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C. C. F. Blake and D. W. Rice

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Phosphoglycerate kinase

By C. C. F. Blake and D. W. Rice
Laboratory of Molecular Biophysics, Department of Zoology, South Parks Road, Oxford OX1 3PS, U.K.

Phosphoglycerate kinase catalyses the high-energy phosphoryl transfer of the acyl phosphate of 1,3-bisphosphoglycerate to ADP to produce ATP, a reaction requiring magnesium ions. The enzyme is widely distributed and apparently highly conserved as a monomer of molecular mass 45000. X-ray studies of the enzymes from horse muscle and yeast, carried out in Oxford and Bristol respectively, have shown that the molecular structures of the two enzymes are almost identical. The most striking aspect of the structure is that the single polypeptide chain is organized into two separated domains composed of the N-terminal and C-terminal halves of the chain. Substrate binding studies and the determination of the complete amino acid sequence of the horse enzyme suggest that the nucleotide substrates and the phosphoglycerate substrates are bound to the C-domain and N-domain, respectively, in sites that are separated by about 12 Å. In order to bring the two substrates together for catalysis, a hinge-bending conformational change involving helix rotation has been proposed, for which there is independent evidence from solution studies. Crystals of the ternary complex of the horse enzyme have been prepared that may contain the folded form of the enzyme.

Phosphoglycerate kinase (PGK) (EC 2.7.2.3) catalyses the high energy phosphoryl transfer reaction 1,3-bisphospho-D-glycerate + ADP $\overset{Mg^{2+}}{\rightleftharpoons}$ 3-phospho-D-glycerate + ATP. The enzyme is required not only for ATP generation in the glycolytic pathways of aerobes and anaerobes, but also for carbon fixation in plants. PGKs isolated from a wide variety of sources are monomeric with molecular masses around 45000 with comparable amino acid compositions and similar catalytic properties. This has led Fifis & Scopes (1978) to propose that the enzyme has a highly conserved molecular and active site structure. This proposal is in agreement with the close structural homology shown by the high-resolution X-ray analyses of the horse muscle (Blake & Evans 1974; Banks *et al.* 1979) and the yeast (Bryant *et al.* 1974) enzymes. In addition, the complete amino acid sequence of the horse PGK (Banks *et al.* 1979) differs from the sequence of the human enzyme (Huang *et al.* 1980) at only 14 positions in the chain of 416 amino acid residues, and the partial sequences of the yeast enzyme (L. A. Fothergill, unpublished) show more than 50 % homology with the mammalian PGKs.

The study of the steady-state kinetics of phosphoglycerate kinase, mostly of the yeast enzyme, has led to the conclusion that it has a rapid-equilibrium random order mechanism in which the binding of one substrate does not affect the binding of the other (Larsson-Ráznikiewicz 1967; 1970). The possibility of a phosphoryl-enzyme intermediate in the catalytic reaction appears to have been definitively ruled out as a tight complex between the enzyme and 1,3-bisphosphoglycerate (Johnson *et al.* 1975; 1976). Several aspects of the kinetic studies of the enzyme from yeast and other sources have proved difficult to understand. For example, many workers have reported biphasic double-reciprocal plots characteristic of negative cooperativity, which have been variously explained as due to multiple substrate binding sites (Schierbeck & Larsson-

Ráznikiewicz 1979; Ali & Brownstone 1976; Beissner & Rudolph 1979), or to complex reactions involving magnesium ions (Orr & Knowles 1974). However, nuclear magnetic resonance (Tanswell *et al.* 1976) and X-ray diffraction studies (Blake & Evans 1974; Bryant *et al.* 1974) reveal only a single, strong binding site for nucleotides, and gel filtration studies have established a single site for phosphoglycerate binding (Scopes 1978*b*). Further investigations of these effects (Scopes 1978*a*) have confirmed the nonlinearity of the kinetic plots for each variable substrate and have revealed that multivalent anions activate the enzyme at low concentrations and inhibit it at high concentrations. Scopes (1978*b*) has suggested that the reason for the anomalous kinetics is direct substrate activation occurring because substrates and anions accelerate the dissociation of 1,3-bisphosphoglycerate from the enzyme, this being the rate-limiting step in the back reaction because of the very tight binding of this ligand ($K_d \approx 5 \times 10^{-8}$ M). Wrobel & Stinson (1978) have investigated anion binding to PGK, finding a single strong site for multivalent ions. If this site is the phosphoglycerate site as suggested by Scopes, their finding that anion binding affected the reactivity of the single thiol group of yeast PGK, which is 30 Å distant, suggests that anions and substrates may cause substantial conformational changes. These suggestions are strongly supported by X-ray studies of the enzyme in crystals and in solution, as described below. An important indication of the catalytic mechanism of phosphoryl transfer used by phosphoglycerate kinase has come from the use of isotopic and chemical labelling of the γ-phosphate group of ATP, which shows that the catalytic reaction proceeds by inversion of configuration (Webb & Trentham 1980). This is consistent with direct transfer of the phosphoryl group from 1,3-bisphosphoglycerate to ADP, but leaves open the question of an associative or dissociative mechanism (Mildvan 1979).

