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STUDIES ON THE ACTIVE SITE OF YEAST PHOSPHOGLYCERATE MUTASE

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SUMMARY

1. Yeast phosphoglycerate mutase (2,3-diphospho-D-glycerate:2-phospho-D-glycerate phosphotransferase, EC 2.7.5.3) was subjected to chemical modification experiments with trinitrobenzenesulfonate in an attempt to identify amino acids in the active site.

2. Trinitrophenylation of amino groups caused a significant loss of the activity, while the presence of the substrate (3- or 2-phosphoglycerate) inhibited strongly trinitrophenylation and protected the enzyme almost completely from loss of the activity.

3. In order to exclude amino groups which were not essential for the activity, the enzyme was carboxymethylated with iodoacetate in the presence of the substrate prior to trinitrophenylation. Trinitrophenylation of the carboxymethylated active enzyme indicated that four amino groups were located in the active site.

4. Although the coenzyme (2,3-diphosphoglycerate) was added at a concentration 10 times the K_m to the reaction mixture for trinitrophenylation, no significant protective effect on the loss of the activity was observed. From these results, it was concluded that four amino groups were located at the substrate site.

5. The similar time courses of trinitrophenylation on component V (the final product of the enzymic modification) and on component I (the native enzyme) indicated that lysine residues liberated by the enzymic modification were not the ones which reacted with trinitrobenzenesulfonate.

INTRODUCTION

Phosphoglycerate mutase (2,3-diphospho-D-glycerate:2-phospho-D-glycerate phosphotransferase, EC 2.7.5.3) catalyzes the reversible transfer of a phosphate group between the 3 and 2 positions of D-glycerate[1]. In the case of the mutase from yeast and animal tissues, the reaction requires the addition of 2,3-diphosphoglycerate

Abbreviations: 2PGA, 2-phospho-DL-glycerate; 3PGA, 3-phospho-D-glycerate; 2,3PGA, 2,3-diphospho-D-glycerate.

as a coenzyme[2]. The results of a kinetic analysis of the yeast mutase reaction pathway were consistent with the sequential mechanism in which an enzyme–substrate (3- or 2-phosphoglycerates)–coenzyme (2,3-diphosphoglycerate) ternary complex was an obligatory intermediate[3]. These results thus suggest that there are separate substrate and coenzyme binding sites on the enzyme molecule. The reagent trinitrobenzenesulfonate[4,5] reacts specifically with amino groups. In the present work it is shown that trinitrophenylation with this reagent reduces the activity very greatly. This loss of activity can be almost completely prevented by the presence of substrate during trinitrophenylation, but the coenzyme does not exert a similar protective action at a concentration which would be expected to be adequate based upon the K_m. Some of these findings have been the subject of a short communication[6].

It was verified that component I, which had the highest activity and the lowest electrophoretic mobility, was the native enzyme in yeast cells and other four components were derived from component I by limited proteolysis caused by phosphoglycerate mutase-modifying enzyme during autolysis[7–10]. About 37 amino acid residues containing 7 lysine residues were liberated during the conversion of component I to component V which had the lowest activity[11].

This paper describes the studies on the function of amino groups of yeast phosphoglycerate mutase by chemical modification experiments.

MATERIALS AND METHODS

Crystalline yeast phosphoglycerate mutase

The procedures of extraction and isolation of the mutase from baker's yeast were as described previously[12]. Native enzyme (component I) was separated chromatographically from the unfractionated enzyme preparation (containing 5 components) prepared from a 3-h autolysate[11]. Component V, the final product of the enzymic modification during autolysis and had the 5% activity of component I was prepared in high yield from a 24-h autolysate.

Other materials

The phosphoglycerate mutase-modifying enzyme preparation converting component I into components II, III, IV, and V was obtained by the method reported[11]. The sodium salts of 3-phospho-D-glycerate and phosphoenolpyruvate and the cyclohexylammonium salt of 2,3-diphospho-D-glycerate were purchased from Boehringer Mannheim Corp. 2-Phospho-DL-glycerate synthesized by the method of KIESSLING[13] and 3-phospho-D-glycerate were purified chromatographically as described by BARTLETT[14]. The barium salt of phosphoglycolate and the dimethylketal cyclohexylammonium salt were kind gifts from Dr. Pizer. Ketal groups of phosphohydroxypyruvate were removed by the method reported[15]. Trinitrobenzenesulfonate was purchased from Tokyo Kasei Kogyo Co. Ltd. and lysozyme, from Sigma Chemical Co. Iodoacetate purchased from Nutritional Biochemicals Corp. was recrystallized from benzene–pentane. α-*N*-acetyllysine was a kind gift from Tanabe Seiyaku Co. Ltd.

