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Crystallization and preliminary X-ray crystallographic studies of *O*-methyltransferase from *Anabaena* PCC 7120

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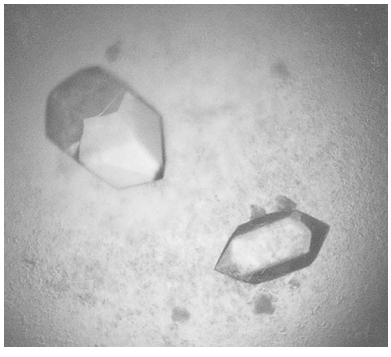

O-Methyltransferase (OMT) is a ubiquitous enzyme that exists in bacteria, plants and humans and catalyzes a methyl-transfer reaction using *S*-adenosyl-L-methionine as a methyl donor and a wide range of phenolics as acceptors. To investigate the structure and function of OMTs, *omt* from *Anabaena* PCC 7120 was cloned into expression vector pET21a and expressed in a soluble form in *Escherichia coli* strain BL21 (DE3). The recombinant OMT protein was purified to homogeneity using a two-step strategy. Crystals of OMT that diffracted to a resolution of 2.4 Å were obtained using the hanging-drop vapour-diffusion method. The crystals belonged to space group $C222_1$, with unit-cell parameters $a = 131.620$, $b = 227.994$, $c = 150.777$ Å, $\alpha = \beta = \gamma = 90^\circ$. There are eight molecules per asymmetric unit.

1. Introduction

Methylation by *S*-adenosyl-L-methionine-dependent *O*-methyltransferases (OMTs; EC 2.1.1) is a common modification in secondary-product biosynthesis that occurs ubiquitously in many organisms including bacteria, fungi, plants and mammals (Cho *et al.*, 2007) and results in methylated metabolites that have different cellular functions (Khosla, 2000). Cation-dependent OMTs constitute a small group of low-molecular-weight enzymes (Ibdah *et al.*, 2003). In mammals, these enzymes play important roles in the modification of catechol neurotransmitters in the brain or may inactivate potentially bioactive metabolites such as quercetin in the liver and kidney (Mannisto & Kaakkola, 1999; Zhu, 2002). In plants, caffeoyl-coenzyme A *O*-methyltransferases (CCoAOMTs), which are named after their preferred *in vitro* substrate, synthesize crucial components of lignin in plant vascular tissues in conjunction with a second group of cation-independent caffeic acid OMTs (Marita *et al.*, 2003; Pakusch *et al.*, 1989). In addition to caffeoyl-CoA, specific subtypes of CCoAOMT-like proteins also methylate other phenylpropanoids, preferentially flavonoids with vicinal dihydroxy groups (Ibdah *et al.*, 2003). The three-dimensional structures of eukaryotic animal and plant OMTs known to date are quite similar despite their otherwise low sequence identities, irrespective of the involvement of bivalent cations and substrates (Coiner *et al.*, 2006; Kopycki, Rauh *et al.*, 2008; Kopycki, Stubbs *et al.*, 2008; Schluckebier *et al.*, 1995; Zubieta *et al.*, 2002). These structures contain alternating β-strands and α-helices, forming a seven-stranded β-sheet with three helices on each side (Martin & McMillan, 2002). Recent studies have identified two new methyltransferase folds. The determination of the structures of several SET domain-containing histone methyltransferases revealed that these proteins have a β-sheet topology which is very different from the classic *S*-adenosylmethionine-dependent *O*-methyltransferase fold described above (Trievel *et al.*, 2002; Zhang *et al.*, 2002; Jacobs *et al.*, 2002; Min *et al.*, 2002; Wilson *et al.*, 2002). These studies have revealed the diversity in the structure and function of OMTs. In order to elucidate the structural and functional characteristics of

