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Crystallization of *Mycobacterium smegmatis* methionyl-tRNA synthetase in the presence of methionine and adenosine

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Methionyl-tRNA synthetase (MetRS) from *Mycobacterium smegmatis* was recombinantly expressed in *Escherichia coli* and purified using Ni^{2+}-affinity and size-exclusion chromatography. Crystals formed readily in the presence of the ligands methionine and adenosine. These two ligands are components of an intermediate in the two-step catalytic mechanism of MetRS. The crystals were produced using the vapour-diffusion method and a full data set to 2.1 Å resolution was collected from a single crystal. The crystal belonged to the monoclinic space group $C2$, with unit-cell parameters a = 155.9, b = 138.9, c = 123.3 Å, $\beta = 124.8^\circ$. The presence of three molecules in the asymmetric unit corresponded to a solvent content of 60% and a Matthews coefficient of 3.1 $Å^3$ Da^{-1}. Structure determination is in progress.

1. Introduction

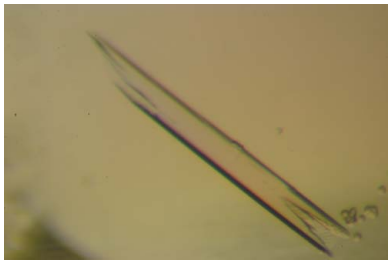
The need for new drugs against tuberculosis has led to intensified research on drug targets in *Mycobacterium tuberculosis* (*Mt*). Some strains of this pathogen have acquired resistance towards the antibiotics used in treatment of this disease. Resistant strains of *Mt* pose a serious threat to the health of future generations worldwide (Loddenkemper *et al.*, 2002).

Aminoacyl-tRNA synthetases (AaRSs) have attracted interest as suitable drug targets in pathogens including *Mt*. Inhibition of these enzymes attenuates protein biosynthesis and leads to the cessation of bacterial growth (Tao *et al.*, 2000; Kim *et al.*, 2003; Schimmel *et al.*, 1998). Mupirocin selectively inhibits isoleucyl-tRNA synthetases and is an example of a drug that is used in clinical practice to treat infections by *Staphylococcus aureus* (Sutherland *et al.*, 1985; Cookson, 1998).

There are several characteristics of AaRSs that make them suitable as drug targets. The evolutionary divergence between eukaryotic and prokaryotic AaRSs makes selective inhibition possible. In addition, the high conservation of AaRSs in prokaryotes opens possibilities for the development of broad-spectrum drugs (Ibba & Soll, 2000; Raczniak *et al.*, 2001). Furthermore, their activity assays are suitable for high-throughput screening of inhibitors (Macarron *et al.*, 2000).

AaRSs are essential in protein biosynthesis. There are 20 AaRSs in a cell and each AaRS is specific for its corresponding amino acid and tRNA. AaRSs have been divided into two classes with ten members each on the basis of their structural characteristics (Eriani *et al.*, 1990; Cusack *et al.*, 1991). AaRSs in class I contain a Rossmann fold and two sequence motifs with consensus sequences HIGH and KMSKS. The class I synthetases can be further divided into three subgroups, a, b and c, depending on the conformation of an insertion in the Rossmann domain. The class II AaRSs have a catalytic core built up by an antiparallel β-sheet containing three consensus sequences.

MetRS belongs to class Ia (Deniziak & Barciszewski, 2001). The enzymes of class Ia are monomeric, with the exception of MetRS, which can physiologically exist in both monomeric and dimeric forms. The catalytic reaction of MetRS is divided into two steps. In the first step, methionine is activated through linkage to ATP by the forma-



tion of an anhydride bond between the methionine carboxylate and the α-phosphate of the AMP molecule. In step two, methionine is transferred from the aminoacyl adenylate to the 3′-end of its cognate tRNA molecule.

Inhibitors of MetRS have been shown to have antibacterial effects and GlaxoSmithKline have developed derivatives of quinolinone that selectively inhibit MetRS in *S. aureus* and *Enterococcus faecalis* (Gentry *et al.*, 2003; Jarvest *et al.*, 2002).

