Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray studies on the molbindin ModG from *Azotobacter vinelandii*

Clare E. M. Williams, Daniel J. White, Laure Delarbre, Lesley A. Mitchenall, Richard N. Pau† and David M. Lawson*

Nitrogen Fixation Laboratory, John Innes Centre, Norwich NR4 7UH, England

† Present address: Department of Biochemistry and Molecular Biology, University of Melbourne, Parkville, Victoria 3052, Australia.

Correspondence e-mail: david.lawson@bbsrc.ac.uk

Received 5 February 1999
Accepted 16 April 1999

Crystals of the molbindin ModG (subunit M_r = 14359 Da), a cytoplasmic molybdate-binding protein from *Azotobacter vinelandii*, were grown by vapour diffusion. Both apo and tungstate-bound forms were crystallized and X-ray data were collected at 100 K. Apo-ModG crystallizes in space group $P6_322$, with unit-cell dimensions $a = b = 90.62$, $c = 79.46$ Å. Native data to a resolution of 2.5 Å were collected from a single crystal, which showed a marked improvement in diffraction quality after annealing. Data from a single-site gold derivative were also collected at 2.7 Å resolution. Crystals of the ligand-bound form of ModG belong to space group $P321$, with unit-cell parameters $a = b = 50.57$, $c = 79.29$ Å. X-ray data to a resolution of 2.0 Å were collected.

1. Introduction

Molybdenum is a component of the cofactors of over 30 molybdoenzymes (Stiefel, 1993). In *Clostridium pasteurianum*, molybdate binds to a small cytoplasmic protein called Mop before being incorporated into molybdoenzymes (Hinton & Mortenson, 1985; Hinton *et al.*, 1987). The amino-acid sequence of ModG, which is encoded in the high-affinity molybdenum transport locus of *A. vinelandii*, consists of a 68 amino-acid tandem repeat of a Mop-like sequence (Mouncey *et al.*, 1995). On the basis of gel-filtration chromatography, ModG is thought to be a homodimer and the Mop protein of *Haemophilus influenzae* appears to be a homotetramer. Thus, the functional unit in both cases consists of four 7 kDa Mop-like domains, and both proteins can bind up to four molybdates with high affinity (K_d < 0.1 μ*M*). As with the periplasmic molybdate-binding protein ModA, they can also bind tungstate but not sulfate (White & Pau, 1998). Secondary-structure analysis indicates that they are all-β proteins and sequence alignments suggest that they make use of both neutral and positively charged protein groups to bind their ligands. Mop and ModG are referred to as molbindins because they represent a fundamental molybdenum-binding structure (Lawson *et al.*, 1997), and they may play a role in intracellular molybdenum homeostasis (Mouncey *et al.*, 1995). Molbindin-like sequences are also present at the C-terminal end of ModC, the ATP-binding component of the high-affinity molybdenum transporter, and in the C-terminal half of ModE, the molybdenum-responsive transcriptional regulator (Pau *et al.*, 1997). ModE is a dimer whose C-terminal half consists of a tandem repeat of amino acids similar to ModG (Anderson *et al.*, 1997). Two crystal forms of *Escherichia coli* ModE have recently been described (Hall *et al.*, 1999).

