Phosphoglucose isomerase (PGI) catalyzes the reversible isomerization between D-fructose 6-phosphate and D-glucose 6-phosphate as part of the glycolytic pathway. PGI from the Archaea Pyrococcus furiosus (Pfu) was crystallized, and its structure was determined by x-ray diffraction to a 2-Å resolution. Structural comparison of this archaeal PGI with the previously solved structures of bacterial and eukaryotic PGIs reveals a completely different structure. Each subunit of the homodimeric Pfu PGI consists of a cupin domain, for which the overall structure is similar to other cupin domain-containing proteins, and includes a conserved transition metal-binding site. Biochemical data on the recombinant enzyme suggests that Fe$^{2+}$ is bound to Pfu PGI. However, as catalytic activity is not strongly influenced either by the replacement of Fe$^{2+}$ by a range of transition metals or by the presence or absence of the bound metal ion, we suggest that the metal may not be directly involved in catalysis but rather may be implicated in substrate recognition.

Phosphoglucose isomerase (EC 5.3.1.9), also referred to as glucose-6-phosphate isomerase, catalyzes the reversible isomerization of D-glucose 6-phosphate to D-fructose 6-phosphate. Comparison of available bacterial and eukaryal sequences and structures (Bacillus stearothermophilus (1), rabbit (2), and human (3)) indicates homologous PGI enzymes that consist of a homodimer of 60–70-kDa subunits. Structural and biochemical studies have established that enzymes belonging to this family are based on an α/β sandwich (3). These studies have led to the proposal of several mechanisms that are based on general acid-base catalysis and are thought to proceed via a cis-enediol intermediate in a metal-independent process involving critical glutamate, histidine, and lysine residues in the isomerization and ring-opening steps of the reaction. One such proposed mechanism from Read et al. (3) is displayed in Fig. 2b.

In the hyperthermophilic Archaea Pyrococcus furiosus (Pfu)1 the metabolism of sugars proceeds via a modified Embden-Meyerhof pathway (4). An analysis of the Pfu genome for counterparts of the bacterial and eukaryal glycolytic enzymes indicated that the archaeal glycolytic pathway is catalyzed by distantly related or unique enzymes. Phosphoglucose isomerase is one of the enzymes for which the corresponding gene could not be initially identified by comparative analyses despite the fact that significant PGI activity could be detected in the soluble fraction of disrupted Pfu cells (4, 5). Recently, a reversed genetics approach has led to the unambiguous identification of the gene encoding this enzyme in both Pfu (6, 7) and the closely related hyperthermophile Thermococcus litoralis (Tli) (8).

The identification of the gene for Pfu PGI revealed that this enzyme comprises a 21.5-kDa polypeptide of 189 amino acids, and gel filtration analysis has suggested that, like the eukaryotic and bacterial enzymes, it forms a homodimer in solution. Biochemical studies have shown that Pfu PGI shows high thermostability with an optimum temperature in excess of 90 °C (6, 7) and a half-life greater than 2 h at this temperature (6). However, sequence comparisons failed to reveal any similarity between the Pfu enzyme and PGIs of eukaryotic and bacterial origin, strongly suggesting that the PGIs from Archaea are based on a completely different three-dimensional structure. The recognition of elements of the consensus sequence belonging to the cupin domain in the amino acid sequence of Pfu PGI suggested that this enzyme belongs to the cupin superfamily (6).

In this paper we report the three-dimensional structure of Pfu PGI as a contribution toward understanding the molecular basis of its substrate specificity and catalytic mechanism. The archaeal PGI is compared with the different class of PGIs present in other domains of life and with proteins that are functionally different but share the same cupin fold.

