The C-helix in CooA Rolls upon CO Binding to Ferrous Heme*

Received for publication, July 9, 2004, and in revised form, August 3, 2004
Published, JBC Papers in Press, August 23, 2004, DOI 10.1074/jbc.M407766200

Taku Yamashita†, Yohei Hoashi‡, Yoshikazu Tomisugi, Yoshinobu Ishikawa, and Tadayuki Uno§
From the Graduate School of Pharmaceutical Sciences, Kumamoto University, Oehonmachi, Kumamoto 862-0973, Japan

CooA is a homodimeric transcriptional activator from *Rhodospirillum rubrum* containing one heme in each subunit. CO binding to the heme in its sensor domain activates CooA, facilitating the binding to DNA by its DNA-binding domain. The C-helix links the two domains and shapes an interface between the subunits. To probe the nature of CO activation, residues at positions 112–121 on the C-helix were replaced by Asn or Gln and their effects were evaluated by resonance Raman spectroscopy and by the measurements of CO binding affinity. The \( \nu(Fe-CO) \) stretching Raman line in CO-bound wild-type CooA was up-shifted by 6 cm\(^{-1} \) in the L116Q, G117N, and L120Q mutants, indicating unequivocally that these residues are close to the bound CO. Residues Leu\(^{116} \) and Leu\(^{120} \) from each subunit form contacts with the corresponding residues in the opposite subunit, enabling hydrophobic interactions in the inactive ferrous form. Thus, in the CO-bound activated form, both C-helices appear to roll to direct these residues toward the heme, forming a hydrophobic pocket for the bound CO. The CO affinity is approximately one order of magnitude higher in the L112Q, I115Q, L116Q, G117N, L120Q, and T121N mutants but reduced in A114N mutant. The variation indicates that these residues are close to the heme in the ferrous and/or CO-bound forms and are responsible for CooA activation. A roll-and-slide mechanism is proposed for CO activation of CooA.

CooA from the photosynthetic bacterium *Rhodospirillum rubrum* is a heme-based transcription factor that is activated upon binding CO (1, 2). CooA regulates the expression of genes whose products are associated with CO metabolism (3). CooA is a homodimer (221 amino acids/monomer) containing a DNA-binding and a sensor domain analogous to those found in the cAMP receptor protein (CRP)\(^{4, 5} \). The crystal structure of ferrous CooA revealed (4) that a \( b \)-type heme is contained within the sensor domain where it is ligated to the His\(^{77} \) side chain (Fig. 1). This ligand is replaced by the Cys\(^{75} \) side chain in the ferric state (6, 7). The ferrous heme ligand *trans* to His\(^{77} \) is the N-terminal proline residue (Pro\(^{2} \)) from the partner subunit in the dimer (4). We have recently established (8) that Pro\(^{2} \) is the ligand that is displaced by the incoming CO as had been proposed previously (9, 10) and that Pro\(^{2} \) coordination fine-tunes the sensing of CO in the media. Because CooA is the first example of a CO sensor protein (1, 2), its CO sensing and activation mechanisms have been attracting the attention of many researchers (11–14).

The signal of CO binding to the heme is transmitted to the DNA-binding domain, directing the protein to its target DNA sequence, which is palindromic (15–17). Because the two domains are linked by a long \( \alpha \)-helix (C-helix), the residues on the C-helix must play a key role in signal transmission from the heme-containing domain to the DNA-binding domain and hence CO activation of CooA. The C-helices form a coiled-coil structure, which shapes the dimer interface in the inactive ferrous form (Fig. 1). As summarized in Table I, the Ca atoms of Ile\(^{113} \), Leu\(^{116} \), Leu\(^{120} \), Cys\(^{123} \), and Ile\(^{127} \) on the respective subunits are in close proximity to each other (<7.0 Å) in CooA as in the optimal leucine zipper alignment. To reveal the activation mechanism, it is potentially informative to inspect the structural difference between CooA and CRP, which is homologous to CooA and whose cAMP-bound activated structure is available (18). The Ca atoms in the C-helix of CRP (residues at position 112–136) are organized in a similar way to the corresponding region of CooA (Table I), and the difference in the distances between the inactive ferrous CooA and active cAMP-bound CRP is not apparent. Thus, it is unclear what kind of structure is required for the activation of these transcription factors.

