In analogous experiments we have found that aniline is also rapidly oxidized to the anilino radical by O^-

$$O^- + C_6H_5NH_2 \longrightarrow OH^- + C_6H_5\dot{N}H \qquad (2)$$

The rate constant for reaction 2 was determined to be $1.6 \times 10^9\ M^{-1}\ sec^{-1}$.[9] In the case of aniline abstraction of the NH_2, hydrogen atoms can contribute, but the very high value of the rate constant again suggests that O^- may well oxidize the aniline directly by attack on the lone pair of electrons on the nitrogen atom. Further studies on these oxidation processes are in progress.

References and Notes

(1) Supported in part by the U.S. Atomic Energy Commission.
(2) B. L. Gall and L. M. Dorfman, *J. Am. Chem. Soc.*, **91**, 2199 (1969).
(3) M. Simic and M. Z. Hoffman, *J. Phys. Chem.*, **76**, 1398 (1972).
(4) P. Neta, M. Z. Hoffman, and M. Simic, *J. Phys. Chem.*, **76**, 847 (1972).
(5) P. Neta and R. H. Schuler, *J. Phys. Chem.*, in press.
(6) L. K. Patterson and J. Lilie, *Int. J. Radiat. Phys. Chem.*, **6**, 129 (1974).
(7) E. J. Land, G. Porter, and E. Strachan, *Trans. Faraday Soc.*, **57**, 1885 (1961).
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(9) A rate constant of $2.4 \times 10^9\ M^{-1}\ sec^{-1}$ is calculated for reaction 2 by correcting the value measured previously (H. Christensen, *Int. J. Radiat. Phys. Chem.*, **4**, 311 (1972)) at pH 13.3 for the partial OH contribution.

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Models for Cytochrome P-450

Sir:

Cytochrome P-450 oxygenases catalyze the hydroxylation of C–H bonds in metabolism, hormone regulation, and drug detoxification.[1,2] These ubiquitous monohydroxylases contain at their active site an oxygen binding hemoprotein referred to as P-450—a term derived from the anomalous Soret band of its ferrous carbonyl derivative. In higher organisms all cytochrome P-450's are membrane bound and are inactivated upon separation; however, Gunsalus[2] has purified and extensively studied a soluble bacterial cytochrome $P\text{-}450_{cam}$ camphor hydroxylase. Four stages of $P\text{-}450_{cam}$ have been characterized in the catalytic cycle (Scheme I):[3] substrate-free low-spin ($S = 1/2$) ferric **1**, substrate-bound high-spin ($S = 5/2$) ferric **2**,[5] deoxy high-spin ($S = 2$) ferrous **3**, and diamagnetic oxygenated ferrous **4**. The nature of the axial bases in P-450 is not well resolved but a cysteinyl thiol is considered to be involved because solutions containing hemin and various mercaptans exhibit the unusual ESR signals characteristic of low-spin $P\text{-}450_{cam}$ **1**.[6] However, well-defined iron porphyrin complexes having axial sulfur ligands have not previously been characterized.[7] Herein, we describe potential models for **1** and **2**.

Scheme I

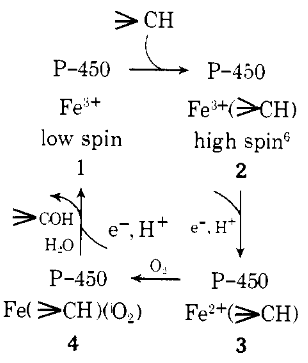


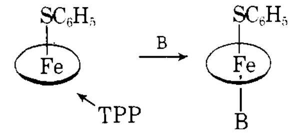
Table I. ESR *g* Values of Ferric Complexes[a]

Complex	g_1	g_2	g_3
2[c]	8	4	1.8
5	8.6	3.4	—[b]
1[c]	2.45	2.26	1.91
6a[e]	2.40	2.25	1.97
6b	2.39	2.26	1.93
6c[d]	2.36	2.27	1.95
6d	2.34	2.25	1.96
6e[d]	2.37	2.27	1.94
6f	2.37	2.26	1.94
6g[e]	2.37	2.22	1.96
6h[e]	2.38	2.22	1.96
6i[e]	2.40	2.26	1.94

[a] Reference 10b and c. [b] Not observable. [c] Reference 2. [d] Unidentified minor species detected. [e] No solvent used.

