Acknowledgment. We thank Takahiro Saito for assistance with the experiment. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan, to which our thanks are due.

Supplementary Material Available: Listings of interatomic distances and angles, positional parameters and isotropic temperature factors for non-hydrogen atoms, positional parameters for hydrogen atoms, and anisotropic temperature factors for non-hydrogen atoms (5 pages). Ordering information is given on any current masthead page.

Elementary Electronic Excitations and the Mechanism of Cytochrome P450

Paul M. Champion

Physics Department, Northeastern University
Boston, Massachusetts 02115

Received November 4, 1988

The class of heme proteins known as cytochromes P450[1] are involved in the cleavage of molecular oxygen and the stereospecific insertion of a single atom of oxygen into a variety of substrates.[2] A specific cytochrome $P450_{cam}$, isolated from *pseudomonas putida*, has been used in a variety of physical–chemical studies[3] in order to elucidate some of the key structure–function relationships common to the broad class of P450 enzymes. In particular, an isotopically sensitive vibrational mode has been observed at 351 cm^{-1} with resonance Raman spectroscopy and assigned to Fe–S stretching involving cysteine.[4] Recent studies of the resonance Raman excitation profile (REP) of this mode have resolved unusual and unexpected S → Fe charge-transfer electronic structure to the blue of the Soret transition.[5] In the present communication, we consider these results in the context of the fundamental steps involved in the mono-oxygenase reaction cycle. (For a recent review see Dawson.[6]) We suggest that these steps are related to specific electronic excitations of the sulfur, iron, porphyrin, and oxygen orbitals.

The samples of cytochrome $P450_{cam}$ used in the Raman studies were prepared as discussed previously.[5,7] Figure 1 shows the REP's of the 1488-cm^{-1} high-spin marker band of the heme and the 351-cm^{-1} Fe–S axial ligand mode of oxidized, substrate-bound $P450_{cam}$. Absorption spectra are also shown for solution (thin solid line) and for *z*-polarized single crystals[8] (thick solid line). Notice the extreme blue shift and structure in the Fe–S REP (solid triangles). We expect the REP's of high-frequency modes to peak to the blue of low-frequency modes when both modes are coupled to the same electronic transitions. Thus, it is quite certain that the 351- and 1488-cm^{-1} modes are coupling to different electronic excitations and that the Fe–S mode is activated by *z*-polarized charge-transfer transitions, one of which is clearly observed in the single-crystal absorption at 323 nm. The other *z*-polarized transition, at 360 nm, was apparently missed in the single-crystal analysis due to its proximity to the Soret band and the difficulty

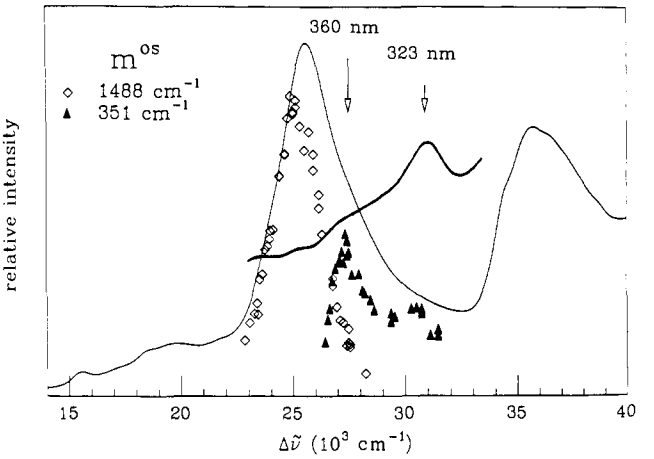
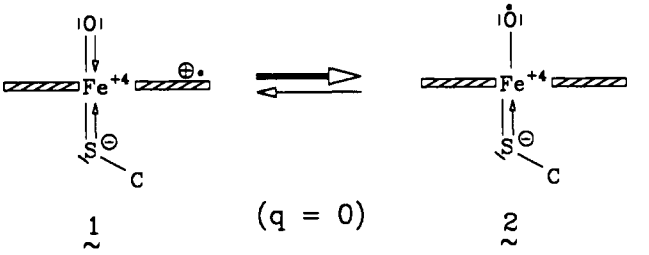
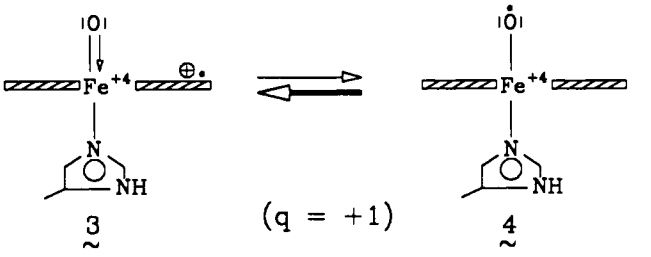


