2842 *Inorg. Chem.* **1998,** *37,* 2842−2843

Ground and Excited State Spectroscopic Probes of $[(TMP)Fe(IV){=}O]^+$: The First Magnetic Circular Dichroism Study of a Model Peroxidase Compound I Intermediate

Robert Jones,[†] Karupiah Jayaraj,[‡] Avram Gold,*[,‡] and Martin L. Kirk*[,†]

Department of Chemistry, The University of New Mexico, Albuquerque, New Mexico 87131-1096, and Department of Environmental Sciences and Engineering, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7400

Received June 26, 1997

A high-valent oxoferryl porphyrin π cation radical species is an intermediate in the catalytic cycle of heme-containing catalases and peroxidases.[1] This intermediate, formally two oxidation equivalents above the ferric porphyrin resting state, is commonly known as “compound I” (cpd I). A similar cpd I intermediate has been postulated as the active oxygen atom donor in cytochrome P450 monooxygenase enzymes.[2] Despite the importance of this catalytic intermediate, our understanding of the relationship between electronic structure and reactivity of cpd I remains incomplete. The consensus approach toward understanding the electronic structure of these enzyme intermediates is through correlation of their unique spectroscopic and magnetic properties with those obtained on structurally defined small molecule model compounds. EPR and Mössbauer spectroscopies have been used to probe the ground state of both enzymic and biomimetic cpd I,[1a,b,3] and these data have been interpreted in the context of an exchange interaction between the ferryl Fe ($S_{Fe} = 1$) and the porphyrin π cation radical ($S_P = {}^1/_2$) and a large zero-field splitting (ZFS), D_{Fe}, of the ferryl Fe.[1b] An admixture of a low-lying ligand field excited state into the ground state has been suggested in order to explain the origin of the large ferryl iron ZFS.[1b] The exchange interaction was determined to range from antiferromagnetic in chloroperoxidase I to weakly ferromagnetic in catalase I, with very weak ferromagnetic exchange present in horseradish peroxidase I (HRP I).[1] The nature of the exchange interaction in the enzymes is in sharp contrast to the *strong* ferromagnetic exchange observed in all meso-substituted cpd I analogues studied to date.[3a,c,d] The electronic communication between the Fe and porphyrin radical spins, coupled with the unique electronic structure of the catalytically relevant oxoferryl moiety, is expected to play a significant role in enzyme catalysis. Thus, a detailed understanding of the electronic origin of excited-state spectral features and ground-state spin-Hamiltonian parameters should provide considerable insight into electronic structure contributions to reactivity. Here we present the first magnetic circular dichroism (MCD) spectroscopic study of a cpd I analogue, which provides a direct probe of the ground and excited-state electronic structure of oxoferryl porphyrin π cation radical intermediates.

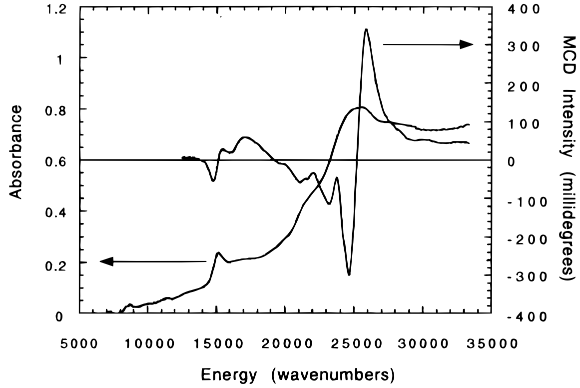

Figure 1. Electronic absorption (8 K) and 7 T MCD (4.2 K) data for $[(TMP)Fe(IV){=}O]^+$ in an optical glass formed from a 77:20:3 mixture of toluene/CH_2Cl_2/methanol.

