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Crystallization, preliminary X-ray diffraction and structure solution of MosA, a dihydrodipicolinate synthase from *Sinorhizobium meliloti* L5-30

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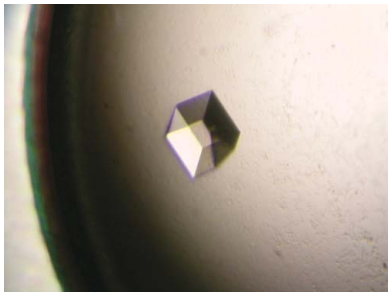

The structure of MosA, a dihydrodipicolinate synthase and reported methyltransferase from *Sinorhizobium meliloti*, has been solved using molecular replacement with *Escherichia coli* dihydrodipicolinate synthase as the model. A crystal grown in the presence of pyruvate diffracted X-rays to 2.3 Å resolution using synchrotron radiation and belonged to the orthorhombic space group $C222_1$, with unit-cell parameters a = 69.14, b = 138.87, c = 124.13 Å.

1. Introduction

MosA is a protein encoded on a pSym megaplasmid of the beneficial soil bacterium *Sinorhizobium meliloti* L5-30. This organism forms a symbiotic relationship with leguminous plants, fixing nitrogen for the plant and exchanging certain nutrients (Dixon, 1969; Lodwig *et al.*, 2003). The *mosA* gene is found in an operon which is required for the synthesis of rhizopines, compounds that have been proposed to act as selective growth substrates for rhizobia such as *S. meliloti* or to give them some competitive advantage in nodulation of plant roots (Murphy *et al.*, 1988; Saint *et al.*, 1993; Rao *et al.*, 1995; Heinrich *et al.*, 2001). The function of MosA has been controversial since it was first proposed. The absence of the *mosA* gene resulted in the isolation of the rhizopine *scyllo*-inosamine (Fig. 1*a*) from bacteroides, but the presence of the gene allowed the isolation of 3-*O*-methyl-*scyllo*-inosamine (Fig. 1*b*). MosA was therefore assigned the role of a methyltransferase (Rao *et al.*, 1995). However, these authors and others (Lawrence *et al.*, 1997; Babbitt & Gerlt, 1997) noted that the sequence of the protein strongly suggests that it is homologous with dihydrodipicolinate synthase (DHDPS), a member of the *N*-acetylneuraminate lyase (NAL) subfamily of type I aldolases. MosA shows no apparent resemblance to any known methyltransferase and no aldolase-like mechanism of methyl transfer has ever been observed.

The first *in vitro* experiments with recombinant purified MosA demonstrated that this protein was an efficient catalyst of the DHDPS reaction (Tam *et al.*, 2004): the aldol condensation of pyruvate with L-aspartate β-semialdehyde (ASA) and the subsequent cyclization of the product to 4-hydroxytetrahydrodipicolinate, which rapidly dehydrates to dihydrodipicolinate (Blickling, Renner *et al.*, 1997; Dobson, Griffin *et al.*, 2004; Dobson, Gerrard *et al.*, 2004; Karsten, 1997) as shown in Fig. 1(*c*).

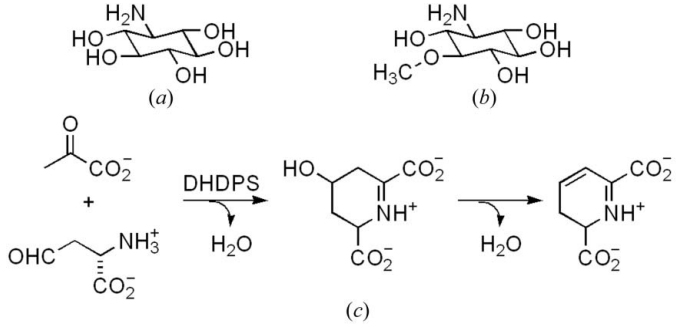

Figure 1
Rhizopine structures: (*a*) *scyllo*-inosamine, (*b*) 3-*O*-methyl-*scyllo*-inosamine. (*c*) The DHDPS-catalyzed condensation of pyruvate and ASA.

DHDPS is found in the diaminopimelate pathway of lysine biosynthesis in bacteria and plants, but not in animals, making it a putative target for antibiotics and/or herbicides. High-resolution crystal structures have been reported for DHDPS from *Nicotiana sylvestris* (Blickling, Beisel *et al.*, 1997) and for wild-type DHDPS from *Escherichia coli* (Blickling, Renner *et al.*, 1997; Dobson *et al.*, 2005) and mutants (Dobson, Valegard *et al.*, 2004). These structural studies have revealed interesting differences between the quaternary structures of plant and bacterial enzymes and lend insight into kinetic studies of the mechanism of DHDPS catalysis and its feedback inhibition by lysine. Structural studies of MosA will allow a further comparison between DHDPS enzymes from different sources and perhaps elucidate how, or if, MosA might interact with rhizopine ligands.