Figure 1. The Raman excitation profiles and absorption spectra of $P450_{cam}$. The REP of the Fe–S mode is shown as solid triangles. The thin solid line is the solution absorption spectrum. The thick solid line is the *z*-polarized single crystal absorption (from ref 8).

Scheme I



Scheme II



of subtracting the in-plane Soret transition moment from the *z*-polarized spectrum (two hemes/unit cell with different orientation). The small inflection at 360 nm is the residual of this transition.

We believe that the two charge-transfer transitions in the high-spin complex involve S → Fe excitations arising from sp^2-hybridized sulfur orbitals[4] that increase the π overlap with the 4-fold symmetric heme while still accounting for the anisotropic esr *g* values.[3] The $S(sp^2) \rightarrow Fe(d\pi)$ excitations are quenched in the low-spin complex, possibly due to filling of the $d\pi$ orbitals when the iron moves into the heme plane (and the *xy* orbital is increased in energy). In this respect, substrate-mediated spin-state equilibria can act as a "switch" to control the S → Fe electron-donation properties of the system.[4]

The sulfur → iron π-electron donation appears to have important mechanistic consequences not only in the dioxygen cleavage step but also in the final hydroxyl insertion step. The high-valent iron porphyrin intermediates that play a key role in the mechanism of both peroxidases and cytochromes P450 have generally been characterized as iron–oxo π cation complexes (Fe^{IV} $S = 1$, porphyrin $S = {}^1/_2$),[9,10] although recent calculations[11] indicate the

(1) *Cytochrome P450*; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1986.
(2) White, R.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 315.
(3) (a) Gunsalus, I. C.; Wagner, G.; Debrunner, P. In *Microsomes, Drug Oxidations and Chemical Carcinogenesis*; Coon, M. J., Ed.; Academic: New York, 1980; p 233. (b) Dawson, J.; Sono, M. *Chem. Rev.* **1987**, *87*, 1255.
(4) Champion, P. M.; Stallard, B.; Wagner, G.; Gunsalus, I. C. *J. Am. Chem. Soc.* **1982**, *104*, 5469.
(5) Bangcharoenpaurpong, O.; Champion, P. M.; Martinis, S.; Sligar, S. *J. Chem. Phys.* **1987**, *87*, 4273.
(6) Dawson, J. *Science* **1988**, *240*, 433.
(7) Gunsalus, I. C.; Wagner, G. *Methods Enzymol.* **1978**, *52*, 166.
(8) Hanson, L. K.; Sligar, S.; Gunsalus, I. C. *Croat. Chem. Acta* **1977**, *49*, 237.
(9) (a) Lowe, G.; Kert, C.; Hjelmeland, L.; Kirchner, R. *J. Am. Chem. Soc.* **1977**, *99*, 3534. (b) Lowe, G.; Herman, Z. *J. Am. Chem. Soc.* **1980**, *102*, 6173.
(10) Groves, J.; Haushalter, R.; Nakamura, M.; Nemo, T.; Evans, B. *J. Am. Chem. Soc.* **1981**, *103*, 2884.

