Communications to the Editor

Mechanism of Autoreduction of Ferric Porphyrins and the Activation of Coordinated Ligands. Detection of One-Electron Oxidized Substrates

Sir:

Although there have appeared scattered reports of the autoreduction of ferric porphyrins in solution,[1,2] in no case has mechanistic information been provided on these novel reactions. Perhaps the best-known example[1] is the formation of the ferrous bispiperidine complex of tetraphenylporphyrin, $TPPFe(pip)_2$. Though this reaction was reported[1] nearly a decade ago, the recent x-ray structural characterization of the reduced complex has emphasized[3] the lack of understanding of the mechanism of the reaction.

The elucidation of the mechanism of iron porphyrin autoreduction takes on added significance when one considers that a variety of hemoproteins (peroxidases,[4] oxygenases,[5] cytochrome P-450[6]) are involved in one-electron valency changes coupled to the oxidation or reduction of substrates. Hence, this "activation" of substrates by such hemoproteins[4-6] could result from intramolecular transfer of a single electron between the iron center and the substrate. One-electron reduction has been confirmed in the formation of the superoxide ion by an oxygenase.[7] Peroxidases oxidize a wide variety of substrates,[4] and, although one-electron oxidation of amines[8] has been proposed to account for the products isolated, the intermediate form of the amine has not been characterized. The elucidation of the nature of the activated substrate is critical to an understanding of the enzyme mechanism; the complexity of such macromolecules, however, suggests that this insight may be more simply provided by studies of model systems.

We report here on some magnetic resonance studies of the autoreduction of ferric porphyrins in the presence of piperidine or cyanide ion which demonstrate that the iron reduction is accompanied by the one-electron oxidation of the substrate. In the reaction of TPPFeCl in chloroform-*d* containing piperidine, reduction of the ferric ion is accompanied by the appearance of a strong ESR signal exhibiting a triplet structure indicative of an alkyl amine radical. No ferric complex of piperidine is detected, and the identity of the free radical must await isotopic labeling experiments.

However, in the case of CN^- as substrate, identification of the reactants and products proved feasible. Addition of excess cyanide in the form of KCN to a 10 mM TPPFeCl solution in Me_2SO-d_6 yields the low-spin ($S = \frac{1}{2}$), ferric biscyanide complex as characterized by its optical and proton NMR spectra and solution susceptibility.[9] Both spectra, however, are found to be time dependent, converting from those typical for low-spin iron(III)[10] to ones characteristic of diamagnetic iron(II).[11] Exclusion of oxygen and light proves the reduction to proceed anaerobically and via a thermal pathway.

In the case of the NMR spectra, the peaks move, with only an averaged resonance per set of equivalent protons for the two oxidation states. The change in averaged shift with time is illustrated in Figure 1. The observation of an averaged resonance for all porphyrin peaks indicates that the same molecular entity exists in both oxidation states, with rapid electron exchange averaging the two spectra.[12]

The ESR spectrum during reaction yields an intense 13-line signal which is identical with that previously reported[13] during anodic oxidation of tetraphenylarsonium cyanide in Me_2SO, and has been interpreted to represent the oxidized cyanide tetramer which is an intermediate in the conversion of cyanide radicals to some unspecified product.

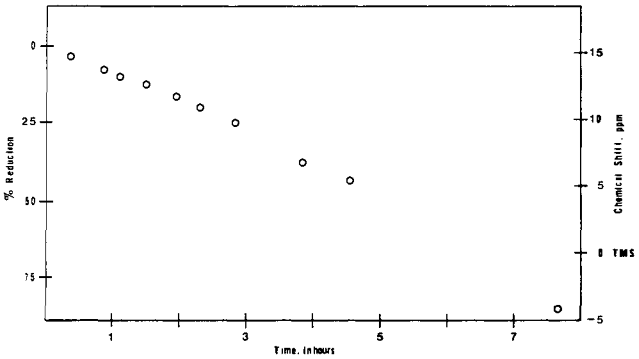


Figure 1. Graph of the position of the averaged pyrrole-H shift of TPP, referenced against Me_4Si, in $TPPFe(CN)_2^{-1,-2}$, vs. time; in Me_2SO-d_6 at 25 °C.

The rate of reduction of $TPPFe(CN)_2^-$ depends on cyanide concentration as well as on the presence of small amounts of water. Increased cyanide concentration speeds up the reduction while small amounts of water inhibit the reaction. These two effects are thought to be related since we have shown[14] elsewhere that cyanide coordinated to ferric porphyrins acts as a strong hydrogen-bond acceptor towards water. Such an interaction would make the coordinated cyanide more difficult to oxidize.

