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Crystallization and preliminary X-ray analyses of catabolite control protein A, free and in complex with its DNA-binding site

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The catabolite control protein (CcpA) from *Bacillus megaterium* is a member of the bacterial repressor protein family GalR/LacI. CcpA with an N-terminal His-tag was used for crystallization. Crystals of free CcpA and of CcpA in complex with the putative operator sequence (catabolite responsive elements, CRE) were obtained by vapour-diffusion techniques at 291 K using the hanging-drop method. CcpA crystals grown in the presence of polyethylene glycol 8000 belong to the hexagonal space group $P6_122$ or $P6_522$, with unit-cell parameters a = 74.4, c = 238.8 Å. These crystals diffract X-rays to 2.55 Å resolution and contain one monomer of the homodimeric protein per asymmetric unit. Crystals of the CcpA–CRE complex were obtained with ammonium sulfate as precipitant and belong to the tetragonal space group $I4_122$, with unit-cell parameters a = 125, c = 400 Å and one complex per asymmetric unit. Although these co-crystals grew to a sufficient size, X-ray diffraction was limited to 8 Å resolution.

1. Introduction

The catabolite control protein is the central regulatory protein for carbon catabolite repression in Gram-positive bacteria (Hueck & Hillen, 1995; Henkin, 1996). It belongs to the GalR/LacI family of bacterial regulatory proteins (Weickert & Adhya, 1992) and shares 26% amino-acid sequence identity with LacI. In the presence of glucose or other favourable carbon sources, HPr kinase is activated and utilizes ATP or GTP to phosphorylate HPr at Ser46 (Deutscher & Saier, 1983; Deutscher *et al.*, 1995; Galinier *et al.*, 1997; Reizer *et al.*, 1998). CcpA binds to HPr by recognizing the phosphorylated Ser46, which results in an increased affinity for its operator site, called the catabolite responsive element (CRE; Deutscher *et al.*, 1995; Gösseringer *et al.*, 1997; Jones *et al.*, 1997; Miwa *et al.*, 1997). CcpA binding to CRE affects repression of a number of carbon-source utilization genes (Grundy *et al.*, 1994; Miwa & Fujita, 1993; Fujita & Miwa, 1994; Wray *et al.*, 1994; Martin *et al.*, 1989; Kraus *et al.*, 1994), as well as the activation of at least one carbon-excretion pathway (Grundy, Waters, Takova *et al.*, 1993). The promoter regions of these genes contain a common sequence element proposed to be a regulatory target site (Weickert & Chambliss, 1990; Jacob *et al.*, 1991; Oda *et al.*, 1992; Grundy, Waters, Allen *et al.*, 1993; Miwa & Fujita, 1993; Grundy *et al.*, 1994; Hueck *et al.*, 1994; Kraus *et al.*, 1994; Wray *et al.*, 1994). Comparison of related genes of CcpA from different bacteria have shown their importance in metabolic gene regulation. Here, we describe the crystallization and preliminary X-ray diffraction analyses of CcpA from *B. megaterium* and CcpA in complex with the DNA-binding site CRE.

2. Methods, results and discussion

2.1. Purification and crystallization of CcpA

CcpA was expressed from recombinant *Escherichia coli* carrying a 17 amino-acid N-terminal extension (MRGSHHHHHHG SDDDDK...; Deutscher *et al.*, 1995). The protein was purified using a Ni–NTA column (QUIAGEN) and concentrated by Amicon ultrafiltration devices (Centricon and Centriprep, 30 kDa cutoff). Protein concentration was determined by absorption spectroscopy with a calculated extinction coefficient ε_{280} = 15 400 M^{-1} cm^{-1}.

