

# Kinetics of the Reaction of Amine Complexes of Cobalt(II) Protoporphyrin IX Dimethyl Ester with Oxygen. Evidence for Hydrogen Bonding with Coordinated Oxygen

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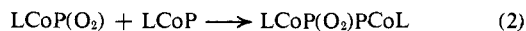
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**Abstract:** The kinetics are reported for reaction of amine complexes of cobalt(II) protoporphyrin IX dimethyl ester with oxygen in toluene to give products believed to be peroxo-bridged complexes. The reaction is first order in cobalt and oxygen and goes from first to zero order in amine (L) with increasing amine concentration. The determined rate law is consistent with the rate-determining step involving a unimolecular distortion of a 1:1 cobalt-oxygen complex. Anomalously rapid rates for L = imidazole and benzimidazole are tentatively explained in terms of hydrogen-bonding effects involving the acidic proton on the imidazole ring. Similarities with hemoglobin and myoglobin are discussed. Thermodynamic data for 1:1 oxygen complexes are presented and discussed in terms of the rate law.

The binding of molecular oxygen to cobalt complexes has been the subject of considerable interest in recent years.<sup>3</sup> In an earlier paper we reported<sup>4</sup> thermodynamic data for the reversible binding of oxygen to cobalt(II) protoporphyrin IX dimethyl ester (abbreviated here throughout simply as CoP) for a series of ligands L, where L was pyridine, 4-*tert*-butylpyridine, and 1-methylimidazole.



As established for many other cobalt complexes, cobalt porphyrin is also thought<sup>5,6</sup> to bind oxygen in a 1:2 fashion



In order to understand fully the binding of oxygen to cobalt, it is necessary to know both the thermodynamics and kinetics of the interactions. For example, with cobalt Schiff base complexes, both the 1:1 and 1:2 complexes may be obtained depending on the temperature, solvent, and axial ligand L.<sup>7-9</sup> Qualitative observations from esr and visible spectroscopy are consistent with a rapid formation of the 1:1 complex followed by a slow reaction to give the 1:2 complex.<sup>7,9</sup> In cases where the preequilibrium reaction (1) has not been observed (*i.e.*, because the equilibrium constant is small), the formation of the binuclear complex has sometimes been interpreted in terms of a slow formation of the 1:1 complex followed by a rapid reaction with a second cobalt moiety.<sup>6</sup> However, this interpretation seems inconsistent with observations that the 1:1 complex is formed rapidly on freezing solutions for esr measurements,<sup>9</sup> and some recent studies from this

laboratory<sup>10</sup> on the oxygenation of bis(salicylaldehyde)-ethylenediiminocobalt(II) are consistent with reaction 1 being rapid, and reaction 2 being slow. Studies of the rates of 1:1 oxygenation of myoglobin<sup>11a</sup> and of isolated hemoglobin chains<sup>11b</sup> by stopped-flow and flash photolysis techniques give second-order rate constants of the order of  $10^7 \text{ M}^{-1} \text{ sec}^{-1}$ .

Kinetic data on the formation of cobalt oxygen complexes have been reported by Wilkins and coworkers<sup>3</sup> for some amino acid complexes. These amino acid systems require the displacement of a ligand prior to formation of the oxygen complex and thus are not directly comparable with porphyrin and Schiff base systems, where oxygenation occurs simply by addition to a vacant coordination site. In this respect, the porphyrin systems may be more related to the heme proteins, although hemoglobin and myoglobin do not undergo a "dimerization" reaction because of steric restrictions of the globin. In addition, reactions of cobalt porphyrin may be carried out in a hydrophobic environment of toluene solutions while the amino acid systems are all aqueous.

The kinetics of the autoxidation of dipyrindineprotoporphyrins (iron porphyrins) have been studied both in aqueous<sup>12</sup> and nonaqueous media.<sup>13</sup> These systems differ from the cobalt porphyrin systems, and hemoglobin and myoglobin, in that the starting iron(II) compound is six coordinate, the rate-determining step in the autoxidation of hemes involves the displacement of a pyridine ligand from iron, and the product is a Fe-O-Fe complex or other iron(III) species.

In this paper we present kinetic data for the reaction of oxygen with amine complexes of cobalt(II) protoporphyrin IX dimethyl ester in toluene; the amine ligands (L) used were imidazole (Im), benzimidazole

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(3) R. G. Wilkins, *Advan. Chem. Ser.*, No. 100, 111 (1971), and references therein.  
(4) H. C. Stynes and J. A. Ibers, *J. Amer. Chem. Soc.*, **94**, 1559 (1972).  
(5) F. A. Walker, *ibid.*, **92**, 4235 (1970).  
(6) K. Yamamoto and T. Kwan, *J. Catal.*, **18**, 354 (1970).  
(7) A. L. Crumbliss and F. Basolo, *J. Amer. Chem. Soc.*, **92**, 55 (1970).  
(8) C. Floriani and F. Calderazzo, *J. Chem. Soc. A*, 946 (1969).  
(9) B. M. Hoffman, D. Diemente, and F. Basolo, *J. Amer. Chem. Soc.*, **92**, 61 (1970).

(10) E. Ochiai, *J. Amer. Chem. Soc.*, submitted for publication.  
(11) (a) Q. H. Gibson, *Progr. Biophys. Biophys. Chem.*, **9**, 1 (1959); (b) R. W. Noble, Q. H. Gibson, M. Brunori, E. Antonini, and J. Wyman, *J. Biol. Chem.*, **244**, 3905 (1969).  
(12) O. H. W. Kao and J. H. Wang, *Biochemistry*, **4**, 342 (1965).  
(13) I. A. Cohen and W. S. Caughey, "Hemes and Hemoproteins," B. Chance, R. W. Estabrook, and T. Yonetani, Ed., Academic Press, New York, N. Y., 1966, p 577; J. O. Alben, W. H. Fuchsman, C. A. Beaudreau, and W. S. Caughey, *Biochemistry*, **7**, 624 (1968).

(BzIm), 1-methylimidazole (CH<sub>3</sub>-Im), and 1,2-dideuterioimidazole (D-Im).

### Experimental Section

**Materials.** Particular care was taken to remove all traces of water from the materials used. Protoporphyrin IX dimethyl ester (grade 1, Lot 100C-2680) was purchased from Sigma Chemical Co. and used without further purification. The CoP complex was prepared as described previously<sup>4</sup> and recrystallized from benzene. *Anal.* Calcd for C<sub>36</sub>H<sub>36</sub>N<sub>4</sub>O<sub>4</sub>Co (647.63): C, 66.77; H, 5.60; N, 8.65. Found: C, 66.89; H, 5.46; N, 8.44. The molar extinction coefficients in toluene, 563 nm ( $\epsilon$  22.8  $\times$  10<sup>3</sup>), 528 (11.3  $\times$  10<sup>3</sup>), and 404 (160  $\times$  10<sup>3</sup>), are in good agreement with those reported in benzene.<sup>14</sup> Imidazole (Aldrich Chemical Co.) and benzimidazole (Eastman Kodak Co.) were recrystallized from benzene and dried *in vacuo*. 1-Methylimidazole (Aldrich) was distilled at reduced pressure from KOH and stored under nitrogen. Oxygen was extra dry grade (Matheson Co.). Toluene was freshly distilled from CaH<sub>2</sub> and degassed three times by the freeze-thaw technique. 1,2-Dideuterioimidazole<sup>15,16</sup> was prepared by equilibrating 1 g of imidazole in 50 ml of D<sub>2</sub>O containing KOH. Solvent was pumped off and the equilibration repeated; solvent was again pumped off and the solid recrystallized twice from benzene. Nmr (CCl<sub>4</sub>-acetone) showed the disappearance of resonances at  $\delta$  12.1 and 7.5 due to the 1 and 2 protons, respectively; only a sharp singlet at  $\delta$  6.95 was observed for the deuterated product due to the 4 and 5 protons, showing the product to be the 1,2-dideuterio derivative.