The low-temperature MCD and electronic absorption spectra of $[(TMP)Fe(IV){=}O]^+$ (TMP = tetramesitylporphyrin) are presented in Figure 1. Particularly noteworthy are two A terms centered at 15 124 cm^{-1} (661.2 nm) and 25 224 cm^{-1} (396.5 nm), indicative of transitions from the A_2 ground state to two different excited states of E symmetry in C_{4v}. The higher energy of the two $A_2 \rightarrow E$ transitions can be roughly equated with the B band and the lower energy transition with the Q band observed in the neutral macrocycle. However, the orbital descriptions of the lowest $A_2 \rightarrow E$ transition is complicated by the presence of significant contributions from one-electron promotions ($e_\pi \rightarrow a_{1\pi}$) originating from low-lying filled porphyrin π orbitals to the singly occupied porphyrin a_1 HOMO.[4] Apart from these transitions, the MCD spectrum of $[(TMP)Fe(IV){=}O]^+$ is quite complex, revealing a combination of temperature independent and field dependent A and B terms in addition to temperature-dependent C terms.[5] Since C term contributions to the MCD are expected to be largest for transitions to spin−orbit split excited states,[6] the C terms should be a sensitive probe of the amount of ferryl Fe character mixed into the excited-state wave functions. The C term contributions to the MCD spectrum of $[(TMP)Fe(IV){=}O]^+$ display only three major features below 26 000 cm^{-1}; namely, a doubly signed pseudo A term[7] (at 25 920 and 24 715 cm^{-1}) and an additional C term (at 23 675 cm^{-1}). The temperature dependence of the B-band pseudo A term is a direct reflection of mixing between ferryl $d_{xz,yz}$ and porphyrin p_{π^*} molecular orbitals of e

---

[†] The University of New Mexico.

[‡] The University of North Carolina at Chapel Hill.

(1) (a) Schultz, C. E.; Devaney, P. W.; Winkler, H.; Debrunner, P. G.; Doan, N., Chiang, R., Rutter, R.; Hager, L. P. *FEBS Lett.* **1979**, *103*, 102−105. (b) Schultz, C. E.; Rutter, R.; Sage, J. T.; Debrunner, P. G.; Hager, L. P. *Biochemistry* **1984**, *23*, 4743−4754. (c) Penner-Hahn, J. E.; Eble, K. S.; McMurry, T. J.; Renner, M.; Balch, A. L.; Groves, J. T.; Dawson, J. H.; Hodgson, K. O. *J. Am. Chem. Soc.* **1986**, *108*, 7819−7825. (d) Roberts, J. E.; Hoffman, B. M.; Rutter, R.; Hager, L. P. *J. Biol. Chem.* **1981**, *256*, 2118−2121. (e) Palaniappan, V.; Terner, J. *J. Biol. Chem.* **1989**, *264*, 16046−16053. (f) Benecky, M. J.; Frew, J. E.; Scowen, N.; Jones, P.; Hoffman, B. M. *Biochemistry* **1993**, *32*, 11929−11933.

(2) Ortiz de Montellano, P. R. In *Cytochrome P450: Structure, Mechanisms and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum Press: New York, 1986; pp 217−272.

(3) (a) Jayaraj, K.; Gold, A.; Austin, R. N.; Mandon, D.; Weiss, R.; Terner, J.; Bill, E.; Müther, M.; Trautwein, A. X. *J. Am. Chem. Soc.* **1995**, *117*, 9079−9080. (b) Boso, B.; Lang, G.; McMurry, T. J.; Groves, J. T. *J. Chem. Phys.* **1983**, *79*, 1122−1126. (c) Ochsenbein, P.; Mandon, D.; Fischer, J.; Weiss, R.; Austin, R.; Jayaraj, K.; Gold, A.; Terner, J.; Bill, E.; Müther, M.; Trautwein. A. X. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1437−1439. (d) Müther, M.; Bill, E.; Trautwein, A. X.; Mandon, D.; Weiss, R.; Gold, A.; Jayaraj, K.; Austin, R. N. *Hyperfine Interact.* **1994**, *91*, 803−808.

