after displacing O_2 with CO, are not identical with the corresponding carboxyhemochrome spectra obtained without prior exposure to O_2. Increased absorbance in the region of 660 nm, particularly evident in the oxidation of I and IIb, is indicative of some oxidation at the methine bridge carbons.[10]

All the Fe(II) complexes described herein were obtained by reduction of the corresponding Fe(III) porphyrin with a mixture of CaH_2 and Pd black. A number of Fe(III) porphyrins have been successfully reduced in DMF, CH_3CN, CH_2Cl_2, benzene, and toluene using this technique. The solution must be deoxygenated prior to the addition of CaH_2 and Pd and must contain a small amount of water to generate H_2 for the reduction. Reduction is accomplished by gentle stirring. Removal of the solution from the solid phase gives an anhydrous solution of the Fe(II) porphyrin free of impurities and excess reducing agent. The latter is of particular importance in studies of reversible O_2 binding.

A dramatic difference is observed in the extent of reaction of I and IIa with O_2. This difference may be due to constraints imposed on orientation of the coordinated imidazole(s) in I by its covalent linkage to the porphyrin ring. The effective concentration of *N*-alkylimidazole in the vicinity of the iron atom is approximately two orders of magnitude higher in I where the imidazoles are covalently linked to the porphyrin ring, than in IIa where the effective concentration of 1-butylimidazole is near the actual concentration, less than 10 m*M*. However, surprisingly, formation of the dioxygen complex is incomplete in IIa as evidenced by a portion of the parent hemochrome α peak remaining after equilibration with pure O_2 (see Figure 2), whereas it is complete in I (see Figure 1). The P_{O_2} required for conversion of 50% of the hemochrome to oxyhemochrome is less than 1 mm in the case of I and approximately 80 mm for IIa. These results suggest that constraints imposed on the orientation of the coordinated imidazole(s) in I favor formation of the dioxygen complex. Small changes in orientation of the imidazole group would alter the overlap between imidazole π orbitals and Fe t_{2g} orbitals. Since O_2 depends on π bonding for complex formation, the extent of π-bonding interaction between imidazole and Fe will necessarily affect the extent of π back-bonding to the other axial ligand. These results provide experimental evidence for a suggestion originally made by Williams[11] that regulation of oxygen affinity in oligomeric hemoglobins could be due at least in part to slight changes in orientation of the proximal imidazole imposed by protein conformational changes. The transition from the high spin five-coordinate (deoxy) to a low spin six-coordinate (oxy, carboxy) results in displacement of the iron relative to the plane of the porphyrin ring,[12] and probably to changes in puckering of the porphyrin ring system as well. Upon the addition of a sixth ligand, adjustment of the protein in response to changes in the iron porphyrin structure could force the imidazole to alter its orientation. Propagation of the protein conformational change to adjacent subunit(s) would induce a similar change in orientation of the proximal imidazole in the unliganded subunit(s) increasing the affinity of the heme for π-bonding ligands.

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Solvent Effects on Reversible Formation and Oxidative Stability of Heme–Oxygen Complexes[1]

Sir:

The stabilities of oxyhemoglobin and oxymyoglobin have been attributed in part to the steric and nonpolar characteristics on the distal side of the heme.[2,3] While this arrangement has been considered to retard both the proposed "binuclear" and "mononuclear" oxidations (eq 1 and 2)[4–8] (B = nitrogen base), it might also affect

$$\mathrm{BFeO_2 + FeB \xrightarrow{k_2} [BFeOOFeB] \longrightarrow 2BFe^{III}X} \quad (1)$$

$$\mathrm{BFeO_2 + H^+ \xrightarrow{k_1} BFe^{III}X} \quad (2)$$

oxygenation itself.

$$\mathrm{BFe^{II} + O_2 \underset{}{\overset{k_3}{\rightleftharpoons}} BFeO_2} \quad (3)$$

$$\mathrm{BFeB + O_2 \overset{k_4}{\rightleftharpoons} BFeO_2 + B} \quad (4)$$

Such an effect has been demonstrated by Stynes and Ibers[9] for cobalt porphyrins. They found that increased solvent polarity favored oxygenation and interpreted this result as indicating a large contribution of $Co^{III}O_2\cdot^-$ to the complex.

