ELSEVIER

Coordination Chemistry Reviews
190–192 (1999) 267–282

COORDINATION CHEMISTRY REVIEWS

www.elsevier.com/locate/ccr

Structure and function of CooA, a novel transcriptional regulator containing a b-type heme as a CO sensor

Shigetoshi Aono *, Hiroshi Nakajima

School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Tatsunokuchi, Nomi-gun, Ishikawa 923-1292, Japan

Accepted 17 February 1999

Contents

Abstract

CooA is a transcriptional activator containing a b-type heme as a prosthetic group. The heme in CooA is in the six-coordinate form in the ferric and ferrous state. Although the ferrous heme is coordinated by two axial ligands, it can react easily with CO to form the CO-bound heme under physiological conditions. Replacement of the heme's axial ligand by CO triggers the activation of CooA, which causes some conformational change around the heme and finally in the whole molecule. In this paper, the coordination structure of the heme in CooA and the mechanism of activation of CooA by CO were studied by spectroscopic analysis of the wild-type and some mutant CooAs. The amino acid residues responsible for

* Corresponding author. Tel.: +81-761-51-1681; fax: +81-761-51-1149.
E-mail address: aono@jaist.ac.jp (S. Aono)

0010-8545/99/$ - *see front matter*
PII: S0010-8545(99)00070-3

the specific binding of CooA to target DNA were elucidated by site-directed mutagenesis.

Keywords: Transcriptional regulator; Heme protein; CO sensor; DNA binding protein; CooA

1. Introduction

A transcriptional regulator CooA in *Rhodospirillum rubrum* has been reported to be a member of the CRP/FNR family of transcriptional regulators [1]. CooA controls the expression of carbon monoxide dehydrogenase, hydrogenase, and some other proteins that are required to metabolize CO and are expressed only in the presence of CO under anaerobic conditions [1–3]. The most interesting feature in transcriptional regulation by CooA is that CO is required for control of the gene expression [1–3], indicating that CO acts as the effector of CooA. If CO is the effector of CooA, CooA should have some prosthetic group to bind CO. In fact, it has been reported that the recombinant CooA can be expressed in *E. coli*, which contains a b-type heme as a prosthetic group [4–6]. The ferrous heme in CooA can bind CO as an axial ligand [4–6]. The DNaseI footprinting analysis with CooA has shown that the protection of the target sequence on DNA is observed only in the presence of CO under anaerobic conditions [2,3,6], indicating that the binding of CO to the heme in CooA causes the specific binding of CooA to the target DNA. These findings demonstrate that the heme in CooA acts as a CO-sensor in vivo and regulates the activity of CooA by binding CO. Though CO is widely used as a probe to study the biochemical and biophysical properties of heme proteins, it generally has no physiological role. CooA is the first example of a heme protein in which CO plays a physiological role.

The function of the heme in CooA, which is the sensor of the effector, is a new one. Two heme proteins, FixL [7–10] and soluble guanylate cyclase (sGC) [11–17], have been reported to be members of heme proteins in which the heme acts as the sensor of the effector. The hemes in FixL and sGC are O_2- and NO-sensors, respectively, and are responsible for regulation of enzymatic activity with the effector [7–17]. CooA is a new member of the 'heme-based sensor proteins'.

2. Experimental

The expression and purification of CooA were carried out as reported previously [4,5]. Site-directed mutagenesis was carried out by using a QuickChange Site-Directed Mutagenesis Kit (Stratagene) or a Chameleon Double-Stranded, Site-Directed Mutagenesis Kit (Stratagene).

To measure the activity of CooA in vivo, the reporter system was constructed as described previously [5,18]. The reporter strain, *E. coli* λCOP, contains a single copy of the reporter gene that is the *cooF-lacZ* transcriptional fusion in the λ

prophage [5,18]. The activity of CooA in vivo was determined by measurement of the specific activity of β-galactosidase expressed in *E. coli* λCOP harboring the expression vector of the wild-type or mutant CooA as previously reported [5,18]. The measurement of the specific activity of β-galactosidase was carried out by the method of Miller [19].

X-band EPR spectra were measured on a JEOL RE1X or RE3X. CooA was dissolved in a 50 mM Tris–HCl buffer (pH 8.0) for measurement of EPR spectra. The ^{1}H-NMR spectra were measured on a Varian Unity 750 (750 MHz) spectrometer at 25°C. Chemical shifts were referenced to 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). For the measurement of NMR, CooA was dissolved in a 50 mM KH_2PO_4–NaOH buffer (pH 7.6) containing 15% D_2O.

3. Results and discussion

3.1. Coordination structure of the heme in CooA

It has been reported that the recombinant CooA can be expressed in *E. coli*, which contains one b-type heme per CooA monomer [4–6,18]. The electronic absorption spectra of CooA purified from *E. coli* are shown in Fig. 1. Ferric CooA showed the Soret, α, β, and δ bands at 423.5, 570.0, 538.5, and 362.5 nm, respectively. In addition to these bands, a weak CT band was observed at 760 nm. A similar CT band, designated a CT band from sulfur to Fe(III), has been observed in some heme proteins containing a thiolate as an axial ligand; these proteins include P450 cytochromes [20] and an M80C-cyt.c mutant in which one of the axial ligands of the heme, Met at position 80, is replaced by Cys [21].

