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Crystallization and preliminary X-ray analysis of CTP:phosphoethanolamine cytidylyltransferase (ECT) from *Saccharomyces cerevisiae*

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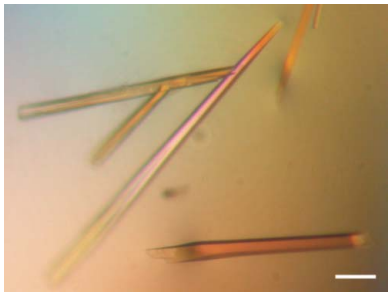

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CTP:phosphoethanolamine cytidylyltransferase (ECT) is the enzyme that catalyzes the conversion of phosphoethanolamine to CDP-ethanolamine in the phosphatidylethanolamine-biosynthetic pathway (Kennedy pathway). ECT from *Saccharomyces cerevisiae* was crystallized by the sitting-drop vapour-diffusion method using PEG 4000 as precipitant. The crystals diffracted X-rays from a synchrotron-radiation source to 1.88 Å resolution. The space group was assigned as primitive tetragonal, $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 66.3$, $c = 150.8$ Å. The crystals contain one ECT molecule in the asymmetric unit ($V_M = 2.2$ Å^3 Da^{-1}), with a solvent content of 43%.

1. Introduction

Phosphatidylethanolamine (PE) is a major glycerophospholipid in both prokaryotes and eukaryotes. Two biosynthetic pathways of PE are known in eukaryotes: the CDP-ethanolamine pathway (Kennedy pathway) and decarboxylation of phosphatidylserine. The CDP-ethanolamine pathway, the main pathway of PE biosynthesis (Arthur & Page, 1991; Tijburg *et al.*, 1989), involves three enzymatic reactions. Firstly, ethanolamine is phosphorylated at its hydroxyl group to form *O*-phosphoethanolamine by ethanolamine kinase (EKI). Secondly, CTP is used for cytidylation of *O*-phosphoethanolamine to synthesize CDP-ethanolamine and a byproduct, pyrophosphate, by CTP: phosphoethanolamine cytidylyltransferase (ECT). Finally, PE is formed from CDP-ethanolamine and diacylglycerol by CDP-ethanolamine:*sn*-1,2-diacylglycerol ethanolaminephosphotranferase (EPT). ECT is proposed to be the key regulator of PE synthesis *via* the CDP-ethanolamine pathway (Sundler, 1975; Infante & Kinsella, 1979). ECT consists of two cytidylyltransferase (CT) domains and each CT domain contains a nucleotide-binding motif H*X*GH (Park *et al.*, 1997) in its N-terminal region. Although ECTs from several organisms such as *Rattus norvegicus* and *Chlamydomonus reinhardtii* have been well characterized enzymatically, no crystallization of an ECT has been reported so far. Here, we report the crystallization and preliminary X-ray analysis of *Saccharomyces cerevisiae* ECT, which is encoded by the MUQ1/ECT1 gene (Min-Seok *et al.*, 1996) and consists of 323 amino-acid residues. In *S. cerevisiae* ECT, the amino-acid sequence identity between the N- and C-terminal CT domains is 21% and the H*X*GH motif in the C-terminal CT domain is replaced by H*X*GD. The most similar protein to ECT with known crystal structures is GCT (*sn*-glycerol 3-phosphate cytidylyltransferase), an enzyme involved in teichoic acid biogenesis in Gram-positive bacteria. Crystal structures of GCTs from *Bacillus subtilis* (TagD) and *Staphylococcus aureus* (TarD), which consist of 129 and 132 amino-acid residues, respectively, comprise a CT domain (Weber *et al.*, 1999; Pattridge *et al.*, 2003; Fong *et al.*, 2006). They are 23 and 24% identical to the N-terminal CT domain and 23 and 21% identical to the C-terminal CT domain of *S. cerevisiae* ECT, respectively.