Molecular structure of phosphoglycerate kinase

X-ray studies of the structure of phosphoglycerate kinase have been carried out in parallel in Oxford, on the horse muscle enzyme, and in Bristol by H. C. Watson and his colleagues on the yeast enzyme. The horse muscle enzyme crystallizes from ammonium sulphate in the monoclinic space group $P2_1$ with cell dimensions $a = 50.8$ Å, $b = 106.9$ Å, $c = 36.3$ Å and $\beta = 98.6°$ (Blake *et al.* 1972), and the yeast enzyme also crystallizes from ammonium sulphate in space group C2 with cell dimensions $a = 126.6$ Å, $b = 54.4$ Å, $c = 93.0$ Å and $\beta = 133.9°$ (Watson *et al.* 1971); both crystals have one enzyme molecule in the asymmetric unit. The structures of the two enzymes were solved initially by multiple isomorphous replacement, first at 6 Å resolution (Blake *et al.* 1972; Wendell *et al.* 1972), then at 3–3.5 Å (Blake & Evans 1974; Bryant *et al.* 1974) and currently at 2.5 Å resolution (Banks *et al.* 1979; Watson, unpublished). Although there was some initial difficulty and disagreement over the conformation and length of the polypeptide chain, the results at 2.5 Å resolution are now in good agreement, showing that the two enzymes have closely homologous structures for the single polypeptide chain of 416 amino acid residues, consistent with a molecular mass of *ca.* 45000. A schematic drawing of the tertiary structure of phosphoglycerate kinase is shown in figure 1, which will stand for either the horse muscle or yeast enzyme. The most striking feature of the molecular structure is the division into two widely separated domains of equal size. The two domains correspond almost exactly to the C-terminal and N-terminal halves of the polypeptide chain, the only exception to this pattern being the 10 C-terminus residues, which form an α-helix that packs on the N-terminal domain. This indicates that there are two connections between the domains: residues

189–202 that form an α-helix (helix 7 in figure 1), and residues 404–408 that link helices 14 and 15. The conformation of the polypeptide chain in each domain is of the same type: a six-stranded parallel β-sheet core surrounded by α-helices, typical of the class of α/β proteins (Levitt & Chothia 1976). The order of the β-strands in the C-domain is CBADEF, which is topologically identical to the dinucleotide-binding fold found in the NAD-domain of the dehydrogenases (Buehner *et al.* 1973); the strand order in the N-domain (CDBAEF) only differs in the location of strand D. The close similarity of the chain topology in the N- and C-

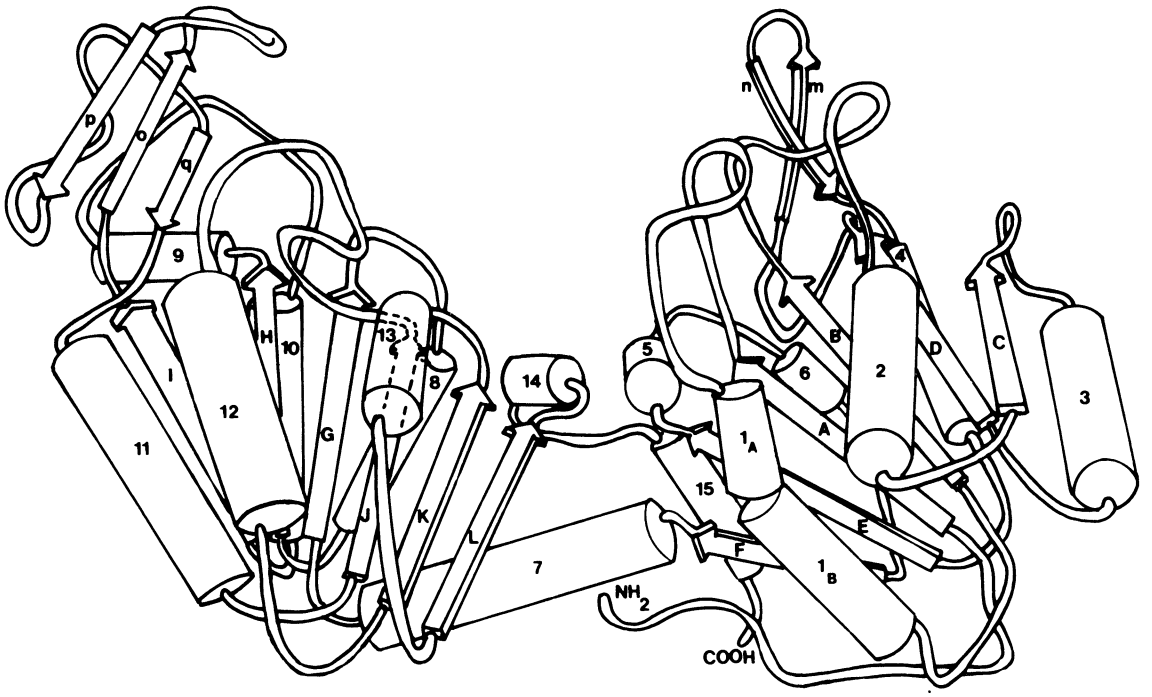

FIGURE 1. A drawing of the structure of the native horse muscle phosphoglycerate kinase molecule. α-Helices are defined by cylinders and the β-strands by arrows that also denote their direction.

domains may argue for the evolution of the enzyme by gene duplication, but the detailed structures of the two domains are not very similar and the amino-acid sequence shows little or no homology between the domains (Banks *et al.* 1979; Huang *et al.* 1980).

