Flavohemoglobin, a Globin with a Peroxidase-like Catalytic Site*

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Biochemical studies of flavohemoglobin (Hmp) from Escherichia coli suggest that instead of aerobic oxygen delivery, a dioxygenase converts NO to NO$_3^-$ and anaerobically, an NO reductase converts NO to N$_2$O. To investigate the structural features underlying the chemical reactivity of Hmp, we have measured the resonance Raman spectra of the ligand-free ferric and ferrous protein and the CO derivatives of the ferrous protein. At neutral pH, the ferric protein has a five-coordinate high-spin heme, similar to peroxidases. In the ferrous protein, a strong iron-histidine stretching mode is present at 244 cm$^{-1}$. This frequency is much higher than that of any other globin discovered to date, although it is comparable to those of peroxidases, suggesting that the proximal histidine has imidazolate character. In the CO derivative, an open and a closed conformation were detected. The distal environment of the closed conformation is very polar, where the heme-bound CO strongly interacts with the B10 Tyr and/or the E7 Gln. These data demonstrate that the active site structure of Hmp is very similar to that of peroxidases and is tailored to perform oxygen chemistry.

Hemoglobin genes have been discovered in organisms from virtually all biological kingdoms. The presence of hemoglobins in unicellular organisms suggests that the gene for hemoglobin is very ancient and that the hemoglobins must have functions other than oxygen transport in view of the fact that O$_2$ delivery in unicellular organisms is a diffusion-controlled process. Based on sequence alignments, two groups of hemoglobins have been identified in several pathogenic and nonpathogenic unicellular organisms (1). The first group consists of small hemoglobins with a novel two-over-two α-helical sandwich motif. These have been termed truncated hemoglobins and are characterized by the absence of the A-helix and the presence of an extended loop substituting for most of the F-helix (1). The second group consists of flavohemoglobins from bacteria and fungi. These consist of a hemoglobin domain with a classical three-over-three α-helical sandwich motif and a flavin-containing reductase domain that is either covalently or noncovalently associated with the hemoglobin domain (2–4). Despite the large differences in the tertiary structures, all the hemoglobins from unicellular organisms discovered to date contain a histidine at the F8 position that coordinates to the heme iron, and a tyrosine at the B10 position in the distal pocket. The distal E7 position, which is occupied by a histidine in mammalian Hbs, is occupied by a variety of different polar or nonpolar residues.

Single-chain flavohemoglobins are found in a variety of prokaryotic and eukaryotic microorganisms (2, 5–9). The C-terminal domain of flavohemoglobins, which resembles ferredoxin reductase, binds NAD(P)H and transfers electrons to the heme in the globin domain via FAD (10, 11). The N-terminal domain, which exhibits high homology to animal and plant hemoglobins (2), contains a single heme b. Biochemical studies demonstrate that deletion of the flavohemoglobin gene from yeast abolishes the NO-consuming activity (12), and, consequently, the growth of these mutant cells is inhibited by a nitrosative challenge that has little effect on the growth of wild-type cells (12). In addition, the expression of flavohemoglobin from Escherichia coli, is inducible by nitric oxide (NO), nitrate, nitrite, or nitroso compounds (13, 14). It is thus generally believed that flavohemoglobins provide protection against NO and related reactive nitrogen species (6, 15–18).

The E. coli flavohemoglobin (Hmp), with a molecular mass of 44 kDa, was the first single-chain flavohemoglobin to be cloned and sequenced (8). Spectroscopic studies showed that Hmp is an oxygen-binding hemoglobin with a histidine as the proximal heme ligand (19). It has also been demonstrated that under aerobic conditions Hmp functions as a nitric-oxide dioxygenase (15, 16) that converts NO to nitrate through the hemoglobin peroxynitrite, Fe$^{2+}$-O–O'–N = O → [Fe$^{3+}$-ONO] → Fe$^{3+}$ + NO$_3^-$.

In this reaction, the heme-bound peroxynitrite, Fe$^{3+}$-ONO$, is a proposed reaction intermediate. Based on kinetic studies, the apparent bimolecular rate of the NO reaction with oxy-Hmp is 40–70 times faster than those for mammalian globins, which makes it almost as efficient as the diffusion-controlled reaction of NO with free O$_2$ (20).