A previous study (19) revealed that the side chains of Ile\(^{113} \) and Leu\(^{116} \) are close to the CO in the ferrous CO-bound CooA. When Leu\(^{116} \) is replaced by the heme-coordinating lysine residue, CooA is active both in the presence and absence of CO (20). The importance of the nearby Gly\(^{117} \) to CooA activation has also been stressed (21). Thus, it is believed that residues in this region are crucial for CooA activation. Although these pieces of evidence are informative, the mutation studies have not been systematic so far and the role of the C-helix in CO activation remains to be fully elucidated.

In our strategy, we at first inspected the distances between the heme iron and each Ca atom on the C-helix with the atomic coordinate set of the inactive ferrous form (4) (Table I). Because CooA is homodimeric, the two Co-Fe distances within the respective subunits (d(Co-Fe)) are averaged and the deviation is given. Similarly, two distances from iron to Ca atoms on the opposite subunit (d(Co-Fe\(_{\text{op}}\))) are evaluated. Although the deviations in the C-terminal half of the C-helix are relatively large, the distances in the N-terminal half are well conserved between the subunits (the deviations are <0.1 Å). The Ca atoms of Leu\(^{112} \) and Leu\(^{116} \) are relatively close to the heme iron within the same subunit (<10 Å), whereas those of Ile\(^{113} \), Ala\(^{114} \), Gly\(^{117} \), Arg\(^{118} \), and Thr\(^{121} \) are close to the iron in the opposite subunit. Thus, in this study, 10 residues at positions from 112 to 121 have been systematically mutated and the mutated proteins have been examined by resonance Raman spectroscopy and by the measurement of CO binding affinity. We conclude that the C-helix rolls to direct residues Leu\(^{116} \), Gly\(^{117} \), and Leu\(^{120} \) toward the heme and that there is a concomitant sliding of the heme with respect to the C-helix. There-
fore, we propose a roll-and-slide model for CO activation of CooA.

MATERIALS AND METHODS

Preparation of CooA Mutants—We have reported a high expression system for CooA, which employs a synthetic gene optimized for expression in Escherichia coli (8). The QuickChange system (Stratagene) was used to introduce mutations into the CooA coding sequence, and the DNA sequence of the plasmid products were confirmed with a Li-Cor Model 4200S2 DNA sequencer. Cell growth and protein purification were performed as described previously (6) with slight modifications. Soluble fractions of the cell extract were applied to a Q-Sepharose FF column (Amersham Biosciences), and the adsorbed proteins were eluted with linear gradient of NaCl in 50 mM Tris-HCl (pH 8.0). The CooA fractions were subsequently purified by Sephacryl S-100 column chromatography (Amersham Biosciences) and concentrated (Amicon Ultra-15, Millipore). Proteins were purified to homogeneity as judged by SDS-PAGE criteria. CooA concentrations were evaluated from the heme content, which was measured by a pyridine hemochrom assay, and hence were based on a monomer unit.

Resonance Raman Spectroscopy—The spectra were recorded using a double monochromator (Jasco R-800) with a slit width of 6 cm⁻¹ following excitation by a krypton ion laser (406.7-nm line, Coherent I-302). A photomultiplier detector was used (Hamamatsu Photonics, R556), and the frequencies were calibrated with indene. The frequencies reported are accurate within 1 cm⁻¹ for sharp and discrete Raman lines. A spinning Raman cell was used throughout the measurements to minimize local heating and sample damage. The samples contained 100 μM protein in 0.1 M Tris-HCl (pH 8.0). Ferrous proteins were prepared by the addition of sodium dithionite after purging extensively with nitrogen gas. The carbonmonoxy forms were prepared by the addition of sodium dithionite under 1 atm CO. The spectra of the CO forms were obtained with a defocused laser beam.

CO Titration—CO titration of ferrous CooA was performed in 0.1 M Tris-HCl (pH 8.0) at 25 °C as described previously (8). A cuvette was filled with a CO solution, which was purged extensively with nitrogen gas stopped with a rubber septum, and the protein was reduced by an excess of sodium dithionite solution, which was injected by a syringe. Full reduction of CooA was confirmed by absorption spectroscopy (Beckman DU640). Aliquots of CO saturated in 0.1 M Tris-HCl (pH 8.0) and dithionite were added to solutions containing ~2 μM protein, and the absorption spectra were recorded. The CO concentrations were calibrated using pig myoglobin. The absorbance changes at the Q-band maxima were traced and analyzed after normalization.