2. Methods and results

2.1. Purification and crystallization

Recombinant *S. cerevisiae* ECT with an N-terminal 6×His tag and thrombin-cleavage site (MGSSHHHHHHSSGLVPRGSHMLEDQ-

FH**M**..., where the M in bold is the N-terminal methionine of *S. cerevisiae* ECT) was overexpressed in *Escherichia coli* strain BL21(DE3) using pET-15b expression vector (Novagen). *E. coli* cells were harvested by centrifugation, resuspended in buffer *A* [25 m*M* HEPES pH 7.7, 500 m*M* NaCl, 5%(*v*/*v*) glycerol and 2 m*M* β-mercaptoethanol] and disrupted by sonication. The cell lysate was centrifuged to remove cell debris and the supernatant was applied onto an Ni–NTA agarose (Qiagen) column. The column was washed with buffer *A* supplemented with 20 m*M* imidazole and 6×His-tagged ECT was then eluted with buffer *A* supplemented with 500 m*M* imidazole. The 6×His tag was cleaved off with thrombin (Amersham Biosciences) and the resulting ECT which has ten extra residues (GSHMLEDQFH) at the N-terminus was separated from 6×His-tagged ECT and 6×His tag using an Ni–NTA agarose column. As the final purification step, gel-filtration chromatography was performed with a HiLoad Superdex 75pg 26/60 column (Amersham Biosciences) equilibrated with buffer *B* (20 m*M* MES pH 6.5, 150 m*M* NaCl and 1 m*M* TCEP–HCl), which indicated that ECT was monomeric in solution. An Apollo centrifugal ultrafiltration device (Orbital Biosciences) was used to remove NaCl from the protein solution and to concentrate purified ECT to 5.0 mg ml^{-1}.

Crystallization trials were performed by the sitting-drop vapour-diffusion method using Crystal Screens 1 and 2 (Hampton Research). In the presence of 1 m*M* CTP, small pillar-shaped crystals appeared under condition No. 9 of Crystal Screen 1, in which polyethylene glycol (PEG) 4000 was used as the precipitant. After refinement of the crystallization conditions, crystals suitable for X-ray analysis were obtained with the reservoir solution 24%(*w*/*v*) PEG 4000, 100 m*M* trisodium citrate pH 5.2, 200 m*M* ammonium acetate. A sitting drop was prepared by mixing 4.0 µl each of protein solution containing 5.0 mg ml^{-1} ECT, reservoir solution, additive solution (10 m*M* $MgCl_2$ and 2 m*M* CTP dissolved in H_2O) and H_2O to give a total drop volume of 16 µl. In order to reduce the number of crystals in a drop, streak-seeding was applied and drops were equilibrated against 1.0 ml reservoir solution at 278 K. Long pillar-shaped crystals grew in 2 d. Crystals thus obtained are shown in Fig. 1.

2.2. X-ray data collection and processing

A crystal of ECT was picked up in a nylon loop (Hampton Research), transferred to a series of cryoprotectant solutions containing 8, 16 and 24%(*v*/*v*) glycerol and mounted for flash-cooling at 100 K using a nitrogen stream. The above-mentioned cryoprotectant solutions were prepared by mixing 80%(*v*/*v*) glycerol and a solution whose composition was almost identical to the crystallization drop [24%(*w*/*v*) PEG 4000, 100 m*M* trisodium citrate pH 5.2, 200 m*M* ammonium acetate, 10 m*M* $MgCl_2$, 2 m*M* CTP, 20 m*M* MES pH 6.5, 1 m*M* TCEP–HCl].

X-ray diffraction data were collected at beamline NW12 at the Photon Factory Advanced Ring, Tsukuba, Japan with 0.5° oscillations, a crystal-to-detector distance of 180 mm and a wavelength of 1.0000 Å; the crystal diffracted X-rays to 1.88 Å resolution (Fig. 2). The diffraction data were indexed, integrated and scaled using *HKL*-2000 (Otwinowski & Minor, 1997). The space group was assigned to be $P4_12_12$ or $P4_32_12$ based on the Laue symmetry and the systematic absences. The Matthews coefficient (V_M = 2.2 Å^3 Da^{-1}; Matthews, 1968) and solvent content (43%) indicated that the crystal contained one ECT molecule per asymmetric unit. The data statistics are summarized in Table 1.

Structural determination by molecular replacement using the coordinates of GCTs (Weber *et al.*, 1999; Pattridge *et al.*, 2003; Fong *et al.*, 2006) as search models is currently under way.

