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Preliminary crystallographic study of *Thermus aquaticus* glycerol kinase

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Glycerol kinase (GlpK) is an important enzyme which catalyzes the rate-limiting step in a central biochemical pathway involving glycerol metabolism. GlpK from the thermophile *Thermus aquaticus* has been overexpressed in *glpK*-deficient *Escherichia coli* and crystallized by the hanging-drop method. The crystal belongs to the cubic space group $I23$, with unit-cell parameters $a = b = c = 163.94$ (3) Å. Native data were collected to 2.87 Å resolution on a Cu $K\alpha$ rotating-anode X-ray source.

1. Introduction

Glycerol kinase (GlpK) catalyzes the first reaction in the bacterial glycerol-utilization pathway. Its activity is inhibited by the glycolytic intermediate fructose-1,6-bisphosphate (FBP; Zwaig & Lin, 1966) and by the unphosphorylated phosphocarrier protein IIA^{Glc} (Postma *et al.*, 1984; Novotny *et al.*, 1985). The crystal structures of the GlpK–IIA^{Glc} and GlpK–FBP complexes from *E. coli* have been solved (Hurley *et al.*, 1993; Feese *et al.*, 1994; Ormö *et al.*, 1998) and the structures of some mutants have been also studied (Feese *et al.*, 1998; Bystrom *et al.*, 1999). However, how this enzyme activity is regulated is still rather unclear. On the other hand, very little is known about the structures of GlpKs other than *E. coli* GlpK. Recently, we have cloned and sequenced *glpK* from *Thermus flavus* and *T. aquaticus* (Huang, Ito *et al.*, 1998; Huang, Kabashima *et al.*, 1998). The thermostable GlpK showed high amino-acid sequence identity (about 80 and 64%, respectively) to *Bacillus subtilis* GlpK (Holmberg *et al.*, 1990) and *E. coli* GlpK (Pettigrew *et al.*, 1988). Biochemical studies showed that GlpKs from mesophilic bacteria lost most of their catalytic activities under high-temperature conditions. For example, after incubation at 338 K for 15 min, the GlpKs from *E. coli* and *B. subtilis* retained approximately 10% of their activities, while the GlpKs from *T. flavus* and *T. aquaticus* showed no change in their activities. We also discovered that when phosphorylated GlpK from *T. flavus* was kept at 333 K about 70% of the GlpK was dephosphorylated within 10 min; however, the remaining 30% of the GlpK was found to retain phosphorylation even after 1 h incubation at 333 K (Darbon *et al.*, 1999). It has been suggested that under physiological conditions GlpK from thermophilic bacteria is converted into a phosphorylated form, which might elevate the enzyme activity. In enterococcal GlpK (the site of phosphorylation was determined as His232), phosphorylation caused a ninefold increase in enzyme activity, which was thought to be mediated *via* elements of protein structure within its subdomain (Charrier *et al.*, 1997). Therefore, the structure determination of this enzyme from thermophilic bacteria will help in our understanding of the mechanisms of protein thermostabilization and provide additional information for further studies of this enzyme. For example, it may explain the structural differences and similarities between this and *E. coli* GlpK and estimate how the phosphorylated residue (His230 in *T. aquaticus* GlpK) links the active sites to the control of the enzyme stabilization. In this paper, we report the crystallization and preliminary X-ray crystallographic analysis of the GlpK from the thermophile *T. aquaticus*.

2. Crystallization

T. aquaticus GlpK was overexpressed in *glpK*-deficient *E. coli*, purified and crystallized as described previously (Huang, Ito *et al.*, 1998). It was suggested that glycerol may promote a major conformational change in *E. coli* GlpK (Thorner & Paulus, 1973). So far, published structures of *E. coli* GlpK were all determined in the presence of glycerol and *E. coli* GlpK could only be crystallized in its presence (Hurley, 1996). In order to obtain crystal forms in both the absence and the presence of glycerol, the initial crystallization trial of *T. aquaticus* GlpK was carried out using the hanging-drop method with a crystallization solution not containing glycerol [0.2 *M* magnesium chloride dihydrate, 0.1 *M* Na HEPES pH 7.5, 28%(*v*/*v*) PEG 400]; however, this crystal type diffracts X-rays only weakly.

Improved crystals (Fig. 1) were obtained by adding glycerol and decreasing the pH of the crystallization solution. Each hanging drop was prepared by mixing 5 µl each of the protein solution (50 mg ml^{-1}) and the crystallization solution [0.2 *M* magnesium chloride dihydrate, 0.1 *M* MES pH 6.8, 15%(*v*/*v*) PEG 400, 5%(*v*/*v*) glycerol]. Cubic crystals appeared in 1 d at 293 K, reached dimensions of 0.3 × 0.3 × 0.3 mm after one week and were stable in storage solution [0.2 *M* magnesium chloride dihydrate, 0.1 *M* MES pH 6.8, 20%(*v*/*v*) PEG 400 and 5%(*v*/v) glycerol] for a few months. These crystals were used to collect native data.

Figure 1
Crystals of GlpK as grown by the hanging-drop method. The average dimensions of these crystals were 0.3 × 0.3 × 0.3 mm.

3. Data collection and processing

Preliminary diffraction data were collected on an R-AXIS IIC image-plate area detector with Cu $K\alpha$ radiation (1.5418 Å) from a Rigaku rotating-anode X-ray generator operating at 40 kV and 130 mA with a monochromator. The crystal was mounted in a glass capillary at 293 K. A total of 30 frames of 1.4° oscillation were measured with the crystal-to-detector distance set to 160 mm. Data were processed using the *PROCESS* package (Rigaku, Japan). Auto-indexing reveals that the crystal belongs to the cubic space group *I*23, with unit-cell parameters $a = b = c = 163.94$ (3) Å, $\alpha = \beta = \gamma = 90°$. One GlpK monomer (54.8 kDa) per asymmetric unit yields a Matthews coefficient of 3.35 Å^3 Da^{-1} and an estimated solvent content of 63%, which is within the 27–65% range commonly observed for protein crystals (Matthews, 1968). A native data set 86.2% complete to 2.87 Å resolution was collected. The final merged data set consisted of 59 992 measurements of 15 021 unique reflections with a $R_{\rm merge}$ of 9.0%. $I/\sigma(I)$ for this data set was 7.5. Details of the data-collection statistics are summarized in Table 1. *T. aquaticus* GlpK shares about 64% homology in its primary sequence with *E. coli* GlpK. An attempt was made to determine a molecular-replacement solution using *AMoRe* (Navaza & Saludjian, 1997) with the known structure of *E. coli* GlpK (Ormö *et al.*, 1998; PDB code 1bot) as a search model. The initial correlation coefficient was 0.211 for the rotation search (data used 15–4 Å) and 0.514 for the translation search (data used 15–3 Å). The appropriately rotated and translated model gave an *R* factor of 46.5%. Crystal structure determination by molecular replacement is now under way.

Table 1
Crystal data and X-ray diffraction data-collection statistics.

Values in square brackets are for the highest resolution shell.

X-ray source	Cu $K\alpha$
Wavelength (Å)	1.5418
Temperature (K)	293
Space group	*I*23
Unit-cell parameters (Å,°)	$a = b = c = 163.94$ (3), $\alpha = \beta = \gamma = 90$
Resolution (Å)	2.87 [3.0–2.87]
Completeness (%)	86.2 [70.7]
$R_{\rm merge}$ (%)	9.0 [30.0]
$I/\sigma(I)$	7.5 [2.2]
Total observations	59992
Independent reflections	15021

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