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Crystal structures of putative phosphoglycerate kinases from *B. anthracis* and *C. jejuni*

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Abstract

Phosphoglycerate kinase (PGK) is indispensable during glycolysis for anaerobic glucose degradation and energy generation. Here we present comprehensive structure analysis of two putative PGKs from *Bacillus anthracis* str. Sterne and *Campylobacter jejuni* in the context of their structural homologs. They are the first PGKs from pathogenic bacteria reported in the Protein Data Bank. The crystal structure of PGK from *Bacillus anthracis* str. Sterne (BaPGK) has been determined at 1.68 Å while the structure of PGK from *Campylobacter jejuni* (CjPGK) has been determined at 2.14 Å resolution. The proteins' monomers are composed of two domains, each containing a Rossmann fold, hinged together by a helix which can be used to adjust the relative position between two domains. It is also shown that *apo*-forms, of both BaPGK and CjPGK adopt open conformations as compared to the substrate and ATP bound forms of PGK from other species.

Keywords

Carbohydrate degradation; Glycolysis; PGK; Phosphoglycerate kinase; Pathogenic organism; Anthrax; Gastroenteritis; Guillain-Barré syndrome; Rossmann fold; *Bacillus anthracis*; *Campylobacter jejuni*

Introduction

The impetus for the molecular investigation of proteins from pathogenic organisms is to inch towards understanding and abating their pathogenic effects. *Bacillus anthracis* is a Gram-positive bacterium which is best known as the causative agent for anthrax [1] and has been prioritized as one of the most lethal human bacteria pathogens (category A) by the National Institute of Allergy and Infectious Diseases (NIAID). *Campylobacter jejuni* is a NIAID Category B food- and water-borne Gram-negative pathogen, and the leading cause gastroenteritis in humans. Certain subspecies have also been associated with Guillain-Barré syndrome (GBS) [2]. There are currently three strains of *Bacillus anthracis* (str. Ames, str. ‘Ames Ancestor’, and str. Sterne) and one subspecies of *Campylobacter jejuni* (subsp. *jejuni* NCTC 11168) under investigation as model organisms for the Center of Structural Genomics for Infectious Disease (CSGID) [3]. The TargetDB [4] currently contains one entry for phosphoglycerate kinase (PGK) from *Bacillus anthracis* str. Sterne (IDP04624) and one from *Bacillus anthracis* str. Ames (OPTIC6700) that encode the same protein, which we shall refer to as BaPGK. The putative PGK from *Campylobacter jejuni* subsp. *jejuni* NCTC 11168 (IDP90717) will be referred to as CjPGK.

Phosphoglycerate kinase is a magnesium-dependent kinase involved in the glycolysis regulation pathway which anaerobically degrades glucose into pyruvate. It controls the first ATP-generating step in glycolysis and thus is a crucial target for disrupting anaerobic metabolism in pathogens [5]. PGK is responsible for transferring the phosphate from 1,3-bisphosphoglycerate to ADP, forming ATP for energy storage and preparing 3-phosphoglycerate (3PG) for further degradation [6]. The reaction catalyzed by PGK ($1,3\text{-bisphosphoglycerate}^{4-} + \text{ADP-Mg}^{2+} \rightarrow 3\text{-phosphoglycerate}^{3-} + \text{ATP-Mg}^{2+}$) is driven by the depletion of ATP in the cell and is repressed by high concentrations of ATP (and low concentration of ADP). This reaction is reversible only in the carbon fixation process in photosynthetic organisms, where the reverse reaction is catalyzed by the same enzyme [7]. The ADP and ATP interacting with PGK are present in magnesium associated forms that neutralize their charge [8].

Since glycolysis abides in the energy generation pathway for all living organisms, PGK is present in virtually all prokaryotic and eukaryotic organisms, and is acting on exactly the same substrate. PGK is highly conserved evolutionarily and there is usually only one PGK encoded per genome [5]. However, in mammalian cells, a second sperm-specific isoform PGK2 exists in addition to the PGK1 found in somatic cells [9].

