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Structure and activity of phosphoglycerate mutase

S. I. Winn,†§ H. C. Watson,† R. N. Harkins‡‖ and L. A. Fothergill‡

† *Department of Biochemistry, University of Bristol, Bristol BS8 1TD, U.K.*

‡ *Department of Biochemistry, University of Aberdeen, Aberdeen AB9 1AS, U.K.*

The structure of yeast phosphoglycerate mutase determined by X-ray crystallographic and amino acid sequence studies has been interpreted in terms of the chemical, kinetic and mechanistic observations made on this enzyme. There are two histidine residues at the active site, with imidazole groups almost parallel to each other and approximately 0.4 nm apart, positioned close to the 2 and 3 positions of the substrate. The simplest interpretation of the available information suggests that a ping-pong type mechanism operates in which at least one of these histidine residues participates in the phosphoryl transfer reaction. The flexible C-terminal region also plays an important role in the enzymic reaction.

1. Introduction

Phosphoglycerate mutase (EC 2.7.5.3) is the glycolytic enzyme that catalyses the interconversion of 2- and 3-phosphoglycerates. The enzyme isolated from animals, yeast and other sources must be phosphorylated to be catalytically active, although there is another type occurring in higher plants that apparently does not require this 'priming' reaction (for review see Rose 1980). The phosphorylation-dependent mutases, with the exception of the yeast enzyme, are dimers of relative molecular mass 56000–60000; yeast phosphoglycerate mutase is a tetramer of relative molecular mass 110000 (Ray & Peck 1972). Thus the molecular masses of the subunits of these enzymes are very similar. For the yeast enzyme both the amino acid sequence (Fothergill & Harkins 1981) and the high-resolution three-dimensional structure (Winn *et al.* 1981) have been determined. These studies have shown that the yeast enzyme is composed of four identical subunits arranged with almost exact 222 symmetry. The polypeptide chain folds to form a subunit consisting of a central core of β-sheet surrounded by α-helices in an arrangement reminiscent of that found in the nucleotide-binding domain of the dehydrogenases (Ohlsson *et al.* 1974). Phosphoglycerate mutase has no known nucleotide binding requirement, although binding of such molecules can occur.

Pizer & Ballou (1959) have studied the specificity of the enzyme for its substrates and have found that for a substrate analogue to participate in the mutase reaction, carbon 1 must carry a substituent with a negative charge and carbons 2 and 3 must carry hydroxyl groups one of which must be phosphorylated. The L-isomer of the substrate will accept a phosphoryl group, although the rate is much slower than for the D-isomer.

In addition to its normal catalytic activity, phosphoglycerate mutase possesses an intrinsic

§ Present address: 70A Gilbert Road, Cambridge CB2 2QH, U.K.
‖ Present address: Genentech, Inc., South San Francisco, California 94080, U.S.A.

phosphatase activity towards 2,3-bisphosphoglycerate. This activity is very much less than the mutase activity (approximately $1:10^5$ for the yeast enzyme (Rodwell *et al.* 1957)), but can be considerably enhanced by substrate analogues such as 2-phosphoglycollate (Sasaki *et al.* 1971). The phosphatase activity is inhibited by the normal substrates of the mutase reaction.

Phosphoglycerate mutase has been reported to catalyse the formation of 2,3-bisphosphoglycerate from 1,3-bisphosphoglycerate in the presence of 3-phosphoglycerate (Laforet *et al.* 1974), the reaction normally catalysed by diphosphoglycerate mutase (EC 2.7.5.4, bisphosphoglycerate synthase). These two mutases have many other similarities. The enzymes from human erythrocytes are both dimers of relative molecular mass 60000 and have very similar amino acid compositions (Sheibley & Hass 1976). Both phosphoglycerate mutase and diphosphoglycerate mutase generate a phosphohistidine intermediate in the reaction pathway (Han & Rose 1979; Hass *et al.* 1980), and amino acid sequence studies show that the enzymes are significantly homologous (Haggarty & Fothergill 1980). Diphosphoglycerate mutase also possesses monophosphoglycerate mutase activity and acts as a phosphatase towards 2,3-bisphosphoglycerate (Sasaki *et al.* 1975; Kappel & Hass 1976). Thus it appears that these two mutases are very closely related and probably function by analogous mechanisms.