Enzyme activity

The phosphoglycerate mutase activity was determined as described previously[16].

When the enzyme was subjected to the chemical modification, an aliquot of the reaction mixture taken at intervals was diluted with a cold 0.1% $(NH_4)_2SO_4$ solution for the determination of the activity.

Trinitrophenylation

The enzyme preparations for the chemical modification were subjected to gel filtration on a Sephadex G-50 column equilibrated with the buffer used for the modification. Details of individual experiments are given in the legends of figures and tables.

It was reported that the removal of SO_3^{2-}, by-product of trinitrophenylation of amino groups, was necessary for the accurate determination of trinitrophenyl amino groups[17]. The effect of SO_3^{2-} can be removed by the acidification of the solution. The reaction mixture for trinitrophenylation was acidified by the addition of an equal volume of the acidic urea solution (8 M urea solution dissolved in 1 M HCl) and the absorbance was read at 348 nm against a blank treated as above but containing water instead of the protein solution. The molar extinction coefficient of trinitrophenyl amino groups was found to be $1.08 \cdot 10^4$ in the acidic urea solution, using lysozyme (mol. wt. 14 300, 7 free amino groups) and α-*N*-acetyllysine as standard.

Protein concentration

The protein concentration of phosphoglycerate mutase was determined from its absorbance at 280 nm, based on a value of $E_{1cm}^{1\%} = 14.2$ for component I and $E_{1cm}^{1\%} = 14.9$ for component V[11]. The protein concentration of the trinitrophenylated enzyme was determined by the method of LOWRY *et al.*[18].

A molecular weight of 110 700 for the mutase was used for calculation[19].

RESULTS

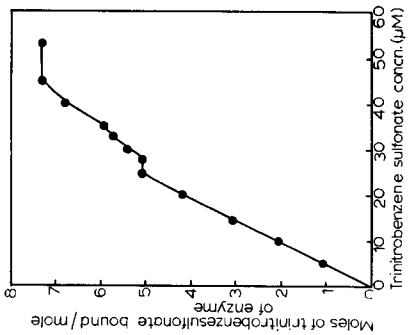
Reactive amino groups

In order to determine the number of reactive amino groups to trinitrobenzenesulfonate in phosphoglycerate mutase (component I), trinitrophenylation was allowed at 37° for 2 h at different concentrations of trinitrobenzenesulfonate. After 2 h incubation, no more change in absorbance at 345 nm was observed. As shown in Fig. 1, phosphoglycerate mutase has 4 or 5 reactive amino groups to trinitrobenzenesulfonate and additional 2 or 3 groups with lower reactivity.

Trinitrophenylation and loss of the enzyme activity

Fig. 2 shows the time-course of trinitrophenylation and loss of the activity. The result indicating that the enzyme (component I) has about 4 reactive amino groups coincided well with that of Fig. 1. The enzyme activity decreased progressively with trinitrophenylation and when 4 amino groups had been trinitrophenylated there was a loss of about 80% of the original activity. The presence of substrate in the reaction mixture repressed strongly the reactivity of these amino groups and protected the enzyme from inactivation.

The reaction rate of trinitrophenylation is very low at pH 5.9 optimal for the enzyme activity[12]. As shown in Fig. 3, the loss of the activity by trinitrophenylation



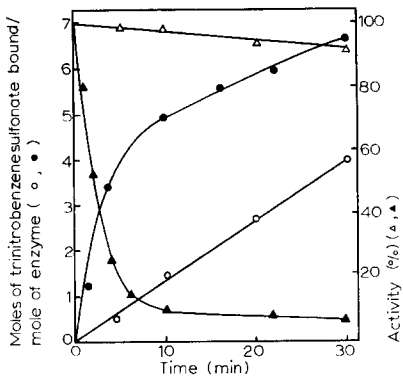


Fig. 1. Reactive amino groups in phosphoglycerate mutase (component I). The reaction mixture contained 5 μM enzyme (component I) and trinitrobenzenesulfonate at the indicated concentrations in 25 mM borate buffer (pH 7.5). After incubation at 37° for 2 h, the reaction mixture was treated with an equal volume of the acidic urea solution and the absorbance at 348 nm was measured to determine the number of trinitrobenzenesulfonate bound per mole of the enzyme.