**Equilibrium and Kinetic Measurements.** The formation constants for 1:1 complexes of the cobalt porphyrin with the various imidazoles were determined at 23° using a standard spectrophotometric procedure, recently used by Halpern and coworkers<sup>17</sup> for similar studies on some cobalt Schiff base systems. The imidazole solutions (10<sup>-4</sup>–5  $\times$  10<sup>-3</sup> M) were added from calibrated syringes through a serum cap to an evacuated degassed solution of the cobalt porphyrin (6  $\times$  10<sup>-6</sup>–6  $\times$  10<sup>-5</sup> M); equilibration was attained rapidly.



Six-coordinate complexes are formed only at much higher ligand concentrations.<sup>18</sup>

Kinetic measurements at 23  $\pm$  1° were made by adding various pressures of O<sub>2</sub> to the above solutions and following the change in the visible spectrum with time. Three different preparations of the cobalt porphyrin gave identical kinetic results.

The technique for the measurement of  $K_{\text{eq}}$  for reaction 1 at low temperature using visible spectroscopy was described previously.<sup>4</sup>

**Instruments.** Visible spectra were recorded on a Cary 14 spectrophotometer, esr on a Varian E3, nmr on a Varian T-60, and mass spectra on an A.E.I. MS9.

### Results

**Esr.** Esr provides an effective tool for the detection of cobalt oxygen complexes, since a characteristic esr spectrum is observed for LCoP(O<sub>2</sub>) systems. Although spectra of numerous oxygen complexes of cobalt porphyrins for a wide variety of ligands, L, have been reported,<sup>5,6</sup> there has been no report of a 1:1 oxygen complex of a cobalt porphyrin where L is imidazole or benzimidazole. This seems somewhat surprising since a corresponding 1-methylimidazole complex is known.<sup>4,5</sup> Imidazole is, of course, involved in coordination in heme proteins. Yamamoto and Kwan<sup>6</sup> concluded that only oxygen-bridged complexes were formed from cobalt tetraphenylporphyrin when L was imidazole or benzimidazole, since no esr signals were detected after oxygenation *at room temperature*.

(14) J. E. Falk, "Porphyrins and Metallo-Porphyrins," Elsevier, New York, N. Y., 1964, p 243.

(15) R. J. Gillespie, A. Grimison, J. H. Ridd, and R. F. M. White, *J. Chem. Soc.*, 3228 (1958).

(16) M. R. Grimmett, *Advan. Heterocycl. Chem.*, **12**, 104 (1970).

(17) L. G. Marzilli, P. A. Marzilli, and J. Halpern, *J. Amer. Chem. Soc.*, **93**, 1374 (1971).

(18) D. V. Stynes, H. C. Stynes, J. A. Ibers, and B. R. James, to be submitted for publication.

We find that the esr spectrum characteristic of LCoP(O<sub>2</sub>) is obtained if solutions of cobalt protoporphyrin IX dimethyl ester containing imidazole or benzimidazole are oxygenated at low temperatures. If the solutions are allowed to warm to room temperature and refrozen, the signal is lost. This result is consistent with the rapid formation at low temperatures of a 1:1 oxygen complex, which at higher temperatures decomposes rapidly to give LCoP(O<sub>2</sub>)PCoL. The complex obtained from the imidazole system analyzes correctly for [C<sub>36</sub>H<sub>36</sub>N<sub>4</sub>O<sub>4</sub>Co(C<sub>3</sub>H<sub>7</sub>N<sub>2</sub>)]<sub>2</sub>O<sub>2</sub>. *Anal.* Calcd: C, 64.02; H, 5.51; N, 11.49. Found: C, 64.54; H, 5.72; N, 11.70. The ImCoP complex is not sufficiently soluble in toluene to follow O<sub>2</sub> uptake, but in dimethylformamide, the quantitative uptake<sup>19a</sup> (0.5 mol of O<sub>2</sub> per Co) is consistent with formation of the bridged complex.<sup>19b</sup>

It should be noted that oxygen-bridged porphyrin complexes of cobalt have not been well characterized. The possibility that the reaction product is the hydroxy-cobalt(III) complex, LCoP(OH), cannot be completely ruled out. The gas uptake and elemental analysis data are consistent with either product. Low solubility excluded molecular weight measurement by osmometry. A mass spectrum at low ionizing voltages ( $\sim$ 10 eV at 300°) showed the highest peak at about 650, probably due to the four-coordinate CoP complex (647.63). Again this could result from decomposition of an oxygen-bridged complex<sup>3</sup> or from a hydroxy-cobalt(III) complex; axial ligands are readily lost from porphyrin complexes under the conditions of the mass spectroscopy measurements.<sup>20</sup>

Of the amine ligand systems studied so far at added amine concentrations of up to 2  $\times$  10<sup>-3</sup> M, the rapid loss of the LCoP(O<sub>2</sub>) complex appears to be unique for imidazole and benzimidazole. Solutions of the 1-methylimidazole system require several days at room temperature for the loss of the LCoP(O<sub>2</sub>) esr signal. The reason for the more rapid reaction does not appear to be due to any unique difference in either LCoP or LCoP(O<sub>2</sub>) since the *g* values and hyperfine splittings in the esr spectrum for the L = imidazole or benzimidazole complexes are similar to those of the 1-methylimidazole analogs (Table I).

**Thermodynamics for the Reversible Oxygenation (1:1) of Cobalt Protoporphyrin IX Dimethyl Ester Systems.** Reaction 1 for L = 1-methylimidazole was found earlier by visible spectroscopy measurements<sup>4</sup> to be reversible at -31°: the spectrum of the deoxygenated species LCoP can also be obtained on pumping off O<sub>2</sub> from a toluene solution of LCoP(O<sub>2</sub>) at -23°. With L = imidazole, the oxygenation is not completely reversible at -23°. In addition spectra of oxygenated solutions of LCoP changed with time at -23° for the imidazole system but not for the 1-methylimidazole system. This change is ascribed to the same irreversible reaction which results in loss of the esr signal. At lower temperatures, this reaction is sufficiently slow to allow thermodynamic data to be obtained for the formation of LCoP(O<sub>2</sub>) at -45°, no change in the visible

(19) (a) B. R. James and G. L. Rempel, *Can. J. Chem.*, **44**, 233 (1966).

