Table I. Relative Reactivities of Homocuban-9-ylidene (**1**) Compared to That of PhCCl[8]

reactant	**1** (25 °C)	PhCCl (25 °C) (k, M^{-1} s^{-1})
methanol	3.3	3.8 (1.3×10^9)
tetramethylethylene	1.9	0.2–0.4 ($(0.7–1.2) \times 10^8$)
pyridine	1.0	1.0 (3.4×10^8)

Stern–Volmer analysis gives the relative reactivities of **1** toward various quenchers (Table I). The selectivity of **1** is comparable to that of chlorophenylcarbene[8] and is rather insensitive to temperature.

The relative reactivity of carbene **1** toward CH_3OD and tetramethylethylene determined by LFP and product analysis is in good agreement; $k_2/k_{tme} = 2.2$ (chemical analysis) and 1.7 (LFP). The relative rate data obtained by LFP used pyridine and CH_3OD concentrations (>0.2 M) sufficient to ensure complete oligomerization of the alcohol and were therefore consistent with the conditions used in the product analysis.

At [pyridine] = 0.05 M, the observed absolute rate constant for formation of ylide **8a**, k_{obs}, is given by eq 6.[12]

$$\frac{k_{pyr}[\text{pyridine}]}{K+1} = k_{obs} \text{ or } K = \frac{k_{pyr}[\text{pyridine}]}{k_{obs}} - 1 \qquad (6)$$

The maximum value of K is defined by the maximum possible value of k_{pyr} ($\leq 5 \times 10^9$ M^{-1} s^{-1})[13] and the minimum value of k_{obs} ($\geq 5 \times 10^7$ s^{-1}) determined by LFP. Thus, at +25 °C, K is bracketed as in eq 7.[15]

$$0.23 \leq K \leq 4 \qquad (7)$$

Supplementary Material Available: Derivations for eqs 4–6, preparation of **5**, plots of the dependence of the products formed from **1** and **2** on [CH_3OD] for the decomposition of both **3** and **5**, transient spectra of **8a,b**, the absolute rate of formation of **8a**, and Stern–Volmer plots (14 pages). Ordering information is given on any current masthead page.

(14) Values of k_{pyr} for several carbenes are known[10] and never exceed 5 $\times$ 10^9 M^{-1} s^{-1}.

(15) Any reaction of **2** with pyridine to produce an invisible dipole (**9**, for example) will raise this value. This is not considered likely as the yield of **9a/9b** is comparable to that realized with other carbenes.

