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Preparation, crystallization and preliminary X-ray analysis of the methionine synthase (MetE) from *Streptococcus mutans*

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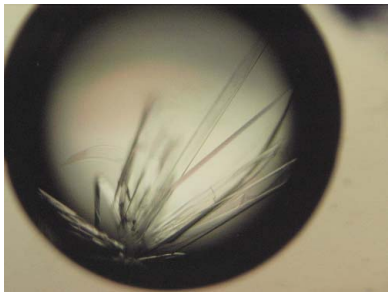

The *Streptococcus mutans metE* gene encodes methionine synthase (MetE), which catalyzes the direct transfer of a methyl group from methyltetrahydrofolate to homocysteine in the last step of methionine synthesis. *metE* was cloned into pET28a and the gene product was expressed at high levels in the *Escherichia coli* strain BL21 (DE3). MetE was purified to homogeneity using Ni^{2+}-chelating chromatography followed by size-exclusion chromatography. Crystals of the protein were obtained by the hanging-drop vapour-diffusion method and diffracted to 2.2 Å resolution. The crystal belongs to space group $P2_1$, with unit-cell parameters a = 52.85, b = 99.48, c = 77.88 Å, β = 94.55°.

1. Introduction

Methionine synthases catalyze the transfer of a methyl group from N^5-methyltetrahydrofolate (CH_3-H_4-folate) to L-homocysteine (Hcy) in the last step of methionine biosynthesis. The major methionine synthases can be classified into two groups: cobalamin-dependent methionine synthases (MetH; EC 2.1.1.13) and cobalamin-independent methionine synthases (MetE; EC 2.1.1.14) (Gonzalez *et al.*, 1992). Although MetH and MetE catalyze the same reaction and both of them require zinc ion for activity, the sequence similarity between them is quite low and their catalytic mechanisms are quite different from each other. MetH first transfers the methyl group of CH_3-H_4-folate to an enzyme-bound cobalamin cofactor, followed by donation of the methyl group to homocysteine, while MetE transfers the methyl group directly to homocysteine from CH_3-H_4-folate (Ferrer *et al.*, 2004). Although both MetH and MetE can be found in bacteria, animals express only MetH, while plants and fungi contain only MetE (Pejchal & Ludwig, 2005). So far, two structures of MetE have been solved, one of which is from *Arabidopsis thaliana* (Ferrer *et al.*, 2004) and the other from *Thermotoga maritima* (Pejchal & Ludwig, 2005). The structures show that MetE contain two $(\beta\alpha)_8$-barrels which form a deep cleft at their interface. The active site of the enzyme is located in the C-terminal domain, facing the cleft.

In *Streptococcus mutans*, the main aetiological agent in human dental caries (Loesche, 1986), the MetE protein is coded by the *metE* gene, located at base pairs 821 698–823 935 of the *S. mutans* genome (Ajdic *et al.*, 2002). The theoretical molecular weight and isoelectric point of the protein are 80 kDa and 5.4, respectively. Here, we report the preparation, crystallization and preliminary X-ray analysis of the *S. mutans* MetE protein. Ultimately, protein structural information may prove helpful in the prevention and cure of dental caries.

2. Experimental procedures and results

2.1. Cloning, expression and purification of MetE

The *metE* gene was cloned from the genomic DNA of *S. mutans* by polymerase chain reaction amplification (PCR; Saiki *et al.*, 1988), using primers 5′-CGCGGATCCATGACAAAAGTTTCAAGCTTGG-3′ and 5′-CCGCTCGAGTTAGTTTCCTAATTTTTGACGAA-3′. The target gene was then inserted into the pET28a vector (Novagen) at the *Bam*HI and *Xho*I restriction sites. An N-terminal fusion His_6 tag was added to the gene product, with the sequence MGSS**HHHHHH**SSGLVPRGSHMASMTGGQQMGRGS. The

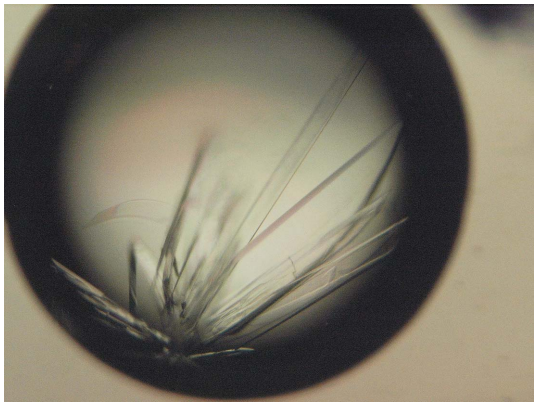

Figure 1
Crystals of *S. mutans* MetE protein.

recombinant vector containing the target gene was transformed into *Escherichia coli* BL21 (DE3).