(4) Edwards, W. D.; Zerner, M. C.*Can. J. Chem.* **1985**, *63*, 1763−1772.

(5) Piepho, S. B.; Schatz, P. N. *Group Theory in Spectroscopy with Applications to Magnetic Circular Dichroism*; Wiley-Interscience Monographs in Chemical Physics; Wiley-Interscience: New York, 1983.

S0020-1669(97)00790-8 CCC: $15.00

symmetry. Therefore, the B band transition formally involves some porphyrin → Fe charge transfer character. The origin of the low-energy, positively signed C term remains undetermined and is the subject of ongoing investigations. That only three transitions display significant C-term behavior is testament to the electronic "decoupling" of the oxoferryl moiety from the porphyrin. This is a manifestation of the high symmetry of the porphyrin core in $[(\mathrm{TMP})\mathrm{Fe(IV)}{=}\mathrm{O}]^+$, which results in negligible overlap between the porphyrin a_1 HOMO and the Fe $d_{xz,yz}$ π orbitals. Although the MCD C term spectrum of $[(\mathrm{TMP})\mathrm{Fe(IV)}{=}\mathrm{O}]^+$ is similar to that reported for HRP I[8] above ~20 000 cm^{-1}, at lower energies HRP I is very temperature dependent. This reflects the relative amount of Fe d−porphyrin $a_1(a_2)$ mixing in low-lying transitions with appreciable porphyrin $e_\pi \rightarrow a_{1\pi}(a_{2\pi})$ character. Since the degree of Fe $d_{xz,yz}$−porphyrin $a_1(a_2)$ overlap is proportional to *antiferromagnetic* contributions to the exchange interaction,[9] MCD spectroscopy can provide critical information with respect to differences in electronic communication between the oxoferryl Fe and porphyrin π cation radical.

We have utilized variable-temperature MCD (VT-MCD) spectroscopy (Figure 2) in order to determine the magnitude of the $|S_{\mathrm{Fe}} = 1, S_{\mathrm{P}} = {}^1/_2, S_{\mathrm{T}} = {}^3/_2\rangle$ quartet ground-state ZFS ($D_{3/2}$) and provide an estimate of Δ, the magnitude of the Fe d_{xy}−$d_{xz,yz}$ orbital splitting.[10] The VT-MCD data for the 25 920 cm^{-1} band of $[(\mathrm{TMP})\mathrm{Fe(IV)}{=}\mathrm{O}]^+$ can be fit with a model[11] which takes into account the ZFS of the quartet ground state. The best fit to the data yields $D_{3/2} = +5.3\ \mathrm{cm}^{-1}$ ($D_{\mathrm{Fe}} = +15.8 \pm 1.0\ \mathrm{cm}^{-1}$), which is within the range of that deduced previously by spectral simulation of Mössbauer data[12] ($D_{\mathrm{Fe}} = +18.6\ \mathrm{cm}^{-1}$) and by fitting the EPR temperature dependence[12] ($+18 \le D_{\mathrm{Fe}} \le +36\ \mathrm{cm}^{-1}$). The experimentally determined value of Δ for $[(\mathrm{TMP})\mathrm{Fe(IV)}{=}\mathrm{O}]^+$ is quite small ($\Delta = 1420\ \mathrm{cm}^{-1}$ for $D_{\mathrm{Fe}} = +15.8\ \mathrm{cm}^{-1}$ and $k\zeta = 300\ \mathrm{cm}^{-1}$) and is contrary to that found in other terminal metal-oxo complexes where the strong π donor properties of the oxo ligand severely destabilize the metal $d_{xz,yz}$ orbitals relative to d_{xy}.[13] Ongoing work in our laboratory[14] indicates that this unusual d orbital splitting pattern arises from interactions with the porphyrin ligand.

