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# Structure of the ADP Complex of the 3-Phosphoglycerate Kinase from *Bacillus stearothermophilus* at 1.65 Å

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## Abstract

The structure of the ADP complex of the enzyme 3-phosphoglycerate kinase (PGK, E.C. 2.7.2.3) from *Bacillus stearothermophilus* NCA-1503 has been determined by the method of molecular replacement. The structure has been refined to an $R$ factor of 0.16 for all data between 10.0 and 1.65 Å resolution, using data collected on the Hendrix-Lentfer imaging plate at the EMBL outstation in Hamburg. The r.m.s. deviations from stereochemical ideality are 0.010 and 0.011 Å for bonds and planes, respectively. Although crystallized in the presence of the nucleotide product MgATP, the high-resolution structure reveals the bound nucleotide to be MgADP reflecting the low intrinsic ATPase activity of PGK. Although the two domains of this enzyme are found to be some 4.5° closer together than is found in the yeast and horse-muscle apo-enzyme structures, this structure represents the 'open' rather than the 'closed', catalytically competent form, of the enzyme.

## Introduction

Phosphoglycerate kinase is the monomeric glycolytic enzyme, $M_r \simeq 43\,000$, responsible for the production of the first ATP molecule during glycolysis,

1,3-bisphosphoglycerate + MgADP ⇆
3-phosphoglycerate + MgATP.

PGK has been extensively studied at both the sequence and structural levels. Over 32 PGK sequences are now known (for a review see Watson & Littlechild, 1990) with about 12% of the residues conserved among all species. The structures of three PGK's have been determined, those from horse muscle (Banks *et al.*, 1979), *Saccharomyces cerevisiae* (Watson *et al.*, 1982) and pig muscle (Harlos, Vas & Blake, 1992). In addition, as part of a study on the nature of thermal stability in proteins, we have recently determined the structure of the PGK from *Bacillus stearothermophilus* (Davies, Gamblin, Littlechild & Watson, 1993).

The structure of PGK consists of two similar domains (Fig. 1), linked by a flexible 'hinge' region, which are believed to undergo a conformation change relative to each other during catalysis. Direct crystallographic evidence for this 'hinge-bending' movement has not been forthcoming. It has been suggested that the sugar phosphate binding site is located in a 'basic patch' on the N-terminal domain, some 10 Å away from the nucleotide site on the surface of the C-terminal domain (Banks *et al.*, 1979; Harlos, Vas & Blake, 1992). Thus, a conformational change must occur if the two substrates are to become sufficiently close for a phosphoryl-transfer event to take place. Evidence for this change in domain orientation during catalysis came initially from small-angle scattering (Pickover, McKay, Engelman & Steitz, 1979). Although the errors in this study were large, the work has since been extended to medium-angle measurements (Ptitsyn, Pavlov, Sinev & Timchenko, 1986; Sinev, Razgulyaev, Vas, Timchenko & Ptitsyn, 1989). These authors report a change in the radius of gyration upon binding of substrates with the greatest change occurring upon ternary-complex formation. A change in sedimentation coefficient upon substrate binding has been reported by Roustan and colleagues (Roustan, Fattoum, Jeanneau & Pradel, 1980) but this work has now been questioned as not only unrepeatable but also theoretically infeasible (Ptitsyn *et al.*, 1986; Mas &

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Resplandor, 1988; Gamblin, 1988). Further evidence that the active form of the enzyme must be a more 'closed' conformation than that seen in the crystal structures determined so far comes from the fact that a lysine in the N-terminal domain may be labelled with a pyridoxal analogue of adenosine (Ladine & Cross, 1991). The 3-phosphoglycerate (3-PGA) complex of pig-muscle PGK reported by Harlos, Vas & Blake (1992) does indeed show a domain orientation some 7° 'closer' than is found in the horse-muscle and yeast enzyme structures but this still leaves the sugar phosphate substrate displaced from the nucleotide-binding site by a large distance.

In order to understand the nature of the proposed conformational change of PGK we have determined the structure of the nucleotide complex of PGK from *B. stearothermophilus* crystallized from polyethylene glycol (PEG). The PGK from *B. stearothermophilus* is a monomer consisting of 394 amino acids (Davies, Littlechild, Watson & Hall, 1991). The structure is interesting in that it shows increased thermal stability compared to the other PGK's of known structure (Davies *et al.*, 1993) and also because it is the first known structure of a prokaryotic PGK. This is the highest resolution structure of a PGK and is also the first structure of this enzyme which has been co-crystallized with its nucleotide substrate.

