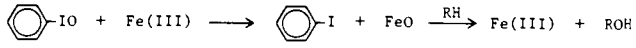
Reaction of Iron(III) Porphyrins and Iodosoxylene. The Active Oxene Complex of Cytochrome P-450

Sir:

Cytochromes P-450 are a unique class of hemoproteins that catalyze the hydroxylation of a wide variety of organic compounds. These enzymes are known to activate molecular oxygen by sequential two-electron reduction followed by O–O and C–H bond scission.[1,2] It has been proposed that an iron-bound carbene like "oxene" is involved in the oxygen-transfer process.[2] This FeO complex, having an overall charge of 3+ and a formal oxidation state of 5+, is formally equivalent to compound I of catalase (CAT) and peroxidase (HRP).[1] Indeed, cytochromes P-450 can function as a peroxidase and can catalyze organic peroxide supported hydroxylation of various substrates in the absence of NADPH and O_2.[3,4] Ullrich et al.[5] further demonstrated that cytochrome P-450 mediated hydroxylation can occur in the presence of iodosobenzene without the requirement for O_2 and NADPH. Presumably transfer of the oxygen atom is achieved via the following processes:



We here present evidence that regiospecific hydroxylation of C–H bonds by oxygen transfer can occur in iron porphyrin model systems.[6]

When a stoichiometric amount of 2-iodoso-*m*-xylene (chosen for solubility reasons) was added to octaethylporphinatoiron(III) chloride in $CHCl_3$ or CH_2Cl_2, the solution turned green within 1 min after mixing. When this was allowed to stand at room temperature, the green color slowly faded; this process can be hastened by using a large excess of iodosoxylene. The green compound has rather broad absorption peaks at 668 and 396 nm (Figure 1A) and the bleached solution has no absorptions in the visible region, indicating that the porphyrin chromophore has been ruptured.[7] Similar behavior can be observed in xylene or CH_2Cl_2–benzene, mixtures but attempts to detect hydroxylated hydrocarbons have not been successful. It appears that under these conditions the porphyrin ring acts as a far more reactive substrate toward the active species. In order to counteract the self-hydroxylation tendency of the hemin and to simulate a proximity effect commonly occurring in enzyme–substrate complexes, model compound **1** was synthesized.[8] In this "strapped" porphyrin, the length of the strap was chosen such that it has enough flexibility to swing aside to allow iodosoxylene molecules to interact with the heme iron. To further direct the iodosoxylene to approach only the strapped side of the hemin, the bare side of this molecule was shielded by taking advantage of a μ-oxo dimer (Fe–O–Fe) structure. Thus when the μ-oxo dimer of **1** was reacted with 2 equiv of iodosoxylene,[9] in addition to ring degradation products, one major pigment,[10] having an R_f value slightly lower than the unreacted starting material, could be isolated by TLC or HPLC. This compound was then subjected to mass spectral, IR, and NMR analyses after iron removal. The new porphyrin (16% overall yield from **1**, after correction for the

