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Crystallization, data collection and phasing of the molybdate-binding protein of the phytopathogen *Xanthomonas axonopodis* pv. *citri*

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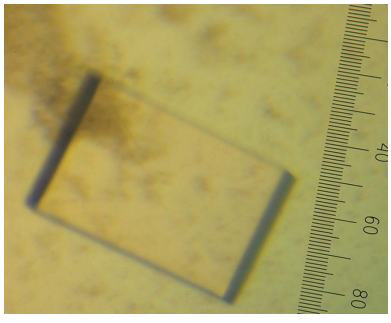


Xanthomonas axonopodis pv. *citri* ModA protein is the ABC periplasmic binding component responsible for the capture of molybdate. The protein was crystallized with sodium molybdate using the hanging-drop vapour-diffusion method in the presence of PEG or sulfate. X-ray diffraction data were collected to a maximum resolution of 1.7 Å using synchrotron radiation. The crystal belongs to the orthorhombic space group $C222_1$, with unit-cell parameters $a = 68.15$, $b = 172.14$, $c = 112.04$ Å. The crystal structure was solved by molecular-replacement methods and structure refinement is in progress.

1. Introduction

Molybdenum is an essential trace metal for most bacteria as well as for plants and animals. Following uptake into the cell, molybdenum is reduced and converted into molybdopterin dinucleotides, which are required as a cofactor for the assembly and functioning of several enzymes, including nitrogenase component 1, nitrate reductase, formate dehydrogenase, dimethylsulfoxide reductase, trimethylamine-*N*-oxide reductase and biotin-sulfoxide reductase (Rajagopalan & Johnson, 1992). Since molybdate is present in the environment at low concentrations, many organisms have developed high-affinity uptake systems to obtain the nutrient. Molybdate transport in *Escherichia coli* is carried out by an ABC-type transport system that generally comprises three proteins encoded by a single operon: ModA, the periplasmic molybdate-binding protein, ModB, the integral membrane channel protein, and ModC, the energizer protein (Self *et al.*, 2001). By analogy to other bacterial periplasmic binding proteins, the ModA protein binds molybdate and transfers it to the ModB protein at the outer surface of the cytoplasmic membrane. ModB, in conjunction with the ModC protein, which contains the ATP hydrolase activity, transports the molybdate into the cell (Rech *et al.*, 1995).

Molybdate transporters similar to those present in *E. coli* have been found in more than 20 bacterial and archaeal species including *Azotobacter vinelandii* (Luque *et al.*, 1993), *Rhodobacter capsulatus* (Leimkuhler *et al.*, 1999), *Haemophilus influenzae* (Fleischmann *et al.*, 1995), *Vibrio cholerae* (Heidelberg *et al.*, 2000), *Staphylococcus carnosus* (Neubauer *et al.*, 1999) and *Pseudomonas aeruginosa* (Stover *et al.*, 2000). However, molybdate transport in plant-associated bacteria remains largely uncharacterized, with no ModA proteins having been functionally or structurally described. Recently, the genomes of *Xanthomonas axonopodis* pv. *citri* (*Xac*) and *X. campestris* pv. *campestris* have been described and similar to the *E. coli* organization, a single *mod* operon formed by three genes (*modA*, *modB* and *modC*) was found in both species (da Silva *et al.*, 2002). *Xac* is the causative agent of citrus canker, a disease affecting several citrus cultivars around the world (Brunings & Gabriel, 2003). The ABC transporters have been described as important systems in the process of pathogenesis and infectivity, partly owing to their function in nutrient uptake (Higgins, 2001).

In this work, we report the crystallization of *Xac* ModA in the presence of sodium molybdate, as well as the collection and preliminary analysis of the crystallographic data. The *Xac modA* gene encodes a periplasmic protein with 256 amino acids (including the 24

amino acids of the signal peptide) which is responsible for the specificity and affinity of the transport system. The mature protein containing 232 amino-acid residues was expressed in *E. coli* BL21 (DE3) with an apparent molecular weight of 25.5 kDa. This recombinant protein preserved the ability to bind molybdate and exhibited increased thermal stability when bound to this ion (Balan *et al.*, unpublished results). To date, the three-dimensional structures of ModA from *E. coli* and *A. vinelandii* have been solved (Hu *et al.*, 1997; Lawson *et al.*, 1998), but phylogenetic analysis suggests that *Xac* ModA belongs to a distinct evolutionary group. Elucidation of the crystal structure of *Xac* ModA protein will shed light on possible conserved structural and functional features shared with other bacterial orthologues and may contribute to a better understanding of the ABC-transport system physiology in this important plant pathogen.