The X-ray model of the horse muscle enzyme was used as a basis for the determination of the complete amino acid sequence of the enzyme, which in turn was used to improve the structure determination by crystallographic refinement. The sequence analysis was carried out by splitting the polypeptide chain into 14 cyanogen bromide peptides, which were individually sequenced by using a Sequenator and the dansyl–Edman technique (Hartley 1970). These sequenced peptides were then aligned end to end on the X-ray map with the use of a number of structural criteria for fitting each individual peptide in its appropriate position along the polypeptide chain. The resultant sequence represented the first complete sequence determination of phosphoglycerate kinase (Banks *et al.* 1979), and is shown in figure 2. The subsequent determination of the sequence of human PGK (Huang *et al.* 1980) by conventional chemical techniques and the assignment of only 14 substitutions in the 416–417 residues proves the validity of the

joint X-ray–chemical sequence of the horse enzyme. The sequence of the yeast enzyme is currently being determined (L. A. Fothergill & H. C. Watson, unpublished).

In parallel with the incorporation of the side chain information from the sequenced peptides, the protein structure was subjected to crystallographic refinement by using the Hendrickson–

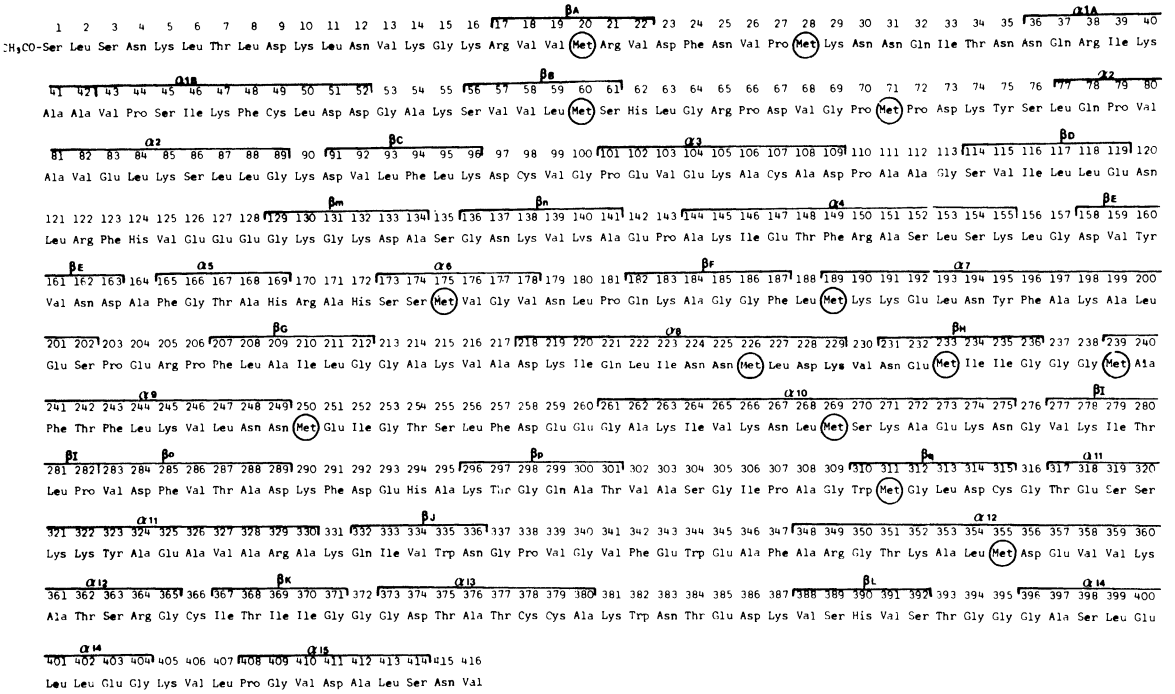

FIGURE 2. Sequence of horse muscle phosphoglycerate kinase determined by ordering the 14 cyanogen bromide peptides on the X-ray map. The methionine residues that terminate each peptide are circled. The position and extent of the α-helices and β-strands are indicated, with the same nomenclature as in figure 1.

TABLE 1. STATISTICS OF X-RAY ANALYSIS OF PGK

phase set	number of atoms	R*	figure of merit
isomorphous	—	—	0.46
combined set 1	1969	0.56	0.54
combined set 2	2472	0.45	0.62
combined set 3	3016	0.32	0.73
combined set 4	3047	0.30	0.74
combined set 5	3167	0.21	0.83

$$R^* = \frac{\Sigma(F_{obs} - F_{calc})}{\Sigma F_{obs}};$$

R* is calculated on all terms from ∞ to 2.5 Å and the data set constitutes some 13 100 reflexions.

