THE TYROSYL RESIDUES OF YEAST 3-PHOSPHOGLYCERATE KINASE: REACTIVITY TOWARD IODINE

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1. Introduction

Recent X-ray crystallography data [1,2] suggest that conformational changes observed in 3-phosphoglycerate kinase are associated with the interaction of this enzyme with its substrates. These conformational changes could be involved in the various stages of catalysis and this could explain kinetic results [3].

The interesting feature of the yeast enzyme is that it possesses only one cysteine residue which is not at the active center [1,4,5]. On the basis of results obtained previously [6,7] a carboxyl residue is implicated in the transphosphorylation process. Furthermore, the carbethoxylation of histidine residue is found to induce a loss of activity concomitant with a weak conformational change [6]. Differential spectrophotometric investigations of the interaction of 3-phosphoglycerate with phosphoglycerate kinase have demonstrated that the binding of the acceptor produces the perturbation of a phenolic chromophore in the enzyme [8]. Since we have found, as mentioned in this paper, that all chromophoric residues are buried, tyrosyl residues have focused our special attention.

Herein, we report some preliminary results obtained by chemical modification of essential tyrosyl residues in yeast 3-phosphoglycerate kinase.

2. Materials and methods

The Boehringer yeast enzyme was used. In some instances this enzyme was chromatographed on carboxymethyl cellulose [7]. Protein concentration was determined from the absorbance at 280 nm using $A_{1\,cm}^{1\%} = 5.0$ [9]. Enzyme samples had a specific activity of 800 to 1000 units at 22°C and pH 7.5 [8,10].

The –SH group was titrated by DTNB [11] in 10% sodium dodecyl sulfate, pH 8, or after carboxymethylation in 6 M guanidine chlorhydrate pH 8.5 at 50°C.

Tryptophane and methionine residues were determined according to [12] and to [13] respectively.

Amino acid analyses were carried out on a TSM_1 Technicon autoanalyser. A complete proteolytic digestion of carboxymethylated 3-phosphoglycerate is obtained after successive treatment at 37°C by pepsin (S/P = 1/20 for 6 hr), pronase (S/P = 1/20 for 15 hr) and aminopeptidase M (S/P = 1/2 for 15 hr).

Separation of ^{125}I derivatives was performed by high electrophoresis at pH 2.1 [14] and paper chromatography [15]. The radioactivity was detected by autoradiography.

3. Results

Perturbation experiments by 20% ethylene glycol or 20% sucrose are performed on yeast 3-phosphoglycerate kinase in 0.05 M Tris–acetate buffer pH 7.5. No spectral effect could be observed, pointing out that chromophoric residues appear not to be exposed to solvent access or at least to molecules of the size of these perturbents. In addition, the same result is obtained after exposure of the enzyme to 8 M urea for 48 hr.

The effects of iodine treatment at pH 7.5 on the catalytic activity of 3-phosphoglycerate are depicted in fig.1. Iodine (2.1 mM) produces an inactivation which increases with time and is affected by temper-

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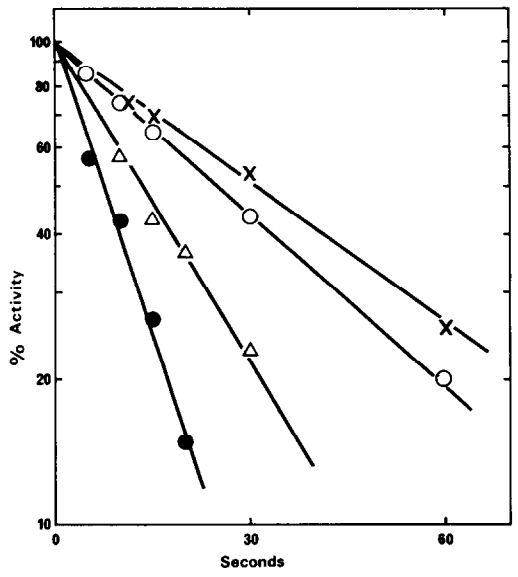

Fig.1. Inhibition of 3-phosphoglycerate kinase by iodine. Enzyme 42 μM, iodine 2.1 mM, in 0.05 M Tris–acetate buffer pH 7.5 at (X–X–X) 8°C, (○–○–○) 12°C, (△–△–△) 16°C and (●–●–●) 20°C. The reaction of iodine was stopped by addition of 0.01 M thiosulfate.

ature. To follow the progress of the iodine incorporation with enzyme inactivation, the amount of bound ^{125}I atoms is determined with an autogamma spectrometer. The data presented in fig.2 show that about