EXPERIMENTAL PROCEDURES

Crystallization and Data Collection—PGI from Pfu was cloned, over-expressed in Escherichia coli, purified, and crystallized as described previously (37). The crystals belong to space group C2 with unit-cell dimensions a = 84.7 Å, b = 42.4 Å, c = 57.3 Å, β = 120.6°, and one molecule in the asymmetric unit. Preliminary x-ray diffraction data were collected on beamline ID29 at the European Synchrotron Radiation Facility.
tion Facility. Subsequently, three-wavelength anomalous diffraction data were collected on a single selenomethionine-incorporated crystal to a maximum resolution of 2 Å using a MAR Research CCD detector on beamline BM14 at the European Synchrotron Radiation Facility. The wavelengths were chosen near the selenium absorption edge based on a fluorescence absorption spectrum obtained from the crystal in order to maximize the \( \frac{f}{H_1} \) component (peak), to minimize the \( \frac{f}{H_2} \) component (inflection), and to maximize \( \frac{f}{H_3} \) (remote). The data for each wavelength were processed individually and scaled in such a way as to preserve the anomalous signal using the DENZO/SCALEPACK (10) package (Table II).

Soaks with metal ions were performed by adding 1 μl of 10 mM MnSO₄ or ZnSO₄ in 1.6 M sodium citrate dihydrate, pH 6.5, to the 2-μl drop containing the crystal for 4 h. The resulting soaked crystal was flash cooled in liquid nitrogen at 100 K, and data were collected to a 2.2- and 1.86-Å resolution, respectively, on a MAR345 image plate detector using platinum/nickel mirror-focused CuK\( \alpha \) x-rays produced by a Rigaku RU200 rotating anode generator. All data were processed and scaled using DENZO/SCALEPACK (10) and subsequently were handled using Collaborative Computing Project 4 software (Table II) (11).

Structure Determination—The multiwavelength anomalous dispersion data were analyzed using XPREP (Bruker AXS, Madison, WI), and the selenium substructure was determined using the “half-baked” approach as implemented in SHELXD (12). Preliminary phases were calculated using SHELXE (12), and a preliminary model was automatically built at a 2-Å resolution using the SOLVE/RESOLVE (13) package. Rounds of rebuilding and refinement using REFMAC5 (14) and TURBO-FRODO (15) resulted in a final model with \( R \) factor of 17.9%.

**Fig. 1.** The molecular structure of *Pfu* PGI. a, a representative portion of the 2-Å \( (2F_o - F_c) \) electron density map centered on Tyr\(^{99} \), contoured at 1.0σ. b, a schematic of the *Pfu* PGI monomer, colored according to secondary structure, with β strands shown in blue and α helices in red. The position of the metal ion-binding site is shown within the cupin domain as a purple sphere. c, stereo representation of the structure of the *Pfu* PGI dimer with one subunit colored according to secondary structure and the symmetry-related subunit shown in orange. β1 and β1\(^\prime\) can be seen to interact with the symmetry-related partner. The β strands that form the major contacts at the interface are labeled. d, electron density of the difference map of the manganese-soaked crystal, contoured at 5σ in the vicinity of the metal ion-binding site.
and $R_{	ext{free}}$ of 25.7% (Table III). The model contains 187 amino acid residues and 230 water molecules/subunit. The two C-terminal residues Lys188 and Lys189 have no density and were omitted. In the final model there are no non-glycine Ramachandran outliers with 91% in favored conformations (PROCHECK, Ref. 16).

A difference Fourier using coefficients ($\Delta F = F_{\text{Native}} - F_{\text{MeSoak}}$) exponentiated contained a single peak of 7 within the protein core, which was close to the side chains of His88, His90, Glu97, and His136 and was thus assigned to a manganese ion. Similarly, for the data from the crystal soaked in 10 mM ZnSO4, a single 5 peak could be seen adjacent to the same side chains and was thus assigned to a zinc ion.

Solvent-accessible areas were calculated using AREAIMOL (17) with a probe radius of 1.4 Å, and ion pairs were determined using CONTACT with a maximum probe radius of 4 Å (18). Figures were prepared using WebLab ViewerPro, Molscript (19), ClustalX (20), GeneDoc 2.6 (21), and Alscript (22).

