Evidence for Displacements of the C-helix by CO Ligation and DNA Binding to CooA Revealed by UV Resonance Raman Spectroscopy*

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The UV and visible resonance Raman spectra are reported for CooA from *Rhodospirillum rubrum, which is a transcriptional regulator activated by growth in a CO atmosphere. CO binding to heme in its sensor domain causes rearrangement of its DNA-binding domain, allowing binding of DNA with a specific sequence. The sensor and DNA-binding domains are linked by a hinge region that follows a long C-helix. UV resonance Raman bands arising from Trp-110 in the C-helix revealed local movement around Trp-110 upon CO binding. The indole side chain of Trp-110, which is exposed to solvent in the CO-free ferrous state, becomes buried in the CO-bound state with a slight change in its orientation but maintains a hydrogen bond with a water molecule at the indole nitrogen. This is the first experimental data supporting a previously proposed model involving displacement of the C-helix and heme sliding. The UV resonance Raman spectra for the CooA-DNA complex indicated that binding of DNA to CooA induces a further displacement of the C-helix in the same direction during transition to the complete active conformation. The Fe-CO and C-O stretching bands showed frequency shifts upon DNA binding, but the Fe-His stretching band did not. Moreover, CO-geminate recombination was more efficient in the DNA-bound state. These results suggest that the C-helix displacement in the DNA-bound form causes the CO binding pocket to narrow and become more negative.

CooA is a transcriptional regulator containing a prosthetic heme group (1–4). The heme in CooA senses CO, which activates coo gene expression, an event required for the growth of *Rhodospirillum rubrum in anaerobic environments containing CO (5). Recently, heme proteins homologous to CooA have been found in other organisms such as *Coxydothermus hydrogenoformans (6, 7). In the presence of CO, CooA adopts an active conformation, promoting specific interactions with the target DNA and an RNA polymerase.

CooA belongs to the cAMP receptor protein (CRP)‡/fumarate nitrate reductase family of transcriptional regulators, which contain helix-turn-helix DNA-binding motifs (8). CooA (2) and CRP (9, 10) are homodimers, whereas fumarate nitrate reductase is a monomer in the inactive state and becomes a dimer in the active state (11). The crystal structure of CooA has been solved only in a CO-free inactive state, as shown in Fig. 1 (12). Crystal structures of CRP are also available in the cAMP-bound active state with (13–15) and without (16–18) bound DNA. CooA and CRP share subunit architecture, including an N-terminal sensor domain and a C-terminal DNA-binding domain. A long α-helix (the C-helix) runs through the length of the protein, and a subsequent hinge region connects the two domains. The dimer interface is formed by the C-helices via a coiled-coil interaction. The two subunits in active (dimeric) CRP are symmetrically bent at the hinge region (15). In contrast, in inactive CooA, one subunit in the dimer was bent (blue in Fig. 1), whereas the other was extended (red). Recently, a small angle x-ray scattering study suggested a different structure in which both subunits are bent even in the inactive state to yield a more compact overall structure (19).

CooA is a member of the newly discovered heme-based gas sensor proteins (20–22). Inactive CooA possesses a six-coordinate low-spin ferrous heme in each sensor domain with His-77 and Pro-2 as axial ligands (12, 23, 24). The N-terminal residue Pro-2 is supplied by the opposite subunit in the dimer (Fig. 1a, inset). When CO binds CooA (CO-CooA), Pro-2 is displaced by CO and moves away from the heme iron in the inactive ferrous state (25, 26). Previous Raman spectroscopic studies of mutant CooA revealed that Ile-113, Leu-116, Gly-117, and Leu-120 are close to the dimer interface of the C-helix, and at a substantial distance from the inactive ferrous state (Fig. 1b). It was therefore proposed that when CO displaces Pro-2, the CO-bound heme slides to the inner side of the protein with concomitant displacement of the C-helix (27, 28); however, it is unknown how the C-helix moves in response to such an axial ligand exchange because the motion of the C-helix was not directly observed.

