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Two crystal forms of ModE, the molybdate-dependent transcriptional regulator from *Escherichia coli*

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The molybdenum-responsive ModE regulatory protein from *Escherichia coli* has been purified and used in crystallization trials. Two crystal forms have been observed. Form I is tetragonal, $P4_12_12$ (or enantiomorph), with $a = b = 72.3$, $c = 246.2$ Å and diffracts to medium resolution. Form II is orthorhombic, $P2_12_12$, with $a = 82.8$, $b = 127.9$, $c = 64.0$ Å and diffraction has been observed beyond 2.8 Å resolution. Structural analysis, in combination with ongoing biochemical characterization, will assist the elucidation of the structure–activity relationship in regulating the uptake of molybdate in bacteria.

1. Introduction

Molybdenum is an essential trace element used in a range of enzyme functions (Rajagopalan, 1996). Specific high-affinity systems have evolved to regulate the uptake of the metal as MoO_4^{2-} and to then utilize its complex chemical properties in biochemical reactions. In *Escherichia coli*, the *modABCD* (molybdenum transport) operon codes for the necessary transport proteins. ModA encodes a periplasmic binding protein, the structure of which has recently been determined (Hu *et al.*, 1997). ModB and ModC are integral membrane-bound proteins, the latter of which binds ATP. Little is known about ModD. Transcription of this operon is regulated by the molybdenum-responsive ModE regulatory protein (Grunden *et al.*, 1996).

ModE has been cloned, overexpressed and biochemically characterized (Anderson *et al.*, 1997). The protein is functional as a homodimer of subunits consisting of 262 amino acids. It has a high affinity for either molybdate or tungstate with a K_d of 0.8 µ*M*, and the binding of the anion is accompanied by a large quenching of the fluorescence attributed to the three tryptophans in the sequence (Anderson *et al.*, 1997). This suggests that upon binding the selected anion a significant conformational change occurs. With anion bound, ModE binds near to the transcription start of the *modABCD* promoter and functions as a repressor. The levels of and availability of molybdenum therefore regulates the conformation of ModE and determines repression of the transport operon, thus regulating the acquisition of this metal.

The ModE sequence suggests two domains, one of which (the C-terminal domain) has significant homologies with domains in a range of bacterial proteins. These domains share the ability to bind MoO_4^{2-} (Anderson *et al.*, 1997; Grunden *et al.*, 1996). A model for this C-terminal molybdate-binding domain has been proposed based on amino-acid sequence homologies (Lawson *et al.*, 1997). The N-terminal domain contains a putative helix–turn–helix motif, which is frequently observed in DNA-binding proteins (Brennan, 1993; Grunden *et al.*, 1996).

We have initiated a crystallographic investigation of ModE to determine how the protein binds specifically to molybdate/tungstate, to determine the fold of the anion-binding domain and the conformational changes that can accompany this binding, and to determine how this in turn regulates the binding to DNA and the repressor function of the protein.

2. Preparation of recombinant ModE.

The *E. coli ModE* gene has (Anderson *et al.*, 1997) been cloned into the T7-promoter based *E. coli* expression pET15b system (Studier *et al.*, 1990; Novagen) creating the plasmid pLAA6. This vector adds a 6-histidine tag to the N-terminus of the protein. The *E. coli* strain BL21(DE3) was transformed with this plasmid and selected on Luria–Bertani (LB) agar plates containing 100 mg ml^{-1} of ampicillin. A positive colony was grown in LB broth with ampicillin to mid-log phase, at which point isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.4 m*M* and cell growth continued with vigorous aeration for 4 h. Cells were harvested by centrifugation (2500*g*) at 277 K, resuspended in 50 m*M* Tris–HCl pH 7.6, 250 m*M* NaCl, 5 m*M* benzamidine and stored frozen in liquid nitrogen. The bacterial cells were broken by passage

through a French press and the insoluble cell debris was pelleted by centrifugation at 277 K (18000*g*) for 15 min. The cell extract was filtered and applied to a 5 ml metal-chelate affinity column (Hi-Trap; Pharmacia) charged with nickel. The unbound proteins were washed from the column with 50 m*M* Tris–HCl pH 7.6 containing 250 m*M* NaCl and 5 m*M* benzamidine. The His-tagged protein was eluted with a 0–500 m*M* imidazole gradient in the same buffer, dialyzed overnight in 50 m*M* Tris–HCl pH 8.0 and then incubated with 15 units of thrombin (Pharmacia) and 2.5 m*M* $CaCl_2$ for 2 h at 301 K to remove the histidine tag. ModE was separated from the thrombin, uncleaved fusion protein and N-terminal peptide by strong anion-exchange chromatography using an HQ (Poros) column on a BioCAD 700E workstation. Fractions were concentrated in 50 m*M* Tris–HCl (pH 7.6) using a Centricon 10 cell. The purity of the sample was checked by SDS–PAGE and by MALDI–TOF mass spectroscopy. The yield of purified protein is around 25 mg per litre of bacterial culture.