RESULTS

Ferric and Ferrous CooA—In our mutation strategy, those residues having long and hydrophobic side chains (Leu and Ile) were replaced by Gln, which has long but polar side chain. On the other hand, small residues (Ala, Gly, and Thr) were replaced by the short and polar Asn, which is analogous to Gln. Arg118, the sole charged residue in our target set, was replaced by the short and polar Asn, which is analogous to Gln. The Pro2-His77 axial ligands as well as the side chains of Ala114 (magenta), Leu116 (cyan), and Leu120 (green) are shown. The Pro2-His77 axial ligands are replaced (8). Thus, some of the C-helix residues affect the axial coordination of the ferric and ferrous hemes in CooA but the effects are slight.

CO Probe—In Fig. 4, resonance Raman spectra of ferrous CO-bound CooA are shown. The ν(Fe-CO) stretching frequency is sensitive to the polarity of the residues surrounding the bound CO, and it therefore represents an excellent probe of the distal environment (25–29). The ν(Fe-CO) stretching line is observed at 490 cm⁻¹ in WT CooA as reported previously (8, 19, 30), indicating that the bound CO is in a hydrophobic distal environment. This line is clearly affected by mutations of some of the C-helix residues, and especially noted are the large up-shifts of 6 cm⁻¹ in the L116Q, G117N, and L120Q mutants. A small but measurable up-shift of 3 cm⁻¹ in the L112Q and A119N mutants is also noted. In these mutants, hydrophobic residues are replaced by polar Gln or Asn residues. The effect of hydrophobic to polar substitutions on the ν(Fe-CO) stretching frequency has been reported in myoglobin (31). In ferrous CO-bound myoglobin, the bound CO is close to the distal His64 (32, 33). The line is observed at 490 cm⁻¹ in H64Q (31). Thus, it is clear that Leu112 and Ala119 may also locate close to the CO but in a rather remote fashion. The frequency of the ν(Fe-CO) stretch and the shift values relative to that in WT protein are summarized in Table II.

Along with the frequency shift, the band shape of the ν(Fe-CO) stretch is slightly affected by the mutations of some of the C-helix residues (Fig. 4). The ν(Fe-CO) lines are relatively broad in the I113Q, A114N, L116Q, and L120Q mutants, suggesting the fluctuation in the Fe-CO geometry. A minor band seems to exist at ~520 cm⁻¹ in the I115Q mutant, and the presence of multiple Fe-CO conformers is suggested. Similar
The effect of these residues on the CO binding affinity was to be remote from the heme in the CO-bound state. It should be noted here that Arg118 are remote from the heme. Ala114 is suggested to play a key role in CO activation of CooA. Because of this low affinity, resonance Raman measurement of CO-bound A114N was very difficult (Fig. 4) and the laser power may partly be affected in the I115Q mutant.

The distances between specific atoms of residues in the C-helix region

| Residue | CooA | CRP | CRP-CooA |
|---------|------|-----|----------|
|         | d(CooA-CooA) Å | d(CooA-CooA) Å | d(CooA-CooA) Å |
|         | d(CooA-CooA) Å | d(CooA-CooA) Å | d(CooA-CooA) Å |
| Met125  | 13.9 | 15.7 ± 0.1 | 17.0 ± 0.1 |
| Ala129  | 7.9  | 12.9 ± 0.0 | 14.6 ± 0.1 |
| Thr121  | 10.1 | 14.5 ± 0.0 | 11.7 ± 0.1 |
| Gly121  | 14.8 | 13.4 ± 0.1 | 13.2 ± 0.1 |
| Leu122  | 10.3 | 9.7 ± 0.1  | 13.0 ± 0.0 |
| Ile113  | 5.6  | 11.2 ± 0.0 | 9.4 ± 0.0  |
| Ala119  | 12.5 | 13.5 ± 0.0 | 8.8 ± 0.0  |
| Leu116  | 13.3 | 11.4 ± 0.0 | 11.4 ± 0.1 |
| Gly117  | 6.2  | 9.4 ± 0.0  | 10.3 ± 0.0 |
| Arg118  | 13.9 | 14.4 ± 0.1 | 9.7 ± 0.0  |
| Ala119  | 10.0 | 12.1 ± 0.0 | 12.1 ± 0.0 |
| Leu116  | 12.0 | 16.6 ± 0.1 | 9.7 ± 0.1  |
| Ser122  | 13.0 | 16.9 ± 0.1 | 13.2 ± 0.1 |
| Cys123  | 6.2  | 16.0 ± 0.1 | 14.6 ± 0.1 |
| Met124  | 9.1  | 18.5 ± 0.0 | 13.3 ± 0.2 |
| Arg125  | 14.2 | 21.0 ± 0.0 | 15.0 ± 0.2 |
| Thr126  | 10.2 | 20.5 ± 0.2 | 18.0 ± 0.1 |
| Ile127  | 6.1  | 21.1 ± 0.0 | 18.4 ± 0.3 |
| Gly128  | 13.5 | 24.1 ± 0.1 | 18.3 ± 0.5 |
| Asp129  | 14.1 | 25.4 ± 0.1 | 21.0 ± 0.4 |
| Leu130  | 7.6  | 25.5 ± 0.4 | 23.1 ± 0.4 |
| Met131  | 10.9 | 27.2 ± 0.0 | 23.2 ± 0.7 |
| Phe132  | 16.6 | 28.7 ± 0.1 | 24.1 ± 0.6 |