The EPR spectrum of ferric CooA showed a rhombic component with g values of 2.46, 2.26, and 1.90 as shown in Fig. 2. These values are typical of low-spin heme proteins and indicate that the heme in ferric CooA is in the six-coordinate, low-spin form. In the resonance Raman spectrum of ferric CooA, the ν_2 and ν_3 bands have been observed at 1580 and 1501 cm^{-1}, respectively [22]. These values are consistent with the model that the ferric heme in CooA is in the six-coordinate, low-spin state. The g values of CooA are almost the same as those of substrate-free ferric P450cam ($g = 2.45$, 2.26, and 1.91) [23] and ferric P420cam ($g = 2.45$, 2.27, and 1.91) [24] cytochromes, in which a thiolate derived from a cysteine is the axial ligand of the heme. These results of electronic absorption and EPR spectroscopy show that a cysteine is the axial ligand of the ferric heme in CooA.

CooA contains five cysteines at positions 35, 75, 80, 105, and 123. There are five Cys-to-Ala mutants of CooA (C35A-, C75A-, C80A-, C105A-, and C123A-CooA), and at one of these positions the cysteine is replaced by alanine; among these, only C75A-CooA has shown the different spectroscopic properties correspondent to those of wild-type CooA [18]. The electronic absorption spectra of C75A-CooA are shown in Fig. 3. The Soret band of the ferric C75A-CooA has been observed at 411.0 nm, though it is blue-shifted by 12 nm as compared with the wild-type. The clear α and β bands in the Q band region have not been observed in the ferric

C75A-CooA [18]. These features are typical of five-coordinate, high-spin heme proteins. EPR spectra of C75A-CooA are shown in Fig. 4. Ferric C75A-CooA showed EPR signals of the high-spin heme in the '$g = 6$' region at 4 K, while it did not show any signals of the low-spin heme in the '$g = 2$' region at 4 and 77 K. The spectroscopic properties of the wild-type and of the C75A-CooA described above indicate that Cys75 is the axial ligand of the heme in ferric CooA. C75A-CooA showed complex EPR signals in the '$g = 6$' region as shown in Fig. 4, which may be the superposition of the signals due to the different conformation around the heme. That is, as the mutation is introduced on the amino acid acting as the axial ligand in C75A-CooA, the arrangement of the heme in CooA will not be fixed.

The sixth axial ligand of the ferric heme is unknown at present. To determine the axial ligands of the heme in CooA, we constructed several mutants by site-directed mutagenesis. All His, Met, and Cys residues were chosen as targets for mutagenesis. For the Lys-to-Ala and Tyr-to-Phe mutants, the residues located in the heme-binding domain (131 residues from Met1 to Met131 [5]) in CooA were chosen as a target for mutagenesis. The electronic absorption spectrum in the ferric state of CooA mutant on His, Met, Cys, Lys, or Tyr (excepting Cys75) is identical to that

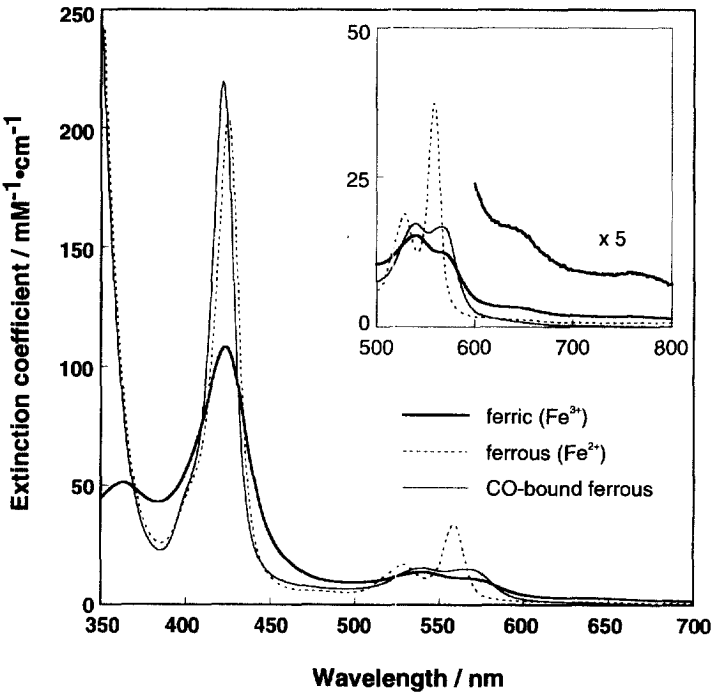

Fig. 1. Electronic absorption spectra of wild-type CooA. The thick solid line, broken line, and thin solid line represent the spectrum of ferric, ferrous, and CO-bound CooA, respectively. CooA (6.8 μM of the dimer) was dissolved in a 50 mM Tris–HCl buffer (pH 8.0). The enlarged spectra in the Q band region are shown in the inset. The extinction coefficients of the Soret band are 108, 200, and 220 mM^{-1} cm^{-1} for ferric, ferrous, and CO-bound CooA, respectively.

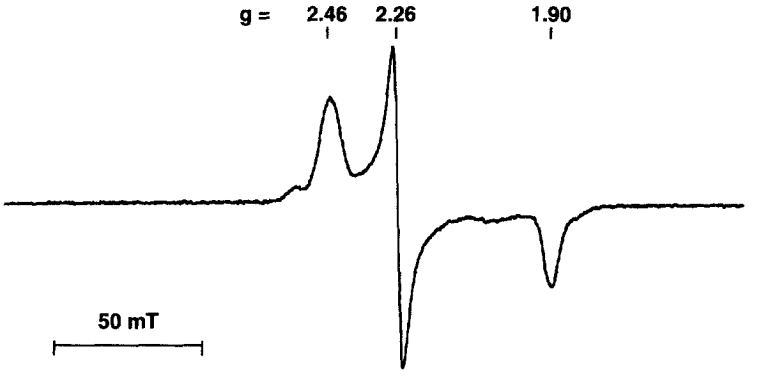

Fig. 2. X-band EPR spectra of wild-type CooA. The microwave power, modulation frequency, modulation amplitude, and measurement temperature are 1 mW, 100 kHz, 1 mT, and 77 K, respectively.

of the wild-type, indicating that these residues are not the sixth axial ligand of the ferric heme in CooA [18]. Although water (or OH^-) is a possible candidate for the sixth axial ligand of the ferric heme, we cannot determine whether water is coordinated to the ferric heme.

