Extended X-ray Absorption Fine Structure Study of *Rhodospirillum rubrum* and *Rhodospirillum molischianum* Cytochromes *c'*: Relationship between Heme Stereochemistry and Spin State†

Z. R. Korszun,*,‡ G. Bunker,§ S. Khalid,§ W. R. Scheidt,∥ M. A. Cusanovich,⊥ and T. E. Meyer⊥

Department of Chemistry and the Biomedical Research Institute, University of Wisconsin—Parkside, Box 2000, Kenosha, Wisconsin 53141, Institute for Structural and Functional Studies, University City Science Center, Suite 320, 3401 Market Street, Philadelphia, Pennsylvania 19104, Department of Chemistry, Notre Dame University, Notre Dame, Indiana 46556, and Department of Biochemistry, University of Arizona, Tucson, Arizona 85721

Received May 27, 1988; Revised Manuscript Received September 28, 1988

ABSTRACT: An EXAFS study on the oxidized and reduced forms of cytochromes *c'* from *Rhodospirillum rubrum* and *Rhodospirillum molischianum* was performed at pH 7. The cytochromes *c'* have an apparent coordination number of 5 in both oxidation states. Average Fe–ligand bond lengths of 2.02 ± 0.025 and 2.06 ± 0.025 Å are obtained in their oxidized and reduced forms, respectively. By use of suitable values for the Fe–N_{His} bond length and Fe out-of-plane displacement, as determined by small molecule crystallographic techniques, the Fe–$N_{pyrrole}$ bond lengths and the porphyrin center-to-$N_{pyrrole}$ distance have been estimated for cytochrome *c'* in both of its oxidation states. With this model, estimates of the Fe–$N_{pyrrole}$ bond lengths are 2.01 ± 0.03 and 2.05 ± 0.03 Å, for the oxidized and reduced cytochromes *c'*, respectively. The center-to-$N_{pyrrole}$ distance is estimated to be 1.99 ± 0.03 Å for oxidized cytochrome *c'* and 2.03 ± 0.03 Å for reduced cytochrome *c'*.

The cytochromes *c'* form a class of proteins containing a protoheme IX prosthetic group covalently bound to the polypeptide via two thioether linkages formed between the heme vinyl groups and protein cysteine residues. These proteins differ from other *c*-type cytochromes in several respects. The hemes are bound to the carboxy-terminal end of the protein, and sequence homology (Ambler et al., 1981) among this class of proteins as well as a crystallographic study of *Rhodospirillum molischianum* cytochrome *c'* (Weber et al., 1980) indicates that the Fe atom is coordinated by a single histidine residue. Also, these proteins exhibit unique EPR and Mössbauer spectra (Maltempo et al., 1974) that have been interpreted to be due to a significant contribution of an intermediate spin state ($S = {}^3/_2$) of the heme in the oxidized form of the molecules. In particular, EPR studies of the *Chromatium vinosum* protein have been interpreted to be due to a quantum mechanical mixture of intermediate- and high-spin ($S = {}^5/_2$) states at pH 7 with the two spin states coupled by spin–orbit coupling. The details of the quantum mechanical mixing depend on several factors including the bacterial source of the protein, pH, oxidation state, and coordination stereochemistry.

Recent crystallographic results on inorganic model hemes have indicated that both 5-coordinate (Reed et al., 1979; Masuda et al., 1980) and 6-coordinate (Summerville et al., 1978) Fe(III) systems can exhibit intermediate-spin-state behavior depending on the nature of the axial ligands. Because of the unique nature of the relationship of heme stereochemistry to spin state and the lack of structural information on the reduced form of the cytochromes *c'* at pH 7, we have performed an EXAFS study on the oxidized and reduced forms of *Rsp. rubrum* and *Rsp. molischianum* cytochrome *c'* at pH 7.

Materials and Methods

Rsp. rubrum cytochrome *c'* (RRCP) and *Rsp. molischianum* cytochrome *c'* (RMCP) were obtained in purified form as previously described (Cusanovich, 1967). The proteins were buffered at pH 7.0 by chromatography on a Sephadex G-25 column equilibrated with 10 mM phosphate buffer, pH 7. The samples were then concentrated in Amicon microconcentrators to a final concentration of 5 mM in heme as determined by optical absorption methods. Model compounds used in this study are [Fe^{III}(*meso*-tetraphenylporphyrin)$(H_2O)_2$]ClO_4 (AQUO), [Fe^{III}(octaethylporphyrin)(2-methylimidazole)]ClO_4 (MONO), and the azide adduct of metmyoglobin (N_3^-Mb).

