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Crystallization and preliminary X-ray crystallographic analysis of deoxyuridine triphosphate nucleotidohydrolase from *Saccharomyces cerevisiae*

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Deoxyuridine triphosphate nucleotidohydrolase (dUTPase) from *Saccharomyces cerevisiae* is essential for cell viability. It has been overexpressed in *Escherichia coli* and has been crystallized at 296 K using polyethylene glycol (PEG) 1500 as a precipitant. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 59.48, b = 138.54, c = 157.91 Å, $\alpha = \beta = \gamma = 90°$. Two molecules of trimeric dUTPase from *S. cerevisiae* are present in the asymmetric unit, giving a crystal volume per protein mass (V_M) of 3.36 $Å^3$ Da^{-1} and a solvent content of 63%. The diffraction limit of the crystals could be significantly extended by the crystal-annealing procedure. A set of native data extending to 2.7 Å resolution has been collected at 100 K using synchrotron X-rays.

1. Introduction

dUTP pyrophosphatase (dUTPase, deoxyuridine 5′-triphosphate nucleotidohydrolase; E.C. 3.6.1.23) catalyses the hydrolysis of dUTP to dUMP and inorganic pyrophosphate (Kornberg & Baker, 1991). This reaction is important as an intermediate step in the synthesis of thymine nucleotides and has an essential role in maintaining sufficiently low dUTP levels to prevent incorporation of uracil into DNA (Dauter *et al.*, 1999). dUTPases are present in organisms ranging from viruses to higher eukaryotes; its activity is essential for the cell viability of *Escherichia coli* (El-Hajj *et al.*, 1988) and *S. cerevisiae* (Gadsden *et al.*, 1993). Five conserved motifs have been revealed by amino-acid sequence alignment (Prasad *et al.*, 1996).

Three-dimensional structures have been determined for the enzymes from *E. coli* (Cedergren-Zeppezauer *et al.*, 1992; Larsson *et al.*, 1996), feline immunodeficiency virus (Prasad *et al.*, 1996), equine infectious anaemia virus (Dauter *et al.*, 1999) and human (Mol *et al.*, 1996). They are all trimers with a similar overall structure, each active site being formed with participation of all three subunits in the trimer. dUTPases from equine infectious anaemia virus and human are potential targets for antiviral and anticancer drug design (Dauter *et al.*, 1999; Harris *et al.*, 1999). dUTPase from *Candida albicans*, a major human pathogen, may also be a potentially useful target for the development of novel antifungal agents (McIntosh *et al.*, 1994). dUTPase from *S. cerevisiae* is a homotrimeric enzyme of 147-residue subunits. Since it is highly similar in its amino-acid sequence to *C. albicans* dUTPase (92 identical residues), the structure of *S. cerevisiae* dUTPase could serve as a template to build the homology model of *C. albicans* dUTPase, the crystallization of which has not yet been reported. Here, we describe the crystallization of dUTPase from *S. cerevisiae* and preliminary X-ray crystallographic data.

2. Experimental

2.1. Protein expression and purification

The gene encoding dUTPase was amplified by the polymerase chain reaction using the *S. cerevisiae* genomic DNA as template. The amplified DNA was inserted into the *Nde*I/*Xho*I-digested expression vector pET-22b. This vector construction adds six histidine residues to the C-terminus of the gene product to facilitate protein purification. The complete nucleotide sequence of the insert was confirmed by dideoxy DNA sequencing performed at the Research Center for Microbiology, Seoul National University. The enzyme was highly overexpressed in soluble form in B834(DE3) cells upon induction by 0.5 m*M* isopropyl-β-D-thiogalactopyranoside (IPTG) at 310 K. Cells were grown in Luria–Bertani medium for 4 h after IPTG induction and were harvested by centrifugation at 6000 rev min^{-1} (Sorvall GSA rotor) for 10 min at 277 K. The cell pellet was resuspended in ice-cold lysis buffer (20 m*M* Tris–HCl pH 7.9, 500 m*M* NaCl, 50 m*M* imidazole and 1 m*M* PMSF) and was then homogenized by sonication. The crude lysate was centrifuged at 36 000*g* (18 000 rev min^{-1}, Hanil Supra 21K rotor) for 30 min at 277 K and the recombinant

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Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (2.75–2.70 Å).

X-ray wavelength (Å)	1.000 (Photon Factory, BL-6A)
Temperature (K)	100
Resolution range (Å)	30–2.7
No. of observations	262682
Unique reflections	35652
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 59.48, b = 138.54, c = 157.91
Completeness (%)	96.8 (92.4)
Mean $I/\sigma(I)$	21.3 (5.2)
$R_{\rm merge}$† (%)	10.4 (35.1)

† $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of reflection h, $\sum_h$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection h.

