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Crystallization and preliminary X-ray analysis of the ligand-binding domain of cAMP receptor protein

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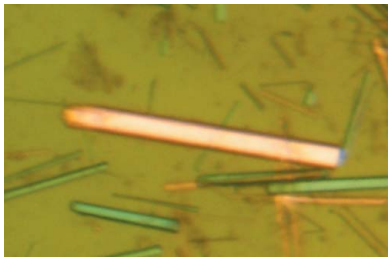
The cyclic AMP receptor protein (CRP) from *Escherichia coli* regulates the expression of a large number of genes. In this work, CRP has been overexpressed, purified and digested by subtilisin and chymotrypsin. The fragments S-CRP (digested by subtilisin) and CH-CRP (digested by chymotrypsin) have been purified and crystallized. Crystals of S-CRP diffracted to 2.0 Å resolution and belonged to space group $P2_1$, with unit-cell parameters a = 59.7, b = 75.1, c = 128.3 Å, β = 91.5°. Crystals of CH-CRP diffracted to 2.8 Å resolution and belonged to space group $P222$, with unit-cell parameters a = 45.8, b = 60.9, c = 205.6 Å.

1. Introduction

The cyclic AMP receptor protein (CRP; Fig. 1) plays a key role in the regulation of the expression of a large number of genes in *Escherichia coli* (Harman, 2001). CRP is a dimeric protein that is composed of two chemically identical subunits consisting of 209 amino-acid residues (Passner *et al.*, 2000; Sharma *et al.*, 2009). Each subunit contains two domains: a larger N-terminal domain (residues 1–133), which contains the cAMP-binding site, and a smaller C-terminal domain (residues 139–209) that binds to DNA through a helix–turn–helix motif. The two functional domains are covalently connected by a stretch of polypeptides denoted as the hinge region (residues 134–138). In the presence of cAMP CRP undergoes a conformational change that leads to the recognition of a specific DNA sequence and interaction with RNA polymerase.

On the basis of structure, the hinge region of CRP seems to be a crucial structural feature of the protein that is involved in both intersubunit and interdomain interactions (Sharma *et al.*, 2009). A contact between loop 3 (residues 53–57) and Phe136 in the hinge region of the adjacent subunit was assumed to result in a reorientation of the C-helices (residues 110–136) and hence in a reorientation of the subunits (Passner *et al.*, 2000). Loss of the hinge region would result in the loss of many interactions between the two subunits, including the removal of Phe136 in the hinge region from contact with the flap of the other subunit (residues 51–59; Passner *et al.*, 2000), and loss of these intersubunit interactions might block communication between domains and subunits.

CRP can be cleaved in the presence of cAMP, producing a dimer of the N-terminal domain (α-CRP) which retains cAMP-binding



10	20	30	40	50
VLGKPQTDPT	LEWFLSHCHI	HKYPSKSTLI	HQGEKAETLY	YIVKGSVAVL
60	70	80	90	100
IKDEEGKEMI	LSYLNQGDFI	GELGLFEEGQ	ERSAWVRAKT	ACEVAEISYK
110	120	130	140	150
KFRQLIQVNP	DILMRLSAQM	ARRLQVTSEK	VGNLAFLDVT	GRIAQTLLNL
160	170	180	▲ ▲190	200
AKQPDAMTHP	DGMQIKITRQ	EIGQIVGCSR	ETVGRILKML	EDQNLISAHG
209				
KTIVVYGTR				

Figure 1
Amino-acid sequence of CRP. Loop 3 is shown in red and the hinge region is shown in blue. The black triangles indicate the cleavage site.

capability (Ebright *et al.*, 1985). An NMR study of the histidine residues indicated that the structure of α-CRP is not dramatically changed relative to that of the intact protein (Clore *et al.*, 1982). Thus, the structures and ligand-binding abilities of these fragments were used to probe the significance of the C-helix in regulating the function of CRP (Li *et al.*, 2002). Here, two proteolytic fragments of the protein, S-CRP (residues 1–134), in which the hinge region is removed, and CH-CRP (residues 1–137), in which residues 134–137 of the hinge region are preserved, were employed in order to study the interdomain and intersubunit communication that leads to co-operativity in ligand binding.

In this work, we report the overexpression, purification and digestion of CRP and the purification, crystallization and preliminary X-ray crystallographic analysis of the α-CRP fragments S-CRP and CH-CRP.

2. Materials and methods

2.1. Overexpression and purification of CRP

The overexpression and purification of CRP have been reported previously (Lin *et al.*, 2002). CRP was overexpressed in BL21 cells. The cells were induced with 1 m*M* IPTG after growth to an OD_{600} of ~0.5–0.8 in Luria broth supplemented with kanamycin at 310 K and were harvested approximately 5 h after induction.