**Elemental Analysis**—A sample of Pfu PGI was subject to multielemental analysis using inductively coupled plasma-mass spectrometry (Agilent Technologies, HP4500). The 15-mg/ml sample was diluted 20-fold in high purity water (Millipore) prior to analysis. The instrument was calibrated using an in-house multielement standard solution (19 elements, 100 µg/liter).

**RESULTS AND DISCUSSION**

**Molecular Structure of Pfu PGI**—The structure of Pfu PGI was solved to a 2 Å resolution by multiwavelength anomalous dispersion phasing using crystals of selenomethionine-containing protein. A representative section of the final electron density map is presented in Fig. 1a. Gel filtration shows that Pfu PGI forms a homodimer (37), and analysis of the crystal contacts showed that two subunits related by the crystallographic 2-fold around the $b$ axis form extensive contacts consistent with a plausible dimer.

The fold of Pfu PGI is based on a cupin domain (Fig. 1b). At the N terminus the chain starts by forming a short stretch of $\alpha$ structure ($\alpha_1$), which forms an additional strand on the edge of the cupin domain of the 2-fold-related subunit (Fig. 1c).
FIG. 3. **Active site location in Pfu PGI.**  
*a* and *b*, stereo views of superimposition demonstrating the overlay between *(a)* the monomer of Pfu PGI (orange) and the auxin-binding protein 1 monomer (green) and *(b)* the dimer of Pfu PGI (orange) and the two cupin domains in the monomer of oxalate decarboxylase (blue).  
*c* and *d*, stereo view of glucose 6-phosphate modeled into the active site of Pfu PGI in two alternative orientations described in the text: *(c)* with the phosphate group close to an arginine-lysine cluster and *(d)* with the phosphate group positioned adjacent to the transition metal center.
two sheets that form the cupin domain have 7 ($\beta_2$, $\beta_3$, $\beta_4$, $\beta_{11}$, $\beta_6$, $\beta_9$, and $\beta_1'$) and 5 ($\beta_{12}$, $\beta_5$, $\beta_{10}$, $\beta_7$, and $\beta_8$) strands, respectively, and between them a deep pocket with approximate dimensions $5 \times 8 \times 8 \text{Å}$ can be observed (Fig. 1c). Following the cupin domain, a short $\alpha$ helix ($\alpha_3$, residues 160–166) and two antiparallel $\beta$ strands ($\beta_{13}$ residues 171–175 and $\beta_{14}$ residues 182–187), which lie on the periphery of the molecule, lead to the C terminus. Overall each subunit contains 14 $\beta$ strands and 3 $\alpha$ helices (Fig. 2), which represent 47 and 12% of the total structure, respectively.

The dimer has dimensions of $\sim80 \times 40 \times 40 \text{Å}$, and the interface between the two subunits is mainly hydrophobic and comprises residues principally from $\beta$ strands $\beta_1'$, $\beta_6$, $\beta_9$, and $\beta_{11}$ and the loop regions between $\beta_{3-4}$ and $\beta_7-\beta_8$. The interface also includes the formation of a small hydrophilic cluster involving interactions between the side chains of Lys$^8$, which lies near the start of $\beta_1'$, Tyr$^{129}$, from $\beta_9$, and Glu$^{59}$ and Asp$^{65}$, from the $\beta_{3-4}$ loop (Fig. 1c). A single subunit of $\text{Pfu PGI}$ has a solvent-accessible area of 10,150 Å$^2$ (figure to the nearest 50 Å$^2$). On forming the dimer 1800 Å$^2$ (17.8%) of the subunit solvent-accessible surface is buried, with this proportion lying in the middle of the range observed for other dimeric proteins (23).