## Crystallization, data collection and processing

Crystals of *B. stearothermophilus* PGK were grown as described previously (Davies, Gamblin, Littlechild & Watson, 1992). The $NaN_3$ concentration was 0.01 m*M* and not 0.1 m*M* as given, incorrectly, in Davies *et al.* (1992). The crystals grown were monoclinic space group $P2_1$, with cell dimensions $a$ = 40.5, $b$ = 74.0, $c$ = 68.5 Å and $\beta$ = 99.8°. There was one molecule of PGK in the asymmetric unit, giving rise to a packing density of 2.40 $Å^3\ Da^{-1}$ and an approximate solvent content of 50% (Matthews, 1968).

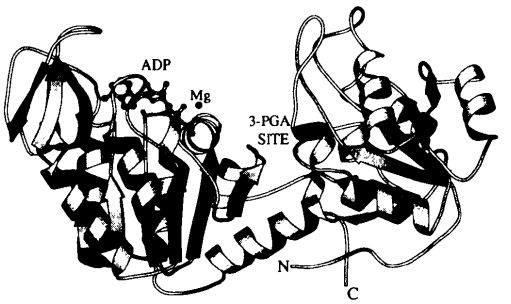


Fig. 1. Ribbon diagram, drawn with the *MOLSCRIPT* program (Kraulis, 1990), showing the structure of *B. stearothermophilus* PGK. The nucleotide substrate atoms are shown in 'ball-and-stick' representation. The 3-PGA site, on the N-terminal domain, as determined by Harlos, Vas & Blake (1992) for the pig-muscle enzyme, is indicated.

Table 1. *Data statistics for PGK from Bacillus stearothermophilus*

| $D_{min}$ (Å) | *R* factor | $R_{Cum}$ | Completeness (%) |
|---|---|---|---|
| 5.99 | 0.046 | 0.046 | 82 |
| 3.97 | 0.047 | 0.046 | 90 |
| 3.17 | 0.054 | 0.050 | 95 |
| 2.72 | 0.064 | 0.053 | 96 |
| 2.42 | 0.077 | 0.056 | 95 |
| 2.20 | 0.038 | 0.056 | 94 |
| 2.03 | 0.042 | 0.055 | 95 |
| 1.90 | 0.054 | 0.055 | 96 |
| 1.79 | 0.068 | 0.056 | 95 |
| 1.65 | 0.126 | 0.056 | 93 |
| Totals | .. | 0.056 | 94 |

Data of 3.3 Å resolution were collected, for the purposes of solving the molecular replacement rotation and translation functions. Reflections were recorded on photographic film using beamline PX 9.6 at the Daresbury synchrotron radiation facility. Further data were collected on beamlines X11 and X31 at the EMBL Hamburg outstation. Data of 1.65 Å resolution were collected from a single crystal, cooled to 279 K, on the imaging-plate scanner (Hendrix & Lentfer, unpublished work) installed on the beamline X11 operating in parasitic user mode at a wavelength of 1.009 Å. In order to record the low-resolution terms, saturated in the first data, low-resolution data (2.5 Å) were collected from a second crystal cooled to 279 K, using the imaging plate on beamline X31. 90° of data were recorded from each of these crystals. Both were mounted with the $a^*$ axis roughly parallel to the camera spindle axis but were offset sufficiently to prevent serious and systematic loss of data in the 'blind region'. Data were processed essentially as outlined in Dauter, Terry, Witzel & Wilson (1990) using a modified version of the *MOSFLM* suite of programs (Leslie, Brick & Wonacott, 1986). All further computation involved the use of the *CCP*4 suite of programs (SERC Daresbury Laboratory, 1979) unless otherwise stated.

The final data set contained 119 169 observations of 44 754 unique data. 135 observations were rejected during the data reduction representing 0.11% of the total number of observations. The completeness of the data and the internal consistency, as judged by the merging *R* factor for common reflections, are shown in Table 1. The final $R_{merge}$ $[\Sigma_{hkl}|I - I_{mean}|/\Sigma_{hkl}(I)]$ was 0.056 for the entire data set consisting of 94% of the unique possible data between 10.0 and 1.65 Å and was only 0.156 for the highest resolution shell (1.7–1.65 Å). The data have an overall $B$ value of 19 $Å^2$ as judged from the Wilson plot (Wilson, 1942).