Sequence alignment of Hmp with other hemoglobins suggests that the proximal ligand to the heme is histidine, and the distal residues at the E7 and B10 positions are glutamine and tyrosine, respectively (2). Although the exact arrangement of these distal residues surrounding the ligand binding site in Hmp is not clear, it is plausible that the distal pocket is large and flexible (2, 21) based on the crystal structure of Alcaligenes (Ralstonia) Hb (the only available crystal structure for flavohemoglobins). We postulate that the large and flexible distal pocket evolved to accommodate both O$_2$ and NO molecules simultaneously for performing O$_2$/NO chemistry. Hmp binds
with Phe increases the O₂ dissociation constant. Replacement of the B10 Tyr with Phe increases the O₂ dissociation constant ~80-fold (20), demonstrating the importance of B10 Tyr in stabilizing the heme-bound O₂. A similar role of the B10 Tyr residue has been found in several nonvertebrate hemoglobins, such as those from Mycobacterium tuberculosis (22), Ascaris suum (23–25), Chlamydomonas eugametos (26), and Cyano bacterium synchocystis (27). In this work, resonance Raman spectroscopy was employed to reveal the structural features of Hmp that underlie its chemical reactivity.

**EXPERIMENTAL PROCEDURES**

Hmp was cloned, expressed, and purified to near homogeneity as described elsewhere (28). The protein was buffered at the desired pH with 50 mM Tris, pH 7.5. For all of the experiments reported here, the protein concentration was 40 μM. The Raman measurements were made with previously described instrumentation (22). The output at 406.7 and 413.1 nm from a krypton ion laser (Spectra Physics), for the measurements of the ferric and the ferrous derivatives, respectively, were focused to a ~30-μm spot (laser power ~ 2 milliwatts) on a rotating cell to prevent photodamage to the sample. The acquisition time was about 20–30 min for each spectrum. The scattered light was collected at right angles to the incident beam and focused on the entrance slit of a 1.25-m polychromator (Spex) where it was dispersed and then detected by a charge-coupled device camera (Princeton Instruments).

**RESULTS**

Resonance Raman spectroscopy has been demonstrated to be extremely informative in probing active site structures of heme proteins (22, 29, 30). In the low frequency region of the spectrum between 200 and 800 cm⁻¹, the specific axial ligands coordinated to the prosthetic heme group can be identified by detecting iron-ligand stretching modes. In the high frequency region of the spectrum between 1300 to 1700 cm⁻¹, the oxidation state, spin state, and the axial coordination state of the iron at the center of the heme can be characterized. In particular, the ν₂ mode, in the region between 1550 and 1600 cm⁻¹, is sensitive to the iron spin state. The line in the 1475–1520 cm⁻¹ region, assigned as the ν₂ mode, is sensitive to both the axial coordination and spin state of the iron. The strong line between 1350 and 1400 cm⁻¹, assigned as the ν₄ mode, is sensitive to the π-electron density of the porphyrin macrocycle and therefore the oxidation state of the iron. The frequency and intensity of these Raman lines are further modulated by the protein environment surrounding the heme and, therefore, provide useful structural information on heme proteins.

The high frequency resonance Raman spectrum of the ferrous deoxy form of Hmp is shown in Fig. 1. The electron density marker line, ν₄, is located at 1353 cm⁻¹ and the spin/coordination sensitive line, ν₂, is located at 1470 cm⁻¹. The spectrum is characteristic of a ferrous five-coordinate heme protein. In the low frequency region (Fig. 2), a very strong line at 244 cm⁻¹ is present that we attribute to an iron-histidine stretching mode. The presence of this mode is consistent with the assignment of the ferrous form being five-coordinate high-spin with histidine as the proximal ligand. In general, the Fe–His stretching frequency of globins, which have a neutral histidine as the proximal ligand, is in the range of 200–230 cm⁻¹ (31–34). On the other hand, that of the peroxidases, which have an imidazolate ligand, is much higher in the range of 240–260 cm⁻¹ (32, 35, 36). Thus, the stretching frequency of 244 cm⁻¹ suggests that the proximal ligand of Hmp has imidazolate character. This conclusion is consistent with the crystallographic data of another flavohemoglobin from Alcaligenes eutrophus, which has high sequence homology with Hmp. This hemoglobin has a Fe–His–Glu grouping on the proximal side of the heme (2), that resembles the catalytic triad (Fe–His–Asp) observed in cytochrome c peroxidase (CCP) (37) as illustrated in Fig. 3.