Konnert constrained least-squares procedure (Konnert 1976), and the resultant calculated phases combined with the isomorphous phases (Hendrickson & Lattman 1970), to produce joint phase sets that were used to calculate improved electron-density maps. This procedure was carried out in four stages with the use of (1) main-chain atoms only; (2) main chain + 200 side chains; (3, 4) main chain + 400 side chains; (5) main chain + 416 side chains. As shown in table 1, at each stage the conventional R-factor for the refinement decreased, the figure of merit

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of the combined phases increased, and the new electron density maps improved markedly in interpretability (Rice 1981). The result of this refinement procedure is that an accurate description of the molecular structure has been obtained, which enables us to define in detail the functional interactions of the horse muscle PGK molecule.

Substrate binding

Crystals of horse muscle PGK grown from ammonium sulphate, when soaked in solutions of three of its four substrates (1,3-bisphosphoglycerate was considered too labile for X-ray experiments), gave variable results: MgADP bound normally; MgATP caused the crystals to crack; and 3-phosphoglycerate did not bind at low concentrations and caused the crystals to dissolve at high concentrations. Similar results were obtained with yeast PGK crystals, which were also grown from sulphate. The reason for these results is almost certainly competition between the substrates and sulphate ions from the mother liquor of crystallization (Scopes 1978*b*). To overcome these difficulties a procedure was developed to transfer the horse muscle PGK crystals from the sulphate in which they were grown to solutions of potassium tartrate. Difference maps of the enzyme in sulphate and tartrate revealed the presence of two bound sulphate ions, one near Lys 219 and the other near Arg 170, corresponding to the nucleotide-binding site and probably the phosphoglycerate-binding site, respectively. The refined maps of the enzyme in tartrate show density probably due to bound tartrate ions at these same two positions.

Horse PGK crystals in tartrate appear to bind all three substrates normally, and allow some conclusions on their position and mode of binding, and associated changes in the enzyme, to be made. MgADP and MgATP (and their Mn adducts) bind at the same site situated on the part of the C-domain that faces the N-domain. Difference maps of MgADP and ATP at 2.5 Å resolution (see figure 3) are sufficiently detailed for the conformation of the bound nucleotide to be determined. The base is in the *anti* conformation with respect to the sugar ($\chi \approx 80°$), the sugar pucker is C2′ *endo*, and the conformation about the exocyclic C4′–C5′ bond is *gauche* (−) (or *trans-gauche*). The triphosphate chain is somewhat coiled and directed away from the adenine group. The major interactions between the enzyme and the nucleotide are shown in figure 4. The adenine ring is almost completely buried in a deep narrow slot that has considerable hydrophobic character, with its amino group innermost, probably making a hydrogen bond to the carbonyl oxygen of Gly 237. The adenine slot is lined by the main-chain segments that immediately follow β-strands G, H and J: Gly 212, Gly 213 and Ala 214; Gly 236, Gly 237 and Gly 238; and Val 339, Gly 340 and Val 341. The side chains of Leu 313 and Leu 256 are close to, or in contact with, the adenine ring. The ribose is located in a shallow depression above the pyrrolidine ring of Pro 338. Ribose binding seems to involve only the side chain of Glu 343, which appears to move and stiffen to form hydrogen bonds with probably both 2′ and 3′ hydroxyls of the sugar. Of the triphosphate chain, only the α-phosphate makes a specific interaction with a protein side chain, the ε-amino of Lys 219 seems to make an ion-pair interaction with it. The site of the α-phosphate is occupied by a sulphate or tartrate ion in the native enzyme. The β- and γ-phosphates of ATP are located about 5 Å from the amino-terminus of helix 13, but do not interact with any side chains. It seems reasonable to conclude therefore that the charged β- and γ-phosphates are interacting with the helix dipole (Hol *et al.* 1978). The MgADP has the same conformation as the ATP and interacts with the protein in exactly the same manner as ATP. The major difference between the two nucleotides seems to lie in the

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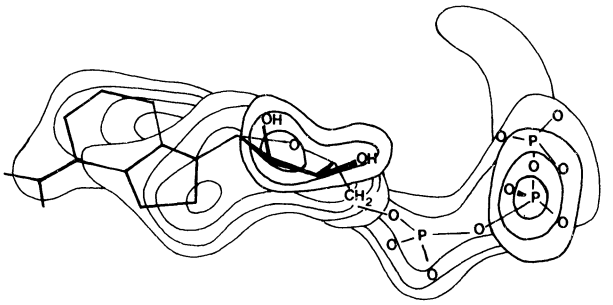

FIGURE 3. Diagram showing the fit of the ATP moiety to its difference electron density.

