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Purification, crystallization and preliminary X-ray data of the transcription factor NtcA from the cyanobacterium *Anabaena* PCC 7120

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NtcA is a transcription factor that acts as a global nitrogen regulator in cyanobacteria. Cyanobacteria are photosynthetic prokaryotic organisms, some genera of which can fix nitrogen under conditions of nitrogen deprivation. NtcA from *Anabaena* PCC 7120 is a dimeric protein that consists of 223 amino acids with a molecular weight of 25 kDa per subunit. It belongs to the cAMP receptor-protein (CAP) family and is involved in the regulation of several of the genes acting in the nitrogen-fixation process. Here, the crystallization and preliminary X-ray data of NtcA are described. The crystallization was made possible by an improved purification method, which provides a stable NtcA protein at concentrations suitable for crystallization. The protein was crystallized using the hanging-drop method. Data were collected to 2.5 Å resolution using synchrotron radiation and the crystals belonged to space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters a = 69.23, b = 69.23, c = 162.15 Å, $\alpha = \beta = \gamma = 90^\circ$. The phases necessary to solve the structure of NtcA could not be obtained by molecular replacement based on the CAP structure using various models.

1. Introduction

NtcA is a transcription factor found in various species of cyanobacteria (Frias *et al.*, 1993; Wei *et al.*, 1993; Lindell *et al.*, 1998). Cyanobacteria are photosynthetic prokaryotes; some genera are capable of fixing nitrogen under conditions of nitrogen deprivation. The process of nitrogen fixation in the filamentous cyanobacterium *Anabaena* PCC 7120 takes place in specialized cells called heterocysts that are dispersed at an interval of approximately every ten cells along the filament (Haselkorn, 1978). These cells provide a micro-aerobic (meaning almost anaerobic) environment for the oxygen-sensitive enzyme nitrogenase that catalyzes the reduction of dinitrogen to ammonia. The differentiation of vegetative cells to heterocysts involves a global change in gene expression and affects a large number of loci (Wolk *et al.*, 1994). Genes involved in nitrogen fixation are up-regulated, while others are down-regulated. Many of these genes are controlled by the transcription factor NtcA (Frias *et al.*, 1993; Lindell *et al.*, 1998).

NtcA belongs to the family of prokaryotic transcription factors represented by the cAMP receptor protein (CAP; Vega-Palas *et al.*, 1992; Wei *et al.*, 1993). A characteristic feature of these transcription factors is that they bind to DNA as dimers with a C-terminal helix–turn–helix motif. Gel-filtration experiments performed on purified NtcA from *Anabaena* PCC 7120 show that the protein is a dimer in solution (Wisén *et al.*, 1999). Furthermore, proteins in the CAP family control gene expression by binding to palindromic sequences in the promoter region (Harrison & Aggarwal, 1990). Even though NtcA is involved in the regulation of many different genes, the target DNA-binding sites are highly conserved. The consensus sequence for NtcA has been suggested to be GTA-N_8-TAC, with some variation in the length of the spacer between the palindromic bases as well as in the flanking regions (Luque *et al.*, 1994; Ramasubramanian *et al.*, 1994; Jiang *et al.*, 2000).

Comparison of the primary structures of NtcAs from different species of cyanobacteria shows a high degree of conservation. Each subunit of the NtcA protein from *Anabaena* PCC 7120 consists of 223 amino acids and has a molecular weight of 25 kDa. Even though the amino-acid sequences as well as other features are known for NtcAs from at least nine species (Herrero *et al.*, 2001), a crystal structure has not been solved for any of them. Recombinant NtcA from *Anabaena* PCC 7120 expressed in *Escherichia coli* gives a yield of approximately 40 mg from 1 l bacterial culture, but after purification it is highly unstable and prone to precipitation.

In this paper, we report an improved purification method as well as the crystallization of and X-ray data from NtcA from *Anabaena* PCC 7120.

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2. Materials and methods

2.1. Expression and purification of NtcA

The high-level expression clone of NtcA with an N-terminal hexahistidine tag developed by Wisén *et al.* (1999) was used to improve the expression and purification methods in order to yield a more stable protein preparation suitable for crystallization. The DNA fragment encoding NtcA was subcloned into the expression vector pET-21a (Novagen) and was co-transformed with pREP4-GroESL, containing the chaperones GroEL-ES, into *E. coli* strain BL-21 (DE3) (Novagen) for overexpression. The chaperones were introduced in order to stabilize NtcA and to facilitate proper folding of the protein following expression in *E. coli*. 3 l of bacterial culture was grown to $OD_{600} \simeq 0.7$, after which expression was induced by adding IPTG to a final concentration of 0.4 m*M*. After 4 h, the bacteria were harvested and centrifuged at 277 K and 5000*g* for 15 min. The bacteria were lysed by dissolving the pellet in 30 ml lysis buffer containing 0.2 mg ml^{-1} lysozyme followed by incubation on ice overnight. The lysis buffer was composed of 20 m*M* sodium phosphate buffer pH 7.0, 10% sucrose, 1 *M* NaCl, 5 m*M* 2-mercaptoethanol and 5 m*M* phenylmethanesulfonyl fluoride (PMSF). The lysed bacteria were diluted approximately fivefold with buffer *A* (20 m*M* phosphate buffer pH 7.0, 2 *M* NaCl) including 50 m*M* imidazole and lysis was completed by ultrasonication for 3 × 20 s.

