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Crystallization and preliminary X-ray diffraction analysis of phosphoglucose/phosphomannose isomerase from *Pyrobaculum aerophilum*

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Phosphoglucose isomerase from the crenarchaeon *Pyrobaculum aerophilum* (PaPGI/PMI) shows virtually no sequence similarity to its counterparts from bacterial and eukaryotic sources and belongs to a unique group within the PGI superfamily. Whereas conventional PGIs show strict substrate specificity for glucose 6-phosphate and fructose 6-phosphate, PaPGI/PMI can also catalyse the isomerization of mannose 6-phosphate. In order to establish its relatedness within the PGI family and to elucidate the structural basis for its broader specificity, this enzyme was crystallized. The crystals belong to space group $P2_1$ and a complete data set extending to 1.6 Å resolution has been collected.

1. Introduction

Phosphoglucose isomerase (PGI; EC 5.3.1.9) is a well known enzyme of sugar metabolism, with roles in glycolysis and gluconeogenesis. The enzyme from mammalian and bacterial sources has been studied extensively. Its reaction, the interconversion of D-glucose 6-phosphate (G6P) and D-fructose 6-phosphate (F6P), is an aldose–ketose isomerization and proceeds *via* a *cis*-enediolate intermediate (Rose, 1975). Given the high degree of sequence similarity between PGIs, the enzymes are likely to share a common mechanism and evidence from crystal structures suggests that Glu357 (in rabbit and human PGIs) is the base responsible for proton abstraction (Jeffery *et al.*, 2001; Read *et al.*, 2001). The enzyme also catalyses the opening of the sugar ring and this is believed to take place *via* an acid-catalysed mechanism using His388 (Lee *et al.*, 2001; Read *et al.*, 2001). The specificity of these 'conventional' PGIs for G6P and F6P is essentially absolute: the enzyme cannot use mannose 6-phosphate as a substrate for isomerization, although it can interconvert the anomeric forms of this sugar (see Rose, 1975).

Phosphoglucose isomerase from the crenarchaeon *Pyrobaculum aerophilum* is a 302-residue protein that, when analysed by ultracentrifugation, exists as a 65 kDa homodimer (Hansen, Urbanke *et al.*, 2004). Its sequence (Fitz-Gibbon *et al.*, 2002) cannot be aligned with those of PGIs from eukaryotic or bacterial sources, yet close examination suggests that some of the highly conserved active-site residues may indeed be present (Fig. 1). We have recently characterized the biochemical properties of this enzyme (Hansen, Urbanke *et al.*, 2004) and related PGI enzymes from *Aeropyrum pernix* and *Thermoplasma acidophilum* (Hansen, Wendorff *et al.*, 2004). All differ from conventional PGIs in their ability to isomerize mannose 6-phosphate to F6P at equal catalytic rates to the conversion of G6P to F6P. The lack of any other detectable *pgi* or *pmi* genes in the genomes of these two archaeal species suggests that this PGI may act as a phosphomannose isomerase (PMI) *in vivo*. Along with putative homologues from *Sulfolobus* species, *T. volcanicum* and *Aquifex aeolicus*, these enzymes constitute a novel PGI/PMI family within the PGI superfamily (Hansen, Wendorff *et al.*, 2004).

In order to establish whether these enzymes are indeed related to the conventional PGI family and to understand their catalytic mechanism, including their broader substrate specificity, we have set out to obtain structural information from a representative of this

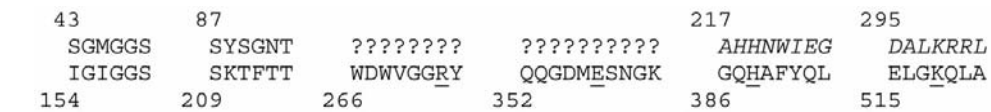

Figure 1
A tentative assignment of six sequence motifs of PaPGI/PMI based on an alignment with those comprising the active site of mammalian PGIs. The top sequence is of PaPGI/PMI and that of human PGI is shown below. Arg272, Glu357, His388 and Lys518, which in conventional PGIs are critical active-site residues, are underlined. The motifs from human PGI are almost totally conserved sequences in eubacteria and eukaryotes. Equivalent motifs for the 266 and 352 regions could not be readily identified in PaPGI/PMI; tentative assignments are shown in italics.

family. Here, we report crystals of PGI/PMI from *P. aerophilum* that diffract to 1.6 Å resolution.