* Distances between α-carbons on the opposite subunit in CooA. The distances of <7.0 Å are shown in boldface.
* Distances between α-carbons and heme iron within the same subunit in CooA. The distances of <10.0 Å are shown in boldface.
* Distances between α-carbons and heme iron within the opposite subunit in CRP. The distances of <7.0 Å are shown in boldface.
* Differences in the distances between α-carbons in CooA and CRP.

**multiple α(Fe-CO) stretching modes were detected in some of the His117 axial ligand mutants of CooA (8), and hence, the Fe-His bond may partly be affected in the I115Q mutant. The main α(Fe-CO) stretching frequency at 489 cm⁻¹, however, is almost the same as that in WT CooA; hence, Ile115 is suggested to be remote from the heme in the CO-bound state.

Because some of the C-helix residues affect the bound CO, the effect of these residues on the CO binding affinity was evaluated. The CO dissociation constants (Kd) for the ferrous-CO bound CooA mutants are given in Table II. The CO affinity of WT CooA (Kd = 11 μM) is similar to that for FixL (9.0 μM) (34) and Dos (10 μM) (35), which are heme-based gas sensor proteins. Although the CO binding kinetics is reported to be multi-step (36, 37), the equilibrium CO binding is a one-step process (8). Thus, binding was analyzed in terms of a simple equilibrium between the ferrous and CO-bound forms of the protein. The Kd values generally decreased in the C-helix mutants, indicating that polar side chains in the mutants facilitate CO binding to the ferrous heme. The logarithm of the ratio of the Kd values for the mutant and WT CooA (i.e. Δlog Kd) is calculated and also summarized in Table II. The CO affinity is less affected in I115Q and R118L, indicating that Ile113 and Arg118 are remote from the heme. It should be noted here that the Kd value greatly increased in the A114N mutant. Thus, Ala114 is suggested to play a key role in CO activation of CooA. Because of this low affinity, resonance Raman measurement of CO-bound A114N was very difficult (Fig. 4) and the laser power had to be reduced to 0.3 milliwatts. Even under this condition, the ferrous heme is not fully coordinating CO because saturated CO concentration is 1 mM. This is the main reason for the relatively weak α(Fe-CO) stretching line in this mutant.

**Table I** Distances between specific atoms of residues in the C-helix region

| Residue | Distance |
|---------|----------|
| Met125  | 13.9 Å    |
| Ala129  | 7.9 Å     |
| Thr121  | 10.1 Å    |
| Gly121  | 14.8 Å    |
| Leu122  | 10.3 Å    |
| Ile113  | 5.6 Å     |
| Ala119  | 12.5 Å    |
| Leu116  | 13.3 Å    |
| Gly117  | 6.2 Å     |
| Arg118  | 13.9 Å    |
| Ala119  | 10.0 Å    |
| Leu116  | 12.0 Å    |
| Thr121  | 13.0 Å    |
| Ser122  | 13.0 Å    |
| Cys123  | 6.2 Å     |
| Met124  | 9.1 Å     |
| Arg125  | 14.2 Å    |
| Thr126  | 10.2 Å    |
| Ile127  | 6.1 Å     |
| Gly128  | 13.5 Å    |
| Asp129  | 14.1 Å    |
| Leu130  | 7.6 Å     |
| Met131  | 10.9 Å    |
| Phe132  | 16.6 Å    |

