, being a monomeric enzyme for which rotation about the bond

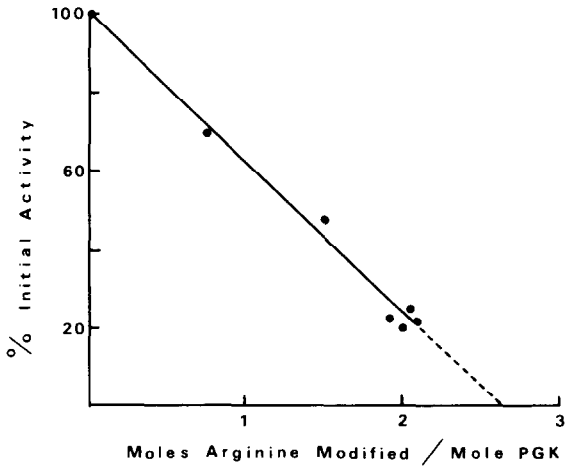

Fig.2. Extent of arginine modification. The number of arginine residues modified/mol PGK was determined by measuring the loss of arginine by amino acid analysis. Extrapolation to zero activity indicates that 2.7 mol arginine/mol PGK were modified.

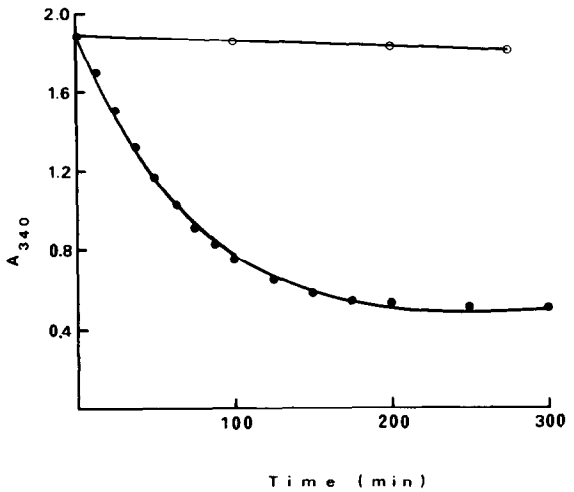

Fig.3. Rate of NADH oxidation by PGK. Purified PGK (•) (0.183 mM) was incubated with ATP (4 mM), NADH (2 mM) and GAPDH (5 μM). The control values (○) were from PGK that had already been treated with ATP, NADH and GAPDH to remove the 3-phosphoglycerate, and to which a second addition of NADH was made. The decrease in A_{340} was 1.3 so the concentration of NADH oxidised was 0.209 mM ($\epsilon_{340} = 6.2 \times 10^3$ M cm^{-1}). There were therefore 1.1 mol 3-phosphoglycerate/mol PGK.

linking its two domains is proposed as part of the catalytic mechanism [13,14]. The exact location of the 3-phosphoglycerate binding site is however based on circumstantial rather than direct evidence from X-ray crystallographic studies [13,15–17] so that further chemical modification studies may be useful. A variety of ligands have been shown to have marked effects on the flow properties of the enzyme [18], and substrates bring about substantial changes in its stability (B. A., in preparation). It is clearly important for these and for kinetic studies that a pure enzyme free from bound ligands is available. The enzyme purification step described here fulfills this requirement.

Acknowledgements

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