## Molecular replacement

Rotation parameters were determined with the *MERLOT* suite of programs (Fitzgerald, 1988). When this study was undertaken two possible search models were avail-

able; the structure of PGK from horse muscle (Banks *et al.*, 1979) and the structure of the yeast enzyme (Watson *et al.*, 1982). Both were tried in the molecular replacement calculations but the horse-muscle structure proved to be more suitable, presumably due to its more advanced state of refinement. Recent calculations with the refined yeast PGK structure (Watson, unpublished) give almost identical results to those obtained with the horse-muscle structure.

The model PGK structure was placed in an artificial *P*1 unit cell with dimensions $a = b = c = 100$ Å and full rotation space maps calculated using the fast rotation function (Crowther, 1972). Data between 8 and 4 Å were used, together with an outer radius of Patterson integration of 25 Å and a 2.5° grid spacing. A single, unambiguous, answer was obtained with an r.m.s. peak height of 6.9. This orientation was refined using the Lattman rotation function (Lattman & Love, 1972) with a 0.5° grid spacing over a 10 × 10 × 10° volume around the original answer.

The translation parameters for the correctly oriented model were determined using an *R*-factor search procedure with the *CCP*4 program *RSEARCH*. The plane perpendicular to the $b^*$ axis was searched using data between 10.0 and 5.0 Å with a 1 Å grid spacing. An *R* factor of 0.49 was obtained for the correct answer, compared with a value of 0.52 for the next highest peak.

The most favourable solution gave rise to a molecular packing arrangement, shown in Fig. 2, which had few steric violations apart from those involving the 'nose' loop region of the eukaryotic search molecule which was known to be absent from *B. stearothermophilus* PGK (Davies *et al.*, 1991). To confirm the molecular replacement solution, phases calculated using the molecular replacement model were used to calculate a difference Fourier with data collected from a crystal soaked in mercury acetate (10 m*M*, 24 h). A single peak was observed in the difference Fourier map and the position of this peak was 4.1 Å from the S atom of the single cysteine residue of the *B. stearothermophilus* PGK. It agreed with the mercury position determined from the difference Patterson map.

## Refinement

### *Rigid-body refinement*

The correct sequence was incorporated into the initial molecular replacement model and the structure was refined as two rigid bodies, corresponding to the N- and C-terminal domains, using the program *CORELS* (Sussmann, Holbrook, Church & Kim, 1977). Initial refinement with 15–7 Å data proved unstable, presumably as a result of the low number of experimental observations, and refinement was eventually achieved with six cycles of constrained rigid-body refinement using data between 10.0 and 5.0 Å. The C-terminal domain moved some 4.5° relative to the N-terminal domain with a concomitant decrease in the *R* factor from 0.51 to 0.47, Fig. 3.

Initial refinement used the *X-PLOR* program (Brünger, 1988) with data in the range 10–3.3 Å. The *R* factor fell to below 0.17 with good model stereochemistry and over 95% of the non-glycine residues had acceptable conformational angles ($\varphi$, $\psi$). Subsequent refinement at higher resolution showed that this refinement, at 3.3 Å resolution, had a deleterious effect on the true quality of the structure. Many atoms moved 'impressive' distances (> 10 Å) away from their true position. Clearly molecular dynamics refinement at intermediate resolutions would appear to be inappropriate, especially if a thorough check of how well the initial model fits the density has not been employed. Refinement, with data between 10.0 and 1.65 Å, continued using conventional stereochemically restrained techniques (Konnert & Hendrickson, 1981) and molecular dynamics methods. At no point was a '$\sigma$ cutoff' applied to the intensities used. The initial model contained many large errors, particularly in the N-terminal domain adjacent to the 'nose' loop (residues 128–140 in the yeast enzyme). The density associated with these sections of the molecule was discontinuous and the refinement required considerable manual intervention. At the start of the refinement procedure, using the model that had been poorly refined at 3.3 Å resolution, up to 35% of the structure (consisting mainly of the 'nose', other loop regions and the nucleotide

