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Crystallization and preliminary X-ray crystallographic study of leucyl-tRNA synthetase from the archaeon *Pyrococcus horikoshii*

Ryuya Fukunaga[a] and Shigeyuki Yokoyama[a,b,c]*

[a]Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Japan, [b]RIKEN Genomic Sciences Center, Japan, and [c]RIKEN Harima Institute at SPring-8, Japan

Correspondence e-mail: yokoyama@biochem.s.u-tokyo.ac.jp

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The leucyl-tRNA synthetase (LeuRS) from the archaeon *Pyrococcus horikoshii* was overexpressed in a C-terminally truncated form in *Escherichia coli*, purified and crystallized by the hanging-drop vapour-diffusion method using ammonium sulfate as a precipitant. The crystals belong to the rhombohedral space group *R*3, with unit-cell parameters $a = b = 186.20$, $c = 91.43$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. The asymmetric unit contains one molecule of LeuRS, with a corresponding crystal volume per protein weight of 3.2 Å^3 Da^{-1} and a solvent content of 60.7%. A data set diffracting to 2.2 Å resolution was collected from a single crystal at 100 K. Selenomethionine-substituted protein crystals were prepared in order to solve the structure by the SAD phasing method.

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. For accurate protein synthesis, high specificity of aaRSs for both the amino acid and tRNA is required. Leucyl-tRNA synthetase (LeuRS) belongs to the class Ia family of aaRSs and is closely related to isoleucyl-tRNA synthetase (IleRS) and valyl-tRNA synthetase (ValRS). These three enzymes have high sequence and structural homology and are thought to have evolved from a common ancestral enzyme as an early divergence from the other class I aaRSs (Brown & Doolittle, 1995). A common characteristic functional feature of these three enzymes is their editing activity, by which misactivated (pre-transfer editing) or mischarged (post-transfer editing) non-cognate amino acids are hydrolyzed (Jakubowski & Goldman, 1992). Biochemical and structural studies have suggested that the hydrolytic activity is associated with an independently folded domain (CP1 domain) that 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). There are some significant differences in LeuRSs between kingdoms. Sequence alignment showed an insertion-point difference of the CP1 domain between the bacteria and the archaea or eukarya (Cusack *et al.*, 2000), while the domains themselves exhibit rather low sequence homology. Furthermore, different tRNA-recognition methods have been suggested between the kingdoms; archaeal/eukaryal LeuRS use a unique long variable loop of $tRNA^{Leu}$ as a recognition determinant for $tRNA^{Leu}$ (Soma *et al.*, 1999), unlike the bacterial LeuRS which does not (Asahara *et al.*, 1993). This implies that the editing mechanisms differ between archaeal/eukaryal LeuRS and bacterial LeuRS. Thus far, the crystal structure of LeuRS from the bacterium *Thermus thermophilus* has been determined (Cusack *et al.*, 2000; Lincecum *et al.*, 2003). The structure revealed the substrate-recognition mechanism in the aminoacylation and editing active sites of this bacterial LeuRS. To gain insight into the functional and evolutional differences of editing mechanisms between different kingdoms, we intend to compare the structures of the editing domains themselves and their connection modes to the catalytic core. For this purpose, we have carried out crystallization and preliminary X-ray crystallographic analysis of LeuRS from the archaeon *Pyrococcus horikoshii*.

2. Methods and results

2.1. Overexpression and purification of the native *P. horikoshii* LeuRS

The full-length *P. horikoshii* LeuRS is composed of 976 amino-acid residues with a molecular weight of 113 kDa. The full-length LeuRS did not crystallize; a mutant with three point mutations crystallized but only diffracted to 5 Å (R. Ishitani, O. Nureki, N. Sakai, I. Tanaka and S. Yokoyama, unpublished results). Therefore, in the present study we truncated the C-terminal 157 residues, since this region is specific to the archaeal LeuRS and even among archaeal LeuRSs the sequence homology in this region is relatively low. In the *T. thermophilus* LeuRS crystal structure the corresponding C-terminal domain is disordered owing to its high flex-