**Ile112**

**Leu113**

**Met114**

**Arg115**

**Leu116**

**Ser117**

**Arg118**

**Arg121**

**Arg122**

**Arg123**

**Arg124**

**Glu125**

**Gly126**

**Val126**

**Thr127**

**Ser128**

**Glu129**

**Lys130**

**Val131**

**Gly132**

**Asn131**

**Leu134**

**Ala135**

**Ala136**

**Phs136**

**Discussion**

**Inactive Ferrous Form**—In Fig. 5, the positions of C-helix residues are schematically drawn in rectangle and in helical wheel projection models. Because the C-helix shapes a coiled-coil structure in the inactive ferrous form (4), a 3.5 residue/turn scheme was adopted. As summarized in Table I, the Co atoms of Ile113, Leu116, Leu120, Cys123, and Ile127 residues on the respective subunits are located close to each other (<7.0 Å) and these residues are marked with small pink circles (Fig. 5, top). It is clear that these residues align on one face of the C-helix and shape a dimer interface. The Co atoms of Leu116 and Leu116 (marked with cyan circles) are close to the ferrous heme within the same subunit, whereas those of Ile113, Ala114, Gly117, Arg118, and Thr121 (marked with green circles) are close to the heme within the opposite subunit. Pro6, one of the heme axial ligands, is provided by the opposite subunit and is shown in green. In the ferric state, the A114N, I115Q, and A119N mutants affected the relative intensity of the v(C≡O) and δ(C≡O=C) bending modes (Fig. 2) and, hence, these residues are probably close to the heme periphery in the WT CooA. In the crystal structure of ferrous CooA (4), the side chain of Ile115 is close to the heme 1-methyl and 2-vinyl groups and Ala119 is close to the 1-methyl group, although Ala114 is remote from the heme periphery (Fig. 1). The proximal ligand in the ferric state is the side chain of Cys75, which is replaced by that of His77 in the ferrous state (6, 7). The redox-linked ligand exchange in CooA may reposition the heme, providing a close contact of heme periphery with Ala114 side chain.

In the ferric state, the mutation of residues Leu116, Gly117, and Leu120 slightly affected the axial coordination of the heme (Fig. 3). Because His77 ligand is opposite to the heme plane when viewed from the C-helix, relatively weak coordination by
observed in our G117N mutant. In these Leu116, Gly117, and Leu120 mutants, the \( K_d \) values for CO dissociation increased by \( \sim 10 \)-fold (Table II). Although many factors may affect CO affinity, this increase may partly be attributed to the weaker Pro\(^2\) coordination in these mutants. Pro\(^2\) coordination greatly reduces the CO affinity, and this coordination is the way in which CooA regulates its CO-sensing level (8). Leu116 and Leu120 locate in the dimer interface in the inactive ferrous state, and the side chain of Leu120 is too far from the Pro2 ligand for direct interaction (Fig. 5, top). Thus, the C-helices must roll relative to each other at least in the L120Q mutant to weaken the Fe-Pro2 bond in the ferrous state. The replacement of these aliphatic residues by the polar Glu should weaken the hydrophobic interaction between the helices. This may promote the rolling of the helices, mimicking the active CO-bound form as described below and facilitating CO binding with high affinity.

**Active Ferrous-CO Form—** As we revealed previously (8), the Pro\(^2\) ligand is replaced by the incoming CO molecule and this replacement triggers CooA activation (11–14). The residues affecting the \( \nu(\text{Fe-CO}) \) stretch (Fig. 4) by \( >3 \text{ cm}^{-1} \) are marked with red circles (Fig. 5). Among these residues, the effects of Leu116, Gly117, and Leu120 mutation were dominant again; hence, these three residues appear to be located close to the CO molecules. To maximize the interaction between these residues and CO, the hemes must slide along the C-helices. Because Pro\(^2\) is provided from the opposite subunit, CO replacement should heave the Pro\(^2\) anchor in CooA. This should increase the freedom for relative movement of C-helices and hemes. Regardless, the movement of both helices as well as both hemes must be symmetrical because CooA is homodimeric and the recognition sequence of CooA is palindromic (15–17).