The two domains in the PGK structure are linked by an α -helix hinge. The phosphoglycerate binding site is in the N-terminal domain and the ADP binding site is located in the C-terminal domain. PGK is a good model enzyme for the study of protein flexibility due to its

well documented mechanism of domain closure during catalysis [10]. The two domains of PGK adopt a closed, catalytically active, conformation upon phosphoglycerate and ADP binding, but assume an open conformation in the absence of substrates [11], with a significant domain rotation of $\sim 33^\circ$ between the two conformations. The phosphoglycerate and ADP binding sites are usually more than 10 Å apart from each other in the open conformation. Upon substrate and ADP binding the active site will close in a “hinge-bending” manner to bring these two sites close enough (~ 4 Å) to each other for the phosphate transfer reaction to proceed [12, 13]. Extensive structural studies on human PGK have recently made the fine-grained illustration of the catalytic mechanism possible [14–17]. These structures include different ligand-bound states of the human PGK (3-phos-pho-D-glycerate, magnesium, ADP, and transition state analogue) in a partially- or fully-closed conformation. A recent synergistic use of SAXS and X-ray crystallography shows that PGK spends most of its time in the open, “resting” state. Binding of both substrate and nucleotide elicit domain closure which exposes a substantial hydrophobic surface that serves as the driving force in a “spring-loaded” release mechanism for domain opening [15].

The ligand-free BaPGK and CjPGK reported in this study both adapt an open conformation, and they are the first PGKs from pathogenic bacteria reported in the PDB. The BaPGK crystal structure was refined at 1.68 Å resolution to an R factor of 17.6% and R_{free} of 21.3%, while the second one was refined at 2.14 Å to an R factor of 19.0% and R_{free} of 23.7%. Compared to the closest structural homolog (PGK from *Bacillus stearothermophilus*), they share 77 and 49% sequence identity respectively. Both crystal structures are monomeric and very similar to other PGK structures determined to date. One of the crystal packing interfaces in the BaPGK structure includes small molecules from the crystallization solution which might assist in crystal growth.

Materials and methods

Cloning, expression and purification

The open reading frames (ORFs) of *pgk* from *Bacillus anthracis* and *Campylobacter jejuni* were amplified by polymerase chain reaction (PCR). These genes were cloned into pMCSG7 plasmids using ligation independent cloning [18–20]. This expression vector encodes an N-terminal tobacco etch virus protease-cleavable hexahistidine tag (MHHHHHHSSGVDLG TENLYFQ/SNA) [21]. Both *pgk* genes were overexpressed in *E. coli* BL21-Codon-Plus(DE3)-RIPL. Cells were grown in selenomethionine media at 37°C, induced with isopropyl- β -D-1-thiogalacto-pyranoside and grown at 20°C afterwards. Harvested cells were sonicated in lysis buffer (300 mM NaCl, 50 mM HEPES pH 7.5, 5% glycerol, 0.5 mM tris(2-carboxy-ethyl)phosphine-HCl (TCEP), 5 mM imidazole, 0.5 mM phenylmethylsulfonyl fluoride and 1 mM benzamidine), clarified by centrifugation, and the supernatant was applied to a nickel chelate affinity resin (Ni-NTA, Qiagen). The resin was washed with wash buffer (300 mM NaCl, 50 mM HEPES pH 7.5, 5% glycerol, 0.5 mM TCEP, 30 mM imidazole) and the tagged protein was eluted using elution buffer (300 mM NaCl, 50 mM HEPES pH 7.5, 5% glycerol, 0.5 mM TCEP, 250 mM imidazole).

The hexahistidine tag was cleaved by addition of 1 mg of recombinant His-tagged TEV protease per 15 mg of eluted protein. EDTA, TCEP and arginine were added to the final

concentrations of 1, 0.5 mM and 0.2 M respectively. The cleavage was performed at 4°C overnight and continued during dialysis into cleavage buffer (300 mM NaCl, 50 mM HEPES pH 7.5, 0.5 mM TCEP). Proteins were separated from TEV protease by running over nickel-chelating resin, dialyzed into crystallization buffer (300 mM NaCl 10 mM HEPES pH 7.5, 5% glycerol, 0.5 mM TCEP), and further concentrated to 40 mg/mL (BaPGK) and 5.3 mg/mL (CjPGK).

Crystallization, data collection and processing

Crystallization was performed using hanging drop vapor diffusion at 20°C. Drops were composed of 1 µL of the reservoir solution with equal volume of the concentrated protein sample. BaPGK (40 mg/mL) was mixed with reservoir solution containing 25% w/v PEG3350, 0.2 M NaCl, 0.1 M Bis-Tris pH5.5 and 2% w/v 1,6-hexanediol. CjPGK (5.3 mg/mL) was mixed with reservoir solution containing 20% w/v PEG3350 and 0.2 M K citrate pH 7. Immediately after harvesting, crystals were transferred into cryoprotectant solution (paratone-N) and flash cooled in liquid nitrogen.

