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Expression, purification and preliminary X-ray characterization of CTP synthetase from *Thermus thermophilus* HB8

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A recombinant form of the CTP synthetase from *Thermus thermophilus* HB8 (tCTPs) was grown as colourless crystals by the hanging-drop vapour-diffusion technique using ammonium sulfate or sodium citrate as a precipitating agent. The crystals belong to space group *I*222, with unit-cell parameters $a = 88.2$, $b = 118.9$, $c = 142.7$ Å, $\alpha = \beta = \gamma = 90^\circ$, and are most likely to contain a monomer in the asymmetric unit with a V_M value of 3.07 Å^3 Da^{-1}. The crystals obtained from ammonium sulfate and sodium citrate solutions diffract X-rays to a resolution of 2.25 Å using synchrotron X-ray sources and to a resolution of 2.35 Å using Cu $K\alpha$ X-rays from a rotating-anode generator.

1. Introduction

Glutamine-dependent amidotransferases are modular enzymes consisting of two domains: glutaminase domains, which have evolved from the same ancestor, and synthetase domains, which are evolutionally unrelated and have functions different from each other (Massiere & Badet-Denisot, 1998; Zalkin & Smith, 1998). The enzymes hydrolyze glutamine to ammonia and glutamate at the glutaminase domain and transfer nascent ammonia to an acceptor substrate to give the aminated product at the synthase domain. Glutamine-dependent amidotransferases are divided into two subfamilies, classes I and II. Class I is characterized by the presence of the triad Cys-His-Glu in the glutaminase domain (subunit) and class II by the presence of the N-terminal catalytic Cys residue. CTP synthetase (CTPs) belonging to a class I glutamine-dependent amidotransferase catalyzes the last step of CTP biosynthesis, in which glutamine is hydrolyzed at the glutaminase domain and nascent ammonia reacts with the ATP-phosphorylated UTP to give CTP at the synthase domain (Koshland & Levitzki, 1974; Weng & Zalkin, 1987). CTP synthetase is inhibited by the product, CTP (Levitzki & Koshland, 1970), and shows negative and positive cooperativity for glutamine and GTP and for ATP and UTP, respectively (Bearne *et al.*, 2001). Since CTPs, which is allosterically regulated, is essential for RNA, DNA and phospholipid biosynthesis, many biochemical and kinetic studies of CTPs have been performed (Ostrander *et al.*, 1998; Bearne *et al.*, 2001).

The CTPs from *Thermus thermophilus* HB8 (tCTPs), which has been cloned and overexpressed in *Escherichia coli*, has 550 residues per subunit with a molecular weight of 60 935 Da. Sequence comparison of tCTPs with the glutamine-dependent amidotranferases indicate that the glutaminase domain is comprised of the C-terminal 250 residues, with a catalytic Cys391–His522 couple (SWISS-PROT; Huang & Raushel, 1999). The synthase domain (N-terminal 300 residues) appears not to have a counterpart in glutamine-dependent amidotransferases, although a 13% sequence identity with the synthase subunit in GMP synthetase is observed. CTPs performs similar chemistry to GMP synthetase, which catalyzes the ATP-dependent amination of xanthosine 5′-monophosphate to form GMP.

Structure determination of tCTPs should help in understanding the mechanism of the catalysis and allosteric regulation of tCTPs. Recent X-ray studies on glutamine-dependent amidotransferases have shown that the ammonia produced in the glutaminase subunit is transferred to the synthase subunit through the channel formed inside a protein molecule (Thoden *et al.*, 1997; Knochel *et al.*, 1999; Spraggon *et al.*, 2001; Parsons *et al.*, 2002). The mechanism of ammonia transfer through the molecular channel in CTPs is an interesting question to be addressed. In this communication, we report the expression, purification, crystallization and the results of preliminary X-ray diffraction studies of tCTPs.

