The Phosphoglucone Isomerase from the Hyperthermophilic Archaeon *Pyrococcus furiosus* Is a Unique Glycolytic Enzyme That Belongs to the Cupin Superfamily*

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*Pyrococcus furiosus* uses a variant of the Embden-Meyerhof pathway during growth on sugars. All but one of the genes that encode the glycolytic enzymes of *P. furiosus* have previously been identified, either by homology searching of its genome or by reversed genetics. We here report the isolation of the missing link of the pyrococcal glycolysis, the phosphoglucone isomerase (PGI), which was purified to homogeneity from *P. furiosus* and biochemically characterized. The *P. furiosus* PGI, a dimer of identical 23.5-kDa subunits, catalyzes the reversible isomerization of glucose 6-phosphate to fructose 6-phosphate, with $K_m$ values of 1.99 and 0.63 mM, respectively. An optimum pH of 7.0 has been determined in both directions, and at its optimum temperature of 90 °C the enzyme has a half-life of 2.4 h. The N-terminal sequence was used for the identification of the *pgiA* gene in the *P. furiosus* genome. The *pgiA* transcription start site has been determined, and a monocistronic messenger was detected in *P. furiosus* during growth on maltose and pyruvate. The *pgiA* gene was functionally expressed in *Escherichia coli* BL21(DE3). The deduced amino acid sequence of this first archaeal PGI revealed that it is not related to its bacterial and eukaryal counterparts. In contrast, this archaeal PGI shares similarity with the cupin superfamily that consists of a variety of proteins that are generally involved in sugar metabolism in both prokaryotes and eukaryotes. As for the *P. furiosus* PGI, distinct phylogenetic origins have previously been reported for other enzymes from the pyrococcal glycolyic pathway. Apparently, convergent evolution by recruitment of several unique enzymes has resulted in the unique *Pyrococcus* glycolysis.

The hyperthermophilic archaeon *Pyrococcus furiosus* is capable of metabolizing sugars via a modified Embden-Meyerhof pathway (1). Novel enzymes and unique control points in this pathway have been elucidated and involve two phosphorylation and an oxidation-reduction reaction (2–5).

A first variation of the pyrococcal glycolysis concerns the unique ADP-dependent sugar kinases, i.e. ADP-dependent glucokinase (ADP-GLK)\(^1\) and ADP-dependent phosphofructokinase (ADP-PFK) have been characterized biochemically, and the paralogous genes were identified on the *P. furiosus* genome (2, 3). The recently determined crystal structure of the ADP-GLK from the related archaeon *Thermococcus litoralis* revealed that the ADP-dependent sugar kinase family (ADP-GLK and most likely ADP-PFK) belong to the ribokinase family (6), whereas their bacterial and eukaryal counterparts belong to the hexokinase and PFK family, respectively (7, 8).

A second variation concerns the glycolytic conversion of glyceraldehyde 3-phosphate to 3-phosphoglycerate in *P. furiosus* that was found to be catalyzed by the unique glyceraldehyde-3-phosphate ferredoxin oxidoreductase enzyme (4, 5). This ferredoxin-dependent, single-step conversion of glyceraldehyde 3-phosphate was shown to represent a novel site of glycolytic regulation in *P. furiosus* (5).

With the increasing number of available sequence data from different species, including bacteria, eucarya, and archaea, and functional characterization of the gene products, most of the genes encoding the other *P. furiosus* glycolytic enzymes (fructose-1,6-bisphosphate aldolase, triose-phosphate isomerase, phosphoglycerate mutase, enolase, and pyruvate kinase) encoding genes could readily be identified in its genome (9). Attempts to identify the gene encoding phosphoglucone isomerase (PGI) by a bioinformatics approach have hitherto been unsuccessful. Although significant PGI activity has previously been detected (0.2 units/mg) in a *P. furiosus* cell-free extract (1, 2, 10, 11), no ortholog of a bacterial/eukaryal PGI could be identified in the *P. furiosus* genome. This suggested that *P. furiosus* might possess a distinct type of PGI. To complete the *P. furiosus* glycolytic pathway and to obtain insight in the anticipated novel type of PGI, we here report on the purification of the PGI enzyme from *P. furiosus*, its characterization, and the isolation of the corresponding *pgiA* gene. This is the first molecular and biochemical characterization of an archaeal PGI, that indeed represents a novel type of this enzyme.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals and enzymes were purchased from Sigma, Merck, or Roche Molecular Diagnostics in analytical grade. *Aspergillus nidulans* mannitol-1-phosphate dehydrogenase was purified from an overproducing *A. nidulans* strain as described previously (12).