The residues that affected the CO affinity more than one order of magnitude are marked by small purple circles (Fig. 5, bottom). The CO affinity increased predominantly in the L112Q, I113Q, L115Q, G117N, L120Q, and T121N mutants. The Leu116, Gly117, and Leu120 side chains are close to one another on one face of the C-helix, thus lining the distal heme pocket and greatly affecting the CO affinity. The remaining Leu112, Ile115, and Thr121 residues are directed outward from the dimer interface in the ferrous form (Fig. 5, top) and may not interact directly with the bound CO. These residues surround the Leu116, Gly117, and Leu120 triad, possibly forming an entrance allowing CO to access the heme.

Among the CooA mutants examined, A114N is the sole mu-

---

**TABLE II**

| Residue      | \( \nu(\text{Fe-CO}) \text{cm}^{-1} \) | \( \Delta \nu(\text{Fe-CO}) \text{cm}^{-1} \) | \( K_d \mu M \) | \( \Delta \log K_d \) |
|--------------|-----------------|-----------------|----------------|-----------------|
| WT           | 490             | 11\(^b\)        |                 |                 |
| L112Q        | 493             | +3.5            | 0.51           | -1.3            |
| I113Q        | 492             | +1.9            | 5.7            | -0.3            |
| A114N        | 491             | +0.8            | 200            | +1.2            |
| L115Q        | 489             | -1.0            | 1.2            | -1.0            |
| L116Q        | 495             | +5.6            | 1.3            | -1.0            |
| G117N        | 496             | +5.7            | 1.1            | -1.0            |
| R118L        | 492             | +2.1            | 5.4            | -0.3            |
| A119N        | 493             | +3.0            | 3.5            | -0.5            |
| L120Q        | 496             | +6.4            | 0.76           | -1.2            |
| T121N        | 489             | -1.0            | 0.95           | -1.1            |

\(^a\) Frequency shift relative to WT CooA. The values greater than 3 \( \text{cm}^{-1} \) are shown in boldface.

\(^b\) From Ref. 8.

\(^c\) The logarithm of the ratio of the \( K_d \) values for the mutant and WT CooA. The values greater than +1 or smaller than –1 are shown in boldface.
tant, which greatly reduced the CO affinity, although Gly\textsubscript{117} and Thr\textsubscript{121}, which lie on the same C-helix face, enhanced the CO affinity when replaced by Asn. It is interesting to note that Ala\textsubscript{114} is sandwiched by Gly\textsubscript{111} and Gly\textsubscript{117}, which have small side chains. Such small residues may facilitate the helical roll in the CO activation. When the small Ala\textsubscript{114} is substituted by the polar and bulkier Asn, it may inhibit C-helix rolling. This inhibition may stabilize the ferrous inactive form so that CO affinity is reduced.

Roll-and-Slide Model—As discussed above, the C-helices must roll, and here, we propose a roll-and-slide model for the CO activation of CooA (Fig. 5, bottom). In this section, we validate the model based on the experimental evidence. Since A114N showed low CO affinity, we propose that Ala\textsubscript{114} could be the fulcrum of the helix rolling to shape the CO-bound form. This means that the heme must slide to the C-terminal side on the helix, and this view is consistent with the close proximity of the Leu\textsubscript{116}, Gly\textsubscript{117}, and Leu\textsubscript{120} triad to the CO-bound heme. Leu\textsubscript{116} is reported to be crucial for CooA activity, and the L116K mutant is active in both ferrous and CO-bound states (20). Leu\textsubscript{116} is close to the heme in our model (Fig. 5, bottom), enabling the coordination of Lys side chain in L116K.

It is proposed (19) that the C-helix relatively moves upon the CO binding and that the heme also moves to the N-terminal side. In our systematic mutation study, however, we favor the movement of the heme toward the C-terminal portion of the C-helices for the reasons outlined below. In the I113Q mutant, the $K_d$ value and $\delta$(Fe-CO) stretching frequency are less affected than in the other mutants examined (Table II), although Ile\textsubscript{113} is one helical turn up from Leu\textsubscript{116} and Gly\textsubscript{117} and is close to the heme (marked with green circle in Fig. 5, top). In contrast, Met\textsubscript{124} is reported to affect iron coordination in the activated form (38). This residue is one helical turn down from Leu\textsubscript{120}, supporting the notion of translation of the heme toward the C terminus of the helix.