2. Kinetic properties of the enzyme

Some of the kinetic properties of yeast phosphoglycerate mutase are summarized in table 1: variability can probably be attributed to differences in the conditions under which the measurements were made. For example, Grisolia & Cleland (1968) showed that for phosphoglycerate mutase from chicken breast muscle the K_m for both 2,3-bisphosphoglycerate and 3-phosphoglycerate increased from 1.4 μM and 0.6 mM respectively at low ionic strength to 42 μM and 4.6 mM in the presence of 400 mM KCl. The pH optimum for the enzyme from all species has been found to be 5.9 when measured by the direct assay procedure.

Attempts to elucidate the mechanism of the mutase reaction by kinetic methods have led to conflicting results. Grisolia & Cleland (1968) suggested a ping-pong or phosphoenzyme mechanism, but Chiba *et al.* (1970), working with the yeast enzyme, proposed a sequential mechanism involving the direct transfer of a phosphoryl group from 2,3-bisphosphoglycerate to a monophosphoglycerate. Britton *et al.* (1972*a*) argued that the available data could not distinguish unequivocally between the two mechanisms, and using an induced transport test they deduced that the reaction did in fact proceed by a ping-pong mechanism. To be consistent with the kinetic properties, any isomerization of the phosphoenzyme must proceed at a rate in excess of $10^6\ s^{-1}$. This led Britton & Clarke (1972) to propose a mechanism in which there was only one phosphorylation site on the enzyme but with the substrate able to bind at the active site in such a way that the phosphoryl group on either the 2 or 3 position could be attacked. It has been shown that phosphoglycerate mutases from yeast, rabbit muscle and pig kidney all function by a ping-pong mechanism (Britton *et al.* 1972*b*, 1973). The other mutase involved in glycolysis, phosphoglucomutase, has been shown to operate with a ping-pong mechanism (Britton & Clarke 1968) but using a phosphoserine (Milstein & Milstein 1968), as opposed to a phosphohistidine, intermediate.

Rose (1970, 1971) isolated ^{32}P-labelled phosphoenzyme, free of bound glycerate, from both rabbit muscle and yeast after incubation with labelled 2,3-bisphosphoglycerate. She found

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that the phosphoryl group on the enzyme can be exchanged with the substrate and is acid labile. Phosphohistidine-containing peptides from yeast phosphoglycerate mutase and from human and horse diphosphoglycerate mutase (Han & Rose 1979; Hass *et al.* 1980) have been sequenced and it has been shown (Rose & Dube 1976) that the rates of phosphorylation and dephosphorylation of the enzyme are consistent with the rate of the mutase reaction. The phosphoglycerate mutase phosphorylated intermediate undergoes fairly rapid dephosphorylation and it has been suggested (Britton *et al.* 1972*a*) that the phosphatase activity of the enzyme may be attributed to the hydrolysis of the complex.

TABLE 1. KINETIC PARAMETERS OF YEAST PHOSPHOGLYCERATE MUTASE

property	value	conditions	reference
K_m (3PGA)	0.2 mM	pH 7.4	B
	0.6 mM	pH 5.9, 25 °C	C
	0.1–0.3 mM	pH 7.5, 25 °C	S
K_m (2PGA)	< 0.1 mM	156 mM 2,3 DPGA	R
K_m (2,3DPG)	0.8 μM	pH 5.9, 25 °C	C
	0.5–0.8 μM	pH 7.5, 25 °C	S
K_i (3PGA)	3.2 mM	pH 5.9, 25 °C	C
	0.2–0.5 mM	pH 7.5, 25 °C	S
K_i (2,3DPG)	0.25 mM	pH 7.5, 25 °C	S
K_{eq} (3PGA:2PGA)	6.3 ± 0.3	pH 4.6–6.65	R

Britton *et al.* (1968), Chiba *et al.* (1970) and Sasaki *et al.* (1971) (references B, C and S respectively) used the enolase coupled assay system. Assays were carried out at 30 °C with 17 mM 3PGA, 3 μM 2,3DPG, 3 mM $MgSO_4$, 33 μM Tris and 10 units of enolase unless otherwise stated. Rodwell *et al.* (1957) (reference R) used the direct assay method at 30 °C, pH 5.9, with 33 μM 2PGA, 0.156 μM 2,3DPG and 100 μM potassium acetate. The following abbreviations are used: 3PGA, 3-phosphoglycerate; 2PGA, 2-phosphoglycerate; 2,3DPG, 2,3-bisphosphoglycerate.