**Organisms and Growth Conditions**—*P. furiosus* was cultivated in artificial seawater medium as described before (3). *Escherichia coli* XL1 Blue was used as a host for the construction of pET24d derivatives. *E. coli* strain SHC 1000 (8) was also used for cloning experiments. *Thermococcus litoralis* (2) was grown in a microfluidic device (11).

* Pyrococcus furiosus was grown in artificial seawater medium as described before (3).

1. The abbreviations used are: GLK, glucokinase; PFK, phosphofructokinase; PGI, phosphoglucone isomerase; PAGE, polyacrylamide gel electrophoresis; PCR, polyacrylamide gel electrophoresis; MOPS, 4-morpholinepropanesulfonic acid.
coli BL21(DE3) was used as an expression host. Both strains were grown in Luria Bertani medium with kanamycin (50 μg/ml) in a rotary shaker at 37 °C.

Preparation of Cell-free Extract from P. furiosus.—P. furiosus cells from a 200-liter culture were harvested by continuous centrifugation (ShakeMaster, 12,000 × g and stored at -70°C). Cell extracts were prepared by homogenization of the harvested cells, in a 1:1 (v/v) ratio of 30 ml Tris/HCl buffer, pH 7.5, and treated with a French press at 100 megapascals. Cell debris was removed by centrifugation for 1 h at 100,000 × g at 10 °C.

Purification of the PGI from P. furiosus Cell-free Extract—To prevent microbial contamination, all buffers contained 0.02% sodium azide. Cell-free extract (27 ml) was filtered (0.45 μm), brought to 1.7 M ammonium sulfate saturation and loaded onto a Phenyl-Sepharose fast flow column (69 ml, Amersham Pharmacia Biotech), equilibrated in 50 mM Tris/HCl buffer, pH 7.8, containing 1.7 M ammonium sulfate. During a 350-ml linear gradient (1.7–0.0 M ammonium sulfate) PGI activity eluted at 1.0 M ammonium sulfate. Active fractions were pooled and desalted by filtration (Microsep, 10-kDa cutoff), using a 50 mM Tris/HCl buffer, pH 8.5. The desalted PGI pool was applied to a Q-Sepharose fast flow column (25 ml, Amersham Pharmacia Biotech) that was equilibrated in the same buffer. The PGI eluted in a 125-ml linear gradient (0.0–0.7 M NaCl) at 0.27 M NaCl. Active fractions were pooled and dia lysed against 20 mM potassium phosphate buffer, pH 7.0. The desalted PGI pool was applied to a hydroxyapatite column (20 ml, Bio-Rad) that was equilibrated in the same buffer. PGI activity eluted in a 200-ml linear gradient (20–500 mM potassium phosphate) at 140 mM potassium phosphate. Active fractions were pooled, the buffer was changed for a 50 mM Tris/HCl buffer, pH 7.6, by dialysis and the pool was loaded onto a Mono-Q HR 5/5 column (1 ml, Amersham Pharmacia Biotech) that was equilibrated in the same buffer. PGI activity eluted in a 30-ml linear gradient (0.0–0.7 M NaCl) at 0.18 M NaCl. Fractions showing PGI activity were pooled and concentrated 10-fold to a final volume of 100 μl. This concentrated pool was applied to a Superdex 200 HR 10/30 gel filtration column (24 ml, Amersham Pharmacia Biotech), equilibrated in 50 mM Tris/HCl buffer, pH 7.8, containing 0.7 M NaCl. Active fractions were pooled and concentrated 10-fold to a final volume of 100 μl using a Microsep filter with a 10-kDa cutoff. The concentrated pool was loaded onto a Superdex 200 HR 10/30 gel filtration column (Amersham Pharmacia Biotech), equilibrated with 50 mM Tris/HCl, pH 7.8, containing 100 mM NaCl, and 1 M urea. The recombinant PGI eluted at 14.5 ml. The purified enzyme was desalted in 50 mM Tris/HCl, pH 7.8, using a Microsep filter with a 10-kDa cutoff.

Protein Concentration and Purity—Protein concentrations were determined with the Coomassie Brilliant Blue G-250 as described before (14) using bovine serum albumin as a standard. The purity of the enzyme was checked by SDS-PAGE as described (15). Protein samples for SDS-PAGE were heated for 5 min at 100 °C in an equal volume of sample buffer (0.1 M citrate-phosphate buffer, 5% SDS, 0.9% 2-mercaptoethanol, 20% glycerol, pH 6.8).

