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Crystallization and preliminary X-ray crystallographic studies of a PduO-type ATP:cob(I)alamin adenosyltransferase from *Bacillus cereus*

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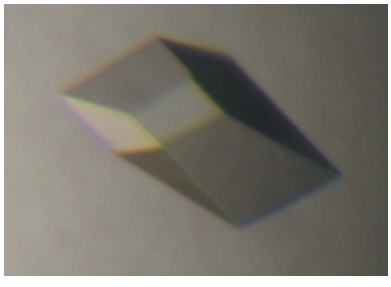


Cobalamin adenosyltransferases transfer a 5′-deoxyadenosyl moiety from ATP and covalently attach it to the cobalt(I) ion of the corrin ring of cobalamin to generate adenosylcobalamin. The PduO-type adenosyltransferase from *Bacillus cereus* was overexpressed in *Escherichia coli*, purified and crystallized as the apoenzyme as well as in complex with Mg^{2+} and ATP (MgATP). Diffraction data were collected to 1.9 Å resolution for the native crystals and 2.0 Å resolution for the complexed crystals. Both crystals belonged to the orthorhombic space group $C222_1$; the native crystals have unit-cell parameters a = 64.93, b = 137.08, c = 158.55 Å. The asymmetric unit contained one trimer, with a corresponding V_M of 2.69 Å^3 Da^{-1}.

1. Introduction

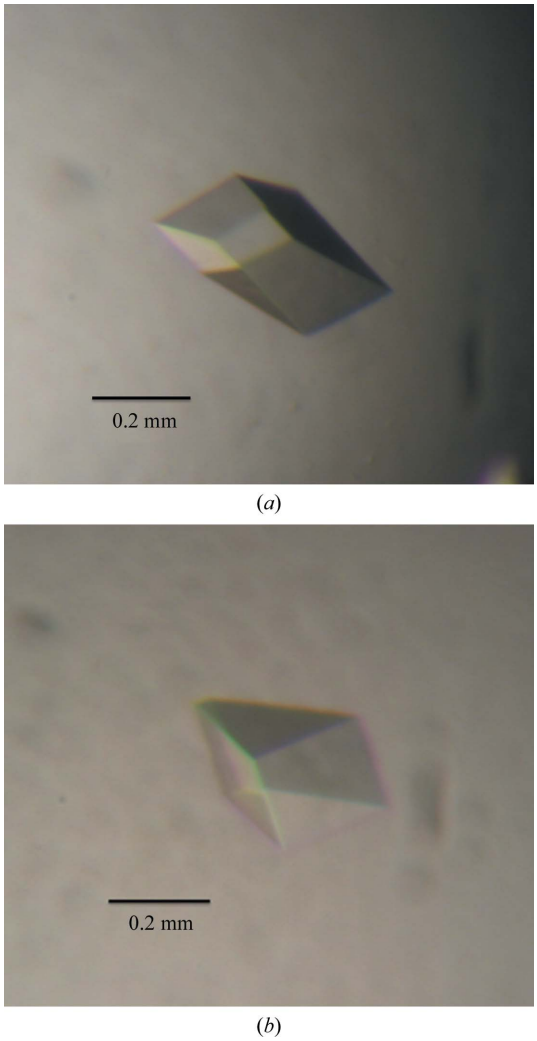
Although vitamin B_{12} (cobalamin, Cbl) is an important nutrient that is necessary for all life forms, it is only synthesized by certain classes of prokaryotes. Adenosylation of Cbl generates coenzyme B_{12} (adenosylcobalamin, AdoCbl), an essential cofactor that is required for acetyl-CoA synthesis, methyl transfer, fermentation and methionine synthesis (Martens *et al.*, 2002; Roth *et al.*, 1996). ATP:cob(I)alamin adenosyltransferase catalyzes the conversion of Cbl to AdoCbl by transferring a 5′-deoxyadenosyl moiety to the cobalt(I) ion in Cbl. Based on their amino-acid sequences, cob(I)alamin adenosyltransferases have been categorized as CobA, PduO or EutT enzymes. CobA enzymes are constitutively expressed and encoded by the *cobA* gene (Suh & Escalante-Semerena, 1995), whereas PduO and EutT enzymes are encoded within large operons whose functions are required to catabolize 1,2-propanediol (Bobik *et al.*, 1997) or ethanolamine (Kofoid *et al.*, 1999), respectively. They share little sequence identity (<20%) and structural determinations to date have revealed that the CobA (Bauer *et al.*, 2001) and PduO (Saridakis *et al.*, 2004) enzymes have three-dimensional structures that are quite different from each other; no structure of an EutT enzyme has yet been described.