Data were collected at the Life Science Collaborative Access Team (LS-CAT) at the Advanced Photon Source (Argonne National Laboratory, Argonne, IL, USA). Data collection at beamline 21ID-F was controlled by MD-2 software from European Molecular Biology Laboratory (EMBL) with LS-CAT developed extensions. Diffraction data were collected at a single wavelength at 100 K and processed with *HKL-2000* [22].

Structure determination, refinement and validation

Structures of the selenomethionine substituted BaPGK and CjPGK were determined using single-wavelength anomalous diffraction (SAD) method. The initial model for BaPGK was built with *HKL-3000* [23]. *HKL-3000* is integrated with *SHELXD*, *SHELXE* [24], *MLPHARE* [25], *DM* [26], *APR/wARP* [27], *CCP4* [28], *SOLVE* [29], *RESOLVE* [30, 31] and *REFMAC5* [32]. The initial phases for CjPGK was determined with *PHENIX* [33], with subsequent model building in *ARP/wARP* [27]. Manual model building and refinement were performed in *COOT* [34]. *TLSMD* web server [35] was used for definition of the TLS groups (six TLS groups for BaPGK and two for CjPGK) during TLS refinement. *MOLPROBITY* [36] and *ADIT* [37] were used for structure validation. Data collection, structure determination, and refinement statistics are summarized in Table 1.

The coordinates and experimental structure factors were deposited to the PDB with the accession code 3uwd for BaPGK and 3q3v for CjPGK. Diffraction images can be obtained through CSGID webpage. (http://www.csgid.org/csgid/pages/diffraction_images).

Structure analysis and visualization

The sequence conservation scores were calculated by the *ConSurf* server [38]. Multiple sequence alignments for conservation scores calculations were done by *ConSurf* server using 150 of the most similar proteins which were restrained by sequence identity in the range of 35–95% and *PSI-BLAST* cutoff E-value 1.0E–04. *PITA* [39] and *PISA* [40] servers were used for prediction of quaternary structure. *NEIGHBORHOOD* database was used to assist the analysis of 3D motifs [41]. An in-house program was used for the preparation of Table 1

from CSGID database (<http://www.csgid.org/>). *PyMOL* [42] was used for the generation of figures of the structure. *TopDrawPrep* and *TopDraw* [43] were used to prepare the topology diagrams.

Structure alignment with PGK homologues

The structure of BaPGK and CjPGK were superimposed with other known PGK structures using the *align* function in *Py-MOL* for both the C-terminal and N-terminal domains separately. The N-terminal domain rotation angle was used to evaluate the conformational changes, and was measured by fixing the BaPGK C-terminal domain orientation with other PGK homologues, and rotating the N-terminal domain of BaPGK to align with the N-terminal domain of each PGK homologue. The distances and angles between domains were used to evaluate the degree of active site opening. The distance was measured between the Arg62 side chain amino group and the C-terminal domain's Asp200 side chain carboxyl group of the superposed BaPGK. The same angle was measured in all PGK structures using the superposed BaPGK as a reference to ensure that side chains used for distance calculations have exactly the same orientation. The angle was measured as Arg62N_{H2}-Val177C_α-Asp200O_{5δ1} (Table 2).

Results and discussion

Protein structure and domains description

The results of domain comparison in both cases clearly confirm that both presented structures belong to PGK family (cd00318: E-value 2.12e-148; COG0126: E-value 2.85e-160; pfam00162: E-value 0). CjPGK shares a sequence identity of 46% with BaPGK, and its crystal structure exhibits a RMSD of 1.6 Å for 344 C-α atoms after superposition with BaPGK. Even though both PGKs are in their “open” conformation, the relative orientations of the two domains are different. The structure alignment can achieve a RMSD of 0.8 Å for 143 C-α atoms in the N-terminal domain and 0.7 Å for 160 C-α atoms in the N-terminal domain if the superposition is done for each domain individually (Table 2).

The structure of BaPGK contains one molecule per asymmetric unit (Fig. 1A). The final model has 329 water molecules, five chloride ions, one magnesium ion, one Bis-Tris molecule and one unknown ligand molecule (Table 1). The six-water coordinated magnesium ion is observed at a non-physiological location. Two residues in the loop that covers the substrate binding site (Lys27, Glu28) cannot be modeled due to poor electron density, presumably caused by high flexibility. A single residue discrepancy with the UniProt sequence (T190A) is observed in the structure and was confirmed by DNA sequencing. The structure of CjPGK contains two molecules per asymmetric unit (Fig. 1B). The final model has 324 water molecules, two sulfate ions, four potassium ions, four formic acid residues, and two PEG fragments (Table 1).