The heme iron of the ferric protein is five-coordinate high-spin at neutral pH, as indicated by the ν₁ and ν₂ lines located at 1491 and 1570 cm⁻¹, respectively, in the resonance Raman spectrum (Fig. 1). These data suggests that the distal binding site of heme is unoccupied. The distal binding site in most ferric globins is occupied by a water molecule at neutral pH, and as a result, the electronic configuration of the heme iron is normally a six-coordinate high-spin and low-spin mixture (38). The absence of a water bound to the heme in the distal site is consistent with the presence of a CCP-like proximal imidazolate ligand for the heme. It has been shown in peroxidases that the imidazolate character of the proximal histidine leads to a strong Fe–His bond. The strong Fe–His bond forces the iron to move out of the porphyrin plane and thereby prevents the coordination of weak distal ligands to the heme (39–41). The ferric protein in most peroxidases thus favors a five-coordinate structure. When the Asp that forms a hydrogen bond with the proximal His in CCP is mutated, the ferric state exhibits a much lower Fe–His stretching frequency, and the ferric state is converted from a five-coordinate to a six-coordinate structure (39). Because Hmp has a similar hydrogen bonding network on the proximal side of the heme as peroxidases, and it contains a strong Fe–His bond as indicated by the high Fe–His stretching frequency, the five-coordinate structure of the ferric state is attributed to the same origin.

In the high frequency region of the resonance Raman spectrum of the CO-coordinated protein, ν₁ is located at 1373 cm⁻¹ and ν₂ is located at 1498 and 1582 cm⁻¹, respectively (Fig. 1). The spectrum is characteristic of a ferrous six-coordinate heme protein. In the low frequency region, two isotope sensitive lines, located at 494 and 535 cm⁻¹, were observed.
that are shifted to 478 and 516 cm$^{-1}$, respectively, in the \textsuperscript{13}C\textsuperscript{18}O derivative (Fig. 2). These two Fe–CO modes are associated with two C–O stretching modes at 1907 and 1960 cm$^{-1}$, respectively, in the \textsuperscript{12}C\textsuperscript{18}O derivative that are shifted to 1825 and 1868 cm$^{-1}$, respectively, in the \textsuperscript{13}C\textsuperscript{18}O derivative (Fig. 4). There is a well established inverse correlation curve linking the Fe–CO stretching frequencies with the associated C–O stretching frequencies (42–44). This correlation is attributed to back-donation of Fe$^{2+}$ d$\sigma$ electrons to the CO $\pi^*$ orbitals. When CO is coordinated to a ferrous heme iron, the $\sigma$ bond formed by donation of electrons from CO to the iron greatly increases the electron density on the iron. A partial double bond resonance (i.e. “back bonding” from iron d$\sigma$ electrons to the CO $\pi^*$ orbitals) is used to decrease the rich electron density added to the iron to stabilize the L-M-CO complex (M and L stand for the ferrous heme iron and the proximal heme ligand, respectively) as is shown in Reaction 2.

\[
\text{L–M–C = O}^{+} \leftrightarrow \text{L–M = C = O}
\]

\textbf{REACTION 2}

The degree of back-donation from CO to the iron depends on the polarity of the distal environment surrounding the heme-bound CO. A positive distal interaction that destabilizes form I thus strengthens the M–CO bond and concomitantly weakens the C–O bond. This inverse correlation has been successfully used to link the Fe–CO and CO frequencies of many heme-containing proteins and model compounds including globins and peroxidases (42). In wild-type myoglobin, the $\nu_{\text{Fe-CO}}$ and $\nu_{\text{C-O}}$ frequencies are located at 508 and 1945 cm$^{-1}$, respectively. As the distal histidine is mutated to various nonpolar residues, $\nu_{\text{Fe-CO}}$ becomes lower and $\nu_{\text{C-O}}$ becomes higher as shown in Fig. 5. Similar trends were observed in horse radish peroxidase and CCP (Fig. 5 and Ref. 45). This inverse correlation also depends on the identity of the proximal ligand such that the line for histidine is distinct from that of thiolate and that of five coordinate species as shown in Fig. 5 (42). Although peroxidases with an imidazolate and globins with a neutral imidazole as the proximal ligands fall on the same line, in general the data from peroxidases are located at the upper left corner of the correlation line with respect to globins because the distal environments of the former are more polar than the latter (Fig. 5).

