Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and X-ray diffraction measurements on recombinant molbindin, MopII, from *Clostridium pasteurianum*

Jennifer A. Harrison, Alexander W. Schüttelkopf, David H. Boxer and William N. Hunter*

School of Life Sciences, The Wellcome Trust Biocentre, University of Dundee, Dundee DD1 5EH, Scotland

Correspondence e-mail: w.n.hunter@dundee.ac.uk

Received 25 July 2001
Accepted 20 August 2001

Clostridium pasteurianum carries three genes termed *mopI*, *II* and *III* encoding three molbindin isoforms, one of which has been cloned, the gene product expressed in high yield and crystallized using the hanging-drop vapour-diffusion method. Well ordered monoclinic crystals in two different crystal forms, both with space group *C*2, were obtained in the presence and absence of Na_2MoO_4 and Na_2WO_4. Ligand-bound MopII crystallized with polyethylene glycol (PEG) 400 as a precipitant, whereas apo MopII required PEG 6000. High-resolution diffraction data were collected for ligand-bound MopII structures using synchrotron radiation to 1.8 and 1.6 Å resolution for the molybdate and tungstate complexes, respectively. Data were collected on apoMopII crystals to a resolution of 1.8 Å in-house.

1. Introduction

Molbindins are a class of low molecular weight proteins that specifically bind molybdate and which are implicated in the molybdenum metabolism of a variety of microorganisms (Grunden & Shanmugam, 1997; Lawson *et al.*, 1997; Pau *et al.*, 1998). Three genes encoding molbindins have been identified in the anaerobic nitrogen-fixing bacterium *Clostridium pasteurianum* (Hinton *et al.*, 1987) and are denoted *mopI*, *II* and *III*. Early studies suggested that Mop proteins bound part of the molybdopterin cofactor; hence, they were first described as molybdopterin-binding proteins (Hinton *et al.*, 1987). Homologues of the Mop sequence have been reported within a variety of bacterial proteins involved in molybdenum metabolism (Wagner *et al.*, 2000; Gourley *et al.*, 2001). Crystal structures of *Escherichia coli* ModE (Hall *et al.*, 1999) and *Azotobacter vinelandii* ModG (Delarbre *et al.*, 2001) have revealed structural details of the molbindin domain and interactions with molybdate, subsequently leading to a classification of molbindin structures (Gourley *et al.*, 2001). Recently, the crystal structure of a tungstate-loaded Mop purified from *Sporomusa ovata* was reported (Wagner *et al.*, 2000). In this study, the authors inadvertently purified and crystallized a different protein to the one they were interested in but were able to make an assignment of sequence and classification of the protein based on a careful interpetation of high-resolution electron-density maps.

At present, there has only been one report of a molbindin domain in the apo-form, that of the regulatory protein ModE (Hall *et al.*, 1999). ModE has a C-terminal oxyanion-binding domain which consists of two Mop-like subdomains termed a DiMop unit, although only one of the subdomains is involved in binding the oxyanion ligand (Gourley *et al.*, 2001). ModE is dimeric and the binding of an oxyanion has been shown to induce conformational changes involving the DiMop oxyanion-binding domain which influence the quaternary structure (Gourley *et al.*, 2001). Such a structural change may contribute to the highly specific biological function of ModE in regulating the oxyanion-dependent regulation of transcription. The Mop proteins are either trimers of DiMop-like subunits as exemplified by ModG (Delarbre *et al.*, 2001) or trimers of Mop dimers as illustrated by the *S. ovata* Mop (Wagner *et al.*, 2000) when loaded with oxyanions. There has not yet been any report of the structure of an apo Mop and our research will ultimately reveal the changes, if any, induced in Mop proteins by ligand binding.

We now describe the cloning of the *C. pasteurianum mopII* gene, the overexpression and purification of the recombinant protein MopII and the crystallization and characterization of crystals of apoMopII together with molybdate and tungstate complexes.

2. Methods and materials

2.1. Cloning and expression of *Clostridium pasteurianum* MopII

The *C. pasteurianum mopII* gene was amplified from genomic DNA by PCR using the 5′-CAA-CTC-GAG-**CAT-ATG**-AGT-ATA-AGT-GCA-AGA-AA-3′ and 5′-CGA-**GGA**-

TCC-AAG-CTT-CAA-CTC-ATA-CTG-TGA-TAA-TTA-AGC-T-3′ specific oligonucleotide primers. These oligonucleotides (Hybaid) introduced restriction sites *Nde*1 and *Bam*H1 (in bold). *Pfu* polymerase, T4 DNA ligase and restriction endonucleases were obtained from Promega. The PCR product was isolated by gel electrophoresis and purified by gel extraction (Qiagen) and cloned into PCR4Blunt TOPO (M13 vector) using the zero blunt TOPO PCR cloning system (Invitrogen). Positive clones were confirmed by DNA sequencing. The *mopII* gene was excized by restriction digest and ligated into the pET15b vector (Novagen). After the identity had been confirmed, the plasmid was heat-shock transformed into *E. coli* BL21(DE3)pLysS (Novagen). A single colony was cultured to an OD_{600} of approximately 0.6 in 1 l of LB broth containing ampicillin (100 µg ml^{-1}) at 310 K, at which point isopropyl-β-D-thiogalactopyranoside (0.01 m*M*) was added to induce protein expression. Cells were grown for a further 16 h at 293 K then harvested by centrifugation (2600*g*) at 277 K.