In the present study, we applied ultraviolet resonance Raman (UVRR) spectroscopy to elucidate the conformational change in CooA during conversion of its inactive ferrous form to its active CO-bound form. Our UVRR spectra provide the first experimental data that permit evaluation of the proposed models for the movement of the C-helix with heme sliding. Furthermore, we extended the measurements to CooA in complex with a target DNA (CO-CooA-DNA) to investigate the conformational change in CooA evoked by the counteraction from the target DNA. Visible RR spectroscopy was also applied to characterize the structure of the heme pocket in CO-CooA-DNA. The results from these experiments suggest that CO-CooA-DNA holds the effector CO in a narrower space in its binding pocket than CO-CooA. We further discuss the implications of our findings with respect to the protein conformational changes in CooA.

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a His-tag was added at the C terminus of CooA. The His-tagged recombinant CooA was prepared as described previously with slight modifications (1, 23). Briefly, a 26-mer oligonucleotides containing P_{i1032}^-/H_{11032}^- and 5’-CCGTCTGTCGGCCAGATGA-3’ were purchased from Operon Biotechnologies and used without further purification. These strands were heated at 80 °C for 5 min and then annealed at room temperature for 30 min. Because CooA-DNA has a K_D of 8 nM for the target DNA in the presence of 10 mM MgCl_2 (29), the concentration of target DNA we employed was enough to allow ~99% of the protein to bind to the target DNA. No additional absorption and Raman spectral changes occurred in the presence of additional DNA.

UVRR Spectroscopy—The 206.5-nm excited RR spectra were measured with the Raman instrument as described in detail elsewhere (30). A CW laser line at 206.5 nm was obtained from a krypton ion laser (BeamLok2060; Spectra Physics). The CW light at 406.7 and 428.7 nm was obtained from a Ti:sapphire laser with a repetition rate of 82 MHz (Tsunami 3950C; Spectra Physics) that was pumped by a 532-nm CW intracavity frequency-doubled Nd:YLF laser (Evolution-X; Spectra Physics). Raman scattered light was collected with a UV microscope objective lens (Ultrafluor ×10; Zeiss) at 90° geometry and focused with a UV achromatic lens onto the entrance slit of a custom-made prism prefiltter (Bunkoh-Keiki) coupled to an 1-m single spectrometer (SPEX 1000M; Jovin Yvon). The prefiltter effectively rejected stray background because of Rayleigh scattering and visible fluorescence. The spectrometer was equipped with a 3600 groove/mm holographic grating and a liquid nitrogen-cooled CCD detector (Spec10:400B/LN; Roper Scientific). The laser power at the sample point was reduced to 0.2 µW to avoid sample photodamage. The sample cell was rotated at 2000 rpm. Sodium sulfate (0.2M) was used as the internal intensity standard. Raman cross-sections were calculated as described previously (31).

UVRR spectra for ferrous CooA and CO-CooA were measured as follows. After data acquisition for ferrous CooA through ~10 exposures (the data were accumulated for 1 min in each exposure), the ferrous sample was replaced with a fresh CO-bound sample, and the data for CO-CooA were then acquired through ~10 exposures. This process was repeated 3 times to yield a final UVRR spectrum as the sum of ~30 exposures for each CooA sample. The spectrum of cyclohexane was finally measured for calibration of Raman shifts, providing an accuracy of ±1 cm⁻¹ for well defined Raman lines. UVRR measurements for CO-CooA and CO-CooA-DNA were also performed using the same procedure. The wavenumber resolution was ~1.2 cm⁻¹/CCD pixel and ~0.9 cm⁻¹/CCD pixel for the 206.5- and 229-nm excited RR spectra, respectively.

Visible RR Spectroscopy—Static RR spectra excited at 406.7 and 428.7 nm were measured with a 75-cm single spectrometer (SPEX750M; Jovin Yvon) equipped with a 2400 groove/mm holographic grating and a liquid nitrogen-cooled CCD detector (Spec10:400B/LN; Roper Scientific). The CW light at 406.7 and 428.7 nm was obtained from a krypton ion laser (BeamLok2060; Spectra Physics) and a diode laser (58-BTLR010; Melles Griot), respectively. To prevent CO photodissociation and sample photodamage, the sample cell was rotated at 2000 rpm, and the laser power at the sample point was reduced to 30 µW for CO-CooA and 300 µW for CO-CooA-DNA.