EXAFS spectra were collected in the fluorescence mode, using a Stern/Heald type ionization chamber on several different occasions on the Cornell High Energy Synchrotron Source (CHESS) beam lines C1 and C2 and on the National Synchrotron Light Source (NSLS) National Biostructures Participating Research Team beam line X9A. In all cases, a sufficient number of scans were collected so that in excess of 10^6 effective counts/data point were accumulated (Stern & Heald, 1979). Low-temperature scans were collected in a cryostat designed by Powers and Chance (Powers et al., 1981), and room temperature scans were collected in a Lucite sample holder fitted with Mylar windows. In no case were scans collected under different conditions added together, but rather data from each individual experiment were analyzed independently. In the case of the proteins, oxidized data were collected first, and then unexposed samples were reduced by the addition of a small amount of buffered sodium dithionite.

The EXAFS spectra of both the proteins and the model compounds were normalized to their edge jumps by using 7.110 KeV as an initial estimate for E_0. Each spectrum was subsequently converted to momentum (k) space and multiplied by k^2 to obtain amplitude functions that peak near the center of the k range utilized. This was followed by background

† Supported by NIH Grants GM 36345, RR 01633, GM 38401, GM 21277, and GM 39612.

* Author to whom correspondence should be addressed.
‡ University of Wisconsin—Parkside.
§ Institute for Structural and Functional Studies.
∥ Notre Dame University.
⊥ University of Arizona.

0006-2960/89/0428-1513$01.50/0

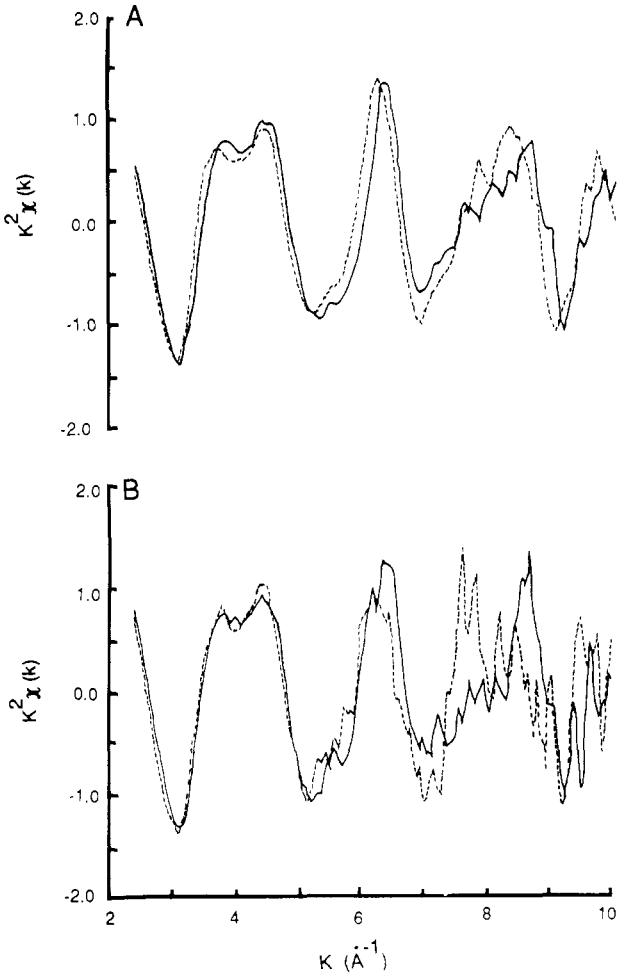

FIGURE 1: $k^2\chi(k)$ vs k spectra for oxidized and reduced RRCP (A) and RMCP (B). Reduced spectra are presented with dotted curves, and oxidized spectra are presented with solid curves. The abscissa has units of Å^{-1}, and the ordinate is in units of Å^{-2}. $k = 0.512(E - E_0)^{1/2}$.

