were not able to isolate or detect **11**. Instead, we found that this reaction gave a 76% yield of **5**. It is presumed that **11** rearranges to **5** via a tight ion-pair mechanism under the conditions of its generation. This again would imply that the α-cyano-substituted cation is more stable than the β-cyano-substituted cation.[12,13]

In summary, we have demonstrated that the H/α-CN rate ratio is approximately 10^3 in contrast to the value of 10^5 observed for a H/β-CN rate ratio in the same system. These experimental results are in agreement with our earlier theoretical calculations. We are continuing our studies in this area.

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Supplementary Material Available: Tables of bond distances, bond angles, thermal parameters, and atom coordinates (5 pages). Ordering information is given on any current masthead page.

(12) An added factor which must be taken into consideration in the discussion is the relative thermodynamic stabilities of the adamantyl and protoadamantyl skeletons.

(13) The extremely mild conditions under which **10** gave **5** indicate that the equilibration of **5** and **11** (via a tight ion pair) occurs with great ease. The failure to isolate any **11** may be due to the selective crystallization of **5** with a resulting total conversion of **11** to **5**. Under equilibrating conditions, the concentration of **11** must be small since its presence is not obvious in solution spectra of **5**.

(14) Proctor and Gamble Fellow, 1977–1978; University of Minnesota Dissertation Fellow, 1978–1979.

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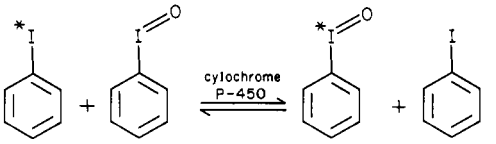
Enzymatic Monooxygenation of Halogen Atoms: Cytochrome P-450 Catalyzed Oxidation of Iodobenzene by Iodosobenzene

Sir:

Cytochromes P-450 are a class of hemoproteins that catalyze the monooxygenation of a variety of organic compounds.[1] The cytochrome P-450 containing mixed-function oxidase systems are thought to activate molecular oxygen via two sequential one-electron reductions, resulting in a highly reactive carbenelike FeO species.[2] A species presumed to be this FeO complex has been obtained with rat liver microsomes without a requirement for O_2 and NADPH, using iodosobenzene as an oxygen atom source.[3] Hydroxylated products have been observed from the reaction of iron porphyrin complexes with iodosobenzene or iodosoxylene.[4] It has been postulated that these hydroxylations also proceed via an FeO species.

Recently we have explored the possibility that cytochrome P-450 mixed-function oxidases metabolize some halogenated compounds via an initial halogen oxidation to form an R—X=O intermediate. Evidence has been presented that at least part of the metabolism of 1,2-dichloroethane by P-450 may proceed through a chlorine oxide (chloroso) intermediate.[5]

A system in which formation of a halogen oxide might easily be demonstrated is the P-450-catalyzed oxygenation of iodobenzene. As mentioned earlier, iodosobenzene has been used as an oxygen atom source for P-450 and, in principle, this reaction should be reversible. To investigate this possibility, we have studied the equilibration between unlabeled iodosobenzene and [^{125}I]-iodobenzene.[6]



One milliliter of a pH 7.4 solution 0.22 μM in P-450 (purified to apparent homogeneity from phenobarbital-induced rat liver),[7] 5 mM in iodosobenzene, 1 mM in [^{125}I]iodobenzene (0.9 μCi/μmol), and 0.1 mM in EDTA was incubated at 37 °C for 2 min. The incubation mixture was then cooled to 0 °C and 50 μL of 1 M $ZnSO_4$ was added. After the mixture stood at 0 °C for 10 min, 100 μL of acetic anhydride was added to convert the iodosobenzene to iodobenzene diacetate [PhI(OAc)$_2$].[8] After this mixture stood at 23 °C for an additional 30 min, a 50-μL aliquot of the mixture was analyzed by high-performance LC by using two 30-cm μ-Bondapak phenyl columns connected in series with 2.5:1 methanol–water (1.75 mL/min) as the mobile phase. Fractions were collected and assayed in a liquid scintillation spectrometer. Under these conditions 14.0 nmol of [^{125}I]PhI(OAc)$_2$ was formed.