Determination of Enzyme Activity—PGI activity was determined in 100 mM MOPS buffer, pH 7.0 (50 °C). Enzyme preparations were added in 5–50 μl. Enzyme activity on fructose 6-phosphate was determined by measuring the formation of NADPH in a coupled assay with yeast fructose-1,6-bisphosphatase (16). Activities were determined in the absence of all known inhibitors, and the activity of the enzyme was measured by monitoring NADPH formation at 340 nm (e = 6.3 mM-1 cm-1) corresponding to the PGI activity.

Determination of Protein Activity—Substrate specificity was investigated using purified PGI. The use of fructose 6-phosphate and glucose 6-phosphate as possible substrates for the PGI was tested using the standard enzyme assay. For the determination of mannose 6-phosphate as a possible substrate the standard enzyme assay for glucose 6-phosphate was used. Glucose, fructose, galactose, and mannose were tested as possible substrates by incubating an appropriate amount of PGI with 5 mM substrate for 30–60 min at 50 °C in 100 mM MOPS, pH 7.0. The reactions were stopped on ice/ethanol and the products were analyzed by high performance liquid chromatography. The effect of cations (MgCl2 and MnCl2, 10 mM) and cofactors (ATP, NAD+ ars enate, and phosphate, 10 mM) on the isomerization of non-phosphorylated monosaccharides was investigated by the standard high performance liquid chromatography assay.

Inhibitors of PGI Activity—Possible inhibitors (mannose 6-phosphate, fructose 1-phosphate, fructose 1,6-bisphosphate, fructose, glucose, mannose, galactose, pyruvate, phosphoenolpyruvate, AMP, ADP or ATP) were tested on the activity of the P. furiosus PGI both in the direction of glucose 6-phosphate and fructose 6-phosphate formation by adding (1.25–10 μM) to the standard enzyme assays at 50 °C.

Kinetic Analysis—Kinetic parameters were determined at 50 °C, in 100 mM MOPS buffer, pH 7.0, by varying the concentration of fructose 6-phosphate (0.05–3.50 mM) or glucose 6-phosphate (0.47–10.0 mM), respectively. 2.0 μg of purified PGI was used for these determinations. Data were analyzed by computer-aided (Program Tablecurve) fit to the Michaelis-Menten curve.

Temperature Optimum and Thermal Inactivation—The temperature optimum was determined in the direction of glucose 6-phosphate formation. Purified PGI (0.0084 mg/ml) was incubated in 1-cm-crimp top quartz cuvetts containing 100 mM MOPS buffer, pH 7.0. The vials were submerged in an oil bath at temperatures varying from 30 to 120 °C, pre-heated for 2 min, and the enzyme reaction was started by injecting 20 mM fructose 6-phosphate. After 1, 2, and 3 min the reaction was stopped by transferring the vials on ice/ethanol, and the amount of glucose 6-phosphate formed was determined spectrophotometrically at room temperature by measuring the reduction of NADP+ (340 nm) in an isomerase-free glucose 6-phosphate dehydrogenase assay for the chemical isomerization of fructose 6-phosphate in the absence of PGI.

Thermal inactivation of PGI was determined by incubating the enzyme (1.28 μg in 200 μl of a pre-heated 100 mM sodium phosphate buffer, pH 7.0, at 60, 70, 80, and 90 °C in crimp-sealed vials, submerged in an oil bath. At certain time intervals, 200-μl aliquots were withdrawn and analyzed for activity in the standard assay. Studies were
performed under V_{max} conditions, since substrate concentrations in the assays are ~30-fold higher than the K_{m}.

**pH Optimum**—The pH optimum was determined at 50 °C in 200 mM Tris maleate buffer over the pH range 6.0–9.5. Buffer pH values were adjusted at this temperature. Except for buffer and temperature, assay conditions were identical to analyze the enzyme's temperature optimum. In the case of fructose 6-phosphate conversion, glucose-6-phosphate dehydrogenase was used as following enzyme. When glucose 6-phosphate was used as substrate, mannitol-1-phosphate dehydrogenase was used as following enzyme.