3. THE ACTIVE SITE OF THE ENZYME

The active site of yeast phosphoglycerate mutase has been located by using crystals of the enzyme soaked in one of its substrates, 3-phosphoglycerate (Winn *et al.* 1981). Although the enzyme used in these studies was inactive, there is evidence to suggest that 3-phosphoglycerate binds at the active site of the unphosphorylated enzyme, both because it is a competitive inhibitor with respect to 2,3-bisphosphoglycerate and also because it provides protection against active site modifying reagents. The crystallographic results show that the active site lies at the bottom of a deep hollow formed entirely by the residues of one subunit. In the tetramer the four sites are well separated (see figure 1) and appear to be freely accessible to the solvent.

In the crystal structure of the native enzyme a sulphate ion appears to bind in the position assumed to be occupied by the phosphoryl group of 3-phosphoglycerate (Winn *et al.* 1981). With the substrate in this position (see figure 2) the phosphoryl oxygen atoms form hydrogen bonds with the hydroxyl groups of a serine and a threonine and the 2′ hydroxyl makes a hydrogen bond with a main-chain carbonyl oxygen atom. The carboxyl group on the 1-carbon of the substrate points towards the back of the active site and is close to an arginine. Such a charge–charge interaction explains why a catalytically active substrate analogue must have a negatively charged group in the 1-position and presumably helps position the substrate for phosphoryl transfer.

Two of the subunit's four histidine residues are located at the C-terminal portion of the

β-sheet and are in close proximity to the substrate. The imidazole rings of histidine 8 from strand B1 and histidine 179 from the adjacent strand B4 of the β-sheet (for a description of the secondary structure see Campbell *et al.* 1974) are parallel and approximately 0.4 nm apart. An arginine forms part of this side chain complex, being above the two histidine residues