Fig. 2. Time-course of trinitrophenylation and loss of the activity. △, ▲, enzyme activity; ○, ●, trinitrophenyl amino groups. ▲, ●, no 3-phosphoglycerate; △, ○, 2 mM 3-phosphoglycerate. The reaction mixture contained 5 μM enzyme (component I) and 0.5 mM trinitrobenzenesulfonate in 25 mM borate buffer (pH 7.5). The temperature was 25°. The enzyme activity was determined by using 2.8 μg of the enzyme.

and the protective effect of the substrate, at pH 5.9, are similar to those observed at pH 7.5. The values of K_m^{3PGA} and K_i^{3PGA} were reported in the previous paper to be 0.6 mM and 3 mM, respectively[3]. The value of K_m^{3PGA} represents a Michaelis constant of 3-phosphoglycerate for the substrate site, while K_i^{3PGA} represents a dissociation constant of 3-phosphoglycerate for the coenzyme site. The strong protection of the activity (Fig. 3) by the presence of 3-phosphoglycerate at the concentration of 5 mM may not be attributable to combination of the substrate with the coenzyme site but to combination of the substrate with the substrate site. These results suggest that some amino groups are located in the active site and have an important role on the substrate binding.

Subtraction of the number of moles trinitrophenylated in the presence of the substrate from the one in the absence of the substrate indicated that 3 or 4 amino groups were protected by the substrate (Fig. 2). However, trinitrophenylation of 8–10 amino groups was required for almost the complete loss of the activity. This observation may offer an ambiguous function to these amino groups. In order to compare enzymic properties of the native enzyme (component I) with those of trinitrophenylated enzymes (component I) which were partially inactivated, kinetic experiments were carried out. The enzymes which had 3.6 and 6 moles of trinitrophenyl amino groups per mole of the enzyme and had 21% and 10% of the original activity, respectively, were used. Typical patterns of kinetic experiments on native and modified enzymes are shown in Fig. 4. The substrate inhibition at high concentrations was competitive with the coenzyme. The modified enzyme exhibited the similar competitive pattern to the native enzyme. The intersecting point on the abscissa gives the value of apparent K_m of the coenzyme. The apparent K_m values

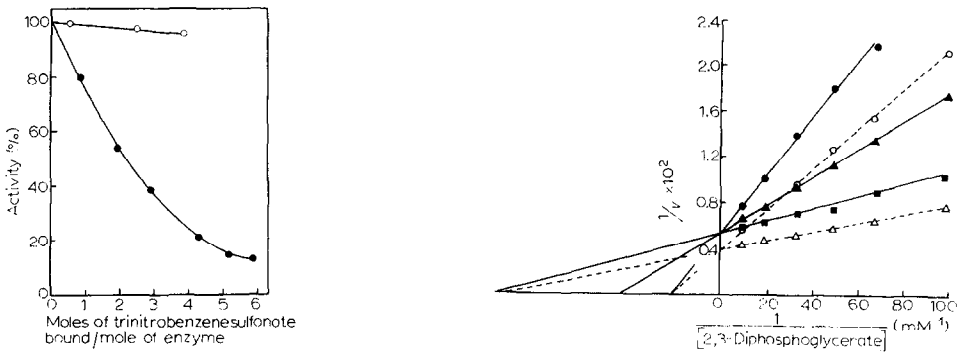


Fig. 3. Loss of the enzyme activity by trinitrophenylation and protective effect of 3-phosphoglycerate at pH 5.9. ●, no 3-phosphoglycerate. ○, 5 mM 3-phosphoglycerate. The reaction mixture contained 15 μM enzyme (component I) and 1.25 mM trinitrobenzenesulfonate in 0.1 M acetate buffer (pH 5.9) containing 0.1 M NaCl. The temperature was 18°. After 5 h incubation in the absence of 3-phosphoglycerate, the binding of 6 moles of trinitrobenzenesulfonate per mole of the enzyme was observed. In the presence of 3-phosphoglycerate, the binding of 4 moles of trinitrobenzenesulfonate was observed after 7 h incubation.

