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Crystallization and Preliminary X-ray Diffraction Studies on the DNA-Binding Domain of the Multidrug Transporter Activation Protein (MtaN) from Bacillus subtilis

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Crystallization and preliminary X-ray diffraction studies on the DNA-binding domain of the multidrug transporter activation protein (MtaN) from \textit{Bacillus subtilis}

The N-terminal DNA-binding domain of the multidrug transporter activation protein (MtaN) was crystallized by the hanging-drop vapour-diffusion method using lithium chloride as a precipitant. The crystals are orthorhombic and belong to the space group \(I\bar{2}_1\bar{2}_1\bar{2}_1\), with unit-cell parameters \(a = 49.4, b = 67.8, c = 115.0\) Å. Diffraction data have been collected at 100 K to 2.75 Å resolution at a synchrotron-radiation source.

1. Introduction

Bacteria express a variety of membrane-bound proteins that extrude toxins from the cell. Because the recognized compounds include many antibiotics, these proteins have been named multidrug transporters (Paulsen et al., 1996; van Veen et al., 1999). In \textit{Bacillus subtilis}, two of these proteins, Bmr and Blt, have been shown to be controlled by specific transcriptional regulators, BmrR and BltR, respectively (Ahmed et al., 1994, 1995). However, both transporters are further regulated by the multidrug transporter activation protein (Mta; Baranova et al., 1999). This protein also controls its own transcription and that of at least one more gene, \(ydfK\), which encodes a putative membrane protein.

Mta, like BmrR and BltR, is a member of the MerR family, named for the mercury-resistance gene regulator (Summers, 1992; Hidalgo et al., 1997). This family has high sequence conservation in the N-terminal DNA-binding domain, which is predicted to contain a helix±turn±helix DNA-binding motif. However, the C-terminal domains of the family are widely divergent in both size and sequence. Beyond the helix±turn±helix motif, there appears to be no sequence or structural homology between the members of the MerR family and any other gene regulators.

Mta contains 257 residues and is a functional dimer. A truncated form of Mta, encompassing the first 109 amino acids (MtaN), is able to bind to the promoter elements and activate the transcription of genes under control of the full-length protein (Baranova et al., 1999). Thus, it appears that the C-terminus contains a regulatory domain, removal of which is sufficient to relieve inhibition of transcriptional activation. \textit{In vivo}, MtaN activates its own transcription, creating a positive feedback loop leading to high levels of intracellular protein. This may be important, as our binding studies have found that MtaN has 30- to 50-fold higher \(K_d\) values for the \(bmr\) and \(blt\) promoters, respectively, than for the \(mia\) promoter (data not shown).

Our interest in MtaN is twofold. The promoters targeted by MerR family members contain an unusually long 19 base-pair spacer between the –10 and –35 elements, and the structural mechanism of transcriptional activation by this family remains unclear. In addition, the known promoter targets of MtaN are quite dissimilar, with only 12 consensus base pairs in a stretch of 31, of which only six are found in the DNase I footprint of the protein (Baranova et al., 1999). Such limited base-pair conservation suggests an unusual mechanism of multiple promoter recognition.

To understand the molecular mechanism of transcription activation by MerR family members and multiple promoter recognition by a single protein, we have undertaken experiments to determine the three-dimensional structure of MtaN by X-ray diffraction methods. In this paper, we report the crystallization and preliminary X-ray diffraction analysis of the DNA-binding domain of the MerR family member Mta.

2. Experimental results and discussion

2.1. Purification and solubilization

Expression and purification of MtaN in \textit{Escherichia coli} was performed essentially as described in Baranova et al. (1999). 1 l of cells expressing MtaN fused to a C-terminal six-histidine tag was pelleted, resuspended in ~25 ml of lysis buffer (20 mM bis-tris propane pH 8.9, 250 mM NaCl) and lysed in a French pressure cell at 68.9 MPa. Lysates clarified by centrifugation were added to 4 ml of a 50% slurry of Talon cobalt-affinity resin (Clontech) and purified by a hybrid batch-gravity flow method. Briefly, the clarified lysates were mixed with a resin slurry, allowed to bind and...
washed several times. The resin was then poured into a small gravity-flow column for stepwise elution. Protein was eluted with 50 mM imidazole and fractions containing MtaN were combined and dialyzed against LiCl, is quite effective for protection.