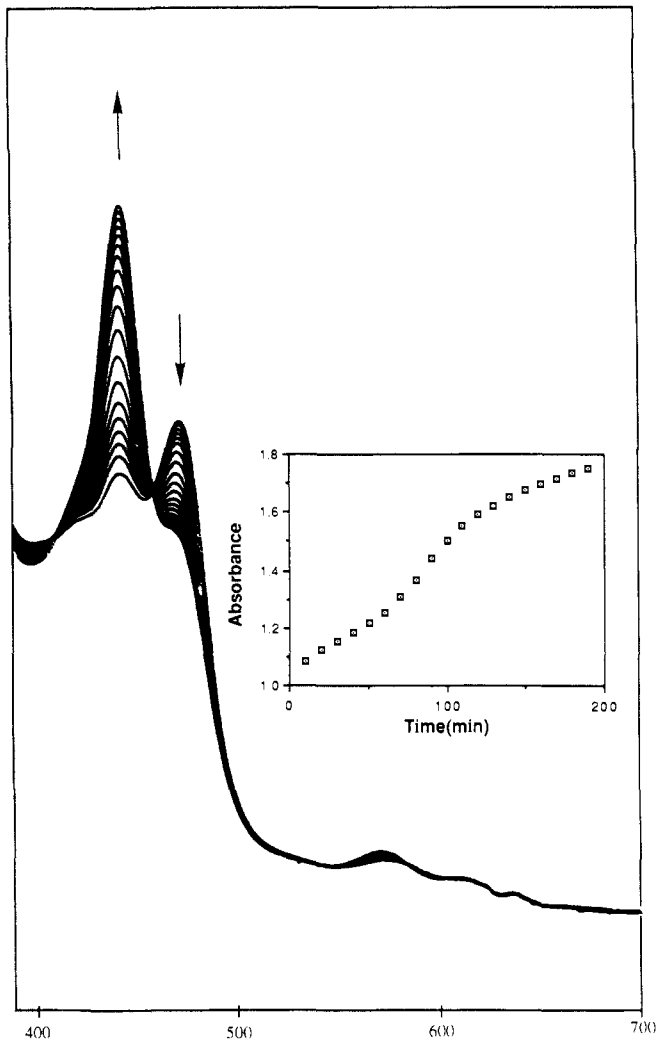

Figure 1. Visible spectral changes observed in a 0.5-cm cuvette during the anaerobic reduction of Mn^{III}(ChPCl) (I) (2.4×10^{-5} M) by a pyruvate (4.2×10^{-2} M)/pyruvate oxidase (4.8×10^{-7} M) system in the presence of amphiphilic flavin (II) (4.8×10^{-5} M) in DPPC (2.4×10^{-3} M) vesicles at pH 6 (1.6 mL of 0.05 M potassium phosphate buffer containing 0.01 M $MgCl_2$ and 0.1 M KCl). Measurements were taken every 10 min after the addition of sodium pyruvate. Inset: time course of the reaction monitored by the increase in absorbance at 440 nm.

Biocompatible Catalysis. Enzymic Reduction of Metalloporphyrin Catalysts in Phospholipid Bilayers

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Received January 8, 1990
Revised Manuscript Received July 2, 1990

Models of the active-site chemistry of heme-containing monooxygenases such as cytochrome P-450 have attracted sustained attention for over a decade. Comparisons of the enzyme and synthetic metalloporphyrins have established that the catalytic cycle involves substrate binding at the active site, reduction of the unusual iron(III) thiolate to iron(II), and binding and reductive cleavage of molecular oxygen to generate a high-valent iron–oxo species responsible for substrate oxidation.[1] The two electrons required for oxygen activation are provided by NADPH through a coupled electron transfer flavoprotein, cytochrome P-450 reductase, and/or cytochrome b_5,[2a]

Cytochrome P-450 and the reductases are integral membrane proteins.[2b] While numerous catalytic systems mimicking the oxygen-transfer step of cytochrome P-450 have been described, model systems designed to understand the electron-transfer steps and the role of the membrane environment in the overall enzymatic process are few in number.[3,4] We have recently reported the design and characterization of a membrane-spanning porphyrin that associates with phospholipid bilayers and catalyzes the oxygenation of sterols and polyunsaturated fatty acids with remarkable regioselectivity.[4] We describe here a multicomponent catalytic vesicular assembly in which electrons derived from the enzymic decarboxylation of pyruvic acid serve to reduce a synthetic, membrane-spanning manganese(III) cholesteryl porphyrin and to mediate subsequent oxygen activation and transfer.

(1) (a) McMurry, T. J.; Groves, J. T. In *Cytochrome P-450, Structure, Mechanism, and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1986; pp 1–28. (b) Sligar, S. G.; Murray, R. I. *Ibid.* p 443–479. (c) Guengerich, F. P.; Macdonald, T. L. *Acc. Chem. Res.* **1984**, *17*, 9–16. (d) White, R. E.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 315–356. (e) Groves, J. T. *Adv. Inorg. Biochem.* **1979**, 119–145.

(2) (a) Peterson, J. A.; Prough, R. A. In *Cytochrome P-450, Structure, Mechanism, and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1986; pp 89–11. (b) Ingelman-Sundberg, M. *Ibid.* p 119. (c) Lambeth, J. D.; Kamin, H. *J. Biol. Chem.* **1976**, *251*, 4299. (d) Pompon, D.; Coon, M. J. *J. Biol. Chem.* **1984**, *259*, 15377.

(3) van Esch, J.; Roks, M. F.; Nolte, R. J. M. *J. Am. Chem. Soc.* **1986**, *108*, 6093.

(4) (a) Groves, J. T.; Neumann, R. *J. Am. Chem. Soc.* **1989**, *111*, 2900. (b) Groves, J. T.; Neumann, R. *J. Org. Chem.* **1988**, *53*, 3891. (c) Groves, J. T.; Neumann, R. *J. Am. Chem. Soc.* **1987**, *109*, 5045.