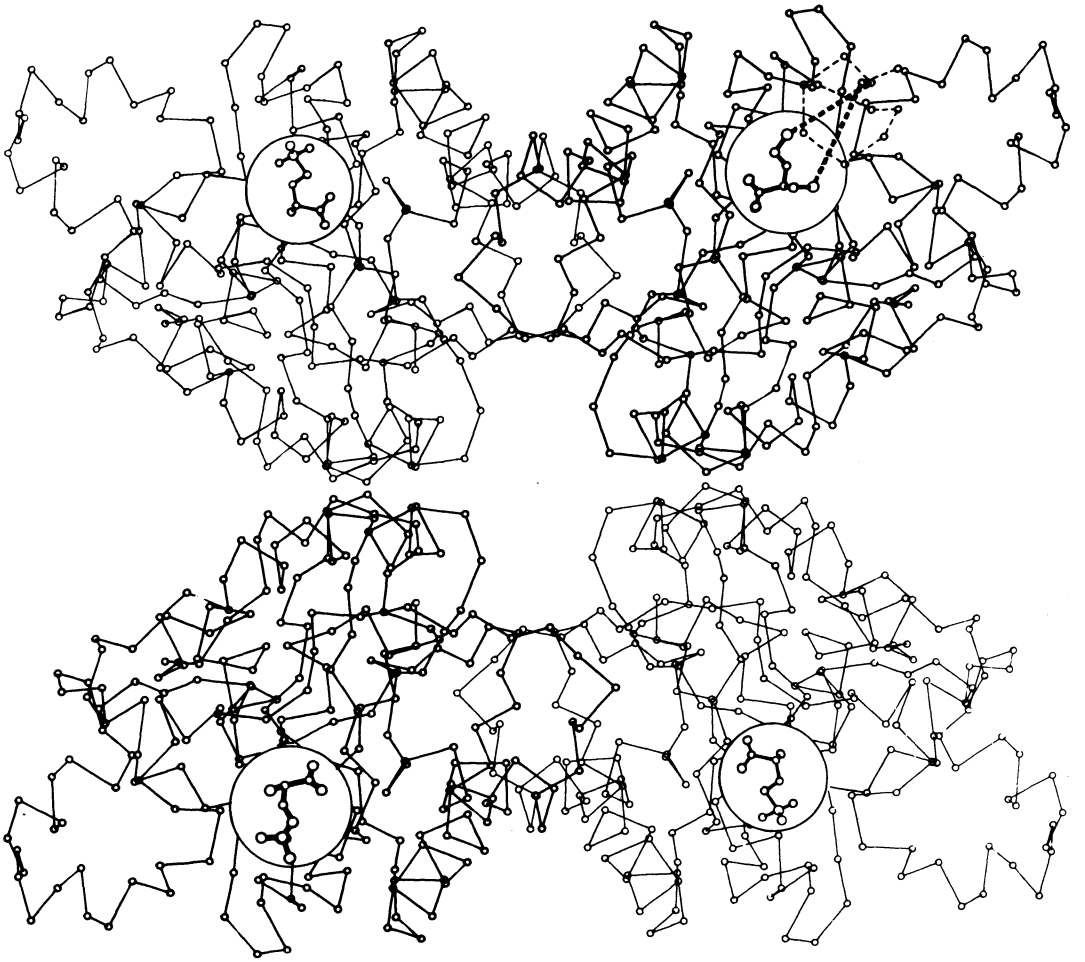

FIGURE 1. An α-carbon drawing of the phosphoglycerate mutase molecule, showing the relative positions of the four active site regions. The substrate shown in the upper right-hand subunit is of 2,3-bisphosphoglycerate (phosphoryl oxygen atoms have been omitted) while the other substrates are 3-phosphoglycerate. The ten C-terminal residues (for detailed explanation see text) are included in the subunit (possible α-carbon atom positions joined by broken lines) with 2,3-bisphosphoglycerate to show how these residues could shield the substrate from the solvent. The heavy broken lines connect the terminal lysines to the substrate's phosphoryl groups, indicating the steric feasibility of the involvement of these side chains in the phosphoryl transfer step. It must be stressed, however, that the C-terminal residues do not take up a well defined conformation in the unphosphorylated enzyme and that crystals soaked in 2,3-bisphosphoglycerate (Winn *et al.* 1981) are unstable, presumably because a structural rearrangement takes place when one of the active centre residues is phosphorylated.

with one of its guanidinium nitrogen atoms near the δ-nitrogens of the two histidines, as shown in figure 2. Histidine 8 is close to the phosphoryl group on the 3-phosphoglycerate molecule and only minor rearrangements would be involved for it to be in the correct position for an attack on the phosphoryl group. Histidine 179 lies close to the hydroxyl on the 2-carbon

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of the substrate and if this hydroxyl were phosphorylated, as in 2-phosphoglycerate or 2,3-bisphosphoglycerate, this phosphoryl group would be close enough to interact with the histidine residue. Thus on the basis of the 3-phosphoglycerate binding observed in the crystal either or both of these two histidine residues could be involved in the phosphoryl transfer.

Several positively charged residues, mostly arginines, line the active site pocket, as might be expected for an enzyme that interacts with negatively charged substrates. Apart from the charge dipole associated with the amino end of a helix (Hol *et al.* 1978), which could help to stabilize a phosphoryl group attached to the substrate's 2′ hydroxyl, the only other notable