**Transcript Analysis**—RNA was isolated from maltose (10 mm) and pyruvate (40 mm) grown *P. furiosus* cells as described previously (16). For Northern blot analysis 15 μg of total RNA was separated on a 1.5% formaldehyde-agarose gel and transferred to a Hybond N\(^*\) membrane. Probes were generated by PCR with the primers BGG902 and BG903. The PCR product was purified by Qiagen (Qiagen) and labeled by nick translation with [α-32P]dATP. The transcription start was determined with a fluorescence (IRD800)-labeled antisense oligonucleotide (5'-CTTTCATGCCCCGTCATCAAC-3', position 103–124 of the pgIA gene).

**Results and Discussion**

### Purification of the PGI from *P. furiosus*

| Purification step | Total activity | Protein | Specific activitya | Purification factor | Recovery |
|------------------|----------------|---------|--------------------|---------------------|----------|
| Cell-free extract | 295.8          | 39.7    | 0.766              | 1.0                 | 100      |
| Phenyl-Sepharose | 93.6           | 3.54    | 0.588              | 2.1                 | 31.6     |
| Q-Sepharose      | 90.2           | 0.670   | 2.99               | 10.8                | 30.5     |
| Hydroxyapatite   | 38.1           | 0.426   | 3.58               | 13.0                | 12.9     |
| Mono-Q           | 25.8           | 9.92    | 2.86               | 14.0                | 8.7      |
| Superdex200      | 10.1           | 0.196   | 14.5               | 52.5                | 3.4      |

*a* Specific activities were determined at 50 °C, with fructose 6-phosphate as substrate.

DNA sequence analysis of pLUW557 confirmed that the cloned pgIA gene showed the expected sequence. SDS-PAGE analysis of a heat-treated cell-free extract of *E. coli* BL21(DE3) harboring pLUW557 revealed an additional band of 23 kDa which was in good agreement with the calculated molecular mass (21.6 kDa) of the gene product. This band was absent in a heat-treated cell-free extract of *E. coli* BL21(DE3) carrying the PET24d vector without insert, in which no PGI activity was detected (not shown). In a heat-treated cell-free extract of *E. coli* BL21(DE3) harboring pLUW557, a PGI activity of 8.3 units/mg was measured at 50 °C, confirming that the cloned *P. furiosus* pgIA gene indeed encoded a PGI. The recombinant PGI was easily purified by two successive chromatographic steps, i.e. anion exchange chromatography and gel filtration. The recombinant enzyme eluted as the native enzyme, and was purified to apparent homogeneity as judged by SDS-PAGE analysis (Fig. 1).

**Physical and Biochemical Characterization of PGI**—The molecular mass of both the native and recombinant PGI as determined by gel filtration was 49.6 ± 0.3 kDa. SDS-PAGE analysis of the two enzymes resulted in identical bands of 23.5 ± 0.2 kDa, suggesting that the PGI is a homodimer. This homodimeric composition has been observed also for bacterial and eukaryal PGIs, although homotetrameric compositions occur as well. Furthermore, the *P. furiosus* PGI differs from all known PGIs by its subunit molecular mass, which is about half of its canonical counterparts (Table II). Moreover, the *P. furiosus* PGI, the first archaeal PGI described to date, exhibits the lowest pH optimum and highest temperature optimum of all known PGIs (Table II).

The specific activities of the native and the recombinant PGI exhibited similar temperature or pH optima. The *P. furiosus* PGI showed reversible isomerization activity with fructose 6-phosphate and glucose 6-phosphate between pH 6.0 and 8.5, with an optimum at pH 7.0 (not shown). PGI showed maximal activity around 90 °C (Fig. 2). From the Arrhenius plot between 30 and 90 °C, an inactivation energy of 41 kJ/mol was calculated. Thermal inactivation was determined at 60, 70, 80, and
addition of cations (Mg\textsuperscript{2+} or Mn\textsuperscript{2+}), nor by addition of 10 mM EDTA to the assay mixture. Under the tested conditions the enzyme did not convert mannose 6-phosphate to fructose 6-phosphate. The PGI from *Escherichia intermedia* has been reported to catalyze the isomerization of non-phosphorylated sugars, like fructose and glucose, but only in the presence of arsenate (26). The purified enzyme from *P. furiosus* was unable to isomerize non-phosphorylated sugars like glucose, fructose, mannose, and galactose both in the absence or presence of cofactors like arsenate and phosphate. This suggests that the phosphoryl group at the C-6 position of fructose 6-phosphate and glucose 6-phosphate plays an important role in substrate recognition of the *P. furiosus* PGI.