Fig. 4. Kinetic studies on the native and trinitrophenylated phosphoglycerate mutases. ●, ▲, ■, native enzyme (component I); ○, △, trinitrophenylated enzyme (3.6 moles of trinitrophenylamino group, 21% of the original activity). ●, ○, 33 mM 2-phosphoglycerate; ▲, 16 mM 2-phosphoglycerate; ■, △, 8 mM 2-phosphoglycerate. The reaction for the determination of the enzyme activity was carried out at 25° in 0.1 M acetate buffer (pH 5.9) containing 2-phosphoglycerate and 2,3-diphosphoglycerate at concentrations indicated in the figure and 2.8 μg of the native enzyme or 19.2 μg of the modified enzyme. v is expressed as μmoles of 3-phosphoglycerate formed per 10 min. Trinitrophenylation of component I was carried out under the following conditions. The reaction mixture for trinitrophenylation contained 5 μM enzyme and 0.5 mM trinitrobenzenesulfonate in 25 mM borate buffer (pH 7.5). The temperature was at 0°. 3 ml of the reaction mixture was taken at 16 min to pass through on a Sephadex G-50 column bufferized with 25 mM borate buffer (pH 7.5). The enzyme had 3.6 moles of trinitrophenylamino groups and the activity of 21% of the original activity.

of the native and modified enzymes are summarized in Table I. The results indicated that the residual activity of the modified enzyme was the same as the native enzyme in the kinetic property. From this observation, it is likely that the residual activity is not due to the enzyme having different enzymic properties from the native enzyme but to the enzyme which was not trinitrophenylated at the active site.

The number of amino groups essential for the enzyme activity

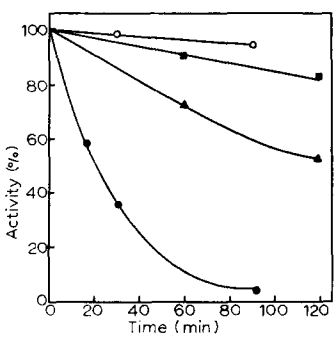
Amino groups which appeared to be reactive but not essential for the enzyme activity were carboxymethylated in the presence of the substrate by iodoacetate prior to trinitrophenylation. As shown in Fig. 5, the time-course of inactivation of the enzyme was highly dependent on pH. A rapid loss of the activity was observed at alkaline pH in the absence of the substrate, while little inactivation was observed in the presence of the substrate. It was reported that the carboxymethylation of ribonuclease was also dependent on pH and amino groups of the enzyme were modified preferentially at alkaline pH[20]. It has been confirmed that the phosphoglycerate mutase protein maintained its native conformation at pH 9.3[19]. The enzyme (component I) carboxymethylated at pH 9.3 in the presence of 3-phosphoglycerate under the conditions as described in the legend of Fig. 6, had the specific activity of 93%

TABLE I

APPARENT MICHAELIS CONSTANTS OF 2,3-DIPHOSPHOGLYCERATE FOR NATIVE AND TRINITROPHENYLATED PHOSPHOGLYCERATE MUTASES

The conditions for trinitrophenylation and determination of the enzyme activity were as in Fig. 4. The enzymes which had 3.6 and 6 moles of trinitrophenyl amino groups had 21% and 10% of the original activity, respectively. The values of apparent $K_m^{2,3PGA}$ were estimated by using plots described in Fig. 4. The enzyme activity was determined by using 2.8 μg of the native enzyme or 19.2 μg of the trinitrophenylated enzyme.

Enzyme	*Moles of trinitrobenzene sulfonate bound per mole of the enzyme*	*Activity (%)*	*2-Phosphoglycerate (mM)*	*Apparent $K_m^{2,3PGA}$ (μM)*
Native enzyme	—	100	33	45
			16	24
			8	10
Trinitrophenylated enzyme	3.6	21	33	45
			8	10
Trinitrophenylated enzyme	6.0	10	33	33
			8	11



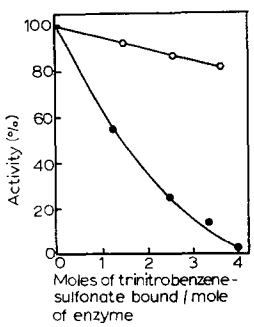


Fig. 5. Effect of pH on inactivation by iodoacetate. The reaction mixture contained 10 μM enzyme (component I) and 0.25 M iodoacetate in 0.1 M acetate buffer (pH 6.0) (■), in 12.5 mM borate buffer (pH 7.5) (▲), in 25 mM veronal buffer (pH 9.3) (●) or in 25 mM veronal buffer (pH 9.3) containing 10 mM 3-phosphoglycerate (○). The temperature was 25°. The activity was determined by using 2.8 μg of the enzyme.

