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Crystallization and preliminary X-ray crystallographic study of UDP-glucose pyrophosphorylase (UGPase) from *Helicobacter pylori*

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UDP-glucose pyrophosphorylase (UGPase) catalyzes the synthesis of UDP-glucose, an essential metabolite in all living organisms. An X-ray crystallographic study of UGPase from *Helicobacter pylori* has been performed in order to elucidate its role in the regulation of this important metabolic pathway. UGPase was crystallized from 0.1 *M* sodium acetate trihydrate pH 4.6, 2.0 *M* ammonium sulfate and 0.1 *M* guanidine–HCl. According to diffraction data collected at a resolution of 2.9 Å using a synchrotron-radiation source, the crystal belongs to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 91.47$, $b = 98.61$, $c = 245.70$ Å, $\alpha = \beta = \gamma = 90.0^\circ$.

1. Introduction

UDP-glucose pyrophosphorylase (UGPase; EC 2.7.7.9) catalyzes the synthesis of UDP-glucose and pyrophosphate from UTP and glucose-1-phosphate. UDP-glucose, an activated form of glucose, is an essential metabolite in almost all cellular processes in living organisms; for example, in the synthesis of glycogen and in the synthesis of the carbohydrate moiety of glycolipids, glycoproteins and proteoglycans (Flores-Diaz *et al.*, 1997). In addition, UGPase is required for the entry of toxic galactose into glycolysis *via* the Leloir pathway (Frey, 1996). UGPase is also an essential protein for virulence in various Gram-negative bacteria (Genevaux *et al.*, 1999), as UGPase is required for the biosynthesis of capsular polysaccharides (CPS), which are major virulence factors. For example, a *Klebsiella pneumoniae* mutant lacking UGPase loses virulence and the mucoid colony phenotype (Chang *et al.*, 1996). In the case of *Streptococcus pneumoniae*, inactivation of the *galU* gene encoding UGPase results in the defective synthesis of capsular polysacchride (Mollerach *et al.*, 1998). It has also been reported that the *galU* mutant of *Escherichia coli* showed reduced adhesion (Genevaux *et al.*, 1999), as the *galU* gene is also involved in lipopolysaccharide synthesis, which has a role in bacterial adhesion.

UGPase is present in all three kingdoms (the eukarya, archaea and bacteria) and belongs to the family of pyrophosphorylases, which includes UDP-*N*-acetylglucosamine pyrophosphorylase, dTDP-glucose pyrophosphorylase, GDP-mannose pyrophosphorylase and ADP-glucose pyrophosphorylase. However, prokaryotic UGPases are completely unrelated to their eukaryotic counterparts, although their sequences are well conserved, with more than 40% sequence identity between prokaryotic UGPases (Mollerach *et al.*, 1998; Mollerach & Garcia, 2000). The UGPase of *Helicobacter pylori* has only 11.2% sequence identity to that of humans. Taken together, these relations suggest that UGPase is an appropriate target for the development of new antibacterial agents (Mollerach *et al.*, 1998).

In order to investigate the structural basis of the catalytic mechanism and identify the active site of UGPase for future application in antibacterial drug development, structural studies are indispensable. As a first step in the structure determination, we report here the overexpression, purification, crystallization and preliminary crystallographic analysis of UGPase from *H. pylori*, a spiral-shaped Gram-negative bacterium that is a major human pathogen, living in the duodenum and stomach.

2. Experimental

2.1. Overexpression and purification

The HP0646 *galU* gene encoding UGPase (273 amino acids) was amplified from the genomic DNA of *H. pylori* strain 26 695 by PCR and inserted into an *Nde*I/*Xho*I-digested pET-21a vector (Novagen Inc., WI, USA). As a result, the recombinant protein has eight extra residues at its C-terminus, comprising Leu-Glu-$(His)_6$. The pET-21a plasmid containing the *galU* gene was transformed into *E. coli* BL21(DE3) competent cells (Stratagene, CA, USA). The transformed cells were inoculated into Luria–Bertani (LB) medium (Difco; Becton, Dickinson and Company, MD, USA) containing 50 µg ml^{-1} ampicillin and cultured at 310 K to an OD_{600} of 0.5. Expression of UGPase was induced by adding 0.5 m*M* isopropyl β-D-thiogalactopyranoside (IPTG)

and the cells were grown for an additional 4 h. The cultured cells were harvested by centrifugation using a Sorvall GS3 rotor (Kendro, NC, USA) at 5000*g* for 15 min. The harvested cell pellet was resuspended in buffer *A* (20 m*M* Tris–HCl pH 7.5, 0.5 *M* NaCl, 20 m*M* imidazole) and sonicated using a VCX 500 Ultrasonic Processor (Sonics and Materials Inc., CT, USA) at an amplitude of 60%. The cell lysates obtained were centrifuged using a Sorvall SS34 rotor at 30 000*g* for 1 h and the supernatant was loaded onto a HiTrap chelating column (Amersham Bioscience, Uppsala, Sweden) pre-equilibrated with buffer *A*. Unbound proteins and other substances in the column bed were washed out with buffer *B* (20 m*M* Tris–HCl pH 7.5, 2 *M* NaCl). UGPase was eluted with a linear gradient of 0.02–1.0 *M* imidazole in buffer *A*. The fractions containing UGPase were desalted using a HiTrap Desalting column (Amersham Bioscience, Uppsala, Sweden) and applied onto a Resource Q anion-exchange column (Amersham Bioscience, Uppsala, Sweden), which was equilibrated with buffer *C* (20 m*M* Tris–HCl pH 7.5, 0.1 *M* NaCl). UGPase was eluted with a linear gradient of 0.1–1.0 *M* NaCl in buffer *C*. The fractions containing UGPase were pooled and used for crystallization without His_6-tag cleavage. The purity of the protein was confirmed by SDS–gel electrophoresis.