PduO enzymes are the most widespread of these cob(I)alamin adenosyltransferases, with homologues in archaea, bacteria and animals (Leal *et al.*, 2003). The MMAB protein, which has been biochemically identified as a human PduO adenosyltranferase, is reportedly responsible for methylmalonic aciduria and metabolic ketoacidosis (Ciani *et al.*, 2000). To date, four crystal structures of PduO enzymes have been reported: those from *Thermoplasma acidophilum* (Saridakis *et al.*, 2004; PDB code 1nog), human (Schubert & Hill, 2006; PDB code 2idx), *Lactobacillus reuteri* (St Maurice *et al.*, 2007; PDB code 2nt8) and *Sulfolobus tokodaii* (Tanaka *et al.*, 2007; PDB code 1woz). Only two of these reports (human and *L. reuteri*) contain cocrystal structures in which the enzyme interacts with Mg^{2+} and ATP (MgATP). The PduO enzyme from *Bacillus cereus* shares 37% sequence identity to the four structurally known PduOs. In order to understand the molecular mechanism employed by ATP:cob(I)alamin adenosyltransferase enzymes and to characterize the conformational changes that accompany ligand binding, we have crystallized the PduO enzyme from *B. cereus* in two forms (with and without bound MgATP).

2. Materials and methods

2.1. Cloning, overexpression and purification

The full-length *pduO* gene was amplified from *B. cereus* genomic DNA by polymerase chain reaction (PCR) using the forward primer 5′-GGAATTC**CATATG**AAATTATATACAAAAACAGGAGATAAAGG-3′ and the reverse primer 3′-ACA**CAGCTG**TTACTCCACTTCCTTCTCTTTCTTATCAC-5′ (*Nde*I and *Xho*I restriction sites are indicated in bold). The PCR product was digested with *Nde*I and *Xho*I and ligated into the pET-28a vector (Novagen, USA), which contains a hexahistidine tag at the N-terminus. The resulting plasmid was transformed into *Escherichia coli* BL21 (DE3) strain (Novagen) and the cells were grown at 310 K in Luria–Bertani medium supplemented with kanamycin (50 µg ml^{-1}). Protein expression was induced by the addition of 0.5 m*M* isopropyl-β-D-1-thiogalactopyranoside (IPTG) once the cells had reached an optical density at 600 nm of about 0.45; the cells were then grown for an additional 12 h at 291 K. Cells were then harvested by centrifugation at 5000*g* for 30 min at 277 K. The pelleted cells were suspended in buffer *A* (20 m*M* Tris–HCl pH 7.9, 500 m*M* NaCl, 5 m*M* imidazole) and lysed by sonication. The crude lysate was centrifuged at 20 000*g* for 1 h at 277 K. The supernatant was loaded onto an Ni^{2+}-chelated HiTrap chelating HP column (GE Healthcare, USA) equilibrated with buffer *A*. The protein was eluted with a linear gradient of buffer *A* containing 1 *M* imidazole. The protein was purified to its final state by gel filtration on a HiLoad 16/60 Superdex 200 column (GE Healthcare, USA) that had previously been equilibrated with buffer *B* (20 m*M* Tris–HCl pH 7.9, 200 m*M* NaCl, 2 m*M* DTT). Prior to crystallization experiments, the purified protein was concentrated to 13 mg ml^{-1} using an Amicon Ultra-15 ultrafiltration device (Millipore, USA). This procedure yielded approximately 15 mg of PduO protein from a 1 l culture. The protein concentration was determined using a Bradford assay and protein purity was examined by 15% SDS–PAGE; the purity was >95%.



(*a*)

(*b*)

Figure 1
Crystals of the PduO protein from *B. cereus*. (*a*) Native crystal; (*b*) cocrystal with Mg^{2+} and ATP. The crystal dimensions of the native and MgATP-complexed crystals were 0.2 × 0.2 × 0.4 and 0.2 × 0.2 × 0.3 mm, respectively.