2. Methods

2.1. Crystallization

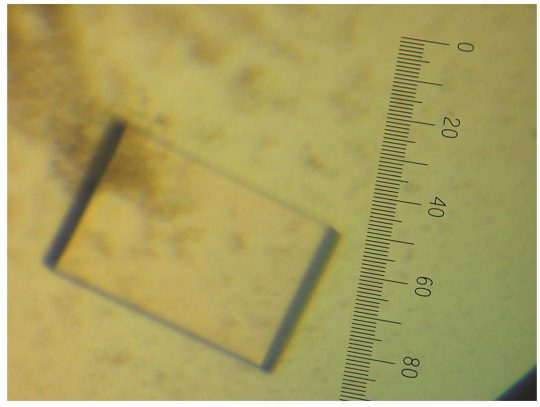
Recombinant *Xac* ModA protein, without the 24 amino acids corresponding to the signal peptide and fused at the N-terminal end to a His_6-thrombin cleavage site tag, was obtained after expression in *E. coli* BL21 (DE3) strain transformed with a pET28a derivative (pETModA) carrying the *modA* gene. The protein was purified from soluble extracts by immobilized nickel-affinity chromatography (Probond resin, Invitrogen) in the same manner described in Balan *et al.* (2005). Samples dialyzed against 10 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl were kept at 253 K at a final concentration of 20 mg ml^{-1}. Samples of recombinant *Xac* ModA protein (12 mg ml^{-1}) in the absence and presence of sodium molybdate (the protein:molybdate molar ratio was 1:3) were submitted to crystallization trials using the hanging-drop vapour-diffusion method, mixing equal volumes (2 µl) of protein and reservoir solution. Initial screening was performed using the Crystal Screen and Crystal Screen 2 kits (Hampton Research), Jena Biosciences 1 and 2 and Wizard I kits according to the instructions of the manufacturers. Conditions showing crystalline structures were refined by varying only the pH and precipitant concentration to yield suitable crystals.

2.2. Data collection, processing and phasing

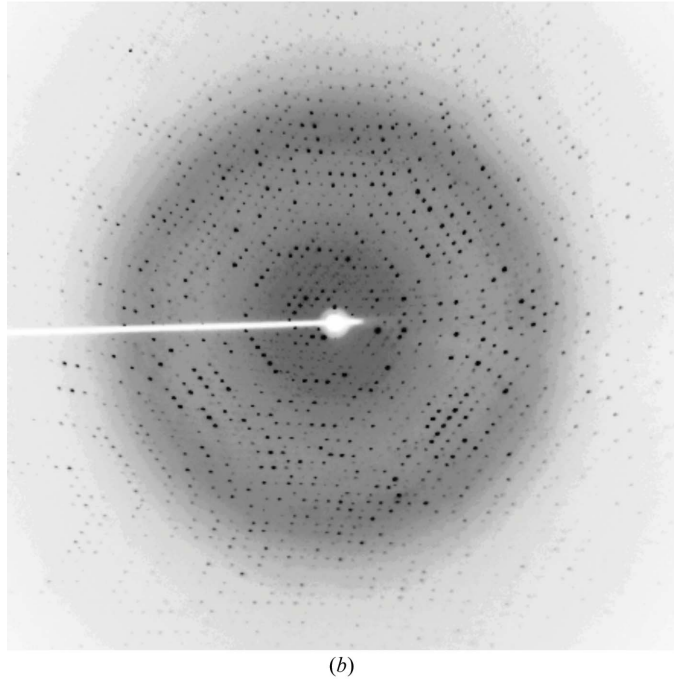
Crystallographic data were collected at protein crystallography beamline D03B-MX1 at the Laboratório Nacional de Luz Síncrotron (LNLS), Campinas, Brazil. The wavelength was 1.421 Å and a MAR CCD detector with a circular X-ray-sensitive surface of 165 mm in diameter combined with a MarDTB goniostat was used to record the oscillation data with $\Delta\varphi = 1.0^\circ$. Crystals were scooped straight from the drop and cooled directly in a stream of nitrogen gas to 110 K in order to minimize radiation damage. The solution in which the crystals were grown provided protection against ice formation. The data set was processed using *HKL*2000 (Otwinowski & Minor, 1997) and the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). Molecular replacement was performed with the *MOLREP* program (Vagin & Teplyakov, 1997) using the *E. coli* K12 ModA structure deposited in the PDB with code 1amf (Hu *et al.*, 1997), which shares a sequence identity of 48% with the *Xac* protein. The molybdate, water molecules, H atoms and atoms with zero occupancy were removed from the 1amf structure to provide a better search model.