Three major factors contribute to cofactor retention in heme proteins (39): (i) covalent bonding between the heme iron and axial ligand residues; (ii) apolar interactions between the heme and surrounding hydrophobic residues; and (iii) polar interac-
tions between the heme propionates and surrounding hydrophilic residues (40). In ferrous CooA, the bonds to the Pro2-His77 ligands may be enough to anchor the heme, although the heme propionate is not surrounded by amino acid side chains. However, in the CO-bound state, Pro2 is replaced by CO, requiring a compensating mechanism for the heme retention. In our model, the helical roll may allow the positively charged Arg118 to interact favorably with the negatively charged heme propionate in the opposite subunit. Although Arg118 interacts with Asp72 in the ferrous form (4), this residue may partly contribute electrostatically to the stabilization of the slipped heme.

In summary, the C-helix in CooA rolls upon CO binding to the heme as revealed by resonance Raman spectra and CO dissociation constants. We propose a roll-and-slide model for the CO activation mechanism of CooA. This model satisfactorily accounts for our observations as well as those reported earlier. Although further studies on the interactions between the C-helices and the DNA-binding domains are required, our roll-and-slide model may be a good starting point for fully understanding the CO activation mechanism.

Acknowledgment—We thank Dr. Anthony J. Wilkinson (University of York) for valuable discussion.

