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Expression, purification, crystallization and preliminary X-ray analysis of the DNA-binding domain of *Rhodobacter capsulatus* MopB

The LysR-type regulator MopB represses transcription of several target genes (including the nitrogen-fixation gene *anfA*) in *Rhodobacter capsulatus* at high molybdenum concentrations. In this study, the isolated DNA-binding domain of MopB (MopB_{HTH}) was overexpressed in *Escherichia coli*. Purified MopB_{HTH} bound the *anfA* promoter as shown by DNA mobility-shift assays, demonstrating the function of the isolated regulator domain. MopB_{HTH} was crystallized using the sitting-drop vapour-diffusion method in the presence of 0.2 M lithium sulfate, 0.1 M phosphate/citrate pH 4.2, 20% (w/v) PEG 1000 at 291 K. The crystal belonged to space group *P*₃₁21 or *P*₃₂21, with unit-cell parameters $a = b = 61.84$, $c = 139.64$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$, and diffracted to 3.3 Å resolution at a synchrotron source.

1. Introduction

Members of the LysR-type transcription regulator (LTTR) family are widely distributed within bacteria and archaea. LTTRs control diverse cellular processes such as virulence, CO₂ fixation, quorum sensing and nitrogen assimilation (Viale *et al.*, 1991; Schlaman *et al.*, 1992; Schell, 1993; Cao *et al.*, 2001; Russell *et al.*, 2004; Bender, 2010). These regulators typically consist of an N-terminal DNA-binding domain with a helix–turn–helix motif and a C-terminal effector-binding domain. LTTRs bind to conserved palindromic sequences called LTTR boxes within their target promoters. The minimal consensus sequence of LTTR boxes is T-N₁₁-A (Parsek *et al.*, 1994). LTTRs mostly form dimers and can both activate and repress the expression of target genes depending on the position of the LTTR box relative to the transcription start site (Maddocks & Oyston, 2008). Binding of LTTRs to target promoters is modulated by different effectors, which can be either metabolites or extracellular factors. Effector binding to the C-terminal domain results in conformational changes that control the affinity of the N-terminal domain for the respective target promoter (Schell, 1993).

Rhodobacter capsulatus is a free-living phototrophic bacterium that is known for its high metabolic versatility (Madigan, 1995). In the absence of ammonium, *R. capsulatus* reduces atmospheric dinitrogen to ammonia via two nitrogenases: a molybdenum-containing nitrogenase (Mo-nitrogenase) and a molybdenum-free alternative iron-only nitrogenase (Fe-nitrogenase). Fe-nitrogenase, which exhibits a lower specific activity than Mo-nitrogenase, is only synthesized in the absence of molybdate (Masepohl & Hallenbeck, 2010). In the presence of molybdate the dimeric regulator MopB represses the transcription of *anfA*, which codes for the transcriptional activator of Fe-nitrogenase genes (Kutsche *et al.*, 1996; Wiethaus *et al.*, 2006). The *anfA* promoter region encompasses a specific LTTR-box (Mo-box) which overlaps the transcription start site. The affinity of MopB for the *anfA* Mo-box is increased upon binding of molybdate (Wiethaus *et al.*, 2006). MopB exhibits 32% sequence identity to the LTTR-like regulator ModE from *Escherichia coli* (Anderson *et al.*, 1997). The crystal structures of ModE and a few other LTTRs have been solved, revealing the exact modular organization of the proteins as well as the conformational changes upon effector binding (Hall *et al.*, 1999;

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Muraoka *et al.*, 2003; Zaim & Kierzek, 2003; Monferrer *et al.*, 2010). In the present study, we demonstrate that the isolated DNA-binding domain of MopB (MopB_{HTH}) retains full binding activity for the *anfA* promoter. MopB_{HTH} was crystallized and preliminary X-ray analyses were carried out.

2. Experimental procedures

2.1. Overexpression and purification of MopB_{HTH}

A DNA fragment coding for the DNA-binding domain of MopB (RRC03910; <http://www.ergo-light.com>; Fig. 1; amino acids 1–120) was PCR-amplified using the primer pair 5'-ATACATATGACGGACGGTGTG-3' and 5'-GTGCTCGAGGGACAGACC-3' with *R. capsulatus* chromosomal DNA as template. The PCR product was digested with *NdeI* and *XhoI* and ligated into vector plasmid pET22b (Novagen). The resulting hybrid plasmid coding for a C-terminally hexahistidine-tagged MopB_{HTH} fusion protein was transformed into *E. coli* BL21 (DE3) (Invitrogen). 2 l cultures were grown at 303 K in Luria–Bertani broth until an OD₅₈₀ of 0.7 was reached. Overexpression of MopB_{HTH} was induced by the addition of 0.5 mM IPTG. After further incubation for 3 h, the cells were harvested by centrifugation at 6000g and stored at 253 K.