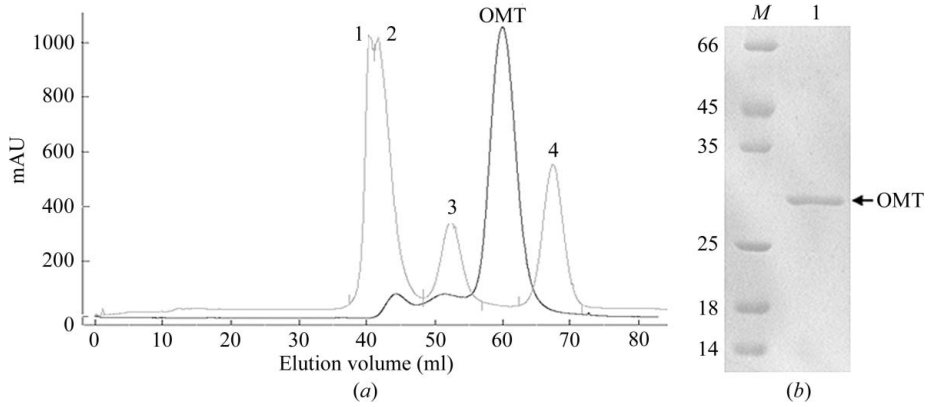

Figure 1
Size-exclusion chromatographic analysis and SDS–PAGE of recombinant OMT. (*a*) Elution profile of OMT on Superdex-75 size-exclusion chromatography. The standard proteins used for molecular-weight calibrations were (1) thyroglobulin (670 kDa), (2) γ-globulin (158 kDa), (3) ovalbumin (44 kDa) and (4) myoglobin (17 kDa). (*b*) OMT protein was purified to near-homogeneity using an Ni^{2+}-chelating column and a Superdex-75 column. Lane *M*, protein molecular-weight markers (kDa); lane 1, purified OMT protein.

OMT from *Anabaena*, here we report the expression, purification and crystallization of the OMT enzyme from *Anabaena* PCC 7120.

2. Experimental results

2.1. Gene cloning, protein expression and purification of OMT

The *omt* gene (GeneID 1108783) containing 663 bp was amplified by the polymerase chain reaction from the genome of *Anabaena* PCC 7120 and cloned into pET21a expression vector with an N-terminal six-His fusion tag. The *omt* sequence in the expression vector was confirmed by DNA sequencing.

The recombinant OMT protein was overexpressed and purified in *Escherichia coli* BL21 (DE3) as follows. Cells were cultured at 310 K overnight in 20 ml Luria–Bertani (LB) medium containing 100 µg ml^{-1} ampicillin and then transferred to 1 l fresh LB medium and grown at 310 K. When the $OD_{600\,nm}$ reached 0.6–0.8, 0.5 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce expression of OMT. After culturing at 291 K for 18 h, the cells were harvested by centrifugation at 5000 rev min^{-1} for 10 min, suspended in binding buffer (20 m*M* HEPES pH 7.5, 500 m*M* NaCl, 5 m*M* imidazole) and sonicated (on for 5 s, off for 10 s; 99 cycles). The lysate was centrifuged at 18 000 rev min^{-1} for 30 min at 277 K and the supernatant was filtered through a 0.22 µm filter and subsequently loaded onto a HiTrap Chelating HP column charged with Ni^{2+}, which was attached to an ÄKTA fast protein liquid chromatography (FPLC) system. The protein was eluted with a linear gradient of 5–500 m*M* imidazole in five bed volumes. The protein peak eluted at 200 m*M* imidazole. To further purify the protein, the concentrated peak fractions were applied onto a HiLoad Superdex-75 gel-filtration column (GE Healthcare, USA) equilibrated with buffer containing 10 m*M* HEPES pH 7.5, 500 m*M* NaCl (Fig. 1*a*). The peak fractions from the elution were pooled and concentrated using an Amicon Ultra centrifugal filter device (Millipore, USA). All purification procedures were performed at 277 K. The recombinant OMT with 6×His tag was estimated to have a molecular weight of about 27 kDa and was used in subsequent crystallization. The protein concentration was measured and the final yield was 10 mg per litre of culture with a purity of over 95% (Fig. 1*b*).