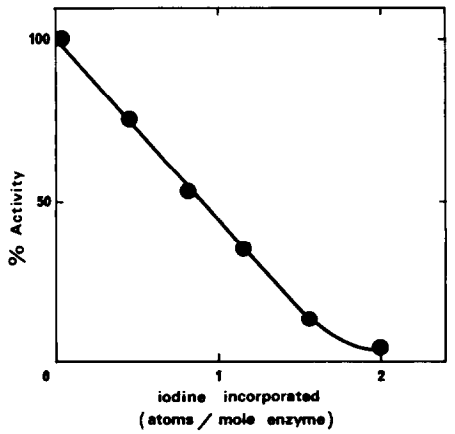

Fig.2. Effect of iodine incorporation on 3-phosphoglycerate kinase activity. Temp. 12°C; for other conditions, see fig.1.

Table 1
Distribution of iodine in the identified iodo amino acids in total enzymatic digest of [^{125}I]carboxymethylated 3-phosphoglycerate kinase

Inhibition (%)	Monoiodotyrosine (residues per mol protein)	Diiodotyrosine (residues per mol protein)
25	0.29	0.06
47	0.57	0.11
66	0.72	0.20
86	0.71	0.40
95	0.66	0.67

2 atoms of iodine are incorporated per mole of enzyme at 12°C concomitar ' ' with complete loss of activity. The rate of inactivation is of pseudo first order (K_{app} = 0.7 min^{-1} at 12°C).

The chemical nature of the iodine-sensitive side chains in protein sample is determined after total proteolytic digestion of ^{125}I carboxymethylated 3-phosphoglycerate kinase by electrophoresis and chromatography. The organic radioactive iodine is present as monoiodotyrosine and diiodotyrosine (table 1).

Apart from tyrosyl residues, different groups of the enzyme such as cysteine, methionine, tryptophane and histidine may react with iodine [16–20]. The single and non-essential thiol group is not oxydised by iodine as judged by measurements with DTNB or

Table 2
Effect of various ligands on the inactivation of 3-phosphoglycerate kinase by iodine

Ligands	Concentration (mM)	Inhibition (%)
None		84
3-phosphoglycerate	20	36
ATP-Mg	10	45
ADP	20	50
3-phosphoglycerate + ADP	20 + 20	36
Pyrophosphate	10	65
Tripolyphosphate	10	54

The enzyme (42 μM) preincubated for 5 min with ligands (at the concentration indicated) is reacted with iodine (2.1 mM) for 1 min at 12°C in 0.05 M Tris–acetate buffer pH 7.5.

iodo acetic acid. Moreover tryptophane and methionine determinations give evidence that no oxidation occurred during iodination of 3-phosphoglycerate kinase. Finally no iodohistidyl residue can be detected. Similar results are obtained with all the enzyme samples before and after further purification.

To test for protection against inactivation, various substrates are added together or separately before the addition of reagent. In all cases, a protection is observed. Moreover, effective protection is observed with pyrophosphate and tripolyphosphate (table 2).

4. Discussion

The specific iodination of tyrosyl residues in 3-phosphoglycerate kinase was undertaken after examination of the environment of the aromatic rings in the enzyme. In contrast to phosphagen kinases [21,22], the results obtained from solvent perturbation show that all the tyrosyl residues are not located on the protein surface. As judged from the results obtained by iodine labeling, treatment of 3-phosphoglycerate kinase with this reagent leads to total loss of the catalytic activity. This inactivation is concomitant with the substitution of about one tyrosyl residue. However, the rate of monoiodotyrosine formation is higher than that of diiodotyrosine. So, up to about 70% inhibition monoiodotyrosine is the predominant derivative, and the amount of monoiodotyrosine produced correlates the degree of inactivation. In the last stages of reaction, we observed the formation of diiodotyrosine.

Furthermore, it may be concluded that the observed change in activity is not related to side reactions; in this case, the effect of iodine appears to be very specific to tyrosyl groups. Since the tyrosyl groups are buried, diiodotyrosine would be formed at a significant rate [23]. Such a result is observed in 3-phosphoglycerate kinase in contrast with phosphagen kinases in wich the most exposed of tyrosyl groups were monoiodinated [14,22].

Either this tyrosyl is located at or near the active site and therefore would be expected to be masked by the substrates, or it is present elsewhere on the protein and becomes buried by virtue of conformational changes when substrates bind to the enzyme.

Further investigations are necessary to determine the exact function of the essential tyrosyl group in yeast 3-phosphoglycerate kinase.

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