Fig. 6. Trinitrophenylation of the carboxymethylated active enzyme. ●, no 3-phosphoglycerate; ○, 10 mM 3-phosphoglycerate. The reaction mixture for trinitrophenylation contained 10 μM active enzyme carboxymthylated in the presence of the substrate and 1 mM trinitrobenzenesulfonate in 25 mM borate buffer (pH 7.5) containing the substrate or not. The temperature was 0°. The enzyme activity was determined by using 2.8 μg of the enzyme. After incubations for 30 min in the absence of the substrate and for 180 min in the presence of the substrate, the binding of 4 moles of trinitrobenzenesulfonate per mole of the enzyme was observed .The method for preparation of the carboxymethylated active enzyme was as follows. The reaction mixture for carboxymethylation contained 48 μM enzyme (component I), 0.25 M iodoacetate, 10 mM 3-phosphoglycerate and 25 mM veronal buffer (pH 9.3). After incubation for 80 min at 29°, the reaction mixture was subjected to passage on a Sephadex G-50 column bufferized with cold 50 mM borate buffer (pH 7.5). The eluted enzyme had the activity of 93% of the native enzyme.

of the native enzyme. The carboxymethylated active enzyme was trinitrophenylated in the absence and presence of the substrate. As shown in Fig. 6, the binding of about 4 moles of trinitrobenzenesulfonate per mole of the enzyme resulted in almost the complete loss of the activity, while loss of the activity was prevented by the presence of the substrate. From these results, it may be concluded that 4 amino groups are essential for substrate binding.

Effect of the coenzyme and other compounds on trinitrophenylation

The yeast enzyme exhibits the 2,3-diphosphoglycerate phosphatase activity, although the activity is very low compared with the mutase activity[11]. Therefore, studies on a protective effect of the coenzyme on inactivation by trinitrophenylation were performed with the dilute concentration of the enzyme (component I). Although the coenzyme at a concentration 10 times the K_m* was added to the reaction mixture of trinitrophenylation, a significant protective effect on the loss of the activity was not observed (Table II). A significant protection was observed in the presence of the coenzyme at a high concentration (1 mM). Requirement of the high concentration of the coenzyme for protection indicates that the presence of amino groups at the

TABLE II

PROTECTIVE EFFECTS OF VARIOUS COMPOUNDS ON INACTIVATION BY TRINITROBENZENESULFONATE AT THE DILUTE ENZYME CONCENTRATION

The reaction mixture contained 0.38 μM of the enzyme (component I), 0.25 mM of trinitrobenzenesulfonate and the indicated compounds in 25 mM borate buffer (pH 7.5). After incubation at 25°, the enzyme activity was determined by using 2.8 μg of the enzyme.

Compound added	*Concn. (mM)*	*Trinitrophenylation time (min)*	*Activity (%)*
No addition		0	100
		5	37
		10	16
		20	12
3-Phosphoglycerate	2	10	100
	1	10	73
2-Phosphoglycerate	4	10	97
2,3-Diphosphoglycerate	1	10	80
	0.1	10	55
	0.01	10	27
Phosphohydroxypyruvate	0.125	10	89
Phosphoglycolate	2	10	100
Phosphoenolpyruvate	2	10	91

* Kinetic parameters at pH 5.9 have been reported[3]. Under the conditions of chemical modification experiments (pH 7.5), 0.1–0.3 mM for K_m^{3PGA}, 0.2–0.5 mM for K_i^{3PGA}, 0.5–0.8 μM for $K_m^{2,3PGA}$, and 0.2–0.5 mM for $K_i^{2,3PGA}$ were obtained by using the coupled method with enolase. Concentrations of free 3-phosphoglycerate and 2,3-diphosphoglycerate were calculated by using dissociation constants of Mg^{2+}–3-phosphoglycerate and Mg^{2+}–2,3-diphosphoglycerate complexes. These constants were determined by using eriochrome black T as a titrator.