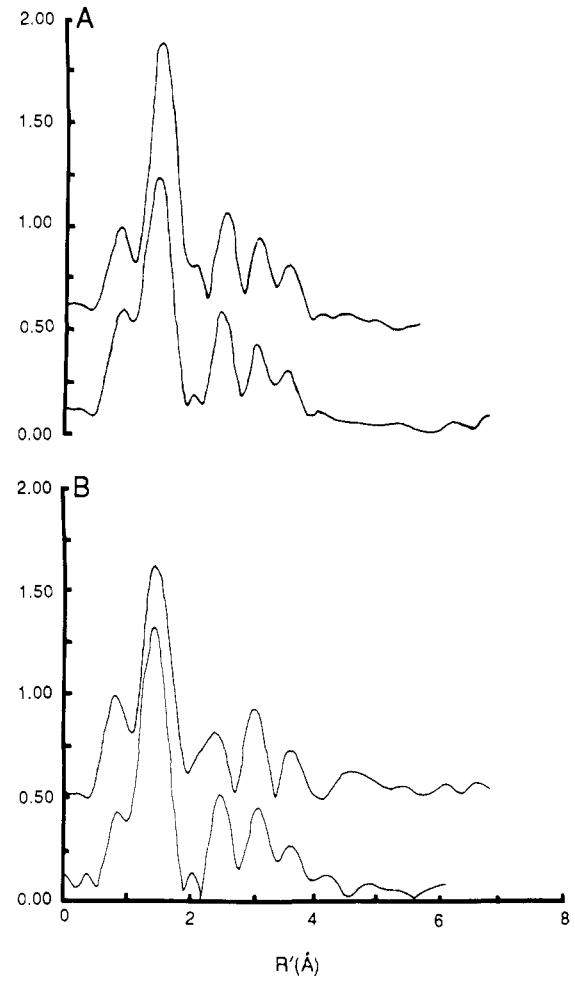

FIGURE 2: Fourier-transformed spectra of RRCP (A) and RMCP (B). The transforms of the reduced molecules are drawn above those of the oxidized molecules. The abscissa has units of Å with $R' = R + {}^1/_2\langle d\alpha(k)/dk\rangle$, where $\alpha(k)$ is the appropriate phase shift function. The magnitudes are directly comparable.

removal where a natural cubic spline was fitted to the data. The background-corrected spectra ranged from 1 to 10.5 Å^{-1}, where they were truncated because signal-to-noise ratios fell to between 2 and 3 (Stern & Heald, 1979). The background-corrected spectra were Fourier transformed into distance (R) space, and a 1.5 Å wide Fourier transform window was used to isolate the first nearest-neighbor backscattering peak. The isolated peak was then back transformed into k space, and the protein data were compared among themselves and the model compounds in a least-squares sense, where R, the average distance of the first nearest neighbors, N, their coordination number, and $\Delta\sigma^2$, the difference in the square of their Debye–Waller factors, were compared. All data analysis was performed by the software developed by Stern and colleagues at the University of Washington.

Results

Parts A and B of Figure 1 present the background-corrected EXAFS spectra of RRCP at pH 7 taken at −100 °C and RMCP at pH 7 taken at room temperature, respectively. Figure 2 shows the Fourier transformed spectra of these proteins. In Figure 1, the oxidized spectra are shown by the solid line and the reduced spectra are shown by the dotted line; in Figure 2 the respective transformed spectra of the reduced species are shown above those for the oxidized species. Numerical results of the analysis are presented in Tables I and II.

Table I: Cytochrome *c'* EXAFS Parameters[a]

protein		least-squares parameters: oxidized	least-squares parameters: reduced
RRCP	N	5.41 ± 0.56	4.90 ± 0.63
	R (Å)	2.03 ± 0.025	2.05 ± 0.025
	$\Delta\sigma^2$	−0.003 ± 0.001	−0.005 ± 0.001
RMCP	N	5.33 ± 0.62	5.63 ± 0.60
	R (Å)	2.01 ± 0.025	2.07 ± 0.025
	$\Delta\sigma^2$	−0.005 ± 0.002	−0.003 ± 0.002

[a] The errors quoted in this table are obtained from the spread in values for the parameters determined from partial sums of the scans. As such, these errors are a measure of the precision of the data. The parameters quoted in this table are the average values obtained by use of the AQUO compound, Fe^{III}(tetraphenylporphyrin) $(H_2O)_2$, as a standard. The stereochemical parameters for the AQUO compound are Fe–$N_{pyrrole}$ average = 2.045 Å and Fe–OH_2 = 2.095 Å.