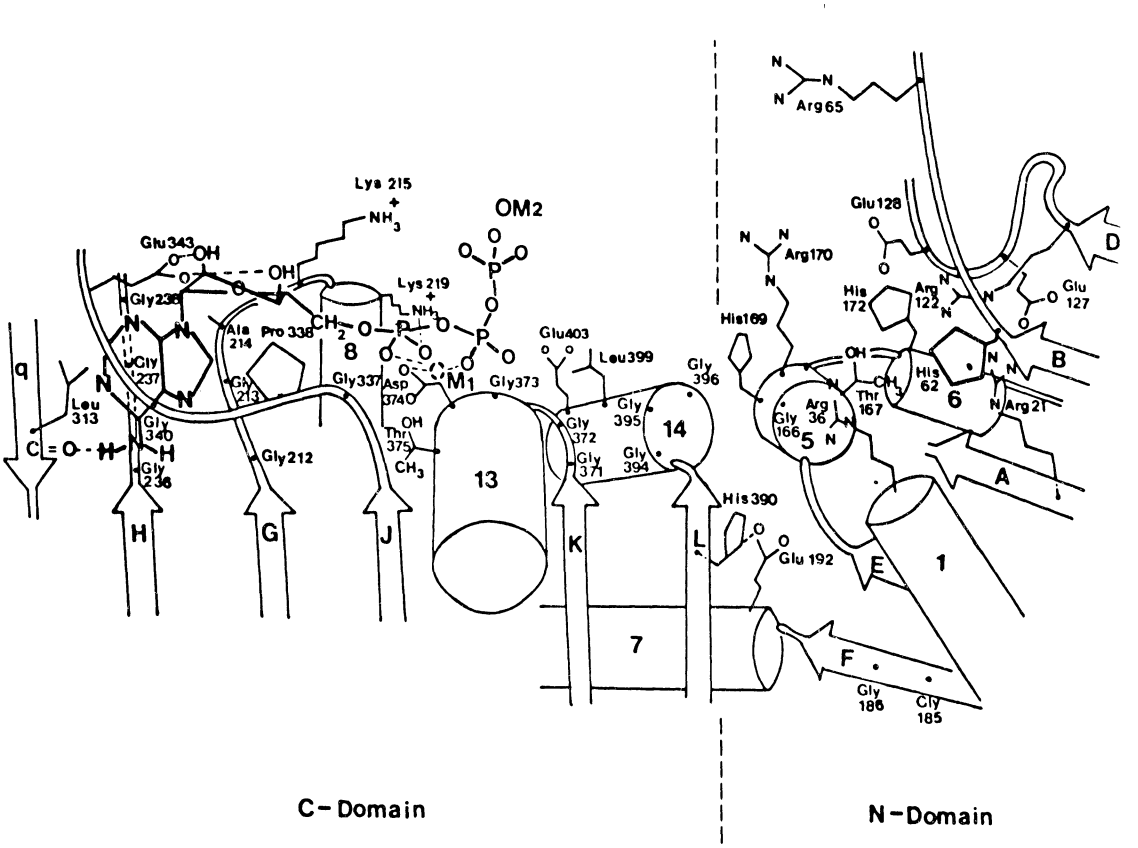

FIGURE 4. A schematic drawing, approximately to scale, of the probable active site of phosphoglycerate kinase, with ATP bound. Bound ADP takes up the same position and conformation. The metal position in the ADP complex is shown by the broken circle (M_1). The metal position in the ATP complex is not as yet completely defined.

location of the Mg^{2+} or Mn^{2+} ion. In ADP the metal is located between the α- and β-phosphates and the carboxylate of Asp 374 on helix 13, as shown in figure 5. The position of the metal in ATP is not as clear, but it does not occupy the site that is seen in the ADP complex, and it must therefore interact with either the β- or the γ-phosphate, or both. This X-ray picture of nucleotide interactions with horse PGK is in very good agreement with the equivalent interaction with yeast PGK determined by X-ray analysis (Bryant *et al.* 1974) and by n.m.r. studies (Tanswell *et al.* 1976).

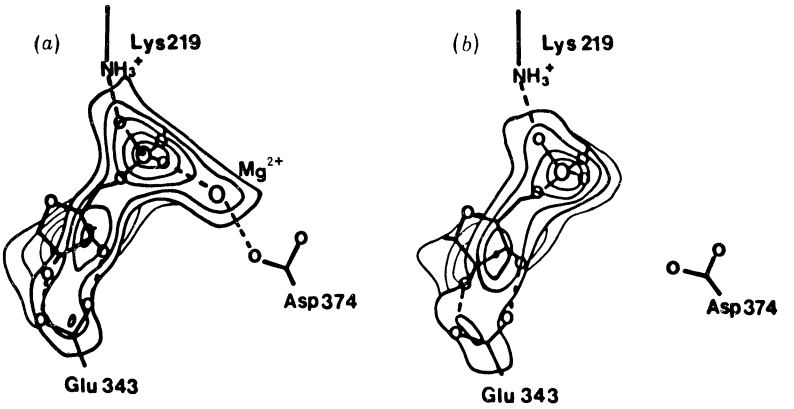

FIGURE 5. Equivalent two sections through the difference maps of (*a*) Mg ADP at 3.0 Å resolution, and (*b*) Mn ATP at 2.5 Å resolution, approximately in the plane of the ribose and α-phosphate (see figure 4). The adenine is below the plane to the left and the remaining phosphates above the plane to the right. The peak corresponding to the metal in ADP can be clearly seen, as can its absence from this position in ATP. The interchange of Mg^{2+} for Mn^{2+} has no effect on the metal position, nor on enzymic activity.