REFERENCES

1. Aono, S., Nakajima, H., Saito, K., and Okada, M. (1996) Biochem. Biophys. Res. Commun. 226, 752–756
2. Shelver, D., Kerby, R. L., He, Y., and Roberts, G. P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11216–11220
3. Kerby, R. L., Hong, S. S., Ensign, S. A., Coppol, L. J., Ludden, P. W., and Roberts, G. P. (1992) J. Bacteriol. 74, 5284–5294
4. Lanzilotta, W. N., Schuller, D. J., Thorsteinsson, M. V., Kerby, R. L., Roberts, G. P., and Poulos, T. L. (2000) Nat. Struct. Biol. 7, 876–880
5. Chan, M. R. (2000) Nat. Struct. Biol. 7, 822–824
6. Shelver, D., Thorsteinsson, M. V., Kerby, R. L., Chung, S. Y., Roberts, G. P., Reynolds, M. F., Parks, R. B., and Burstyn, J. N. (1999) Biochemistry 38, 2669–2678
7. Aono, S., Ohkubo, K., Matsuo, T., and Nakajima, H. (1998) J. Biol. Chem. 273, 25757–25764
8. Yamashita, T., Hoashi, Y., Watanabe, K., Tomisugi, Y., Ishikawa, Y., and Uno, T. (2004) J. Biol. Chem. 279, 21394–21400
9. Yamamoto, K., Ishikawa, H., Takahashi, S., Ishimori, K., Morishima, I., Nakajima, H., and Aono, S. (2001) J. Biol. Chem. 276, 11473–11476
10. Uchida, T., Ishikawa, H., Ishimori, K., Morishima, I., Nakajima, H., Aono, S., Mizutani, Y., and Kitagawa, T. (2000) Biochemistry 39, 12747–12752
11. Rodgers, K. R. (1999) Curr. Opin. Chem. Biol. 3, 158–167
12. Chen, M. K. (2001) Curr. Opin. Chem. Biol. 5, 216–222
13. Aono, S. (2003) Acc. Chem. Res. 36, 825–831
14. Aono, S., and Nakajima, H. (1999) Coord. Chem. Rev. 190–192, 267–282
15. He, Y., Shelver, D., Kerby, R. L., and Roberts, G. P. (1996) J. Biol. Chem. 271, 120–123
16. Fox, J. D., He, Y., Shelver, D., Roberts, G. P., and Ludden, P. W. (1996) J. Bacteriol. 178, 6290–6298
17. Aono, S., Takasaki, H., Unno, H., Kamiya, T., and Nakajima, H. (1999) Biochem. Biophys. Res. Commun. 261, 270–275
18. Passner, J. M., Schultz, S. C., and Steitz, T. A. (2000) J. Mol. Biol. 304, 847–859
19. Cole, C. M., Puranik, M., Yoon, H., Nielsen, S. B., Williams, R. D., Kerby, R. L., Roberts, G. P., and Spiro, T. G. (2003) J. Biol. Chem. 278, 35384–35393
20. Youn, H., Kerby, R. L., Thorsteinsson, M. V., Clark, R. W., Burysten, J. N., and Roberts, G. P. (2002) J. Biol. Chem. 277, 33616–33625
21. Youn, H., Kerby, R. L., Thorsteinsson, M. V., Conrad, M., Staples, C. R., Serate, J., Beack, J., and Roberts, G. P. (2001) J. Biol. Chem. 276, 41603–41610
22. Spiro, T. G., and Li, X.-Y. (1988) in Biological Applications of Raman Spectroscopy (Spiro, T. G., ed) Vol. 3, pp 1–38, John Wiley & Sons, Inc., New York
23. Kneizd, J. R. (2000) in The Purpury Handbook (Kadish, K. M., Smith, K. M., and Guillard, R., eds) Vol. 7, pp 227–291, Academic Press, San Diego
24. Hu, S., Smith, K. M., and Spiro, T. G. (1996) J. Am. Chem. Soc. 118, 12658–12666
25. Uno, T., Nishimura, Y., Tsuobi, M., Makino, R., Iizuka, T., and Ishimura, Y. (1987) J. Biol. Chem. 262, 4549–4556
26. Li, T., Quillin, M. L., Phillips, G. N., Jr., and Olson, J. S. (1994) Biochemistry 33, 1433–1446
27. Ray, G. B., Li, X.-Y., Ibers, J. A., Sessler, J. L., and Spiro, T. G. (1994) J. Am. Chem. Soc. 116, 162–167
28. Oldfield, E., Guo, K., Augspurger, J. D., and Dykstra, C. E. (1991) J. Am. Chem. Soc. 113, 7537–7541
29. Yu, N.-T., Kerr, E. A., Ward, B., and Chang, C. K. (1983) Biochemistry 22, 4534–4540
30. Uchida, T., Ishikawa, H., Takahashi, S., Ishimori, K., Morishima, I., Ohkubo, K., Nakajima, H., and Aono, S. (1998) J. Biol. Chem. 273, 19888–19992
31. Sakan, Y., Ogura, T., Kitagawa, T., Frauenfelter, F. A., Mattera, R., and Kerby, R. L. (1999) J. Am. Chem. Soc. 121, 12638–12646
32. Tsubaki, M., Srivastava, R. B., and Yu, N.-T. (1982) Biochemistry 21, 1132–1140
33. Uno, T., Nakamoto, R., Tomisugi, Y., Ishikawa, Y., and Wilkinson, A. J. (2003) Biochemistry 42, 10191–10199
34. Gilles-Gonzalez, M. A., Gonzalez, G., Perutz, M. F., Kiger, L., Marden, M. C., and Poyart, C. (1994) Biochemistry 33, 8067–8073
35. Delgado-Nison, V. M., Gonzalez, G., and Gilles-Gonzalez, M. A. (2000) Biochemistry 39, 2685–2691
36. Puranik, M., Nielsen, S. B., Yoon, H., Hvitveed, A. N., Bourassa, L. J., Case, M. A., Teugroth, C., Balakrishnan, G., Thorsteinsson, M. V., Groves, J. T., McLendon, G. L., Roberts, G. P., Olson, J. S., and Spiro, T. G. (2004) J. Biol. Chem. 279, 21096–21108
37. Kumazaki, S., Nakajima, H., Sakaguchi, T., Nakagawa, E., Shinohara, H., Yoshihara, K., and Aono, S. (2000) J. Biol. Chem. 275, 38378–38383
38. Thorsteinsson, M. V., Kerby, R. L., Yoon, H., Conrad, M., Serate, J., Staples, C. R., and Roberts, G. P. (2001) J. Biol. Chem. 276, 26807–26813
39. Hargrove, M. S., Wilkinson, A. J., and Olson, J. S. (1996) Biochemistry 35, 11300–11309
40. Uno, T., Yukimura, A., Moriyama, Y., Ishikawa, Y., Tomisugi, Y., Brannigan, J. A., and Wilkinson, A. J. (2001) J. Am. Chem. Soc. 123, 512–513
41. DeLano, W. L. (2002) The PyMOL Molecular Graphics System, DeLano Scientific, San Carlos, CA
The C-helix in CooA Rolls upon CO Binding to Ferrous Heme
Taku Yamashita, Yohei Hoashi, Yoshikazu Tomisugi, Yoshinobu Ishikawa and Tadayuki Uno

J. Biol. Chem. 2004, 279:47320-47325.
doi: 10.1074/jbc.M407766200 originally published online August 23, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M407766200

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 39 references, 14 of which can be accessed free at
http://www.jbc.org/content/279/45/47320.full.html#ref-list-1