Table II: Model Compound EXAFS Parameters

model compound		least-squares parameters
MONO	N	5.01 ± 0.50
	R (Å)	2.05 ± 0.025
	$\Delta\sigma^2$	−0.002 ± 0.002
azidometmyoglobin	N	5.69 ± 0.55
	R (Å)	2.01 ± 0.025
	$\Delta\sigma^2$	−0.004 ± 0.002

The first nearest-neighbor backscattering peaks of the model compounds were compared among themselves to determine the accuracy of the EXAFS results. As shown in Table II,

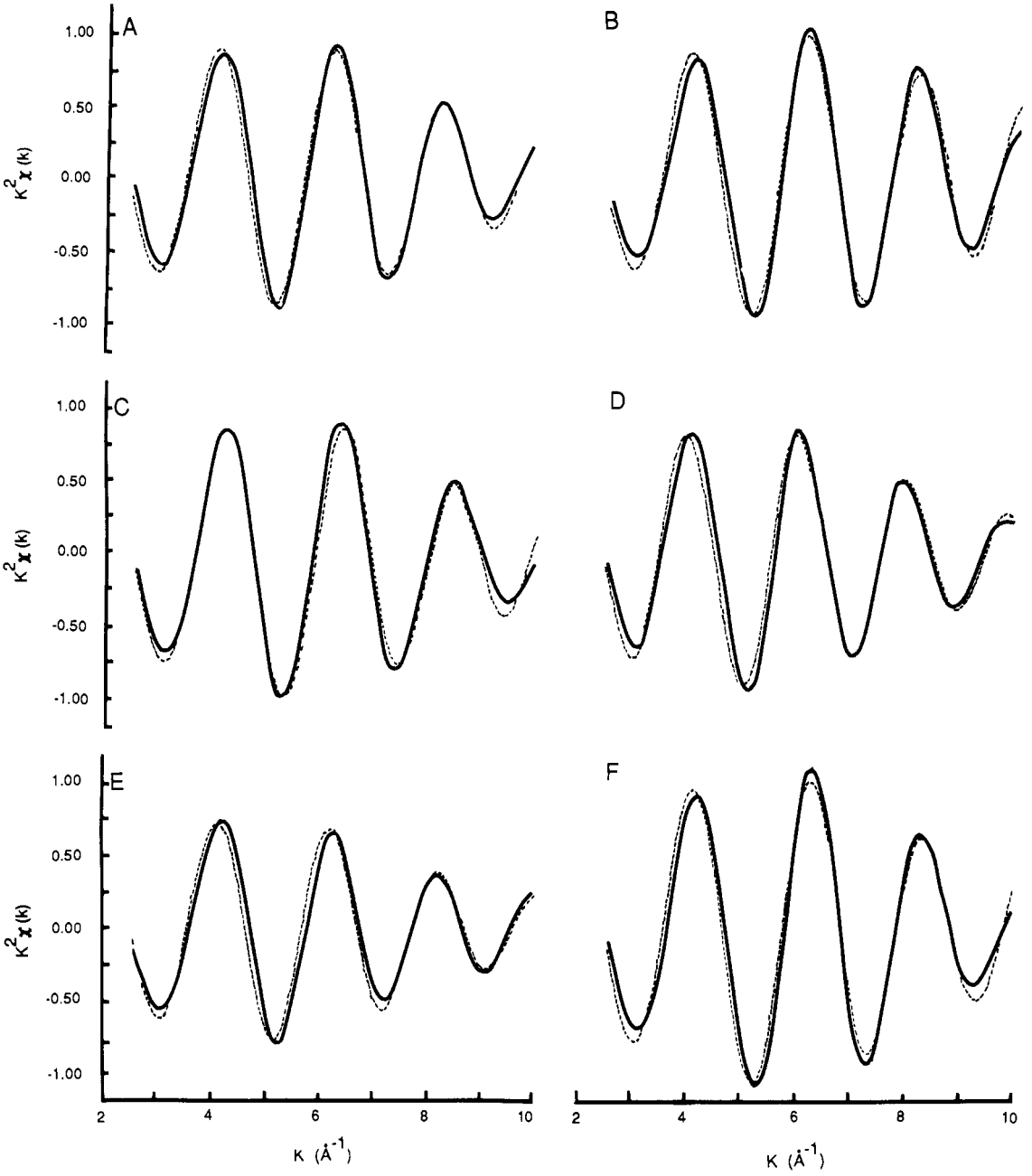

FIGURE 3: Fit of the back-transformed filtered first shell backscattering contributions to the EXAFS spectra of oxidized RRCP (A), reduced RRCP (B), oxidized RMCP (C), reduced RMCP (D), MONO (E), and N_3^-Mb (F) using the AQUO model as a reference. The fitting parameters used are those presented in Tables I and II.