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(4) J. H. Wang, *Accounts Chem. Res.*, **3**, 90 (1970).
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(7) J. P. Collman, R. R. Gagne, T. R. Halbert, J. C. Marchan, and C. A. Reed, *J. Amer. Chem. Soc.*, **95**, 7868 (1973); *Chem. Eng. News*, **52** (3), 20 (1974).
(8) J. E. Baldin and J. Huff, *J. Amer. Chem. Soc.*, **95**, 5759 (1973).
(9) H. C. Stynes and J. A. Ibers, *J. Amer. Chem. Soc.*, **94**, 5125 (1972).

Table I. Effects of Solvent on Oxygenation or Oxidation of Heme–Nitrogen Base Complexes under 1 atm of O_2[g]

	Solvent system							
Heme	CH_2Cl_2–CH_3OH–H_2O[a] 65:30:5 −45°	DMF[b] −45°	DMF[b] 25°	1-Methyl pyrrolidone[b] 25°	CH_2Cl_2[a] −45°	CH_2Cl_2[a] 25°	Toluene[b] −45°	Toluene[b] 25°
1a	C (~10 min)[c]	C (>6 hr)[c]	C (~5 min)[c]	C (~15 min)[c]	C (>1 hr)[c]	Oxid[e]		
1b		C (>6 hr)[c,d]	C (~5 min)[c]		C (>1 hr)[c]	Oxid[e]		
1c		C (>6 hr)[c]	Oxid[e]	Oxid[e]	C (>10 min)[c]	Oxid[e]	Partially C	Oxid[e]
2		C (>30 min)[c]	C (~5 min)[c]	C (~5 min)[c]	Oxid[e]	Oxid[e]	NC (>1 hr)[f]	NC (~30 min)[f]

[a] Reduced with sodium dithionite. [b] Reduced with Pd and calcium hydride. [c] An O_2 complex was formed immediately and changed to oxidized hemin in the time shown in parentheses. [d] The mass spectrometric technique described previously[6a] was used to show a 1:1 heme–oxygen stoichiometry in solution. [e] The heme was oxidized immediately and therefore it was not possible to determine the extent of oxygenation. [f] The heme did not form an O_2 complex but changed to oxidized hemin in the time shown in parentheses. [g] Key: C, complex formed; oxid, oxidized; NC, no complexformed. DMF was vacuum distilled from calcium hydride prior to use. Heme concentrations were about 5×10^{-5} *M*.

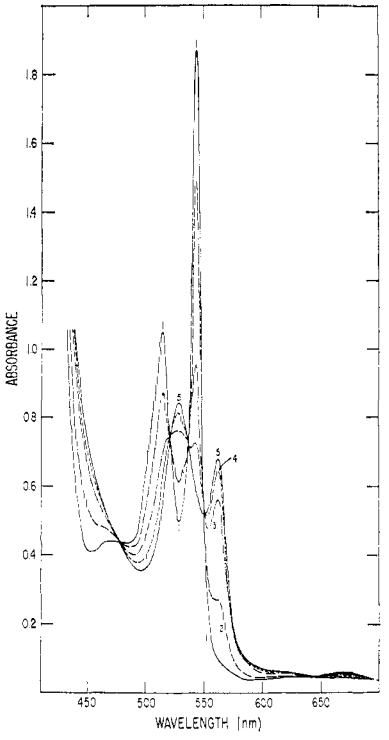
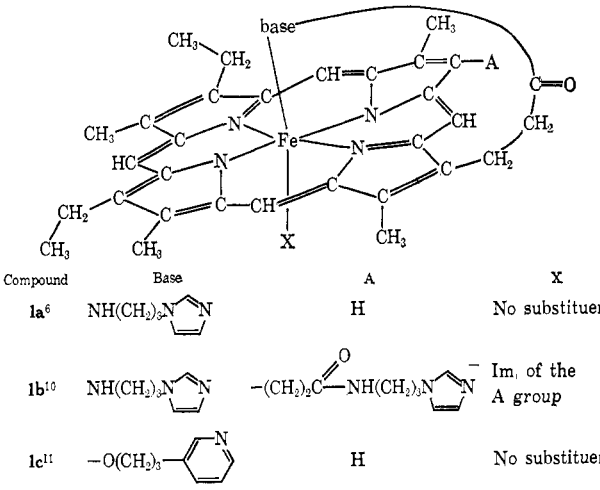


Figure 1. Spectra of reduced **1c** in dry DMF at −45°: 1 (—), under vacuum; 2 (— —), 3.79 Torr of O_2; 3 (—·—), 7.58 Torr of O_2; 4 (— – – —), 11.37 Torr of O_2; 5 (—) 150 Torr of O_2 (air).