2. Materials and methods

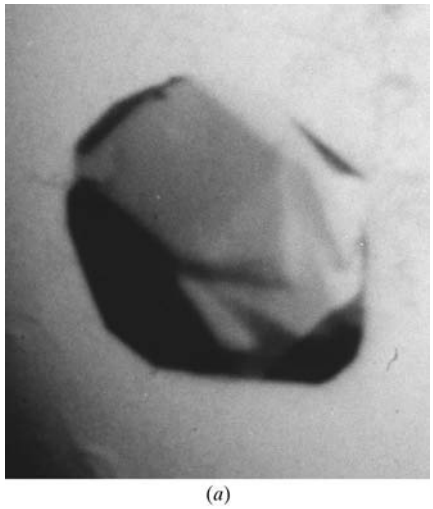
2.1. Expression and purification

The coding sequence of the *modG* gene, amplified by PCR using the plasmid pLAM97212 as a template, was cloned into the expression vector pET15b (Novagen) to give plasmid pDJW373. This was transformed into *E. coli* strain BL21(DE3)pLys (Studier & Moffatt, 1986), which was grown at 310 K in a New Brunswick Micros fermentor using 20 l Luria–Bertani medium containing 0.4% glucose. Protein overexpression was induced at A_{600} = 0.4–0.5 by addition of 0.5 m*M* isopropyl-β-D-thiogalactopyranoside and was continued for 4 h. Harvested cells were suspended in 25 m*M* Tris–HCl pH 7.5, 0.1% Tween 20 and 2 mg ml^{-1} DNAase I and were lysed by freeze-thawing with liquid nitrogen. The soluble cell lysate, obtained by centrifugation at 20000 rev min^{-1} for 2 h, was loaded on an Ni^{2+}-charged Hi-Trap metal-chelation column (Pharmacia) and unbound proteins were removed by washing with 20 m*M* imidazole in 50 m*M* Tris–HCl pH 8.0. His-tagged ModG which bound to the column was eluted with a 0–500 m*M* imidazole gradient in 50 m*M* Tris–HCl pH 8.0. The peak which contained His-tagged ModG was dialysed against 20 m*M* Tris–HCl pH 8.4, 150 m*M* NaCl, 2.5 m*M* $CaCl_2$ and concentrated with a Centriprep-10 concentrator (Amicon). The N-terminal His tag was cleaved by digestion with thrombin (ICN) at 296 K for 16 h. ModG was further

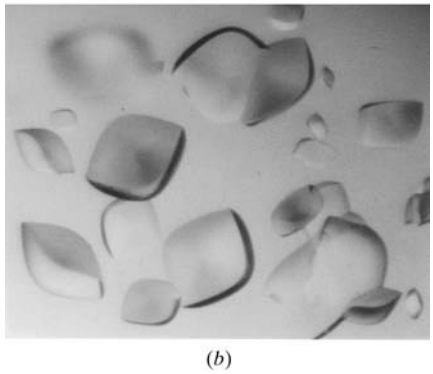
purified by gel-filtration chromatography on a Superdex-75 16/60 column (Pharmacia), being eluted with 50 m*M* Tris–HCl pH 7.6, 100 m*M* NaCl. ModG was then buffer-exchanged into 10 m*M* Tris–HCl pH 7.6 and concentrated to 15 mg ml^{-1} (assuming $A_{280} \simeq 0.5$ for a 1 mg ml^{-1} solution) using Centriprep-10 and Centricon-10 concentrators (Amicon). The protein was centrifuged through a 0.1 µm Ultrafree filter (Millipore) before use.

2.2. Crystallization and X-ray diffraction analysis

Crystallization trials were performed by vapour diffusion in hanging drops using VDX plates (Hampton Research) at a constant temperature of 291 K. Drops consisted of 2 µl protein solution mixed with 2 µl well solution (well volume was 1.0 ml). Initial crystallization conditions were established using sparse-matrix screens (Jancarik & Kim, 1991) and subsequently optimized. Heavy-atom derivatives of the apo-form were produced by soaking crystals in mother liquor containing heavy-atom salts. Crystals were cryoprotected by soaking for up to 5 min in mother liquor containing either glycerol or ethylene glycol in place of 25–30% of the buffer volume. They were then mounted in cryo-loops (Hampton Research) and flash-cooled to 100 K using an Oxford Cryosystems Cryostream cooler. Diffraction data were collected at the Synchrotron Radiation Source at Daresbury on station PX 7.2 (λ = 1.488 Å) using a 300 mm MAR Research image plate. X-ray data were processed with *DENZO* and merged using *SCALEPACK* (Otwinowski & Minor, 1997). All subsequent downstream processing and statistical analysis was effected using programs from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994).