Approximately 50 g of cells was resuspended in 60 ml buffer *A* (50 m*M* Tris–HCl pH 7.8, 1 m*M* EDTA, 100 m*M* KCl, 1 m*M* DTT, 0.2 m*M* PMSF, 5% glycerol), homogenized at 277 K and then lysed using a French Press. 35 g BioRex70 (Bio-Rad Laboratories Inc.) was weighed and added to 50 ml buffer *A*. The pH of the solution was adjusted to 7.5 and was rechecked the following day. The buffer *A* was removed before use. The lysate was clarified by centrifugation; ~35 g BioRex70 was then added and gently mixed for 30 min. The supernatant was removed and replaced by 200 ml fresh buffer *A*. After gentle stirring, the supernatant was again removed. This procedure was repeated approximately five times until the OD_{278}/OD_{260} was >1.5.

The matrix was slowly packed into a column and washed with ~100 ml buffer *A*. CRP was gradient-eluted with 120 ml buffer *A*/120 ml 1.0 *M* KCl. After elution, the OD of each tube was examined and the protein was pooled. The protein solution was dialyzed overnight against buffer *A*.

The protein sample was centrifuged to remove any precipitate. Approximately 15 g hydroxyapatite (Fluka) was suspended in double-distilled water (ddH_2O) and packed into a column. The column was loaded with the centrifuged CRP sample and washed with ~50 ml buffer *B* (50 m*M* K_2HPO_4/KH_2PO_4 pH 7.5, 1 m*M* EDTA, 1 m*M* DTT, 10% glycerol). CRP was gradient-eluted with 120 ml buffer *B*/120 ml 0.50 *M* K_2HPO_4/KH_2PO_4 pH 7.5. The protein samples were pooled according to SDS–PAGE. Solid ammonium sulfate was added to a final concentration of 1.2 *M*. The sample was then loaded onto a Phenyl Sepharose (GE Healthcare) column that had been pre-washed with ~1 l ddH_2O and equilibrated with ~300 ml buffer *C* (1.2 *M* ammonium sulfate, 50 m*M* Tris–HCl pH 7.8, 100 m*M* KCl, 1 m*M* EDTA, 1 m*M* DTT). After sample loading, the column was washed with ~50 ml buffer *C* and the protein was gradient-eluted with 100 ml buffer *C*/100 ml ddH_2O and pooled. 20% glycerol was added to the sample, which was kept at 253 K.

After purification, the CRP solution was dialyzed against TEK buffer (50 m*M* Tris–HCl pH 7.8, 1 m*M* EDTA, 100 m*M* KCl and 1 m*M* DTT) before use. The concentration of stock CRP was determined using an extinction coefficient of 0.91 ml mg^{-1} cm^{-1} at 278 nm (Lin *et al.*, 2002). The absorbance ratio at 278/260 nm was greater than 1.85, indicating the removal of contaminating DNA.

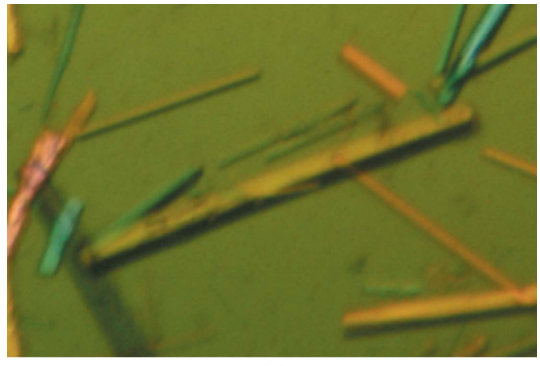
2.2. Preparation and purification of S-CRP and CH-CRP

The procedures that were used for CRP digestion and fragment purification were based on previous work (Li *et al.*, 2002). Approximately 30 ml 0.8 mg ml^{-1} CRP in TEK buffer containing 200 μM cAMP was digested with either 8 μg ml^{-1} (final concentration) subtilisin (Sigma) for 120 min or 15 μg ml^{-1} (final concentration) chymotrypsin (Sigma) for 150 min at 310 K. The reactions were stopped with 2 m*M* PMSF and the reaction mixtures were absorbed onto a 20 ml BioRex70 column, which was then washed with ~1 l buffer *D* (50 m*M* Tris–HCl pH 7.8, 50 m*M* KCl, 1 m*M* EDTA, 1 m*M* DTT) to remove cAMP. The α-CRP fragments were eluted with a linear gradient of 0.05–1 *M* KCl; SDS–PAGE was used to check the purity of each fraction. The fractions were pooled and concentrated by centrifugation using an Amicon Ultra-4 (Millipore Corporation, Billerica, USA). The α-CRP fragments (>8 mg ml^{-1}) were collected and again dialyzed against TEK buffer.