Both CO derivatives of Hmp fall on the histidine correlation curve (Fig. 5), confirming the assignment that the proximal ligand is a histidine for both species. The modes located at 535 and 1907 cm$^{-1}$ are similar to those reported in peroxidases and are thus assigned as originating from a closed conformation in which the distal residues strongly interact with the CO. The modes located at 494 and 1960 cm$^{-1}$ are similar to mammalian globins (38, 46, 47) and are assigned as an open conformation without any strong positive polar interactions between the distal environment and the CO. The relative population of the open and closed conformation is highly pH-dependent. The intensity ratio of the 494/535 cm$^{-1}$ lines decreases as the pH decreases. A pH$_{\text{Ka1}}$ of 8.3 is obtained by plotting the ratio of $I_{494}/(I_{494}+I_{535})$ as a function of pH (Fig. 6), where $I_{494}$ and $I_{535}$ are the intensities of the 494 and 535 cm$^{-1}$ modes, respectively. Based on this observation, we attribute the positive polar interaction in the closed conformation to the hydrogen bonding with the hydroxide on the B10 Tyr and/or the amine group of the E7 Gln, and the observed pH$_{\text{Ka1}}$ at 8.3 is attributed to the protonation/deprotonation of either one or both of these residues.

\section*{DISCUSSION}

It is widely accepted that the O–O bond activation in peroxidases is facilitated by a “push-pull” (37, 48–51) mechanism that is illustrated in the middle panel of Fig. 3. In this model, the presence of a strong hydrogen bond between the proximal histidine and an aspartate leads to partially anionic character in the proximal histidine that supplies a better electronic push for the heterolytic cleavage of the O–O bond (48–51). On the other hand, the pull effect on the distal side of the heme is created by an arginine to polarize the O–O bond and a histidine functioning as an acid/base catalyst in proton transfer during the catalytic cycle. Although recent new evidence suggests that the push effect from the proximal side of the heme is not required for some peroxidase activity (50–54), the structural features of Hmp revealed by resonance Raman spectroscopy suggest that the push-pull mechanism may underlie the enzymatic reactivity of Hmp.

In Fig. 3 we show a proposed structure of Hmp based on the crystal structure of \textit{A. eutrophus} that has high sequence homology and functional similarity with Hmp (2, 55). On the proximal side of the heme, the Fe-His-Glu triad is analogous to that in CCP (Fe-His-Asp). Our data suggest that the Glu forms a very strong H-bond with the proximal histidine giving it imidazolate character as in CCP. This structural feature accounts for the very high frequency of the Fe–His mode (244 cm$^{-1}$) and the five-coordinate structure in the ferric form of the protein, which are characteristics of peroxidases (39). This proximal hydrogen bonding interaction in Hmp provides a strong electronic push for the activation of the O–O bond during its catalytic reaction with NO.

The distal structural information of Hmp is provided by the resonance Raman spectra of the CO complex. Two distinct structures are evident from the two sets of the Fe–CO and C–O
stretching modes. One set of frequencies is characteristic of heme proteins in which there are no polar interactions between the CO and the distal residues (an open structure) whereas the other set corresponds to a structure in which there are strong H-bonding interactions (a closed structure). The frequencies of the closed structure are similar to those of peroxidases and the inverse correlation between $n_{\text{Fe-CO}}$ and $n_{\text{C-O}}$ indicates that the distal pocket of Hmp is as polar as peroxidases. The distal environment in Hmp thus may provide a strong electronic pull for the O–O bond activation just like that in peroxidases. The most likely residues offering the pull effect in the distal pocket are the B10 Tyr and the E7 Gln as may be seen in Fig. 3. It has been shown that the reaction rate of oxy-Hmp with NO decreases by a factor of $\frac{1}{30}$ when the B10 Tyr is mutated to Phe (20), demonstrating the importance of this residue in modulating the reaction.