Protein solution containing 20 mg ml^{-1} CcpA, 5 mM glucose-6-phosphate, 10 mM Tris–HCl buffer pH 7.5 was screened for crystallization conditions at 291 K using a protein crystal screen (Jancarik & Kim, 1991) and the hanging-drop vapour-diffusion method. In a typical crystallization experiment, 2.5 µl of protein solution was mixed with the same volume of reservoir solution and allowed to equilibrate against 1 ml of reservoir. The best crystals, which had a hexagonal bipyramidal shape, were obtained in the presence of 3% polyethylene glycol 8000, 100 mM $CaCl_2$, 10%(v/v) glycerol, 100 mM Tris–HCl buffer

pH 8.0. They grew to dimensions of 0.6 mm along the hexagonal axis and 0.3 mm at the bipyramidal base. Prior to X-ray diffraction data collection, crystals were transferred to cryoprotectant buffer containing 25% glycerol and flash-frozen in liquid propane.

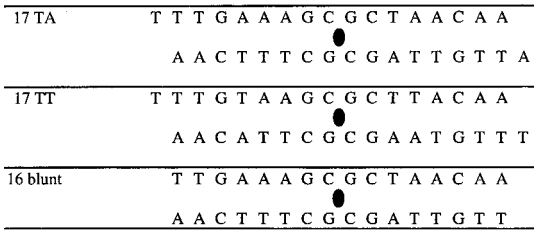

Figure 1
Oligonucleotide sequences used for crystallization with CcpA.

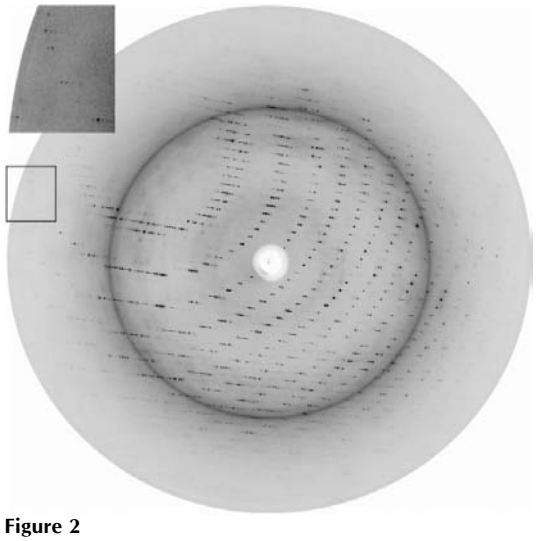

Figure 2
X-ray diffraction pattern of a single crystal of free CcpA. The image was recorded using the X-ray beam at beamline 9.5 (SRS, Daresbury) on a MAR Research imaging plate with an oscillation angle of 2°.

Figure 3
X-ray diffraction pattern of a single crystal of CcpA in complex with 17 TA. The image was recorded using the X-ray beam at beamline X11 (EMBL Outstation, Hamburg) on a MAR Research imaging plate with an oscillation angle of 2°. The arrow corresponds to 8.5 Å resolution.

2.2. CcpA–CRE complex preparation and crystallization

Complementary oligonucleotides with 16–18 bases per single strand were purchased from TIB-Molbiol/Berlin, separately purified and hybridized (Heinemann & Alings, 1991). The DNA duplexes contained blunt ends or single overhanging nucleotides at each end. They were concentrated to 1.3 m*M* using a Centricon concentrator (3 kDa cutoff) in 100 m*M* NaCl, 20 m*M* Tris–HCl pH 7.5. The concentrations were determined using calculated extinction coefficients. The CcpA–CRE complex was formed by mixing the two components with 1:1 to 5:2 molar ratios of oligonucleotide to protein. Solutions of the CcpA–CRE complexes were screened for crystallization conditions using a protein–DNA complex crystal screen (Scott *et al.*, 1995) and the hanging-drop vapour-diffusion method. For crystallization, 3.0 µl of the CcpA–CRE solution was mixed with the same volume of reservoir solution and was allowed to equilibrate against 1 ml reservoir. Thin plate-like crystals (0.2 × 0.2 × 0.05 mm) were obtained in 2 *M* ammonium sulfate, 30 m*M* $MgSO_4$, 7%(*v*/*v*) glycerol, 50 m*M* sodium cacodylate buffer pH 6.2. Spectroscopic analysis of dissolved crystals indicated the presence of DNA in the crystals (data not shown). Oligonucleotide sequences used for crystallization experiments are shown in Fig. 1. The best CcpA–CRE crystals were obtained using a 16 base-pair CRE fragment with overhanging T and A (17 TA).

