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Isolation, crystallization and preliminary crystallographic analysis of *Salmonella typhimurium* uridine phosphorylase crystallized with 2,2′-anhydrouridine

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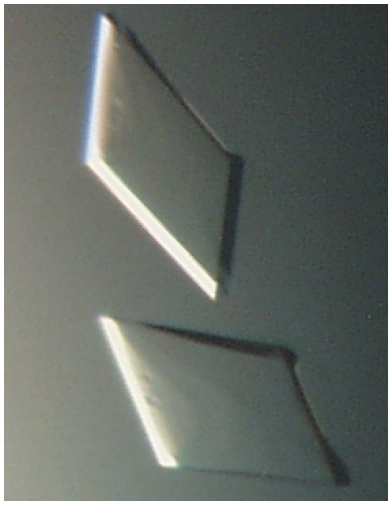

Uridine phosphorylase (UPh; EC 2.4.2.3) is a member of the pyrimidine nucleoside phosphorylase family of enzymes which catalyzes the phosphorolytic cleavage of the C—N glycoside bond of uridine, with the formation of ribose 1-phosphate and uracil. This enzyme has been shown to be important in the activation and catabolism of fluoropyrimidines. Modulation of its enzymatic activity may affect the therapeutic efficacy of chemotherapeutic agents. The structural investigation of the bacterial uridine phosphorylases, both unliganded and complexed with substrate/product analogues and inhibitors, may help in understanding the catalytic mechanism of the phosphorolytic cleavage of uridine. *Salmonella typhimurium* uridine phosphorylase has been crystallized with 2,2′-anhydrouridine. X-ray diffraction data were collected to 2.15 Å. Preliminary analysis of the diffraction data indicates that the crystal belongs to space group $P2_12_12_1$, with unit-cell parameters $a = 88.52$, $b = 123.98$, $c = 133.52$ Å. The solvent content is 45.51%, assuming the presence of one hexamer molecule per asymmetric unit.

1. Introduction

Salmonella enterica subspecies I, serovar Typhimurium (*S. typhimurium)*, is a leading cause of human gastroenteritis and is also a murine pathogen, causing a disease similar to typhoid fever in humans. Uridine phosphorylase (Uph; EC 2.4.2.3), a member of the pyrimidine nucleoside phosphorylase family of enzymes ubiquitously present in a variety of organisms, catalyzes the phosphorolytic cleavage of the uridine C—N glycoside bond with the formation of ribose 1-phosphate and uracil (Leer *et al.*, 1977; Vita *et al.*, 1986). The amount of this enzyme is frequently elevated in tumours (Leyva *et al.*, 1983). Uridine phosphorylase has been shown to be important in the activation and catabolism of fluoropyrimidines. Modulation of its enzymatic activity may affect the therapeutic efficacy of chemotherapeutic agents (Chu *et al.*, 1984; Ishitsuka *et al.*, 1980; Cao *et al.*, 2005).

The amino-acid sequences of bacterial and eukaryotic uridine phosphorylases show few similarities. Indeed, the identity between the sequences of the uridine phosphorylases from *S. typhimurium* and *Homo sapiens* is only 35%. However, the amino acids involved in the formation of the active centre appear to be conserved in bacterial and eukaryotic enzymes. This observation suggests a similarity in the spatial organization of the active site and the catalytic mechanism in all known uridine phosphorylases.

The crystal structure of the native *S. typhimurium* uridine phosphorylase (*St*UPh) has been determined previously (Dontsova *et al.*, 2005) and found to be hexameric. Here, we describe the preparation of new crystal form of the enzyme grown in the presence of the ligand 2,2′-anhydrouridine (ANU).

2. Materials and methods

2.1. Enzyme purification

The *St*UPh gene was cloned into pBluescript II SK plasmid and used for transformation of *Escherichia coli* BL21(DE3). The cells were grown on LB agar supplemented with 100 µg ml^{-1} ampicillin at

Table 1
Crystallographic data.

Values in parentheses are for the highest resolution shell (2.25–2.15 Å).

Space group	$P2_12_12_1$ [No. 19]
Unit-cell parameters (Å)	a = 88.52, b = 123.98, c = 133.52
Molecular weight (kDa)	165
Molecules per ASU	1 hexamer
X-ray source	Consortium beamline X13, DESY, Hamburg
Wavelength (Å)	1.05
Resolution of experimental set (Å)	88.52–2.15 (2.25–2.15)
No. of observed reflections	276750 (8452)
No. of unique reflections	75645 (1875)
Redundancy	3.7
Completeness (%)	99.6 (98.1)
Mosaicity (°)	0.287
Average $I/\sigma(I)$	17.87 (8.11)
$R_{\rm merge}$	4.7 (15.76)
$V_{\rm M}$ (Å^3 Da^{-1})	2.26
Solvent content (%)	45.51