(*a*)



(*b*)

Figure 1
Crystals of *A. vinelandii* ModG. (*a*) Single crystal of apo-ModG, with a largest dimension of approximately 0.5 mm. (*b*) Crystals of tungstate-bound ModG. The largest crystals are approximately 0.3 mm in the maximum dimension.

3. Results and discussion

3.1. Apo-ModG X-ray data

Crystals of apo-ModG grew from 45% saturated ammonium sulfate in 0.1 *M* HEPES buffer at pH 8.0 after about 2 d. They formed short hexagonal rods (Fig. 1*a*). For the first data collection, a crystal was cryoprotected by soaking in mother liquor containing 25%(*v*/*v*) ethylene glycol for 30 s. The diffraction pattern for a 0.5° oscillation image showed diffuse and misshapen diffraction peaks to a maximum resolution of 3.4 Å with an estimated mosaicity of 0.8°. Subsequently, the crystal was removed from the cryogenic nitrogen-gas stream and returned to the cryoprotectant solution at room temperature for 4 min, during which time several small fragments broke off from the crystal. The remaining piece of crystal was remounted and flash-cooled again. This time, a 0.5° oscillation image showed sharp circular spots in an ordered lattice to a diffraction limit of 2.5 Å and with a mosaicity of 0.4°. The symmetry was primitive hexagonal, with unit-cell parameters $a = b = 90.62$, $c = 79.46$ Å. A full 180° of data were collected and initially processed in space group *P*6. After inspection of pseudo-precession photographs, the space group was assigned as $P6_322$ and the data were reprocessed in this space group. The resultant data set was 99.9% complete to 2.5 Å resolution, with a redundancy of 19.9 and an $R_{\rm merge}$[1] of 10.3%. The data for the outer resolution shell (2.59–2.50 Å) gave an $R_{\rm merge}$ of 34.7% and an $I/\sigma\langle I\rangle$ of 8.5. A similar crystal soaked in 10 m*M* potassium dicyanoaurate [$KAu(CN)_2$] for 5 h was cryoprotected as above, but for 5 min initially. This gave good diffraction without the need for an annealing step. A 99.8% complete data set was collected to a resolution of 2.7 Å, having an overall redundancy of 18.7 and an $R_{\rm merge}$ of 12.2% (outer shell $R_{\rm merge}$ = 37.4%, $I/\sigma\langle I\rangle$ = 7.3). This data set was scaled to the native set using *SCALEIT*, giving a mean fractional isomorphous change[2] of 14.9%. Inspection of an isomorphous difference Patterson map calculated from data in the 10.0–4.0 Å resolution range revealed several clear peaks on the Harker sections, which resulted from a single major site located at $x = y = 0.33$, $z = 0.40$ (fractional coordinates).

3.2. Ligand-bound ModG X-ray data

Ligand-bound ModG was produced by adding Na_2WO_4 to a final concentration of 2 m*M* to the protein. Crystals were grown from 75% saturated KH_2PO_4 in 0.1 m*M* Tris–HCl buffer at pH 8.5 and took up to 10 d to grow to full size. They typically adopted a rounded teardrop-shaped morphology and were highly birefringent under cross-polarized light (Fig. 1*b*). Prior to flash cooling, crystals were soaked transiently in cryoprotectant comprised of 30%(*v*/*v*) glycerol and 60% saturated KH_2PO_4 in 0.1 m*M* Tris–HCl buffer at pH 8.5 containing 2 m*M* Na_2WO_4. Despite the high mosaicity of 1.3°, X-ray data to a resolution of 2.0 Å were collected from a single crystal rotated through 90°. These data could be processed in a primitive trigonal cell with parameters $a = b = 50.57$, $c = 79.29$ Å. Subsequent analysis confirmed the space group to be *P*321. The resultant data set was 99.8% complete to 2.0 Å resolution, with a redundancy of 4.2 and an $R_{\rm merge}$ of 7.3%. The data for the outer resolution shell (2.07–2.00 Å) gave an $R_{\rm merge}$ of 31.2% and an $I/\sigma\langle I\rangle$ of 3.4. Unfortunately, the wavelength of the incident X-rays (1.488 Å) was unsuitable for the measurement of anomalous dispersion effects resulting from the bound tungstate. Thus, the heavy-atom positions could not be determined from an anomalous difference Patterson.