0002-7863/89/1511-3433$01.50/0

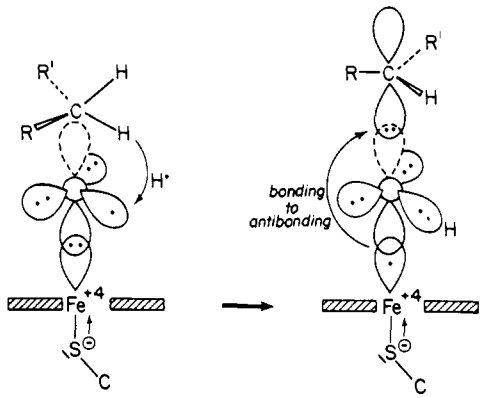


Figure 2. The product formation step in the mono-oxygenase reaction. The inversion of the oxygen orbitals is driven by a concerted stabilization of the carbon radical in the Fe–O antibonding orbital, along with a low-energy bonding to antibonding excitation.

presence of an oxy radical species within 0.7 kcal/mol of the ground state. This species is apparently stabilized as the iron–oxygen bond is lengthened.[11] In Scheme I we show the equilibrium between two isoelectronic iron–oxo structures involving thiolate ligated heme. We suggest that, since the overall complex has neutral charge and S → Fe π electron donation occupies the iron dπ orbitals (reducing the iron–oxygen bond order), the system will tend to the oxygen radical configuration **2** instead of the "compound I" like cation radical **1**. The overall neutral charge of the thiolate system ensures that, barring heme pocket polarization effects, **1** must lie at higher energy, since it corresponds to the spontaneous creation of an electric dipole in a non-polar environment. Thiolate ligated heme proteins such as chloroperoxidase,[12] which apparently utilize compound I intermediates and have a lower probability for hydroxylation reactions, must have polar heme pockets[13] that tend to stabilize the porphyrin cation radical of **1**.

Scheme II displays the analogous isoelectronic states in histidine-ligated heme proteins. Here the net charge on the complex is +1 and the positively charged "hole" is stabilized through delocalization in the π-cation radical. In addition, the absence of the thiolate π-electrons allows the Fe(dπ) orbitals to couple more effectively with the oxygen. Thus, as suggested by recent Raman[14] and EXAFS[15] data, species **3** is energetically favored in histidine-ligated complexes and the porphyrin radical is poised for electron reduction at the heme periphery[16] in the peroxidase catalytic cycle. Note, however, that the thermodynamic probability of finding the system in **4** is non-zero and formation of hydroxylated product may be possible under certain circumstances. Thus, the "branching ratio" in the chemistry of iron–oxo complexes of heme proteins may be determined by the energetics such as in Schemes I and II. Additional factors, such as substrate alignment and H_2O occupancy in the distal pocket, will also affect the product distributions.[17]

In order to be more specific about the oxo radical **2** and the hydroxylation reaction, we show in Figure 2 a sketch of the various orbitals of substrate and thiolate–iron–oxo radical. Abstraction of one[18] of the hydrogen atoms into the oxygen radical orbital would be followed by a rehybridization of the substrate carbon atom. The carbon radical can be stabilized (and oriented) by the vacant Fe–O antibonding orbital. This results in an inversion of the oxygen orbitals, analogous to the inversion of ammonia. The inversion of the orbitals is accompanied by a low-energy (Fe–O) bonding to antibonding electronic excitation that stabilizes the C–OH bond and repels the hydroxylated product away from the ferric porphyrin. It should be noted that the inversion of the oxygen orbitals will stabilize the Fe–O antibonding orbital so that the electronic excitation energy is thermodynamically favorable. It is not inconceivable that specific protein fluctuations involving the sulfur, iron, oxygen, and substrate nuclei could also help to induce the transition. Events analogous to those described above, but involving electron (rather than hydrogen atom) transfer, might also be useful in describing the epoxidation of carbon–carbon double bonds.[19]

Acknowledgment. This work was supported by NIH Grant DK 35090 and NIH Research Career Development Award DK 01405. The author is especially indebted to John Dawson and Steve Sligar for numerous stimulating discussions.