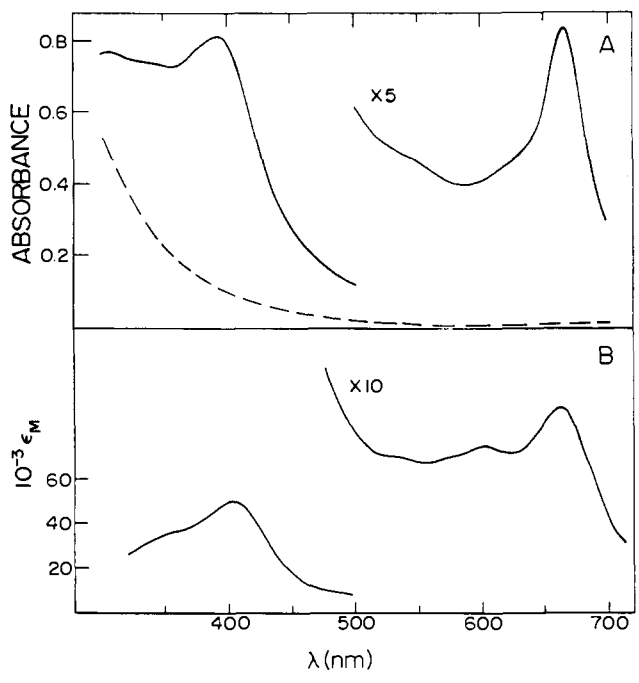


Figure 1. A: OEP Fe(III)Cl was mixed with excess 2-iodoso-*m*-xylene in CH_2Cl_2; the solutions was chilled at 1.5 min after mixing and the spectrum recorded at −45 °C (—) and after the solution was allowed to stand for 2 h at 23 °C (- - -) (protohemin chloride also gives similar spectra). B: spectrum of compound I of horse erythrocyte catalase.[12]

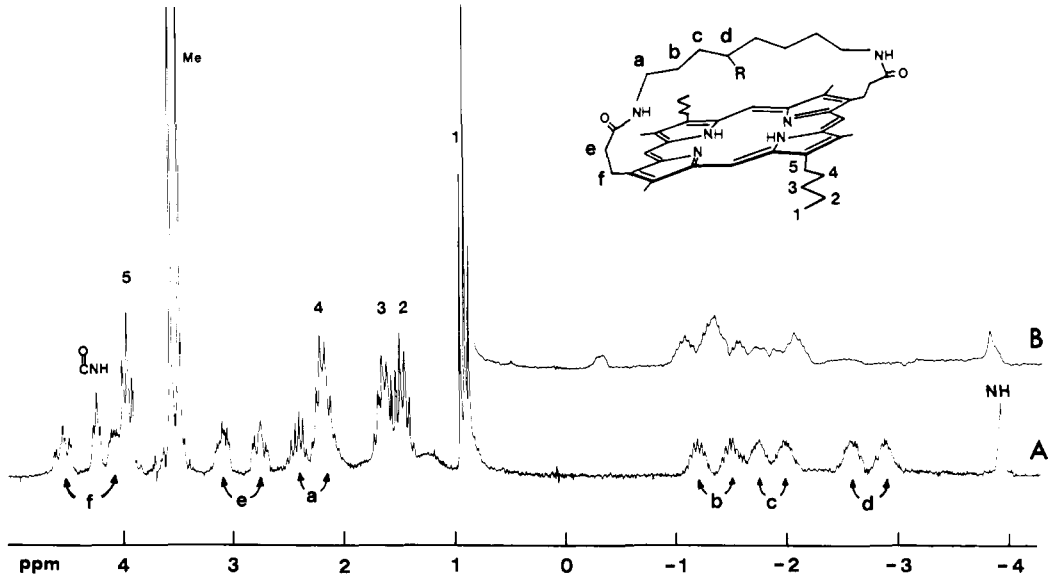


Figure 2. ^{1}H NMR ($CDCl_3$) of **1** (A, R = H) and the hydroxylated porphyrin (B, R = OH). Once the OH group attached to d, the methylene protons on the neighboring carbons were shifted downfield. The single α-H appeared at δ−0.3 but the hydroxy proton was not observed, probably obscured by the pentyl peaks. Spectra were recorded on a Bruker WH-180. The overlapping peaks of a and **4** have been clearly separated using a Bruker 360-MHz instrument at Brookhaven National Laboratory.