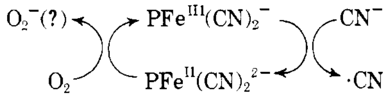
The generation of ·CN during the reduction of Fe(III) suggests that the mechanism involves homolytic bond cleavage, i.e.

$$PFe^{III}(CN)_2^- \longrightarrow PFe^{II}(CN)^- + \cdot CN \qquad (1)$$

$$PFe^{II}(CN)^- \xrightarrow{CN^-} PFe^{II}(CN)_2^{2-}$$

with the product complex rapidly picking up another CN^-, as verified by the electron-exchange averaged NMR spectra. Oxidation of cyanide by intramolecular electron transfer is supported by observations that the rate of reduction is decreased significantly as the porphyrin is made more basic.[15]

Introduction of molecular oxygen qualitatively reverses the process depicted in Figure 1, indicating that $TPPFe(CN)_2^{2-}$ is oxidized directly without the formation of the oxo-bridged dimer usually encountered in the oxidation of ferrous porphyrins.[16] Furthermore, this reaction can be cycled repeatedly as represented below. The presence of the superoxide ion has not yet been confirmed. The piperidine reaction[1] is not catalytic, but terminates with the formation of the oxo-bridged dimer. The activation process, however, is also likely to involve homolytic bond cleavage, as for cyanide. Autoreduction of ferric porphyrins has also been detected[15] optically for alkyl thiols and phosphines.



We have thus demonstrated that the "activated" substrates are the product of the one-electron oxidation by the ferric porphyrin. More detailed investigations into the identity of the piperidine radical and the scope of these reactions are in progress and will be reported in the future.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (HL-16087); the authors are indebted to A. L. Balch for numerous valuable discussions.

References and Notes

(1) L. M. Epstein, D. K. Straub, and C. Maricondi, *Inorg. Chem.*, **6**, 1720 (1967).

(2) J. A. Weightman, N. J. Hoyle, and R. J. P. Williams, *Biochim. Biophys. Acta*, **244**, 567 (1971); D. K. Straub and W. M. Connor, *Ann. N.Y. Acad. Sci.*, **206**, 383 (1973); L. A. Constant and D. G. Davis, *Anal. Chem.*, **47**, 2253 (1975).

(3) L. J. Radonovich, A. Bloom, and J. L. Hoard, *J. Am. Chem. Soc.*, **94**, 2073 (1972).

(4) B. C. Saunders in "Inorganic Biochemistry", Vol. 2, G. L. Eichhorn, Ed., Elsevier, Amsterdam, 1973, Chapter 28.

(5) P. Feigelson and F. O. Brady in "Molecular Mechanisms of Oxygen Activation", O. Hayaishi, Ed., Academic Press, New York, N.Y., 1975, p 87.

(6) I. C. Gunsalus, J. R. Meeks, J. D. Lipscomb, P. Debrunner, and E. Munch, ref 5, p 561.

(7) S. Yamamoto and O. Hayaishi, *J. Biol. Chem.*, **242**, 5260 (1967); F. O. Brady, H. J. Forman, and P. Feigelson, *ibid.*, **246**, 7119 (1971); F. Hirata and O. Hayasishi, *ibid.*, **246**, 7825 (1971); H. W. Strobel and M. J. Coon, *ibid.*, **246** (1971).

(8) P. J. G. Mann and B. C. Saunders, *Proc. R. Soc. London, Ser. B*, **119**, 47 (1935).

(9) Bands are observed at 334, 434, 576, 613, and 687 nm. The susceptibility was found to be 1.7 μ_B.

(10) J.-H. Fuhrhop, *Struct. Bonding (Berlin)*, *18*, 1 (1974).

(11) Bands were observed at 436, 516, 558, and 596 nm.

(12) As the percent reduction approaches ~75%, there is evidence that both the ferric and ferrous forms exist in another form, as evidenced by the appearance of second averaged pyrrole-H signal. Since the intensity of this second signal is suppressed by $\mathrm{CN^-}$, we suggest it to be the $\mathrm{TPPFe(CN)(Me_2SO)^{0,-}}$ species.

(13) S. Andreades and E. W. Zahnow, *J. Am. Chem. Soc.*, **91**, 4181 (1969).

(14) J. S. Frye and G. N. La Mar, *J. Am. Chem. Soc.*, **97**, 3561 (1975).

(15) J. Del Gaudio and G. N. La Mar, unpublished observations.

(16) J. P. Collman, R. R. Gagne, C. A. Reed, T. R. Halbert, G. Land, and W. T. Robinson, *J. Am. Chem. Soc.*, **97**, 1427 (1975); the observation that introduction of $\mathrm{O_2}$ yields the oxo-bridged dimer in dry solvent indicates that water is not necessary for dimer formation.

(17) Fellow of the Alfred P. Sloan Foundation 1972–1976; Fellow of the John Simon Guggenheim Foundation 1975–1976.