2.3. Data collection and crystal characterization

X-ray diffraction data were collected using a MAR Research imaging-plate detector and synchrotron radiation on beamlines 9.5 (SRS, Daresbury) and X11 (EMBL Outstation, Hamburg). Native CcpA crystals were measured at 100 K (Fig. 2). The CcpA–CRE co-crystals were mounted in glass capillaries and data collection was performed at 277 K (Fig. 3). The diffraction data were evaluated using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

Table 1
Data-collection statistics for free CcpA.

Values in parentheses refer to the last resolution shell (2.65–2.55 Å).

Space group	Hexagonal $P6_122$ or $P6_522$
Unit-cell parameters (Å)	a = 74.4, c = 238.8
Temperature (K)	100
Wavelength (Å)	0.95
Resolution limits (Å)	30–2.55
Measured reflections	92728
Independent reflections	14161
Completeness (%)	98.6
Mean $I/\sigma(I)$	24.8 (3.7)
R_{merge}† (%)	5.1 (43.6)

† $R_{merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of reflection h and $\sum_h$ and $\sum_i$ are the summations over all reflections and all measurements i, respectively.

Processing data of native CcpA crystals revealed a primitive hexagonal crystal system with unit-cell parameters a = 74.4, c = 238.8 Å (Table 1). The space group of native CcpA crystals was determined to be $P6_122$ or $P6_522$, based on the symmetry of the diffraction pattern and systematic absences along 00*l*. Assuming one CcpA monomer (38.6 kDa) per asymmetric unit, the Matthews coefficient is calculated to be 2.40 $Å^3\ Da^{-1}$ (Matthews, 1968), corresponding to a solvent content of 48%.

The CcpA–CRE crystals were extremely susceptible to X-rays and could not be frozen without shattering. Co-crystals with the 16 bp oligonucleotide containing either 5′-overhanging T or A nucleotides (17 TA) showed diffraction to a resolution of 8 Å. Modification of the crystallization conditions (pH, salt concentrations and addition of supplements) did not improve the crystal quality. Owing to crystal damage, only incomplete data sets could be collected. Processing these data revealed a body-centered tetragonal crystal system with unit-cell parameters a = 125, c = 400 Å. On merging the data from several crystals, the space group could be determined to be $I4_122$, based on the symmetry in the diffraction pattern and on systematic absences along 00*l*. Assuming one complex formed by a CcpA dimer (77.3 kDa) and one CRE molecule (17 TA, M_W = 10.5 kDa) to be located in the asymmetric unit, the Matthews coefficient is 4.45 $Å^3\ Da^{-1}$. The corresponding solvent content is about 72%;

if two complexes in the asymmetric unit are assumed, the solvent content is about 45%.

CcpA in complex with the 16 bp palindromic oligonucleotide (16 blunt) crystallized in a different space group. The data were indexed in a primitive tetragonal crystal system with unit-cell parameters $a = 120$, $c = 175$ Å. This complex presumably crystallized in space group $P4_12_12$ or $P4_32_12$, with a lower solvent content (V_m = 3.63 Å^3 Da^{-1}; solvent 65%) in comparison with CcpA–17 TA.

Molecular-replacement studies using the proteins PurR (Schumacher *et al.*, 1994), LacI (Lewis *et al.*, 1996) or TreR (Hars *et al.*, 1998) as a model have been unsuccessful. This might be explained by the differences in the relative orientation of the DNA-binding domain and the two subdomains of the protein core. A search for heavy-atom derivatives for structure solution *via* isomorphous replacement has therefore been initiated.

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