3.3. Comparison of data sets

A survey of the Protein Data Bank (Bernstein *et al.*, 1977) reveals that both $P6_322$ and *P*321 are relatively uncommon space groups for macromolecular crystals.

[1] $R_{\rm merge} = \sum(|I_j - \langle I_j\rangle|)/\sum\langle I_j\rangle$, where I_j is the intensity of an observation of reflection *j* and $\langle I_j\rangle$ is the average intensity for reflection *j*.

[2] The mean fractional isomorphous change between the native structure factors ($F_{\rm P}$) and the derivative structure factors ($F_{\rm PH}$) is defined as $\sum(|F_{\rm PH} - F_{\rm P}|)/\sum F_{\rm P}$.

As of January 1999, there were 46 entries for each in the database, which actually reduce to a non-redundant set of only 24 entries for $P6_322$ and 15 entries for $P321$. Crystallographic threefold axes parallel to the c axis are common to both these space groups, and it is this axial length which is conserved between the two ModG crystal forms, being approximately 80 Å in both cases. A cross-rotation function calculated between the native apo and tungstate-bound data sets using *ALMN* on data in the range 15–6 Å gave a single significant peak at 5.8σ. This corresponds to the alignment of the two c axes and may be indicative of similar molecular packing in the two crystal forms. Solvent-content estimations indicate that there is one subunit per asymmetric unit in both cases, giving solvent-content values of 63% for the $P6_322$ cell and 41% for the $P321$ cell (Matthews, 1968).

The determination of the crystal structures of both ligand-bound and apo forms of ModG will enable us to describe the conformational changes associated with anion binding. Furthermore, we will be able to compare and contrast the anion-binding sites of ModG with those of the periplasmic molybdate-binding proteins. Finally, we hope that these structures will shed more light on the role of ModG and molbindins in general.

This work is funded by the BBSRC. We would like to thank the staff at Daresbury for assistance during X-ray data collection, which was supported by the CCLRC. DJW and LD are funded by studentships from the John Innes Foundation. We are grateful to M. Chan for large-scale bacterial cultures and E. J. Dodson for assistance with data analysis.

References

Anderson, L. A., Palmer, T., Price, N. C., Bornemann, S., Boxer, D. H. & Pau, R. N. (1997). *Eur. J. Biochem.* **246**, 119–126.

Bernstein, F. C., Koetzle, T. F., Williams, G. J., Meyer, E. F. Jr, Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T. & Tasumi, M. (1977). *J. Mol. Biol.* **112**, 535–542.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Hall, D. R., Gourley, D. G., Duke, E. M. H., Leonard, G. A., Anderson, L. A., Pau, R. N., Boxer, D. H. & Hunter, W. N. (1999). *Acta Cryst.* D**55**, 542–543.

Hinton, S. M. & Mortenson, L. E. (1985). *J. Bacteriol.* **162**, 485–493.

Hinton, S. M., Slaughter, C., Eisner, W. & Fisher, T. (1987). *Gene*, **54**, 211–219.

Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.

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. 3981–3984.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Mouncey, N. J., Mitchenall, L. A. & Pau, R. N. (1995). *J. Bacteriol.* **177**, 5294–5302.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Pau, R. N., Klipp, W. & Leimkühler, S. (1997). *Iron and Related Transition Metals in Microbial Metabolism*, edited by G. Winkelmann & C. J. Carrano, pp. 217–234. Amsterdam: Harwood Academic Publishers.

Stiefel, E. I. (1993). *Molybdenum Enzymes, Cofactors and Model Systems*, edited by E. I. Stiefel, D. Coucouvanis & W. E. Newton, pp. 1–18. Washington DC: American Chemical Society.

Studier, F. W. & Moffatt, B. A. (1986). *J. Mol. Biol.* **189**, 113–130.

White, D. J. & Pau, R. N. (1998). Personal communication.