3. Crystallization

Initial crystallization experiments were based around the sparse-matrix sampling approach (Jancarik & Kim, 1991) using Crystal Screen I purchased from Hampton Research (USA). Hanging-drop geometry was used and trials were duplicated at 277 and 293 K. Small crystals were obtained from one condition and several other conditions gave promising crystalline precipitates. Further experimentation varying protein and precipitant concentrations resulted in crystals of well defined morphology and of a size suitable for diffraction studies. Such crystals were grown from 3 µl of protein solution (incubated for 1 h with either 20 m*M* Na_2WO_4 or Na_2MoO_4) mixed with 1 µl of reservoir solution on the cover slip and then sealed over the reservoir using vacuum grease. The reservoirs used were 250 µl of 10–18% PEG 8000, 50 m*M* cacodylic acid pH 6.0, 100 m*M* magnesium acetate. Crystals grew in two days to a size of 0.4 × 0.3 × 0.3 mm (Fig. 1).

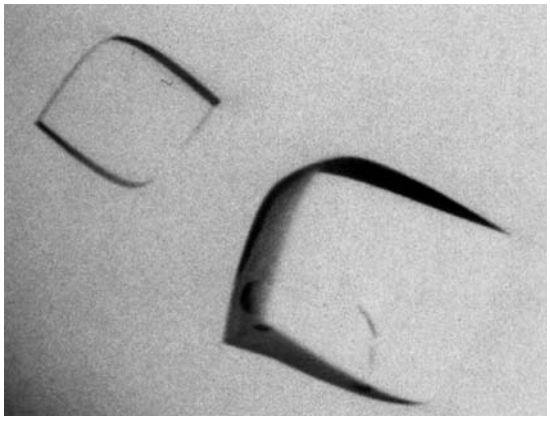

Figure 1
Crystals of ModE, form II.

4. X-ray diffraction and unit-cell characterization

Two crystal forms have been obtained which display similar morphology and can grow in the same drops. Crystals were sealed in glass capillaries for exposure to X-rays on beamline PX9.5 at Daresbury Laboratory with wavelength 1.2 Å and sample temperature 277 K, using a MAR300 image-plate detector. Autoindexing and data processing was carried out with *HKL* (Otwinowski & Minor, 1997).

The first crystals obtained (Form I) display Laue group 4/*mmm*, with unit-cell dimensions $a = b = 72.3$, $c = 246.2$ Å. Systematic absences suggest the space group is $P4_12_12$ or enantiomorph. The larger crystals, with dimensions of up to 0.3–0.5 mm, diffract strongly to 3.5 Å, after which diffraction falls away sharply. These larger crystals were often mechanically twinned and in addition give rise to highly mosaic diffraction. Smaller specimens (0.1 × 0.2 × 0.2 mm) give good low-resolution data. One crystal, grown in the presence of MoO_4^{2-}, has been used to obtain 6085 unique reflections from 30367 measurements to 3.8 Å resolution with an $R_{\rm merge}$ of 7.3%. This represents about 85.5% coverage, with an $\langle I/\sigma(I)\rangle$ of 18.2.

Further crystallization experiments produced a second form (II) which is orthorhombic with $a = 82.8$, $b = 127.9$, $c = 64.0$ Å. Using a crystal of maximum dimension 0.3 mm grown in the presence of WO_4^{2-}, we obtained 14113 unique reflections ($\lambda = 1.0$ Å, 92% complete) from 103431 measurements to 2.9 Å resolution, with an $R_{\rm merge}$ of 8.0% and an $\langle I/\sigma(I)\rangle$ of 26.2. Systematic absences suggest space group $P2_12_12$.

The protein is functional as a homodimer with a subunit of 262 amino acids and a molecular mass of approximately 28 kDa. The form I crystals give a Matthews coefficient, V_m, of 2.9 Å^3 Da^{-1} for a homodimer in the asymmetric unit with about 57% solvent volume (Matthews, 1968). Form II has a V_m of 3.0 Å^3 Da^{-1}, again for a homodimer in the asymmetric unit, with about 59% solvent volume. These are reasonable values given that the range of typical V_m values observed for protein crystals is 1.68–3.53 Å^3 Da^{-1} and 27–65% solvent volume. We have been unable to identify any non-crystallographic twofold axes on the basis of self-rotation functions using data from either crystal form. The form II crystals display improved diffraction quality and strength compared with form I, and recent experiments indicate that they diffract to near 2.0 Å using station BM14 at ESRF. These crystals are most suitable for a structure determination that will require experimentally derived phase information.

5. Conclusions

We have obtained two crystal forms of *E. coli* ModE, a tetragonal and an orthorhombic form. The orthorhombic crystals are well ordered and should facilitate structure determination providing results to compliment ongoing biochemical and genetic studies of molybdenum uptake and utilization in bacteria.

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