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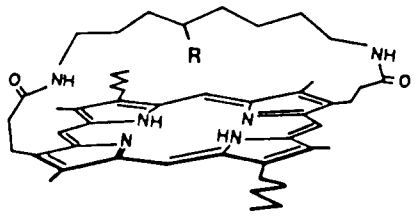

1 R= H

2 R= OH

unreacted starting material) has an absorption spectrum identical with that of **1**; it exhibits a molecular ion of mass number 774 (**1** + 16) and reacts with acetyl chloride to give an acetate (IR 1740 cm^{-1}), all indicating that it is a monohydroxyl derivative of the parent porphyrin. The position of hydroxylation was established by NMR. The strapped porphyrin **1** has a unique NMR spectrum in that the protons on b, c, and d carbons all have upfield chemical shifts (Figure 2A). The porphyrin diamagnetic ring current also causes all of the rigidly held methylene protons on the pendant chain to split into pairs. We have assigned all of the protons based on results obtained from exhaustive homonuclear decoupling. The spectrum of the hydroxylated product (Figure 2B) clearly shows that the signals arising from the d protons have been eliminated, and the overall pattern suggests that the OH group must attach to either of the two equivalent d carbons. This assignment is further strengthened by comparison with an authentic sample of **2** synthesized by an unambiguous route.[11] Analytical results including TLC R_f values and mass and NMR spectra showed that the two porphyrins are essentially identical.

When hemin chloride instead of the μ-oxo dimer of **1** was used, the yield of **2** was reduced in half (~9%), while the yield of degradation products increased substantially. We attribute this to the lack of protection structure in hemin chloride, which resulted in the formation of active oxygen atom at the bare side of the hemin. Molecular oxygen exerts no appreciable effect on the reaction; controlled reactions carried out under air, under carefully deoxygenated conditions, and in the presence of small amount of ethanol all produced the same amount of **2**. It is therefore concluded that the source of oxygen in the hydroxylated product must arise from iodosoxylene.

The success of this hydroxylation provides an unprecedented opportunity to examine the postulated iron-bound "oxene" species. Since the strapped hemin reacts with iodosoxylene to give also a green solution which turns brownish when hydroxylation goes to completion, it is safe to assume that the green intermediate may be the active oxene complex. Although the green compound is not stable in solution at room temperature, its stability is greatly enhanced when the temperature drops below −20 °C. The formation of this complex can be shown to be reversible: addition of degassed pyridine solution containing sodium dithionite yields the typical pyridine hemochrome spectrum. More significantly, the green compound has a remarkable spectral resemblance to compound 1 of catalase[12] (Figure 1B), suggesting that the electron configuration of the two may be the same.[13] Preliminary magnetic susceptibility measurements[14] of the green intermediate showed a μ_{eff} of 4.9 BM, which is consistent with a high-spin Fe(IV) structure.[15] It is interesting to note that recent NMR studies on compound 1 of HRP also reached a conclusion of having a high-spin ferryl ion.[16] While our present result that a compound-**1**-like species capable of transferring oxygen atom seems to suggest that a heme-bound oxygen atom with seven electrons

$(Fe^{IV}{:}\ddot{O}\cdot)$

could account for all the oxidizing equivalents and could indeed be the general structure for compound 1 intermediares of P-450, CAT, HRP, and chloroperoxidase,[17] the distinction among various possible "oxidation states" such as

$$(P)^{+\cdot}Fe^{IV}{:}\ddot{O}{:},^{18}\ (P)Fe^{IV}{:}\ddot{O}\cdot,\ \text{and}\ (P)Fe^{III}{:}\ddot{O}$$

must await further experiments.[20] It may be tempting to speculate that, since these forms differ merely in the localization of electron, the shift in electron density from porphyrin, through iron, to oxygen is a continuous function and should be strongly influenced by trans ligand effects as well as by medium effects. Studies directed along these lines should give insights concerning the identity of the active species.