(b) The solid reaction product certainly has coordinated imidazole; whether or not the base is coordinated in the solution product has yet to be established.

(20) L. Edwards, D. M. Dolphin, and M. Gouterman, *J. Mol. Spectrosc.*, **35**, 90 (1970), and references therein.

**Table I.** ESR Parameters of the Five-Coordinate Imidazole Complexes and the Six-Coordinate Imidazole-O<sub>2</sub> Adducts of Cobalt(II) Protoporphyrin IX Dimethyl Ester in Toluene at 77°K

Compound	$g_{\perp}$	$g_{\parallel}$	$A_{\parallel}^{\text{Co}}, \text{G}$	$A_{\parallel}^{\text{N}}, \text{G}$
ImCoP	2.32	2.033	77	16.5
(CH <sub>3</sub> -Im)CoP	2.32	2.033	76.5	17.0
(BzIm)CoP	2.34	2.033	82	Unresolved
ImCoP·O <sub>2</sub>	1.99 ± 0.005 <sup>a</sup>	2.08	16.4	
(CH <sub>3</sub> -Im)CoP·O <sub>2</sub>	1.99 ± 0.005 <sup>a</sup>	2.083	16.7	
(BzIm)CoP·O <sub>2</sub>	1.99 ± 0.005 <sup>a</sup>	2.08	Unresolved	

<sup>a</sup> By inspection of the spectra; no consideration is given for nonaxial behavior.

**Table II.** Thermodynamic Data for Reversible O<sub>2</sub> Binding to Cobalt(II) Protoporphyrin IX Dimethyl Ester Base

Compound	Temp, °C	Log $K_{\text{eq}}, \text{mm}^{-1}$	$\Delta H^{\circ}, \text{kcal/mol}$	$\Delta S^{\circ}, \text{eu}$	$\text{p}K_{\text{a}}^b$
pyCoP <sup>c</sup>	-45	-2.84 ± 0.03			
	-57.5	-2.25 ± 0.06	-9.2 ± 0.6 <sup>e</sup>	-53 ± 3 <sup>e</sup>	5.27
	-63.5	-2.09 ± 0.05			
<i>(t</i> -Bu-py)CoP <sup>c</sup>	-37.5	-3.12 ± 0.03			
	-45	-2.77 ± 0.02	-10.0 ± 0.5	-57 ± 3	5.99
	-63.5	-1.97 ± 0.03			
(CH <sub>3</sub> -Im)CoP <sup>c</sup>	-31	-2.36 ± 0.03			
	-37.5	-2.04 ± 0.03	-11.5 ± 0.5	-58 ± 3	7.25
	-45	-1.70 ± 0.02			
ImCoP <sup>d</sup>	-31	-2.50 ± 0.03			
	-45	-1.84 ± 0.03	-11.2 ± 0.5	-58 ± 3	6.95
	-57.5	-1.25 ± 0.03			
(BzIm)CoP <sup>d</sup>	-45	-3.14 ± 0.03	-9.6 ± 0.6	-56 ± 3	5.48
	-63.5	-2.30 ± 0.03			

<sup>a</sup> Standard state of 1 Torr. <sup>b</sup> At 25°; from K. Hoffman, *Chem. Heterocycl. Compounds*, 6, 3, 247 (1953), and K. Schofield, "Hetero-Aromatic Nitrogen Compounds," Plenum Press, New York, N. Y., 1967, p 146. <sup>c</sup> Reference 4. <sup>d</sup> Present work. <sup>e</sup> Error of estimate corresponding to two standard deviations (>95% of normal curve).

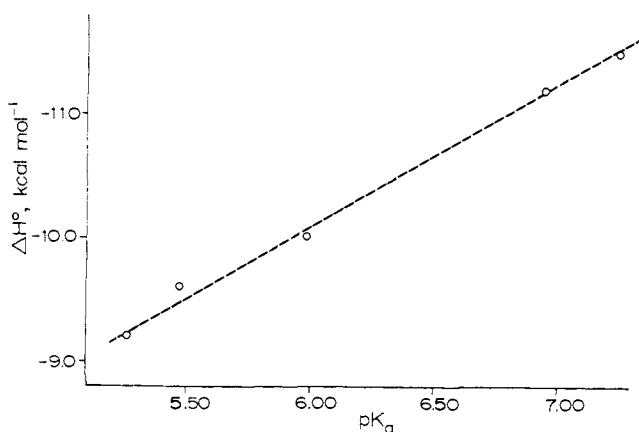


Figure 1. Correlation between the enthalpy change of reaction 1 and  $\text{p}K_{\text{a}}$  of the ligand L in aqueous solution (Table II).

spectrum of an oxygenated solution occurs over 2 hr, and the oxygenation is completely reversible.

Values of  $K_{\text{eq}}$  for reaction 1 and the corresponding thermodynamic parameters are given in Table II for the imidazole and benzimidazole systems together with the data reported earlier<sup>4</sup> for other base systems. A reasonable correlation exists between the enthalpy of the reaction and the  $\text{p}K_{\text{a}}$  of the protonated base in aqueous solution (Figure 1); as noted previously,<sup>4,5</sup> there is a general increase in the stability of the oxygen adduct with increasing basicity.

The spectrophotometrically determined  $K'$  values for reaction 3 are given in Table III. The trend of stronger L coordination with increasing basicity was observed for the related Schiff base systems.<sup>17</sup>

**Kinetic Measurements.** The reaction rates at 23°

**Table III.** Equilibrium Constants for  $\text{CoP} + \text{L} \xrightleftharpoons{K'} \text{LCoP}$  Reaction in Toluene at 23°

Ligand	$10^{-3}K', \text{M}^{-1}$	$10^{-3}K', \text{M}^{-1}$
Imidazole	4.5 ± 1.0	~2
1-Methylimidazole	5.1 ± 1.0	
Benzimidazole	3.0 ± 1.0	
Dideuterioimidazole		~2

<sup>a</sup> Determined spectrophotometrically. <sup>b</sup> Determined kinetically according to eq 7.

were studied generally by following changes in the intensity of the Soret band at 404 nm due to equilibrium mixtures of CoP and LCoP (determined by  $K'$  and [L] for eq 3). The extinction coefficients for the Soret band of the five-coordinate LCoP species are lower than that of the four-coordinate species, but the differing original intensities of the 404-nm band do not affect the kinetic treatment. For ImCoP the molar extinction coefficient at 404 nm is  $108 \times 10^3$ . Extrapolation of the  $K_{\text{eq}}$  data of Table II to 23° (see below) shows that no detectable amount of LCoP(O<sub>2</sub>) is present at O<sub>2</sub> pressures up to 760 mm. The reaction rates could also be followed by an increase in the absorption band at about 428 nm due to the product, which has absorption maxima at 428 ( $\epsilon 93 \times 10^3$ ), 542 ( $\epsilon 13.0 \times 10^3$ ), and 575 nm ( $\epsilon 11.8 \times 10^3$ ) (Figure 2). Kinetic runs using cobalt solutions at the higher concentrations were more conveniently studied by following optical density changes at about 560 nm where the product has an absorption minimum ( $\epsilon 9.1 \times 10^3$ ; Figure 3). The absorption maximum of the LCoP species in this region was around 555 nm with a lower extinction coefficient than the CoP complex, but again the kinetic treatment is unaffected by the differing initial optical densities.