the average distance obtained for the inorganic hemes is in good agreement with the crystallographically determined average Fe–ligand bond lengths. Figure 3 graphically illustrates the fit of the first nearest-neighbor backscattering peak of the proteins and the MONO and azidometmyoglobin model compounds to the AQUO standard using the parameters quoted in Table I. Inspection of Figure 3 shows that the fit of the periodicity of the back-transformed spectra is acceptable, with the major source of discrepancy being in the amplitudes of the reconstructed spectra. In particular, the fit to the MONO model compound is good when compared to the average Fe–ligand bond lengths determined for these compounds by small molecule crystallography, where average Fe–ligand bond lengths of 2.045 Å are obtained. In a separate analysis of the model compounds, where coordination numbers were constrained to their true values, no significant variations in average Fe–ligand bond lengths compared to those in Table II were observed, indicating that bond length and coordination number are not strongly correlated. Although the AQUO compound used as a standard exhibits small amplitude differences due to the oxygen ligands as compared to the other molecules studied (Bunker et al., 1982), an analysis using MONO as a standard (data not shown) did not produce any significant differences in our results, and the uncertainties resulting from small amplitude variations are implicitly included in our error estimates.

As a final check on our data collection and analysis procedure, azidometmyoglobin was analyzed as a model compound. Although the structure of azidomethemoglobin has been solved (Moffat et al., 1979), it was not refined but fit with the known structure parameters of $Fe^{III}(TPP)(N_3^-)$-(pyridine) (Adams et al., 1979), where $Fe–N_{pyrrole}$ and $Fe–N_3^-$ bond lengths of 1.99 and 1.93 Å, respectively, were used. The EXAFS analysis has yielded average Fe–ligand bond lengths of 2.01 Å, approximately 0.02–0.03 Å larger than those expected. Taken together, the results of our analysis of the model

compounds suggests that the EXAFS data collected on the cytochromes *c'* can conservatively be interpreted with errors in accuracy of the average Fe–ligand bond lengths of ±0.03 Å and errors in the accuracy of the coordination number of ±20%.

Discussion

In view of the fact that solution EXAFS spectra yield average metal–ligand distances, it is possible that several different stereochemical models can be consistent with the EXAFS results. Nevertheless, plausible models can be discussed in terms of the small molecule crystallographic results that have established generalized coordination groups for intermediate- and high-spin hemes. In particular, in the intermediate- and high-spin Fe(III) system, as well as in the high-spin Fe(II) system, the small molecule crystallographic results suggest that good estimates of the axial Fe–N_{Im} bond length and the Fe out-of-plane displacement can be made. By use of these estimates, reasonable values for the average Fe–$N_{pyrrole}$ and porphyrin center-to-$N_{pyrrole}$ distance can be obtained.