Picosecond time-resolved RR spectra were obtained using a homemade pump/probe system, in which the wavelength of the pump and probe beams were set to 540 and 442 nm, respectively. Details of the apparatus were described previously (32–34). The laser power at the...
sample point was 17 and 0.3 μl for the pump and probe beams, respectively. The wavenumber resolution was −0.5 and −0.7 cm⁻¹/CCD pixel for static and time-resolved RR spectra, respectively. The Raman shifts were calibrated within an accuracy of ±1 cm⁻¹ for visible RR spectroscopy.

RESULTS

α-Helical Content of Ferrous CooA and CO-CooA—The RR spectra excited around 200 nm are mainly composed of bands arising from the backbone amide modes (amides I, II, and III and Cα-Cϵ bending) as well as ring modes for aromatic residues such as Phe, Trp, and Tyr (35–37). The amide bands correlate with the protein secondary structure and can be used to estimate the α-helical content (38–40). The RR spectra excited at 206.5 nm for ferrous CooA and CO-CooA and their difference spectrum are shown in Fig. 2, a–c, respectively. The frequencies, intensities, and band shapes of the amide bands in spectra a and b were essentially identical. Therefore, the α-helical content of CooA, which is estimated to be ~35% in the ferrous form based on the crystal structure (12), is changed little by CO binding.

Conformational Changes Around Trp and Tyr Induced by CO Binding—Raman bands arising from Trp and Tyr are selectively enhanced upon excitation around 220–250 nm and can be used as the structural probes for their surroundings (35–37). The CooA subunit has one Trp (Trp-110) in the C-helix, two Tyr (Tyr-55 and Tyr-67) in the sensor domain, and two Tyr (Tyr-192 and Tyr-201) in the DNA-binding domain (Fig. 1a). Fig. 3, a and b, show the 229-nm excited RR spectra for ferrous CooA and CO-CooA, respectively. The bands arising from Trp and Tyr are marked by W and Y, respectively, and are followed by individual mode numbers (35–37). The spectra were dominated by the Trp bands (W1, W3, W7 doublet, W16, W17, and W18) and the Tyr bands (Y7a, Y8a, Y8b, and Y9a). The difference spectrum c, which was calculated with the SO²⁺ band at 981 cm⁻¹ as an internal intensity standard, indicates that the intensity of the Trp bands increased upon CO binding, whereas there was no appreciable difference for the Tyr bands. In particular, the increase of the W16 intensity was most pronounced. The UVRR intensity of Trp is sensitive to both the hydrogen-bond strength at the indole nitrogen and the environmental hydrophobicity around the indole ring (41, 42).

W17 is a marker of hydrogen bonding at the indole nitrogen (42, 43). The frequency of W17 for ferrous CooA was observed at 878 cm⁻¹, which indicates a medium-strength hydrogen bond, similar to that formed with solvent water. In fact, the indole nitrogen of Trp-110 is exposed to solvent in the crystal structure (12). CO-CooA showed the same W17 frequency as ferrous CooA, indicating that the hydrogen bond to a water molecule is maintained after CO binding. Therefore, the increase in intensity of the Trp bands upon CO binding is ascribed to an increase in the hydrophobicity around Trp-110, whose indole ring has one side accessible to solvent in the ferrous state (12).

W3 is known to serve as a marker for the conformation of Trp. Its frequency correlates well with the torsion angle (\(\chi^2\)) about the Cα-Cϵ linkage (42, 44), which is one of parameters describing the side chain orientation. The \(\chi^2\) angle of Trp-110 in ferrous CooA was estimated from the observed W3 frequency (1554 cm⁻¹) to be 104°, which is the same as that of Trp in water (Fig. 25 in Ref. 36). Thus, the tension imposed on the side chain of Trp-110 from its surroundings is very small; however, the \(\chi^2\) angle of Trp-110 in the crystal structure was about 87° (12), which is expected to give a W3 band at 1547 cm⁻¹. The large deviation from our estimation is probably because of the crystal packing force. Upon CO binding, the W3 band was upshifted by ~1 cm⁻¹ (approximately one CCD pixel) (Fig. 3, inset), whereas the peak positions of the other bands, including the internal intensity standard band, were unchanged within the half of the CCD pixel. Similar results were obtained when the experiments were repeated on different days, with a W3 band shift from 1554 ± 0.4 to 1555 ± 0.5 cm⁻¹. Therefore, we judged that the small upshift in the W3 band is physically meaningful, although the increase in the \(\chi^2\) angle was only ~2°.