The amount of labeled PhI(OAc)$_2$ detected was a nonlinear function of the cytochrome P-450 concentration. Thus 0.11 μM P-450 yielded 10 nmol of [^{125}I]PhI(OAc)$_2$, and 1.1 μM P-450 produced 62 nmol of the labeled diacetate.[9] At higher P-450 concentrations, however, the high-performance LC peak corresponding to PhI(OAc)$_2$ was diminished and the radioactivity became more diffusely spread throughout the chromatogram. An incubation with a P-450 concentration of 2.6 μM gave no more than 30 nmol of labeled PhI(OAc)$_2$. Since iodosobenzene is a strong oxidizing agent, it is to be expected that some will be lost as a result of protein or heme oxidation. As the concentration of P-450 was increased (and therefore the concentration of possible reducing agents for iodosobenzene increased), the amount of iodosobenzene surviving the incubation decreased. In the absence of cytochrome P-450 no radioactivity was detected in the PhI(OAc)$_2$ peak.

Iodobenzene diacetate has also been used as an oxygen source for P-450.[3,10] Presumably the diacetate is hydrolyzed in situ to iodosobenzene. When unlabeled PhI(OAc)$_2$ was substituted for iodosobenzene in the incubation mixture containing 0.22 μM P-450, 178 nmol of [^{125}I]PhI(OAc)$_2$ was detected. However, if acetic anhydride was not added in the workup of the incubation mixture, there was no radioactivity in the PhI(OAc)$_2$ peak. Gustafsson et al.[10] report the (diacetoxyiodo)benzenes to be more efficient oxygen donors than the corresponding iodosobenzene.

Denaturing the P-450 by heating in 1% sodium dodecyl sulfate and removing the detergent blocked the oxygen transfer. Also, when the heme was removed from the protein by treatment with

(1) Coon, M. J.; Vermilion, J. L.; Vatsis, K. P.; French, J. S.; Dean, W. L.; Haugen, D. A. *A.C.S. Symp. Ser.* **1976**, *No. 44*, 46–71. Sato, R.; Omura, T. In "Cytochrome P-450"; Academic Press: New York, 1978.

(2) Hamilton, G. A. In "Molecular Mechanisms of Oxygen Activation"; Hayaishi, Ed.; Academic Press: New York, 1974; pp 405–451.

(3) Lichtenberger, F.; Nastainczyk, W.; Ullrich, V. *Biochem. Biophys. Res. Commun.* **1976**, 939. Gustafsson, J.-Å.; Bergman, J. *FEBS Lett.* **1976**, *70*, 276.

(4) Chang, C. K.; Kuo, M.-S. *J. Am. Chem. Soc.* **1979**, *101*, 3413. Groves, J. T.; Nemo, T. E.; Myers, R. S. *Ibid.* **1979**, *101*, 1032.

(5) Guengerich, F. P.; Crawford, W. M., Jr.; Domoradzki, J. Y.; Macdonald, T. L.; Watanabe, P. G. *Toxicol. Appl. Pharmacol.* **1980**, *53*, 303.

(6) Prepared by adding K^{125}I to diazotized aniline. The [^{125}I]iodobenzene was isolated by short-column chromatography on silica gel with pentane as solvent. The material was homogeneous by high-performance LC analysis.

(7) Guengerich, F. P. *J. Biol. Chem.* **1978**, *253*, 7931.

(8) This was done since we were unable to separate iodobenzene cleanly from iodosobenzene with any high-performance LC system. The conversion to PhI(OAc)$_2$ resulted in a 7.5-min separation of PhI and PhI(OAc)$_2$ peaks.

(9) Iodophenols, the expected hydroxylation products, could not be detected with certainty in the high-performance LC as they eluted too near the void volume. Although there was no clearly discernible peak, from the amount of radioactivity in the region of the *p*-iodophenol retention time, there could be as much as 9 nmol of iodophenol formed in the 1.1 μM P-450 incubation.

(10) Gustafsson, J.-Å.; Rondahl, L.; Bergman, J. *Biochemistry* **1979**, *18*, 865.