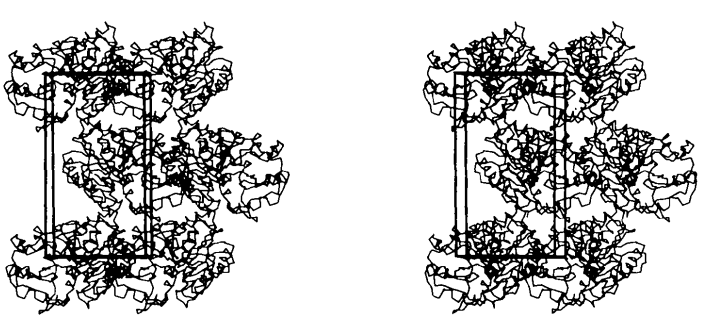

Fig. 2. Stereo diagram illustrating the molecular packing of *B. stearothermophilus* PGK in the crystal. The view is down the $c^*$ axis. The main chain is indicated as linked $C_\alpha$ atoms.

phosphate binding helix XII) had to be omitted to help avoid phase bias. These regions were omitted as they were both poorly defined in the initial omit maps and also because the quality of the density, in the $2F_o - F_c$ maps was highly correlated with which residues were included in the phase calculation. At a particular point, corresponding to a drop in the crystallographic *R* factor (10–1.65 Å) below 0.29, the quality of the entire map improved dramatically and further refinement proved straightforward. Unrestrained *B*-factor refinement was performed with the *CCP*4 program *SFALL*. The difficulty of refinement from the starting horse PGK model structure can be judged from the large differences between the initial and final structures. The r.m.s. deviation in the positions of the equivalent $C_\alpha$ atoms of the horse (after *CORELS* refinement) and *B. stearothermophilus* PGK structures was 1.89 Å. A plot of the main-chain r.m.s. deviations between the two structures indicated differences as large as 10 Å in some areas, Fig. 4. The final electron density of the *B. stearothermophilus* enzyme was of extremely high quality, however, allowing features such as the single *cis*-proline residue to be clearly identified, Fig. 5. The water structure was clearly visible and the nucleotide substrate, included in the crystallization media as MgATP, was shown to be MgADP (see below). The nucleotide substrate was included in the later stages of the refinement (with the crystallographic *R* factor below 0.24) when the density for the whole of the substrate was clear and unambiguous. The conformation of the furanose ring was not restrained during the refinement.

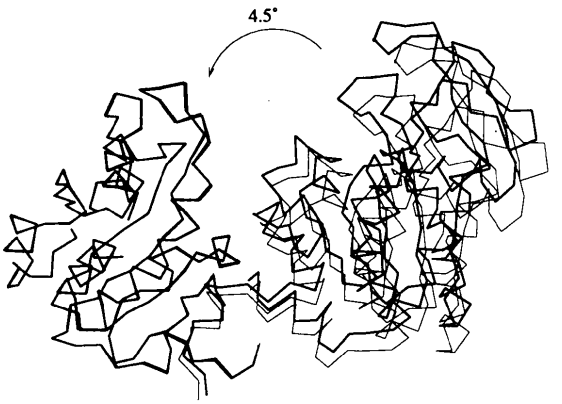


Fig. 3. Change in domain orientation from the starting model achieved with six cycles of constrained rigid-body refinement using the *CORELS* program (Sussman *et al.*, 1977).

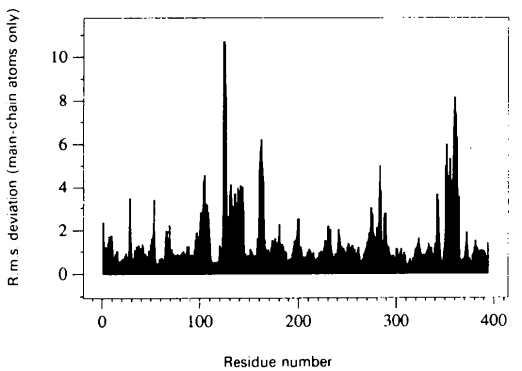


Fig. 4. Root-mean-square deviations in the positions of the main-chain atoms, as a function of residue, for the superposed *B. stearothermophilus* and horse PGK structures. The structures were superposed (N- and C-terminal domains independently) using the *QUANTA* program (Molecular Simulations Inc., USA).