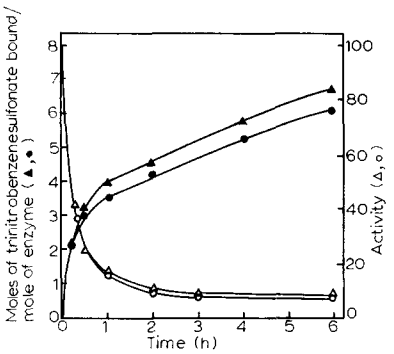
coenzyme site may be excluded. At a high concentration of the coenzyme, inhibition competitive with the substrate was observed with $K_i^{2,3PGA}$ of 0.2–0.5 mM. Therefore, the protective effect by the coenzyme at a high concentration may be attributable to the binding of the coenzyme at the substrate site.

Such phosphate compounds as phosphohydroxypyruvate, phosphoglycolate and phosphoenolpyruvate prevented the enzyme from inactivation by trinitrophenylation (Table II). These compounds were found to be competitive inhibitors of the substrate with K_i values of the order of 0.1 mM. As reported in the accompanying paper[21], kinetic studies on the activation of the phosphatase activity by these phosphate compounds indicated that these compounds combined with the enzyme at the substrate site and activated the hydrolysis of a phosphoester bond in 2,3-diphosphoglycerate bound at the coenzyme site. Therefore, it may be concluded that these compounds combine with the enzyme at the substrate site and protect the enzyme against inactivation by trinitrobenzenesulfonate.

The almost complete protection by inorganic phosphate, pyrophosphate and citrate was observed, when each compound was added in a final concentration of 10 mM.

Trinitrophenylation of component V

Enzymic properties of component V are similar to component I except that the activity of component V is about 5% of component I[8,9]. Therefore, the presence of essential 4 amino groups must be observed in component V. Furthermore, in order to investigate the state of lysine residues in component I which were liberated during the enzymic conversion of component I to component V, trinitrophenylation of both



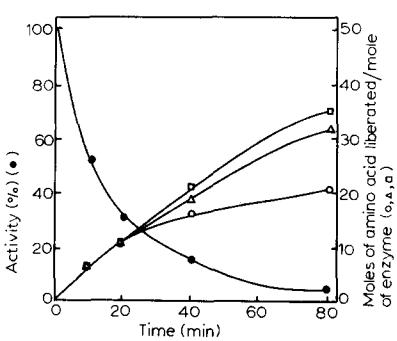


Fig. 7. Trinitrophenylation of component I and V. △, ○, enzyme activity; ▲, ●, trinitrophenyl amino groups. △, ▲, component I; ○, ●, component V. The conditions were as in Fig. 2. except that the reaction for trinitrophenylation was carried out at 0°. The enzyme activity was determined by using 2.8 μg of component I and 36 μg of component V.

Fig. 8. Liberation of amino acids from native and trinitrophenylated phosphoglycerate mutase by phosphoglycerate mutase-modifying enzyme. ○, ●, native enzyme (component I); △, enzyme (component I) having 4.8 moles of trinitrophenyl amino groups; □, enzyme (component I) having 6.8 moles of trinitrophenylamino groups. ●, enzyme activity; ○, △, □, amino acid liberated. The reaction mixture for the enzymic modification contained 6.25 μM native component I or trinitrophenylated component I and 50 μg/ml phosphoglycerate mutase-modifying enzyme in 20 mM sodium phosphate buffer (pH 7.0). After incubation at 25°, the 25% trichloroacetic acid solution was added in a final concentration of 5%. Amino acids and peptides in the supernatant solution were estimated as leucine equivalent by the ninhydrin method[22].

components was carried out under the same conditions. As shown in Fig. 7, the time-course of trinitrophenylation and loss of the activity of component V were substantially similar to those of component I. The presence of the substrate in the modification mixture of component V prevented the activity from inactivation as in the case of component I. These results indicate that the enzymic modification of component I gives little effect on the reactivity of amino groups at the substrate site. Furthermore, these results suggest that lysine residues which are liberated from component I by the enzymic modification and attributed to the increase of electrophoretic mobility of component V, are not so reactive to trinitrobenzenesulfonate.