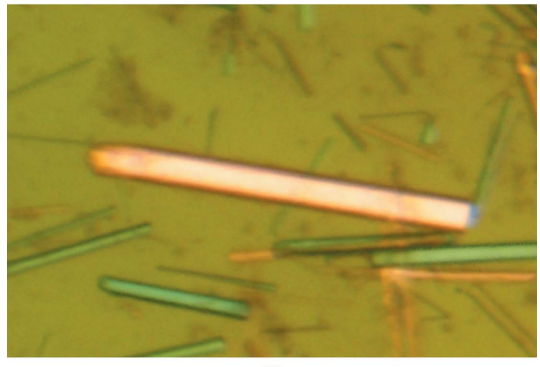
2.3. Crystallization and X-ray analysis

The crystals were obtained serendipitously: 20 m*M* cAMP was slowly titrated into 500 µl of 200 μM α-CRP fragments (S-CRP and CH-CRP) to give a final cAMP concentration of 200 μM. The mixture was stored at 277 K. Crystals appeared in the tube after 25 d.

The crystals were soaked in cryoprotectant solution (TEK buffer containing 20% glycerol) and flash-frozen in liquid nitrogen. Diffraction experiments were conducted at 100 K using an X-ray wavelength of 0.9791 Å on beamline 17U of the Shanghai Synchrotron Radiation Facility.



(*a*)

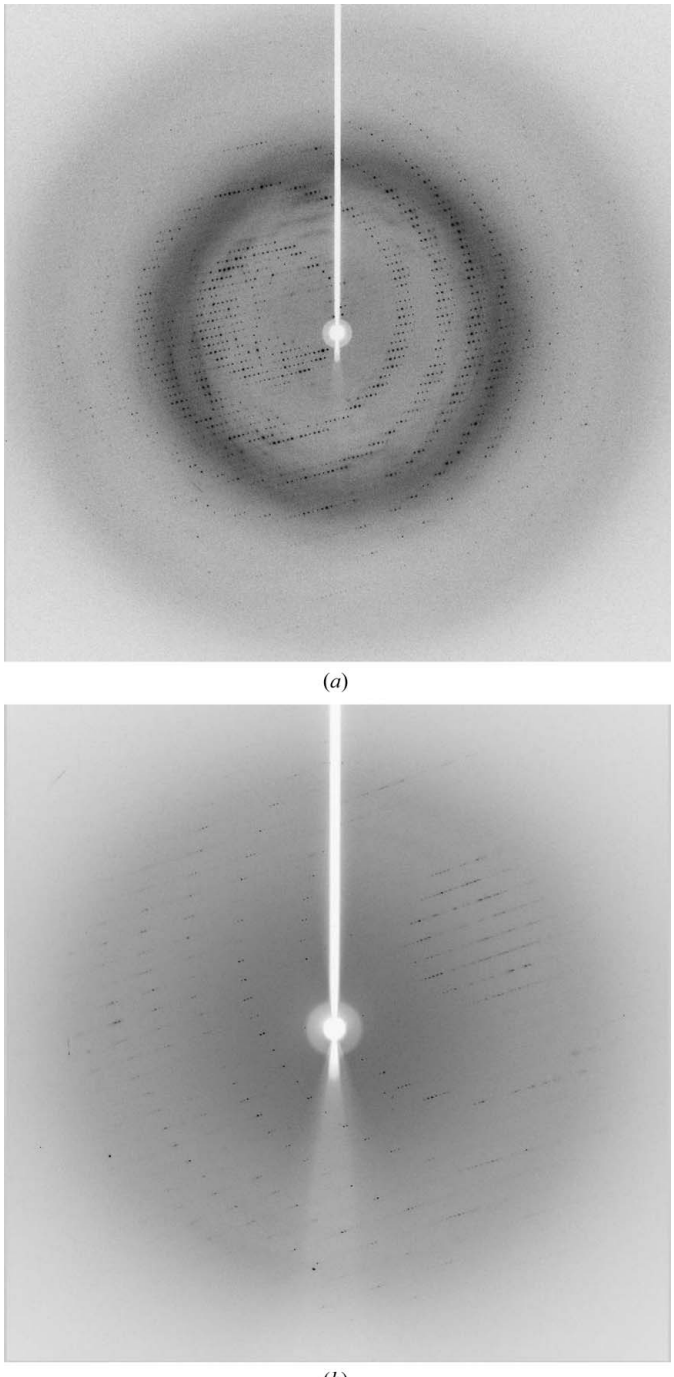


(*b*)

Figure 2
Crystals of (*a*) S-CRP and (*b*) CH-CRP. Both crystals had approximate dimensions of 0.1 × 0.1 × 0.8 mm.