0002-7863/90/1512-7796$02.50/0

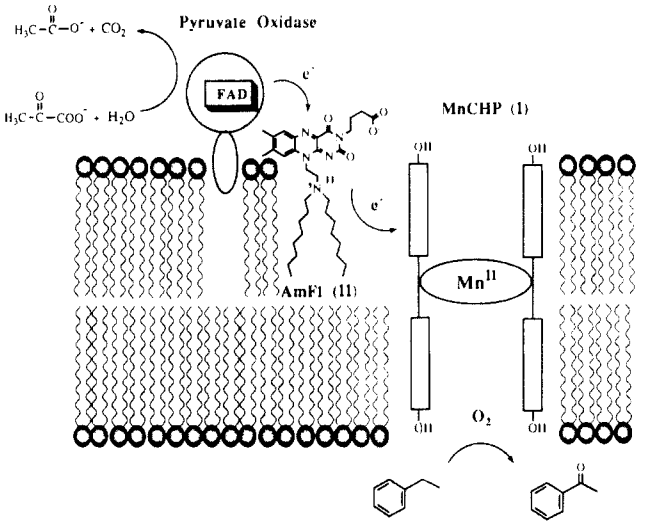

Figure 2. Schematic representation of biocompatible catalysis in a synthetic multicomponent redox membrane containing pyruvate, pyruvate oxidase (PO), amphiphilic flavin (II), Mn^{III}ChPCl (I), ethylbenzene, and DPPC.

The membrane-spanning tetrakis[*o*-(3-hydroxy-5-cholenoylamino)phenyl]porphyrin (H_2ChP) and its manganese(III) derivative (MnChPCl, I) were prepared as we have previously described.[4] The amphiphilic flavin (AmFl, II) was synthesized from 6,7-dimethyl-9-formylisoalloxazine[5a] by reductive amination with *N,N*-dioctylamine, subsequent N-alkylation with ethyl 4-bromobutyrate, and acid hydrolysis of the ethyl ester.

The reduction of Mn^{III}ChPCl to Mn(II) (I) in dipalmitoylphosphocholine (DPPC) vesicles[4] was followed by changes in the visible absorption spectrum following the injection of oxygen-free sodium pyruvate (Figure 1). Under these conditions the time course of the reduction of Mn(III) to Mn(II) was found to be triphasic (Figure 1 inset). No reduction of Mn(III) was observed in the absence of the enzyme, and only traces of Mn(II) were observed over a 24-h period if the amphiflavin (II) was omitted or was replaced by flavin adenine dinucleotide (FAD). Furthermore, in a separate experiment, we found that the pyruvate oxidase/pyruvate system reduced the amphiphilic flavin (II) rapidly in vesicles lacking Mn(ChP)Cl. Thus, we conclude that the rate-determining step in this reduction is electron transfer from AmFl (II) to Mn^{III}ChPCl.

Next we investigated the enzymic reduction of I in the presence of molecular oxygen and ethyl benzene. Vesicles containing 40 μmol of DPPC, 10 μmol of ethylbenzene, 0.1 μmol of Mn(ChP)Cl, 0.2 μmol of amphiflavin (II), and 4 μmol of *N*-methylimidazole were prepared by sonication in 4 mL of buffer solution. Following the addition of 2 nmol of pyruvate oxidase, the reaction mixture was allowed to stand at 30 °C for 15 h. Products were isolated as we have previously described and analyzed by GC and GC–MS vs authentic samples. Acetophenone was obtained in 20% conversion, representing 20 turnovers of the Mn(III) catalyst. In separate experiments we found that no oxygenation took place when any of the components was omitted.

These results can be explained as follows. Pyruvate oxidase catalyzes the oxidative decarboxylation of pyruvate to acetate and carbon dioxide and the concomitant reduction of the tightly bound FAD cofactor to $FADH_2$.[6,7] Thiamine pyrophosphate and Mg^{2+} are known to be cofactors of this reaction. The enzyme has been shown to bind to phospholipid vesicles when the cofactors and the substrate are simultaneously present.[8] It has been suggested that a change in the conformation of the protein exposes a membrane-binding hydrophobic peptide segment.[9] It is expected that the amphilic flavin (II) will bind to the vesicle wall such that one edge of the isoalloxazine ring penetrates into the bilayer interior while the other edge is near the lipid–water interface to accommodate the two alkyl groups and the amino acid ion pair. This component thus serves not only as the initial electron acceptor for the reduced enzyme but also as the reductant for the membrane-spanning Mn^{III}(ChP)Cl located at the center of the bilayer.[4] Figure 2 depicts the sequence of chemical events that take place upon introduction of pyruvic acid to the multicomponent catalytic system: (1) binding of the enzyme to the vesicles, (2) oxidative decarboxylation of pyruvic acid with the concomitant reduction of enzyme-bound FAD to $FADH_2$, (3) electron transfer from $FADH_2$ to AmFl (II), (4) reduction of Mn^{III}ChPCl (I) to Mn(II) by reduced AmFl, and (5) binding and reductive activation of molecular oxygen to produce a high-valent manganese oxo species responsible for hydrocarbon oxidation.[10]

Thus we have shown that a properly arranged catalytic bilayer assembly can productively harvest electrons from a membrane-bound redox enzyme. This approach can also be used to probe the relative position of membrane components and the pathways for trans-membrane electron transfer.