(11) Lowe, G.; Collins, J.; Luke, B.; Waleh, A.; Pudzianowski, A. *Enzyme* **1986**, *36*, 54.
(12) Bangcharoenpaurpong, O.; Champion, P. M.; Hall, K.; Hager, L. P. *Biochemistry* **1986**, *25*, 2374.
(13) Sono, M.; Dawson, J.; Hall, K.; Hager, L. *Biochemistry* **1986**, *25*, 347.
(14) Paeng, K.; Kincaid, J. *J. Am. Chem. Soc.* **1988**, *110*, 7913.
(15) Penner-Hahn, J.; Eble, K.; McMurry, T.; Renner, M.; Balch, A.; Groves, J.; Dawson, J.; Hodgson, K. *J. Am. Chem. Soc.* **1986**, *108*, 7819.
(16) Ator, M.; David, S.; Ortiz de Montellano, P. *J. Biol. Chem.* **1987**, *262*, 14954.
(17) Atkins, W.; Sligar, S. G. *J. Am. Chem. Soc.* **1987**, *109*, 3754.
(18) Gelb, M.; Heimbrook, D.; Malkonen, P.; Sligar, S. G. *Biochemistry* **1982**, *21*, 370.
(19) Traylor, T. G.; Nakano, T.; Dunlap, B. *J. Am. Chem. Soc.* **1986**, *108*, 2782.

The Methyl Group Geometry in Trichloromethyltitanium: A Reinvestigation by Gas Electron Diffraction

Peter Briant and Jennifer Green

Inorganic Chemistry Laboratory
South Parks Road, Oxford OX1 3QE, Great Britain

Arne Haaland,* Harald Møllendal, Kristin Rypdal, and Janos Tremmel

Department of Chemistry, University of Oslo
P.B. 1033, 0315 Oslo 3, Norway

Received December 8, 1988

A recent preliminary communication on the molecular structure of Cl_3TiCH_3 as determined by gas electron diffraction[1] suggested an unusual methyl group geometry: C–H bond distance 115.8 (1.6) pm and valence angle $\angle$TiCH = 101.0 (2.2)°, presumably due to partial donation of C–H bonding electrons into vacant d orbitals on Ti. Such an interaction is also consistent with the observation of an unusually large positive H,H coupling constant of +11.3 Hz and an unusually low CH_3 rocking mode of 580 cm^{-1} as compared to 825 cm^{-1} in Cl_3GeCH_3.[1]

Very recently Williamson and Hall have reported the results of extensive SCFMO and GVB calculations on Cl_3TiCH_3.[2] On the basis of structure optimizations at different levels they predict a normal C–H bond distance of 110 ± 1 pm and a slightly less-than-tetrahedral angle of 107 ± 1°.[2] The calculations reproduce the lowering of the rocking mode relative to Cl_3GeCH_3 but provide no indication for Ti···(C–H) interactions.

We prepared Cl_3TiMe (Me = CH_3 or CD_3) with the intention of determining the molecular structure by MW spectroscopy. We have, however, been unable to record a MW spectrum, probably because Cl_3TiMe decomposes rapidly on the metal walls of the waveguide. It was then decided to record the GED data for both compounds with use of an all-glass inlet system. These experiments proceeded without difficulty. We hope to record MW spectra

(1) Berry, A.; Dawoodi, Z.; Derome, A. E.; Dickinson, J. M.; Downs, A. J.; Green, J. C.; Green, M. L. H.; Hare, P. M.; Payne, M. P.; Rankin, D. W. H.; Robertson, H. E. *J. Chem. Soc., Chem. Commun.* **1986**, 520.
(2) Williamson, R. L.; Hall, M. B. *J. Am. Chem. Soc.* **1988**, *110*, 4428.
(3) For a discussion of the rocking mode of H_3TiCH_3 see also: Eisenstein, O.; Jean, Y. *J. Am. Chem. Soc.* **1985**, *107*, 1177. Shiga, A.; Kojima, J.; Sasaki, T.; Kikuzono, Y. *J. Organomet. Chem.* **1988**, *345*, 275.

0002-7863/89/1511-3434$01.50/0