ibility (Cusack *et al.*, 2000). The C-terminally truncated *P. horikoshii* LeuRS is composed of 810 amino-acid residues with a molecular weight of 96 kDa. The recombinant truncated *P. horikoshii* LeuRS was overexpressed in *Escherichia coli* strain BL21 CodonPlus (DE3) (Stratagene) using the pET system (Novagen). Expression of *P. horikoshii* LeuRS was induced by adding 1 m*M* IPTG. After induction, the *E. coli* culture was incubated for an additional 4 h and harvested by centrifugation. The cells were suspended in 50 m*M* Tris–HCl buffer pH 8.0 containing 5 m*M* $MgCl_2$, 500 m*M* NaCl, 1 m*M* DTT and 0.5 m*M* PMSF and then disrupted by sonication on ice. After centrifugation at 20 000*g* for 30 min at 277 K, the supernatant was incubated at 353 K for 30 min to denature the *E. coli* proteins. After centrifugation at 20 000*g* for 30 min at 277 K, 3.2 *M* ammonium sulfate was added to the supernatant to a final concentration of 0.8 *M*. The sample was applied to a phenyl-Toyopearl column (Tosoh) equilibrated with 50 m*M* Tris–HCl buffer pH 8.0 containing 5 m*M* $MgCl_2$, 1 m*M* DTT and 0.8 *M* ammonium sulfate. The sample was eluted with a linear gradient of 0.8–0 *M* ammonium sulfate. LeuRS-containing fractions were dialyzed against 20 m*M* Tris–HCl buffer pH 8.0 containing 2 m*M* $MgCl_2$ and 1 m*M* DTT, applied to a ResourceQ column (Amersham Biosiences) and eluted with a linear gradient of 0–1.0 *M* NaCl. LeuRS-containing fractions were dialyzed against 50 m*M* Tris–HCl buffer pH 7.5 containing 5 m*M* $MgCl_2$ and 1 m*M* DTT and were applied to a HiTrap Heparin column (Amersham Biosiences) and eluted with a linear gradient of 0–1.0 *M* NaCl. LeuRS-containing fractions were dialyzed against 10 m*M* Tris–HCl buffer pH 8.0 containing 5 m*M* $MgCl_2$ and 5 m*M* β-mercaptoethanol and were concentrated to a final concentration of 10 mg ml^{-1} with a Centricon YM-30 filter (Millipore). The leucylation activity of the purified C-terminally truncated *P. horikoshii* LeuRS towards the *in vitro* transcribed $tRNA^{Leu}$ was checked essentially as described by Soma *et al.* (1999) and Kobayashi *et al.* (2003). The result showed that the leucylation activity of the truncated LeuRS is much lower than that of the full-length enzyme (unpublished results). The C-terminal region may function to recognize tRNA, as in the case of many other aaRSs (Silvian *et al.*, 1999; Delagoutte *et al.*, 2000; Fukai *et al.*, 2000; Yaremchuk *et al.*, 2002). Nevertheless, we continued the structural study of this C-terminally truncated *P. horikoshii* LeuRS. The structure should provide plenty of information about, for example, the overall structure of the editing domain and the editing and aminoacylation active-site architectures, as well as the functions of the truncated C-terminal region. The structure could also be used as a starting model in the molecular-replacement method when we try to solve the structure of the full-length enzyme or its complex with tRNA.

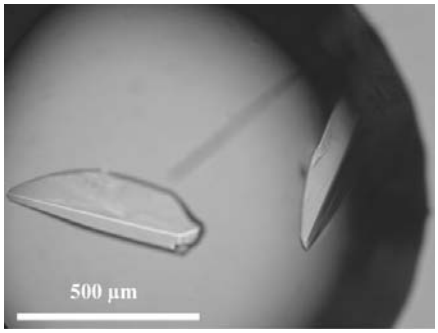

Figure 1
Native crystals of *P. horikoshii* LeuRS.

Table 1
Data-collection statistics.

Values in parentheses are for data in the highest resolution shell.

	Native	SeMet peak
Wavelength (Å)	1.0000	0.97903
Resolution range (Å)	50–2.2 (2.28–2.2)	50–2.65 (2.74–2.65)
Measured reflections	282903	172407
Unique reflections	59695	33782
Completeness (%)	99.5 (98.0)	99.1 (97.2)
Mean $I/\sigma(I)$	24.5 (3.2)	17.2 (2.4)
R_{merge}† (%)	5.8 (23.9)	7.7 (25.0)

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

2.2. Crystallization and X-ray data collection

Crystals suitable for X-ray analysis were obtained in a day by mixing 1 µl protein solution and 1 µl reservoir solution consisting of 1.9 *M* ammonium sulfate, 5%(*w*/*v*) PEG 400 and 50 m*M* ADA–NaOH buffer pH 6.5. Drops were equilibrated against 500 µl reservoir solution at 293 K. Fig. 1 shows a typical crystal.

For cryoprotection, crystals were soaked in reservoir solution containing 20% glycerol. Native X-ray diffraction data sets were collected using cryocooled (100 K) crystals at BL26B1 of SPring-8 (Harima, Japan). The diffraction data were collected using 1.0° oscillations with a crystal-to-detector distance of 190 mm. The crystals diffracted X-rays to 2.2 Å. The data were indexed and scaled with *HKL*2000 (Otwinowski & Minor, 1997). The statistics of the data collection are summarized in Table 1. The crystals belong to the rhombohedral space group *R*3, with unit-cell parameters $a = b = 186.20$, $c = 91.43$ Å, $\alpha = \beta = 90$, $\gamma = 120°$. The asymmetric unit contains one molecule of LeuRS, with a corresponding crystal volume per protein weight of 3.2 $Å^3$ Da^{-1} and a solvent content of 60.7%. The high resolution (2.2 Å) of the present crystals of the C-terminally truncated form of *P. horikoshii* LeuRS presents a striking contrast to the results of the previous full-length *P. horikoshii* LeuRS samples. Our attempts to solve the structure of *P. horikoshii* LeuRS by molecular-replacement procedures using *T. thermophilus* LeuRS as a model with *MOLREP* (Collaborative Computational Project, Number 4, 1994) failed. The quality of the electron-density maps did not allow tracing of the polypeptide chain.

2.3. Preparation and analysis of selenomethionine-labelled crystals

Therefore, in order to solve the structure by the SAD phasing method, selenomethionine-labelled crystals were prepared. For the purification of selenomethionine-labelled *P. horikoshii* LeuRS, the transformed methionine auxotrophic *E. coli* strain B834 CodonPlus (DE3) cells were grown in minimal medium in which the methionine was substituted with selenomethionine. The labelled protein was purified and crystallized in the same way as the native protein.

The diffraction data sets of the selenomethionine-substituted *P. horikoshii* LeuRS crystals were collected at BL26B1 of SPring-8. The diffraction data were collected using 1.0° oscillations with a crystal-to-detector distance of 245 mm. The crystals diffracted X-rays to 2.65 Å. The data were indexed and scaled with *HKL*2000. The data-collection statistics are summarized in Table 1. The crystals belong to the rhombohedral space group *R*3, with unit-cell

parameters $a = b = 185.80$, $c = 91.18$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$.

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