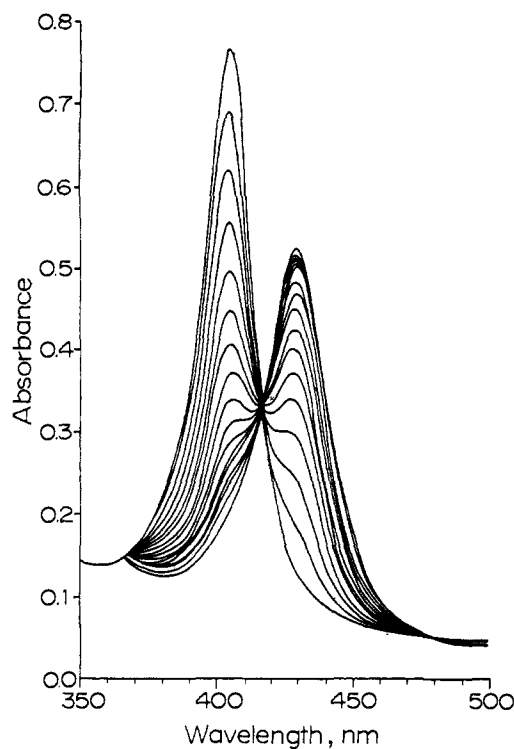


Figure 2. Changes in absorption spectra in Soret region during reaction; band at 404 nm decreases with corresponding increase at 428 nm:  $[\text{Co}]_{\text{total}} = 5.6 \times 10^{-6} M$ ,  $[\text{Im}] = 2.26 \times 10^{-4} M$ , 570 mm of  $\text{O}_2$ .

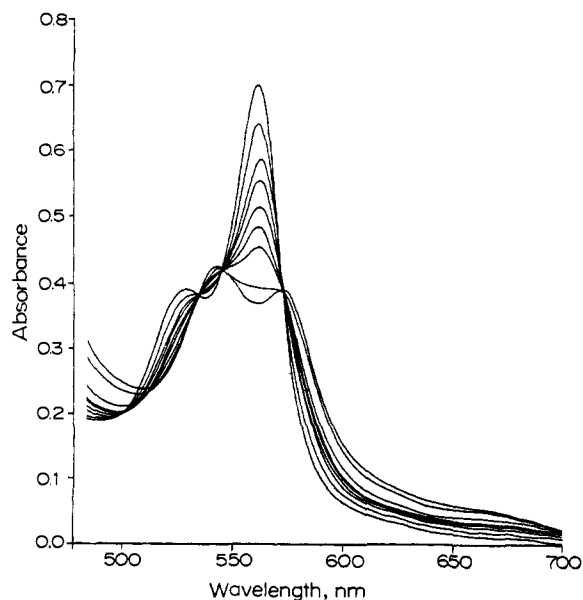


Figure 3. Changes in absorption spectra in 500-600-nm region during reaction; absorption at 560 nm decreases with time:  $[\text{Co}]_{\text{total}} = 3.2 \times 10^{-6} M$ ,  $[\text{Im}] = 1.16 \times 10^{-4} M$ , 190 mm of  $\text{O}_2$ .

For ImCoP,  $\epsilon$  at 555 nm is  $17.5 \times 10^3$ . During any one kinetic run, good isosbestic points were obtained, as expected for a fixed ratio of CoP:LCoP species reacting with  $\text{O}_2$  to give one product. All reactions were found to go to completion.

Plots of  $\log \{[\text{CoP}] + [\text{LCoP}]\}$  vs. time are shown in Figures 4 and 5 and indicate a first-order dependence on total cobalt; the plots were linear over at least three half-lives. Kinetic runs at cobalt concentrations

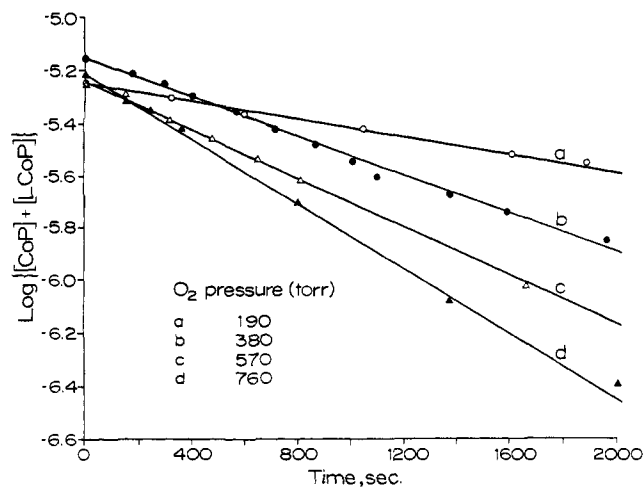


Figure 4.  $\log \{[\text{CoP}] + [\text{LCoP}]\}$  vs. time plots for various oxygen pressures;  $[\text{Co}]_{\text{total}} = 6 \times 10^{-6} M$ ,  $[\text{Im}] = 2.26 \times 10^{-4} M$ .

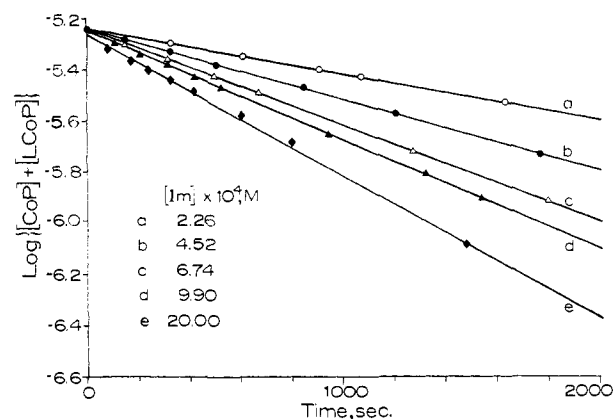


Figure 5.  $\log \{[\text{CoP}] + [\text{LCoP}]\}$  vs. time plots for various imidazole concentrations;  $[\text{Co}]_{\text{total}} = 6 \times 10^{-6} M$ , 190 mm of  $\text{O}_2$ .

varying from  $6 \times 10^{-6}$  to  $6 \times 10^{-5} M$  confirm the first-order dependence on cobalt. Pseudo-first-order rate constants,  $k_{\text{obsd}}$ , obtained from the slopes of the log plots, are given in Table IV for the imidazole and di-deuterioimidazole systems at the conditions noted. A

Table IV. Kinetic Data<sup>a</sup> for the Imidazole System in Toluene at 23°

	$\text{O}_2$ pressure, mm	$10^4[\text{Im}]$ , M	$10^3 k_{\text{obsd}}$ , $\text{sec}^{-1}$
Imidazole	190	2.26	0.39
	380	2.26	0.85
	570	2.26	1.07
	760	2.26	1.42
	190	0.98	0.14
	190	2.92	0.58
	190	3.40	0.72
	190	4.52	0.62
	190	6.74	0.84
	190	8.50	0.99
1,2-Dideuterio- imidazole	190	20.0	1.33
	190 <sup>b</sup>	2.26	0.40
	190 <sup>b</sup>	1.16	0.16
	190	4.50	0.54
	190	8.80	0.69
	190	20.0	1.06

<sup>a</sup> $[\text{Co}]_{\text{total}} = 6 \times 10^{-6} M$  unless stated otherwise. <sup>b</sup> $[\text{Co}]_{\text{total}} = 6 \times 10^{-5} M$ .