Conformational Changes Around Trp and Tyr Induced by Binding of the Target DNA—We also applied UVRR spectroscopy to CO-CooA-DNA. Fig. 4, a and b, show the 229-nm excited RR spectra for CO-CooA-DNA and CO-CooA, respectively. Their difference, shown in spectrum c, contained the bands arising from nucleotide residues of the target DNA (45, 46) as well as Trp and Tyr residues of CooA. The spectrum of the target DNA was measured separately and is shown in spectrum d. Thus, the net difference of the Trp and Tyr bands caused by binding of the target DNA, shown in spectrum e, was obtained from the difference between spectra c and d. The double-difference spectrum e resembled the difference spectrum between ferrous CooA and CO-CooA-DNA (Fig. 3c). Specifically, the intensity of the Trp bands increased upon binding of the target DNA without a frequency shift in W17, whereas the Tyr bands exhibited no clear change. In addition, W3 was again upshifted very slightly (~1 cm⁻¹). These results suggest that the transition from CO-CooA to CO-CooA-DNA involves a conformational change around Trp-110 similar to that occurring during the transition from ferrous CooA to CO-CooA.

To compare the Trp band intensity among ferrous CooA, CO-CooA, and CO-CooA-DNA, we calculated Raman cross-sections. The Raman cross-section for W16 was 1.5, 1.8, and 2.1 barns/(molecule steradian) for ferrous CooA, CO-CooA, and CO-CooA-DNA, respectively. The magnitude of increase in the Raman cross-section upon binding of the target DNA was comparable with that observed upon CO binding, indicating that the increase in hydrophobicity around Trp-110 induced by binding of the target DNA is as large as that induced by CO binding.

Structural Change of the Proximal Heme Pocket Induced by Binding of the Target DNA—The electronic absorption spectra of CO-CooA and CO-CooA-DNA are depicted in Fig. 5. The Soret band was red-shifted by 0.6 nm (34 cm⁻¹) by binding of target DNA, which is indicative of a small structural rearrangement in the vicinity of heme. We next examined the Fe-His stretching mode, \(\nu\) (Fe-His), to probe the structural change of the heme pocket by a more sensitive method. Because the gernimate recombination of CO to CooA is very rapid (discussed below), we observed RR spectra of the immediate five-coordinate photoproducts after the CO photodissociation using a picosecond time-resolved
technique. The time-resolved RR spectra of photodissociated CO-CooA are displayed in Fig. 6a. Although we previously reported the \( \nu(\text{Fe-His}) \) of photodissociated CooA to be 211 cm\(^{-1} \) (25), the current experiments, which had a higher signal to noise ratio, determined the frequency to be 216 cm\(^{-1} \). Binding of the target DNA to CooA did not change the \( \nu(\text{Fe-His}) \) frequency as shown in Fig. 6b.

The band intensity of \( \nu(\text{Fe-His}) \) decreased with time because of the CO-geminate recombination. Previous reports indicated that more than \( \sim 90\% \) of the photodissociated CO from CO-CooA recombines geminately with time constants of \( \sim 70 \) and \( \sim 300 \) ps (47, 48). This is compatible with our current results. In the presence of the target DNA, we note that the \( \nu(\text{Fe-His}) \) band for CO-CooA-DNA decayed faster and vanished completely within 1 ns, indicating a higher rate of geminate recombination by CO along with a high quantum yield close to unity in the DNA-bound form. The modulation of CO geminate recombination without a change in the Fe-His bond strength suggests that binding of the target DNA to CooA alters the heme pocket structure on the distal side rather than on the proximal side.

