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# Crystallization and preliminary X-ray crystallographic study of the editing domain of *Thermus thermophilus* isoleucyl-tRNA synthetase complexed with pre- and post-transfer editing-substrate analogues

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The CP1 domain (the editing domain) of isoleucyl-tRNA synthetase (IleRS) hydrolyzes misactivated Val-AMP in pre-transfer editing and mischarged Val-tRNA$^{Ile}$ in post-transfer editing. The CP1 domain of *Thermus thermophilus* IleRS was expressed in isolation, purified and cocrystallized with Val-AMS (a Val-AMP analogue) and with Val-2AA (a Val-tRNA$^{Ile}$ analogue). Two different expression constructs were used for each cocrystallization. The complex crystals with Val-AMS belong to the tetragonal space group $P4_12_12$, with unit-cell parameters $a = b = 102.00$, $c = 84.88$ Å. The asymmetric unit contains two molecules of the CP1 domain, with a corresponding crystal volume per protein weight of 2.7 Å$^3$ Da$^{-1}$ and a solvent content of 53.5%. The complex crystals with Val-2AA belong to the tetragonal space group $P4_122$, with unit-cell parameters $a = b = 72.59$, $c = 83.68$ Å. The asymmetric unit contains one molecule of the CP1 domain, with a corresponding crystal volume per protein weight of 2.8 Å$^3$ Da$^{-1}$ and a solvent content of 55.8%. Data sets diffracting to 1.7 Å resolution were collected from each single crystal at 100 K.

## 1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) are a family of enzymes that catalyze the specific attachment of an amino acid to the 3′-end of its cognate tRNA. This reaction proceeds in two steps: the synthesis of an aminoacyladenylate as an activated intermediate from the amino acid and ATP and the transfer of the aminoacyl moiety to the 3′-terminal of the cognate tRNA to yield the aminoacyl-tRNA (aa-tRNA) (Fersht & Kaethner, 1976). To maintain accurate protein biosynthesis, each aaRS must discriminate between its cognate amino acid and other similar amino acids (Freist *et al.*, 1988). Some aaRSs, including the isoleucyl-, leucyl- and valyl-tRNA synthetases (IleRS, LeuRS and ValRS, respectively), have a specific editing activity that hydrolyzes the misactivated aminoacyladenylates ('pre-transfer editing') or the misaminoacylated tRNAs ('post-transfer editing') (Baldwin & Berg, 1966; Fersht, 1977; Englisch *et al.*, 1986; Jakubowski & Goldman, 1992; Hale *et al.*, 1997). For example, in addition to the cognate isoleucine, the IleRS aminoacylation active site also recognizes valine, which is smaller than isoleucine by only one methylene group, misactivates it to Val-AMP and subsequently mischarges it to tRNA$^{Ile}$, producing Val-tRNA$^{Ile}$. The misactivated Val-AMP is then hydrolyzed to valine and AMP in the pre-transfer editing pathway, while in the post-transfer editing pathway the mischarged Val-tRNA$^{Ile}$ is hydrolyzed to valine and tRNA$^{Ile}$ (Fersht, 1977; Hale *et al.*, 1997). The pre-transfer editing proceeds only in the presence of tRNA$^{Ile}$. Biochemical and structural studies have suggested that the hydrolytic activity is associated with an independently folded domain (the CP1 domain, also called the editing domain), which is inserted into the catalytic Rossmann-fold domain (Schmidt & Schimmel, 1995; Lin *et al.*, 1996; Nureki *et al.*, 1998; Fukai *et al.*, 2000; Lincecum *et al.*, 2003; Fukunaga *et al.*, 2004). Thus far, we have solved the crystal structures of the *Thermus thermophilus* IleRS CP1 domain in both its apo form and its complexed form with valine (Fukunaga *et al.*, 2004). The structure revealed the valine-specific recognition mechanism by the editing active site. However, it is yet to be clarified whether this single valine-binding site actually recognizes the valyl moiety of both the pre- and the post-transfer editing substrates as in the case of *T. thermophilus* LeuRS (Lincecum *et al.*, 2003) or whether there are two distinct sites for valyl moiety recognition (Fukai *et al.*, 2000; Hendrickson *et al.*, 2002). To understand the editing reaction precisely, editing-domain structures complexed with the pre- and post-transfer editing-substrate analogues are necessary. In the present study, we prepared a longer construct of the *T. thermophilus* IleRS CP1 domain in addition to the previously prepared construct (Fukunaga *et al.*,

2004) and carried out the crystallization and preliminary X-ray crystallographic analysis of their complexes with either the pre-transfer editing-substrate analogue or the post-transfer editing-substrate analogue.

## 2. Methods and results

### 2.1. Overexpression and purification of the two IleRS CP1-domain constructs

We prepared two expression constructs with different amino-acid residue lengths. The first (CP1-185) was composed of 185 amino acids: residues 201–384 of *T. thermophilus* IleRS with the initiating Met residue. The second (CP1-194) was composed of 194 amino acids: residues 196–388 of the full-length enzyme with the initiating Met residue. Both the CP1-domain constructs were overexpressed, purified and concentrated by previously described methods (Fukunaga *et al.*, 2004) with slight modifications: for protein purification by column chromatography, Butyl-Toyopearl (Tosoh), ResourceQ (Amersham Biosciences) and UnoQ (BioRad) columns were used. The purified sample protein was dialyzed against 10 m*M* Tris–HCl buffer pH 8.0 containing 5 m*M* $MgCl_2$ and 5 m*M* $\beta$-mercaptoethanol and concentrated to 10 mg ml$^{-1}$ with Centricon YM-30 (Millipore). The final yields were about 80 and 10 mg of pure CP1-185 and CP1-194, respectively, from 1 l LB culture.