3. Results and discussion

CRP was overexpressed in *E. coli* and purified and was successfully digested by subtilisin and chymotrypsin to obtain the fragments S-CRP and CH-CRP, respectively. However, the results of mass-spectrometric analysis showed that the cleavage sites (at residues 134–135 and 137–138) differed from those reported previously (Heyduk *et al.*, 1992). Crystals of S-CRP and CH-CRP appeared serendipitously in a mixture of 500 µl 200 µ*M* α-CRP and 200 µ*M* cAMP after 25 d storage.



(*a*)

(*b*)

Figure 3
Diffraction images of (*a*) S-CRP and (*b*) CH-CRP. The crystal-to-detector distances for the S-CRP and CH-CRP crystals were set to 200 and 300 mm, respectively. Oscillation images of 1.0° were recorded for both crystals with an exposure time of 6 s.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

	S-CRP	CH-CRP
Experimental conditions		
Beamline	17U	17U
Wavelength (Å)	0.9791	0.9791
Temperature (K)	100	100
Detector	MAR225	MAR225
Oscillation angle (°)	1.0	1.0
No. of images	180	180
Crystal parameters		
Space group	$P2_1$	$P222$
Unit-cell parameters (Å, °)	a = 59.7, b = 75.1, c = 128.3, β = 91.5	a = 45.8, b = 60.9, c = 205.6
Solvent content (%)	48.87	47.45
Monomers in asymmetric unit	8	4
Data processing		
Software	*HKL*-2000	*HKL*-2000
Resolution range (Å)	128–2.0	102.6–2.8
$R_{\rm merge}$† (%)	6.9 (29.6)	12.4 (63.8)
Completeness (%)	98.9	97.1
Mean $I/\sigma(I)$	12.3 (2.5)	8.9 (2.2)
No. of unique reflections	68161	13726
No. of observed reflections	75710	14521

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith observation and $\langle I(hkl)\rangle$ is the mean intensity of the reflection.

Diffraction data were obtained from S-CRP (Fig. 2*a*) and CH-CRP (Fig. 2*b*) crystals in the resolution ranges 128–2.0 and 102.6–2.8 Å, respectively, and were processed using *HKL*-2000 (Otwinowski & Minor, 1997). Crystal parameters and diffraction data statistics are summarized in Table 1. The space groups of the crystals were determined to be $P2_1$ for S-CRP, with unit-cell parameters a = 59.7, b = 75.1, c = 128.3 Å, β = 91.5°, and $P222$ for CH-CRP, with unit-cell parameters a = 45.8, b = 60.9, c = 205.6 Å. From a total of 75 710 reflections measured for S-CRP, 68 161 independent reflections were obtained with an $R_{\rm merge}$ of 6.9% (29.6% in the last shell) and the data set was 98.9% complete at the resolution limit of 2.0 Å (Fig. 3*a*). From a total of 14 521 reflections measured for CH-CRP, 13 726 independent reflections were obtained with an $R_{\rm merge}$ of 12.4% (63.8% in the last shell) and the data set was 97.1% complete at the resolution limit of 2.8 Å (Fig. 3*b*). The Matthews coefficient (Matthews, 1968) was 2.4 Å^3 Da^{-1} for the S-CRP crystal, corresponding to a solvent content of 48.87% and indicating the presence of eight monomers in the asymmetric unit. Similarly, the Matthews coefficient for the CH-CRP crystal was 2.34 Å^3 Da^{-1}, corresponding to a solvent content of 47.45% and indicating the presence of four monomers in the asymmetric unit. Further refinement is currently in progress and we are also attempting to obtain a better quality data set using properly strategized data collection for the crystals, particularly for CH-CRP.

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References

Clore, G. M. & Gronenborn, A. M. (1982). *Biochemistry*, **21**, 4048–4053.
Ebright, R. H., LeGrice, S. F. J., Miller, J. P. & Krakow, J. S. (1985). *J. Mol. Biol.* **182**, 91–107.
Harman, J. G. (2001). *Biochim. Biophys. Acta*, **1547**, 1–17.
Heyduk, E., Heyduk, T. & Lee, J. C. (1992). *Biochemistry*, **31**, 3682–3688.
Li, J., Cheng, X. & Lee, J. C. (2002). *Biochemistry*, **41**, 14771–14778.

Lin, S.-H., Kovac, L., Chin, A. J., Chin, C. C. & Lee, J. C. (2002). *Biochemistry*, **41**, 2946–2955.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Passner, J. M., Schultz, S. C. & Steitz, T. A. (2000). *J. Mol. Biol.* **304**, 847–859.
Sharma, H., Yu, S., Kong, J., Wang, J. & Steitz, T. A. (2009). *Proc. Natl Acad. Sci. USA*, **106**, 16604–16609.