2. Experimental

2.1. Crystallization

PGI/PMI from *P. aerophilum* (referred to here as PaPGI/PMI) was expressed in *Escherichia coli* and purified by hydrophobic interaction and gel-filtration chromatography, as described elsewhere (Hansen, Urbanke *et al.*, 2004). Prior to crystallization trials, the protein was dialysed against a solution containing 10 m*M* Tris–HCl pH 7.5 and concentrated to 30 mg ml^{-1} by ultrafiltration. The search for crystallization conditions used the hanging-drop vapour-diffusion method in which the drop volume was 4 µl (2 µl protein solution and 2 µl reservoir solution) and the drop was inverted over 1 ml well solution. In initial trials, the protein was screened against Crystal Screen and Crystal Screen II sparse-matrix kits (Hampton Research) and incubated at 295 K.

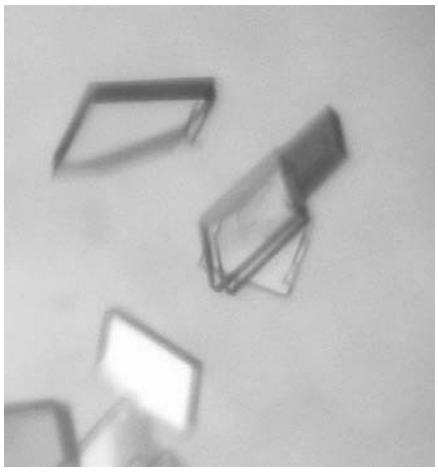

Figure 2
Crystals of phosphoglucose isomerase/phosphomannose isomerase from *P. aerophilum* grown using the hanging-drop method with polyethylene glycol 8000 as a precipitant. Their approximate dimensions are 0.20 × 0.15 × 0.05 mm.

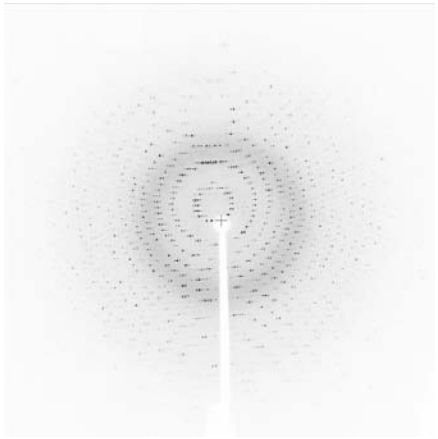

Figure 3
A diffraction image collected from crystals of PGI/PMI from *P. aerophilum*. The edge of the plate corresponds to 1.7 Å resolution.

2.2. Diffraction analysis

The diffraction properties of the crystals were analyzed using a Rigaku RU-H3R copper rotating-anode generator, operating at 50 kV and 100 mA, fitted with Confocal Maxflux optics (Osmic Inc.). The crystals were cryoprotected over a period of several hours by passage through a series of mother-liquor solutions containing increasing amounts of glycerol in 2% increments up to a maximum of 26%. The crystals were then flash-frozen *in situ* in the cryostream (X-Stream 2000; Rigaku-MSC). 360° of native data were recorded on an R-AXIS-IV^{++} imaging-plate system in 1.0° oscillations with an exposure time of 3 min per frame. The crystal-to-detector distance was 120 mm. These data were processed using *CrystalClear*/*d*Trek* (Pflugrath, 1999). In order to introduce heavy atoms for the purposes of phasing the data, crystals were soaked in mother-liquor solutions each containing a particular heavy-atom salt. Data were collected from these crystals in the same way as for the native data and scaled to the native data; Patterson functions were then calculated using *PHASES* (Furey & Swaminathan, 1996).