Transformed cells were grown overnight in 20 ml Luria–Bertani (LB) medium containing 50 µg ml^{-1} kanamycin at 310 K. The overnight culture was then inoculated into 1 l fresh LB medium containing 50 µg ml^{-1} kanamycin and grown at 310 K for about 3 h. When the OD_{600} of the cell culture reached 0.6, isopropyl β-D-thiogalactoside was added to the culture to a final concentration of 0.5 m*M* for induction. After further growth for 3 h at 310 K, cells were harvested by centrifugation and then suspended in 20 ml buffer *A* (20 m*M* Tris–HCl pH 7.5, 500 m*M* NaCl).

The resuspended cells were lysed by sonication on ice and the cell lysate was centrifuged at 3500*g* for 40 min to remove the debris. The supernatant was filtered and applied onto an Ni^{2+} chelating affinity column (HiTrap, GE Healthcare, USA) previously equilibrated with buffer *A*. The column was washed using a linear gradient of imidazole from 0 to 0.5 *M* in buffer *A*. Fractions containing target protein were pooled and concentrated to about 1.5 ml. The target protein was further purified by size-exclusion chromatography on an S200 column. The protein eluted as a sharp peak at a location consistent with a protein monomer. The purity of target protein was above 95% by SDS–PAGE analysis.

2.2. Crystallization

Purified protein was concentrated to about 15 mg ml^{-1} in 20 m*M* Tris–HCl pH 7.5, 150 m*M* NaCl. Crystallization screening was carried out at 298 K using the hanging-drop vapour-diffusion method. Hampton Research Crystal Screen, Crystal Screen 2 and Index Screen (Hampton Research, CA, USA) were selected for initial screening. 1 µl protein solution was mixed with 1 µl reservoir solution and equilibrated against 500 µl reservoir solution. After two weeks, crystals appeared under ten different conditions. After optimization, the crystals most suitable for diffraction experiments were obtained using the exact conditions of condition No. 15 from the Index Screen, which contains 0.2 *M* Li_2SO_4, 0.1 *M* Bis-Tris pH 6.5 and 25%(*w*/*v*) PEG 3350 (Fig. 1). The volumes of the protein and reservoir solutions and the protein concentration in the final crystallization conditions are same as those used in the screening. The crystal dimensions are about 0.7 × 0.2 × 0.05 mm.

Table 1
Data-collection statistics of MetE.

Values in parentheses are for the highest resolution shell.

Resolution (Å)	50–2.2 (2.33–2.2)
Completeness (%)	95.9 (77.2)
R_{sym} (%)	10.09 (28.18)
Mean $I/\sigma(I)$	5.96 (2.01)
Redundancy	3.51 (1.89)
Mosaicity	0.56
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 52.85, b = 99.48, c = 77.88, β = 94.55
No. of observed reflections	129466
No. of unique reflections	39151
Molecules per ASU	1
V_M (Å^3 Da^{-1})	2.42
Solvent content (%)	49.2

† $R_{sym} = \sum |I_{obs} - I_{avg}| / \sum I_{avg}$, where the summation is over all reflections.

2.3. X-ray analysis

X-ray diffraction data were collected on a Bruker SMART 6000 CCD detector using Cu $K\alpha$ radiation from a Bruker–Nonius FR591 rotating-anode generator operating at 45 kV and 100 mA. The crystal-to-detector distance was set to 5 cm. 1000 frames were collected with 0.2° φ oscillation per frame and an exposure time of 60 s per frame. The crystal was first flash-cooled without any cryoprotectant and was maintained at 100 K using nitrogen gas during data collection. Diffraction data were processed using the Bruker *PROTEUM* software suite.

The crystal diffracted to 2.2 Å and belongs to space group $P2_1$, with unit-cell parameters a = 52.85, b = 99.48, c = 77.88 Å, β = 94.55°. Assuming the presence of one molecule per asymmetric unit, the V_M value is 2.42 Å^3 Da^{-1} (Kantardjieff & Rupp, 2003), corresponding to a solvent content of 49.2%. The crystallographic parameters and data-collection statistics are listed in Table 1. Structure determination by the molecular-replacement method is on going.

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