### *Quality of the final model structure*

The final model structure has a crystallograpic *R* factor of 0.16 for the 45 193 reflections between 10.0 and 1.65 Å, constituting 94% of the unique data. The model contains 3009 protein atoms, 265 solvent water molecules, a single bound magnesium ion and 27 nucleotide substrate atoms. Distinct double conformation has only been modelled for a single side chain, that of the single cysteine residue Cys18. The final model shows deviations from stereochemical ideality of 0.010 Å for bonds, 0.011 Å for planes and 0.029 Å for bond angles (1-3 bonding distances). With the exception of four residues adjacent to the 'left-handed turn' region of the Ramachandran plot, all non-glycine residues have conformational angles ($\varphi$, $\psi$) within the allowed regions

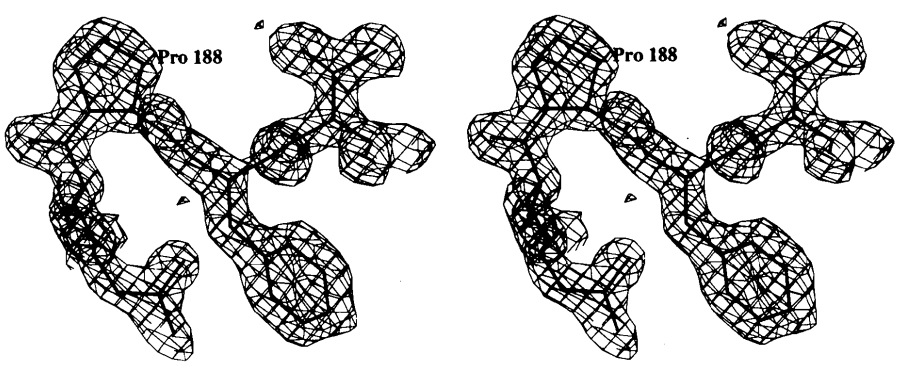


Fig. 5. The single *cis*-proline residue (Pro188) in the *B. stearothermophilus* PGK. This *cis*-proline is a totally conserved residue at what may be considered to be the end of the N-terminal domain (see text). The map shown is a $2F_{obs} - F_{calc}$ synthesis contoured at a level of 0.55 e Å$^{-3}$. The figure was drawn with the *QUANTA* program.

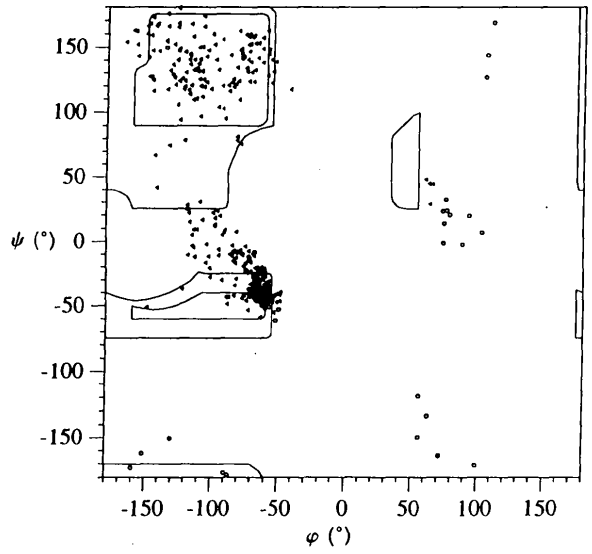


Fig. 6. Ramachandran plot (Ramakrishnan & Ramachandran, 1965) for the refined *B. stearothermophilus* PGK. Energetically favourable regions for the values of $\varphi$ and $\psi$ are indicated. Glycine residues are indicated by circles and non-glycine residues by triangles. Four non-glycine residues may be found adjacent to the 'left-handed turn' region of the plot with $\varphi \simeq 50°$ and $\psi \simeq 30°$. These are residues Asn2, Gln28, Lys64 and Asn126.

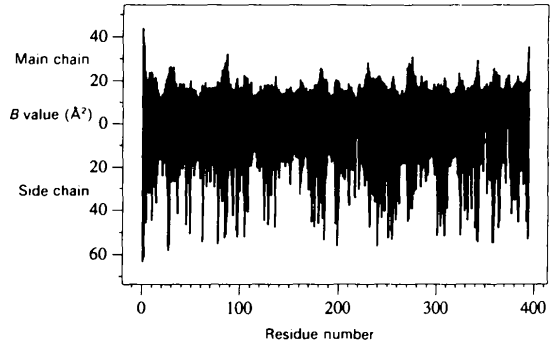


Fig. 7. Average main-chain and side-chain $B$ values (Å$^2$) for refined *B. stearothermophilus* PGK as a function of residue number after unrestrained $B$-value refinement.