Table 1
Crystal parameters of yeast ECT.

Values in parentheses are for the highest resolution shell.

X-ray source	PF-AR NW12
Wavelength (Å)	1.0000
Space group	$P4_12_12$ or $P4_32_12$
Unit-cell parameters (Å)	$a = b = 66.3$, $c = 150.8$
Resolution range (Å)	50.0–1.88 (1.91–1.88)
Reflections observed	91691
Unique reflections	28316
Redundancy	3.3 (3.0)
Data completeness (%)	97.4 (92.0)
Mosaicity (°)	0.67
R_{merge}†	0.072 (0.291)
$\langle I\rangle/\langle\sigma(I)\rangle$	14.6 (3.68)

† $R_{merge} = \sum_{hkl}[(\sum_i |I_i - \langle I\rangle|)/\sum_i |I_i|]$, where I_i is the ith intensity measurement of reflection hkl, including symmetry-related reflections, and $\langle I\rangle$ is its average.

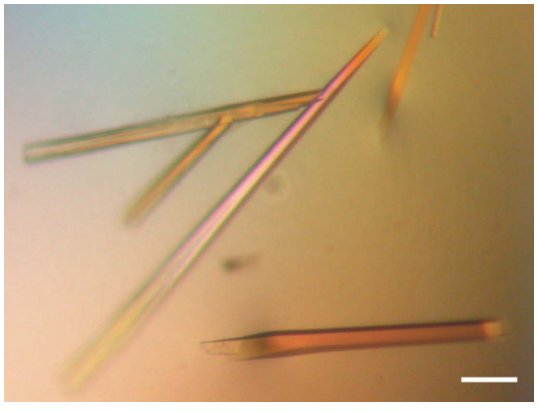

Figure 1
Crystals of *S. cerevisiae* ECT in the presence of magnesium ion and CTP with approximate dimensions 1.0 × 0.3 × 0.3 mm. The scale bar corresponds to 0.1 mm.

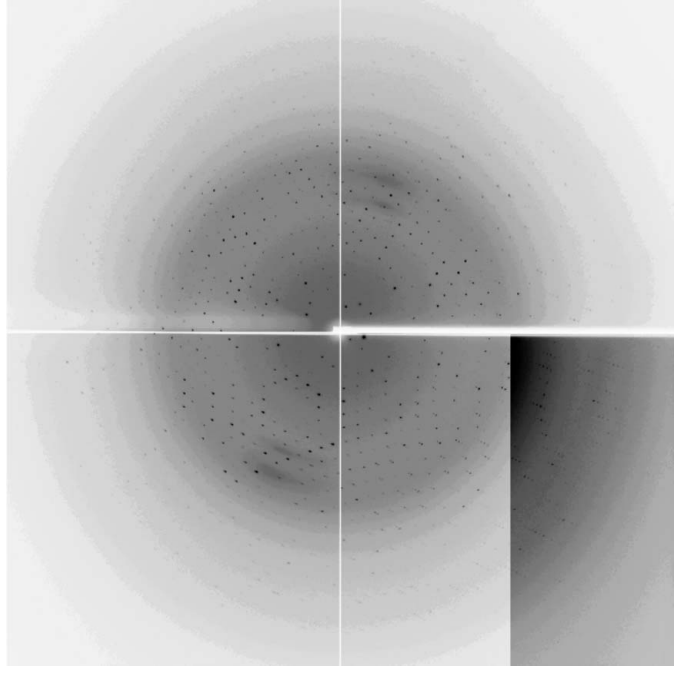

Figure 2
An X-ray diffraction image (0.5° oscillation) of a crystal of *S. cerevisiae* ECT. The edge of the diffraction image corresponds to a resolution of 1.92 Å.

The synchrotron-radiation experiments were performed at NW12 at Photon Factory Advanced Ring (PF-AR, Tsukuba, Japan) with the approval of Photon Factory, KEK (Proposal No. 2003S2-002). This work was partly supported by the National Project on Protein Structural and Functional Analysis of the Ministry of Education, Culture, Sports, Science and Technology of Japan and by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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