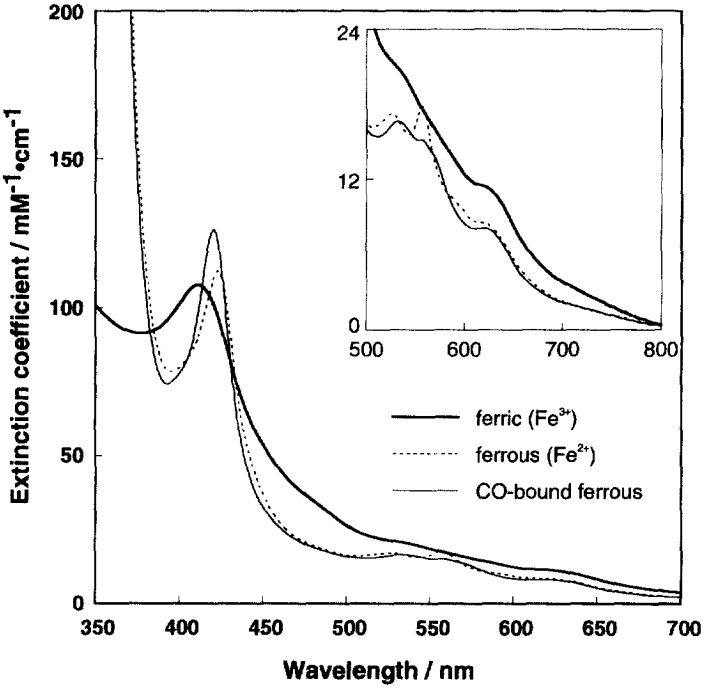

Fig. 3. Electronic absorption spectra of C75A-CooA. The thick solid line, broken line, and thin solid line represent the spectrum of ferric, ferrous, and CO-bound CooA, respectively. The enlarged spectra in the Q band region are shown in the inset.

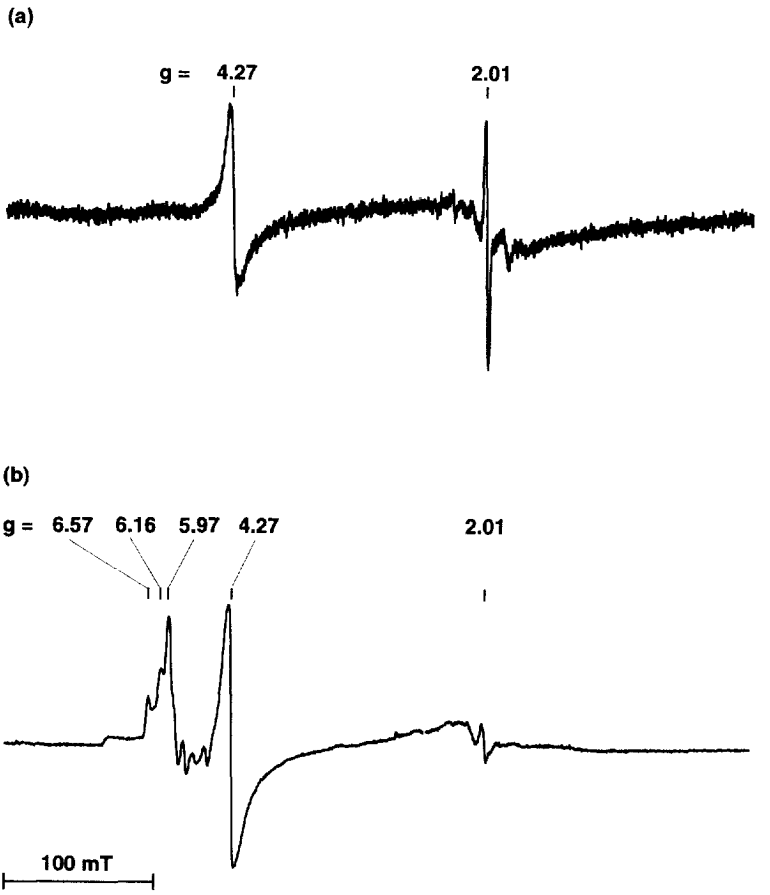

Fig. 4. X-band EPR spectra of C75A-CooA at (a) 77 K and (b) 4 K. The microwave power, modulation frequency, and modulation amplitude are 1 mW, 100 kHz, and 1 mT, respectively. The signals at $g = 2.01$ and 4.27 seem to be due to unknown contaminants in the sample and to an adventitious Fe^{3+}, respectively.

Ferrous CooA showed the Soret, α, and β bands at 424.5, 557.5, and 528.5 nm, respectively, which is typical of six-coordinate, low-spin heme proteins. In the resonance Raman spectrum of ferrous CooA, the ν_2 and ν_3 bands have been observed at 1579 and 1491 cm^{-1}, respectively [25]. These values are similar to those of the ferrous cytochrome b5 ($\nu_2 = 1583$ and $\nu_3 = 1493$ cm^{-1} [26]), in which the heme is in the six-coordinate, low-spin form. These results indicate that the heme in ferrous CooA is in the six-coordinate, low-spin form.