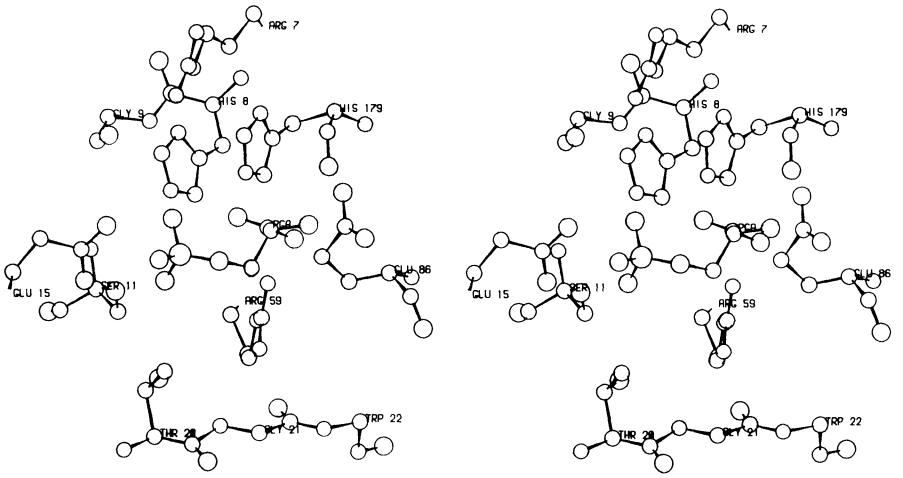

FIGURE 2. A stereo view of the active site region of the phosphoglycerate mutase subunit. Only those amino acid residues adjacent to the substrate have been included. The 3-phosphoglycerate molecule is shown at the centre of the drawing and is labelled PGA. Note that the two active centre imidazole groups appear to be locked in a position below but on either side of the guanidinium group by main-chain atoms. Although the viewpoint for this drawing was chosen to illustrate the structural features of the substrate binding site, it also serves to indicate the general direction of approach of the substrate on entering the active site region from the solvent.

feature of the active site region concerns the glutamate residues 15 and 86. The carboxyl groups of these residues are positioned on either side of the substrate binding site and could easily move to act as proton-withdrawing groups from the 3′ and 2′ hydroxyls respectively.

4. The mechanism of the enzymic reaction

The reaction sequence shown schematically in figure 3 has been proposed as the simplest interpretation of the available data. An alternative, more complicated scheme is given in figure 4. Britton *et al.* (1972*a*) observed an initial burst in the amount of monophosphoglycerates formed corresponding to the amount of enzyme present when 2,3-bisphosphoglycerate was added to the system and they interpreted this as the formation of phosphoenzyme. According to the scheme in figure 3 the enzyme would be phosphorylated on histidine 8 by 2,3-bisphosphoglycerate. For 3-phosphoglycerate to be converted to 2-phosphoglycerate, histidine 8 would donate its phosphoryl group to the 2′ hydroxyl of the substrate, and an enzyme–2,3-bisphosphoglycerate complex would be formed as an intermediate in the reaction. Thus the 'priming' of the enzyme by the action of 2,3-bisphosphoglycerate would be identical to part of the normal mutase reaction rather than a separate initial step.

The results of isotope exchange experiments show that 2,3-bisphosphoglycerate becomes radioactively labelled if the mutase reaction is carried out with ^{32}P-labelled 3-phosphoglycerate (Cascales & Grisolia 1966), the amount of label incorporated into 2,3-bisphosphoglycerate being small at low ionic strength but increasing at high salt concentrations. Since the enzyme can be phosphorylated by 1,3-bisphosphoglycerate, the dissociation of 2,3-bisphosphoglycerate from the enzyme would explain the small amount of diphosphoglycerate mutase activity displayed by the enzyme.