The overview of both BaPGK and CjPGK structures are presented on Fig. 1. They both adopt very similar overall structures in the open conformation (Figs. 2B, 3A). Both PGK monomers are comprised of two domains with a linking helix referred to as the hinge region (α-helix 11 in Fig. 1C; BaPGK: Gly168-Asn184, CjPGK: Gly172-Ile186, marked in red on

Fig. 2A). Each domain is composed of a central β -sheet sandwiched by two α -helical layers (Rossmann fold) (Fig. 1A). A pre-proline peptide bond is observed in the *cis* conformation at the border between the N-terminal domain and the C-terminal domain (BaPGK: Arg187-Prol88, CjPGK: Arg191-Prol92) and is believed to be conserved [44]. The C-terminal domain ends with a helix (BaPGK: Leu385-Cys390, CjPGK: Leu388-Ala393, also marked in red on Fig. 2A) followed by a C-terminal loop (α -helix 23 in Fig. 1C; BaPGK: Leu391-Lys394, CjPGK: Leu394-Ser396). This C-terminal loop is in contact with the N-terminal domain, and comes into the proximity of Met1-Asn2-Lys3-Lys4 (BaPGK) and Met1-Ser2-Asp3-Ile4-Ile5 (CjPGK) thus is considered as a second “hinge” [13].

The topologies of both PGK structures within each domain are almost identical, with the only exception being helix 7, which is a seven-residue α -helix in BaPGK but a five-residues 3_{10} helix in CjPGK (Fig. 1C). The N-terminal domain is composed of a central β -sheet of six parallel strands (A1–A6) surrounded by two α -helices in the front (helices 2 and 4) and three α -helices at the back (helices 5, 7, 8) plus five 3_{10} helices (helices 1, 3, 6, 9, 10) (Fig. 1). The C-terminal domain is formed by a major six parallel stranded central β -sheet (B1–B6), and a minor β -sheet of three anti-parallel strands (C1–C3). These two β -sheets are surrounded by three α -helices in the front (helices 17, 19, 20) and four α -helices at the back (helices 12, 13, 15, 22) plus four 3_{10} helices (helices 14, 16, 18 21).

Two highly conserved motifs around a potential salt bridge

Phosphoglycerate kinase proteins with substrate and nucleotide bound adopt a completely “closed” conformation in the active state as compared to the “open”, resting conformation observed when either reactant is absent. Upon transition to the active, fully closed state, a salt bridge is formed between Arg62 from the N-terminal domain on top of the 3PG binding site and Asp200 from the C-terminal domain on top of the nucleotide binding site [45]. This creates a cover to fully close the cleft and form a catalytically active environment. The BaPGK and CjPGK structures determined here are both an *apo*-forms, without either substrate or nucleotide bound. They adopt a typical “open” conformation with 15 and 13 Å distance between the end of the side chains of Arg62 and Asp200 respectively, which gives an opening angle of 37° and 31° relative to the middle of the linker helix on the right.

DALI [46] and *FATCAT* [47] searches using the BaPGK structure identifies more than a dozen PGK structures, including those from bacteria/archaea (thermophilic bacteria/archaea) and eukaryotic organisms (human, mouse, pig, horse, yeast, malaria parasite, and sleeping sickness parasite) (Table 2). While most other known structures share a 40–50% sequence identity with BaPGK, the closest homologue to *Bacillus anthracis* PGK with a known structure is *Bacillus stearothermophilus* PGK (PDB code: 1php) [44] which shares 75% sequence identity (Fig. 3A). Using this subset of PGK structures, two strongly evolutionarily preserved motifs around the salt-bridge forming residues Asp62 and Asp200 in BaPGK were identified. Namely, “SHLGRPK” that contains Arg62 (BaPGK) and “GG(A)KV(K)DKX” (where X is usually Ile or Leu) that contains Asp200 (BaPGK) (Fig. 2C). Interestingly, although Arg62 is conserved in CjPGK, a glycine is present in the position corresponding to Asp200. The lack of Asp200 may influence the aforementioned domain closure in CjPGK. It is possible that CjPGK structure adopts another way of

stabilizing its closed conformation for the enzymatic reaction or does not possess PGK activity. Further biochemical data is needed to validate either hypotheses.