(Ramakrishnan & Ramachandran, 1965), Fig. 6. The plot of $R$ factor against resolution (Luzzati, 1952), or the $\sigma_a$ method of Read (1986), gives an upper estimate of coordinate error of approximately 0.18 Å. The average main-chain and side-chain $B$ values are plotted, as a function of residue number, in Fig. 7. Both the N and C termini show rather high $B$ values (in excess of 45 Å$^2$) but apart from these termini no regions of the polypeptide chain, including helix 'XII' (horse PGK nomenclature) disordered in the horse-muscle enzyme structure, show excessive mobility.

## Discussion

### *Description of the structure*

The overall topology of the *B. stearothermophilus* PGK is very similar to that described for the enzyme from horse muscle (Banks *et al.*, 1979; Blake & Rice, 1981), yeast (Watson *et al.*, 1982) and pig muscle (Harlos, Vas & Blake, 1992). The structure consists of two similar-sized domains connected by a 'waist' region. Each domain consists of a core of six parallel $\beta$-strands surrounded by $\alpha$-helices, similar to the NAD-binding domain of dehydrogenases (Buehner, Ford, Moras, Olsen & Rossmann, 1973). The ten C-terminal residues cross back to the N-terminal domain, *via* a short helical segment leaving the $C_\alpha$ atoms of the N- and C-terminal residues only 8 Å apart. The structures of the two domains are shown in Fig. 1. The strand order in the N-terminal domain (residues 1–188) is CDBAEF, different to that found in the NAD-binding domain of dehydrogenases. The first helix is broken into two segments by a proline residue (Pro42, *B. stearothermophilus* numbering). This residue is conserved in all known prokaryotic and eukaryotic PGK sequences (Watson & Littlechild, 1990). The effect of this change of direction in the helix is to allow residues, such as Arg36, to interact with the 3-PGA substrate in the 'basic patch'.

This 'basic-patch' region, on the N-terminal domain, is known to be the binding site for general anions and

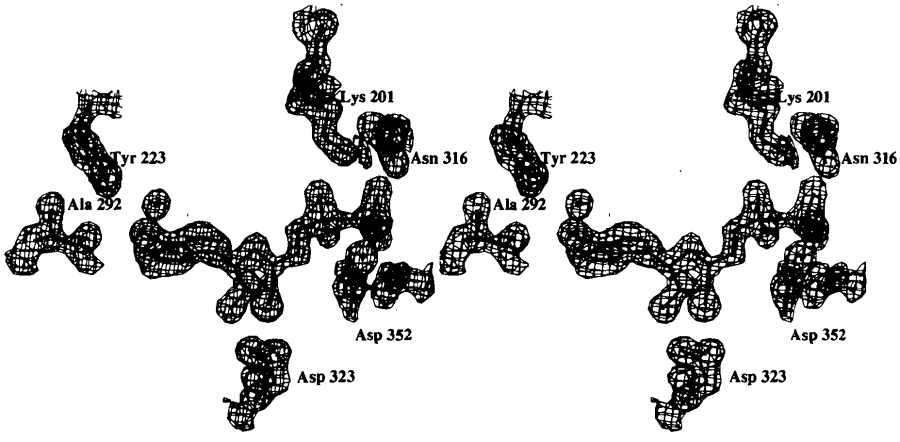


Fig. 8. Residues in the vicinity of the nucleotide substrate-binding site on the C-terminus of the enzyme. The map is a $2F_{obs} - F_{calc}$ synthesis contoured at a level of 0.7 e Å$^{-3}$. The ribose ring has an unusual O4′-*endo* conformation which allows the O2′ and O3′ hydroxyl groups to make optimum hydrogen bonds with the side chain of Asp323.

for the sugar substrate 3-PGA. Crystallographic studies on the pig-muscle enzyme have identified the residues responsible for interaction with 3-PGA in an 'open' state of the enzyme (Harlos, Vas & Blake, 1992). Kinetic studies on mutants of the yeast enzyme have helped to elucidate the role of these residues in binding and catalysis (Barber, Gamblin, Watson & Littlechild, 1993). The majority of the residues known to interact with 3-PGA in the pig-muscle structure are similarly located in the (3-PGA free) *B. stearothermophilus* structure. A notable exception is Arg62, corresponding to Arg65 in the pig-muscle enzyme. There is little density, beyond $C_\alpha$, for this residue in the *B. stearothermophilus* PGK structure implying that in the absence of the sugar substrate it exhibits an intrinsic flexibility. In the pig-muscle structure this residue interacts with the sugar substrate *via* an unusual contact in which the guanidinium group of the arginine side chain lies in a plane above the phosphate group of the sugar. The 3-PGA binding site of the *B. stearothermophilus* PGK is occupied by a number of discrete water molecules.