Acknowledgment. Support of this research by the National Institutes of Health (GM-36928) is gratefully acknowledged.

(5) (a) Fall, H. H.; Petering, H. G. *J. Am. Chem. Soc.* **1956**, *78*, 377. (b) Spectral properties of II: ^{1}H NMR (270 MHz in $CDCl_3$) δ 12.45 (1 H, br), 8.64 (1 H, s), 8.04 (1 H, s), 5.32 (2 H, t), 4.15 (2 H, t), 3.47 (2 H, br), 3.23 (4 H, br), 2.72 (3 H, s), 2.45 (3 H, s), 2.39 (2 H, t), 2.07 (2 H, m), 1.40 (4 H, br), 1.30 (20 H, br), 0.87 (6 H, t). Methyl ester of II: MS [EI, m/e (rel intensity)] 609 (10), 370 (24), 266 (45), 252 (70), 168 (100).

(6) Pyruvate oxidase (*E. coli*) was a generous gift of Dr. Y. Y. Chang and Professor J. E. Cronan at the University of Illinois.

(7) Gennis, R. B.; Hager, L. P. In *The Enzymes of Biological Membranes*; A. Martonosi, Ed.; Plenum: New York, 1976; Vol. 2, pp 493-504.

(8) Koland, J. G.; Miller, M. J.; Gennis, R. B. *Biochemistry* **1984**, *23*, 445.

(9) Grabau, C.; Chang, Y.; Cronan, J. E. *J. Biol. Chem.* **1989**, *264*, 12510 and references therein.

(10) (a) Groves, J. T.; Stern, M. K. *J. Am. Chem. Soc.* **1988**, *110*, 8628. (b) As we have shown elsewhere, the oxidation of methylene groups directly to ketones by manganese porphyrins results from the capture of the intermediate secondary radicals by oxygen. Cf.: Groves, J. T.; Viski, P. *J. Org. Chem.* **1990**, *55*, 3628.

Competing Reaction Pathways in Protonation Reactions of a Low-Valent Tungsten Alkylidyne Isocyanide Complex: Formation of Nitrilium and Aminoalkyne Ligands

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Received May 29, 1990

Protonation reactions of low-valent alkylidyne, or Fischer-type carbyne, complexes have been observed to lead to alkylidyne hydride metal complexes or alkylidene complexes or to result in further transformations of the alkylidyne ligands,[1,2] The outcome of these reactions depends strongly on the nature of the ancillary ligands.[3] For example, we obtained the complexes [W(CPh)-Br_2(H)(PMe_3)$_3$] (**1**), [W(CHPh)Cl_2(CO)(PMe_3)$_2$] (**2**), and [W-(CHPh)Cl_2(PhC_2Ph)(PMe_3)$_2$] (**3**) from protonation reactions of alkylidyne metal complexes.[4] These complexes differ overall only in a single ligand (PMe_3, CO, and PhC_2Ph, respectively, neglecting the difference in the halides, yet **1** is an alkylidyne hydride metal

(1) (a) Holmes, S. J.; Clark, D. N.; Turner, H. W.; Schrock, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6322. (b) Bottrill, M.; Green, M.; Orpen, A. G.; Saunders, D. R.; Williams, I. D. *J. Chem. Soc., Dalton Trans.* **1989**, 511.

(2) (a) Kreissl, F. R.; Sieber, W. J.; Keller, H.; Riede, J.; Wolfgruber, M. *J. Organomet. Chem.* **1987**, *320*, 83. (b) Kim, H. P.; Kim, S.; Jacobson, R. A.; Angelici, R. J. *Organometallics* **1984**, *3*, 1124. (c) Howard, J. A. K.; Jeffery, J. C.; Laurie, J. C. V.; Moore, I.; Stone, F. G. A.; Stringer, A. *Inorg. Chim. Acta* **1985**, *100*, 23. (d) Garrett, K. E.; Sheridan, J. B.; Pourreau, D. B.; Feng, W. C.; Geoffroy, G. L.; Staley, D. L.; Rheingold, A. L. *J. Am. Chem. Soc.* **1989**, *111*, 8383.

(3) Mayr, A. *Comments Inorg. Chem.* **1990**, *10*, 227.

(4) (a) Mayr, A.; Asaro, M. F.; Kjelsberg, M. A.; Lee, K. S.; Van Engen, D. *Organometallics* **1987**, *6*, 432. (b) Mayr, A.; Lee, K. S.; Kjelsberg, M. A.; Van Engen, D. *J. Am. Chem. Soc.* **1986**, *108*, 6079.

0002-7863/90/1512-7797$02.50/0