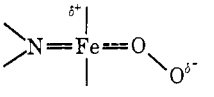
We wish to report similar solvent effects upon both oxidation (eq 1 and 2) and oxygenation (eq 3 and 4) of hemes. Three hemes having either one or two covalently bound bases (**1a, b, c**) and protoheme dimethyl ester with external *N*-*n*-butylimidazole (100-fold excess) (**2**) were used. The solutions of heme–base complexes were prepared either by reduction with sodium dithionite as previously described or by reduction with a mixture of Pd black and calcium hydride.[10] Spectral changes occurring upon the sequential introduction to the heme solutions of O_2 and CO are similar to those previously described. Oxidations were observed by the change to hemin type spectra.[6] The behavior of these four heme–base combinations in several solvents is shown in Table I.

(10) W. S. Brinigar and C. K. Chang, unpublished results.



In addition to these qualitative results we have also determined by oxygen titration (see Figure 1) the pressure of oxygen for half saturation of **1c** by eq 3 in DMF ($P_{1/2}$ = 5 Torr) in 10% *N*-methylpyrrolidone–90% toluene ($P_{1/2}$ = 28 Torr) and in toluene ($P_{1/2}$ ≈400 Torr) at −45°.[12] Compare these data with those for 1-methylimidazolecobalt protoporphyrin[9] in DMF ($P_{1/2}$ = 12.6 Torr) and toluene ($P_{1/2}$ = 417 Torr). These and the other data in Table I clearly show that oxygenation is markedly increased and oxidation retarded in polar aprotic solvents. In the polar, protic solvents (CH_2Cl_2–MeOH–H_2O) oxygenation is favored but oxidation (presumably by reaction 2) is rapid even at −45°.

The similarity of our results to those of Stynes and Ibers[9] leads us to draw similar conclusions and to formulate the Fe–OO bond as a highly dipolar[13] complex similar to their formulation for the cobalt–oxygen



(11) Compound **1c** was prepared from the acid chloride of pyrroporphyrin as previously described.[6] Nmr and analytical data are consistent with the indicated structure.

(12) Our failure to observe oxygenation of pyridine–heme compounds similar to **1c** in polystyrene films can now be understood as a combination of a poor proximal base[6d] and a poor solvent environment for oxygenation.

(13) J. J. Weiss, *Nature (London)*, 202, 83 (1964).

bond. This formulation is also consistent with the proposed synergistic π-base effect on oxygenations.[5b,6d]

These results indicate that the heme–oxygen complexes are more stable and more easily prepared than has been suspected. While minimal requirements for reversible oxygenation remain to be established, some factors which contribute to optimal binding are now apparent. Current results suggest that an ideal oxyheme would have a single "proximal" imidazole (or other equally good π and/or σ base) having the "distal" side protected from other hemes or from protic solvents and a polar aprotic "solvent" environment.[14,15]

The effect of local environment on the reactivity of coordinated O_2 in oxyheme proteins is apparently more complex than has been previously indicated. Our results agree with the suggestion of Cole, Curthoys, and Magnusson[19] that the distinctions among oxygen carriers and oxidases might be due in part to subtle differences in the polarity as well as protic environment in the immediate vicinity of the heme group.

(14) The "picket fence" iron porphyrin of Collman, *et al.*,[7] has amide groups near the Fe–OO bond and possibly owes some of its oxygen binding ability to a local "polar solvent" environment.

(15) Previously reported heme–oxygen complexes[6-8,16,17] incorporated some of these properties but none has yet incorporated all of them. The previously synthesized heme cyclophane[18] should incorporate most of these effects.

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Reversible Reaction of Simple Ferrous Porphyrins with Molecular Oxygen at Low Temperatures

Sir:

Currently there is great interest in studying model compounds of myoglobin and hemoglobin. In recent years this has led to the preparation of cobalt oxygen carriers whose properties have been extensively studied.[1,2] However, when similar iron compounds, such as porphyrins and Schiff bases, are allowed to react with oxygen under analogous conditions, irreversible oxidation to a μ-oxo dimer results.[3,4] In order to prevent dimer formation, sterically hindered iron complexes have been prepared, which react reversibly with oxygen.[5,6] Traylor and Chang have prepared a section of the myoglobin active site which also reacts reversibly with oxygen.[7] This is attributed to the neighboring group effect of an attached imidazole and to the low temperature (−45°) retardation of irreversible oxidation.