**Thermal Stability**—The cupin domain has been identified as a stable fold and has been utilized as a platform for the construction of proteins involved in stress response in bacterial and eukaryotic systems (24). Thus, for example, barley oxalate oxidase is remarkably resistant to proteolysis (25). Like many proteins from hyperthermophiles, $\text{Pfu PGI}$ displays exceptional thermal stability (6). Analysis of the structures of a number of hyperthermophilic proteins has so far failed to reveal a single mechanism that is responsible for stabilizing proteins at high temperatures. A striking structural feature found in many hyperthermophilic proteins compared with their mesophilic counterparts is the presence of networks of ion pairs (26). This correlates with the bias for a higher proportion of charged versus polar (non-charged) amino acids in the proteomes of hyperthermophilic organisms with optimum growth temperatures above 80 °C (27). In $\text{Pfu PGI}$, there are 13 ion pair interactions/monomer, of which 11 are intrasubunit and 2 are intersubunit. Overall the number of ion pairs/residue (0.07) within $\text{Pfu PGI}$ is not significantly greater than that seen in an analysis of mesophilic proteins (0.04) (Ref. 18) and is less than that observed in some hyperthermophilic proteins (0.13) (Ref. 26). However, an analysis of the nature of the ion pairs in $\text{Pfu PGI}$ shows that 5 of the ion pairs are found in two small networks involving three (Lys$^{180}$-Glu$^{175}$, Lys$^{180}$) and four (Asp$^{94}$-Arg$^{96}$-Glu$^{95}$-Lys$^{96}$) residues. The two closely related hyperthermophilic $\text{Pfu}$ and $\text{Tli}$ have optimal growth temperatures of 100 (28) and 80 °C (29), respectively, with the enzymes from the latter generally being less stable (27). The sequences of the $\text{Pfu}$ and $\text{Tli}$ PGIs are 84% identical, implying that their three-dimensional structures will be very similar. Although comparison of the sequences would suggest the 4-residue network ion pair is conserved, the substitution of Lys$^{60}$ and Lys$^{180}$ for Asn and Val, respectively, in $\text{Tli}$ PGI would lead to the loss of the 3-residue network. This network stabilizes the contact between $\beta$ strands $\beta_{13}$ and $\beta_{14}$ with the loop between $\beta_4$ and $\beta_5$ of the cupin domain, and the loss of this interaction could possibly lead to fraying of the chain at the C terminus and subsequent unfolding. However, whether the sequence differences are related to any difference in stability between these two enzymes and more generally whether the ion pairs are related to increased thermal stability are yet to be tested experimentally.

**Comparison with Other Enzymes**—The fold of $\text{Pfu PGI}$, based as it is on a cupin domain, is completely different from the $\alpha/\beta$ sandwich of the much larger subunit that forms the structure of the eukaryotic (3) and bacterial (1) PGIs. Comparison of the structure of $\text{Pfu PGI}$ with all of the proteins in the Protein Data Bank (October 2002 release) (30) using the program PROTEP (31) and the DALI Version 2.0 server (32) detected similarities with structures containing cupin domains. The best four hits were found to be against maize auxin-binding protein 1 (Protein Data Bank code 1LR5) (33), *Candida albicans* phosphomannose isomerase (Protein Data Bank code 1PME) (34), *Bacillus subtilis* oxalate decarboxylase (Protein Data Bank code 1J58) (35), and barley oxalate oxidase (Protein Data Bank code 1F2) (36) with root mean square deviations of 1.32, 1.24, 1.42, and 1.47 Å between the $\alpha$ carbons of 83, 60, 87, and 92 equivalent residues, respectively (e.g. Fig. 3, a and b). Structural overlaps between $\text{Pfu PGI}$ and these proteins primarily involve residues from the cupin domains. Only oxalate decarboxylase showed any significant sequence similarity when compared with $\text{Pfu PGI}$, with 35% identity between the two sequences. Two enzymes, phosphomannose isomerase (34) and dTDP-4-dehydrooxamnolase 3,5-epimerase (9), which catalyze reactions with similar chemistry to PGI, share a common cupin domain architecture; however, despite the similarity in chemistry, there is no significant sequence similarity between them and $\text{Pfu PGI}$. Moreover, whereas phosphomannose isomerase requires a bound divalent metal ion for catalytic activity (34),