Oxidized Cytochrome c'. As indicated in Table I, both RRCP and RMCP have identical average Fe–ligand bond lengths in their oxidized forms at pH 7 with an apparent coordination number of 5. Despite the rather large, although not unusual, errors quoted for the coordination number in the EXAFS experiments, this result is consistent with the protein crystallographic observation on RMCP (Finzel et al., 1985). Recent crystallographic studies of intermediate spin Fe^{III}(tetraphenylporphyrin)($OClO_3^-$) (Reed et al., 1979) and Fe^{III}(octaethylporphyrin)($OClO_3^-$) (Masuda et al., 1980) have shown Fe–$N_{pyrrole}$ bond lengths of 2.00 Å and Fe–O–ClO_3^- bond lengths of 2.03 and 2.07 Å, respectively. These intermediate-spin complexes show an Fe displacement of 0.27 Å from the mean plane of the pyrrole nitrogens toward the perchlorate ligand. In a crystallographic study of high-spin Fe^{III}(octaethylporphyrin)(2-methylimidazole)ClO_4^- as the $CHCl_3$ and CH_2Br_2 solvates, Scheidt et al. (1985) report Fe–$N_{pyrrole}$ bond lengths of 2.04 Å and Fe–N_{Im} bond lengths of 2.09 and 2.07 Å, respectively, with Fe displacements of 0.35 Å from the mean plane of the heme. The average Fe–ligand bond lengths measured in this EXAFS experiment are somewhat shorter than those expected for the high-spin monoimidazole Fe(III) hemes and are consistent with those observed for the intermediate-spin hemes. Taking the Fe–N_{His} bond lengths to be 2.07 Å, as indicated by the protein and model heme studies, the EXAFS results suggest that the average Fe–$N_{pyrrole}$ bond lengths should be 2.01 ± 0.03 Å. By use of a displacement of 0.27 Å for the Fe(III) atom, based on small molecule crystallographic results [a slightly smaller displacement has been reported for RMCP by Finzel et al. (1985)] a porphyrin center-to-$N_{pyrrole}$ distance of 1.99 ± 0.03 Å is calculated. The calculated values of Fe–$N_{pyrrole}$ and porphyrin center-to-$N_{pyrrole}$ distances agree well with the small molecule crystallographic observations and the resonance Raman spectra (Strekas & Spiro, 1975; Kitagawa et al., 1977; Felton & Yu, 1978) where core-expansion-sensitive Raman bands suggest a 1.99–2.01-Å core size.

The EXAFS results are best explained in terms of a predominantly intermediate-spin state heme in the oxidized cytochromes *c'* with an essentially unpopulated Fe $d_{x^2-y^2}$ orbital, producing a $^4A_2$ state with an Fe electronic configuration of $d_{xy}^2 d_{yz}^1 d_{xz}^1 d_{z^2}^1$ as suggested by Maltempo et al. (1974). Specifically, the Fe atom is thought to be approximately 0.25–0.3 Å out of the plane of the heme toward the imidazole ligand with Fe–$N_{pyrrole}$ bond lengths of 2.01 ± 0.03 Å and an $N_{pyrrole}$–center distance of 1.99 ± 0.03 Å. The long Fe–N_{His} bond is consistent with that observed for the high-spin Fe(III) monoimidazole hemes which also have a single electron in the Fe d_{z^2} orbital.

Reduced Cytochrome c'. At pH 7, Mössbauer (Moss et al., 1968) magnetic susceptibility (Ehrenberg & Kamen, 1965; Tasaki et al., 1967) and resonance Raman (Strekas & Spiro, 1974; Kitagawa et al., 1977) spectra show that the reduced cytochromes *c'* are electronically similar to deoxymyoglobin and several Fe(II) model hemes. Therefore, their hemes are expected to be stereochemically similar. Despite the rather large errors associated with the determination of the coordination number for RMCP and RRCP, the EXAFS results are consistent with a 5-coordinate heme. In this regard, it is expected that the heme will belong to the high-spin monoimidazole coordination group with the Fe(II) atom displaced from the mean plane of the heme toward the histidine ligand.

In discussing possible models for the stereochemistry of the reduced cytochromes *c'* in terms of our EXAFS results, it is informative to discuss the details of the heme stereochemistry of several Fe(II) model hemes whose structures have been crystallographically determined. In particular, one finds that for the Fe(II) model hemes the Fe–$N_{pyrrole}$ and Fe–N_{Im} bond lengths of [Fe^{II}(α,α-5,15-bis(2-octanediamidophenyl)-α,α-10,20-bis(*o*-pivalamidophenyl)porphyrin(1-MeIm)] fall between the values reported for [Fe^{II}(tetraphenylporphyrin)(2-MeIm)] (Hoard, 1975) and [Fe^{II}(*meso*-tetra($\alpha,\alpha,\alpha,\alpha$-*o*-pivalamidophenylporphyrin)(2-MeIm)] (Jameson et al., 1978). Momenteau et al. (1988) argue that the effects of steric hindrance of the 2-methyl group in the latter two compounds do not manifest themselves in a systematic change in either the Fe–$N_{pyrrole}$ bond lengths (2.068–2.086 Å) or the Fe–N_{Im} bond length (2.10–2.16 Å) but rather in the Fe(II) out-of-plane displacement. In the two model hemes with a hindering 2-methyl group, the Fe out-of-plane displacement (measured with respect to the pyrrole nitrogens) is greater than 0.4 Å, while in the molecule without the hindering 2-methyl group, this displacement drops to 0.3 Å.