Reaction of $[Fe(TPP)]_2O$ and benzenethiol affords the low-spin ferric complex **6a**, $Fe(TPP)(SC_6H_5)(HSC_6H_5)$,[8] which loses benzenethiol upon heating under vacuum to give the high-spin (μ(25°) = 5.8 BM), oxygen sensitive ferric complex, $Fe(TPP)(SC_6H_5)$ (**5**).[8] Both compounds decompose in benzene at 25° affording Fe(TPP) and $(C_6H_5S)_2$. In toluene glass (77°K) **5** gives an ESR spectrum[9] whose *g* values (Table I) are similar to substrate-bound high-spin P-450, **2**.[1,2] Of special note is the extremely rhombic character of the high-spin ferric spectra in **5** and **2**. Small differences in the ESR of **2** and **5** may be due to the porphyrins employed, protoporphyrin IX and TPP, respectively, and differences in the nature of the mercaptide, presumably the conjugate sulfur base of cysteine in the case of **2**.

At 25° in the presence of virtually any Lewis base, the ferric complex **5** is rapidly reduced to the ferrous complex $Fe(TPP)B_2$ forming as a by-product the disulfide $(C_6H_5S)_2$. However, in solution at low temperature this reaction is very slow and ESR spectra characteristic of low-spin $P\text{-}450_{cam}$ **1** can be obtained (Table I) suggesting formation of metastable six-coordinate complexes **6b–f**, eq **1**.



(1)

5 high spin

6 low spin

a, B = C_6H_5SH
b, B = N–MeIm
c, B = THT
d, B = THF
e, B = cam
f, B = acetone
g, B = NH_3
h, B = CH_3NH_2
i, B = Ⓟ–Im

In the solid state, crystalline **5** also reacts with gaseous amines affording **6g, h.** The similarity and unusual rhombic character of the *g* values for high spin **5** and the high-spin component in substrate-bound P-450, **2**, leave no doubt that the single axial base is a mercaptide. However, for the series of model low-spin complexes **6a–h**, the *g* values (Table I) are too similar to assign the nature of the second axial base in low-spin **1**. This second axial ligand could be a cysteine thiol, a histidine imidazole, a methionine thioether, a water molecule,[10] or a lysine amino group. It is clear that this second ligand is displaced upon substrate binding.[11]

Although the low-spin complexes **6a–f** are quite unstable in solution at 25°, such complexes are dramatically stabilized by immobilization. For example, **6a** is stable in the crystalline state, and **6i** in which the imidazole ligand is N-bonded to cross-linked polystyrene is unchanged after prolonged storage as a solvent-free solid at 25°. These results could provide an explanation for the membrane stabilization of P-450's.[12]

Acknowledgments. This research was supported by National Institutes of Health Grants GM17880 and HL13531 and by National Science Foundation Grant MPS70-01722-A03.

References and Notes

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(2) I. C. Gunsalus, J. R. Meeks, J. D. Lipscomb, P. Debrunner, and E. Munck in "Molecular Mechanisms of Oxygen Activation," O. Hayaishi, Ed., Academic Press, New York, N.Y., 1974, p 561.

(3) The following abbreviations are used in this paper: >CH = a hydrocarbon substrate, >COH = hydroxylated product, TPP = *meso*-tetraphenylporphyrin, N-MeIm = *N*-methylimidazole, THT = tetrahydrothiophene, THF = tetrahydrofuran, cam = camphor, Ⓟ-Im = polystyrene-bonded imidazole.[4]

(4) J. P. Collman, R. R. Gagne, J. Kouba, and H. L. Wahren, *J. Am. Chem. Soc.*, **96**, 6800 (1974).

(5) Actually, this substrate bound ferric form **2** exhibits an equilibrium between high and low spin forms.[2] We suggest the possibility that this equilibrium arises from competitive coordination of the camphor carbonyl.

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(7) During the course of our studies, we learned of similar work by R. H. Holm to whom we are indebted for making his results available to us prior to publication: S. Koch, S. C. Tang, R. H. Holm, and R. B. Frankel, *J. Amer. Chem. Soc.*, following paper; J. C. Tang, S. Koch, R. H. Holm, R. B. Frankel, and J. A. Ibers, *ibid.*, following paper.

(8) These compounds have been characterized by elemental analysis. These results were provided to the referees. A single-crystal X-ray diffraction structural analais of **6a** is in progress.

(9) (a) All such solutions of **5** exhibit an additional unidentified ferric porphyrin signal at $g = 6$ whose intensity increases slowly with time. In freshly prepared samples this impurity is <10%. (b) With the exception of **6a**, **e**, **g**, and **h** (determined at 77°K) all ESR spectra in the present work were determined at both 77 and 20°K and showed no apparent change indicating the absence of a spin equilibrium such as that reported[2] for **1** and **2** (see ref 6). (c) Axial bases were used in excess to form **6b–f**, especially using camphor to form **6e**. This may explain the apparent discrepancy between our work and that of Holm.