Conformation changes in PGK between “resting” and “active” states

The set of PGK structures identified in the *DALI* and *FAT-CAT* searches described in the previous section is analyzed further in terms of conformational changes. The RMSD values from the *DALI* search do not account for different conformation states amongst these structures. These differences can be quite significant and cannot be neglected. Therefore N-terminal and C-terminal domains were superposed separately. PGK structures adopt an almost identical fold amongst all species. For both C-terminal and N-terminal domains, more than 70–80% of the homologues can be aligned with an RMSD of around 1 Å (C-terminal domain) or less (N-terminal domain), which is no more than the flexibility between different structures of the same protein. The difference in length of structure alignment is mostly due to missing residues in incomplete structure models. Exceptionally high RMSDs are observed for only two structures (2pgk: RMSD = 2.6–2.9 Å, 3pgk: RMSD = 1.7–2.3 Å) that were determined almost three decades ago at relatively low resolution. These differences may reflect the quality of the structure and/or the tools available for structure refinement/validation at that time, rather than species-specific variations among PGK structures.

The degree of cleft opening can be evaluated using the distance between Arg62 and Asp200. In a structure with a typical “open” conformation this distance is between 13 and 18 Å, and in structures with a typical “closed” conformation it is less than 5–6 Å (Table 2). The actual distance between Arg62 and Asp200 will be closer to 3 Å upon domain closure due to side chain rearrangements that accompany salt bridge formation. The angle of cleft opening, which is measured between Arg62 and Asp200 relative to the middle of the linker helix, is around 40° and 12° for “open” and “closed” conformation, respectively. The rotating motion is also measured for BaPGK N-terminal domain while immobilizing C-terminal domain, and embodies a rotation in the range of 30°–40° upon switching to the “closed” conformation (Table 2). Some domain rotations which are not in the direction of domain closing are also observed in the range of 10°–20°. Conformational changes within the substrate binding site associated with substrate binding are shown by superimposing BaPGK with three other partially closed or fully closed PGK structures (Fig. 3). During the transition from the fully open to fully closed conformation, the substrate 3PG associated with the N-terminal domain is brought closer to, and eventually contacts, the phosphate moiety from the nucleotide, exemplifying a significant domain rotation of around 37°.

Substrate binding site

The residues along the cleft between the two domains are highly conserved, while other surface exposed regions are highly variable (Fig. 4). The conserved region corresponds well with the substrate binding site in the N-terminal domain and the ADP-Mg/ATP-Mg binding site in the C-terminal domain. The buried residues of the linker helix and its surrounding helices are also highly conserved. These residues form the hydrophobic patch which will be partially exposed to the solvent upon substrate binding, thereby making the closed conformation less energetically favorable, which has to be compensated by strong

interactions in the active site during the catalytic process. This agrees with the proposed spring-loaded release mechanism proposed in a recent study [15].

The PGK substrate and ATP binding site is well-defined and resides in the cleft between the two domains [16]. Compared to human PGK in closed conformation (PDB code: 2wzc) the BaPGK model exhibits some different side chain conformations (Arg62, Asp200, Lys197) and main chain conformation (Gly349–351) within its potential binding environment (Fig. 5). Arg62 and Asp200 presumably adopt different orientations upon forming a salt bridge in the closed conformation. Lys197 and Arg36 which form part of the coordination sphere for the transferred phosphate have highly exposed side chains compared to the corresponding residues in the active state of human PGK. The substrate binding site is highly positively charged, with Arg62, Arg118, Arg151 coming close to form a phosphate clamp that precisely defines the location for the phosphate moiety from 3PG. Two main-chain alternative conformations (Gly349-Gly350-Gly351) are also modeled at the beginning of helix Gly351-Phe360. This motif forms the base below the phosphate moiety of the putative nucleotide binding site and is likely to be stabilized upon nucleotide binding.

Crystal packing interfaces

The active biological unit of PGK is most likely a monomer. Analysis of crystal contacts for both BaPGK and CjPGK structures reveal three crystal packing interfaces with buried interface area approximately 600 Å² in each structure. *PISA* result indicates that all three interfaces in CjPGK structure are not stable, but for BaPGK structure the interface with symmetry operator $(-x, y, -z)$ in the same cell is predicted to be stable in solution and could be treated as a putative dimer interface. It involves four hydrogen bonds and six salt bridges between two C-terminal domains. Modeled small molecules, including a Bis-Tris molecule and an unidentified carbohydrate-like molecule, contribute to this crystal interface. However, when the Bis-Tris and the unidentified molecule from crystallization condition are removed from the model, this interface is no longer predicted to be stable by *PISA*. Although this interface does not appear to be biologically relevant, the small molecules may play an important role for crystal formation.