Structural information is available for several MetRSs from the species *Aquifex aeolicus*, *Thermus thermophilus*, *Pyrococcus abyssi* and *Escherichia coli* (Sugiura *et al.*, 2000; Mechulam *et al.*, 1999; Crepin *et al.*, 2004; Serre *et al.*, 2001). The sequence identities between *Mycobacterium smegmatis* (*Ms*) MetRS and the abovementioned homologues of MetRS are 43, 39, 28 and 24%, respectively. We have initiated structural work on MetRS from *Mt* and from the closely related bacterial strain *Ms*. Our aim is to provide structural information as an aid to the discovery of new drugs against tuberculosis. In this study, we describe the cloning, expression, purification, crystallization and preliminary crystallographic data of *Ms* MetRS in the presence of methionine and adenosine.

2. Materials and methods

2.1. Cloning and expression

The gene *metG* (*MSMEG_5441* locus) from *Ms* MC2 155 coding for MetRS was isolated by PCR. The primers 5′-ATGAGCGAGCCCTTTTACATCACCACGGCCATC-3′ (forward) and 5′-TCACTTGCCTTCGGGTGGTTGCGGCGGCTG-3′ (reverse) were used in the first PCR. A His_6 tag and a linker sequence for directional ligation to the vector pET 101/D-TOPO (Invitrogen) were attached to the N-terminal in two subsequent PCRs. The forward primers in these steps were 5′-CATCATGGCGGCAGCGAGCCCTTTTACATCAC-3′ and 5′-CACCATGGCTCATCATCATCATCATCATGGCGGCAGCGAGC-3′.

Cloning was performed in *E. coli* Top10 cells (Invitrogen). Plasmids were prepared according to the Qiagen Spin Miniprep kit protocol (Qiagen) and expression was performed in *E. coli* BL21 Star (DE3) cells (Invitrogen). The transformant was cultured at 310 K in Luria broth medium supplemented with 50 µg ml^{-1} ampicillin. Induction was performed with 100 µg ml^{-1} isopropyl β-D-1-thiogalactopyranoside (Sigma) at an OD_{600} of ∼0.9.

2.2. Purification

The pelleted cells were suspended in lysis buffer consisting of 50 m*M* NaH_2PO_4 pH 8.0, 300 m*M* NaCl, 10 m*M* imidazole, 10%(*v*/*v*) glycerol, 0.01 mg ml^{-1} RNase, 0.02 mg ml^{-1} DNase, 0.01 mg ml^{-1} lyzosyme, 5 m*M* PMSF and 0.5%(*v*/*v*) Triton X-100. Lysation was performed in a Constant Cell Disruptor (Constant Systems Ltd). The lysate was centrifuged (39 000*g*) for 20 min at 277 K and the supernatant was incubated with 1 ml Ni–NTA agarose (Qiagen) for 20 min at 277 K. The protein was eluted with lysis buffer supplemented with 25 m*M* imidazole in 48 column volumes. The eluate was concentrated and subjected to size-exclusion chromatography on a HiLoad 16/60 Superdex-200 column (GE Healthcare Biosciences). The chromatography buffer was 50 m*M* HEPES pH 7.0. The protein was concentrated to 11 mg ml^{-1} in a Vivaspin concentrator (Vivascience; 10 kDa molecular-weight cutoff).

2.3. Crystallization

Screening of crystallization conditions was performed by the sitting-drop vapour-diffusion method using Wizard I and II screens (Emerald BioSystems) at 293 K. Drops were prepared by mixing 0.5 µl protein solution with 0.5 µl reservoir solution. The protein solution contained 6 mg ml^{-1} MetRS, 2.5 m*M* adenosine, 2.5 m*M* methionine, 2.5 m*M* tetrasodium pyrophosphate, 50 m*M* HEPES pH 7.0. The drops were equilibrated against 100 µl reservoir solution consisting of the crystallization condition 1.2 *M* NaH_2PO_4, 800 m*M* K_2HPO_4, 200 m*M* Li_2SO_4 and 100 m*M* CAPS pH 10.5. The final pH in the crystallization droplets was 7.0. This condition was repeated with hanging-drop vapour diffusion using 2 µl drops and 800 µl reservoir solution. These drops were streak-seeded with nuclei from the crystals produced in the screen.