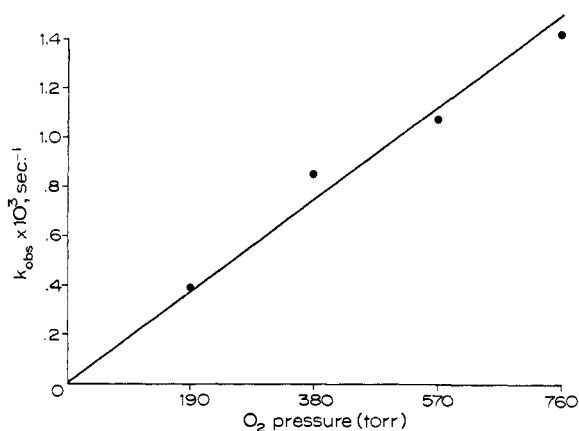


Figure 6. Pseudo-first-order rate constant as a function of  $O_2$  pressure;  $[Co]_{total} = 6 \times 10^{-6} M$ ,  $[Im] = 2.26 \times 10^{-4} M$ .

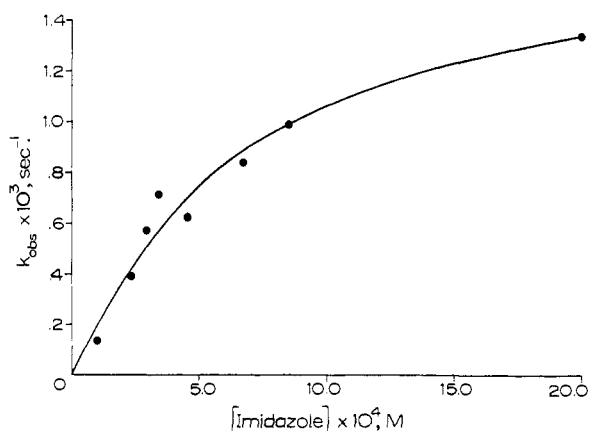


Figure 7. Pseudo-first-order rate constant as a function of imidazole concentration;  $[Co]_{total} = 6 \times 10^{-6} M$ , 190 mm of  $O_2$ .

plot of  $k_{obs}$  vs. oxygen pressure (Figure 6) indicates a reasonably first-order dependence on oxygen. It should be noted that the oxygen concentration (*ca.*  $10^{-3} M$  at 1 atm) remains effectively constant during a kinetic run. The dependence on ligand concentration (Figure 7) is first order at low concentrations and decreases toward zero with increasing concentration; studies at much higher imidazole concentrations were not carried out because of the problem of its aggregation, which becomes significant at concentrations above *ca.*  $2 \times 10^{-3} M$ .<sup>21,21a</sup>

Kinetic data for the 1-methylimidazole, dideuterioimidazole, and benzimidazole systems also analyzed for pseudo-first-order log plots (Figure 8) and some determined rate constants ( $k_{obs}$ ) are given in Table V.

(21) K. Hoffman, "The Chemistry of Heterocyclic Compounds," Vol. 6, A. Weissberger, Ed., Interscience, New York, N. Y., 1953, pp 3, 247.

(21a) NOTE ADDED IN PROOF. We have recently learned of studies on the reaction of the imidazole complex of cobalt(II) deuterioporphyrin dimethyl ester with oxygen in ethyl acetate [M. Rougee, D. Prince, V. Favaudon, and M. Momenteau, *Colloq. Int. Centre Nat. Rech. Sci.*, No. 191, 335 (1970); through *Chem. Abstr.*, 77, 118752 (1972)]. The kinetics were first order in oxygen, between zero and second order in imidazole, and second order in cobalt. However, the imidazole concentration range used (0.01–0.25 *M*) was much higher than ours, and the cobalt dependence was determined at the 0.25 *M* value. Aggregation of the amine was not considered. Their interpretation did not consider 1:1  $O_2$  complexation and was based on decomposition of a steady-state concentration of a  $LCoP(O_2)PCoL$  species to unknown six coordinate products.

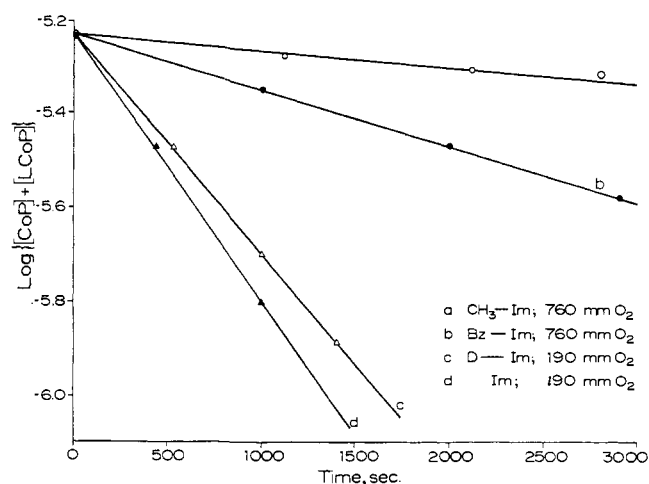


Figure 8.  $\log \{[CoP] + [LCoP]\}$  vs. time plots for reactions with different imidazole ligands;  $[Co]_{total} = 6 \times 10^{-6} M$ ,  $[ligand] = 2.0 \times 10^{-3} M$ .

Table V. Kinetic Data<sup>a</sup> for Various Imidazole Systems in Toluene at 23°

Axial ligand L	$O_2$ pressure, mm	$10^3 \cdot k_{obs}$ , sec <sup>-1</sup>	$k^*K_{eq}$ , sec <sup>-1</sup> mm <sup>-1</sup>
Imidazole	190	1.33	$4.4 \times 10^{-6}$
1,2-Dideuterioimidazole	190	1.06	$3.8 \times 10^{-6}$
1-Methylimidazole	760	0.06	$(4.4 \pm 0.1) \times 10^{-8}$
Benzimidazole	760	0.32	$(2.5 \pm 0.1) \times 10^{-7}$

<sup>a</sup>  $[Co]_{total} = 6 \times 10^{-6} M$ ; initial  $[L] = 2.0 \times 10^{-3} M$ .