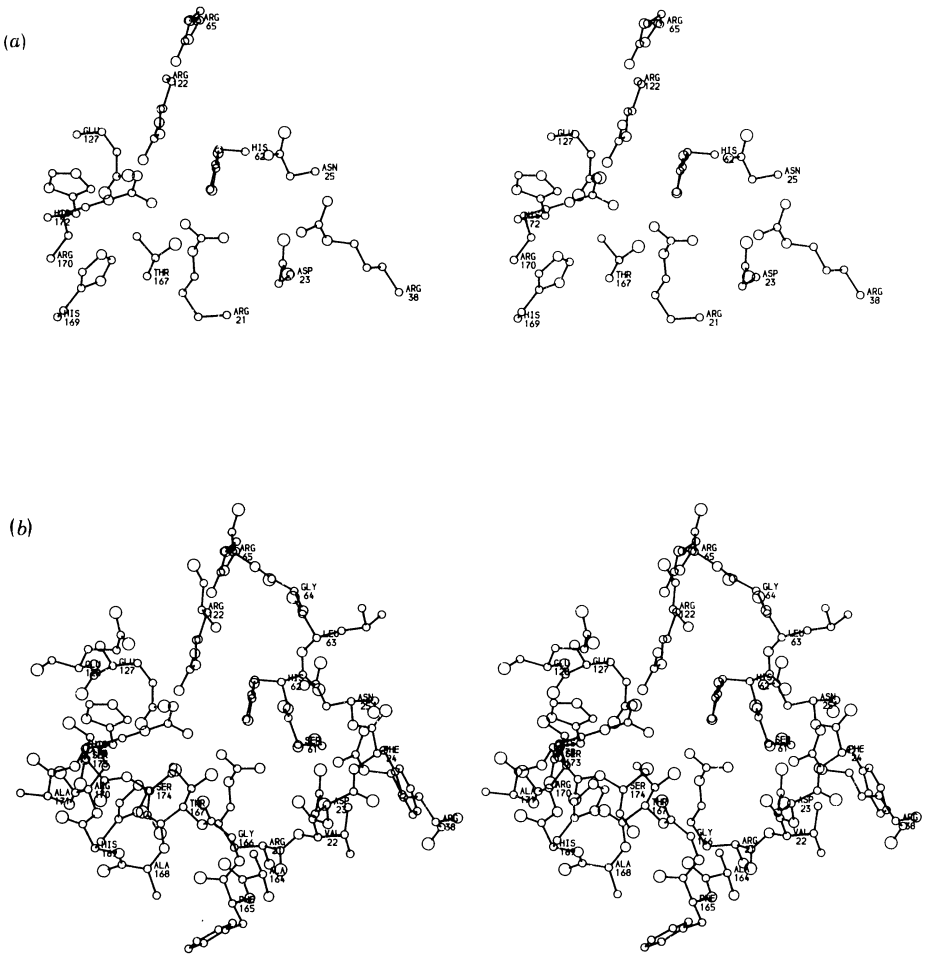

FIGURE 6. The cluster of basic residues in the face of the N-terminal domain shown as a stereo diagram: (*a*) side chains only; (*b*) the environment of the residues.

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Whereas the protein conformational changes that accompany ADP or ATP binding to phosphoglycerate kinase are slight and local, difference maps of 3-phosphoglycerate binding are dominated by features corresponding to conformational changes that encompass the whole enzyme. Although these conformational movements have yet to be fully analysed, it seems probable that they correspond to a change in the relative orientation of the two domains. Among the numerous difference features corresponding to these conformational changes, it has proved difficult to identify with certainty any specific feature as corresponding to the bound 3-phosphoglycerate. The most plausible feature is situated on that part of the N-domain that faces the C-domain and its bound nucleotide, and is located between two arginines, residues 38 and 170. These two arginines are part of a very striking cluster of basic residues that occupies this part of the N-domain, which includes in all five arginine and three histidine residues. This cluster is shown as a stereo diagram in figure 6. No other part of the protein surface has such a concentration of basic, functional residues and it seems reasonable therefore to identify this cluster with the anion-binding site noted by Scopes (1978*b*) and Wrobel & Stinson (1978). In view of the fact that the ADP/ATP site appears totally unsuitable for phosphoglycerate binding, containing neither functional groups capable of interacting with the phosphoglycerate nor basic residues that are surely necessary to bring the two highly negatively charged substrates together, there seems no alternative to this site on the N-domain being the phosphoglycerate site. Although the evidence that this site is the phosphoglycerate site is not yet definitive, the site has all the appropriate characteristics of such a binding site, and probably of the site of the catalytic apparatus as well. For example, Arg 38 and Arg 170 face one another some 6–8 Å apart, in a very suitable arrangement to bind an extended phosphoglycerate moiety, which is about 6 Å long with negative charges at each end. If positioned between these two arginines the phosphoglycerate would be able to interact with a number of other functional residues, such as Asp 23, His 62, which unusually is hydrogen-bonded to a buried arginine, residue 21, Arg 122, His 172 and Thr 167. In contrast to this rich variety of charged and functional residues, the protein surface near the γ-phosphate of the bound ATP is barren of functional groups, which with the exception of one leucine are exclusively glycines as can be seen in figure 4.

