

Hirofumi Komori,^{a,b} Kensuke Satomoto,^a Yasufumi Ueda,^a Naoki Shibata,^{a,b} Sayaka Inagaki,^{c,d} Shiro Yoshioka,^c Shigetoshi Aono^c and Yoshiki Higuchi^{a,b*}

^aDepartment of Life Science, Graduate School of Life Science, University of Hyogo, Japan,

^bRIKEN SPring-8 Center, Japan, ^cOkazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences, Japan, and

^dDepartment of Structural Molecule Science, The Graduate University for Advanced Studies, Japan

Correspondence e-mail: hig@sci.u-hyogo.ac.jp

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Crystallization and preliminary X-ray analysis of CooA from *Carboxydothemus hydrogenoformans*

CooA, a homodimeric haem-containing protein, is responsible for transcriptional regulation in response to carbon monoxide (CO). It has a b-type haem as a CO sensor. Upon binding CO to the haem, CooA binds promoter DNA and activates expression of genes for CO metabolism. CooA from *Carboxydothemus hydrogenoformans* has been overexpressed in *Escherichia coli*, purified and crystallized by the vapour-diffusion method. The crystal belongs to space group $P2_1$, with unit-cell parameters $a = 61.8$, $b = 94.7$, $c = 92.8$ Å, $\beta = 104.8^\circ$. The native and anomalous difference Patterson maps indicated that two CooA dimers are contained in the asymmetric unit and are related by a translational symmetry almost parallel to the c axis.

1. Introduction

Transcription regulators, which are widely distributed throughout bacteria, play an important role in their metabolism. The catabolite activator protein (CAP) is a well characterized transcription regulator and many proteins have been identified as members of the CAP family (Busby & Ebright, 1999). They regulate the expression of specific genes in response to distinct external signals (effectors). CooA, a member of the CAP family, was first identified as a carbon monoxide (CO) dependent transcription factor in the photosynthetic bacterium *Rhodospirillum rubrum* (Aono *et al.*, 1996; Shelver *et al.*, 1997). CooA is a homodimeric haem protein composed of about 200 amino acids per monomer and the haem plays an important role in activating CO-dependent transcription. In the absence of CO, CooA has a six-coordinated ferrous haem with two endogenous ligands. Previous studies have proposed that CO binds to haem as an axial ligand and induces a conformational change of the DNA-binding domain for DNA binding. The only structure known for CAP is the effector (cAMP) bound form, whereas the structure of CooA has been determined as the inactive effector-free form for the protein from *R. rubrum* (Rr-CooA; Lanzilotta *et al.*, 2000; McKay & Steitz, 1981; Schultz *et al.*, 1991). Although the overall structure of the inactive form of Rr-CooA has a global structural similarity to the active form of CAP, the placement of the DNA-binding domains is completely different between them. This has suggested that a substantial conformational change of the DNA-binding domains occur upon CO binding to CooA. Owing to the lack of structural information on the effector-bound form of CooA, the details of this allosteric mechanism are still unclear. Here, we report the crystallization of CooA from *Carboxydothemus hydrogenoformans* (Ch-CooA; Inagaki *et al.*, 2005; Youn *et al.*, 2004) bound to the exogenous ligand imidazole.

2. Expression and purification

C-terminal 6×His (LEHHHHHH) tagged Ch-CooA was purified from an overexpressing strain of *Escherichia coli* using a Talon affinity column and a heparin column. The purity was assessed by Coomassie-stained SDS-PAGE (Fig. 1). The purified protein solution was applied onto a 1 ml Hi-Trap Heparin column and eluted with a



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high salt concentration (1 M NaCl). All purification steps were carried out under aerobic conditions at 277 K. The absorption spectra showed that the haem in Ch-CooA was in the ferric state. During purification, we noticed a marked change in the UV spectrum and concluded that the cause was binding of imidazole to the protein. The fractions eluted from the Talon column at a high concentration (400 mM) of imidazole contained Ch-CooA bound to imidazole in the ferric state (Fig. 2). Crystallization experiments were performed using the protein sample (10 mg ml⁻¹) in 20 mM MES-NaOH buffer pH 6.0 containing 1 M NaCl).

3. Crystallization

Initial crystallization trials were carried out using commercially available screening kits from Hampton Research (Crystal Screen, Grid Screen, Natrix and MembFac) implementing the sitting-drop vapour-diffusion technique in 96-well plates. Protein droplets prepared by mixing 1 µl protein solution and 1 µl reservoir solution were equilibrated against 100 µl reservoir solutions at 283 K. Clusters

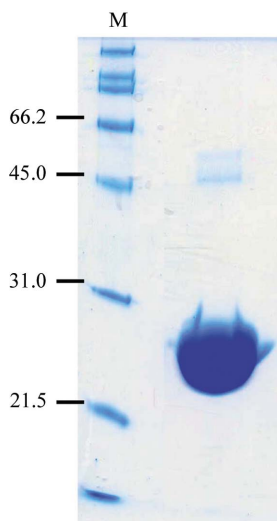


Figure 1
SDS-PAGE of purified Ch-CooA. Lane M, molecular-weight markers (kDa).