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References and Notes

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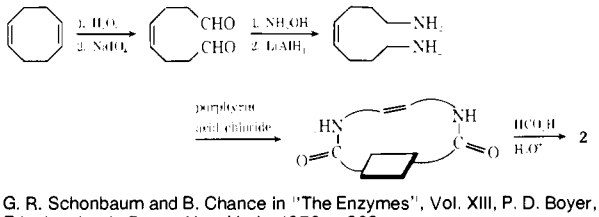
(2) G. A. Hamilton in "Molecular Mechanisms of Oxygen Activation", O. Hayaishi, Ed., Academic Press, New York, 1974, p 405.
(3) A. D. Rahimtula and P. J. O'Brien, *Biochem. Biophys. Res. Commun.*, **60**, 440 (1974).
(4) J. Gustafsson, E. G. Hrycay, and I. Ernster, *Arch. Biochem. Biophys.*, **174**, 440 (1976).
(5) F. Lichtenberger, W. Nastainczyk, and V. Ullrich, *Biochem. Biophys. Res. Commun.*, **70**, 939 (1976).
(6) For related nonenzymatic hydroxylation model systems, see J. T. Groves and M. Van der Puy, *J. Am. Chem. Soc.*, **98**, 5290 (1976). See also ref 21.
(7) Porphyrin degradation is a result of hydroxylation at the meso positions, followed by processes probably very similar to natural heme degradation pathways. A small amount of *meso*-hydroxy OEP has been detected in this reaction.
(8) The porphyrin was synthesized by condensation of 1,8-diaminooctane and the acid chloride of 2,6-dipentyldeuteroporphyrin II prepared by similar procedures reported earlier. C. K. Chang, *J. Am. Chem. Soc.*, **99**, 2819 (1977); C. B. Wang and C. K. Chang, *Synthesis*, in press.
(9) A typical reaction procedure is given here. The hemin (400 mg) was added to 80 mL of CH_2Cl_2 and then 200 mg of iodosoxylene was added. The mixture was stirred at room temperature for 2 h. The solution was then concentrated to proper volume for chromatography. Alternatively, the mixture was treated with $FeSO_4$ in hot HOAc to remove iron; the resultant porphyrins were then separated on silica gel. Normally 40% of the unreacted starting material can be recovered.
(10) Another hydroxylated product (2% yield) was also isolated. The visible spectrum of this green porphyrin is in accord with those of *meso*-hydroxy porphyrins. See P. S. Clezy in "The Porphyrins", Vol. II, D. Dolphin, Ed., Academic Press, New York, 1978, p 103. Its mass spectrum has an intense peak at mass number 773, arising from the stable oxophlorin ion. Presumably this compound was produced by hydroxylation of 1 through intermolecular reactions.
(11) Synthesized by the scheme shown (C. B. Wang and C. K. Chang, unpublished results). The dialdehyde is a known compound (A. Barco, S. Benetti, G. P. Pollini, and R. Taddia, *Org. Prep. Procedures Intl.*, 6, 217 (1974)).



(12) G. R. Schonbaum and B. Chance in "The Enzymes", Vol. XIII, P. D. Boyer, Ed., Academic Press, New York, 1976, p 363.
(13) In addition to the FeO structure, two possibilities exist for the green intermediate. Although coordination through iodine (Fe–I(O)–Ph) is highly unlikely for steric reasons, a heme–iodosoxylene complex with O coordination (Fe–OIPh) cannot be easily ruled out.
(14) Determined by the Evans method. Parallel spectral measurements indicated that the compound was at least 90% pure.
(15) A Fe(III) with $S = 3/2$ configuration is also possible (with spin-orbit coupling): J. Peisach, W. E. Blumberg, B. A. Wittenberg, and J. B. Wittenberg, *J. Biol. Chem.*, **243**, 1871 (1968).
(16) I. Morishima and S. Ogawa, *Biochem. Biophys. Res. Commun.*, **83**, 946 (1978); also *J. Am. Chem. Soc.*, **100**, 7125 (1978). These authors argued against the porphyrin π-cation-radical formulation[18] on the assumption that, if there were unpaired electron on the porphyrin ring, the ring methyl

peaks should be shifted and broadened, not sharp peaks as observed. This argument, however, is erroneous. According to the original proposal,[18] the porphyrin cation radical of compound I of HRP is in the $^2A_{2u}$ state which has spin density only on the meso carbons and the nitrogen atoms;[19] therefore, the methyl groups attached to the β positions should not be affected (J. Fajer, L. K. Hanson, and C. K. Chang, manuscript in preparation.)