2. Experimental

2.1. Expression and purification

The tCTPs gene amplified by PCR was subcloned into the *Nde*I and *Bam*HI sites of pET11a. The sequence of the primers were as follows: 5′-primer, 5′-ATATcatatgAATGGGAGCGCCGACGCGGGTCCCAGGC-3′ (*Nde*I

site in lower case); 3′-primer, 5′-ATATagatctTTATTAGGCCCTTTCCTGGTAGGCCAGCGCC-3′ (*Bgl*II site in lower case). *E. coli* BL21(DE3) cells carrying the resultant expression plasmid were grown overnight in a medium containing 1.0% polypeptone, 0.5% yeast extract and 0.5% NaCl (adjusted to pH 7.0 with NaOH) supplemented with 50 µg ml^{-1} ampicillin at 310 K.

The cells were suspended in 20 m*M* Tris–HCl buffer pH 8.0 containing 50 m*M* NaCl and 5 m*M* 2-mercaptoethanol and disrupted by sonication. The lysate was incubated at 343 K for 10 min, kept on ice for 12 min and ultracentrifuged (200 000*g*) for 60 min at 277 K. The following procedures were all performed at room temperature. Ammonium sulfate was added to the resultant supernatant to make a 27% saturated ammonium sulfate solution. This solution was applied onto a Resource ISO column (Amersham Bioscience) equilibrated with 50 m*M* sodium phosphate buffer pH 7.0 containing 27% saturated ammonium sulfate and eluted with a linear gradient of ammonium sulfate from 27 to 0% saturation in 50 m*M* sodium phosphate buffer pH 7.0. The fractions containing tCTPs were pooled, desalted using a HiPrep 26/10 desalting column (Amersham Bioscience) and applied onto a Resource Q column (Amersham Bioscience) equilibrated with 20 m*M* Tris–HCl buffer pH 8.0. tCTPs was eluted with a linear gradient of NaCl from 0 to 0.5 *M* in the same buffer. Fractions containing tCTPs were pooled and applied onto a CHT-2I column (Bio-Rad) equilibrated with 10 m*M* sodium phosphate buffer pH 7.0. The flow-through fractions containing tCTPs were pooled and concentrated by ultrafiltration. The concentrated solution was applied onto a HiLoad 16/60 Superdex 200pg column (Amersham Bioscience) equilibrated with 20 m*M* Tris–HCl pH 8.0 containing 150 m*M* NaCl. The fractions containing tCTPs were pooled and then stored at 277 K.

2.2. Crystallization

tCTPs was crystallized by the hanging-drop vapour-diffusion method at 293 K. The initial screening for the crystallization conditions was performed using the sparse-matrix screens Crystal Screen I and II from Hampton Research (Jancarik & Kim, 1991). Minute crystals appeared and the crystallization conditions were optimized. Crystals were obtained from two different crystallization conditions, (*a*) and (*b*). Condition (*a*) involves equilibration of a mixture containing 3 µl protein solution (11.0 mg ml^{-1} protein, 10 m*M* Tris–HCl pH 8.5) and 3 µl reservoir solution [1.4 *M* ammonium sulfate, 10%(*v*/*v*) dioxane, 20%(*v*/*v*) glycerol, 100 m*M* Tris–HCl, pH 8.5] against 400 µl of reservoir solution. Condition (*b*) is the same as condition (*a*) except that 0.6 *M* trisodium citrate was used as the precipitating agent instead of 1.4 *M* ammonium sulfate.