Mechanism of action

If we are correct about the location of the nucleotide and phosphoglycerate binding sites, a most interesting implication occurs. As situated on the native enzyme, the γ-phosphate of ATP is 12–15 Å from the phosphoglycerate, when the mechanism implied by Webb & Trentham (1980) requires this distance to be 3–5 Å (Mildvan 1979). The most obvious way to reconcile these facts, and at the same time to explain the extraordinary bidomain structure of PGK, is to introduce a hinge bending motion in which the two domains, together with their bound substrates, are brought together in the catalytic reaction. This logical imperative of the single-crystal X-ray studies has received strong independent support from solution X-ray studies of PGK. Pickover *et al.* (1979) have observed that the radius of gyration of the PGK molecule decreases by 1.09 Å on the formation of the ternary substrate complex. This change is consistent with, and has been interpreted as, a substrate-induced conformational change that brings the two domains together in precisely the way demanded by the single-crystal X-ray studies.

Although it is obvious that the bidomain structure of PGK will permit such a structural change, the possible nature of the hinge is of some interest. There is no evidence in the X-ray

maps of any real, or incipient, flexibility in the domain interface, which seems to imply a specific hinge point that is triggered in some way by substrate binding. The domain interface is composed of two helices, helix 7 and helix 14, that are involved in a more or less parallel helix–helix interaction. Analyses of such interactions (Cohen 1980) suggest that they nucleate around the normal to the helix axes, which can be regarded as notional internal degree of freedom. An analysis has been carried out of the effect of treating the contact normal of helix 7 and helix 14 as the hinge (Blake *et al.* 1981). This model treated the two domains as rigid bodies that were rotated about the helix normal, while the distance between the two substrates and the number of internal bad contacts introduced by the rotation were monitored. This model is shown in figure 7. It was found that a rotation of 20–25° about the helix normal reduced the

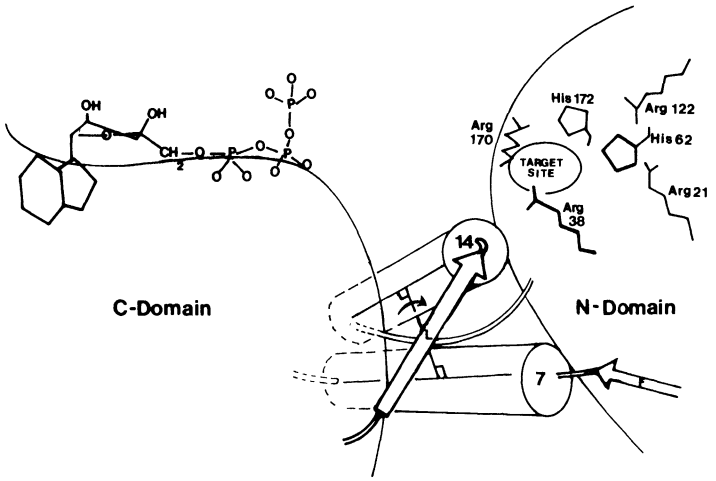

FIGURE 7. A schematic diagram illustrating the proposed 'hinge' in PGK along with the relative positions of the ATP and the target site.

distance between the substrates from 12 Å to about 5 Å, a suitable distance for a dissociative mechanism (Mildvan 1979), at the same time introducing unsatisfactorily close interactions between only six amino acid residues, all associated with a single segment of polypeptide chain. The change in the radius of gyration calculated from this model was consistent with the change observed in the low-angle X-ray studies by Pickover *et al.* (1979). Stereo drawings of the PGK molecule before and after this conformational change are shown in figure 8.

CONCLUSIONS

The studies of phosphoglycerate kinase by a variety of techniques have now brought together the unusual molecular structure of the enzyme, its anomalous kinetic behaviour and its biochemical requirements into a single coherent picture, centred on the hinge-bending conformational change. As Koshland (1959), and later Anderson *et al.* (1979), have pointed out, kinases have a particular need to exclude water from their active sites. If their active sites were contiguous like most other enzymes the binding of ATP would result in the catalytic attack of a water molecule on the γ-phosphate, with the result that the enzymes would function as ATPases. With enzymes such as PGK that catalyse high-energy phosphoryl transfer, attack by