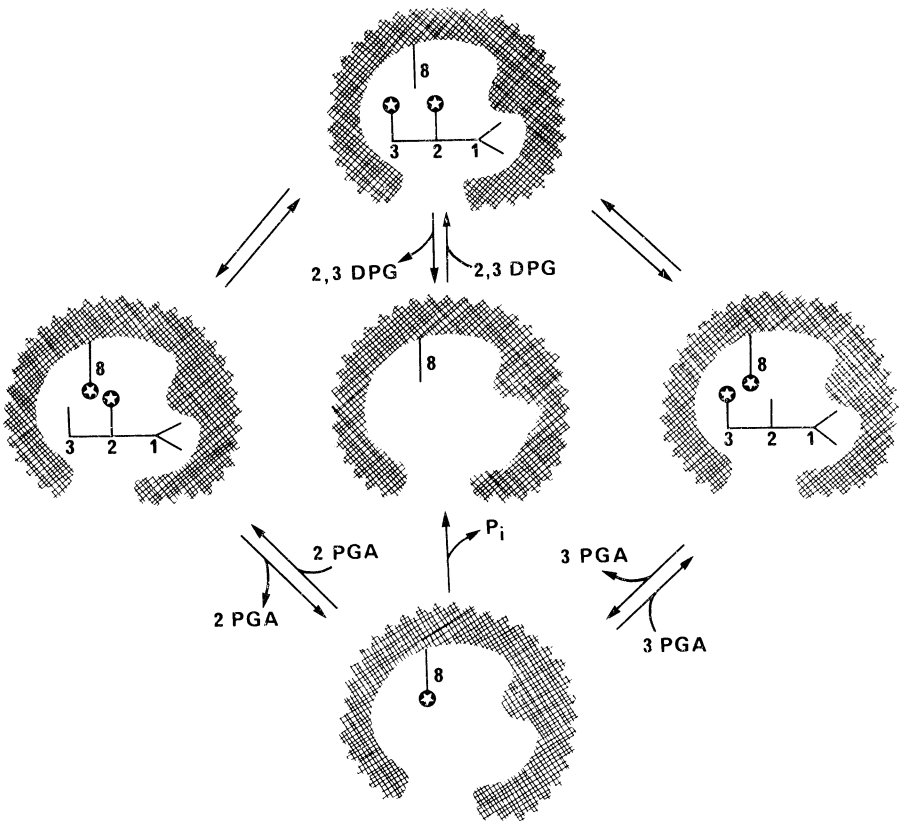

FIGURE 3. A schematic drawing of the proposed mutase reaction sequence. The histidine residue is represented by its residue number (8). The substrate carbon positions are marked 1, 2 and 3, and the circles with stars attached either to the substrate or the enzyme indicate phosphoryl groups. Abbreviations used are defined in table 1.

The structural work on phosphoglycerate mutase has shown the presence of two histidine residues at the active site in suitable positions to participate in phosphoryl transfer and it is tempting to postulate a mechanism involving both residues. The participation of both histidines appears to be unlikely, however, for a number of reasons. Phosphohistidine-containing peptides have now been isolated from phosphoglycerate mutase and diphosphoglycerate mutase from several species (Han & Rose 1979; Hass *et al.* 1980), and invariably contain histidine 8. There is no evidence from this experimental approach that histidine 179 is phosphorylated, but the existence of such a phosphorylated form with a very short half-life cannot be completely excluded.

Blättler & Knowles (1980) have shown that the rabbit muscle phosphoglycerate mutase

reaction proceeds with overall retention of the configuration at the phosphorus, which implies that an even number of phosphoryl transfer steps have occurred. The scheme shown in figure 3 involves two phosphoryl transfer steps and is therefore the simplest possibility. An alternative, more complicated scheme is that in which both histidine residues are involved in the phosphoryl transfer and in which an isomerization step regenerates the active site in a form suitable for