Conclusion

We present herein two new crystal structures of putative PGKs from pathogenic organisms. They both conform to the canonical PGK fold. Their overall structures are consistent with an “open” conformation typical for other PGKs in *apo* form. Comparison with other substrate and ATP bound structures highlights the flexibility of the hinge between two domains, reaffirming that the cleft is closing upon substrate and/or ATP binding, with both domains moving toward each other. Some of the residues around the substrate binding site are less ordered due to the lack of substrate, as compared to the active state seen in other structures. Nevertheless, as shown in our surface conservation analysis, these residues are still highly conserved. We have also identified two PGK specific motifs not described previously. These two motifs contain residues Arg62 and Asp200 that form a salt bridge participating in the mechanism of domain movements upon substrate binding.

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Abbreviations

BaPGK	Phosphoglycerate kinase from <i>Bacillus anthracis</i>
CjPGK	Phosphoglycerate kinase from <i>Campylobacter jejuni</i>
PGK	Phosphoglycerate kinase
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
3PG	3-Phospho-D-glycerate
SAXS	Small-angle X-ray scattering
ORF	Open reading frame
HEPES	2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
TCEP	Tris(2-carboxyethyl)phosphine-HCl
TEV	Tobacco etch virus
EDTA	Ethylenediaminetetraacetic acid
PEG	Polyethylene glycol
LS-CAT	Life Science Collaborative Access Team
EMBL	European Molecular Biology Laboratory
SAD	Single-wavelength anomalous diffraction
TLS	Translation/libration/screw
PDB	Protein Databank
RMSD	Root mean square deviation
GBS	Guillain-Barré syndrome
NIAID	National Institute of Allergy and Infectious Diseases
CSGID	Center of Structural Genomics for Infectious Disease

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Fig. 1. Wall-eyed stereo view of both pathogenic PGKs in cartoon representation. **A** The BaPGK structure (3uwd) with helices colored in *green*, β -sheets in *red* and loops in *gray* **B** The CjPGK structure (3q3v) with helices colored in *light green*, β -sheets in *red* and loops in *gray* **C** The topology of both PGKs with the same color coding as in **A**

Fig. 2.

Atlas of PGK architecture. **A** Presents crystal structure of BaPGK, N-terminal domain is coloured in *light green*, the hinge region in *red* and C-terminal domain in *dark green*. The substrate (3PG) binding site in the N-terminal domain and nucleotide (ADP/ ATP) binding site in the C-terminal domain are indicated by *arrows*. The side chains of Arg62 and Asp200 that form a salt bridge upon closing are shown in stick representation. The distance between the ends of the side chains (15 Å) and the opening angle (37°) as defined in the text are shown using *black lines*. **B** A superposition of CjPGK (*gray*) and BaPGK (coloured as on **A**),

with two highly conserved motifs *circled*. C A multiple sequence alignment of the highly conserved motifs indicated in B, with Arg62 and Asp200 marked by an *asterisk*. Sequences used for multiple alignment are ordered the same way as in Table 2, with BaPGK and CjPGK highlighted in a *red boxed* frame

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Fig. 3. Conformational change of PGK during catalysis. Structures have been superimposed on the C-terminal domain of BaPGK and separated into **A**(*apo*-PGK in *open* forms) and **B**(*semi*-close and *fully*-closed forms) for clarity. **A** BaPGK in green CjPGK in red, and PGK from *Bacillus stearothermophilus* in yellow (PDB code: 1php). **B** PGK from *Homo sapiens* in light blue (PDB code: 3c39), PGK from *Thermotoga maritima* in navy blue (PDB code:lvpe), and *fully*-closed PGK from *Homo sapiens* in orange (PDB code: 2wzc). All *semi*-close and *fully*-closed forms of PGKs are shown with nucleotide/substrate or their analogues (in *ball* and *stick* representation) bound in the structures

Fig. 4. Sequence conservation analysis for BaPGK (A) and CjPGK (B). Conservation score mapped on both surface and cartoon representations are colored according to *ConSurf* conservation score (*dark red*: the most conserved to *cyan*: variable)