The catalytic role of B10 Tyr in nonvertebrate hemoglobin is intriguing. For example, in a bacterial hemoglobin (HbN) from M. tuberculosis that belongs to the truncated hemoglobin family (1), the B10 Tyr interacts strongly with the heme-bound O2 and OH ligands, and it causes the CO adduct to exhibit both an open and a closed conformation (22). As observed in Hmp, the closed conformation has spectroscopic properties similar to those of peroxidases as may be seen from the Fe–CO versus C–O correlation curve in Fig. 5. When the B10 Tyr is mutated to Phe, only the open conformation is observed, and the O2 off-rate increases by a factor of 150 (22). Similarly interactions between the B10 Tyr and the heme-bound ligands have been identified in A. suum (23–25), C. eugametos (1, 26), and C. synechocystis (27) hemoglobins based on both spectroscopic and crystallographic studies. Like Hmp, it was proposed that
HbN and Ascaris Hb may perform NO/O₂ chemistry physiologically (22, 56). A Tyr at the B10 position thus may play an essential role in this chemistry.

Recently, a hemoglobin, DHP, with peroxidase activity, was discovered from a marine worm, Amphitrite ornate (57, 58). This hemoglobin catalyzes the oxidative dehalogenation of polyhalogenated phenols in the presence of H₂O₂ at a rate at least 10 times faster than that of all known halohydrolases of bacterial origin. The catalytic mechanism involves the binding of H₂O₂ to the heme iron in the distal site and the heterolytic cleavage of the O–O bond, as that observed in peroxidases. Surprisingly, the structure of DHP has a classic globin fold with only small variations on the distal and proximal heme side (57). Two structural features of DHP are important for its efficient peroxidase activity. First, the Fe–His bond is stronger than in typical globins as evidenced by a high Fe–His stretching frequency at 233 cm⁻¹ (53), presumed to originate from a strong H-bond between the proximal histidine and a peptide carbonyl oxygen. Second, the distal histidine is one Å further away from the heme iron than in typical globins and readily swings out of the heme pocket enabling the substrate to enter the distal pocket and undergo the oxidation reaction (57). Although the physiological function of Hmp is distinct from that of DHP, the presence of a strong proximal Fe–His bond, and the ability to convert between an open and a closed structure in Hmp suggests that both features may be critical for globins to carry out enzymatic functions.

An intriguing structural feature of Hmp that is distinct from other globins is the orientation of the proximal histidine (assuming that the structure of Hmp is similar to the flavohemoglobin from A. eutrophus) as illustrated in Fig. 7. In most globins, including DHP, the imidazole plane of the proximal histidine is almost coincident with the heme normal (i.e. a tilt angle of 0°), in contrast to a large tilt angle of ~10° in CCP. The tilt of the imidazole ring appears to be a consequence of the strong hydrogen bond between its Nδ and a carboxylate side chain of an aspartate (Asp-235) located in a nearby helix that packs against the proximal helix, based on the fact that this tilt angle becomes zero when Asp-235 is mutated to an alanine or a glutamate (37). In Hmp, this tilt angle is even larger (~25°). We attribute it to the strong hydrogen bonding between the Nδ and the carboxylate side chain of a glutamate in another helix. The tilt of the imidazole ring could generate strain in the molecule and affect the covalent bonding between the proximal histidine and the heme iron, which consequently influences the reactivity of the distal ligands.