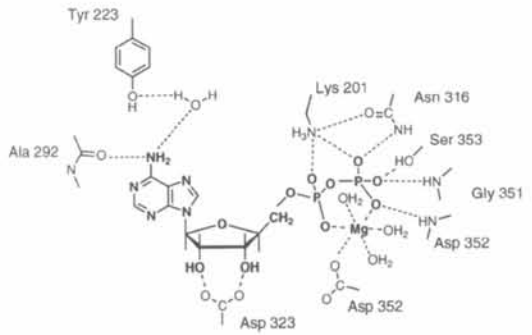


Fig. 9. Schematic diagram illustrating the main interactions of the nucleotide substrate, ADP, with the enzyme.

The N-terminal domain may be considered to terminate at the end of the interdomain helix at residue 188. Interestingly, this totally conserved residue is the only *cis*-proline found in the structure, Fig. 5. The interconversion of *cis-trans* isomers of proline is known to have a high activation energy barrier and is thus a rate-limiting step in protein folding (Brandts, Halvorson & Brennan, 1975). The existence of the single *cis*-proline in PGK at the domain interface thus suggests a method of allowing independent folding of the two domains prior to the correct prolyl isomerization event. The C-terminal domain has a strand topology (IHGMNO: *B. stearothermophilus* PGK nomenclature) more reminiscent of that found in the NAD-binding domain of the dehydrogenases (Buehner *et al.*, 1973). In *B. stearothermophilus* PGK there is an additional sheet of three anti-parallel $\beta$-strands as in the horse-muscle enzyme and also observed in the refined structure of the yeast enzyme. The C-terminal domain is also the site of the nucleotide binding as discussed below.

### *The nucleotide substrate-binding site*

The density for the nucleotide substrate present in the crystals clearly indicated that the nucleotide was MgADP, Figs. 8 and 9. Presumably this is due to the low intrinsic phosphatase activity of PGK. It is likely that over the course of the 14 d crystallization, the MgATP was hydrolysed to MgADP. Many of the nucleotide substrate-enzyme interactions are as described for yeast PGK following Mn-adenylimidodiphosphate (MnAMP-PNP) soaking experiments described in Watson *et al.*

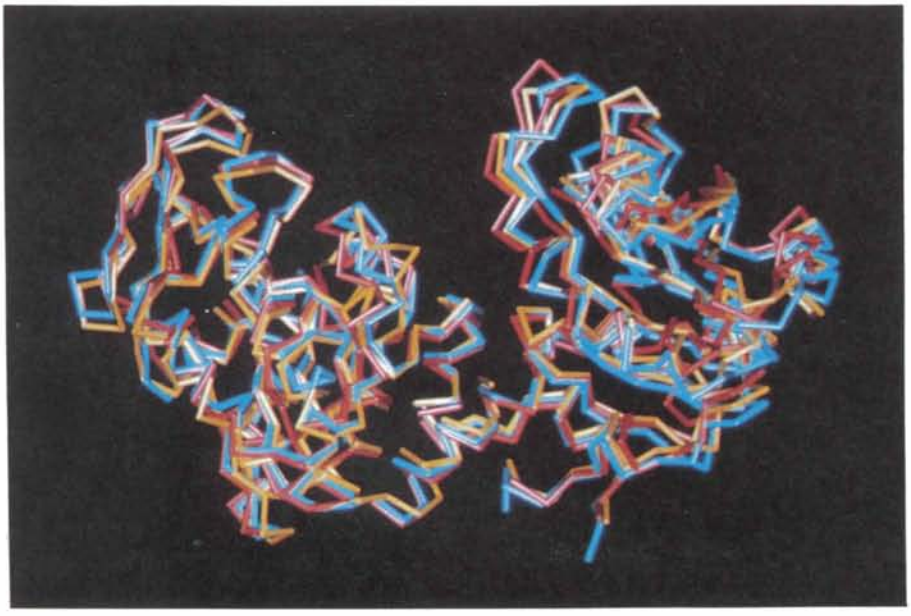

Fig. 10. Superposition of the horse-muscle (yellow), yeast (blue) and *B. stearothermophilus* (red) PGK structures. Despite different biological origin, crystallization conditions and the state of ligation, the structures are extremely similar.