**Structural Change in the Distal Heme Pocket Induced by Binding of the Target DNA**—To clarify the structural change in the distal heme pocket, we examined \( \nu(\text{Fe-CO}) \) and \( \nu(\text{C-O}) \) (the Fe-CO and C-O stretching modes, respectively). The RR spectra excited at 406.7 nm for CO-CooA and CO-CooA-DNA are shown in Fig. 7. Traces a, b, and c show the spectra of \( ^{12}\text{CO}-\text{CooA}, \ 13\text{CO}-\text{CooA}, \text{and the } b - a \text{ difference, respectively. Traces } c, d, \text{ and } f \text{ show the spectra for } ^{12}\text{CO}-\text{CooA-DNA}, \ 13\text{CO}-\text{CooA-DNA}, \text{and the } d - c \text{ difference, respectively.}
In the absence of the target DNA, a band at 487 cm$^{-1}$ exhibited an isotopic frequency shift by 3 cm$^{-1}$ upon $^{13}$CO substitution, whereas two bands at 1979 and 1963 cm$^{-1}$ shifted by 45 and 42 cm$^{-1}$ (Fig. 7, a and b), respectively, in agreement with previous studies (27, 28, 49). This indicates that there are two υ(C-O) bands but a single υ(Fe-CO) band. In the presence of the target DNA, on the other hand, two υ(Fe-CO) bands appeared at 486 and 519 cm$^{-1}$, along with two υ(C-O) bands at 1984 and 1960 cm$^{-1}$ (Fig. 7c), although the relative intensity of the small υ(Fe-CO) band at 519 cm$^{-1}$ was somewhat variable. The peak positions of the porphyrin υ$_s$ band (351 cm$^{-1}$), the propionate bending band (379 cm$^{-1}$), and the vinyl bending band (417 cm$^{-1}$) were unchanged by addition of the target DNA.

The RR spectra were also measured with excitation at 428.7 nm. Spectra g and h in Fig. 7 show the 428.7-nm excited RR spectra of $^{12}$CO-CooA and $^{13}$CO-CooA-DNA, respectively. Only one strong υ(Fe-CO) band and one strong υ(C-O) band were found: υ(Fe-CO) = 487 cm$^{-1}$ and υ(C-O) = 1979 cm$^{-1}$ for CO-CooA; and υ(Fe-CO) = 486 cm$^{-1}$ and υ(C-O) = 1984 cm$^{-1}$ for CO-CooA-DNA. These findings show that the minor υ(Fe-CO) and υ(C-O) bands could be detected only upon excitation at shorter wavelengths. Therefore, although the protein structure appeared to be inhomogeneous, the predominant species are the molecules with υ(Fe-CO) = 487 (±0.4) cm$^{-1}$ and υ(C-O) = 1979 cm$^{-1}$ in the absence of the target DNA and υ(Fe-CO) = 486 (±0.5) cm$^{-1}$ and υ(C-O) = 1984 cm$^{-1}$ in the presence of the target DNA. Note that a frequency shift for υ(Fe-CO) or υ(C-O) was not observed for CO-CooA in the presence of DNA with a random sequence (data not shown).

The backbonding correlation between υ(Fe-CO) and υ(C-O) is a good probe for the electrostatic field around the bound CO in the heme pocket (50–53). Upon binding of the target DNA, υ(Fe-CO) was downshifted by 1 cm$^{-1}$ and υ(C-O) was upshifted by 5 cm$^{-1}$. On the other hand, the donor strength of the trans ligand did not change, as described above. These results indicate that the electrostatic field near the bound CO is more negative in CO-CooA-DNA.