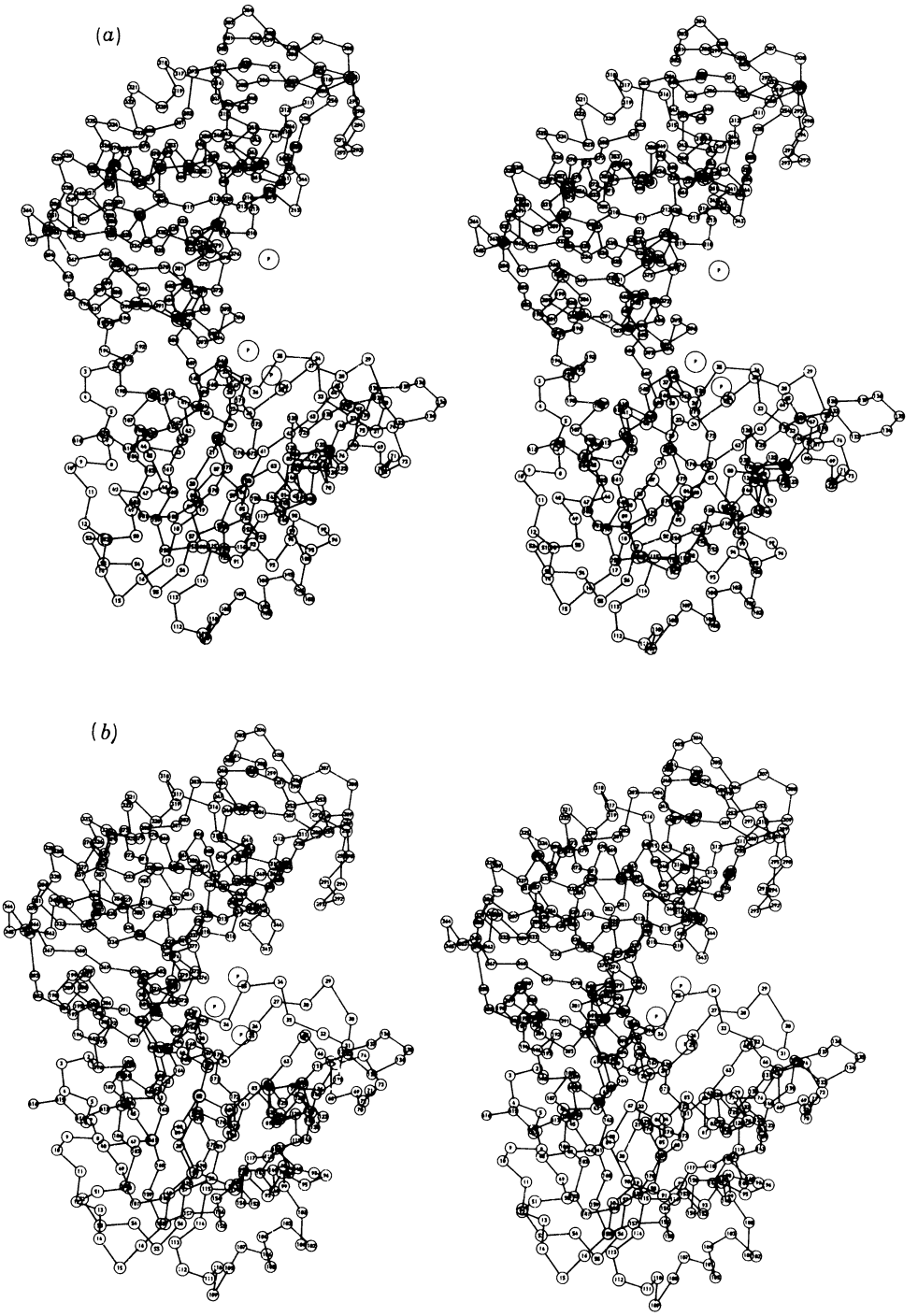

FIGURE 8. Stereo drawings of the α-carbon atoms in PGK: (*a*) before rotation, (*b*) after rotation of 0.45 rad. The large open circles labelled P are the phosphate positions: the upper is the β-phosphate in ADP; the lower two correspond to the 1-phosphate of the 1,3-bisphosphoglycerate moiety in two orientations.

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water molecules could also be promoted on the second substrate as well. One solution to this problem, the one apparently used by PGK, hexokinase and perhaps other kinases, is to divide the active site and the substrate binding sites into two separate parts, which only come together when *both* substrates are bound and at the same time squeeze the water out of the active site. Thus not only is the water prevented from interfering with the catalytic process, its exclusion will result in a low dielectric environment needed for the nucleophilic attack of the phosphoryl acceptor on the phosphoryl group to be transferred. As in hexokinase (Anderson *et al.* 1978), adenylate kinase (Pai *et al.* 1977) and phosphofructokinase (Evans & Hudson 1979), phosphoglycerate kinase has an aspartic acid residue, 23, that could be hydrogen-bonded to the phosphoryl acceptor and which may function as a general base catalyst. Removal of the Asp and the phosphoryl acceptor from the solvent will enhance their nucleophilicity. The molecular structure of the enzyme is clearly appropriate to this kind of behaviour, and it is tempting to believe that the substrate and anion activation effects are also associated effects.

In broad outline, the catalytic process in phosphoglycerate kinase seems to be understood. What is needed now is confirmation of these events and a more detailed knowledge of the catalytic mechanism. From the point of view of X-ray analysis, structures of the PGK–3-phosphoglycerate complex and the ternary (folded) complex are required. The former is under way at present, while an attempt to obtain crystals of the ternary complex has been made by salting out mixtures of PGK, ATP and 3-phosphoglycerate with non-ionic solvents. This has resulted in the formation of large plates belonging to the orthorhombic system that are suitable for X-ray analysis. If these crystals contain the folded form of the enzyme, their analysis could be extremely fruitful.

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