If a 2.10-Å Fe–N_{His} bond length is assumed for the reduced cytochromes *c'*, average Fe–$N_{pyrrole}$ bond lengths of 2.05 ± 0.03 Å are calculated. On the basis of the structure of [Fe^{II}-(Piv_2C_8)(1-MeIm)], it is not unreasonable to expect the Fe out-of-plane distance in reduced cytochrome *c'* to be 0.3 Å since histidine is sterically similar to 1-methylimidazole. This yields a center-to-$N_{pyrrole}$ distance of 2.03 ± 0.03 Å. This distance is somewhat shorter than expected on the basis of core-expansion-sensitive resonance Raman frequencies (Spiro, 1983) that predict a core-expansion distance of approximately 2.05 Å but is close to that observed for [Fe^{II}(TPivP)(2-MeIm)]. The reasons for the apparent discrepancy between our EXAFS results and the resonance Raman prediction are not clear. Part of the discrepancy is undoubtedly due to the uncertainty in the EXAFS distance determination and part is possibly due to an anomaly in the resonance Raman core–sensitive bands that significantly overestimate the core expansion in [Fe^{II}(TPP)(2-MeIm)] (Spiro, 1983). In any event, our EXAFS results are consistent with a high-spin heme stereochemical model, with an out-of-plane Fe(II) and a center-to-$N_{pyrrole}$ distance larger than that observed in Fe(III) porphyrins (Scheidt et al., 1985).

Registry No. Heme, 14875-96-8.

References

Adams, K. M., Rasmussen, P. G., Scheidt, W. R., & Hatano, K. (1979) *Inorg. Chem. 18*, 1892–1899.

Ambler, R. P., Bartsch, R. G., Daniel, M., Kamen, M. D., McLellan, L., Meyer, T. E., & Van Beeuman, J. (1981)

Proc. Natl. Acad. Sci. U.S.A. 78, 6854–6857.
Bunker, G., Stern, E. A., Blankenship, R. E., & Parson, W. W. (1982) *Biophys. J. 37*, 539–55.
Cusanovich, M. A. (1967) Ph.D. Thesis, University of California—San Diego.
Ehrenberg, A., & Kamen, M. D. (1965) *Biochim. Biophys. Acta 102*, 333–340.
Felton, R. H., & Yu, N.-T. (1978) in *The Porphyrins* (Dolphin, D., Ed.) Vol. III, pp 347–388, Academic Press, New York.
Finzel, B. C., Weber, P. C., Hardman, K. D., & Salemme, F. R. (1985) *J. Mol. Biol. 186*, 627–743.
Hoard, J. L. (1975) in *Porphyrins and Metalloporphyrins* (Smith, K. M., Ed.) p 317, Elsevier, Amsterdam.
Jameson, G. B., Molinaro, F. S., Ibers, J. A., Collman, J. P., Brauman, J. I., Rose, E., & Suslick, K. S. (1978) *J. Am. Chem. Soc. 100*, 6769–6770.
Kitagawa, T., Ozaki, Y., Kyogoku, Y., & Horio, T. (1977) *Biochim. Biophys. Acta 495*, 1–11.
Maltempo, M. M., Moss, T. H., & Cusanovich, M. A. (1974) *Biochim. Biophys. Acta 342*, 290–305.
Masuda, H., Taga, T., Osaki, K., Sugimoto, H., Yoshida, Z.-I., & Ogoshi, H. (1980) *Inorg. Chem. 19*, 950–955.
Moffat, K., Deatherage, J. F., & Seybert, D. W. (1979) *Science 206*, 1035.
Momenteau, M., Scheidt, W. R., Eigenbrot, C. W., & Reed, C. A. (1988) *J. Am. Chem. Soc. 110*, 1207–1215.
Moss, T. H., Bearden, A. J., Bartsch, R. G., & Cusanovich, M. A. (1968) *Biochemistry 7*, 1583–1596.
Powers, L. P., Chance, B., Ching, Y., & Angiolillo, P. (1981) *Biophys. J. 34*, 465–497.
Reed, C. A., Mashiko, T., Bentley, S. P., Kastner, M. E., Scheidt, W. R., Spartalian, K., & Lang, G. (1979) *J. Am. Chem. Soc. 101*, 2948–2958.
Scheidt, W. R., Geiger, D. K., Lee, Y. J., Reed, C. A., & Lang, G. (1985) *J. Am. Chem. Soc. 107*, 5693–5699.
Spiro, T. (1983) in *Iron Porphyrins* (Lever, A. P. B., & Gray, H. B., Eds.) pp 89–159, Addison-Wesley, Reading, MA.
Stern, E. A., & Heald, S. M. (1979) *Rev. Sci. Instrum. 50*, 1579.
Strekas, T. C., & Spiro, T. G. (1974) *Biochim. Biophys. Acta 351*, 237–245.
Summerville, D. A., Cohen, I. A., Hatano, K., & Scheidt, W. R. (1978) *Inorg. Chem. 17*, 2906–2910.
Tasaki, A., & Kamen, M. D. (1965) *Biochim. Biophys. Acta 140*, 284–290.
Weber, P. C., Bartsch, R. G., Cusanovich, M. A., Hamlin, R. C., Howard, A., Jordan, S. R., Kamen, M. D., Meyer, T. E., Weatherford, D. W., Xuong, Ng.-h., & Salemme, F. R. (1980) *Nature (London) 286*, 302–304.