2.3. Data collection

For preliminary characterization, crystals were mounted in glass capillaries with a small amount of mother liquor and the intensity data for unit-cell parameter and space-group determination were collected on a Rigaku R-AXIS IV++ image-plate detector. Data collection for the crystal obtained from condition (*a*) was performed at 100 K using a wavelength of 1.00 Å from the synchrotron-radiation source at SPring-8 BL44B2 and a MAR CCD165 detector system (Hyogo, Japan). The crystal was mounted in a 0.5 mm cryoloop (Hampton Research) and flash-frozen in a cold nitrogen stream at 100 K. The data were processed using *HKL*2000 (Otwinowski & Minor, 1997). Data collection for the crystal obtained from condition (*b*) was performed at 100 K on an R-AXIS IV++ image-plate detector equipped with Osmic Maxflux optics using a wavelength of 1.54 Å (Cu $K\alpha$) from a Rigaku rotating-anode generator operated at 40 kV and 100 mA. The data were processed using *Crystalclear* (Molecular Structure Corporation, a Rigaku company).

3. Results and discussion

In both conditions (*a*) and (*b*) crystals of tCTPs appeared within a week of incubation and grew to maximum dimensions of 0.4 × 0.2 × 0.2 mm (Fig. 1). From the diffraction data collected on the R-AXIS IV++, unit-cell parameters were determined to be

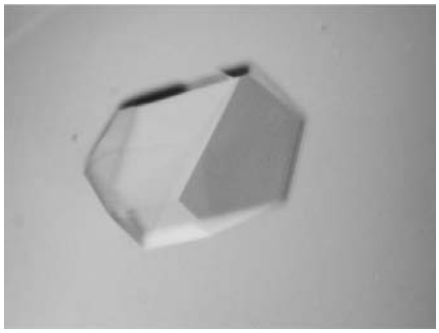

Figure 1
An orthorhombic crystal of CTP synthetase obtained from ammonium sulfate solution.

Table 1
Data-collection and processing statistics.

Values for the highest resolution shell are given in parentheses

Data set	Condition (*a*)	Condition (*b*)
Space group	*I*222	*I*222
Unit-cell parameters (Å)	*a* = 90.4, *b* = 117.1, *c* = 142.3	*a* = 86.0, *b* = 120.7, *c* = 143.1
Temperature (K)	100	100
Wavelength (Å)	1.00	1.54
Resolution range (Å)	50.0–2.25 (2.33–2.25)	46.7–2.35 (2.43–2.35)
No. of reflections	245096	106978
No. of unique reflections	36023	31395
Completeness (%)	99.2 (99.0)	99.5 (99.5)
$R_{\rm merge}$† (%)	4.0 (30.7)	5.2 (29.9)
Mean $I/\sigma(I)$	30.0 (2.4)	9.7 (2.0)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_{hkl,i} - \langle I_{hkl}\rangle|/\sum_{hkl}\sum_i I_{hkl,i}$, where I is the observed intensity and $\langle I\rangle$ is the average intensity for multiple measurements.

a = 90.4, b = 117.1, c = 142.3 Å and a = 86.0, b = 120.7, c = 143.1 Å for the crystals obtained from conditions (*a*) and (*b*), respectively, with the an orthorhombic *I*222 space group. It is very likely that the different conditions for crystallization, cryogenic experiments and data collection caused the differences in these two sets of unit-cell parameters. Assuming one monomer in the asymmetric unit, the Matthews coefficient ($V_{\rm M}$) values were calculated as 3.09 and 3.05 Å^3 Da^{-1}, respectively, indicating solvent contents of approximately 54 and 53% in the unit cell for the crystals obtained from conditions (*a*) and (*b*). These values are within the range for typical protein crystals (Matthews, 1968). A data set for the crystal obtained from condition (*a*) has been collected with 36 023 unique reflections, giving a data-set completeness of 99.2% in the resolution range 50.0–2.25 Å, with an $R_{\rm merge}$ of 4.0% (Table 1). A data set for the crystal obtained from condition (*b*) has been collected with 31 395 unique reflections, giving a data-set completeness of 99.5% in the resolution range 46.7–2.35 Å, with an $R_{\rm merge}$ of 5.2% (Table 1). These data indicate good-quality crystals for X-ray structural analysis. The crystals showed no significant decay upon exposure.

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