Furthermore, we found a remarkable difference between CO-CooA and CO-CooA-DNA in the CW laser power required to observe the CO photoproduction (weaker for CO-CooA). Fig. 8 shows the porphyrin υ$_s$ band of CO-CooA (a) and CO-CooA-DNA (b) at six different laser powers and two excitation wavelengths (406.7 and 428.7 nm). The υ$_s$ band of CooA can be well resolved into contributions from the CO-bound state (1371 cm$^{-1}$), Pro-2-bound state (1359 cm$^{-1}$), and the unligated five-coordinate state (1356 cm$^{-1}$) (54). In the absence of the target DNA, the population of the CO-photodissociated, Pro-2-bound state increased with increasing laser power above sub-mW levels (Fig. 8a). Although geminate recombination of the photodissociated CO from
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CO-CooA occurs rapidly with a very high quantum yield (47, 48), Pro-2 can compete for the rest of the photogenerated five-coordinate heme with bimolecular rebinding of CO. Once Pro-2 binds to the heme, very little of it dissociates because Pro-2 is not photodissociable by CW lasers, as confirmed by the shape of the νa band for ferrous CooA irradiated with strong CW light (28). This probably explains why the Pro-2-bound fraction accumulated. In the presence of the target DNA, a laser power of 5 mW was not sufficient to observe the CO-photoproduction (Fig. 8b). This was found at both excitation wavelengths. Notably, the absorption coefficient of CO-CooA-DNA was slightly lower at 406.7 nm but slightly higher at 428.7 nm than that of CO-CooA (Fig. 5, inset). Previously, we reported that there is no change in the quantum yield of CO photodissociation upon binding of the target DNA (49). Therefore, necessity of higher laser power to observe CO photodissociation for CO-CooA-DNA than for CO-CooA is likely because of the modulation of the ligand binding kinetics.

DISCUSSION

Conformational Changes Induced by CO Binding—The central issue in understanding the dynamics of CooA is elucidating how the C-helix responds to the CO-sensing signal from the heme. To examine this, we used UVRR spectroscopy because it can provide unique information about protein structures in solution based on the bands from the polypeptide backbone and from aromatic residues like Trp and Tyr (35–37, 55, 56). Trp-110 in the C-helix, the only Trp residue in CooA, is ~10 Å away from the heme iron in the opposite subunit and close to the residues comprising the CO-binding pocket (Ile-113, Leu-116, Gly-117, and Leu-120, Fig. 1). Thus, Trp-110 serves as a good structural probe for the signal transduction between the distal heme pocket and DNA-binding domain through the C-helix.

Based on the distance between the heme and the CO-binding pocket in the ferrous state, Coyle et al. (27) and Yamashita et al. (28) proposed that CO-bound heme slides into the protein interior (Fig. 1b). An alternative possibility is that the binding pocket residues slide toward the solvent. This possibility, however, can be ruled out by our current UVRR findings, which indicate that the region near Trp-110 in the C-helix becomes more deeply buried upon binding of CO. Coyle et al. (27) and Yamashita et al. (28) further expanded their own models to include displacement of the C-helix. The model of Yamashita (28) assumed that CO interacts with Ile-113, Leu-116, Gly-117, and Leu-120 in the same subunit, which requires a more than 90° rotation of each C-helix about its helical axis because Ile-113, Gly-117, and Leu-120 are located close to the heme in the opposite subunit in the ferrous state; however, considering that the side chain of Trp-110 is only slightly deformed, as revealed by the W3 frequency, such dramatic rotations of the C-helices would be unlikely. As proposed earlier in the model of Coyle (27), it is more plausible that CO interacts with Ile-113, Gly-117, and Leu-120 in the opposite subunit and Leu-116 in the same subunit, which can be achieved by modest displacement of the C-helices. Fig. 9 shows the overall motion of the model of Coyle (27). This motion is essentially a rotation of both hemes and both C-helices around the 2-fold symmetry axis of the dimer. The overall rotation in the model of Coyle (27) seems to partially agree with the activation model proposed by Akiyama et al. (19) based on small-angle x-ray scattering data, wherein one domain rotates relative to the other by ~8° around the 2-fold symmetry axis. Our current results support the model of Coyle (27) in which Trp-110 moves to the inside of the protein by C-helix displacement.

The CooA subunit has two Tyr residues in the sensor domain and two Tyr residues in the DNA-binding domain (Fig. 1a). Although the four Tyr residues are distributed over the entire protein (Fig. 1a), we found no substantial change in the environments around them in the UVRR spectra excited at 229 nm. This result was also obtained from the UVRR spectra excited at 244 nm (data not shown). Furthermore, we found no obvious change in the α-helical content in the UVRR spectra excited at 206.5 nm. Therefore, the structure within each domain may already be well organized and may not change. It is more likely that the activation of CooA is regulated by alteration of the relative positions between domains and/or between subunits because of C-helix displacement, which may be followed by a motion of the flexible hinge region.