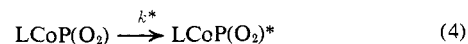
## Discussion

### (1) Kinetics of the Reaction in the Imidazole System.

It seems reasonable to assume that the initial reaction product is the oxygen bridged species  $LCoP(O_2)PCoL$  as established for other cobalt systems. Whether this is the isolated product, or whether it is the precursor to some hydroxycobalt(III) product, has not been established unequivocally; hydrogen abstraction from imidazole, or, less likely, the toluene, is necessary in order to form a hydroxy product (see below also).

The observed first-order dependence on both cobalt and oxygen is consistent with mechanisms in which the formation of the 1:1 oxygen complex is rate determining. But this is ruled out, since the equilibria shown in eq 3 and 1 are established rapidly at low temperature (as shown by both the esr and visible spectroscopy measurements) and hence also at the ambient temperature of the kinetic measurements.

The cobalt dependence requires that only one cobalt species be involved in the rate-determining step. In order to account for this, and the slow formation of the product relative to  $LCoP(O_2)$ , it is necessary to invoke some slow rearrangement or distortion of the 1:1 oxygen complex for the rate-determining step.



The overall mechanism then is outlined by reactions 3 and 1, followed by reactions 4 and 5. This leads to the rate law shown in eq 6, which is consistent with the ob-

served kinetics

$$\frac{-d\{[\text{CoP}] + [\text{LCoP}]\}}{dt} = 2k^*[\text{LCoP}(\text{O}_2)] = \frac{2k^*K'K_{\text{eq}}[\text{Co}]_{\text{total}}[\text{O}_2][\text{L}]}{1 + K'[\text{L}]} \quad (6)$$

Thus

$$k_{\text{obsd}} = 2k^*K'K_{\text{eq}}[\text{L}][\text{O}_2]/(1 + K'[\text{L}]) \quad (7)$$

The data of Figure 7 can be analyzed by plotting  $(k_{\text{obsd}})^{-1}$  vs.  $[\text{L}]^{-1}$  to give a straight line (Figure 9) which yields values of  $K'$  and  $k^*K_{\text{eq}}$ . Values of  $K'$  (given by intercept/slope) for the imidazole and dideuterioimidazole systems determined in this way are given in Table III; a value of about  $2 \times 10^3 \text{ M}^{-1}$  for the imidazole system is considered in reasonable agreement with the spectrophotometric value. Values of  $k^*K_{\text{eq}}$ , determined from the intercepts of the lines drawn in Figure 9, are given in Table V, together with those calculated (eq 7) for the 1-methylimidazole and benzimidazole systems using the  $K'$  values given in Table III.

One can only speculate about the nature of the rate-determining step. It could be related to a charge transfer process from cobalt to oxygen, which results in an increased O-O bond length and a decrease in the O-O bond order. Structural data<sup>22</sup> on  $\text{Co}(\text{bzacen})(\text{py})\text{O}_2$ , where bzacen = *N,N'*-ethylenebis(benzoylacetonimide) and py = pyridine, confirm conclusions from esr<sup>9</sup> that the complex is best regarded as a superoxo  $\text{Co}(\text{III})$  species; the O-O bond length is 1.26 Å, similar to that of the superoxide ion, 1.28 Å. Bond lengths reported<sup>23</sup> for the diamagnetic oxygen dimers are 1.34–1.65 Å, and these are usually discussed in terms of peroxo-bridged species. Thus, in going from the 1:1 to the 1:2 complex, a considerable change in the geometry and bonding characteristics ( $\text{sp}^2 \rightarrow \text{sp}^3$ ) of the coordinated oxygen is required. Our kinetic findings would require that a large amount of this distortion occurs in a slow step prior to a more rapid attack by another cobalt molecule.

It seems unlikely that the rate-determining step involves some shift of the cobalt position relative to the porphyrin plane. Although no crystal structures of cobalt(II) porphyrin or cobalt(II) porphyrin·L complexes have been published, sufficient data are available in the literature<sup>24</sup> to enable us to estimate these Co-N distances. The  $\text{Co}^{11}\text{-N}$  distance in the high-spin  $\text{Co}(\text{NH}_3)_6^{2+}$  complex is 2.114 Å, while the  $\text{Co}^{11}\text{-N}$  distance, determined from structures of low-spin cobalt(III) complexes, is  $1.955 \pm 0.015$  Å. Generally, the radius of a high-spin metal(II) ion exceeds that of the low-spin metal(II) ion by ca. 0.12 Å, and the radius of a metal ion decreases by ca. 0.04 Å on increasing the oxidation state from II  $\rightarrow$  III. We therefore expect, from both the spin and oxidation state trends, that the  $\text{Co}^{11}\text{-N}$  distance in the low-spin cobalt porphyrin complexes will be ca. 1.99 Å, which would place the cobalt atom within 0.05 Å of the plane<sup>25</sup> of the four nitrogen

(22) G. A. Rodley and W. T. Robinson, *Nature (London)*, **235**, 438 (1972).

(23) M. Calligaris, G. Nardin, L. Randaccio, and A. Ripamonti, *J. Chem. Soc. A*, 1069 (1970), and references therein.

(24) (a) H. C. Stynes and J. A. Ibers, *Inorg. Chem.*, **10**, 2304 (1971), and references therein; (b) D. M. Collins, R. Countryman, and J. L. Hoard, *J. Amer. Chem. Soc.*, **94**, 2066 (1972), and references therein.

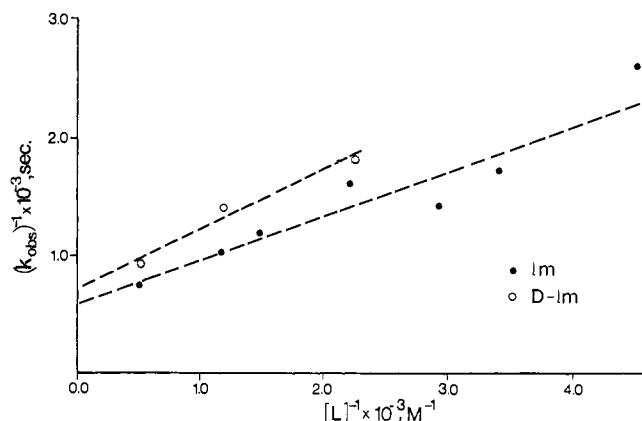


Figure 9. Analysis of pseudo-first-order rate constant data according to eq 7.

atoms.<sup>24b,26</sup> Changes to the superoxo and peroxo complexes with concomitant increase of charge on the cobalt will shorten the Co-N bond length, and the cobalt will remain effectively in the plane of the porphyrin molecule.

The data in Table V show a dramatic difference in reaction rates for the different axial ligands. From the thermodynamic data in Table II, we calculate  $\log K_{\text{eq}}$  at 23° to be very approximately -4.20, -4.40, and -5.25 for the 1-methylimidazole, imidazole, and benzimidazole complexes, respectively. Using these values give corresponding  $k^*$  values of ca.  $0.7 \times 10^{-3}$ ,  $1.1 \times 10^{-1}$ , and  $0.4 \times 10^{-1} \text{ sec}^{-1}$ . The value for the 1-methylimidazole system is thus a factor of 100 less, which corresponds to a difference in free energy of activation of about 3 kcal at ambient temperatures.