2.2. Purification of *C. pasteurianum* MopII

Cells were resuspended in 50 m*M* Tris–HCl pH 7.6 and broken open by passage through a French press. The cell extract was filtered to 0.45 µm and applied to a metal-chelating column (5 ml HiTrap, Amersham Pharmacia Biotech) which had been loaded with divalent nickel (using a nickel chloride solution) on a BioCAD 700E workstation. The column was washed with 50 m*M* Tris–HCl pH 7.6 to remove any unbound protein. The histidine-tagged protein was eluted in a gradient (0–500 m*M*) of imidazole in the same buffer. As MopII lacks a detectable chromophore, the protein was detected by Bradford assay and SDS–PAGE analysis. The fractions containing the protein were dialysed against 50 m*M* Tris–HCl pH 7.6, then incubated with thrombin (Amersham Pharmacia Biotech, 10 µg per milligram of protein) in 50 m*M* phosphate-buffered saline at room temperature overnight to remove the histidine tag. The protein was charged to a strong anion-exchange (HQ Poros) column (Amersham Pharmacia Biotech) and eluted in a gradient of NaCl (0–500 m*M*). The fractions containing Mop were identified (Bradford assay and SDS–PAGE), dialysed against Tris–HCl pH 8 overnight and the protein solution concentrated to 10 mg ml^{-1} for crystallization trials. The yield of protein was ∼30 mg l^{-1} of culture; the purity was confirmed by SDS–PAGE (single band on a Coomassie-stained gel) and by MALDI-TOF mass spectrometry.

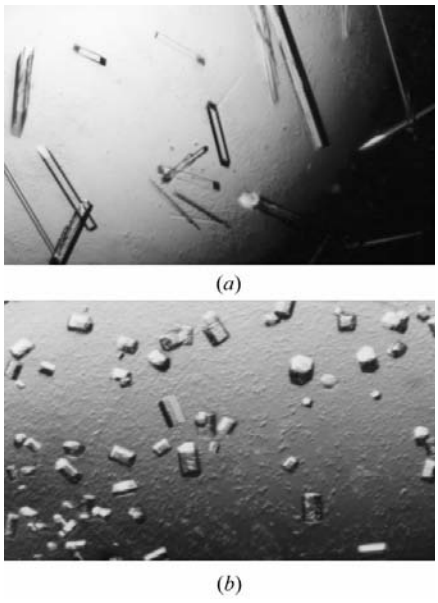

Figure 1
Typical crystals (*a*) of oxyanion-bound MopII and (*b*) apo MopII from *C. pasteurianum*.

2.3. Crystallization of oxyanion-bound and apo *C. pasteurianum* MopII

Crystallization trials were initiated using commercially available screens (Hampton Research, USA) at different temperatures. The best results, which were subsequently optimized, for the oxyanion-bound protein were observed in Crystal Screen Cryo condition 14 and for the apo-protein were observed in Crystal Screen 2 condition 1.

Crystals of MopII complexed with tungstate (Fig. 1) were grown at 293 K overnight from hanging drops consisting of 2 µl of protein solution, 2 µl of reservoir, 1 µl of 8 m*M* Na_2WO_4 (fivefold molar excess relative to MopII) in 95 m*M* HEPES pH 7.5, 26.6%(*w*/*v*) polyethylene glycol 400, 5%(*w*/*v*) glycerol and 190 m*M* $CaCl_2$. Well formed blocks, with maximum dimensions of 0.70 × 0.05 × 0.05 mm, are monoclinic in space group *C*2, with unit-cell parameters a = 56.37, b = 78.51, c = 94.84 Å, β = 90.01°. The molybdate–MopII complex crystals were grown under similar conditions and are isomorphous, with unit-cell parameters a = 56.81, b = 78.38, c = 95.27 Å, β = 90.01°. Crystals of the apo MopII (Fig. 1) grew overnight at 293 K in hanging drops consisting of 2 µl of protein stock solution and 2 µl of a reservoir solution consisting of 2 *M* NaCl and 10%(*w*/*v*) polyethylene glycol 6000. These crystals are also monoclinic with space group *C*2, but represent a different crystal form with unit-cell parameters a = 79.14, b = 82.47, c = 57.14 Å, β = 93.38°.

Table 1
Data collection and processing statistics of oxyanion-bound MopII and apoMopII from *C. pasteurianum*.