2.3. X-ray diffraction

Preliminary intensity data for unit-cell parameter and space-group determination were collected on an R-AXIS IV image-plate system equipped with double-focusing mirrors and using Cu Kα radiation from a Rigaku RU-300 rotating-anode generator operated at 50 kV and 100 mA. These data revealed an orthorhombic space group, either I222 or P2_12_12_1, which are indistinguishable from each other based on diffraction patterns. A V_m value of 3.6 Å³ Da⁻¹ (Matthews, 1968) and a solvent content of 63% are consistent with a single molecule in the asymmetric unit. There is a band of diffuse scattering in the diffraction pattern at resolutions between 4.0 and 3.0 Å (Fig. 2). The cause of this is unclear, although it is likely to be a consequence of thermal motion in the crystal lattice. All experimental attempts to eliminate this feature have failed. However, the thermal diffusion does not seem to interfere significantly with successful data collection and intensity integration.

Although the crystals are very unstable in the X-ray beam at room temperature, they are extremely stable in the crystallization drop. As an example, the best intensity data have been collected from a crystal over 18 months old (Table 1). For intensity data collection, a crystal was removed directly from the drop in which it was grown, flash-frozen in the N_2 stream of an X-stream cryo-cooling system (Molecular Structure Corporation) and stored until data collection. Intensity data were collected at 100 K on beamline 7-1 at the Stanford Synchrotron Radiation Laboratory (SSRL) using a MAR 345 detector and has yielded data-quality set has been collected using MOSFLM (Collaborative Computational Project, Number 4, 1994).

Heavy-atom soaks have been unsuccessful in derivatizing the protein, although this may in part be a consequence of the presence of the high salt concentration in the crystallization conditions. As an alternative strategy, selenomethionyl-substituted protein has been prepared (Van Duyne et al., 1993) for use in multiwavelength anomalous diffraction (MAD) experiments (Hendrickson, 1991) and has yielded data-quality crystals. A MAD intensity data set has been collected using beamline 1-5 at SSRL (Table 1).

Patterson map analysis of the MAD data using SOLVE (Terwilliger & Berendzen, 1999) has located four of the five possible selenium sites and shown the space group to be \( \text{P}_2_1\text{2}_1\text{2}_1 \), rather than the far more commonly observed space group I222. Initial electron-density

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**Table 1**

<table>
<thead>
<tr>
<th>Crystal</th>
<th>Native</th>
<th>Se-Met</th>
</tr>
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<tbody>
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<td>SSRL beamline</td>
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<td>1:5</td>
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<tr>
<td>Unit-cell parameters (Å)</td>
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<td>( a = 50.1, b = 67.6, c = 116.1 )</td>
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<td>Resolution limits (Å)</td>
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<td>Outer shell (Å)</td>
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<td>Wavelength (Å)</td>
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<tr>
<td>Unique reflections</td>
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<td>0.9226</td>
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<tr>
<td>Compl. (%)</td>
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<td>97.3 (100)</td>
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<tr>
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<tr>
<td>Rmerge (%)</td>
<td>7.9 (2.7)</td>
<td>4.2 (1.4)</td>
</tr>
</tbody>
</table>

\[ R_{merge} = \frac{\sum|I_i - \langle I \rangle_i|}{\sum I_i} \]

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**Figure 1**

A crystal of MtaN. This crystal is approximately 0.8 mm long.

**Figure 2**

A diffraction image of MtaN. Insets show a threefold magnification of diffuse scattering in the 4.0-3.7 Å resolution shell.
maps show clear protein and solvent regions. Solvent flattening in PHASES (Furey & Swaminathan, 1997) has significantly improved the quality of these maps and model building is under way.

References