The large differences in rate are not readily explained in terms of donor properties of the coordinated ligand in  $\text{LCoP}(\text{O}_2)$ . The esr spectra do not indicate any unusual differences in either  $\text{LCoP}$  or  $\text{LCoP}(\text{O}_2)$  for the different ligands. We tentatively propose that the differences arise from hydrogen bonding effects.<sup>27</sup>

The ability of imidazole and benzimidazole to hydrogen bond is well documented.<sup>21,28</sup> Viscosity, molecular weight data, and dipole moment measurements all indicate substantial aggregation at concentrations  $\geq 2 \times 10^{-3} \text{ M}$  in aromatic solvents<sup>21</sup> (e.g., the molecular weight of 0.1 M imidazole in benzene is 1500). Under the same conditions, 1-methylimidazole, which does not contain an acidic proton, is monomeric.

The relative rates of the imidazole systems may be understood if hydrogen bonding of the imidazole and benzimidazole to the superoxide complex accelerates the distortion of the oxygen complex in the direction of a

(25) The cobalt atom could be somewhat further out of the plane in a five-coordinate tetragonal-pyramidal structure (0.20 Å is the maximum out-of-plane distance observed thus far in cobalt(II) Schiff base complexes; see M. Calligaris, G. Nardin, and L. Randaccio, *Coord. Chem. Rev.*, **7**, 385 (1972)).

(26) J. L. Hoard, *Science*, **174**, 1295 (1971).

(27) A recent paper by R. J. Sundberg and coworkers, *J. Amer. Chem. Soc.*, **94**, 6558 (1972), has reported on the rates of aequation of  $[(\text{NH}_3)_5\text{Ru}^{11}\text{L}]^{2+}$ , which are 500 times greater for L = imidazole than for L = pyridine. The imidazole is considered to be bound *via* C-2 of the imidazole ring, although benzimidazole and 4-methylimidazole form primarily nitrogen bonded complexes. Alternative bonding modes of the imidazoles were not considered in our discussion, since the faster rates correlated with the imidazoles capable of hydrogen bonding.

(28) S. Martinez-Carrera, *Acta Crystallogr., Sect. B*, **20**, 783 (1966); A. Santoro, A. D. Mighell, M. Zouchi, and C. W. Riemann, *ibid.*, **25**, 842 (1969); K. S. Lundberg, *ibid.*, **21**, 901 (1966).

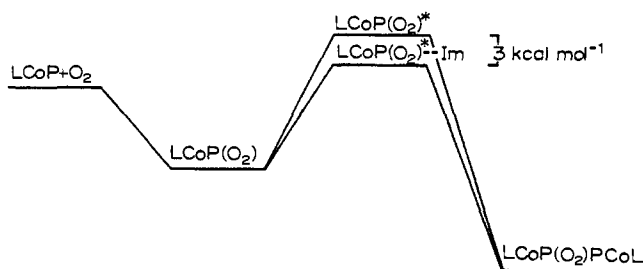
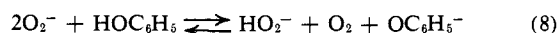


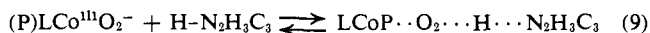
Figure 10. Energy level diagram for oxygenation of LCoP showing possible effect of hydrogen bonding on the reaction rate.

peroxo species. The fact that the ligand dependence in the rate law results solely from the preequilibrium reaction (1) (*i.e.*, the slow step (reaction 4) involves no further ligand molecule) implies that, once formed, the LCoP(O<sub>2</sub>) complex becomes weakly associated with the hydrogen bonding imidazole ligands, even at the lower ligand concentrations used. Hydrogen bonding would be expected to be more important in the transition state than in the ground state since a greater charge separation is involved in the transition state. Typical values cited for the energy of a hydrogen bond interaction<sup>29</sup> are *ca.* 4 kcal mol<sup>-1</sup>. The differences estimated above for the activation free energies are of the correct order of magnitude for the postulated hydrogen bonding effects (see Figure 10). The proposed role of hydrogen bonding is related to that invoked to explain shifts of carbonyl  $\pi-\pi^*$  transitions to lower energy in hydrogen bonding solvents.<sup>30</sup> A 100-fold increase in rate of internal conversion from excited singlet to ground state, due to hydrogen bonding effects of ortho-substituted amide groups, has been observed in fluorescence studies of isomers of 2-(acetoamidophenyl)-2,1,3-benzotriazole.<sup>31</sup>

The anomalous behavior of imidazole seems closely related to observations that electrochemically generated superoxide ion is unstable in the presence of proton sources.<sup>32</sup> Electrochemical reduction of O<sub>2</sub> in aqueous solution gives peroxide by a two-electron process. In aprotic solvents, such as dimethylformamide and dimethyl sulfoxide, a one-electron reduction to superoxide ion is observed; however, only the two-electron process is detected in aprotic solvents containing phenol, and this was attributed to a disproportionation of the superoxide ion



Such an interaction might occur between the cobalt oxygen complex and imidazole.



A hydroperoxide species LCoP(O<sub>2</sub>H) could then react with a further LCoP moiety to give either LCoP(O<sub>2</sub>)PCoL by loss of a proton, or two molecules of LCoP(OH) by a gain of one proton. Whether reaction 9 would involve cleavage of the H-N bond (homolytic or heterolytic), or would be promoted merely by a hydrogen bond interaction, is subject to question. The

(29) W. C. Hamilton and J. A. Ibers, "Hydrogen Bonding in Solids," W. A. Benjamin, New York, N. Y., 1968.

(30) N. Mataga and T. Kubota, "Molecular Interactions and Electronic Spectra," Marcel Dekker, New York, N. Y., 1970, Chapter 7.

(31) J. R. Merrill and R. G. Bennett, *J. Chem. Phys.*, **43**, 1410 (1965).

(32) D. L. Maricle and W. G. Hodgson, *Anal. Chem.*, **37**, 1562 (1965).

isotope effect observed with dideuterioimidazole is more consistent with the latter. The extent of bond breaking in the rate-determining step may be sensitive to the proton source and to the solvent.<sup>33</sup> Similarly, the nature of the final product may depend on the experimental conditions.

We have found that, while addition of phenol to a toluene solution of the four-coordinate cobalt porphyrin under O<sub>2</sub> does not result in a rapid reaction similar to that observed in the imidazole system, such a reaction does occur in the presence of a ligand (pyridine) and phenol. These results indicate that phenol does not coordinate, since without an axial ligand the 1:1 oxygen complex does not form, and no subsequent decomposition can occur. In the presence of pyridine, PyCoP(O<sub>2</sub>) forms, and indeed its subsequent decomposition is promoted by the presence of the phenol.<sup>34</sup>

It is difficult to make any definitive statement about the differences in hydrogen bonding ability between imidazole, benzimidazole, and 1,2-dideuterioimidazole. Although benzimidazole is slightly less aggregated than imidazole,<sup>21</sup> this may be due more to a difference in nitrogen basicity than hydrogen bonding ability. Interpretation of the slightly lower rate on deuterium substitution into imidazole is also difficult since there are differing views as to whether deuterium or hydrogen forms the stronger hydrogen bond.<sup>29, 35, 36</sup> If we ignore complications from secondary isotope effects, the present data indicate a slightly weaker hydrogen bond in the deuterium compound.