Among the mutation on His, Met, Cys, Lys, or Tyr, only the mutation on Cys75 and His77 has been reported to affect the electronic absorption spectrum of ferrous CooA [18]. Ferrous C75A- and H77A-CooA show the Soret, α, and β bands at 423.0, 557.0, and 527.0 nm, and 422.5, 557.5, and 528.0 nm, respectively [18]. The Soret bands of these mutants are slightly blue-shifted by ca. 2 nm, and the molar

extinction coefficients are different compared with those of wild-type CooA. Given that His77 is thought to be the proximal ligand of CO-bound CooA as described below, it is a possible candidate for the axial ligand of ferrous CooA.

The thiolate derived from Cys75 is thought to be coordinated to the ferric heme in CooA as described above. This poses the question whether it is coordinated to the ferrous heme as well. Ferrous low-spin, thiolate-ligated heme proteins such as H450 (a soluble iron protoporphyrin IX-containing protein of unknown function [27]) typically exhibit the Soret peak at ca. 445 nm [27–30]. Ferrous CooA shows the Soret peak at 424.5 nm, indicating that no thiolate-ligand exists in the ferrous heme in CooA. A blue-shift has been reported in the wavelength of the Soret peak of ferrous H450 from near 450 nm to about 425 nm upon lowering the pH to 6, which requires that the thiolate (cysteinate) ligand is either protonated or displaced upon lowering the pH [27,29,30]. In the case of ferrous CooA, therefore, Cys75 will be protonated or replaced by another amino acid residue.

CO-bound ferrous CooA showed the Soret, α, and β bands at 422.0, 568.5, and 540.5 nm, respectively. In the resonance Raman spectrum of CO-bound CooA, the stretching modes of Fe–CO and C=O, ν(Fe–CO) and ν(C=O) have been observed at 487 and 1969 cm^{-1}, respectively [25]. There is a well-known correlation between the frequencies of the Fe–CO and C=O stretching modes, which can be used to estimate the strength of the axial ligand *trans* to CO in CO-bound heme proteins [31–33]. The resonance Raman spectrum of CO-bound CooA has revealed that a neutral histidine is the proximal ligand of the heme in CO-bound form, and that the Fe–CO unit has a conformation similar to that of the A_0 conformer of Mb [34], which indicates the absence of any significant interaction between the bound CO and the distal heme pocket [25].

CooA contains five histidines at positions 28, 77, 133, 146, and 200. Among these five His-to-Ala mutants (H28A-, H77A-, H133A-, H146A-, and H200A-CooA), only H77A-CooA has shown the different spectroscopic properties corresponding to those of the wild-type CooA [18]. Other mutants, excepting H77A-CooA, show identical electronic absorption spectra in the ferric, ferrous, and CO-bound form [18]. The results of the electronic absorption and resonance Raman spectroscopies described above indicate that His77 is the axial ligand of the CO-bound heme in CooA.

The transcriptional activator activity of the mutant CooA proteins constructed as part of the work herein are summarized in Table 1. H77A-CooA is inactive as the transcriptional activator in vivo even in the presence of CO, though other mutants showed transcriptional activator activity (data shown in Table 1). The activity of C75A-CooA is almost same as that of the wild-type CooA. Given that only CO-bound CooA has been considered to assume the active form as the transcriptional activator, the proximal ligand of the CO-bound heme should be essential to the activity of CooA. The mutation of the amino acid that is the proximal ligand in the CO-bound heme, will cause a drastic change in the activity of CooA. The above results support the proposition that His77 is coordinated to the CO-bound heme in CooA, though Cys75 is not.

Based on the above discussion, we propose a model of the coordination structure of the heme in CooA (shown in Fig. 5). Although the complete coordination structure of the heme in CooA is not elucidated at present, the proposed model shows some interesting features, described below.

1. The heme in CooA is in the six-coordinate form in the ferric, ferrous, and CO-bound form. The six-coordinated ferrous heme in CooA can bind CO easily under physiological conditions. Although some six-coordinated ferrous heme proteins that are partially denatured have been reported to react with CO to form a CO-bound heme [35], CooA is the first example of the formation of a CO-bound heme by reaction of a six-coordinated heme with CO under physiological conditions. The replacement of the axial ligand by CO is thought to trigger the activation of CooA by CO as described in Section 3.3.
2. The thiolate derived from Cys75 is one of the axial ligands in the ferric CooA. Chloroperoxidase from *Calariomyces fumago* [36,37], NO synthase [38], and P450 cytochromes [20,39,40] are well-known heme proteins possessing a cysteine-derived thiolate ligand to the heme iron. In these enzymes, the strong

Table 1
The activity of wild-type and mutant CooAs

	LacZ/units mg protein^{-1}	
CooA	+CO[a]	−CO[b]
Control[c]	0.05	0.05
Wild-type	15.7	0.23
H28A	9.9	0.16
H77A	0.34	0.32
H133A	6.7	0.05
H146A	12.0	0.59
H200A	14.7	0.28
M73L	10.8	0.21
M76L	9.6	0.20
M108L	15.1	0.27
M124L	14.4	0.25
M131L[d]	286	311
C35A	9.5	0.20
C75A	13.4	0.40
C80A	13.4	0.80
C105A[e]	247	222
C123A	6.2	0.15

[a] *E. coli* λCOP was grown in the presence of CO.
[b] *E. coli* λCOP was grown in the absence of CO.
[c] pKK223-3/*E. coli* λCOP was used.
[d] The electronic absorption spectra in the ferric, ferrous, and CO-bound form of M131L-CooA are identical to those of the wild-type, which shows that the change in the activity of this mutant is not caused by the change in the coordination structure of the heme [5].
[e] C105A-CooA is active regardless of the presence or absence of CO, and its activity is up-regulated though the electronic absorption spectra data on it are the same as those of the wild-type [18].