The native *P. furiosus* PGI showed Michaelis-Menten kinetics at 50 °C, *K_m* values of 0.63 ± 0.07 and 1.99 ± 0.11 mM for fructose 6-phosphate and glucose 6-phosphate, respectively, and *V_{max}* values of 20.1 ± 0.73 and 34.3 ± 0.71 units/mg for fructose 6-phosphate and glucose 6-phosphate, respectively. *K_m* values and *V_{max}* values determined for the recombinant PGI were in the same order of magnitude, with *K_m* values of 0.42 ± 0.03 and 2.00 ± 0.17 mM for fructose 6-phosphate and glucose 6-phosphate, respectively, and *V_{max}* values of 19.2 ± 0.37 and 47.7 ± 1.40 units/mg for fructose 6-phosphate and glucose 6-phosphate, respectively. The *k_{cat}*/*K_m* values for fructose 6-phosphate and glucose 6-phosphate conversion of the native PGI were 11.5 and 6.2 s\(^{-1}\) mM\(^{-1}\), and of the recombinant PGI 16.5 and 8.6 s\(^{-1}\) mM\(^{-1}\).

The effect of potential inhibitors was tested on the activity of the recombinant PGI (5 mM substrate). The addition of fructose, glucose, mannose, galactose (10 mM), pyruvate, phosphoenolpyruvate (10 mM), AMP, ADP, or ATP (3.5 mM), did not show any effect on the PGI activity neither in the fructose 6-phosphate formation, nor in the glucose 6-phosphate formation. Typical PGI inhibitors like mannose 6-phosphate, fructose 1-phosphate, and fructose 1,6-bisphosphate negatively affected the PGI activity in both directions. Residual activities of 18 and 38% were monitored in the presence of 1.25 mM mannose 6-phosphate, in the direction of fructose 6-phosphate and glucose 6-phosphate formation, respectively. In the presence of 2 mM fructose 1-phosphate residual activities of 50 and 69% were measured, respectively. Finally, the addition of 10 mM fructose 1,6-bisphosphate to the assay mixture resulted in residual activities of 41 and 53%, respectively. Hence, the activity of the *P. furiosus* PGI is inhibited by classical PGI inhibitors (27), and the affinity of the *P. furiosus* enzyme for fructose 6-phosphate and glucose 6-phosphate (determined at 50 °C) was in the same order of magnitude as that of the classical PGIs (Table II).

Hence, catalytic properties of the *P. furiosus* PGI resemble that

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**FIG. 2.** Dependence of PGI activity on temperature. Activity of native PGI was determined by measuring the amount of glucose 6-phosphate formed after incubation for 1, 2, and 3 min at the desired temperature. *Inset*, Arrhenius plot of the data from 30 to 90 °C. Both native and recombinant PGI showed similar behaviors to temperatures (not shown).

**FIG. 3.** Thermal stability of PGI. The native enzyme (0.0064 mg/ml) was preincubated at 90 °C in 100 mM sodium phosphate buffer, pH 7.0. Residual activity was measured at 50 °C using fructose 6-phosphate as substrate. The 100% activity corresponds to 18.6 units/mg for fructose 6-phosphate, and 9.5 units/mg for glucose 6-phosphate, respectively. Inset, Arrhenius plot of the data from 30 to 90 °C. Both native and recombinant PGI showed similar behaviors to temperatures (not shown).
of the classical PGIs in most respects. When this paper was being evaluated, Hansen et al. (28) independently described a biochemical characterization of the phosphoglucose isomerase from *P. furiosus*, in general revealing features as reported in this study.

**Transcript Analysis**—For an accurate assignment of the promoter region in *P. furiosus* the transcription start of the *pgiA* mRNA was determined by primer extension. The transcription is initiated at the thymine (T) 11 base pairs upstream of the ATG start codon (Fig. 4A). A putative ribosomal binding site was identified at position +2 to +6. A putative TATA box is positioned around −24/−25 of the transcription start, and a clear transcription factor B Recognition Element (BRE site, consensus sequence (A/G)N(A/T)AA(A/T)) (29) is positioned around −33/−34 (Fig. 4B).

Northern blot analysis revealed a strong hybridization signal at 0.7 kilobase pairs with the *pgiA* probe, indicating the presence of a monocistronic transcript (Fig. 4C). As shown by primer extension (4-fold) and Northern blot analysis (1.5-fold), *pgiA* transcription is slightly higher under catabolic (maltose) than under anabolic (pyruvate) conditions. Moreover, a 1.7-fold increase of PGI activity was detected when grown on maltose (0.32 units/mg) compared with pyruvate (0.19 units/mg). Similar observations were made for the reversible fructose-1,6-bisphosphate aldolase and phosphoenolpyruvate synthetase from *P. furiosus* (30, 31). This might suggest a different flux through the pathway when used in the anabolic or in the catabolic direction.