2.2. Crystallization

Purified UGPase was concentrated to 7 mg ml^{-1} using a Centricon YM-10 (Millipore Corporation, MA, USA). Initial crystallization was performed by the hanging-drop vapour-diffusion method using commercially available screening kits (Hampton Research, CA, USA) at 295 K. Although a number of crystals were obtained using different conditions, rod-shaped crystals obtained using screen No. 47 from Crystal Screen I were selected for further optimization by using Additive Screens 1, 2 and 3 and Detergent Screens 1, 2 and 3 (Hampton Research, CA, USA).

2.3. X-ray data collection and analysis

Initial diffraction testing was performed using a Rigaku IV^{++} area detector and Cu $K\alpha$ X-rays (Rigaku, Japan). Complete data from a crystal flash-frozen at 100 K were collected on a Quantum 4R CCD detector using synchrotron radiation at beamline 38B1 of SPring-8, Japan. Prior to freezing in a cold nitrogen stream, crystals were immersed in cryoprotectant composed of crystallization solution containing 25% glycerol. The wavelength of the synchrotron radiation was 1.0000 Å and the distance between the crystal and detector was 210 mm. Using a 1° oscillation range and 30 s exposure per frame, a total of 116° of data were collected. Data were processed and integrated using *DENZO* and scaled using *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results and discussion

3.1. Crystallization

Several small crystals with different shapes were produced using the conditions described above over a period of a week. Of these, rod-shaped crystals grown from a solution containing 0.1 *M* sodium acetate trihydrate pH 4.6 and 2 *M* ammonium sulfate showed diffraction to medium resolution (Fig. 1*a*). However, these crystals were unsuitable for diffraction study since their diffraction pattern indicated that the crystals could be twinned and their growth rate could not be controlled.

Further optimization of the initial crystallization conditions using additive and detergent screens resulted in the growth of large single crystals from 0.1 *M* sodium acetate trihydrate pH 4.6, 2.0 *M* ammonium sulfate and 0.1 *M* guanidine–HCl at 295 K (Fig. 1*b*). Of 144 different conditions, guanidine–HCl was found to be the most effective additive for reducing crystal twinning. The crystals grew to typically 0.2 × 0.3 × 0.8 mm within 7 d.

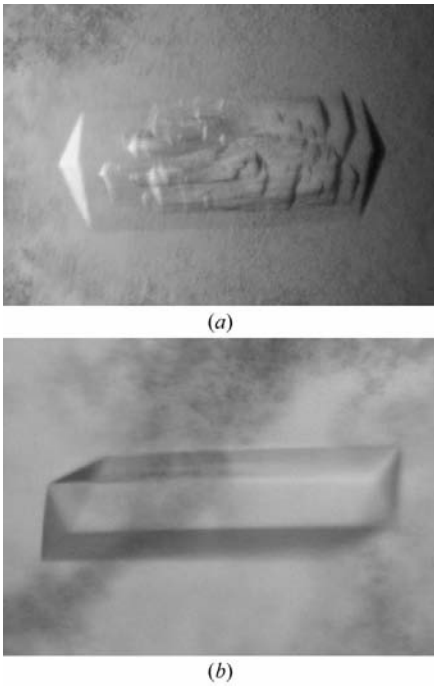

Figure 1
Crystals of UDP-glucose pyrophosphorylase from *H. pylori* grown in a solution containing 0.1 *M* sodium acetate trihydrate pH 4.6 and 2 *M* ammonium sulfate (*a*) in the absence and (*b*) in the presence of 0.1 *M* guanidine–HCl.

Table 1
X-ray data-collection and processing statistics.

Values in parentheses refer to the highest resolution shell.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 91.47, b = 98.61, c = 245.70
Resolution range (Å)	50–2.9 (3.0–2.9)
No. unique reflections	47 890 (4638)
Multiplicity	4.32
Data completeness (%)	95.5 (94.0)
R_{sym}† (%)	7.7 (31.0)
$I/\sigma(I)$	19.4 (3.5)
B factor (Å^2)	66.68

† $R_{sym} = \sum_{hkl} |I - \langle I \rangle| / \sum_{hkl} I$.

3.2. Diffraction data analysis

X-ray diffraction data were collected at a resolution of 2.9 Å. The data sets revealed that the crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 91.47, b = 98.61, c = 245.70 Å. The data consisted of 48 890 unique reflections merged from a total of 209 317 reflections with an R_{merge} of 7.7%. Assuming a molecular weight of 31 799 Da and eight molecules in the asymmetric crystallographic unit, the crystal volume per protein mass (V_M) and the solvent content of the crystal were estimated to be 2.18 Å^3 Da^{-1} and 43.5%, respectively. This V_M value is within the range commonly observed for protein crystals (Matthews, 1968). Table 1 summarizes the data-collection and processing statistics. Several similar structures have been deposited in the PDB, of which TDP-glucose pyrophosphorylase (TGPase) showed the highest sequence identity (27%). However, we failed in solving the structure of UGPase by the molecular-replacement (MR) method using TGPase. Therefore, we intend to solve the structure of *H. pylori* UGPase using multiple anomalous dispersion (MAD) methods.

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