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acetone–HCl[11] no radioactivity was found in the $PhI(OAc)_2$ peak. Three other hemoproteins, cytochrome P-450 from β-naphthoflavone-induced rat livers,[12] horseradish peroxidase, and catalase, caused no detectable increase in radioactivity in the $PhI(OAc)_2$ peak. Free heme (ferriprotoporphyrin IX) did not catalyze the reaction. Thus, the oxygen transfer appears to be specific for cytochrome P-450 and specificity within the group of cytochromes P-450 exists. Specificity among the cytochromes P-450 has been observed for some substrates but not others.[1,13]

The results of these experiments indicate that oxygen transfer from cytochrome P-450 to a halogen is, in fact, a possible mode of reaction. In aromatic systems epoxidation of the ring is probably favored over oxygenation of bromine or chlorine substituents and there seems to be no reason to postulate halogen oxides as intermediates in aryl hydroxylation. However, halogen oxidation may offer an alternative to oxygen insertion into carbon–hydrogen bonds in aliphatic halocarbons. The iodobenzene–iodosobenzene equilibration described here offers further proof that the actual oxygenating species in cytochrome P-450 is a monoxygenated and not a dioxygenated species. However, an iodine coordinated species, similar to that suggested by Groves et al.[4] for the iron porphyrin system, cannot be ruled out as the intermediate in the oxygen transfer from iodosobenzene to iodobenzene.[14]

(11) Guengerich, F. P.; Ballou, D. P.; Coon, M. J. *J. Biol. Chem.* **1975**, *250*, 7405.
(12) This protein is apparently identical with the major form of P-450 isolated from 3-methylcholanthrene-treated rat liver.[7]
(13) Haugen, D. A.; van der Hoeven, T. A.; Coon, M. J. *J. Biol. Chem.* **1975**, *250*, 3567. Guengerich, F. P. *Ibid.* **1977**, *252*, 3970.
(14) This work was supported by Grants ES00267 and ES01590 to Vanderbilt University from the U.S. Public Health Service.

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Enhanced Stability of the Imidazolate-Bridged Dicopper(II) Ion in a Binucleating Macrocycle

Sir:

There is great current interest in metal complexes of binucleating ligands[1,2] as models[3] for the coordination environments of metallobiomolecules such as hemocyanin,[4] cytochrome *c* oxidase,[5] and bovine erythrocyte superoxide dismutase (BESOD).[6] Previously we reported[7] the synthesis and structure of $[Cu_2(imH)_2(im)\subset A](ClO_4)_3$ (**1**)[8] which has features analogous to BESOD. Here we show two important new aspects of the chemistry and stability of the related complex $[Cu_2(im)\subset A']^{3+}$ (**2**). The first is the marked hydrolytic integrity of the imidazolate bridge in **2** compared to the corresponding $[(TMDT)_2Cu_2(im)]^{3+}$ complex. The second, and the more unusual, aspect is the spontaneous formation of **2** from the 1:1 complex of A′ with copper(II) in the presence of 1 equiv of imidazole. This insertion of two copper(II) ions into the same binucleating macrocycle to form the imidazolate-bridged dicopper(II) unit is closely parallel to the pH-dependent migration of copper(II) to the vacant zinc-binding site observed for zinc-free BESOD.[9]

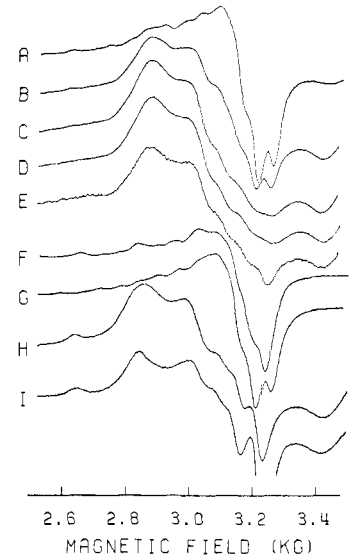
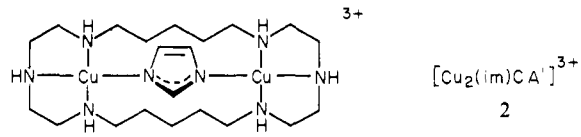


Figure 1. Electron spin resonance spectra of frozen 50% aqueous Me_2SO solutions of $[Cu_2(im)\subset A'](ClO_4)_3 \cdot H_2O$ at 115 K as a function (see ref 20) of pH [(A) 5.6, (B) 6.2, (C) 7.1, (D) 10.4, (E) 11.5] and at 77 K at various Cu–A′–imH–H^+ ratios [(F) 1:1:0:0, (G) 2:1:0:0, (H) 1:1:1:0, (I) 1:1:1:1]. Samples were run in degassed solvents using ~5 mM macrocyle concentrations on a Varian E line X-band spectrometer. Instrument settings were 10 mW of microwave power, 5-G modulation amplitude, and 125 G min^{-1} scan rate.