2.2. Crystallization and X-ray analysis

Preliminary crystallization screens were performed using the sitting-drop vapour-diffusion method (0.2 µl protein solution and 0.2 µl reservoir solution equilibrated against 100 µl reservoir solution) using the Hydra II Plus One crystallization robot (Matrix Technologies Ltd, UK) to set up 96-well Intelliplates (Art Robbins Instruments, USA) at 295 K. Commercial screening kits from Hampton Research were used. Crystals were obtained under conditions consisting of 100 m*M* MES pH 6.5, 1.6 *M* ammonium sulfate and 10%(*v*/*v*) dioxane. Crystal growth was scaled up using the hanging-drop vapour-diffusion method in 24-well VDX plates (Hampton Research, USA); each hanging drop was prepared by mixing 1 µl protein solution and 1 µl reservoir solution [100 m*M* MES pH 6.5, 1.52 *M* ammonium sulfate, 9%(*v*/*v*) dioxane] and equilibrated over 500 µl reservoir solution. For cocrystallization, the protein solution was mixed with ATP and $MgCl_2$ (4 m*M* each) and complexed crystals were obtained at 295 K by the hanging-drop vapour-diffusion method in 100 m*M* MES pH 6.5, 1.57 *M* ammonium sulfate and 9.7%(*v*/*v*) dioxane. Single crystals were obtained within 10 d and were used for X-ray diffraction. The crystal dimensions of native and MgATP-complexed crystals were 0.2 × 0.2 × 0.4 and 0.2 × 0.2 × 0.3 mm, respectively. Prior to data collection, the crystals were transferred to cryoprotection solution containing 50 m*M* MES pH 6.5, 0.76 *M* ammonium sulfate, 4.5%(*v*/*v*) dioxane and 1.7 *M* sodium malonate pH 7.0. Data sets were collected from the native and MgATP-complexed crystals on beamline 6C at the Pohang Light Source (Pohang, Republic of Korea) using an ADSC Quantum 210 CCD detector. A total range of 120° was covered with 1.0° oscillations and 10 s exposure per frame. The wavelength of the synchrotron X-rays was 1.23986 Å. The crystal-to-detector distance was set to 150 mm. X-ray diffraction data were collected to 1.9 Å resolution from native crystals and 2.0 Å resolution from MgATP-complexed crystals. All data sets were indexed, processed and scaled using the *HKL*-2000 software package (Otwinowski & Minor, 1997).

3. Results and discussion

The gene encoding a PduO protein from *B. cereus* was cloned. The protein was overexpressed in *E. coli* and purified for structural studies. Crystals suitable for X-ray analysis were obtained using the following optimized crystallization conditions: 100 m*M* MES pH 6.5 containing ammonium sulfate and dioxane as precipitants (Fig. 1). The native crystal belonged to the *C*-centred orthorhombic space group $C222_1$, with unit-cell parameters a = 64.93, b = 137.08, c = 158.55 Å, $\alpha = \beta = \gamma = 90°$. The MgATP-complex crystal also belonged to space group $C222_1$, with unit-cell parameters a = 64.93, b = 137.04, c = 158.26 Å, $\alpha = \beta = \gamma = 90°$. X-ray diffraction data were collected to 1.9 Å resolution for the native crystals and 2.0 Å reso-

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shells.

	Native	MgATP complex
Space group	$C222_1$	$C222_1$
Unit-cell parameters (Å, °)	a = 64.93, b = 137.08, c = 158.55, $\alpha = \beta = \gamma = 90$	a = 64.93, b = 137.04, c = 158.26, $\alpha = \beta = \gamma = 90$
Resolution range (Å)	50–1.9 (1.90–1.97)	50–2.0 (2.00–2.07)
Total reflections	205795	157325
Unique reflections	53001	43832
Redundancy	3.9 (2.2)	3.6 (2.5)
Completeness (%)	94.4 (69.0)	91.1 (65.4)
$R_{\rm merge}$† (%)	4.0 (17.6)	8.1 (31.2)
$\langle I/\sigma(I)\rangle$	26.7 (3.4)	11.1 (1.9)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where I represents the observed intensity, $\langle I\rangle$ represents the average intensity and i counts through all symmetry-related reflections.

lution for the MgATP-complexed crystals. Each asymmetric unit contained three protein molecules, yielding a crystal volume per protein weight ($V_{\rm M}$) of 2.69 Å^3 Da^{-1} and a solvent content of 54.3% (Matthews, 1968) for both the native and the MgATP-complexed crystals. The data-collection statistics are summarized in Table 1. The crystal structure was solved by the molecular-replacement method using the *CNS* package (Brünger *et al.*, 1998) with the *B. halodurans* putative cobalamin adenosyltransferase (PDB code 2ah6) as a search model. After obtaining a good molecular-replacement solution, initial crystallographic refinement was performed using rigid-body refinement in the resolution range 50–3.5 Å, resulting in a model with an R factor of 42.15% and an $R_{\rm free}$ of 44.92%. Complete refinement of the structure is in progress.

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