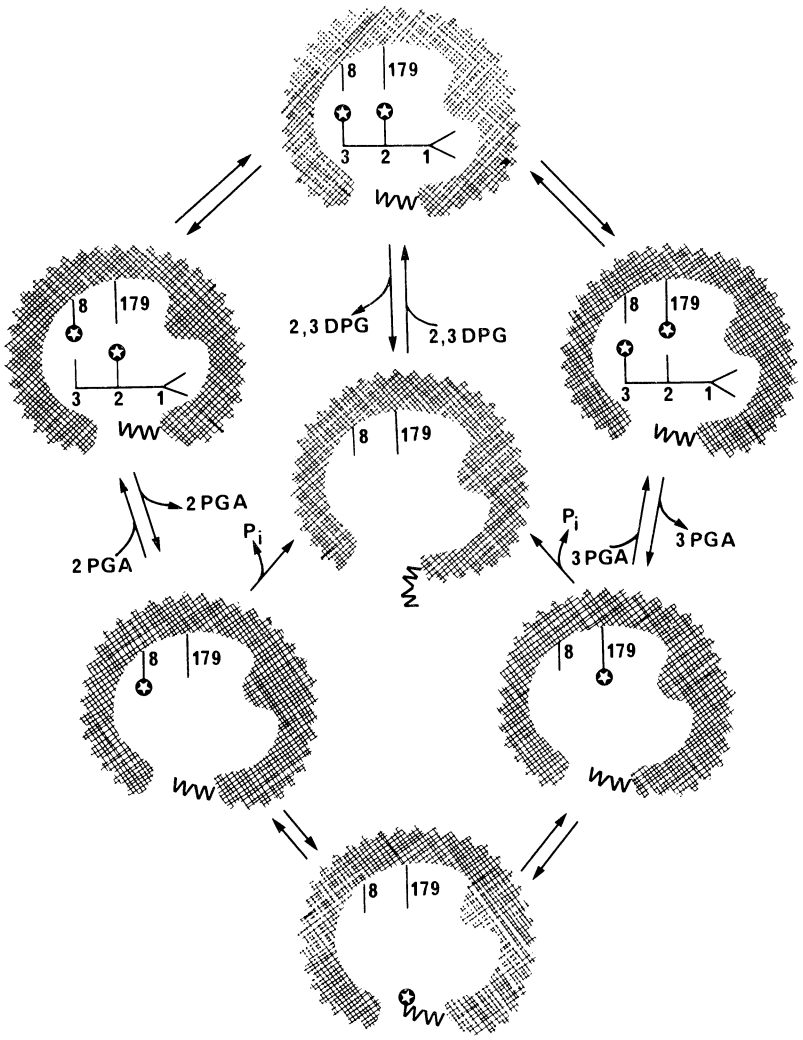

FIGURE 4. A schematic drawing of the mutase reaction sequence involving histidine residues 8 and 179. The C-terminal tail (including lysine residues 239 and 240) is represented by WW. The substrate and phosphoryl identification scheme is as in figure 3.

a new round of catalysis (see figure 4). Such an isomerization step would have to proceed at a rate in excess of 10^6 s^{-1} (Britton *et al.* 1972*a*). Examination of the active site shows that the conformation of the two histidine residues is sterically unfavourable for direct phosphoryl transfer and it becomes necessary to invoke the participation of an additional residue. A lysine residue near the C-terminus of the enzyme may fill this role (see next section). This reaction scheme would also be consistent with the observed retention of configuration at the phosphorus.

According to Britton *et al.* (1972*a*) the yeast phosphoenzyme has a half-life of between 1 and

2 min. The hydrolysis of the phosphoenzyme is therefore sufficient to account for the observed phosphatase activity of phosphoglycerate mutase. The rate of this hydrolysis is enhanced by substrate analogues (Sasaki *et al.* 1971) and may be caused by the analogues binding to the enzyme with their phosphoryl group close to the histidine residue, thus mimicking the substrate and facilitating the release of the phosphoryl group from the enzyme, which is accepted by a free hydroxyl ion instead of the substrate. The analogue would then be released, leaving the enzyme free to be rephosphorylated.

5. The carboxy-terminal residues

Early preparations of crystalline yeast phosphoglycerate mutase were often found to be inhomogeneous and could be separated by electrophoresis into five components, each with a different activity (Chiba & Sugimoto 1959; Chiba *et al.* 1960*a*). Component I, the species with the lowest electrophoretic mobility, had the highest specific activity and component V a very low specific activity. There was a change in specific activity of approximately one quarter that of component I between successive components, suggesting that the effect was due to the various possible combinations of active and inactive subunits.

It was found by Chiba *et al.* (1960*b*) that the formation of components with reduced activity was dependent on the autolysis time of the yeast during the enzyme preparation. They concluded that component I was the fully active form of the enzyme and that the other components arose from modification of the enzyme during autolysis. Sasaki *et al.* (1966) partly purified the enzyme responsible for the modification and showed that it caused the release of nine or ten amino acids per subunit from phosphoglycerate mutase. They also showed that the optical rotatory dispersion properties of phosphoglycerate mutase did not vary during the course of the modification and suggested that the enzyme conformation had not altered on removal of these residues. The amino acid sequence shows that the modification involves the removal of the peptide

Gly-Ala-Ala-Val-Ala-Asn-Gln-Lys-Lys-Gly

from the carboxyl terminus of each subunit. Although the modified enzyme is inactive as a mutase, the phosphatase activity towards 2,3-bisphosphoglycerate is unaffected. However, the stimulation of the phosphatase activity by substrate analogues is not observed for component V (Sasaki *et al.* 1971).

The ten carboxyl terminal residues are not observed in the electron density map (see Winn *et al.* 1981), presumably because they form a flexible 'tail' which, in the dephosphorylated form of the enzyme, has no well defined conformation. Model-building studies with these residues show that they can adopt a conformation that will hinder access to the active site as shown in figure 1. It is possible that the function of these residues is to exclude water from the active site during the mutase reaction, thus ensuring that the phosphoryl group on the enzyme is transferred to the substrate rather than to a water molecule. In addition, the two consecutive lysine residues, the penultimate residues in the chain, could approach the two histidine residues or phosphoryl groups attached either to one of the histidine residues or to a substrate bound in the active site. These lysine residues could be involved in the phosphoenzyme isomerization step and would conserve the configuration of the phosphoryl group, but would need to function very rapidly to be consistent with the required reaction rate.

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