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Crystallization and preliminary X-ray crystallographic analysis of the catalytic domain of pyrrolysyl-tRNA synthetase from the methanogenic archaeon *Methanosarcina mazei*

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Pyrrolysyl-tRNA synthetase (PylRS) from *Methanosarcina mazei* was overexpressed in an N-terminally truncated form PylRS(c270) in *Escherichia coli*, purified to homogeneity and crystallized by the hanging-drop vapour-diffusion method using polyethylene glycol as a precipitant. The native PylRS(c270) crystals in complex with an ATP analogue belonged to space group $P6_4$, with unit-cell parameters $a = b = 104.88$, $c = 70.43$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$, and diffracted to 1.9 Å resolution. The asymmetric unit contains one molecule of PylRS(c270). Selenomethionine-substituted protein crystals were prepared in order to solve the structure by the MAD phasing method.

1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) are a family of enzymes that catalyze the specific esterification of an amino acid to the 3′ CCA end of its cognate tRNA and thus play a role in protein synthesis (Ibba & Söll, 2000). The aaRSs have been divided into two distinct classes of ten enzymes each based on sequence similarity and catalytic domain architecture (Eriani *et al.*, 1990). The class I aaRSs possess a Rossmann-fold active-site domain, while the class II enzymes have active-site domains constructed from antiparallel β-sheets (Cusack *et al.*, 1990; Ruff *et al.*, 1991). In addition to the 20 canonical aaRSs, pyrrolysyl-tRNA synthetase (PylRS) was found as the 21st natural aaRS, a class II aaRS showing the closest structural similarities to phenylalanyl-tRNA synthetase. PylRS esterifies the amber suppressor $tRNA^{Pyl}$ with the 22nd amino acid, pyrrolysine (Blight *et al.*, 2004; Polycarpo *et al.*, 2004). Pyrrolysine is a non-canonical amino acid found in the monomethylamine methyltransferase from the methanogenic archaeon *Methanosarcina barkeri*, where it is incorporated at a UAG codon (Hao *et al.*, 2002; Srinivasan *et al.*, 2002; Krzycki, 2005). The crystal structures of the 20 canonical aaRSs have provided detailed structural information on their substrate specificity and evolution, but the structure of PylRS has not yet been available. Here, we report the purification, crystallization and preliminary X-ray diffraction analysis of the catalytic domain of PylRS from *M. mazei*. This is the first report of the crystallization of PylRS.

2. Methods and results

2.1. Overexpression and purification of the catalytic domain of PylRS

The full-length *M. mazei* PylRS consists of 454 amino-acid residues, with a molecular weight of 51 kDa. Attempts to obtain diffracting crystals of full-length PylRS failed. Limited proteolysis revealed that PylRS is mainly composed of two domains: the N-terminal RNA-binding domain and the C-terminal aaRS catalytic domain. The gene encoding an N-terminally truncated PylRS [PylRS(c270); residues 185–454; 270 amino-acid residues] was PCR-amplified from the *M. mazei* strain JCM9314 genomic DNA (RIKEN Bioresource Center, Japan) and cloned into the pET28c vector (Novagen). The PylRS(c270) protein was expressed as a fusion with an N-terminal hexahistidine tag in *Escherichia coli* BL21(DE3) Codon Plus-RIL (Stratagene). Transformants were grown in 1 l LB

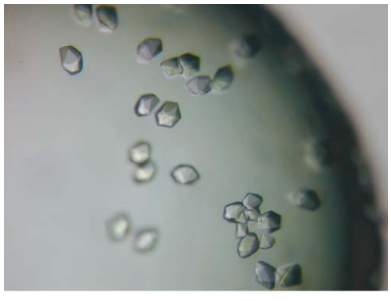

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

	SeMet, AMPPNP complex	Native, AMPPNP complex	Native, apo form
X-ray source	SPring-8 BL26B1	PF AR-NW12	SPring-8 BL41XU
Wavelength (Å)	0.9791	1.0000	1.0000
Unit-cell parameters	$a = b = 104.90$, $c = 70.51$	$a = b = 104.88$, $c = 70.43$	$a = b = 104.13$, $c = 71.23$
Resolution (Å)	50–2.4 (2.44–2.4)	50–1.9 (1.93–1.9)	50–2.64 (2.69–2.64)
$I/\sigma(I)$	17.56 (2.34)	25.01 (1.60)	34.11 (2.33)
Completeness (%)	99.6 (98.5)	99.8 (99.5)	96.7 (67.6)
Observed reflections	169149	279223	97782
Unique reflections	17362	34806	12559
Redundancy	9.75	8.0	7.8
R_{sym}	0.103 (0.427)	0.089 (0.45)	0.052 (0.318)