Mop	WO_4^{2-}-bound	MoO_4^{2-}-bound	Apo MopII
No. of unique reflections	53578	36043	33112
Resolution range (Å)	20–1.60	20–1.83	25–1.80
Completeness (%)	93.6 (90.4)	97.4 (98.8)	97.6 (93.9)
Redundancy	3.5	2.8	1.9
R_{sym}	6.8 (26.4)	5.5 (33.6)	4.1 (28.0)
Average $I/\sigma(I)$	14.7 (4.3)	16.7 (3.7)	16.8 (2.6)

2.4. X-ray measurements and data processing

The oxyanion-loaded MopII crystals were removed directly from the mother liquor and maintained in a nitrogen-gas stream at 100 K. High-resolution X-ray diffraction data were collected at the Synchrotron Radiation Facility, beamline PX9.6 (λ = 0.89 Å) at Daresbury, England from the tungstate-bound MopII crystals to a resolution of 1.6 Å and from molybdate-bound MopII to a resolution of 1.8 Å. The apoMop crystals were cryoprotected by soaking in 33%(*v*/*v*) glycerol for approximately 5 s before being placed in a nitrogen-gas stream at 100 K. X-ray diffraction data were collected in-house using an RU-200 Cu rotating-anode X-ray source (λ = 1.5418 Å) and an R-AXIS IV image-plate detector. Data were collected for the apo MopII to a resolution limit of 1.8 Å.

All data were processed with *DENZO* and scaled with *SCALEPACK* (Otwinowski & Minor, 1997); details are given in Table 1. The Matthews coefficients (Matthews, 1968) for MopII are calculated based on the assumption of six subunits, each of approximate molecular mass 7 kDa, per asymmetric unit. For the oxyanion-bound MopII, a Matthews coefficient of 2.4 Å^3 Da^{-1} with ∼50% solvent content is estimated, whilst for apo MopII the coefficient is 2.1 Å^3 Da^{-1} with a solvent content of ∼42%. Self-rotation function calculations (using *POLARRFN*; Collaborative Computational Project, Number 4, 1994; data not shown) indicate that a non-crystallographic three-fold axis of symmetry is present in both crystal forms. This observation is consistent with the quaternary structure observed for the oxyanion-loaded Mop of *S. ovata* (Wagner *et al.*, 2000).

Several approaches are available to solve the structures of these crystal forms.

Molecular-replacement methods using the *S. ovata* WO_4^{2-}-bound Mop (Wagner *et al.*, 2000) or the *E. coli* ModE DiMop (Gourley *et al.*, 2001; Hall *et al.*, 1999) as initial search models could be attempted. However, an alternative approach, and one we favour since it could provide experimentally derived phase information, would exploit the anomalous scattering signal of tungsten (from the tungstate complex) either by itself or combined with the isomorphous differences with the molybdate complex. A difference Patterson function calculated on the basis of the tungstate and molybdate complex data (Table 1) shows well defined maxima (data not shown), which suggests that a single isomorphous replacement with anomalous scattering approach to phase determination should be successful.

In summary, we have obtained crystals of recombinant *C. pasteurianum* MopII complexed with the oxyanions molybdate or tungstate and also crystals of the apo protein. The crystals are well ordered, diffract to high-resolution and are suitable for full structural characterization of free and ligand-bound *C. pasteurianum* MopII to complement ongoing biochemical studies on the storage and utilization of molybdate.

We thank the Biological and Biotechnology Sciences Research Council, UK and the Wellcome Trust for funding, C. Bond and D. Gourley for discussions and encouragement, and G. Sawers for a gift of genomic DNA.

References

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Delarbre, L., Stevenson, C. E. M., White, D. J., Mitchenall, L. A., Pau, R. N. & Lawson, D. M. (2001). *J. Mol. Biol.* **308**, 1063–1079.
Gourley, D. G., Schüttelkopf, A. W., Anderson, L. A., Price, N. C., Boxer, D. H. & Hunter, W. N. (2001). *J. Biol. Chem.* **276**, 20641–20647.
Grunden, A. M. & Shanmugam, K. T. (1997). *Arch. Microbiol.* **168**, 345–354.
Hall, D. R., Gourley, D. G., Leonard, G. A., Duke, E. M. H., Anderson, L. A., Boxer, D. H. & Hunter, W. N. (1999). *EMBO J.* **18**, 1435–1446.
Hinton, S. M., Slaughter, C., Eisner, W. & Fisher, T. (1987). *Gene*, **54**, 211–219.
Lawson, D. M., Williams, C. E., White, D. J., Choay, A. P., Mitchenall, L. A. & Pau, R. N. (1997). *J. Chem. Soc. Dalton Trans.*, pp. 1–4.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Pau, R. N., Klipp, W. & Leimkuhler, S. (1998). *Iron and Related Transition Metals in Microbial Metabolism*, edited by G. Winkelmann & C. J. Carrano, pp. 217–234. Harwood Academic Publishers.
Wagner, U. G., Stupperich, E. & Kratky, C. (2000). *Structure*, **8**, 1127–1136.