The rotation angle of the imidazole ring along the Fe–His bond is also very interesting. It is rotated by ~60°, 90°, and 180° for DHP, Hmp, and CCP, respectively, with respect to that in Mb (Fig. 7). In DHP, this rotation is made possible by the replacement of the last helix turn of the proximal helix with a short 3₁₀ helix (57). It allows the proximal histidine to form a hydrogen bond with a peptide carbonyl that is six residues away in sequence. The resulting hydrogen bond is stronger than that in Mb as reflected by the Fe–His stretching frequency at 233 cm⁻¹ versus 220 cm⁻¹ in Mb (57). In Hmp, the 90° rotation of the proximal histidine is permitted by the shift of the F helix away from the center of the heme (see the projection view in Fig. 7). It allows the proximal histidine to form a hydrogen bond with a carboxylate side chain of a glutamate in another helix (2). This hydrogen bond strength is comparable with that in CCP as indicated by the Fe–His stretching frequency at 244 cm⁻¹ versus 248 cm⁻¹ in CCP (35). In CCP, the proximal helix is rotated 90° and shifted away from the center of the heme with respect to that in Mb (37). This allows the proximal histidine to form a hydrogen bond with a neighboring aspartate. It is noteworthy that the orientation of the proximal helix in DHP and Hmp lies somewhat between that in Mb and CCP, which may have implications for the evolution of hemoglobin from an oxygen activator to an oxygen carrier (59).
It is well established that hemoglobin is an oxygen carrier, and the heme pocket structure of hemoglobin is optimized to bind and release oxygen reversibly. In contrast, in peroxidases the residues lining the heme pocket are tailored to activate heme-bound oxygen species. The discovery of the dioxygenase activity of Hmp, the nitric oxide-activated deoxygenase activity of Ascaris Hb, and DHP from A. ornatia, clearly demonstrate that globins share a wide spectrum of catalytic activities with peroxidases. The results from this work suggest that Hmp has a peroxidase-like active site structure that may promote O₂/NO chemistry instead of oxygen delivery. This finding presents a paradigm shift in our understanding of hemoglobin structure and function. Hmp, along with Ascaris Hb, DHP, and HbH from M. tuberculosis offer excellent examples that appropriate modification of the distal residues and subtle control of the proximal ligand in hemoglobins can alter their catalytic reactivity drastically. The essential role of the Tyr at the B10 position on the enzymatic activities is now becoming clear. The understanding of the structural and functional relationships in these heme proteins will help us to reconcile the old view of an oxygen transport and storage function for globins and a wide range of new enzymatic functions in invertebrate hemoglobins that were discovered in recent years.