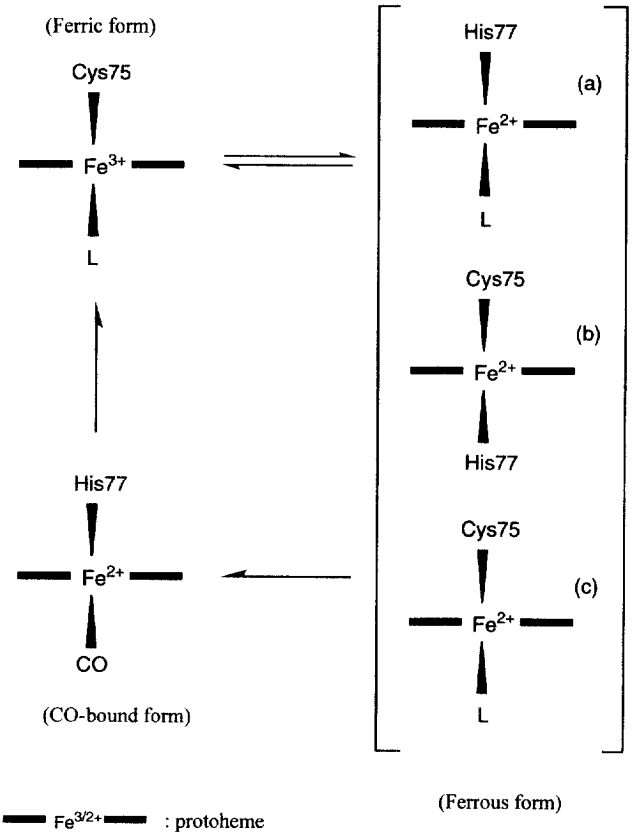

Fig. 5. The proposed model of the coordination structure of the heme in CooA. L represents an unidentified ligand. The coordination structure of the ferrous heme is tentative. The three possible models are shown in the figure. Cys75 will be protonated if it is coordinated to the ferrous heme (see the text).

electron-donating contribution of the thiolate ligand has been proposed to play an important role in catalysis, especially in the activation of bound hydrogen peroxide or oxygen [20,40]. On the other hand, neither redox nor cleavage reaction of the substrate takes place on the heme in CooA. Since the only CO-bound CooA is active as the transcriptional activator, the dissociation of CO from the heme is required to deactivate CooA. The CO-bound form remains intact by degassing the sample solution containing CO-bound CooA by a vacuum pump [18], which suggests that decreasing the partial pressure of CO would not be sufficient to dissociate CO from the heme in vivo. As the oxidation of CO-bound CooA gives ferric CooA in vitro [4,6], the oxidation of CO-bound CooA may be involved in the deactivation process of CooA. The coordination of Cys75 in the ferric heme may regulate the redox potential at which the oxidation of CO-bound CooA to ferric CooA proceeds efficiently in vivo.

3. CooA has the unique property that the axial ligand is exchanged at the time of the change in the redox state of the heme iron. Cys75 is coordinated to the ferric heme, but not to the CO-bound heme. On the contrary, His77 is coordinated to the CO-bound heme, but not to the ferric heme. A similar redox-controlled ligand exchange has been reported for cytochrome cd1 from *Thiosphaera pantotropha* [41,42]. In the case of *T. pantotropha* cytochrome cd1, the exchange of the axial ligands has been considered responsible for the adjustment of the redox potential to regulate the internal electron transfer and for the release of the reaction product, NO, from the d1 heme [41,42]. In the case of CooA, the exchange of the axial ligand at the time of the change in the redox state of the heme may play a partial role in the regulation of the redox potential of the heme.

3.2. Primary and domain structure of CooA

Roberts et al. have reported the cloning of the *cooA* gene and proposed that CooA is a member of the CRP/FNR family of transcriptional regulators on the basis of its amino acid sequence homology [1]. CRP is a homodimer of a 209 amino acid monomer that is composed of two domains [43–48]. The small carboxyl-terminal domain contains a helix-turn-helix (HTH) DNA binding motif; the large amino-terminal domain is responsible for subunit–subunit contact for dimerization, and binds cAMP as the effector [46–50]. The two domains are connected by a hinge region (residues 135–139) [46–48].

CooA has been reported to be a homodimer of a 222 amino acid monomer in the ferric, ferrous, and CO-bound ferrous state [6,18]. We have reported that the amino-terminal region from Met1 to Met131 is the heme-binding domain of CooA [5]. The region of the heme-binding domain of CooA corresponds well to that of the cAMP-binding domain of CRP, which supports the proposition that CooA is a member of the CRP/FNR family of transcriptional regulators [1].

3.3. The activation of CooA by CO

CooA is active as the transcriptional activator in *E. coli* when the reporter strain was grown in the presence of CO (shown in Table 1). The expressed CooA has been reported to exist in the ferrous form in *E. coli* [5,18]. As ferrous CooA reacts readily with CO to form CO-bound CooA, CooA in *E. coli* cells grown in the presence of CO should exist in CO-bound ferrous form. Therefore, the results shown in Table 1 indicate that ferrous CooA is not active as the transcriptional activator, though CO-bound ferrous CooA is. It has been reported that CooA exhibits sequence-specific DNA-binding and binds DNA only in the presence of CO under anoxic, reducing conditions [2,3,6], which shows that only CO-bound CooA can bind the target DNA and thus be active as the transcriptional activator. These results indicate that the binding of CO to the heme activates CooA as the transcriptional activator.