The phenomena of aggregation and dimerization of porphyrin complexes<sup>5, 37</sup> appear unimportant at the concentrations at which we have worked. The equilibrium constants of reaction 3 were independent of the concentration of the cobalt(II) porphyrin complex from  $6 \times 10^{-6}$  to  $6 \times 10^{-5}$  M. The kinetic data are not readily interpreted if the LCo species are aggregated.

**(2) Factors Influencing Formation of 1:1 Monomeric Oxygen Adducts vs. 1:2 Oxygen-Bridged Complexes.** The thermodynamic and kinetic data presented here for the cobalt oxygen complexes, in conjunction with esr and structural data on both 1:1 and 1:2 oxygen complexes, provide a reasonably consistent explanation for the reactions of a wide variety of oxygen complexes. Generally, the 1:1 complexes are stable at low temperatures and are formed rapidly. The formation constants of the adducts in polar aprotic solvents, such as dimethylformamide, acetonitrile, and dimethyl sulfoxide, are much higher than in toluene solution,<sup>8, 38</sup> and this is consistent with available data that describe the bonding in the 1:1 complex in terms of a superoxo

(33) Consistent with this discussion are the recent findings of D. A. White, *Inorg. Chem.*, **11**, 2160 (1972), that reaction of Co(CN)<sub>5</sub><sup>3-</sup> with oxygen in the aprotic solvent acetonitrile yields [Co(CN)<sub>5</sub>O<sub>2</sub>]<sup>3-</sup>, while the corresponding reaction in water yields the binuclear complex [(CN)<sub>5</sub>CoO<sub>2</sub>Co(CN)<sub>5</sub>]<sup>4-</sup>.

(34) This observation tends to suggest that the rate-determining step in the imidazole system (reaction 4) should involve a further imidazole molecule, and this would seem intuitively more correct; however, this leads to a ligand dependence of the form  $[L]^2/(1 + K[L])$ , and the data do not analyze well for such a dependence.

(35) G. Dahlgren and F. A. Long, *J. Amer. Chem. Soc.*, **82**, 1303 (1960).

(36) D. W. Thompson and A. L. Allred, *J. Phys. Chem.*, **75**, 433 (1971).

(37) S. J. Cole, G. C. Curthoys, and E. A. Magnusson, *J. Amer. Chem. Soc.*, **92**, 2991 (1970).

(38) H. C. Stynes and J. Ibers, *ibid.*, **94**, 5125 (1972).

cobalt(III) species. The instability of the 1:1 complex at ambient temperatures is due to the dominance of the unfavorable entropy term for the relatively weak interactions ( $\Delta H^\circ \approx 10$  kcal). The temperature at which appreciable amounts of 1:1 adduct exist will be a function of  $\Delta H^\circ$ , which will depend on the planar ligand environment (porphyrin, Schiff base, etc.), the basicity of the axial ligand, and the solvent. In the Schiff base complexes,  $\Delta H^\circ$  values are more negative than in the porphyrins,<sup>4,39</sup> presumably because the Schiff base is less able to delocalize electron density from cobalt and this results in stronger  $\pi$  back-bonding to oxygen. Increased axial base strength has the effect of increasing  $-\Delta H^\circ$  for both the Schiff base and porphyrin systems.<sup>4,7</sup>

Whether the 1:1 or the 1:2 complex is observed depends both on  $K_{eq}$  and on the subsequent kinetics of reaction 2. Detailed discussion must await activation parameter data and kinetic studies on the Schiff base systems. However, the general trend seems clear. If  $K_{eq}$  is small, only the reaction to give the 1:2 complex is detected; if  $K_{eq}$  is large, the rapid reaction to give the 1:1 oxygen complex and the subsequent reactions can be observed. Low temperatures can be used to detect the 1:1 complex when subsequent decomposition is fast at ambient conditions.

Steric constraints presumably prevent formation of the peroxo bridged species in coboglobin<sup>40,41</sup> and vitamin B<sub>12</sub> systems.<sup>42</sup>

**(3) Comparison with Hemoglobin and Myoglobin.** The enhanced rate of reaction for the imidazole system is of particular interest in comparison with the reversible oxygen binding process in proteins. In hemoglobin<sup>43</sup>

(39) G. Amiconi, M. Brunori, E. Antonini, G. Tauzher, and G. Costa, *Nature (London)*, **228**, 549 (1970).

(40) B. M. Hoffman and D. H. Petering, *Proc. Nat. Acad. Sci. U. S.*, **67**, 637 (1970).

(41) B. M. Hoffman, C. A. Spilburg, and D. H. Petering, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 343 (1971).

(42) J. H. Bayston, N. K. King, F. D. Looney, and M. E. Winfield, *J. Amer. Chem. Soc.*, **91**, 2775 (1969).

(43) M. F. Perutz, H. Muirhead, J. M. Cox, and L. C. G. Goaman, *Nature (London)*, **219**, 131 (1968), and references therein.

and myoglobin,<sup>44</sup> the ligand in the fifth coordination site is an imidazole from a proximal histidine residue. X-Ray studies on methemoglobin<sup>43,45</sup> and metmyoglobin<sup>44,46</sup> (ferri form) show that the sixth coordination site is occupied by a water molecule to which a second histidine (called the distal one) is hydrogen bonded. In deoxymyoglobin, X-ray analysis has shown that the oxygen binding site is vacant; however, the distal histidine is still in a position where it can interact with the coordinated oxygen in oxymyoglobin.<sup>47</sup>

Although there has been considerable speculation on the importance of this hydrogen bond in stabilizing the coordinated oxygen, no definite evidence has been presented for such an interaction. In our model system we find no evidence for an increase in  $K_{eq}$  with ligand concentration at low temperatures, nor do we find larger  $K_{eq}$  values for systems with hydrogen bonding ligands. Thus we rule out a thermodynamic effect of hydrogen bonding in the cobalt porphyrin model system at the concentrations used. This does not indicate that such an effect is unimportant in coboglobin or hemoglobin where the distal histidine is held in close proximity to the coordinated oxygen. On the contrary, we believe that the kinetic data of the cobalt porphyrin model systems, *if* our tentative interpretation in terms of hydrogen bonding effects is correct, would add support to the possibility of a thermodynamic effect in the protein systems.

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(44) J. C. Kendrew, *Brookhaven Symp. Biol.*, **15**, 216 (1962); *Science*, **139**, 1259 (1963), and references therein.

(45) M. F. Perutz, *Nature (London)*, **228**, 726 (1970).

(46) L. Stryer, J. C. Kendrew, and H. C. Watson, *J. Mol. Biol.*, **8**, 96 (1964).

(47) R. E. Dickerson and I. Geis, "The Structure and Action of Proteins," Harper and Row, New York, N. Y., 1969, p 50.