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REFERENCES

1. Pesce, A., Couture, M., Dewilde, S., Guertin, M., Yamauchi, K., Ascenzi, P., Moens, L., and Bolognesi, M. (2000) EMBO J. 19, 2424–2434
2. Ermler, U., Siddiqui, R. A., Cramm, R., and Friedrich, B. (1995) EMBO J. 14, 6067–6077
3. Tarricone, C., Calogero, S., Galizi, A., Coda, A., Ascenzi, P., and Bolognesi, M. (1997) Proteins 27, 154–156
4. Tarricone, C., Galizi, A., Coda, A., Ascenzi, P., and Bolognesi, M. (1997) Structure 5, 497–507
5. Iwashita, H., Takagi, T., and Shikama, K. (1992) J. Mol. Biol. 227, 948–954
6. Cramm, R., Siddiqui, R. A., and Friedrich, B. (1994) J. Biol. Chem. 269, 7349–7354
7. Zhu, H., and Riggs, A. F. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 5015–5019
8. Vasudevan, S. G., Armargeo, W. L., Shaw, D. C., Lilley, P. E., Dixon, N. E., and Poole, R. K. (1991) Mol. Gen. Genet. 226, 49–58
9. Poole, R. K., and Hughes, M. N. (2000) EMBO J. 19, 4770–4780
10. Poole, R. K., and Hughes, M. N., and Stewart, V. (1996) J. Biol. Chem. 271, 12581–12589
11. Ollesch, G., Kaunzinger, A., Juchelka, D., Schubert-Zsilavecz, M., and Ermpler, U. (1999) Eur. J. Biochem. 266, 4752–4764
12. Yeh, S. R., Couture, M., Ouellet, Y., Guertin, M., and Rousseau, D. L. (2000) J. Biol. Chem. 275, 1679–1684
13. Kloc, A. K., Yang, J., Mathews, P. S., Frieden, C., and Goldberg, D. E. (1994) J. Biol. Chem. 269, 2377–2379
14. De Baere, I., Perutz, M. F., Kiger, L., Marden, M. C., and Poyart, C. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1594–1597
15. Yang, J., Kloc, A. K., Goldberg, D. E., and Mathews, P. S. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4224–4228
16. Couture, M., Das, T. K., Lee, H. C., Peisach, J., Rousseau, D. L., Wittenberg, B. A., Wittenberg, J. B., and Guertin, M. (1999) J. Biol. Chem. 274, 6898–6910
17. Couture, M., Das, T. K., Savid, P. Y., Ouellet, Y., Wittenberg, J. B., Wittenberg, B. A., Rousseau, D. L., and Guertin, M. (2000) Eur. J. Biochem. 267, 4770–4780
18. Mills, C. E., Sedelnikova, S., Seballe, B., Hughes, M. N., and Poole, R. K. (2001) Biochem. J. 353, 207–213
19. Wang, J., Caughey, W. S., and Rousseau, D. L. (1996) Resonance Raman Scattering: A Probe of Heme Protein-bound Nitric Oxide. Methods in Nitric Oxide Research (Feelisch, M., and Stamler, J. S., eds), pp. 427–454, John Wiley & Sons Inc., NY
20. Ha, S., Smith, K. M., Frei, H., and Spiro, T. G. (1996) J. Am. Chem. Soc. 118, 12638–12646
21. Argade, P. V., Sassaroli, M., Rousseau, D. L., Inubushi, T., Ikeda-Saito, M., and Lapidot, A. (1984) J. Biol. Inorg. Chem. 2, 43–57
22. Smulevich, G., Su, S., Rodgers, K. R., Goodin, D. B., Smith, K. M., and Spiro, T. G. (1996) Biospectroscopy 2, 365–376
23. Terakawa, J., and Kitagawa, T. (1983) J. Biol. Chem. 258, 3969–3977
24. Van Steelandt-Freuntrup, J., Salmen, I., and Babcock, G. T. (1981) J. Am. Chem. Soc. 103, 5981–5982
25. Dasgupta, S., Rousseau, D. L., Anni, H., and Yonetani, T. (1989) J. Biol. Chem. 264, 654–662
26. Kitagawa, T., Ondrias, R. M., Rousseau, D. L., Ikeda-Saito, M., and Yonetani, T. (1982) Nature 298, 869–871
27. Goodin, D. B., and McRee, D. E. (1990) Biochemistry 32, 3133–3134
28. Feis, A., Marzocchi, M. P., Pauli, M., and Smulevich, G. (1994) Biochemistry 33, 4577–4583
29. Smulevich, G. (1998) Biospectroscopy 4, 83–17
30. Ann, H., and Yonetani, T. (1988) Prog. Clin. Biol. Res. 274, 437–449
31. Yonetani, T., and Ann, H. (1987) J. Biol. Chem. 262, 9547–9554
32. Vogel, K. M., Kozlowski, P. M., Zgiesiers, M. Z., and Spiro, T. G. (2000) Inorg. Chem. Acta 297, 11–17
33. Li, X. Y., and Spiro, T. G. (1988) J. Am. Chem. Soc. 110, 6024–6033
34. Ray, G. B., Li, X. Y., Ibers, J. A., Sessler, J. L., and Spiro, T. G. (1994) J. Am. Chem. Soc. 116, 162–175
35. Goodin, D. B., and Lapidot, A. (1984) J. Am. Chem. Soc. 106, 12581–12589
36. Minning, D. M., Gow, A. J., Bonaventura, J., Braun, R., Dewhirst, M., Goldberg, D. E., and Stamler, J. S. (1999) Nature 401, 497–502
37. LaCourt, M. W., Zhang, E., Chen, Y. P., Han, K., Whitten, M. M., Lincoln, D. E., Woudin, S. A., and Lebioda, L. (2000) J. Biol. Chem. 275, 18712–18716
38. Lebioda, L., LaCourt, M. W., Zhang, E., Chen, Y. P., Han, K., Whitten, M. M., Lincoln, D. E., and Woudin, S. A. (1999) Nature 401, 445
39. McRee, D. E., and Lapidot, A. (1982) J. Struct. Bio. 109, 13–17
40. Li, T., Quillin, M. L., Phillips, G. N., Jr., and Olson, J. S. (1994) Biochemistry 33, 1433–1446
41. Das, T. K., Friedman, J. M., Kloc, A. P., Goldberg, D. E., and Rousseau, D. L. (1999) Biochemistry 38, 837–842