Since MopB_{HTH} precipitated under standard conditions, protein purification was optimized with regard to buffer composition and pH value. The best results were obtained using buffer A (5 mM MES, 5 mM CHES, 5 mM CAPSO, 10 mM NaH₂PO₄, 10 mM Tris–HCl pH 9.0, 100 mM NaCl) with 10 mM imidazole. The cell pellet was resuspended and cell disruption was performed using a French pressure cell (Thermo Scientific) at 13.8 MPa. After centrifugation at 277 K and 22 548g the lysate was further clarified using a 0.22 µm filter (Millipore). The lysate was applied onto an Ni–NTA chromatography column (Qiagen). The column was washed with two column volumes of buffer A containing 50 mM imidazole before the protein was eluted using buffer A containing 250 mM imidazole. Pooled protein-containing fractions were further purified by size-exclusion chromatography. For this purpose, fractions were applied onto a Superdex 75 HR 10/30 column (Amersham Biosciences) pre-equilibrated with buffer B (20 mM CHES, 100 mM NaCl pH 9.0). Separation was performed as described previously (Wiethaus *et al.*, 2009). MopB_{HTH} eluted at 12.9 ml, which corresponds to a molecular weight of 26 kDa (Fig. 2a) and suggests that MopB_{HTH} (like the full-length MopB protein) forms dimers. After pooling, proteins were concentrated using Amicon 15 kDa molecular-weight cutoff devices (Millipore), resulting in a final protein concentration of 15 mg ml⁻¹. The purity of MopB_{HTH} was demonstrated by SDS–PAGE (Fig. 2b).

Figure 1
Domain organization of *R. capsulatus* full-length MopB and MopB_{HTH}. The DNA-binding domain harbours a helix–turn–helix (HTH) motif. The effector (Mo-binding) domain consists of two Mop subdomains. MopB and MopB_{HTH} consist of 261 and 120 amino acids, respectively.

2.2. Functional analysis of MopB_{HTH} by DNA mobility-shift assays

In order to test whether the isolated DNA-binding domain of MopB (MopB_{HTH}) was sufficient for target promoter binding, DNA mobility-shift assays were performed as described previously (Müller *et al.*, 2010). Briefly, a 209 bp ³²P-labelled *anfA* promoter fragment was incubated with increasing amounts of purified MopB_{HTH} prior to separation of promoter–MopB_{HTH} complexes from free promoter by polyacrylamide gel electrophoresis. Radioactively labelled bands were visualized using Amersham Hyperfilm MP (GE Healthcare). MopB_{HTH} bound the *anfA* promoter (Fig. 2c), indicating that the isolated DNA-binding domain was correctly folded and active. MopB_{HTH} bound the *anfA* promoter even more strongly than the full-length MopB protein (Müller *et al.*, 2010). As expected for a MopB protein lacking the Mo-binding effector domain, the affinity of MopB_{HTH} for its target promoter was independent of Mo availability (Wiethaus *et al.*, 2006; data not shown).

2.3. Crystallization

Crystallization of MopB_{HTH} was performed in 96-well format by applying the sitting-drop vapour-diffusion method at 291 K. Using a Phoenix crystallization robot (Art Robbins Instruments, Sunnyvale, USA), 0.1 µl MopB_{HTH} solution was mixed with 0.1 µl of crystallization screening solutions (Qiagen). A single MopB_{HTH} crystal was observed in JCSG+ Suite condition A6 [0.2 M lithium sulfate, 0.1 M phosphate/citrate pH 4.2, 20% (w/v) PEG 1000]. The trigonal crystal, with dimensions of about 50 × 50 × 25 µm (Fig. 3), was briefly soaked in mother liquor supplemented with 7.5% PEG 400 and 100 mM NaCl and flash-cooled in liquid nitrogen.