(17) L. P. Hager, D. Doubek, and P. Hollenberg in "Molecular Basis of Electron Transport", J. Schultz and B. F. Cameron, Ed., Academic Press, New York, 1972, p. 367.

(18) D. Dolphin, A. Forman, D. G. Borg, J. Fajer, and R. H. Felton, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 614 (1971).

(19) J. Fajer, D. C. Borg, A. Forman, D. Dolphin, and R. H. Felton, *J. Am. Chem. Soc.*, **92**, 3451 (1970).

(20) EPR and Mössbauer measurements of the green intermediate are in progress.

(21) J. T. Groves, T. E. Nemo, and R. S. Myers, *J. Am. Chem. Soc.*, **101**, 1032 (1979), have recently reported similar hydroxylation reactions using iodosobenzene. It may be worthwhile to point out that Fe^{III} TPP is a superior catalyst to Fe^{III} OEP since TPP is more stable toward meso cleavage.

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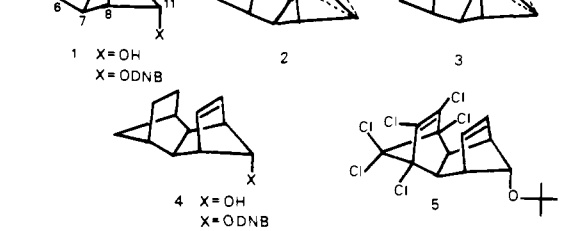
Nonconjugated Charge Delocalization by Remote π Electrons via Relay through a Double Bond Proximate to a Solvolysis Center

Sir:

Diene **1** is of considerable fundamental interest regarding our understanding of factors which influence chemical reactivity because of its remarkably high reaction rate in solvolysis. Comparison of **1** with the historic *anti*-7-norbornenyl and 7-norbornyl systems[1] shows the relative rate ratio to be 10^{14}:10^{11}:1 (at 25 °C), respectively.[2] The enormous reactivity of **1** was attributed to π-electron participation of the remote C_4C_5 double bond by relay through the C_9C_{10} double bond to give the extensively charge delocalized carbocation **2**.[2] Theoretical calculations agree with this and predict **2** to have considerable extra stabilization.[3] However, it has been claimed by Paquette and Dunkin[4] that, owing to leveling of the rate ratios, the three-carbon $C_9C_{10}C_{11}$ network shown in **3** prevails and $C_4{=}C_5$ *does not anchimerically assist ionization.*

We find the latter interpretation to be highly questionable. It appears that "leveling of rate ratios" refers to the observation that a diene–monoene combination like **1** and **4** has the same rate within a factor of ~2.[4] However, the Paquette cases[4] and **1** and **4** (vide infra) all show *large rate* enhancements of 200–1000 (depending on the temperature of comparison) over the *anti*-7-norbornenyl system. Apparently this fact was not



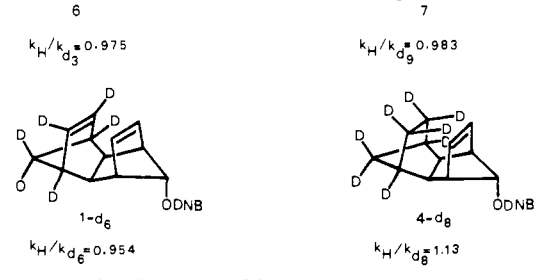
considered to be important.[4] Our reevaluation of the status of the whole problem has prompted us to initiate a detailed investigation seeking new experimental information.