2.2. Crystallization of OMT

Crystallization screening experiments were performed at 293 K by the hanging-drop vapour-diffusion method using Hampton Research kits including Crystal Screen, Crystal Screen 2, Grid Screen, PEG/Ion and Index (Hampton Research, USA). Typically, each drop was prepared by mixing 1 µl protein solution with 1 µl reservoir solution. Microcrystals were obtained under many initial conditions. After optimizing the pH and the concentration of precipitants, diamond-shaped crystals (0.5 × 0.3 × 0.2 mm) that yielded the best diffraction quality were obtained within 7 d from 30% PEG 550, 200 m*M* $MgCl_2$, 100 m*M* Tris pH 8.0 using a protein concentration of 12 mg ml^{-1} (Fig. 2).

2.3. Diffraction data collection

X-ray diffraction data were collected on a MAR 325 CCD detector at Beijing Synchrotron Radiation Facility (beamline 3W1A) with a wavelength of 1.04361 Å. The crystal was flash-cooled and maintained at 100 K in cooled nitrogen gas during data collection, with 20% glycerol as the cryoprotectant. The crystal-to-detector distance was 165 mm; a φ scan was performed with 1° φ oscillation per frame. 180 frames were collected; the exposure time per frame was 20.3 s. The data were processed with *MOSFLM* (Powell, 1999) and the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). The crystal diffracted to 2.4 Å resolution. The OMT crystals belonged to space group $C222_1$, with unit-cell parameters a = 131.620, b = 227.994, c = 150.777 Å, $\alpha = \beta = \gamma = 90°$. The Matthews coefficient predicts eight molecules per asymmetric unit. The data-collection

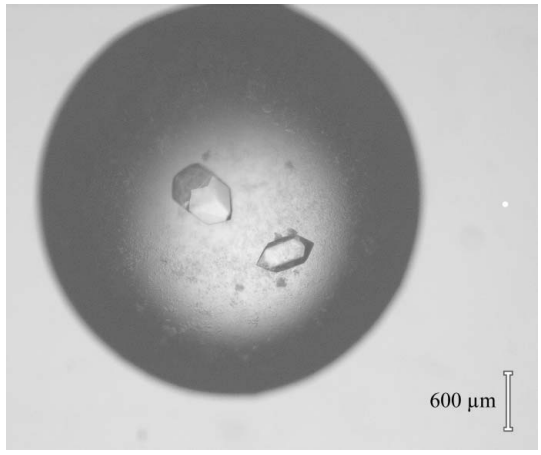

Figure 2
Crystals of OMT obtained in 30% PEG 550, 200 m*M* $MgCl_2$, 100 m*M* Tris pH 8.0.

Table 1
Crystallographic parameters and data-collection statistics.

Values in parentheses are for the last resolution shell.

Wavelength (Å)	1.04361
Resolution (Å)	30–2.4 (2.49–2.4)
Completeness (%)	99.9 (99.7)
R_{merge} (%)†	0.067 (0.715)
$I/\sigma(I)$	28.457 (1.915)
Space group	$C222_1$
Unit-cell parameters (Å, °)	a = 131.620, b = 227.994, c = 150.777, $\alpha = \beta = \gamma = 90$
No. of observed reflections	617670
No. of unique reflections	39401
Redundancy	7.0 (5.8)
Molecules per ASU	8
V_M (Å^3 Da^{-1})	2.83
B factor from Wilson plot (Å^2)	39.4

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the average intensity over symmetry-equivalent reflections

statistics are listed in Table 1. Since we could not obtain a useful result by molecular replacement using the structure of *Mesembryanthemum crystallinum* PFOMT (PDB code 3c3y; Kopycki, Rauh *et al.*, 2008) as a search model, we have therefore started screening heavy-atom derivatives and the structure of OMT will be reported in the future.

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