3. Results

An initial crystallization 'hit' was obtained over wells containing 30%(*w*/*v*) PEG 8000, 0.2 *M* ammonium sulfate and 0.1 *M* sodium cacodylate pH 6.5. The crystals were optimized on the basis of their visual appearance by varying the concentration of the precipitant, the pH of the buffer and by the presence of different additive salts. The best crystals were obtained over solutions containing 25%(*w*/*v*) PEG 8000 and 0.22 *M* ammonium sulfate buffered with 0.1 *M* Tris–HCl pH 8.5. These appeared after a period of around 3–4 weeks and grew as rhomboid-shaped plates with approximate dimensions of 0.20 × 0.15 × 0.05 mm (Fig. 2).

Table 1
The data-collection statistics for the native data.

Values in parentheses are for the outer shell of data.

Resolution (Å)	45.0–1.60 (1.66–1.60)
No. observations	487708
No. unique reflections	68525
Completeness	92.9 (82.4)
$I/\sigma(I)$	16.9 (2.4)
$R_{\rm merge}$†	0.074 (0.30)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$.

Analysis of diffraction images revealed that the crystals belonged to space group $P2$ or $P2_1$, with unit-cell parameters a = 55.1, b = 100.8, c = 55.8 Å, β = 113.2°. Assuming the presence of one 65 kDa dimer in the asymmetric unit, the crystal density $V_{\rm M}$ is 2.1 Å^3 Da^{-1}. This lies within the normal range for protein crystals and corresponds to a solvent content of 42% (Matthews, 1968). A diffraction image of the native data is shown in Fig. 3. These data reduced with an $R_{\rm merge}$ of 7.4% and are 92.9% complete to 1.6 Å resolution (Table 1). Although two of the crystal unit-cell parameters are very similar, the data would only reduce in the monoclinic system. Of the 28 reflections recorded in the 0k0 zone for which k was an odd number, 21 had intensities lower than the σ of the intensity [$\sigma(I)$] and seven had intensities just slightly higher than $\sigma(I)$. In contrast, the intensities of those reflections where k was an even number were on average several-fold higher than $\sigma(I)$. On this basis, the space group was assigned as $P2_1$. This is also supported by the presence of two strong peaks on the v = 0.5 section of a difference Patterson function calculated using data collected from crystals soaked in potassium tetrabromoaurate(III) (Fig. 4).

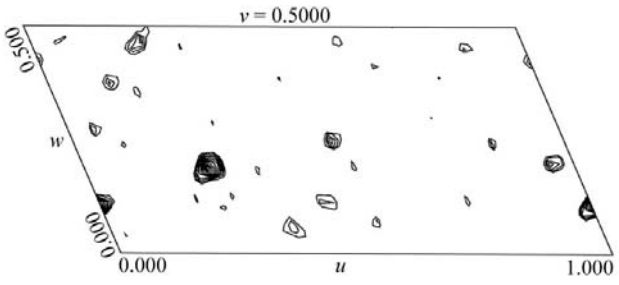

Figure 4
A difference Patterson calculated at 2.5 Å resolution using native data and data from a gold derivative of PaPGI/PMI and contoured in 0.5σ increments starting at 2σ. The Harker section for space group $P2_1$ is plotted and shows two major peaks.

Excluding the origin peak, no significant peaks were seen in the $v = 0$ section, but a cross-peak was observed at $v = 0.075$ corresponding to the vector between the two heavy-atom sites. This derivative will be used to determine the structure by a single-crystal method such as multiple-wavelength anomalous dispersion (MAD) or in combination with a second derivative by multiple isomorphous replacement (MIR).

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