This work represents the first effort at understanding the unique electronic structure of a model oxoferryl porphyrin π cation radical intermediate by MCD spectroscopy and form a basis for extension to enzymic cpd I catalytic intermediates. The VT-MCD analysis of $[(\mathrm{TMP})\mathrm{Fe(IV)}{=}\mathrm{O}]^+$ indicates an unusual d orbital splitting pattern similar to that observed for HRP I. This is postulated to arise from Fe $d_{xz,yz} \rightarrow$ porphyrin p_{π^*} back-bonding interactions where the porphyrin p_{π^*} orbitals of e symmetry play a π acceptor role, lowering the energy of the Fe $d_{xz,yz}$ orbital set and reducing their antibonding character. The effect of this back-bonding interaction is to *strengthen* the Fe=O bond by partially delocalizing the π antibonding $d_{xz,yz}$ electron density onto the macrocycle, resulting in a more electrophilic and reactive oxo ligand. This indicates that nonplanar macrocycle distortions and nature's choice of a particular prosthetic group may play subtle roles in fine-tuning the reactivity of oxoferryl species. We have also shown that the low-energy C term MCD reveals critical differences between $[(\mathrm{TMP})\mathrm{Fe(IV)}{=}\mathrm{O}]^+$ and HRP I which parallel differences in the nature of the exchange interaction (strongly ferromagnetic for $[(\mathrm{TMP})\mathrm{Fe(IV)}{=}\mathrm{O}]^+$ and weakly ferromagnetic for HRP I). Theoretical calculations indicate that many of the low-lying states of porphyrin π cation radicals contain appreciable $e_\pi \rightarrow a_{1\pi}(a_{2\pi})$ character.[4] Therefore, the magnitude of the low-energy MCD C terms reflects the degree of Fe $d_{xz,yz}$−porphyrin $a_1(a_2)$ orbital overlap and probes the electronic origin of the ground-state exchange interaction. Fe $d_{xz,yz}$−porphyrin $a_1(a_2)$ orbital overlap provides a potential super-exchange pathway for electron transfer between the ferryl Fe and neutral macrocycle in one-electron reduced cpd II. This electron transfer pathway is hypothesized to be important in transferring the remaining oxidizing equivalent from the ferryl Fe to the porphyrin periphery,[8b] where reaction with substrate occurs. Finally, the formation of a strong Fe=O bond is central to all heme enzymes which form cpd I catalytic intermediates, and a detailed understanding of the nature of Fe=O bonding is crucial to developing a comprehensive and detailed understanding of electronic structure contributions to reactivity in heme-containing monooxygenases, peroxidases, and catalases.

Acknowledgment. We gratefully acknowledge helpful discussions with Dr. Eckhard Bill and the generous financial support of the National Science Foundation (CHE-9316557) (M.L.K.), Sandia National Laboratories (M.L.K.), The University of New Mexico (M.L.K.), and the National Institutes of Health (ES03433) (A.G.).

Supporting Information Available: Figures showing the MCD saturation data for the 23 675 and 25 920 cm^{-1} bands, the VTVH MCD saturation data for the 25 920 cm^{-1} band, and the C term MCD data (2 pages). Ordering information is given on any current masthead page.

IC970790M

---

(6) (a) Gamelin, D. R.; Bominaar, E. L.; Kirk, M. L.; Wieghardt, K.; Solomon, E. I. *J. Am. Chem. Soc.* **1996**, *118*, 8085−8097. (b) Rowe, M. D.; McCaffery, A. J.; Gale, R.; Copsey, D. N. *Inorg. Chem.* **1972**, *11*, 3090−3098. (c) Gewirth, A. A.; Solomon, E. I. *J. Am. Chem. Soc.* **1988**, *110*, 3811−3819. (e) LaCroix, L. B.; Shadle, S. E.; Wang, Y.; Averill, B. A.; Hedman, B.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1996**, *118*, 7755−7768. (f) Breton, J. L.; Farrar, J. A.; Kennedy, M. C.; Beinert, H.; Thomson, A. J. *Biochem. J.* **1995**, *311*, 197−202. (g) George S. J.; Thomson, A. J.; Crabtree, D. E.; Meyer, J.; Moulis, J. M. *New J. Chem.* **1991**, *15*, 455−465.