protein in the supernatant fraction was purified by four chromatographic steps. The first step utilized the C-terminal histidine tag by metal-chelate chromatography on Ni-NTA resin (Qiagen). Next, gel filtration was performed on a HiLoad XK 16 Superdex 200 prep-grade column (Amersham-Pharmacia), which was previously equilibrated with buffer *A* (25 m*M* Tris–HCl pH 8.5, 1 m*M* $MnCl_2$, 1 m*M* β-mercaptoethanol) containing 100 m*M* sodium chloride. Further purification was achieved by an ion-exchange chromatographic step on Mono-Q resin packed in a HR10/10 column (Amersham-Pharmacia) which was previously equilibrated with buffer *A*. Before loading the protein sample onto this ion-exchange column, the salt concentration was lowered to 25 m*M* by dialyzing against buffer *A* containing 25 m*M* sodium chloride. The protein was eluted with a linear gradient of 0–1.0 *M* sodium chloride in buffer *A*. The purified dUTPase was homogeneous as judged by polyacrylamide gel electrophoresis in the presence of 0.1%(*w*/*v*) sodium dodecyl sulfate (Laemmli, 1970). This procedure yielded approximately 15 mg of homogeneous enzyme from a 2 l culture.

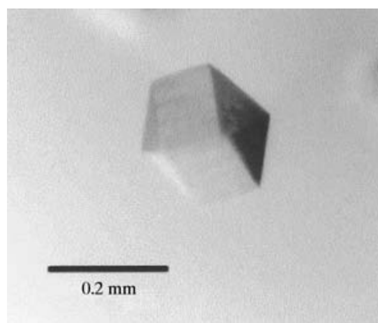

Figure 1
An orthorhombic crystal of dUTPase from *S. cerevisiae*. The largest crystals have approximate dimensions of 0.2 × 0.15 × 0.15 mm.

2.2. Crystallization and X-ray data collection

The protein solution was concentrated using a YM10 membrane (Amicon). The protein concentration was estimated by measuring the absorbance at 280 nm, assuming a correspondence of 0.83 mg $\rm ml^{-1}$ concentration to the unit absorbance for 1.0 cm path length. Crystallization was performed by the hanging-drop vapour-diffusion method using 24-well tissue-culture plates (Hampton Research) at 297 K. Each hanging drop was prepared by mixing 2 µl each of the protein solution and the reservoir solution and was placed over 1 ml of the reservoir solution. Results from the initial searches for crystallization conditions performed using Screen I (Jancarik & Kim, 1991) and Screen II kits (Hampton Research) were optimized.

A crystal of *S. cerevisiae* dUTPase was directly transferred to a solution of 40%(*w*/*v*) PEG 1500, 100 m*M* *N*-(2-acetamido)-2-iminodiacetic acid (ADA) at pH 6.5 before being flash-frozen. The crystal annealing was performed according to Harp *et al.* (1998), with a 5 min incubation time. The diffraction data were collected at 100 K using a Weissenberg camera for macromolecular crystallography at the BL-6A experimental station of Photon Factory, Tsukuba, Japan (Sakabe, 1991). The wavelength of the synchrotron X-rays was 1.000 Å and a 0.1 mm collimator was used. One image plate (20 × 40 cm, Fuji BAIII) was placed at a distance of 429.7 mm from the crystal. The oscillation range per image plate was 3.5°, with a speed of 2.0° $\rm s^{-1}$ and a coupling constant of 1.0° $\rm mm^{-1}$. An overlap of 0.5° was allowed between contiguous image plates. The diffraction patterns recorded on the image plates were digitized by the off-line scanner Fuji BA100. The raw data were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results

Crystals of recombinant dUTPase from *S. cerevisiae* have been obtained using PEG 1500 as precipitant. The optimized reservoir solution consisted of 23.4%(*w*/*v*) PEG 1500, 100 m*M* ADA pH 6.5. Crystals grew to dimensions of approximately 0.2 × 0.15 × 0.15 mm within 4 d (Fig. 1). The flash-frozen crystals diffracted initially to ∼3.0 Å using synchrotron X-rays. Therefore, crystal annealing was attempted and the diffraction limit was significantly improved to ∼2.2 Å resolution. Owing to the limitation on beam time, data were collected to 2.7 Å resolution at 100 K using 1.000 Å X-rays. Table 1 summarizes the data-collection statistics. The autoindexing procedure performed with *DENZO* indicated that the crystals belong to a primitive orthorhombic space group, with unit-cell parameters a = 59.48 (14), b = 138.54 (33), c = 157.91 (61) Å and $\alpha = \beta = \gamma = 90°$, where estimated standard deviations are given in parentheses. The space group was determined to be $P2_12_12_1$ on the basis of systematic absences. The asymmetric unit contains two homotrimeric molecules of dUTPase, giving a crystal volume per protein mass ($V_{\rm M}$) of 3.36 $\rm Å^3\,Da^{-1}$ and a solvent content of 63%, which are within the frequently observed ranges for protein crystals (Matthews, 1968). A molecular-replacement solution could be found using the human enzyme, which shows 51.0% sequence identity, as a model, but it could not be refined. Enzymes from *E. coli*, equine infectious anaemia virus and feline immunodeficiency virus show lower levels of sequence identity (27.2, 26.5 and 23.8%, respectively). Therefore, the structure will be solved by multiple isomorphous replacement or multiwavelength anomalous diffraction.

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