### Table I

**Specific activity assays**

| Protein sample | Assay temperature | Specific activity |
|----------------|-------------------|------------------|
|                | °C | units/ml         |
| Native PGI     | 50 | 20               |
| Rec PGI        | 50 | 19               |
| Rec PGI        | 60 | 21               |
| Rec PGI + Zn$^{2+}$ (4 mM) | 60 | 22               |
| Rec PGI + Mn$^{2+}$ (5 mM) | 60 | 100              |
| Rec PGI        | 40 | 12               |
| Rec PGI + Fe$^{3+}$ (1 mM) | 40 | 19               |

*Rec PGI is the recombinant enzyme expressed in E. coli.*

### Table II

**Data collection statistics for the multiwavelength anomalous diffraction selenomethionine data**

| Data set | Peak ($\lambda_1$) | Inflection ($\lambda_2$) | Remote ($\lambda_3$) | Mn soak | Zn soak |
|----------|--------------------|--------------------------|---------------------|---------|---------|
| Wavelength (Å) | 0.979 ± 0.001 | 0.979 ± 0.001 | 0.898 ± 0.002 | 1.54    | 1.54    |
| Resolution (Å) | 5.0 ± 0.2 | 5.0 ± 0.2 | 5.1 ± 0.2 | 20 ± 0.2 | 20 ± 0.2 |
| Unique reflections | 10,188 (648) | 10,167 (668) | 13,143 (900) | 4809 (223) | 12,354 (771) |
| Multiplicity | 3.7 (3.2) | 3.7 (3.0) | 3.7 (3.1) | 1.5 (1.5) | 2.0 (1.5) |
| Completeness (%) | 96.5 (66.6) | 96.3 (64.2) | 96.5 (66.7) | 57.6 (42.5) | 81.5 (66.7) |
| I/|Io| > 3% (%) | 91.6 (85.0) | 90.5 (84.5) | 85.4 (72.6) | 89 (80.5) | 95.2 (97.1) |
| $R_{merge}$ | 0.064 (0.140) | 0.055 (0.121) | 0.057 (0.162) | 0.052 (0.174) | 0.037 (0.141) |

*Where appropriate the values in parentheses are for data in the highest resolution shell.

*The percentage of reflections with I/|Io| > 3%.

$R_{merge} = \frac{\sum |I - \langle I \rangle|}{\sum |I|}$, where $I$ is the integrated intensity of a given reflection.
dTDP-4-dehydrorhamnose 3,5-epimerase does not (9).

The dimeric structure of auxin-binding protein 1 closely resembles that of Pfu PG1, and dimerization of both enzymes is achieved via interactions mainly between residues belonging to the larger of the β sheets, which makes up the cupin domain. The arrangement of the dimers of auxin-binding protein 1 (33) and Pfu PG1 is also similar to that seen between the 2-fold-related subunits in the homohexamer of oxalate oxidase, the quaternary structure of which is based on six subunits arranged in D3 symmetry (36). This structural similarity extends to the interaction of the larger β sheet of the cupin domain with the N-terminal β strand from a symmetry-related subunit (β' in Pfu PG1). The disposition of the two cupin domains in the dimer of Pfu PG1 also resembles that between the two non-identical cupin domains found in the much larger monomeric bicupin enzyme phosphomannose isomerase (34) and in the hexameric bicupin oxalate decarboxylase (Fig. 3b) (35).