(7) (a) A pseudo A term consists of energetically similar, oppositely signed C or B terms. See ref 5. (b) The C term spectrum is included in the Supporting Information.

(8) (a) Browett, W. R.; Gasyna, Z.; Stillman, M. J. *J. Am. Chem. Soc.* **1988**, *110*, 3633−3640. (b) Cheesman, M. R.; Greenwood, C.; Thompson, A. J. *Adv. Inorg. Chem.* **1991**, *36*, 201−255.

(9) The $a_1(a_2)$ label reflects the nature of the hole in the ground state of the porphyrin. This corresponds to an $(a_2^2a_1^1)_{\mathrm{porphyrin}}(b_2^2e^2)_{\mathrm{Fe}}$ configuration for meso-substituted models and an $(a_1^2a_2^1)_{\mathrm{porphyrin}}(b_2^2e^2)_{\mathrm{Fe}}$ configuration for β-substituted models and proteins; see: Hay, J. P.; Thibeault, J. C.; Hoffman, R. *J. Am. Chem. Soc.* **1975**, *97*, 4884.

(10) The axial ZFS parameter $D_{3/2}$ can be related to the intrinsic D_{Fe} using tensor operator methods, yielding $D_{3/2} = D_{\mathrm{Fe}}/3$ (Scaringe, R. P.; Hodgson, D. J.; Hatfield, W. E. *Mol. Phys.* **1978**, *35*, 701−713. Gatteschi, D.; Bencini, I. *EPR of Exchanged Coupled Systems*; Springer-Verlag: Berlin, 1990.). Ligand field theory can then be used to relate D_{Fe} and Δ yielding $D_{\mathrm{Fe}} = (k\zeta)^2/\Delta 4$ (Oosterhuis, W. T.; Lang, G. *J. Chem. Phys.* **1973**, *58*, 4757−4765.).

(11) (a) Browett, W. R.; Fucaloro, A. F.; Morgan, T. V.; Stephens, P. J. *J. Am. Chem. Soc.* **1983**, *105*, 1868−1872. (b) The exchange Hamiltonian used in this analysis is $H = -2J(\mathbf{S}_1 \cdot \mathbf{S}_2)$. Application of the strong exchange limit has been confirmed by Mössbauer and EPR spectroscopies (see ref 12). This isolated quartet arises from strong ferromagnetic exchange between the Fe ($S_{\mathrm{Fe}} = 1$) and porphyrin π cation radical ($S_{\mathrm{P}} = {}^1/_2$) spins. (c) Incorporation of the exchange-coupled doublet $|S_{\mathrm{Fe}} = 1, S_{\mathrm{P}} = {}^1/_2, S_{\mathrm{T}} = {}^1/_2\rangle$ did not lead to improved fits to the data, leading to a lower limit of $J \ge +62\ \mathrm{cm}^{-1}$.

(12) Bill, E.; Ding, X.-Q.; Bominaar, E. L.; Trautwein, A. X.; Winkler, H.; Mandon, D.; Weiss, R.; Gold, A.; Jayaraj, K.; Hatfield, W. E.; Kirk, M. L. *Eur. J. Biochem.* **1990**, *188*, 665−672.

(13) For example, the d_{xy}−$d_{xz,yz}$ orbital splitting is on the order of ~12 000−15 000 cm^{-1} for simple oxovanadium(IV) and oxochromium(V) complexes; see: Lever, A. B. P. *Inorganic Electronic Spectroscopy:* Elsevier: New York, 1984; pp 390, 394, and references therein.

(14) Jones, R.; Dewitt, J.; Gold, A.; Kirk, M. L. Manuscript in preparation.