(10) J. A. Peterson and B. W. Griffin, *Drug Metab. Dispos.*, **1**, 14 (1973).

(11) Competition experiments show that N-MeIm is bound preferentially over THF, THT, or cam. This raises the question as to how camphor substrate could replace a histidyl imidazole in going from **1** to **2**.

(12) In P-450$_{\mathrm{cam}}$ which is not membrane bound the protein may afford such protection as is found in oxygenated hemoglobin.

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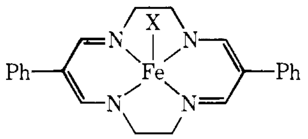
The [Fe^{III}N$_4$SR] Coordination Unit. Synthesis and Ground State Electronic Properties of Macrocyclic Ferric Thiolates

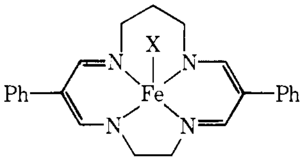
Sir:

The structural, electronic, and reactivity properties of natural and synthetic Fe(II, III) porphyrin complexes are significantly dependent upon the interactions of heme-iron with its axial ligand(s). In this regard the generalized [FeN$_4$S] coordination unit of idealized tetragonal microsymmetry is of particular current interest because of its possible occurrence in one or more of the reaction states of cytochrome P-450 enzymes.[1] In, for example, the P-450 camphor hydroxylase system, four reaction states of the enzyme have been identified[2] and the occurrence of sulfur-ligated forms is a matter of active speculation.[1,2] Although there have been reports of the electronic absorption and EPR spectra[1-6] of complexes described as ferric porphyrin thiolates, the great majority of these species were generated in situ, usually in the presence of a nitrogenous base, and were not isolated or otherwise characterized. In order to probe the properties of this donor atom arrangement, we report here and in the following communication[7] the synthesis and certain ground state electronic features of species containing the [Fe^{III}N$_4$SR] unit, previously unknown in stable isolable complexes.[8] This work has utilized both porphyrin and other types of synthetic diprotic macrocycles[9] as N$_4$ ligands as a means of detecting any modifying effects of supporting ligand structure on the properties of this unit.

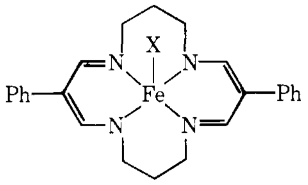
Reaction of 14-, 15-, and 16-membered metal-free bis(β-iminoamine) macrocycles[9] with ferrous and basic ferric acetate in hot DMF solution gave in good yield the corresponding Fe(II) (X absent) and Fe(III) macrocycles (X = OAc) **1a–3a**,[10] respectively, which serve as precursors for thiolate complexes. The latter were synthesized by the substitution reaction (1) and the oxidative addition reaction (2).



Fe(6565)X
X = OAc (**1a**)
SPh (**1b**)
Br (**1c**)



Fe(6566)X
X = OAc (**2a**)
SPh (**2b**)
Br (**2c**)



Fe(6666)X
X = OAc (**3a**)
SPh (**3b**)
SCH$_2$Ph (**3c**)
Br (**3d**)
OCOPh (**3e**)

$$\mathrm{Fe^{III}N_4OAc + RSH \longrightarrow Fe^{III}N_4SR + HOAc} \tag{1}$$

$$\mathrm{Fe^{II}N_4 + \tfrac{1}{2}RSSR \longrightarrow Fe^{III}N_4SR} \tag{2}$$

Both reactions yield **1b–3b** while **3c** was obtained by the first method. In a synthesis typical of reaction 1, 1.1 equiv of benzenethiol was added to an ethanol suspension of 1.0 equiv of **1a**. After 1 hr reaction time at 25°, filtration, and recrystallization (DMF–methanol, 25°), **1b** was obtained in 90% yield as brown crystals. As an example of reaction 2, Fe(6566) and diphenyl disulfide were allowed to react in DMF solution for 1 hr at 25°. Work-up and recrystallization afforded black crystalline **2b** (50%). The reactive Fe(II) macrocycles also undergo facile oxidative addition with other reagents including halogens and dibenzoyl peroxide, affording the complexes **1c**, **2c**, **3d**, and **3e**. Ferric porphyrin thiolates were most conveniently prepared in high analytical and magnetic[10] purity by the cleavage of μ-oxo dimers with relatively acidic thiols[11] in toluene solution at