310 K for 10 h. The cells were then subcultured into liquid LB supplemented with ampicillin and were grown at 310 K with intensive mixing (200–250 rev min^{-1}) until they reached an OD_{595} of 0.5. Protein synthesis was induced with isopropyl-β-D-1-thiogalactopyranoside for 3 h, after which the cells were pelleted and resuspended in buffer containing 100 m*M* Tris–HCl pH 8.0, 1.5 *M* NaCl, 4 m*M* EDTA and 1 m*M* β-mercaptoethanol. The cells were sonicated by ultrasound and cell debris was removed by centrifugation (15 000*g*). A solution containing 20 m*M* NaH_2PO_4 pH 7.0, 10%(*w*/*v*) polyethylenamine, 0.5 *M* NaCl and 0.5 m*M* β-mercaptoethanol was added to the supernatant and after 2 h at 277 K the mixture was centrifuged (10 000*g*; Beckman JA-10 rotor, 277 K, 15 min). The proteins were isolated from the supernatant by ammonium sulfate precipitation. The proteins were then dissolved in 50 m*M* KH_2PO_4 pH 7.2, 2 *M* ammonium sulfate and 0.5 m*M* β-mercaptoethanol for further chromatographic purification on butyl-Sepharose (Sigma). The protein was eluted from the column with ammonium sulfate (a gradient from 2 to 0 *M*). Fractions containing *St*UPh were pooled, dialyzed against 50 m*M* Tris–HCl pH 7.5 and loaded onto a Q-Sepharose (Sigma) column. This column was washed with a 0–1 *M* NaCl gradient. The eluted *St*UPh was dialyzed against 10 m*M* Tris–HCl pH 7.3 and concentrated to 10 mg ml^{-1}. The purity of *St*UPh was determined by electrophoresis in a 6% native (SDS-free) polyacrylamide gel with Coomassie staining. From SDS-containing gel electrophoresis, the estimated molecular weight of the *St*UPh molecule was 28 kDa. The molecular weight of *St*UPh estimated by native gel electrophoresis was 170 kDa, suggesting that the native form of the protein is a hexamer.

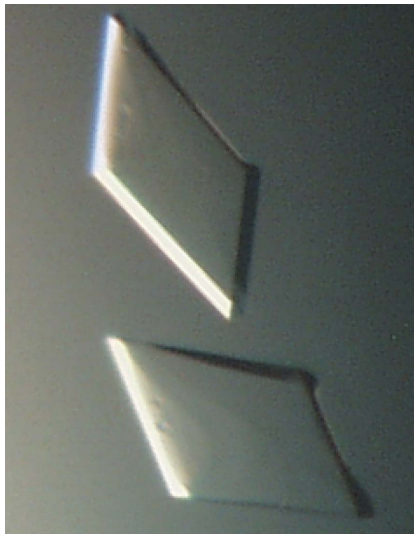

Figure 1
Crystals of StUPh.

2.2. Crystallization

Crystallization was performed using the hanging-drop vapour-diffusion method on siliconized glass slides in Linbro plates at 294 K. The complex of *St*UPh with 2,2′-anhydrouridine was obtained by cocrystallization. Initial screening for crystallization conditions was performed using commercially available sparse-matrix screens from Hampton Research. Optimization of crystallization conditions was performed by varying the pH and the concentrations of both *St*UPh and PEG. For the X-ray experiment, a crystal obtained as described below was used.

The reservoir solution (1 ml) contained 100 m*M* Tris–maleate/NaOH buffer pH 5.2 and 19%(*w*/*v*) PEG 3350. The crystallization drop contained 4 µl *St*UPh solution (9 mg ml^{-1}), 1.5 µl reservoir solution, 0.5 µl 10 m*M* ANU and 0.1 µl 1 m*M* potassium acetate. Well formed crystals were detectable after 2–4 d and grew to 0.2–0.3 mm (Fig. 1).

2.3. Data collection

X-ray data were collected from crystals of the complex of *St*UPh with 2,2′-anhydrouridine to 2.15 Å under cryogenic conditions (100 K) at the consortium beamline XI3 (DESY, Hamburg, Germany). Prior to freezing in liquid nitrogen, the crystals were transferred into a cryosolution buffer containing 60 m*M* sodium acetate trihydrate pH 5.2, 30%(*w*/*v*) PEG 400 and 10%(*w*/*v*) PEG 6000. All data were processed using the program *XDS* (Kabsch, 1988). The crystallographic data and statistics are summarized in Table 1.

3. Results and discussion

The crystals of *St*UPh with ANU belong to space group $P2_12_12_1$, with one *St*UPh hexamer in the asymmetric unit. The structure was solved by molecular replacement. The previously reported hexamer structure of *S. typhimurium* uridine phosphorylase at 2.5 Å resolution (Dontsova *et al.*, 2005; PDB code 1sj9) was used as the starting model. The amino-acid sequence was obtained from the SWISS-PROT database (CAA74658). Molecular-replacement calculations were performed using the program *Phaser* (McCoy, 2007) as implemented in the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). X-ray diffraction data from 25 to 2.5 Å were used for the rotation and translation functions. The best solution gave a correlation coefficient of 0.652 for the top peak (*R* factor = 0.344). Several cycles of rigid-body refinement were performed using the maximum-likelihood refinement option in *Phaser*.

The space group and unit-cell parameters of these crystals (Table 1) are strikingly different from those of the native (Dontsova *et al.*, 2005; space group $P6_1$; a = 91.37, c = 266.38 Å), despite the fact that the native crystals were obtained under similar conditions with a reservoir solution consisting of 100 m*M* sodium acetate pH 5.0, 7%(*w*/*v*) PEG 8000. We anticipate that the structure determination in progress will reveal that these are crystals of the complex of *St*UPh with ANU.

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