In order to confirm this concept, the enzymes (component I) which had 4.8 and 6.3 moles of trinitrophenyl amino groups per mole of enzyme were subjected to the enzymic modification by phosphoglycerate mutase-modifying enzyme. The amino acids and peptides released from native and trinitrophenylated component I by the enzymic modification are presented as leucine equivalent in Fig. 8. The activity of the native component I was decreased to 5% of the original activity accompanying with release of about 20 amino acids as leucine equivalent. In early steps of the enzymic modification, almost the same amounts of amino acids were released from both native and trinitrophenylated component I. This result indicates that the trinitrophenylated component I has the same conformation as the native component I. However, after incubation for 180 min, liberation of 30–35 amino acids as leucine equivalent was observed in the case of the trinitrophenylated enzymes. It seems that the hydrolysis of specific peptide bonds in the trinitrophenylated component I, which are to be hydrolyzed during conversion of component I to component V, may facilitate access of phosphoglycerate mutase-modifying enzyme to peptide bonds which are not to be hydrolyzed in the case of the native component I.

Absorbance at 348 nm of 5% trichloroacetic acid-soluble fractions was measured to ascertain whether trinitrophenyllysine was liberated during the enzymic modification or not. Little liberation of trinitrophenyllysine from trinitrophenylated enzymes was observed. This result was consistent with that in Fig. 7 indicating that lysine residues liberated from component I by the enzymic modification were buried in the interior of the protein molecule inaccessible to trinitrobenzenesulfonate.

DISCUSSION

Although yeast phosphoglycerate mutase has about 92 lysine residues per mole of the enzyme[23], there are 7 or 8 amino groups which react rapidly with trinitrobenzenesulfonate (Fig. 1). It was found that 4 amino groups were protected from trinitrophenylation in the presence of the substrate. However, trinitrophenylation of 8–10 amino groups was required for almost the complete loss of the enzyme activity. Amino groups which were reactive to trinitrobenzenesulfonate but not essential for the activity were carboxymethylated in the presence of the substrate without loss of the activity. Trinitrophenylation of 4 amino groups of the carboxymethylated active enzyme caused the complete loss of the activity. From these results, it has been concluded that 4 amino groups are indispensable for the enzyme activity.

Experimental results of the protective effects of the substrate and the coenzyme

on the loss of the activity by trinitrophenylation indicated that these 4 amino groups were located at the substrate site.

Some results in this paper can be explained by a ping pong mechanism[24], provided that a phosphoenzyme has the similar reactivity with trinitrobenzenesulfonate to a dephosphoenzyme. However, kinetic studies on the yeast enzyme were consistent with the idea that there were separate substrate and coenzyme binding sites on the enzyme molecule[3]. This result was further supported by the protective effect of 3-phosphoglycerate in Fig. 2. If the mechanism of the yeast enzyme is a ping pong one and the yeast enzyme is normally present as a dephosphoenzyme, a protective effect can not be more than 50% when the concentration of 3-phosphoglycerate is equal to its K_i value. As shown in Fig. 2, a almost complete protection by the presence of 3-phosphoglycerate at the concentration approximately equal to its K_i value suggests that a phosphoenzyme mechanism may be excluded.

The similar time course of trinitrophenylation in both components suggests that the enzymic modification does not give an influence on the environment of amino groups at the substrate site.

From kinetic studies on the 2,3-diphosphoglycerate phosphatase activity of both components as described in the accompanying paper[21], it is suggested that protein conformation required for hydrolysis of 2,3-diphosphoglycerate at the coenzyme site is induced more efficiently in component I than in component V and that this hydrolysis step is the first step in the mutase reaction pathway. Therefore, it is likely that amino acids and peptides liberated by the enzymic modification play an important role on the efficient formation of the active conformation.

It was reported that phosphoglycerate mutase protein was composed of 4 polypeptide chains with almost the same molecular weight[19]. It is likely that 4 amino groups essential for the substrate binding correspond to the number of subunits and each polypeptide chain has one binding site of the substrate. These amino groups may be attributable to ε-amino groups of lysine, since the N-terminal groups could not be detected by using the dinitrophenylation method of LEVY AND LI[25]. It appears that N-terminal amino acid residues are buried in the interior of the molecule inaccessible to dinitrofluorobenzene.

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