high-energy E_b band is now split, although the two bands are not well resolved. This split E_b band has been observed in the trans isomer of tris(*N*-methyl-*l*-menthoxyacetohydroxamato)chromium(III) and was speculated to arise from the lower symmetry of the trans relative to the cis isomer. Thus we assign the second isomer as a mixture of trans isomers, both Λ and Δ in which the Δ isomers predominate, and the first isomer as a cis isomer in which again the Δ optical isomer predominates. Both iron and the chromium complexes therefore exist predominantly as the Δ *optical isomers.*

Summary

The complexes of rhodotorulic acid with Fe^{3+}, Al^{3+}, and Cr^{3+} have been prepared and characterized. From pH 4 to 10 the ferric complex exists only as a dimer of composition Fe_2RA_3. The two ferric ions are octahedrally coordinated by the hydroxamate groups of three rhodotorulic acid molecules and both ferric ions are in Δ-cis absolute configurations. Below pH 3.5 the dimer rapidly dissociates to a monomeric cation $[Fe(RA)]^+$ in which both hydroxamate groups of the RA are coordinated to one ferric ion.

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References and Notes

(1) For references to previous papers in this series see part 9: A. Avdeef, S. R. Sofen, T. L. Bregante, and K. N. Raymond, *J. Am. Chem. Soc.*, preceding paper in this issue.
(2) W. M. Latimer, "Oxidation Potentials", Prentice-Hall, Englewood Cliffs, N.J., 1952.
(3) J. B. Neilands, Ed., "Microbial Iron Metabolism", Academic Press, New York, N.Y., 1974.
(4) C. E. Lankford, *CRC Crit. Rev. Microbiol.*, **2**, 273 (1973).
(5) K. N. Raymond, "Bioinorganic Chemistry-II", *Adv. Chem. Ser.*, **No. 162** (1977).
(6) V. Prelog, *Pure Appl. Chem.*, **6**, 327 (1963).
(7) J. Nüsch and F. Knüsel, "Antibiotics", D. Gottlieb, Ed., Springer-Verlag, New York, N.Y., 1967.
(8) C. L. Atkin and J. B. Neilands, *Biochemistry*, 7, 3734 (1968).
(9) C. J. Carrano and K. N. Raymond, submitted for publication.
(10) R. L. Rawls, *Chem. Eng. News*, **55**, 24 (1977).
(11) A. Zalkin, J. D. Forrester, and D. H. Templeton, *J. Am. Chem. Soc.*, **88**, 1810 (1966).
(12) R. Norrestam, B. Stensland, and C. I. Brändén, *J. Mol. Biol.*, **99**, 501 (1975).
(13) E. Hough and D. Rogers, *Biochem. Biophys. Res. Commun.*, **57**, 73 (1974).
(14) C. J. Carrano, S. R. Cooper, and K. N. Raymond, submitted for publication.
(15) R. J. Angelici, "Synthesis and Technique in Inorganic Chemistry", W. B. Saunders, Philadelphia, Pa., 1977.
(16) D. F. Evans, *J. Chem. Soc.*, 2003 (1959).
(17) F. J. C. Rossotti and H. Rossotti, "The Determination of Stability Constants", McGraw-Hill, New York, N.Y., 1961.
(18) K. Abu-Dari and K. N. Raymond, *J. Am. Chem. Soc.*, **99**, 2003 (1977).
(19) J. Leong and K. N. Raymond, *J. Am. Chem. Soc.*, **97**, 293 (1975).
(20) J. Leong and K. N. Raymond, *J. Am. Chem. Soc.*, **96**, 6628 (1974).
(21) J. Leong and K. N. Raymond, *J. Am. Chem. Soc.*, **96**, 1757 (1974).

The Relationship of Thermodynamic Data for Base Adduct Formation with Cobalt Protoporphyrin IX Dimethyl Ester to the Corresponding Enthalpies of Forming Dioxygen Adducts with Implications to Oxygen Binding Cooperativity

Russell S. Drago,* Tony Beugelsdijk, John A. Breese, and J. Patrick Cannady

Contribution from the School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801. *Received December 2, 1977*

Abstract: Enthalpies for the binding of a wide variety of axial bases to cobalt(II) protoporphyrin IX dimethyl ester, CoPPIXDME, have been determined by spectrophotometric titration methods. The enthalpies for the subsequent binding of dioxygen to the resulting base CoPPIXDME adducts have also been determined. For similar donor types, enthalpies of dioxygen binding are found to increase with the base-binding enthalpies. Implications of the above result to the complex problem of cooperative effects in hemoglobin are presented and discussed in terms of a "modified restraint theory". The *E* and *C* model has been successfully used to correlate enthalpies of base adduct formation to CoPPIXDME. We have shown theoretically and experimentally that the *E* and *C* equation can be extended to include the enthalpies of dioxygen binding enabling us to predict the O_2-cobalt bond strength for some 50 base adducts. The EPR spectra of several adducts have been investigated and interpreted in terms of the electron transfer model we proposed earlier.