The ligand was prepared by reaction of equimolar quantities of the disodium salt of *N,N′,N″*-tris(*p*-tolylsulfonyl)diethylenetriamine[10,11] and 6,9,12-tris(*p*-tolylsulfonyl)-6,9,12-triazaheptadecane-1,17-bis(methanesulfonate)[12] (**4**) in DMF at 90 °C for 2 h. The crude product, isolated upon addition of H_2O, was chromatographed with $CHCl_3$ on silica to give 1,4,7,13,16,19-hexatosylhexaazacyclotetracosane as a white solid in 20% yield. Detosylation[11] was achieved by using 97% H_2SO_4 at 100 °C for

(1) Groh, S. E. *Isr. J. Chem.* **1976–1977** *15*, 277–307.
(2) (a) Gagné, R. R.; Kreh, R. P.; Dodge, J. A. *J. Am. Chem. Soc.* **1979**, *101*, 6917–6927. (b) Gunter, M. J.; Mander, L. N.; McLaughlin, G. M.; Murray, K. S.; Berry, K. J.; Clark, P. E.; Buckingham, D. A. *Ibid.* **1980**, *102*, 1470–1473. (c) Drew, M. G. B.; McCann, M.; Nelson, S. M. *J. Chem. Soc., Chem. Commun.* **1979**, 481–482. (d) Bulkowski, J. E.; Burk, P. L.; Ludmann, M-F.; Osborn, J. A. *Ibid.* **1977**, 489–499. (e) Agnus, Y.; Louis, R.; Weiss, R. *J. Am. Chem. Soc.* **1979**, *101*, 3381–3384. (f) Gisselbrecht, J. P.; Gross, M.; Alberts, A. H.; Lehn, J. M. *Inorg. Chem.* **1980**, *19*, 1386–1388.
(3) Ibers, J. A.; Holm, R. H. *Science* **1980**, *209*, 223–235.
(4) Himmelwright, R. S.; Eickman, N. C.; Lubien, C. D.; Solomon, E. I. *J. Am. Chem. Soc.* **1980**, *102*, 5378–5388.
(5) Palmer, G.; Babcock, G. T.; Vickery, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73* 2206–2210.
(6) Richardson, J. S.; Thomas, K. A.; Rubin, B. H.; Richardson, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 1349–1353.
(7) Coughlin, P. K.; Dewan, J. C.; Lippard, S. J.; Watanabe, E-i.; Lehn, J.-M. *J. Am. Chem. Soc.* **1979**, *101*, 265–266.
(8) Abbreviations: A = 1,4,7,13,16,19-hexaaza-10,22-dioxacyclotetracosane; A′ = 1,4,7,13,16,19-hexaazacyclotetracosane; imH, imadazole; im, imidazolate anion; TMDT, 1,1,7,7-tetramethyldiethylenetriamine; DMF, dimethylformamide; Me_2SO, dimethyl sulfoxide.
(9) Valentine, J. S.; Pantoliano, M. W.; McDonnell, P. J.; Burger, A. R.; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 4245–4249.
(10) Richman, J. E.; Atkins, T. J. *J. Am. Chem. Soc.* **1974**, *96*, 2268–2270.
(11) Atkins, T. J.; Richman, J. E.; Oettle, W. F. *Org. Synth.* **1978**, *58*, 86–97.
(12) Reaction of 2 equiv of 5-(tetrahydropyran-2-yloxy)pentane-1-methanesulfonate (**5**) with **3** in DMF gave the ditetrahydropyranyloxy derivative of **4**. Hydrolysis of the tetrahydropyranyl groups with *p*-toluenesulfonic acid in 95% EtOH[13] afforded the diol, which was then allowed to react with methanesulfonyl chloride[14] to generate **4**. Compound **5** was obtained by reaction of 2,3-dihydropyran with threefold excess of 1,5-pentanediol.[15] Extraction into benzene followed by vacuum distillation gave the pure monotetrahydropyranyloxy derivative, which was then mesylated to give **5**.
(13) Dauben, W. G.; Bradlow, H. L. *J. Am. Chem. Soc.* **1952**, *74*, 559–560.
(14) Crossland R. K.; Servis, K. L. *J. Org. Chem.* **1970**, *35*, 3195–3196.
(15) Cowie, J. S.; Landor, P. D.; Landor, S. R. *J. Chem. Soc., Perkin Trans. 1.* **1973**, 720–724.

0002-7863/80/1502-7616$01.00/0