A proximal pathway for the intramolecular signal transduction was also proposed in a previous study (27). Heme sliding involves a simultaneous movement of the proximal His-77, and it breaks a hydrogen bond between His-77 and Asn-42 (Fig. 9). This hydrogen-bond cleavage was proposed to trigger some conformational change in the sensor domain associated with the activation mechanism; however, our UVRR spectra detected no significant change in the environments of the two Tyr residues in the sensor domain. Therefore, our results did not confirm the proximal pathway in the present study.

Conformational Changes Induced by Binding of the Target DNA—Because the biological function of CooA is to bind the specific DNA sequence and recruit RNA polymerase through protein-protein interactions, structural characterization of the CooA-DNA complex is indispensable for fully understanding the activation mechanism of CooA. The present application of UVRR spectroscopy enabled, for the first time, observation of the protein conformational change accompanying formation of the CooA-DNA complex. We found that the indole ring of Trp-110 in the C-helix becomes more deeply buried with a slight change in its side chain orientation upon binding of the target DNA, but it maintains a hydrogen bond at the indole nitrogen. This movement of Trp-110 bears close resemblance to that induced by CO binding. In addition, the visible RR spectra allowed characterization of the structure in the vicinity of the heme. The time-resolved measurements showed that CO-geminate recombination is more efficient in the DNA-bound state, suggesting that the CO binding pocket is narrower. Furthermore, the ν(Fe-CO) and ν(C-O) frequencies revealed that the electrostatic field near the bound CO becomes more negative.

The CO-binding pocket consists of hydrophobic residues, but two charged residues, Asp-72 and Arg-118, are located in the region surrounding the hydrophobic pocket (Fig. 9). These two residues are hydrogen-bonded to each other in the ferrous state. According to the UVRR spectra, binding of the target DNA is predicted to induce a further displacement of the C-helix in the same direction as that induced by CO binding. This displacement of the C-helix would cause Arg-118
to move away from the surrounding of the hydrophobic pocket, and as a result it produces a less positive electrostatic field around the bound CO. This idea agrees with the results from visible RR spectroscopy. Therefore, we speculate that the activation dynamics of CooA is accomplished by the further displacement of the C-helix because of binding of the target DNA to CooA.

Based on observations of the CO photoproduc produced by the CW laser, the further C-helix displacement is expected to modulate the ligand binding kinetics after CO photodissociation (summarized in Fig. 10). In the presence of the target DNA, the photogenerated Pro-2-bound state was not observed under strong CW irradiation (Fig. 8). Although the CO-geminate recombination ($k_{\text{CO, geminate}}$) becomes more efficient as revealed in Fig. 6, its change seems to be insufficient to generate a significant difference as seen in the Raman spectra in Fig. 8. Bimolecular rebinding of CO to CooA ($k_{\text{CO}}$) is slightly slowed by binding of the target DNA (49). Therefore, it is possible that the affinity of Pro-2 is considerably lower in the DNA-bound state than in the DNA-free state. Because Pro-2 acts as an inhibitor of CO binding, a lower affinity of Pro-2 is an advantage for the physiological function of CooA, which must maintain the CO-bound conformation while the protein activates gene transcription.

In the heme proximal side, on the other hand, the frequency of (Fe-His) did not change upon binding of the target DNA. The possibility of further heme sliding is therefore excluded. The structure in the proximal side of heme would be little influenced by the further displacement of the C-helix occurring in the distal side.

In summary, the structural information obtained from the UV and visible RR spectra demonstrates that the C-helix is displaced in such a way that its N-terminal region around Trp-110 is more deeply buried in response to CO binding. The C-helix is further displaced presumably in the same direction in response to specific DNA binding for complete protein activation. In the complete active conformation, movement of CO is restricted strongly within a narrower space of its binding pocket and may be less affected by the endogenous inhibitor Pro-2.

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