After centrifugation at 40 000*g* for 60 min, the lysate was added to ∼15 ml Ni–IMAC Sepharose pre-treated with buffer *A* for batchwise binding of NtcA to the Ni–IMAC for 1 h. The binding was followed by washing of the resin with approximately 0.5 l buffer *A* supplemented with 100 m*M* imidazole on a glass-filter funnel to remove unbound proteins. The washed resin was transferred to a column and the NtcA protein was eluted with buffer *A* containing 300 m*M* imidazole. The fractions including the NtcA protein were detected by absorbance measurements at 280 nm followed by pooling of the protein-containing fractions. The approximate yield of pure NtcA is 35–40 mg per litre of bacterial culture.

2.2. Crystallization of NtcA

In order to obtain suitable conditions for crystallization, purified NtcA was concentrated to ∼35 mg ml^{-1} using an Amicon cell. To avoid precipitation of NtcA, the concentration step requires the presence of 300 m*M* imidazole and 2 *M* NaCl, which were included in the elution buffer. The concentration was therefore performed directly on the pooled NtcA after the addition of 2 m*M* DTT and 1 m*M* EDTA. Following concentration, the detergent *n*-octyl β-D-glucopyranoside (OBG) was added [0.1%(*w*/*v*)] before changing the buffer on a PD-10 gel-filtration column to 10 m*M* Tris–HCl pH 7.8, 1.2 *M* NaCl, 1 m*M* EDTA, 2 m*M* DTT, 0.1%(*w*/*v*) OBG and 0.02%(*w*/*v*) NaN_3. The eluate containing NtcA with a concentration of ∼30 mg ml^{-1} was supplemented with additional OBG to a final concentration of 0.3%(*w*/*v*). Single crystals of NtcA were obtained using the hanging-drop vapour-diffusion method. The screening kit used was a CryoII sparse-matrix crystallization screen (Emerald BioStructures Inc.). The crystals of highest quality were grown from protein solution of concentration 10 mg ml^{-1}. The drop contained 5 µl of this protein solution plus 5 µl reservoir buffer consisting of 50–60%(*v*/*v*) PEG 300 (Fluka), 0.1 *M* sodium acetate pH 4.5, 0.2 *M* NaCl and 2 m*M* DTT, giving a protein concentration of 5 mg ml^{-1} in the drop. The volume of the buffer in the reservoir was 1 ml.

3. Results

Crystals grew to approximate dimensions of 0.2 × 0.1 × 0.1 mm within a couple of days (Fig. 1). The crystals were cooled directly from the mother liquor and stored in liquid nitrogen until subjected to X-ray diffraction analysis.

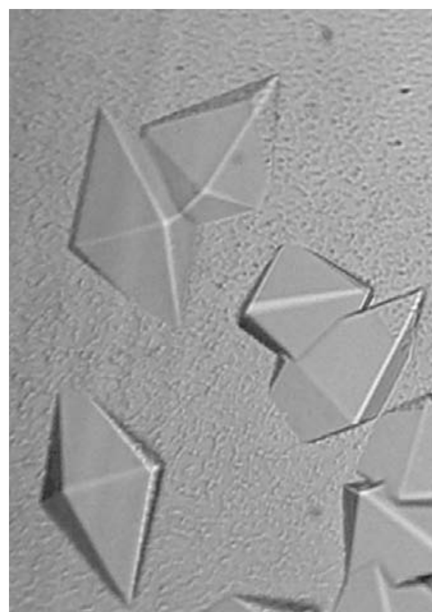

Figure 1
Crystals of NtcA from *Anabaena* PCC 7120.

Table 1
Data-collection statistics.

Values in parentheses refer to data in the highest resolution shell.

Beamline	ESRF ID14-EH1
Wavelength (Å)	0.934
Space group	$P4_12_12$ or $P4_32_12$
Unit-cell parameters (Å, °)	*a* = 69.23, *b* = 69.23, *c* = 162.15
No. observations	80259
Unique reflections	14293
Resolution range (Å)	30–2.5 (2.56–2.50)
Multiplicity	5.6 (5.2)
$\langle I/\sigma(I)\rangle$	28.5 (2.9)
Completeness (%)	99.3 (99.9)
R_{merge}	0.051 (0.303)

Data were collected to 2.5 Å resolution at 100 K using synchrotron radiation. The diffraction images displayed substantial amounts of diffuse scattering and the diffraction pattern was anisotropic, with weaker scattering along the crystallographic fourfold axis. Table 1 summarizes the data-collection statistics. The space group is either $P4_12_12$ or $P4_32_12$.

The unit-cell parameters are a = 69.23, b = 69.23, c = 162.15 Å, $\alpha = \beta = \gamma = 90°$. The presence of one molecule in the asymmetric unit gives a crystal volume per protein weight (V_M; Matthews, 1968) of 3.97 Å^3 Da^{-1} and a solvent content of 69%. Assuming the presence of two molecules in the asymmetric unit would yield a solvent content of 37% and a V_M of 1.99 Å^3 Da^{-1}. However, there is no peak in the self-rotation function corresponding to a second molecule. Moreover, the low solvent content calculated assuming two molecules in the asymmetric unit is unlikely considering the poor diffraction. From solution studies (Wisén *et al.*, 1999) NtcA is anticipated to occur as a dimer in the crystal, in which case the dimer is expected to be created from symmetry-related monomers. Despite extensive efforts, phases could not be obtained by molecular replacement based on the available CAP structures with PDB codes 3gap (Weber & Steitz, 1987) and 1run (Parkinson *et al.*, 1996) using polyalanine models, homology models and models representing the whole monomer or individual domains.

In order to solve the structure, experimental phases derived from multiple isomorphous replacement or anomalous dispersion will be required. We are currently exploring the possibilities of obtaining other crystal forms using NtcA containing selenomethionine instead of methionine, as well as co-crystallization with DNA and various additives that may possibly influence the activation of NtcA *in vivo*.

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