Determination of the Chirality of the Saturated Pyrrole in Sulfmyoglobin Using the Nuclear Overhauser Effect†

W. O. Parker, Jr., Mariann J. Chatfield, and Gerd N. La Mar*
Department of Chemistry, University of California, Davis, California 95616
Received July 27, 1988; Revised Manuscript Received September 14, 1988

ABSTRACT: The interproton nuclear Overhauser effect (NOE) and paramagnetic dipolar relaxation rates for hyperfine-shifted resonances in the proton NMR spectra of sperm whale met-cyano sulfmyoglobin have led to the location and assignment of the proton signals of the heme pocket residue isoleucine 99 (FG5) in two sulfmyoglobin isomers. Dipolar relaxation rates of these protein signals indicate a highly conserved geometry of the heme pocket upon sulfmyoglobin formation, while the similar upfield direction of dipolar shifts for this residue to that observed in native sperm whale myoglobin reflects largely retained magnetic properties. Dipolar connectivity of this protein residue to the substituents of the reacted heme pyrrole ring B defines the stereochemistry of the puckered thiolene ring found in one isomer, with the 3-CH_3 tilted out of the heme plane proximally. The chirality of the saturated carbons of pyrrole ring B in both the initial sulfmyoglobin product and the terminal alkaline product is consistent with a mechanism of formation in which an atom of sulfur is incorporated distally to form an episulfide across ring B, followed by reaction of the vinyl group to yield the thiolene ring that retains the C_3 chirality.

Recent studies on sulfmyoglobin (SMb)[1] formation based largely on NMR have provided some of the first quantitative characterization of the chemical nature of the modified green prosthetic group (Chatfield et al., 1986a–c, 1987, 1988a,b; Bondoc et al., 1987). Early optical studies had shown that the characteristic green color of SMb is likely due to a chlorin-like structure, where the insertion of an atom of sulfur into the carbon skeleton leads to saturation of a pyrrole; the addition of sulfur across the β–β bond of hemin (**1**) to yield an episulfide, **2**, has been proposed (Berzofsky et al., 1972). ^{1}H NMR studies demonstrated that SMb is generally heterogeneous, where the apparently unique initially formed species (designated S_AMb) can rapidly form several major terminal equilibration products, including an alkaline product designated S_CMb (Chatfield et al., 1986a,c, 1987). While S_AMb possesses a prosthetic group unstable with respect to extraction (with likely structure **2**), the prosthetic group from S_CMb has been extracted and its structure (Chatfield et al., 1986c), as well as that of one of its derivatives (Bondoc et al., 1986), deter-

† This research was supported by a grant from the National Institutes of Health (GM 26226).
* Address correspondence to this author.

[1] Abbreviations: SMb, sulfmyoglobin; S_AMb, S_CMb, isomeric forms of sulfmyoglobin; Mb, myoglobin; metMb, ferric myoglobin; NMR, nuclear magnetic resonance; ppm, parts per million; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate; NOE, nuclear Overhauser effect.

0006-2960/89/0428-1517$01.50/0