2. Experimental results

2.1. Enzyme expression and purification

The *MosA* gene encoding a protein consisting of 292 amino-acid residues was cloned, expressed with an N-terminal His tag and sequenced as previously described (Tam *et al.*, 2004). Briefly, the *mosA* gene from the plasmid pPM1062 was cloned into the expression vector pET-28b (Novagen). The recombinant His-tagged protein was then expressed in *E. coli* BL-21(DE3) and the pelleted cells lysed into buffer containing 50 m*M* Tris–HCl pH 8.0, 10 m*M* imidazole, 500 m*M* NaCl and 12.5%(*v*/*v*) glycerol. The MosA protein was purified by passing the lysed solution through a Chelating Sepharose FF column (Amersham Biosciences) charged with Ni^{2+} and eluted with EDTA. The elution fractions containing MosA were pooled and dialyzed into buffer *A* (10 m*M* Tris–HCl pH 8.0, 50 m*M* KCl, 2 m*M* 2-mercaptoethanol, 5 m*M* pyruvate and 2 m*M* EDTA) at 278 K overnight. Dialysis was continued the next day with fresh buffer *A* without EDTA and dialyzed for another 18 h at 278 K. The resulting solution of MosA was judged to be homogeneous by SDS–PAGE with an approximate molecular weight of 33 kDa. The protein was concentrated to 5.4 mg ml^{-1} using Millipore 5000 MWCO centrifugal concentrators and stored at 278 K. The His tag was not cleaved from the protein.

2.2. Dynamic light scattering

MosA protein in buffer *A* without EDTA was filtered through a 0.1 µm Anodisc 13 filter (Whatman) and 20 µl was placed in the testing cuvette of the dynamic light-scattering instrument (DynaPro-MS800). Data were processed using the software supplied with the instrument (*Dynamics* v. 5.26.60, Protein Solutions Inc.). Measurements of the hydrodynamic radius were recorded at intervals from 277 to 318 K.

2.3. Crystallization

MosA protein in solution with buffer *A* was screened at room temperature against The Classics and The PEGs screening kits (Nextal Biotechnologies) using the methods of microbatch-under-oil and vapour diffusion (sitting drops), respectively. The protein readily crystallized from several of the cocktails from both screening kits. Crystallization conditions were subsequently optimized using vapour diffusion in hanging drops at room temperature. Drops were formed by mixing an equal volume of the protein solution (5.5 mg ml^{-1} protein, 2.5 m*M* sodium pyruvate, 25 m*M* KCl, 5 m*M* Tris–HCl pH 8.0 and 1 m*M* 2-mercaptoethanol) and well solution (12.5% PEG 3350, 0.1 *M* buffer pH 8.5 and 0.2 *M* $MgCl_2$). The buffer in the well solution was a tri-buffer system (Newman, 2004) composed of L-malic acid, MES and Tris in a ratio of 1:2:2.

Thick plate-like and block-shaped crystals appeared within several days and continued to grow over the next few weeks to approximately 0.2 mm in their largest dimension (Fig. 2). The crystals were harvested into cryosolution (40% ethylene glycol, 0.18 *M* $MgCl_2$, 20% PEG 3350, 0.08 *M* buffer pH 8.5) prior to cooling in liquid nitrogen.

Solutions of MosA containing pyruvate and/or one of three other ligands, L-lysine (an inhibitor of DHDPS enzymes), 2,6-pyridine dicarboxylic acid (PDC; dipicolinic acid, an analog of 4-hydroxy-tetrahydrodipicolinate, the product of the MosA reaction) and the trifluoroacetate salt of ASA, were also prepared and the successful conditions from the screening plates were replicated. Plate-like and block-shaped crystals formed within several days by vapour diffusion in hanging drops at room temperature. These were subsequently harvested into cryosolutions containing ethylene glycol and cooled with liquid nitrogen.

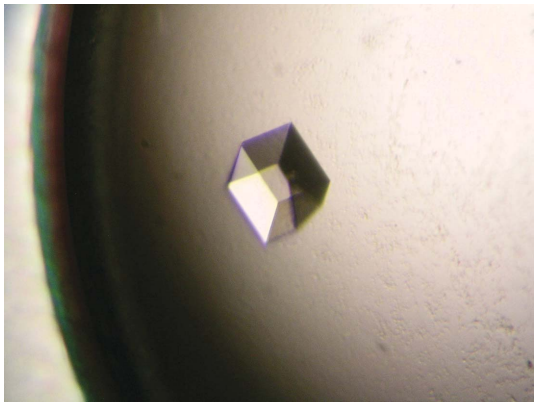

Figure 2
MosA crystal. The largest dimension is approximately 0.2 mm.