CO should replace one of the axial ligands to bind the ferrous heme because the ferrous heme in CooA is in the six-coordinate form as described in Section 3.1. CO displaces one of the axial ligands from the ferrous heme in CooA and causes some conformational change around the heme by means of the ligand exchange, which will be a trigger of activation of CooA by CO. This conformational change around the heme caused by the binding of CO could be observed by NMR spectroscopy. The ^{1}H-NMR spectra of the ferrous and CO-bound CooA are shown in Fig. 6. Ferrous CooA showed several signals that were shifted by the ring current of the heme in the $-2 \sim -6$ ppm region (shown in Fig. 6(a)), which were due to protons of the amino acid(s) functioning as the axial ligand(s) of the heme and/or those

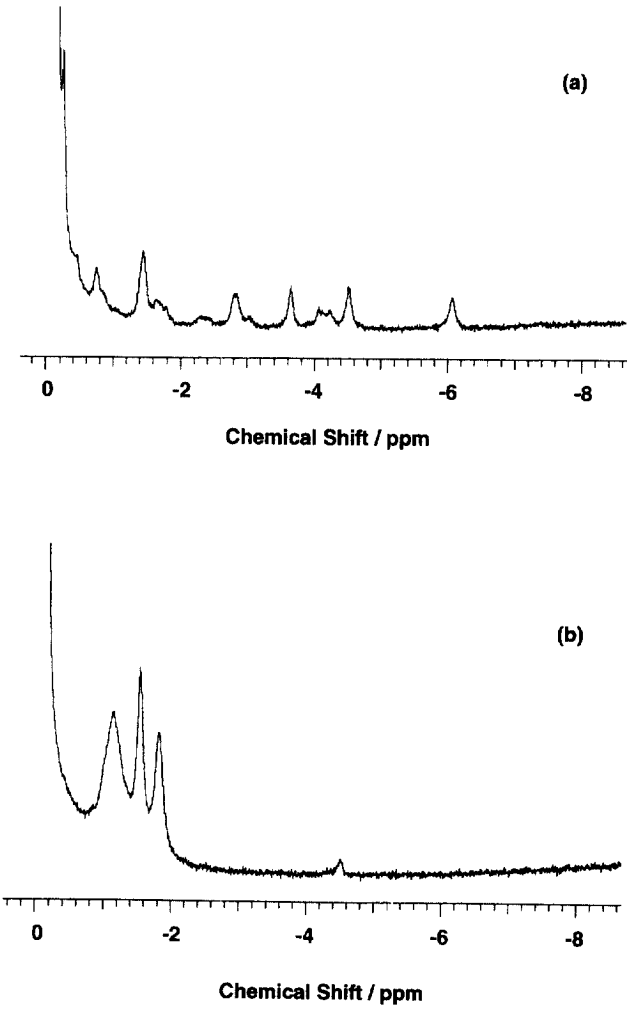

Fig. 6. ^{1}H-NMR spectra of (a) ferrous CooA and (b) CO-bound CooA. CooA (700 μM of the dimer) was dissolved in a 50 mM KH_2PO_4–NaOH buffer (pH 7.6).

located nearby above the heme plane. These signals observed in the $-2 \sim -6$ ppm region in ferrous CooA disappeared when CO bound to the heme (shown in Fig. 6(b)). These results indicate that the amino acid residue(s) giving the signals that are shifted by the ring current of the heme in ferrous CooA, move away from the vicinity of the heme to be free from the ring current effect upon the binding of CO. The movement of the amino acid residue(s) as described above, may cause the conformational change around the heme. This signal of the conformational change around the heme, which is induced by the binding of CO, will finally change the conformation of the whole molecule to adapt it to specific binding to the target DNA.

The details of the activation mechanism of CooA by CO is not completely elucidated at the present time, though it is certain that the binding of CO to the heme is the trigger of the activation process. Mutant CooA proteins that are constitutively active or inactive in the absence or presence of CO, respectively, when available, will give some useful information on the mechanism for the activation of CooA by CO. One mutant CooA that is constitutively active regardless of the presence or absence of CO has been isolated by a random mutagenesis [5]. In this mutant, Met131 is replaced by Leu [5]. Since Met131 is located at the carboxyl-terminal end of the heme-binding domain and adjacent to the hinge region which connects the heme-binding domain and the DNA-binding domain as described in Section 3.2, the replacement of Met by Leu at position 131 will cause a change in the relative orientation of the two domains by means of a change in the steric hindrance of the residue at position 131. M131L-CooA is active even in the absence of CO probably because the conformation of M131L-CooA in the absence of CO will be the same as that of the wild-type CooA in the presence of CO. A similar effect from mutations around the hinge region has been reported in the case of CRP [51–53]. The activity of M131L-CooA is up-regulated compared with that of the wild-type, but the reason for this up-regulation is presently unclear.

3.4. The recognition of the target sequence on DNA by the HTH motif in CooA

The direct hydrogen-bonding and van der Waals interactions between protein side chains and the exposed edges of base pairs in the major groove of B-DNA are an important source for the specific binding of the transcription regulators to the target DNA [54]. For example, the X-ray crystallography and site-directed mutagenesis of CRP have revealed that Arg180 and Glu181, on the recognition helix in the HTH motif, are responsible for the specific binding of CRP to the target DNA [47].