Previously it had been suggested that catalysis by Pfu PG1 was metal-independent (6). However, many cupin domains contain metal ion-binding sites for first row transition metals (35), with auxin-binding protein 1 (33) and phosphomannose isomerase (34) binding Zn²⁺ and oxalate decarboxylase (35) and with oxalate oxidase (36) binding Mn²⁺. Furthermore, despite differences in the metal ions bound by these proteins, the ligands used for coordinating these ions are often identical in sequence and are spatially conserved between auxin-binding protein 1 (His57, His59, Glu63, and His106), oxalate oxidase (His88, His90, Glu95, and His137), and oxalate decarboxylase (His95, His97, Glu101, and His140). In phosphomannose isomerase the four residues that form the metal-binding ligands (Gln111, His113, Glu138, and His285) differ only in the replacement of one of the histidines by a glutamine. Moreover, despite the apparent differences in sequence numbers, these residues are structurally equivalent, as phosphomannose isomerase has two large insertions compared with these other proteins. Comparison of these structures with Pfu PG1 revealed that the latter conserves each of the four metal-binding ligands (His88, His90, Glu97, and His136), strongly suggesting the presence of a metal ion-binding site in this enzyme. However, examination of the electron density map provided no evidence for a bound metal.

**PG1 Binds First Row Transition Metals**—The close similarity of the structure of Pfu PG1 to the metalloenzymes described above together with the apparent identification of the metal-binding ligands led us to consider the possibility that Pfu PG1, like other members of the cupin superfamily, might bind a first

### Table III

| Data set | Remote |
|----------|--------|
| Resolution (Å) | 20–2 |
| Unique reflections | 11,087 |
| R cryst | 0.175 |
| R free | 0.257 |
| No. of protein atoms | 1503 |
| No. of water molecules | 220 |
| Root mean square deviation Bond length (Å) | 0.018 |
| Bond angle (degrees) | 1.9 |
| Average temperature factor (Å²) Protein atoms | 24 |
| Water molecules | 36 |

**FIG. 4.** Sequence alignment of PGIs from Pfu (PfuPGI), Tli (TliPGI), and the putative PGIs from S. meliloti (SmPGI1 and -2), M. mizei (MmPGI), and M. acetivorans (MaPGI). Highlighted residues are the metal-binding ligands present in Pfu PG1.
row transition metal. Analysis of the electron density map for Pfu PGI clearly indicated that the potential metal-binding site was unoccupied. However, this could be explained by the purification protocol of the enzyme, which included EDTA (37). Inductively coupled plasma-mass spectrometry analysis of recombinant Pfu PGI, purified in the absence of EDTA, showed the clear presence of iron in the protein sample. Despite this, the specific activity of the metal-depleted enzyme is comparable with that of Pfu PGI purified in the absence of EDTA either from Pfu or from the overexpressing E. coli strain (Table I). Incubation of the EDTA-purified recombinant enzyme with Zn$$^{2+}$$ (4 mM), Fe$$^{3+}$$ (1 mM), or Mn$$^{2+}$$ (5 mM) gave rise to enhancements to the specific activities of −5, 50, and 500%, respectively (Table I). Although inductively coupled plasma data would suggest that the enzyme preferentially binds Fe$$^{3+}$$ in the metal-binding site as outlined above, activity is not necessarily maximal with iron, and further analysis of the protein isolated from Pfu is required. More recently Jeong et al. (8) have reported the identification of iron as the bound metal in recombinant Tli PGI, expressed in E. coli.

The position of the metal-binding site within Pfu PGI was confirmed by difference Fourier analysis using data from crystals purified in the presence of EDTA and soaked with either Mn$$^{2+}$$ (Fig. 1d) or Zn$$^{2+}$$ (data not shown) (Table II). This confirmed that these cations bind as predicted, making interactions with His$$^{88}$$ (3.1 Å), His$$^{90}$$ (2.9 Å), Glu$$^{97}$$ (2.0 Å), and His$$^{138}$$ (2.3 Å) (Fig. 1d).