medium containing kanamycin (50 µg ml^{-1}) and protein expression was induced by adding IPTG to a final concentration of 0.5 m*M*. The cells were harvested and suspended in buffer *A* [50 m*M* potassium phosphate buffer pH 7.4 containing 0.5 *M* NaCl, 25 m*M* imidazole, 5 m*M* β-mercaptoethanol, 10% glycerol and protease inhibitors (Complete-EDTA free, Roche)]. The PylRS(c270) protein was purified in four column chromatographic steps. The cells were sonicated and the cell debris was removed by centrifugation. The supernatant was applied onto a HisTrap column (Amersham Biosciences) equilibrated with buffer *A*. After the column had been washed, the protein was eluted with buffer *A* containing 0.3 *M* imidazole. The HisTrap fraction was dialyzed against buffer *B* (50 m*M* potassium phosphate buffer pH 7.4 containing 0.5 *M* NaCl, 1 m*M* DTT, 10% glycerol and protease inhibitors) and $(NH_4)_2SO_4$ was then added to a final concentration of 1 *M*. This solution was loaded onto a phenyl-Superose column (Amersham Biosciences) equilibrated with buffer *B* containing 1 *M* $(NH_4)_2SO_4$. After the column had been washed with buffer *B* containing 1 *M* $(NH_4)_2SO_4$, the proteins were eluted with a linear gradient of 1–0 *M* $(NH_4)_2SO_4$ in buffer *B*. The PylRS(c270) fractions of the eluate were collected and dialyzed against buffer *C* (25 m*M* potassium phosphate buffer pH 7.4 containing 1 m*M* DTT, 10% glycerol and protease inhibitors). The dialyzed sample was applied onto an Uno Q column (BioRad). The flowthrough fractions containing PylRS(c270) were pooled and loaded onto a HiTrap Heparin column (Amersham Biosciences). The column was washed and the proteins were eluted with a linear gradient of 0–0.8 *M* NaCl. The protein fractions were pooled, dialyzed against 20 m*M* potassium phosphate buffer pH 7.4 containing 0.3 *M* NaCl, 5 m*M* $MgCl_2$ and 10 m*M* β-mercaptoethanol and then concentrated with an Amicon-15 filter (Millipore). SeMet-labelled PylRS(c270) was overexpressed from the methionine auxotroph *E. coli* B834(DE3) and was purified as the wild-type enzyme. The final yields were about 120 and 11 mg of native and SeMet PylRS(c270) proteins, respectively, from 1 l LB cultures.

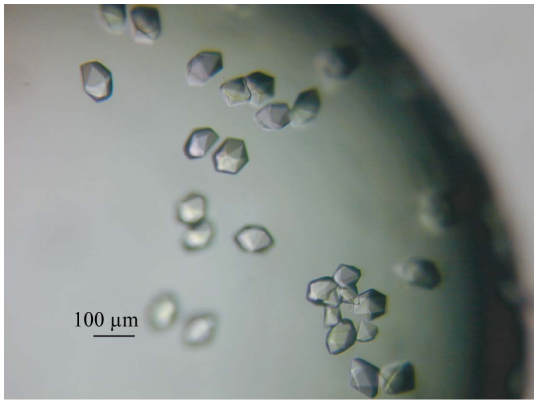

Figure 1
SeMet crystals of *M. mazei* PylRS in complex with an ATP analogue.

2.2. Crystallization and X-ray data collection

The initial screening of crystallization conditions was conducted using commercially available screening kits (Hampton Research, Emerald Biostructures and Molecular Dimensions Ltd). We could not obtain diffracting crystals of the apo form of PylRS(c270). Limited proteolysis of PylRS(c270) (33 kDa) by V8 protease yielded two fragments (26 and 7 kDa; data not shown). The proteolytic cleavage of this site was inhibited in the presence of ATP, indicating that ATP binds near the proteolytic site of PylRS(c270) and protects the Glu or the Asp residue from proteolytic attack (data not shown). We cocrystallized PylRS(c270) (9.58 mg ml^{-1} protein) with the ATP analogue AMPPNP (10 m*M*) and obtained hexagonal crystals at 293 K by the hanging-drop vapour-diffusion method in 100 m*M* sodium cacodylate buffer pH 6.8 containing 0.25 *M* NaCl, 5 m*M* $MgSO_4$ and 5%(*w*/*v*) PEG 4000. For cryoprotection, the crystals were gradually soaked in reservoir solution containing 35% ethylene glycol. A 2.4 Å three-wavelength multiwavelength anomalous dispersion (MAD) data set from the cryocooled (100 K) SeMet crystals was obtained at SPring-8 beamline BL26B1. The diffraction data were collected using 1° oscillations with a crystal-to-detector distance of 240 mm. Data-collection statistics are summarized in Table 1. The selenomethionylated PylRS(c270) crystal (Fig. 1) belongs to the hexagonal space group $P6_4$, with unit-cell parameters $a = b = 104.90$, $c = 70.51$ Å, $\alpha = \beta = 90$, $\gamma = 120°$, and the asymmetric unit contains one PylRS(c270) molecule, with a corresponding crystal volume per protein weight (V_M) of 3.39 Å^3 Da^{-1} and a solvent content of 63.69%. All data were processed using the *DENZO* and *SCALEPACK* programs from the *HKL*-2000 package (Otwinowski & Minor, 1997); other crystallographic calculations were carried out with the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). The 1.9 Å native data set from the PylRS(c270) crystal in complex with AMPPNP was collected at Photon Factory beamline AR-NW12. PylRS(c270)–AMPPNP crystals were back-soaked in AMPPNP-free reservoir solution for 6 h in order to remove the ATP analogue from the crystals. The 2.64 Å native data set from the apo form of the PylRS(c270) crystals was collected at SPring-8 beamline BL41XU.

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