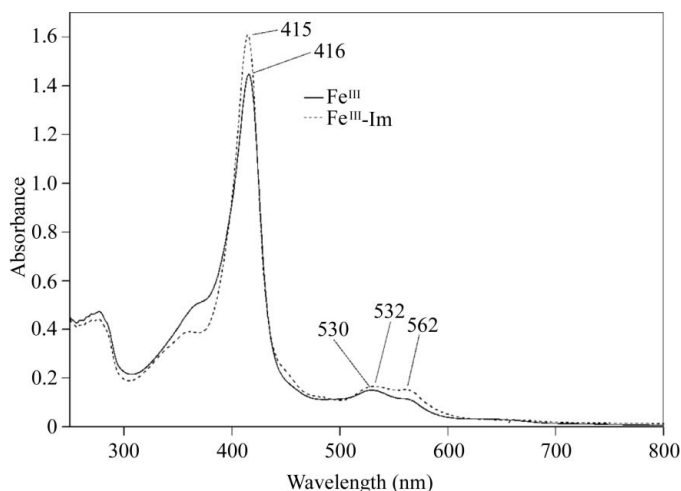


Figure 2
Absorption spectra of purified Ch-CooA in imidazole-bound and imidazole-free forms. Fe^{III} and Fe^{III}-Im indicate the imidazole-free (ferric) and imidazole-bound (ferric) Ch-CooA, respectively.

of small crystals were obtained with a reservoir solution consisting of 100 mM Bis-Tris buffer pH 5.5 and 25% PEG 3350. The crystallization conditions were refined and single crystals were obtained with a reservoir solution consisting of 100 mM Bis-Tris buffer pH 6.0, 30% PEG 3000, 200 mM NaCl, 5% glycerol and 5% dioxane at 293 K. Plate-like crystals appeared after 3–5 days and grew to approximate dimensions of 0.5 × 0.4 × 0.1 mm within one week (Fig. 3).

4. Data collection and crystallographic analysis

For data collection, the crystal was soaked in a cryoprotectant solution (30% PEG 3000, 500 mM NaCl, 10% glycerol) for a few minutes prior to freezing in a nitrogen cold stream. X-ray diffraction data sets were collected at 100 K on an R-Axis imaging-plate camera system (Rigaku Co. Ltd) using Cu K α radiation generated by a rotating-

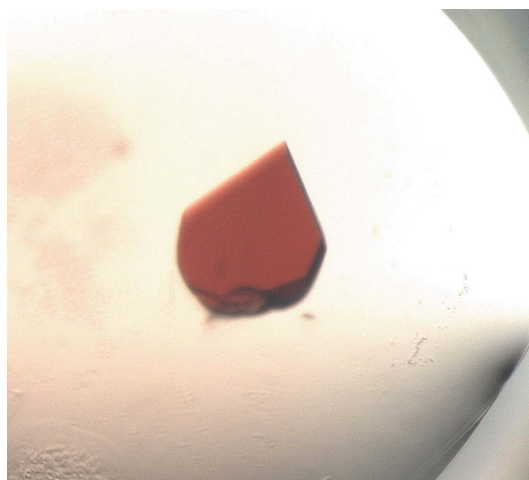


Figure 3
Photograph of a Ch-CooA crystal; the largest dimension is 0.5 mm.

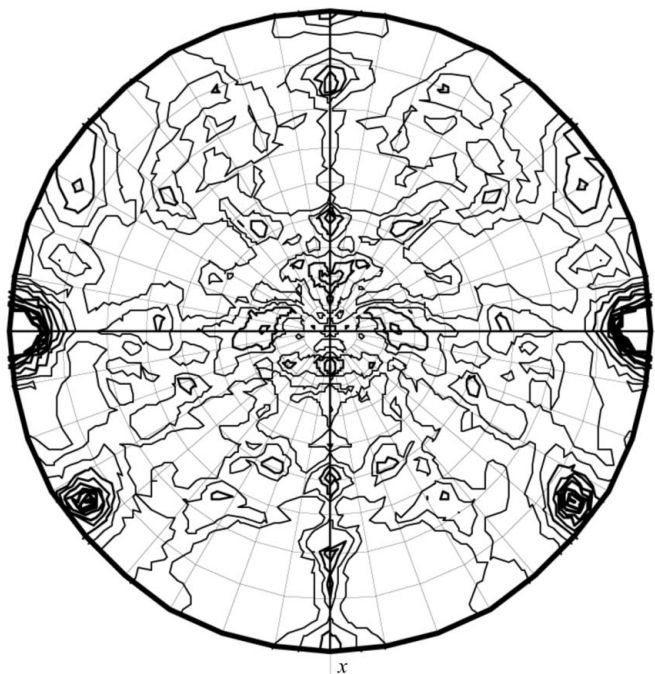


Figure 4
A $\chi = 180$ section of the self-rotation function calculated using *MOLREP* in the resolution range 15–3.0 Å, integration radius 5–30 Å.