Fig. 5. Side chain conformations around the active site that are important for substrate binding are shown after independently superposing the N-terminal (*magenta*) and C-terminal (*light blue*) domains of BaPGK on the closed conformation of human PGK (*white*) with 3-phosphoglyceric acid (3PG), trifluoroaluminate (ALF, a phosphate analogue), and ADP bound (PDB code: 2wzc). Side chains are shown in *stick* representation for residues that form the salt bridge (Arg62, Asp200), the phosphate clamp (Arg62, Arg118, Arg151) and coordinates phosphate (Arg36, Lys197). The Gly349-Gly350-Gly351 loop below the phosphate analogue that has a main chain modelled with alternative conformations is shown in *green*

Table 1

Summary of data collection, structure determination, and refinement statistics. Data for the highest-resolution shell are given in parentheses. Bijvoet pairs were merged for R_{merge} calculation. Ramachandran plot statistics were calculated by *MOLPROBITY*

PDB accession code	3UWD	3Q3V
Data collection		
Wavelength (Å)	0.9790	0.9787
Space group	C 1 2 1	P 21 21 2
Unit-cell parameters (Å, °)	a= 119.7, b = 45.3, c = 68.6, $\alpha = 90.00$, $\beta = 97.95$, $\gamma = 90.00$	a= 110.3, b= 165.2, c = 54.6, $\alpha = 90.00$, $\beta = 90.00$, $\gamma = 90.00$
Resolution (Å)	50.00-1.68 (1.69-1.68)	30.00-2.15 (2.19-2.15)
No. of unique reflections	41,651 (996)	55,178
Completeness (%)	99.9 (99.4)	99.3 (97.4)
Redundancy	4.1 (4.0)	5.0 (4.9)
Mean I/σ	24.38 (2.2)	17.97 (2.4)
Molecules in asymmetric unit	1	2
Matthews coefficient (Å ³ Da ⁻¹)	2.15	2.80
Solvent content (%)	42.77	56.09
R_{merge} (%)	3.9 (39.8)	10.3 (63.7)
Structure refinement		
Resolution range (Å)	50.00-1.68 (1.73-1.68)	91.72-2.15 (2.20-2.15)
$R_{\text{work}}/R_{\text{free}}$ (%)	17.6/21.3	19.0/23.7
No. of residues/protein atoms	392/3,047	786/6,012
No. of water atoms	329	324
Average B factors (Å ²)		
Main chain	25.2	18.5
Side chains	28.7	21.3
Overall	25.9	24.0
Waters	29.0	25.2
Ligands/ion	22.8	34.4
Ramachandran plot (%)		
Most favoured	98.97	98.30
Allowed	1.03	1.70
Disallowed	0.00	0.00
RMS deviations		
Bond lengths (Å)	0.013	0.015
Bond angles (°)	1.59	1.42