CooA has been proposed to have a HTH motif in its carboxyl-terminal domain [1]. In Fig. 7(a), the amino acid sequence of the putative HTH motif of CooA, which is deduced from the sequence homology between CooA and CRP [1], is shown along with those of CRP and FNR. To determine which amino acid residues in the HTH motif are responsible for the specific binding of CooA to the target DNA, the mutant CooA proteins, in which the mutation was introduced at each amino acid on the recognition helix, were prepared and their activity as the transcriptional activator was measured, respectively. The results are summarized in

(a)

	Helix	Turn	Recognition Helix
CRP	169 RQEIGQI	176 VGCS	180 RETVGRILK
FNR	197 RGDIGNY	204 LGLT	208 VETISRLLG
CooA	166 VEEIANL	173 IGSS	177 RQTTSTALN

(b)

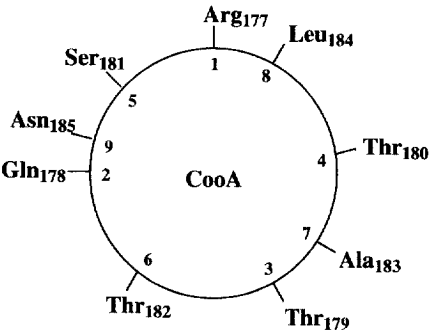

Fig. 7. (a) Amino acid sequence of the HTH motif of CRP, FNR, and CooA. (b) The helical wheel model for the DNA recognition helix of CooA.

Table 2. Three mutants, T179A-, T180V- and T182R-CooA, were active only in the presence of CO as the wild-type. The three mutants in which the mutation was introduced at position 177, 178, or 181 (R177V-, Q178E-, and S181G-CooA) were inactive regardless of the presence or absence of CO. When the mutation was introduced at position 183, an unexpected result was obtained. A183T- and A183I-CooA were active regardless of the presence or absence of CO.

Since the mutation at position 179, 180, and 182 does not affect the activity of CooA, it is clear that Thr179, Thr180, and Thr182 do not play any evident role in the specific binding of CooA to the target DNA. Arg177, Gln178, and Ser181 are possible candidates for the amino acid residues responsible for the specific interaction between CooA and the target DNA because the activity is lost when mutation occurs on these residues. The side chains of these three residues at position 177, 178, and 181 are thought to be located on the same face of the helix as shown in Fig. 7(b). On the basis of the sequence homology shown in Fig. 7(a), the positions of Arg177 and Gln178 of CooA are thought to correspond to those of Arg180 and Glu181 of CRP, respectively. The side chains of Arg180 and Glu181 of CRP have been reported to engage in hydrogen bonding directly to the base pairs in the major groove of the target DNA [47]. These results suggest that the surface of the helix on

Table 2
The activity of the mutant CooAs in which the mutation is introduced at the amino acid on the DNA binding recognition helix[a]

CooA	LacZ/units mg protein^{-1}	
	+CO	−CO
Wild-type	15.7	0.23
R177V	0.07	0.01
Q178E	0.14	0.06
T179A	11.3	0.13
T180V	9.38	0.03
T180I	9.33	0.23
S181G	0.15	0.08
T182R	9.15	0.09
A183T	9.97	9.73
A183I	10.5	8.30

[a] The experimental conditions are the same as those described in Table 1.

which Arg177, Gln178, and Ser181 are located will face the major groove of the target DNA when CooA binds it, and that these three residues will be responsible for the specific binding of CooA to the target DNA. However, it is not clear at present whether all of these three residues interact directly to the base pairs in the target DNA.

A183T- and A183I-CooA are active as the transcriptional activator even in the absence of CO as described above, indicating that these mutants can bind the target DNA regardless of the presence or absence of CO. The activation of CooA by CO will be caused by the conformational change of CooA, which is triggered by the binding of CO to the heme, as described in Section 3.3. These results suggest that the replacement of Ala183 by Thr or Ile will cause a conformational change similar to that induced by the binding of CO to wild-type CooA. The mutation at position 183 seems to cause the change in the relative orientation of the two helices in the HTH motif and/or of the two domains in CooA.

Acknowledgements

The authors thank Professor I. Okura and Dr T. Kamachi of the Tokyo Institute of Technology for their help with the measurement of EPR spectra. They also acknowledge Professor T. Ohkubo of the Japan Advanced Institute of Science and Technology for the measurement of NMR spectra. Work on the resonance Raman spectroscopy of CooA described herein is the product of collaboration with Dr S. Takahashi, Professor K. Ishimori, and Professor I. Morishima of Kyoto University. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports, and Culture in Japan (10129209 and 10145215). The author (S.A.) thanks the Sumitomo Foundation and the Asahi Glass Foundation for their financial support.