The logical first step is to see if the $C_4{=}C_5$ moiety is involved in the rate-determining step of the solvolysis reaction. Study of secondary deuterium isotope effects has proven to be a very informative investigative tool in this connection.[5] Accordingly, **1**-ODNB and 3,4,5,5,6,12,12-**1**-ODNB-d_6 were prepared for such examination. Monoenes **4**-ODNB and 3,4,4,5,5,6,12,12-**4**-ODNB-d_8 also were obtained for comparison purposes.

Known compound **5**[6] was converted to **1**-OH and **4**-OH. It was found to be advantageous to remove the *tert*-butyl group from **5** with $HClO_4$ in THF and protect the OH function as the tetrahydropyranyl ether by reaction with dihydropyran in dry ether containing a trace of HOTs. Dechlorination of the pyranyl ether with sodium in dry THF–*t*-BuOH, removal of the protecting group with aqueous $HClO_4$–THF, washing of a combined ether solution of crude product with water and then aqueous $NaHCO_3$, followed by evaporation of the ether, and recrystallization of the oil from hexane gave **1**-OH.[7] An ether solution of the residue was extracted with aqueous $AgNO_3$ and treated with aqueous $HClO_4$, followed by aqueous $NaHCO_3$, and the ether was removed. Recrystallization of this concentrate from hexane afforded **4**-OH.[7] Preparation of **1**-OH-d_6 and **4**-OH-d_8 was achieved by using sodium in dry THF–*t*-BuOD[8] in the dechlorination step; deuterium incorporation was >97%.[7] The desired dinitrobenzoates were obtained from reaction of the corresponding alcohols with 3,5-dinitrobenzoyl chloride in pyridine.[7]

Solvolysis studies were carried out in 80% aqueous dioxane at 85.00 ± 0.02 and 87.00 ± 0.01 °C for **1**-ODNB and **4**-ODNB, respectively. Product analyses after ~10 reaction half-lives by GC and NMR showed only formation of anti-retained alcohols from both diene and monoene systems. Kinetic measurements were made by following the changes in conductivity with a Radiometer Model CDM3 conductivity meter.[9] Five concurrent **1**-ODNB and **1**-ODNB-d_6 runs were made in the same constant-temperature bath with *carefully* matched conductivity cells which had been degassed (N_2) and sealed.[10a] In each successive run the cells used for **1**-ODNB and **1**-ODNB-d_6 were switched. The rate constants were calculated from the standard first-order rate law using a least-squares computer program. Average values were $k_H = 6.627 \times 10^{-5}$ s^{-1} and $k_D = 6.946 \times 10^{-6}$ s^{-1} with $k_H/k_D = 0.954 \pm 0.001$.[10a] For **4**-ODNB and **4**-ODNB-d_8 four separate concurrent rate measurements were made in the same way.[10b] Average values were $k_H = 6.662 \times 10^{-5}$ s^{-1} and $k_D = 5.920 \times 10^{-5}$ s^{-1} with $k_H/k_D = 1.13 \pm 0.02$.[10b] A rate constant for **4**-ODNB also was determined at 85.00 °C for comparison purposes; $k = 4.23 \times 10^{-5}$ s^{-1}. The **1**-ODNB:**4**-ODNB rate ratio is 1.57:1.

Rate effects on limiting solvolysis reactions caused by deuteration of remote saturated C–H positions not conjugated with the reaction site ordinarily show inverse isotope effects.[5a,11] Solvolyses of **6**[11] and **7**[5a] in 80% aqueous EtOH at 25 °C illustrate typical γ and δ effects. These kinds of k_H/k_D



values are ascribed to normal inductive interaction which attenuates by a factor of ~3 for each intervening saturated carbon and becomes minuscule per D at the δ position and almost nil beyond that.[5a] Calculations based on this for **1**-ODNB-d_6 predicts k_H/k_D to be essentially unity (>0.998). It is evident that the inverse isotope effect found with **1**-ODNB-d_6 is significantly larger than can be accounted for by an inductive factor.

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