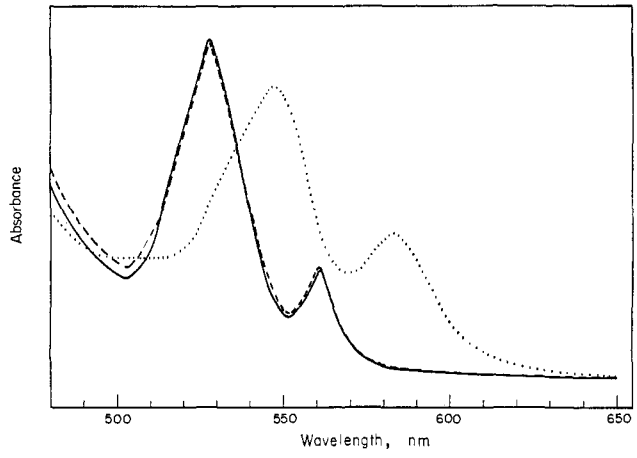

Figure 1. Visible spectrum of ~1 × 10^{-4} *F* Fe(py)$_2$TPP in methylene chloride at −78°: (—) under nitrogen, (····) under oxygen, (- - - -) under nitrogen after one oxygenation–deoxygenation cycle.

We have preliminary evidence that at low temperatures simple ferrous porphyrins also react reversibly with molecular oxygen. The complexes examined in this work are *meso*-tetraphenylporphyrinbis(pyridine)iron(II), Fe(py)$_2$TPP,[8] *meso*-tetraphenylporphyrinbis(1-methylimidazole)iron(II), Fe(1-Me(imid))$_2$TPP,[9] and *meso*-tetraphenylporphyrinbis(piperidine)iron(II), Fe(pip)$_2$TPP.[10] A water free methylene chloride solution of Fe(py)$_2$TPP, prepared under nitrogen without any added pyridine, is exposed to oxygen at −78°. Over a period of 20 min the visible spectrum[11] is shifted into the red region (Figure 1). Removal of oxygen (effected by bubbling rigorously dried and deoxygenated nitrogen through the solution) returns the spectrum to that characteristic of Fe(py)$_2$TPP. Three oxygenation–deoxygenation cycles can be accomplished with less than 10% irreversible oxidation occurring. The spectrum of the solution in the presence of oxygen is very similar to that reported by Collman, *et al.*,[6] for 1:1 binding of dioxygen by iron(II) in Fe(O_2)(1-Me(imid))($\alpha,\alpha,\alpha,\alpha$-TpivPP) in benzene. This compound is a substituted iron *meso*-tetraphenylporphyrin whose spectral changes should be similar to those of the unsubstituted complex. Oxygen uptake of Fe(py)$_2$TPP–methylene chloride solutions indicates that 0.96 ± 0.10 mol of O_2 are taken up per mole of Fe(py)$_2$TPP initially present.[12] Hence we formulate the observed oxygen adduct as Fe(O_2)(py)TPP. Similar spectral changes and reversible behavior are observed for Fe(1-Me(imid))$_2$TPP and Fe(pip)$_2$TPP in methylene chloride at −78°.

(1) R. G. Wilkins, *Advan. Chem. Ser.*, No. 100, 111 (1971).

(2) (a) F. A. Walker, *J. Amer. Chem. Soc.*, 95, 1154 (1973); (b) D. V. Stynes, H. C. Stynes, B. R. James, and J. A. Ibers, *ibid.*, 95, 1796 (1973); (c) M. J. Carter, D. P. Rillema, and F. Basolo, *ibid.*, 96, 392 (1974).

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(8) H. Kabayashi and Y. Yanagawa, *Bull. Chem. Soc. Jap.* 45, 450 (1972). The preparative procedure followed involves direct reaction of the porphyrin with ferrous acetate. Hence the observed reversibility cannot be ascribed to incomplete removal of a reductant.

(9) Reference 3. Our sincere thanks to Professor J. P. Collman and Dr. R. R. Gagne for generously providing us with a sample of this complex.

(10) L. M. Epstein, D. K. Straub, and C. Maricondi, *Inorg. Chem.*, 6, 1970 (1967).

(11) Spectral measurements were made using a Pyrex cell mounted in a dewar. See ref 2c.

(12) Determined using a 2-cm^{-3} gas buret at constant temperature (solution at −45°) and pressure (1 atm); complex contained in a glass ampoule prior to measurement. We are grateful to Professor J. E. Baldwin for helpful suggestions regarding this technique.