John Del Gaudio, Gerd N. La Mar*[17]
Department of Chemistry, University of California
Davis, California 95616

Received September 15, 1975

Cotton Effect Behavior of Skewed 1,3-Cyclohexadienes. Evidence for Dominance of Homoannular Allylic Chirality Contributions

Sir:

According to the widely accepted diene chirality rule,[1] the inherent dissymmetry or skew sense of a nonplanar conjugated diene controls the long-wavelength (lowest-energy) $\pi \rightarrow \pi^*$ transition Cotton effect (positive for a right-handed diene helicity; negative for a left-handed one). Although apparently successful in explaining many cases, the rule does not adequately correlate rotational strength with skewness,[2] nor does it allow for effects of substituents.[3] Likewise, it cannot be applied without modification[4] to heteroannular cisoid dienes,[5] nor does it predict the nonconforming behavior of a number of substituted cyclohexadienes of unambiguous chirality, including several steroidal 19-nor-5(10),6-dienes[6] and various C-16 epimeric pentacyclic steroidal ring-E dienes.[7]

Here, as an extension of the allylic chirality concept[8] (see signs and arrows in drawings below) previously applied to heteroannular,[5] allylic oxygenated,[3b] and planar[9] dienes, we present evidence that the long-wavelength Cotton effects of skewed 1,3-cyclohexadienes *are determined primarily by chirality contributions of the homoannular allylic axial substituents or bonds according to their size or polarizability and not by the helicity or amount of twist of the chromophore.* Such a predominant role for homoannular allylic chirality is probably related to the electronic features of the 5,6 σ-bond that produce the characteristic bathochromic shifts in the uv absorption maxima of 1,3-cyclohexadienes.

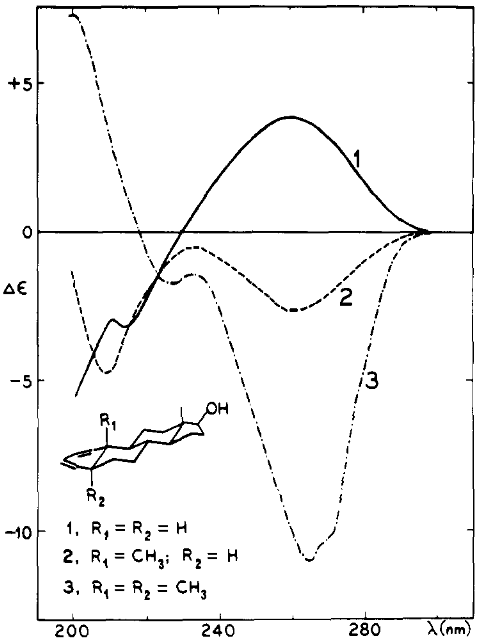
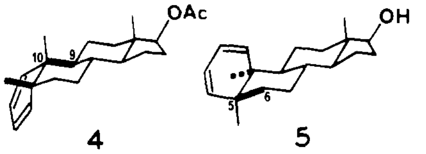


Figure 1. CD curves of 5α-estra-1,3-dien-17β-ol (**1**), 5α-androsta-1,3-dien-17β-ol (**2**), and 5α-methylandrosta-1,3-dien-17β-ol (**3**); measurements were made in hexane in a 1.0-cm cell with a Cary 6001 CD accessory unit on a Cary 60 recording spectropolarimeter.

P chirality M chirality
(enantiomeric 1,3-cyclohexadienes)

As seen in Figure 1, the 260-nm region Cotton effects of steroidal 5α 1,3-dienes show a direct dependence on the axial substituents at C-5 and C-10. The 5α-methyl diene **3**[10] exhibits a much more intense CD band ($\Delta\epsilon_{264}$ −11.1) than either the parent 5α diene **2**[3a,11] ($\Delta\epsilon_{260}$ −2.7) or the 19-nor 5α diene **1**[11] ($\Delta\epsilon_{260}$ +3.8), even though all three compounds probably have nearly the same C-2–C-3 diene torsional angle of ca. −17°.[12] These widely divergent Cotton effects indicate a significant difference in the chirality contributions by methyl and hydrogen, perhaps because of the greater anisotropic polarizability of an allylic C–alkyl bond compared to that of an allylic C–H bond.[13]

In the A/B cis steroid series even greater chirality effects are observed for allylic axial substituents larger or more polarizable than a methyl group. From their ORD amplitudes[10] dienes **4** and **5** can be estimated[14] to have $\Delta\epsilon_{264}$ +27.6 and +14.7, respectively. Both compounds have comparable P-diene chirality, but in the former the bulky C-9 tertiary allylic axial substituent at C-10 evidently makes an especially strong positive chirality contribution. In the latter



4 **5**
(allylic axial bonds intensified)