2.4. Data collection and processing

A data set was collected from a single crystal on beamline ID23-1 at the European Synchrotron Radiation Facility (ESRF; Grenoble, France) using an ADSC Q315R CCD detector. The data were

Figure 2
Purification of MopB_{HTH} and DNA-binding assays. (a) MopB_{HTH} eluted as a symmetric peak of 26 kDa indicating dimeric organization of the native protein. (b) MopB_{HTH} appeared as a single band of ~13 kDa after SDS–PAGE. Precision Plus Protein Standards All Blue (Bio-Rad) were used as molecular-mass standards. (c) Binding of MopB_{HTH} to the *anfA* promoter was shown by DNA mobility-shift assays as described previously for the full-length MopB protein (Müller *et al.*, 2010). Increasing amounts (0–7.5 µM) of MopB_{HTH} were incubated with ³²P-labelled promoter.

Table 1Data-collection statistics for MopB_{HTH}.

Values in parentheses are for the outermost resolution shell.

Wavelength (Å)	0.97625
Oscillation range (°)	1
Space group	<i>P</i> ₃ 21 or <i>P</i> ₃ 21
Unit-cell parameters (Å, °)	<i>a</i> = <i>b</i> = 61.84, <i>c</i> = 139.64, α = β = 90, γ = 120
Resolution (Å)	42.49–3.3 (3.4–3.3)
Total reflections	51107
Unique reflections	8991
Multiplicity	10.1 (9.6)
<i>I</i> /σ(<i>I</i>)	17.03 (4.23)
Completeness (%)	99.7 (99.3)
<i>R</i> _{merge} [†] (%)	6.9 (45.6)

[†] $R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$, where $I_i(hkl)$ is the intensity of an individual measurement and $\langle I(hkl) \rangle$ is the corresponding mean value.

Figure 3

Trigonal crystal of MopB_{HTH}. Crystallization was performed in 0.2 M lithium sulfate, 0.1 M phosphate/citrate pH 4.2, 20% (w/v) PEG 1000.

obtained to a resolution of 3.3 Å at 100 K using a wavelength of 0.97625 Å. The data set consisted of 180 frames with an oscillation range of 1°. It was integrated with *XDS* (Kabsch, 2010) and scaled using *XSCALE* (Kabsch, 2010). The statistics of the resulting data are shown in Table 1.

3. Results and discussion

The isolated DNA-binding domain of MopB (MopB_{HTH}) was successfully expressed in *E. coli* with a hexahistidine tag and purified via Ni-NTA and size-exclusion chromatography (Figs. 1 and 2). DNA mobility-shift assays with purified MopB_{HTH} showed that the isolated DNA-binding domain was sufficient for promoter binding (Fig. 2). MopB_{HTH} was crystallized using the sitting-drop vapour-diffusion method at 291 K. After 25 d, a diffracting crystal with dimensions of 50 × 50 × 25 μm was obtained (Fig. 3). A data set was collected from a single crystal on beamline ID23-1 at the European Synchrotron Radiation Facility (ESRF; Grenoble, France). After scaling, the data were 99.7% complete to 3.3 Å resolution, with an overall *R*_{merge} of 6.9%.

The crystal belonged to the trigonal space group *P*₃21 or *P*₃21. A Matthews coefficient of 2.96 Å³ Da^{−1} was estimated, assuming the presence of two molecules per asymmetric unit, with a solvent content of 58.54%, which is consistent with a dimeric organization of

MopB_{HTH} as determined by size-exclusion chromatography (Fig. 2). In both cases a twin fraction of 0.02–0.3 corresponding to the twin law (−*h*, −*k*, *l*) was estimated using the merohedral twinning server (Yeates, 1997), *phenix.xtriage* (Zwart *et al.*, 2005) or *TRUNCATE* (French & Wilson, 1978).

Molecular replacement of MopB_{HTH} was performed using the structures of the molybdate-dependent transcriptional regulator ModE from *E. coli* (PDB entries 1b9n and 1o7l; Hall *et al.*, 1999; Schüttelkopf *et al.*, 2003) and a putative ModE from *Agrobacterium tumefaciens* (PDB entry 2ijl; Midwest Center for Structural Genomics, unpublished work). Molecular-replacement searches were executed for all possible space groups. No clear solution could be identified. Therefore, we are currently attempting to solve the structure of MopB_{HTH} using both the MIR method with heavy-atom soaks and sulfur single anomalous dispersion.

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