**Location of the Putative Active Site**—Thus far, we have been unable to identify the substrate-binding site in Pfu PGI by difference Fourier analysis on crystals soaked with substrates or inhibitors (Table III). Furthermore, the relatively high sequence similarity of the related enzymes from Pfu and Tli is such that the active site cannot be predicted to any degree of certainty from the pattern of sequence conservation. Analysis of the sequence conservation between these PGIs and the putative PGIs identified by analysis of genome sequence data from Methanosarcina mazei, Methanosarcina acetivorans, and Sinorhizobium meliloti reveals striking conservation at one end of the pocket formed between the two β sheets of the cupin domain and involving the four metal-binding ligands (Fig. 4). Also seen is significant conservation of the hydrophilic residues, which line this pocket and include the conserved tryrosines (Tyr$$^{12}$$, Tyr$$^{99}$$, and Tyr$$^{160}$$) and threonines (Thr$$^{71}$$ and Thr$$^{86}$$). However, a number of inward-facing hydrophobic residues are substituted, particularly Ala$$^{89}$$, Phe$$^{148}$$ and Ala$$^{150}$$. Finally His$$^{138}$$, on the edge of the pocket, is substituted by serine or glutamine. Despite the uncertainty of the reaction catalyzed by these other enzymes, this analysis would seem to point to the active site being in this region of the structure, in line with the position of the active sites in other enzymes based on the cuff fold.

The size of the pocket between the two β sheets in Pfu PGI is consistent with binding of a glucose 6-phosphate (Glc-6-P) or a fructose 6-phosphate moiety. Moreover, analysis of the structure reveals that there are a number of residues that could form hydrogen bonds to the hydroxyl groups of the sugar and two groups of positively charged residues that could neutralize the negative charge on the phosphate group of the substrate. Modeling studies suggest that Glc-6-P can be oriented in the pocket in two distinctly different orientations. In one of the orientations the phosphate group is bound by an arginine-lysine cluster (Lys$$^{21}$$, Arg$$^{23}$$, and Lys$$^{65}$$). This would place the C1 and C2 of Glc-6-P adjacent to the transition metal center and nearby the side chains of Tyr$$^{122}$$ and His$$^{138}$$ and the more remote side chain of Glu$$^{98}$$ (Fig. 3c). In the alternative orientation the phosphate group lies adjacent to the transition metal center, with the C1 and C2 of Glc-6-P adjacent to side chains of Lys$$^{21}$$, Ser$$^{22}$$, Arg$$^{25}$$, Lys$$^{68}$$, His$$^{158}$$, and Tyr$$^{160}$$ (Fig. 3d). In either orientation the residues flanking the C1 and C2 hydroxyls are not completely conserved, except in Pfu and Tli PGIs, but the hydrophilic side chains that line the pocket are in positions that could form hydrogen bonds to the hydroxyl groups of the glucose. In the absence of experimental data, we cannot be certain of the orientation of the substrate, and indeed orientations other than the ones described are possible. Given that catalysis does not depend strongly on the presence or absence of transition metal ions, we would favor a binding mode in which the catalytic center is on the outer lip of the cupin domain with the phosphate group binding adjacent to the metal center. However, this is not consistent with the pattern of sequence conservation seen between Pfu and Tli PGI and the other putative PGIs. This analysis is further complicated by the lack of biochemical verification of the true activity of these putative PGIs which, although clearly related in structure, are not necessarily related in function. This ambiguity can only be resolved by the independent identification of the substrate-binding site of Pfu PGI and further studies on the function of the putative PGIs.

The structure of Pfu PGI described here clearly reveals a further example of the diverse role to which a cupin domain can be applied. Furthermore, the structure reveals a completely different fold from that found in the PGIs belonging to the eukaryotic and bacterial kingdoms. Further studies on this enzyme will allow us to define the enzyme mechanism in more detail and, should the mechanism prove to be related to that of the bacterial and eukaryotic enzymes, to provide a new example of convergent evolution at work.

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