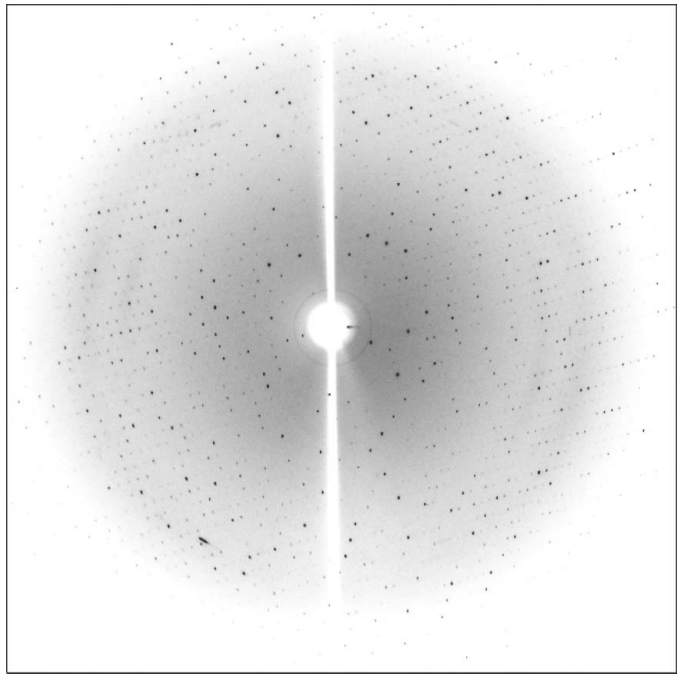

Figure 3
Diffraction image from a crystal of MosA.

Table 1
Data-collection statistics for crystal of MosA–pyruvate.

Temperature (K)	150
Beamline	X9A, NSLS
Detector	MAR CCD
Space group	$C222_1$
Unit-cell parameters (Å)	a = 69.14, b = 138.87, c = 124.13
Matthews coefficient (Å^3 Da^{-1})	2.33
Solvent content (%)	47.1
Unit-cell volume (Å^3)	1191875
No. of molecules in the asymmetric unit	2
No. of measured reflections	165560
Total No. of unique reflections collected	26960
Resolution range (Å)	30–2.30 (2.38–2.30)
Completeness (%)	99.9 (100)
Redundancy	6.1 (6.2)
$R_{\rm merge}$†	0.036 (0.092)
$\langle I/\sigma(I)\rangle$	41.5 (15.7)

† $R_{\rm merge} = |I - \langle I\rangle|/\sum I$, where $\langle I\rangle$ is the average intensity over symmetry-related reflections and I is the measured intensity. Statistics for the highest resolution shell are given in parentheses.

All ligand stock solutions were prepared with water, with the pH of the PDC solution adjusted with NaOH. Sodium pyruvate, PDC and L-lysine were purchased commercially. ASA was synthesized by the method of Roberts *et al.* (2003).

2.4. Data collection and processing

Crystals of MosA grown in four different ligand-containing solutions (pyruvate, pyruvate plus L-lysine, pyruvate plus ASA and PDC) were used to diffract X-rays (Fig. 3) at the NSLS (Upton, NY, USA) during the RapiData 2005 course. Data were collected from four crystals. The data for the MosA–pyruvate crystal complex are presented in Table 1. Intensity data were indexed, integrated and scaled with the *HKL*2000 programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Structure solution

A molecular-replacement solution for the diffraction data set from the MosA–pyruvate crystal was found using *AMoRe* (Navaza, 1994) from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). The structure of dihydrodipicolinate synthase from *E. coli* (PDB code 1dhp), which has 45% sequence identity to MosA, was used as the model. The initial solution gave a correlation factor of 0.52 and an *R* factor of 0.48. After one round of refinement with *CNS* (Brünger *et al.*, 1998), the correlation factor increased to 0.62 and the *R* factor improved to 0.34. The structure continued to be improved through cycles of manual building followed by refinement. Solutions for the three other data sets will be attempted using the MosA–pyruvate structure as a starting model.

4. Results and discussion

Analyses of the MosA protein using dynamic light scattering and X-ray diffraction add to the evidence that MosA is a DHDPS and a member of the NAL subfamily of enzymes.

Dynamic light scattering performed on MosA indicated a highly homogenous solution that was likely to be composed of tetramers, as are other DHDPS. The data revealed a bimodal distribution with one highly monodisperse (<15% polydispersivity) peak comprising 99% of the mass and having an approximate hydrodynamic radius of 4.2 nm, corresponding to a 130 kDa tetramer. The protein solution tolerated a change of temperature from 277 to 303 K. Above 318 K, the detector recorded an overload indicating a temperature limit had been reached, which was likely to coincide with denaturation of the protein. The DLS results also agree with the results (P. H. Tam and D. R. J. Palmer, unpublished work) from gel-filtration chromatography, which indicate that MosA is a tetramer.

The molecular-replacement solution of the X-ray diffraction data revealed two molecules of MosA in the asymmetric unit related by twofold non-crystallographic symmetry. The two molecules along with two symmetry-related molecules form a tetramer. The overall tertiary structure of each monomer resembles DHDPS and is the characteristic $(\beta/\alpha)_8$ TIM barrel.

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