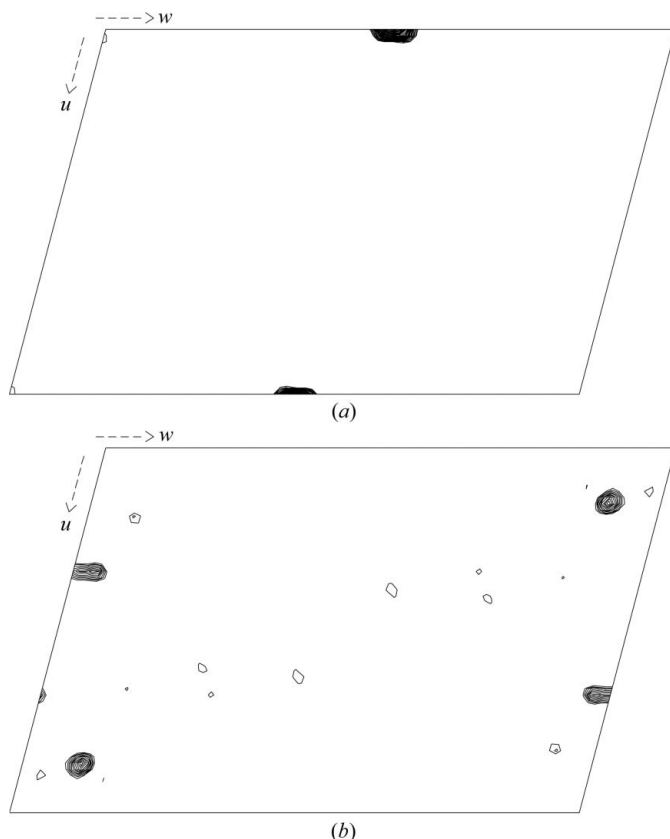


Figure 5
 (a) Native Patterson map ($0 < u < 1, v = 0.05, 0 < w < 1$). (b) Anomalous Patterson map ($0 < u < 1, v = 0.5, 0 < w < 1$).

anode generator operated at 40 kV and 100 mA with a fine focus filament. The crystal-to-detector distance was maintained at 200 mm with an oscillation range per image of 1° , covering a total oscillation range of 360° . Diffraction from the crystals extends to 2.3 \AA resolution. Determination of the unit-cell parameters and integration of reflections were performed using the program *d*TREK* (Pflugrath, 1999). The crystal is monoclinic and belongs to space group $P2_1$. The unit-cell parameters were determined to be $a = 61.8, b = 94.7, c = 92.8 \text{ \AA}, \beta = 104.8^\circ$. A total of 227 281 reflections were integrated to a resolution of 2.3 \AA and were then merged to obtain 35 094 unique reflections with an overall R_{merge} and completeness of 0.036 and 76.3%, respectively (Table 1). Anisotropic diffraction spots are the main reason for the low completeness. The Matthews equation (Matthews, 1968) indicates that four Ch-CooA protein per asymmetric unit would yield a solvent content of 53%. Native Patterson and self-rotation functions were calculated using the programs *FFT* (Read & Schierbeek, 1988) and *MOLREP* (Vagin & Teplyakov, 1997). The self-rotation function showed the presence of peaks corresponding to a non-crystallographic twofold axis (Fig. 4). The native Patterson was analyzed for off-origin peaks that would indi-

Table 1
 Summary of crystallographic data.

Values in parentheses are for the outer resolution shell.

Source	Rigaku RA-Micro7
Wavelength (\AA)	1.5418
Space group	$P2_1$
Unit-cell parameters ($\text{\AA}, ^\circ$)	$a = 61.8, b = 94.7,$ $c = 92.8, \beta = 104.8$
V_M ($\text{\AA}^3 \text{ Da}^{-1}$) (two dimers in ASU)	2.5
Resolution (\AA)	50.00–2.30 (2.38–2.30)
Total No. of reflections	227281
Unique reflections	35094
Multiplicity	6.48 (5.02)
Completeness (%)	76.3 (45.2)
R_{merge}^\dagger (%)	3.6 (10.0)

$^\dagger R_{\text{merge}} = \sum \sum |I_i - \langle I_i \rangle| / \sum \langle I_i \rangle$, where I_i is the observed intensity and $\langle I_i \rangle$ is the average intensity over symmetry-equivalent measurements.

cate translational symmetry (Fig. 5a). A large peak was detected on the v plane ($v = 0.05$) in the native Patterson map at $u = 0$ and $w = 0.5$. These results indicate that two Ch-CooA dimers are present in the asymmetric unit, related by a translational symmetry almost parallel to the c axis. The anomalous difference Patterson map showed clear peaks corresponding to the haem Fe atoms in the Harker section $v = 1/2$ (Fig. 5b). The structure of Ch-CooA in the exogenous ligand (imidazole) bound form will make an important contribution to understanding the molecular mechanism of the CAP family.

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