References

[1] D. Shelver, R.L. Kerby, Y. He, G.P. Roberts, J. Bacteriol. 177 (1995) 2157.
[2] Y. He, D. Shelver, R.L. Kerby, G.P. Roberts, J. Biol. Chem. 271 (1996) 120.
[3] J.D. Fox, Y. He, D. Shelver, G.P. Roberts, P.W. Ludden, J. Bacteriol. 178 (1996) 6200.
[4] S. Aono, H. Nakajima, K. Saito, M. Okada, Biochem. Biophys. Res. Commun. 228 (1996) 752.
[5] S. Aono, T. Matsuo, T. Shimono, K. Ohkubo, H. Takasaki, H. Nakajima, Biochem. Biophys. Res. Commun. 240 (1997) 783.
[6] D. Shelver, R.L. Kerby, Y. He, G.P. Roberts, Proc. Natl. Acad. Sci. USA 94 (1997) 11216.
[7] M.A. Gilles-Gonzalez, G.S. Ditta, D.R. Helinski, Nature 350 (1991) 170.
[8] E.K. Monson, M. Weinstein, G.S. Ditta, D.R. Helinski, Proc. Natl. Acad. Sci. USA 89 (1992) 4280.
[9] M.A. Gilles-Gonzalez, G. Gonzalez, M. Perutz, Biochemistry 34 (1995) 232.
[10] E.K. Monson, G.S. Ditta, D.R. Helinski, J. Biol. Chem. 270 (1995) 5243.
[11] P.A. Craven, F.R. DeRubertis, J. Biol. Chem. 253 (1978) 8433.
[12] P.A. Craven, F.R. DeRubertis, D.W. Pratt, J. Biol. Chem. 254 (1979) 8213.
[13] L.J. Ignarro, P.J. Kadowitz, W.H. Baricos, Arch. Biochem. Biophys. 208 (1981) 75.
[14] L.J. Ignarro, J.B. Adams, P.M. Horwitz, K.S. Wood, J. Biol. Chem. 261 (1986) 4997.
[15] J.R. Stone, M.A. Marletta, Biochemistry 33 (1994) 5636.
[16] J.R. Stone, M.A. Marletta, Biochemistry 34 (1995) 14668.
[17] J.R. Stone, M.A. Marletta, Biochemistry 35 (1996) 1093.
[18] S. Aono, K. Ohkubo, T. Matsuo, H. Nakajima, J. Biol. Chem. 273 (1998) 25757.
[19] J.H. Miller, A Short Course in Bacterial Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1992.
[20] J.H. Dawson, M. Sono, Chem. Rev. 87 (1987) 1257.
[21] A.L. Raphael, H.B. Gray, J. Am. Chem. Soc. 113 (1991) 1038.
[22] T. Uchida, S. Takahashi, K. Ishimori, I. Morishima, K. Ohkubo, H. Nakajima, S. Aono, unpublished result.
[23] J.H. Dawson, L.A. Andersson, M. Sono, J. Biol. Chem. 257 (1982) 3606.
[24] S.A. Martinis, S.R. Blanke, L.P. Hager, S.G. Sligar, G.H.B. Hoa, J.J. Rux, J.H. Dawson, Biochemistry 35 (1996) 14530.
[25] T. Uchida, H. Ishikawa, S. Takahashi, K. Ishimori, I. Morishima, K. Ohkubo, H. Nakajima, S. Aono, J. Biol. Chem. 273 (1998) 19988.
[26] T. Kitagawa, Y. Kyogoku, T. Iizuka, M. Ikeda-Saito, T. Yamanaka, J. Biochem. 78 (1975) 719.
[27] J.H. Dawson, L.A. Andersson, M. Sono, J. Biol. Chem. 258 (1983) 13637.
[28] E.W. Svastits, J.A. Alberta, I. Kim, J.H. Dawson, Biochem. Biophys. Res. Commun. 165 (1989) 1170.
[29] T. Hasegawa, H. Sadano, T. Omura, J. Biochem. 96 (1984) 265.
[30] T. Omura, H. Sadano, T. Hasegawa, Y. Yoshida, S. Kominami, J. Biochem. 96 (1984) 1491.
[31] X.-T. Li, T.G. Spiro, J. Am. Chem. Soc. 110 (1988) 6024.
[32] N.-T. Yu, E.A. Kerr, in: T.G. Spiro (Ed.), Biological Application of Raman Spectroscopy III, Wiley, New York, 1988.
[33] E. Okdfiekd, K. Guo, J.D. Augspurger, C.E. Dykstra, J. Am. Chem. Soc. 113 (1991) 7537.
[34] D. Morikis, P.M. Champion, B.A. Springer, S.G. Sligar, Biochemistry 28 (1989) 4791.
[35] L.V. Belovolova, L.A. Bliumenfel'd, D. Sh. Burbaev, A.F. Vanin, Mol. Biol. (Mosk) 9 (1975) 934.
[36] M. Sono, L.P. Hager, J.H. Dawson, Biochim. Biophys. Acta 1078 (1991) 351.
[37] S.R. Blanke, S.A. Martinis, S.G. Sligar, L.P. Hager, J.J. Rux, J.H. Dawson, Biochemistry 35 (1996) 14537.
[38] K.A. White, M.A. Marletta, Biochemistry 31 (1992) 6627.
[39] T.L. Poulos, B.C. Finzel, I.C. Gunsalus, G.C. Wagner, J. Kraut, J. Biol. Chem. 260 (1985) 16122.
[40] J.H. Dawson, Science 240 (1988) 433.
[41] P.A. Williams, V. Fülop, E.F. Garman, N.F.W. Saunders, S.J. Ferguson, J. Hajdu, Nature 389 (1997) 406.
[42] V. Fülop, J.W.B. Moir, S.J. Ferguson, J. Hajdu, Cell 81 (1995) 369.

[43] W.B. Anderson, A.B. Schneider, M. Emmer, R.L. Perlman, I. Pastan, J. Biol. Chem. 246 (1971) 5928.
[44] H. Aiba, S. Fujimoto, N. Ozaki, Nucl. Acids Res. 10 (1982) 1345.
[45] P. Cossart, N. Ozaki, Nucl. Acids Res. 10 (1982) 1363.
[46] I. Weber, T.A. Steitz, J. Mol. Biol. 198 (1987) 311.
[47] S. Schultz, G. Shields, T.A. Steitz, Science 253 (1991) 1001.
[48] G. Parkinson, C. Wilson, A. Gunasekera, Y. Ebright, R. Ebright, H. Berman, J. Mol. Biol. 260 (1996) 395.
[49] J.S. Krakow, I. Pastan, Proc. Natl. Acad. Sci. USA 70 (1973) 2529.
[50] E. Eilen, C. Pampeno, J.S. Krakow, Biochemistry 17 (1978) 2469.
[51] S. Garges, S. Adhya, Cell 41 (1985) 745.
[52] J. Kim, S. Adhya, S. Garges, Proc. Natl. Acad. Sci. USA 89 (1992) 9700.
[53] S. Ryu, J. Kim, S. Adhya, S. Garges, Proc. Natl. Acad. Sci. USA 90 (1993) 75.
[54] T.A. Steitz, Q. Rev. Biophys. 23 (1990) 205.