(1982). The adenine ring is bound in a hydrophobic pocket on the surface of the C terminus of the enzyme. The adenine ring is flanked by Val321 and Leu237. Surprisingly, the $NH_2$ group associated with the adenine ring forms only weak hydrogen bonds with the protein molecule, mainly *via* the carbonyl O atom of Ala292 but also with an additional interaction mediated *via* a water to the hydroxyl group of Tyr223. The absence of a direct interaction between the adenine amino group and the protein explains the observations that both ITP and GTP are also quite reasonable substrates for *B. stearothermophilus* PGK (Suzuki & Imahori, 1974). It is significant that the ribose ring does not adopt either of the typical, and expected, C2′- or C3′-*endo* conformations. Instead it appears to be nearer an O4′-*endo* conformation. This conformation is not uncommon in small-molecule sugar structures and is only slightly less energetically favourable than the usual C2′- or C3′-*endo* conformations (Murray-Rust & Motherwell, 1978; Olson & Sussman, 1982). This ring conformation is determined by the location of the adenine ring and phosphate groups, both of which are clearly defined in the final electron-density map. In addition, it allows both the O2′ and O3′ hydroxyl groups to form excellent hydrogen bonds with the two carboxyl O atoms of Asp323.

The magnesium ion exhibits tetragonally distorted octahedral coordination. The axial ligands are a single water molecule and one of the carboxylate O atoms of Asp352. The equatorial ligation is provided by two water molecules and two phosphate O atoms, one from each of the $\alpha$- and $\beta$-phosphates. With the exception of the interaction with the aspartate, most of the coordinating atoms lie between 2.27 and 2.29 Å from the magnesium. The aspartate carboxylate O atom lies only 1.96 Å from the magnesium ion, reflecting the charged nature of this interaction.

Further interaction of the phosphate groups is provided by residues Lys201 and Asn316 for the $\alpha$- and $\beta$-phosphates, respectively. The hydroxyl group of Ser353 makes an additional hydrogen bond to the $\beta$-phosphate. Finally, the $\beta$-phosphate lies close into the amino end of an $\alpha$-helix (helix XII in the yeast nomenclature) to interact with the characteristic Gly-Gly-Gly motif where it is presumably stabilized by the $\alpha$-helix dipole (Hol, van Duijnen & Berendsen, 1978) and *via* hydrogen-bonding interactions with the main-chain amide H atoms of residues Gly351 and Asp352, Fig. 9.

*Concluding remarks*

The superposition of the horse-muscle, yeast and *B. stearothermophilus* PGK structures is shown in Fig. 10. The three structures are extremely similar despite differing crystallization conditions, state of ligation and biological origin. 4–5° differences in the relative domain orientations can be observed but none of these structures represent a fully 'closed', and therefore catalytically competent, form of the enzyme. The binary 3-PGA pig-muscle PGK complex of Harlos, Vas & Blake (1992) again shows an approximate 7° closing of the domains but also leaves the sugar substrate too distant from the ATP site for phosphoryl transfer to occur. The conclusions drawn as to the relevance of these small domain orientation differences must be treated with some caution. The small 'closings' observed in the binary pig-muscle structure and the binary MgADP structure, reported here, are only of the order of the difference observed between the unliganded structures from different origins such as horse muscle and yeast. It is clear that a series of structures in various states of ligation from a single species is required before the nature of the ligand-induced domain closure can be rigorously dissected. Further experiments are underway with the *B. stearothermophilus* enzyme to address these questions.

The refined coordinates and observed structure-factor amplitudes for the *B. stearothermophilus* 3-phosphoglycerate kinase have been deposited with the Protein Data Bank.*

The authors would like to thank Eleanor Dodson and Johan Turkenburg (University of York) for help with the initial *X-PLOR* refinement of the *B. stearothermophilus* PGK and for many useful discussions. GJD was the recipient of an EMBO short-term fellowship for data collection at the EMBL Hamburg outstation. This work was funded, in part, by the SERC protein engineering initiative.

* Atomic coordinates and structure factors have been deposited with the Protein Data Bank, Brookhaven National Laboratory. Free copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England (Supplementary Publication No. SUP 370105). A list of deposited data is given at the end of this issue.

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