**Structural Analysis**—The amino acid sequence of PGI has full-length homologs with high levels of sequence identity (90 and 91% for *Pyrococcus abyssi* and *Pyrococcus horikoshii*, respectively) in the other two *Pyrococci*, suggesting that these genes most likely also function as PGIs. Homology with other sequences is limited to positions 66 to 152 of the *P. furiosus* PGI (Fig. 5). Using profile based sequence comparisons (PSI-Blast, 9 iterations, E < 0.002) this area can be shown to be homologous to a wide range of proteins belonging to the cupin superfamily, that consists of a variety of proteins that are generally involved in sugar metabolism in both prokaryotes and eukaryotes (33). The molecular function of this cupin domain (consensus, PG(X)_5HXH(X)_4E(X)_7G and G(X)_5P(X)_2H(X)_3N) is generally the binding of carbohydrates, and in some cases apparently to establish an interaction with other proteins (33, 34). Among the homologs are two additional hypothetical proteins from *Pyrococcus* itself (PF_396648 and PF_62346), as well as several type-2 mannose-6-phosphate isomerases, oxalate de-
The question remains whether or not a complete glycolytic pathway existed at the time that the non-homologous enzymes evolved independently from the classic PGI from bacteria (Fig. 6), and hence most likely is an example of convergent evolution.

Recruitment of Enzymes in Unique "Top" Glycolysis

The identification of PGI allowed a comparison of the nine-enzyme glycolysis in Pyrococcus with the classical 10-enzyme glycolysis in bacteria and eucarya. Notably four of the nine pyrococcal enzymes, that were identified experimentally, are non-homologous to their classical counterparts. Here we have shown, based on sequence comparisons and on structural data, that the P. furiosus PGI (the second step in glycolysis) is not homologous to the bacterial and eukaryal PGI. The other five enzymes (fructose-1,6-bisphosphate aldolase, triose-phosphate isomerase, phosphoglycerate mutase, enolase, and pyruvate kinase) have been predicted on the basis of orthology with bacterial proteins (9). Four of these five are orthologous to their bacterial counterparts in the glycolysis. The fifth, fructose-1,6-bisphosphate aldolase, is not homologous to the standard bacterial class II aldolase (35). This aldolase has recently been proposed to constitute a new family of aldolases, archaeal type Class I aldolase (Class IA), that is rare in bacteria and abundant in archaea, and only distantly related to Class I fructose-1,6-bisphosphate aldolases (31).

The structure of carboxylases, oxalate oxidases (germin), seed storage protein, canavalin (Figs. 5 and 6), as well as sugar-binding transcriptional regulators of the AraC family (33). No proteins with PGI activity have been reported to belong to this family before.

For a number of sequences in this family a crystal structure has been determined (e.g., carovlin), revealing that the cupin domain has a typical double-stranded \( \beta \)-helix, forming a barrel (32, 33). Based on an alignment of PGI with its closest homolog, a secondary structure prediction has been performed using Profile-based neural network system from HeiDelberg (20), confirming that PGI is homologous to canavalin (Fig. 5).
ent: (i) three of the unique glycolytic steps in Pyrococcus are specifically catabolic (ADP-GLK, ADP-PFK, and glyceraldehyde-3-phosphate ferredoxin oxidoreductase); (ii) the first three unique steps (catalyzed by ADP-GLK, PGI, and ADP-PFK) form the part of the pathway that is rather specific for glucose degradation, whereas the more conserved part of the pathway (the interconversion of glyceraldehyde-3-phosphate and pyruvate) is made up by a more general set of enzymes that are potentially involved in numerous metabolic routes. This would argue for an independent invention of the glycolytic pathway in the lineage leading to Pyrococcus. Although non-homologous displacement of enzymes in Pyrococcus central carbohydrate metabolism has been observed before (36), this would be, to our knowledge, the first example of such excessive replacement of enzymes in a pathway, and is a compelling example of convergent evolution.

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The Phosphoglucone Isomerase from the Hyperthermophilic Archaeon *Pyrococcus furiosus* Is a Unique Glycolytic Enzyme That Belongs to the Cupin Superfamily

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