3. Results and discussion

Single-step purification by affinity chromatography was sufficient to produce crystallization-quality protein. Initial crystallization trials of *Xac* ModA in the absence of molybdate resulted in spherulites and crystals smaller than 20 µm in the longest dimension, in accordance with the common absence of periplasmic binding protein structures without their cognate ligands. Attempts to improve the quality of the crystals were successful when the protein was crystallized in the presence of sodium molybdate, indicating possible rearrangements in the structure upon ligand binding. Crystal growth usually occurred in one to two weeks, leading to crystals with dimensions between 100 and 500 µm. Crystals were obtained in 12 different conditions containing polyethylene glycol (PEG or PEG monomethyl ether) or sulfate. In most cases both compounds were used in the crystallization conditions. The best diffraction pattern was obtained from a crystal grown in 0.2 *M* lithium sulfate, 0.1 *M* Tris–HCl pH 7.5 and 32% PEG 4000 (Fig. 1). This crystal diffracted to a maximum resolution of 1.7 Å and showed the symmetry and systematic absences of the ortho-



(*a*)



(*b*)

Figure 1
(*a*) Crystal of *X. axonopodis* pv. *citri* ModA protein in the presence of molybdate. Crystallization conditions are described in the text. (*b*) Diffraction pattern of *Xac* ModA protein crystal.

Table 1
Data-collection and processing statistics.

Values in parentheses correspond to data in the highest resolution shell (1.76–1.70 Å).

Space group	$C222_1$
Unit-cell parameters (Å)	a = 68.15, b = 172.14, c = 112.04
Mosaicity (°)	0.5
Temperature (K)	110
Wavelength (Å)	1.421
Oscillation (°)	1
Crystal-to-detector distance (mm)	70.0
No. of frames	150
Resolution limits (Å)	40.00–1.70 (1.76–1.70)
$I/\sigma(I)$ after merging	26.6 (3.3)
Completeness (%)	99.8 (99.3)
Multiplicity	5.7 (5.7)
R_{sym}	0.062 (0.415)
No. of reflections	417669
No. of unique reflections	72786 (7166)

rhombic space group $C222_1$. Table 1 summarizes the data-collection statistics.

The Matthews coefficient (Matthews, 1968) for two and three protein molecules in the asymmetric unit was calculated to be 3.2 and 2.1 Å^3 Da^{-1}, respectively, giving solvent contents of 61 and 42%. Although two molecules in the asymmetric unit was also a possibility according to the Matthews coefficient, three molecules with no packing clashes were found by a molecular-replacement procedure using a modified *E. coli* ModA structure as the search model. Each molecule corresponded to one of the three highest correlation values of the translation function. After fixing the first molecule, a search for the second molecule was performed and the correlation coefficient difference between the correct and incorrect peaks increased. The same happened in the search for the third molecule. The final R factor of 0.514 and correlation coefficient of 0.351, calculated in the 34.3–3.0 Å resolution range, were convincingly better than the second highest peak values of 0.552 and 0.234, respectively. The initial maps produced are encouragingly good, clearly showing electron density for the molybdate and the changes in the amino-acid side chains. The model is being rebuilt and refined.

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