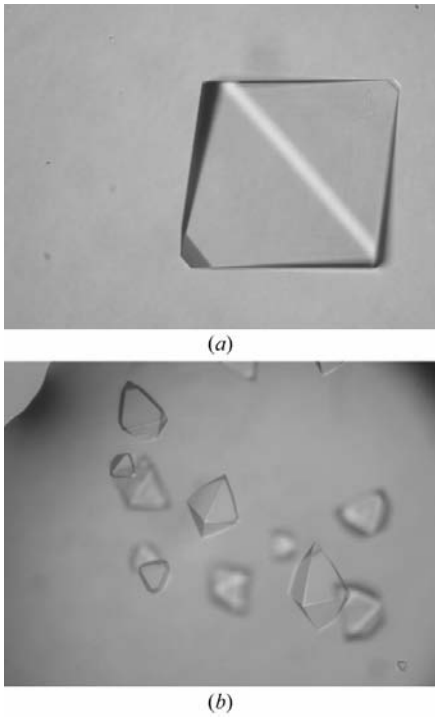

**Figure 1**
(*a*) A cocrystal of CP1-185 and Val-2AA. (*b*) Cocrystals of CP1-194 and Val-AMS.

**Table 1**
Data-collection statistics.

Values in parentheses indicate data in the highest resolution shell. Combination pairs, in which electron density corresponding to the substrate analogue was observed in the editing active site, are highlighted in bold.

| | CP1-185 + Val-AMS | **CP1-185 + Val-2AA** | **CP1-194 + Val-AMS** | **CP1-194 + Val-2AA** |
|---|---|---|---|---|
| Beamline station | BL26B1 | BL38B1 | BL26B1 | BL26B1 |
| Wavelength (Å) | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| Resolution range (Å) | 50–1.7 (1.76–1.7) | 50–1.7 (1.76–1.7) | 50–1.7 (1.76–1.7) | 50–2.0 (2.07–2.0) |
| Space group | $P4_12_12$ | $P4_122$ | $P4_12_12$ | $P4_12_12$ |
| Unit-cell parameters (Å) | | | | |
| $a = b$ | 102.61 | 72.59 | 102.00 | 102.36 |
| $c$ | 83.19 | 83.68 | 84.88 | 84.02 |
| Measured reflections | 557588 | 250762 | 494096 | 273431 |
| Unique reflections | 49428 | 24960 | 49549 | 30999 |
| Completeness (%) | 99.8 (99.7) | 98.8 (98.7) | 99.7 (99.5) | 99.5 (98.2) |
| Mean $I/\sigma(I)$ | 35.0 (2.5) | 50.0 (2.9) | 40.0 (2.7) | 28.2 (1.9) |
| $R_{\rm merge}$† (%) | 8.2 (36.8) | 4.7 (39.8) | 5.3 (44.2) | 7.0 (43.9) |

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_{hkli} - \langle I_{hkl}\rangle|/\sum_{hkl}\sum_i \langle I_{hkl}\rangle$.

### 2.2. Cocrystallization with analogues and X-ray data collection

For the cocrystallization, the pre-transfer editing-substrate analogue {5′-*O*-[*N*-(valyl)-sulfamoyl]adenosine, Val-AMS} (RNA-TEC) and the post-transfer editing-substrate analogue [2′-(L-valyl)amino-2′-deoxyadenosine, Val-2AA] (RNA-TEC) were each added to CP1-185 and CP1-194 to the final concentration of 1 m*M* substrate analogue. For all four combinations of the two kinds of CP1 domains and the two kinds of analogues, crystals suitable for X-ray analysis (Fig. 1) were obtained in a few days by mixing 1 µl protein solution and 1 µl reservoir solution consisting of 2.0 *M* ammonium sulfate, 5%(*w*/*v*) 2-propanol and 50 m*M* ADA buffer pH 6.5 using the hanging-drop vapour-diffusion method. Drops were equilibrated against 500 µl reservoir solution at 293 K.

Crystals were soaked in a cryoprotectant reservoir solution containing 1 m*M* of each analogue and 22% glycerol. X-ray diffraction data sets were collected using cryo-cooled (100 K) crystals at BL26B1 and BL38B1 of SPring-8 (Harima, Japan). The data were indexed and scaled with *HKL*2000 (Otwinowski & Minor, 1997). The data-collection statistics are summarized in Table 1.

Our attempts to solve the structures by molecular-replacement procedures using *T. thermophilus* IleRS CP1-185 in its apo form (Fukunaga *et al.*, 2004) as a model with *MOLREP* (Collaborative Computational Project, Number 4, 1994) were successful. For each of the four cocrystals, molecular-replacement solutions were obtained under different conditions with respect to the space group and/or the number of monomers in the asymmetric unit. Their comparison revealed that the space groups and numbers of monomer(s) in the asymmetric unit listed in Table 1 are much more likely than the other tested possibilities. The quality of the electron-density maps was good. In the editing active site, electron density corresponding to Val-AMS was visible in the CP1-194/Val-AMS cocrystallization data and electron density corresponding to Val-2AA was visible in the CP1-185/Val-2AA and CP1-194/Val-2AA cocrystallization data, while the CP1-185/Val-AMS data exhibited no electron density corresponding to the analogue. Structural details will be analyzed and described elsewhere.

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