Table 2

Summary of different conformations for known PGK structures compared to BaPGK

Complex status	Organism	pdbid [seq. ident. (%)]	Ligand ^a	C-term. RMSD ^c	N-term. RMSD ^c	N-term. rotation	Distance (Å) (R62-D200)	Angle (°) (R62-V177-D200)
apo-PGK	<i>B. anthracis</i>	3uw d (100)		0.0 (170)	0.0 (184)	0	15	37
	<i>T. thermophilus</i>	lv6 s (50)		0.9 (160)	0.7 (122)	12	15	35
	<i>T. caldophilus</i>	2ie8 (50)		0.9 (159)	0.7 (127)	18	12	28
	<i>S. cerevisiae</i>	lfw8 (49)		1.1 (159)	0.6 (132)	12	15	36
	<i>M. musculus</i>	2p9q (48)		1.2 (150)	0.8 (138)	4	15	35
	<i>E. coli</i>	lzm r (47)		1.0 (163)	0.8 (127)	1	16	39
	<i>E. caballus</i>	2pgk (47)		2.6 (157)	2.9 (148)	5	14	32
	<i>C. jejuni</i>	3q3v (46)		0.7 (160)	0.8 (143)	15	13	31
	<i>G. stearoherm</i>	lph p (75)	ADP	0.5 (159)	0.4 (154)	3	15	36
	<i>P. falciparum</i>	lltk (50)	AMP	1.1 (159)	0.9 (141)	5	17	41
Binary: PGK, nucleotide	<i>T. brucei</i>	16pk (48)	BIS ^b	0.8 (153)	0.6 (140)	17	14	33
	<i>S. scrofa</i>	lvjc, lvjd (47)	ATP	1.1 (162)	0.7 (132)	5	15	36
	<i>H. sapiens</i>	2zgv (47)	ADP	1.0 (150)	0.7 (133)	6	15	35
		3c3b (47)	CDP	1.1 (152)	0.9 (134)	17	13	31
	<i>M. musculus</i>	2p9t (48)	3PG	1.2 (164)	0.8 (140)	16	14	33
	<i>H. sapiens</i>	2xe6, 3c39 (47)	3PG	1.1 (163)	0.8 (135)	14-23	12-14	29-33
	<i>P. horikoshii</i>	2cun (37)	3PG	2.5 (140)	0.8 (127)	20	11	26
	<i>S. cerevisiae</i>	3pgk (49)	3PG, ATP	2.3 (147)	1.7 (131)	9	18	44
Tertiary: PGK, substrate, nucleotide ^d	<i>M. musculus</i>	2paa (48)	3PG, ATP	1.1 (160)	0.7 (139)	4	15	36
	<i>T. brucei</i>	13pk (48)	3PG, ADP	0.9 (156)	0.7 (141)	33	6	13
	<i>S. scrofa</i>	lhdi (47)	3PG, AMP	1.1 (161)	0.9 (137)	7	15	35
	<i>H. sapiens</i>	2xe7, 3c3a, 3c3c, 2x13 (47)	3PG, ADP/CDP	1.0-1.3 (152-164)	0.8-0.9 (133-135)	15-23, 37 (2xe7)	12-13, 6 (2xe7)	29-31, 13 (2xe7)
Tertiary: PGK, 3PG, ADP/ATP/Pi analogue ^b	<i>T. maritima</i>	lvpe (59)	3PG, ANP	0.9 (160)	0.6 (148)	29	8	19
	<i>S. cerevisiae</i>	lpgg (49)	3PG, MAP	1.1 (161)	0.6 (137)	7	16	37

Complex status	Organism	pdbid [seq. ident. (%)]	Ligand ^a	C-term. RMSD ^c	N-term. RMSD ^c	N-term. rotation	Distance (Å) (R62-D200)	Angle (°) (R62-V177-D200)
	<i>S. scrofa</i>	1kfo (47)	3PG, ACP	1.1 (162)	0.9 (135)	9	15	35
	<i>H. sapiens</i>	2x14, 2ybe, 2y3i, 2xe8(47)	3PG, LA8(+ALF)/ACP	1.0–1.3 (160–166)	0.8–1.0 (133–142)	37–38, 14(2xe8)	5–6, 14(2xe8)	12–13, 32(2xe8)
		2wzb, 2wzc, 2wzd (47)	3PG, ADP, Pi analogue ^b	1.3 (159–164)	0.9 (134–136)	37–38	5–6	12–13

RMSD to BaPGK is calculated for C-terminal (Pro188-Thr371) and N-terminal (Met1-Leu170) domains individually. The rotation angle is defined by the rotation necessary to superpose N-terminal domains after previously superposing C-terminal domains. The distance is measured between the NH2 atom of Arg62 from the superposed N-terminal domain and the Oδ1 atom of Asp200 from the fixed C-terminal domain. The angle is measured as Arg62NH2-Vall77 Cα-Asp200Oδ1, while Vall77 is at the center of the connecting hinge helix between N-terminal and C-terminal domains. Both distance and angle are used to indicate the cleft opening

^a 3-Letter ligand code from PDB is used, only substrate and nucleotide cofactors are listed, magnesium or other metal cofactors are not indicated

^b ADP analogue: LA8 (L-adenosine-5'-diphosphate); ATP analogues: ACP (phosphomethylphosphonic acid-adenylate ester), ANP (phosphoaminophosphonic acid-adenylate ester), MAP (magnesium-5'-adenylyl-imido-triphosphate), BIS (1,1,5,5-tetrafluorophosphopentylphosphonic acid-adenylate ester; Pi (phosphate) analogues: MGF (trifluoromagnesium), ALF (trifluoroaluminate), AF3 (aluminum fluoride)

^c RMSD for N-terminal and C-terminal domains are calculated for main-chain CH atoms only and in the unit of Å, the number in parenthesis indicates the number of C_α atoms being aligned for RMSD calculation. Length of alignment could be different for different models from the same species due to model completeness. If there are more than one model of the same organism and complex status, the RMSD value for higher number of aligned Ca atoms is reported

^d In glycolysis, substrate is 3PG (3-phosphoglyceric acid), and product is XI5 (1,3-bisphosphoglyceric acid); substrate and product is reversed for carbon fixation process in photosynthetic organisms