2.4. Data collection

Crystals were soaked for 20 s in a cryogenic solution consisting of 16.6% glycerol, 3.75 m*M* methionine, 3.75 m*M* tetrasodium pyrophosphate, 3.75 m*M* adenosine, 540 m*M* K_2HPO_4, 820 m*M* NaH_2PO_4, 70 m*M* CAPS, 140 m*M* Li_2SO_4 pH 7.0 and flash-cooled in liquid nitrogen before being mounted on the beamline. Diffraction data were collected at MAX-lab, Lund, Sweden on beamline I911-2. The crystal-to-detector distance was 140 mm, the oscillation angle was 1.0° and the exposure time was 40 s per frame. A 2.1 Å data set consisting of 200 diffraction images was collected from a single crystal. The data set was processed with *MOSFLM* (Leslie, 1992) and scaled with *SCALA* (Evans, 1993) from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

Ms MetRS was efficiently expressed in the *E. coli* BL21 Star (DE3) cells and SDS–PAGE analysis of the total lysate showed expression of the 58 kDa protein. In the class Ia MetRSs, the physiological form is either monomeric or dimeric. In the latter case the enzymes contain a C-terminal domain that triggers the dimerization (Kim *et al.*, 1998). *Ms* MetRS does not have this dimerization domain and as expected migrated as a monomer in size-exclusion chromatography.

Crystals appeared after two weeks in one of the 96 conditions of the crystallization screen. In the repeated crystallization experiments with streak-seeded drops crystals appeared after 48 h. The crystals grew to dimensions of 0.5 × 0.08 × 0.08 mm within two weeks (Fig. 1). A 97% complete X-ray diffraction data set could be collected from a single crystal. The data-collection statistics are summarized in Table 1.

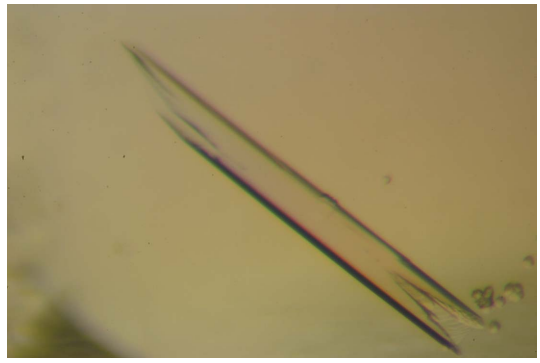


Figure 1
A crystal of *Ms* MetRS with dimensions 0.5 × 0.08 × 0.08 mm produced in a hanging drop.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell.

Wavelength (Å)	1.038
Resolution range (Å)	30.0–2.10 (2.21–2.10)
Space group	*C*2
Unit-cell parameters (Å, °)	a = 155.9, b = 138.9, c = 123.3, β = 124.8
No. of measured reflections	488513 (63155)
No. of unique reflections	121579 (17231)
Completeness (%)	97.1 (94.4)
Mean $I/\sigma(I)$	10.1 (1.9)
$R_{\rm merge}$† (%)	11.0 (65.0)
$R_{\rm p.i.m.}$‡ (%)	6.1 (37.0)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$. ‡ $R_{\rm p.i.m.} = \sum_{hkl}[1/(N-1)]^{1/2} \times \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

The program *Phaser* (McCoy *et al.*, 2007) from *CCP*4 was used to calculate the Matthews coefficient. The calculations suggested the presence of three molecules in the crystal asymmetric unit, a $V_{\rm M}$ of 3.1 Å^3 Da^{-1} and a solvent content of 60%. Structure determination is in progress.

In parallel with the work on *Ms* MetRS, experiments were also performed with the related enzyme from *Mt*. However, to date we have not been able to produce crystals of this variant. The high similarity, 74% identity and the fact that all the active-site residues are identical except for amino acid 294, which is a Trp in the *Ms* enzyme and a Phe in *Mt*, makes the *Ms* enzyme a suitable model for the development of new drugs against tuberculosis.

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