Introduction

Understanding the processes whereby the reactivity of dioxygen is enhanced or inhibited by metal ions has important implications in fuel cell design, improving commercial catalytic oxidations, and understanding oxidations as well as oxygen transport in biological systems. The factors that influence the nature and strength of interaction between the metal and the dioxygen as well as the nature of the bound dioxygen are essential features for understanding the above problems. One of the key features regarding the nature of the bound dioxygen is the amount of metal electron density transferred into it upon coordination. This is an important property that is expected to influence the susceptibility of attack on dioxygen by nucleophiles or electrophiles. Variation of the metal and coordinated ligands is expected to have a pronounced effect on the electron density transfer into the bound dioxygen. In view of both the relative stability of the dioxygen adducts of cobalt(II) toward irreversible decomposition and the EPR probe provided by the existence of one unpaired electron in the molecule, most of the research in the area of dioxygen binding has involved this metal center.

We have recently proposed a spin-pairing model to account for the binding of dioxygen to a series of cobalt(II) complexes.[1]

The essential bonding interaction involves the coupling of the spins of a cobalt(II) unpaired electron in d_{z^2} with an electron in a dioxygen π-antibonding orbital. The EPR spectra were interpreted to indicate a wide range of electron transfer into the O_2 fragment as the ligand field strength of the groups coordinated to cobalt(II) is varied. The essential features of the bonding model are schematically illustrated in Figure 1. This model is in contrast to a superoxide description[2,3] of O_2 binding which arose from an EPR and infrared examination of these adducts. In the reanalysis of the EPR, we pointed out[1] that there are substantial differences in the interpretation of the cobalt hyperfine coupling constant in the two models. Direct delocalization of the unpaired electron on superoxide ion into the d_{yz} orbital of cobalt is proposed in the superoxide model.[2] This would lead to positive spin density on cobalt. This sign spin density leads to fractional unpaired electron density greater than one in the system when combined with the ^{17}O hyperfine results. On the other hand, the spin polarization mechanism which we proposed involves the unpaired electron in ψ_2 (mainly oxygen p orbital) (see Figure 1) polarizing the pair of electrons in the bonding molecular orbital ψ_1 by an indirect mechanism. This would give rise to negative spin density on cobalt. Thus, there are seen to be some real differences in the interpretation of the EPR with the inconsistency regarding the number of unpaired electrons in the system remaining unexplained in the superoxide model. As far as the chemical consequences of the two models are concerned, the essential difference is in the extent of electron transfer into the bound O_2, i.e., the partial negative charge on O_2. The spin pairing model can accommodate a bound dioxygen over a range of zero to a full electron transfer.[4] In the extreme of $Co^{II}O_2$, the unpaired electron is mainly on O_2 with $g_\parallel > g_\perp$, A_{aniso} is large, and cobalt has negative spin density on it. (For examples of $0.1e^-$ transfer, see ref 1.) The superoxide model, as originally proposed, transfers the electron back to the metal via the d_{yz}–$O_{(\pi)}$ interaction as we approach a $Co^{II}O_2$ limit. The unpaired electron now becomes localized mainly in a metal orbital with $g_\perp > g_\parallel$. A_{aniso} is large and there is positive spin density on cobalt(II).

The spin pairing model has not been properly represented in subsequent literature.[5] In spite of the very substantive differences in the two models that were discussed in ref 1 and have been summarized in qualitative terms above, the subsequent literature[3b,5] has focused on the oxidation state of the cobalt. For example, in ref 5 (footnote 68), an experiment and spectral measurements are suggested "to confirm that the oxidation state is three". In all the cobalt-dioxygen complexes studied to date, there is some electron transfer into the bound dioxygen so the oxidation state of cobalt is obviously three. It was not claimed to be otherwise in ref 1.[6] The assignment of oxidation state is often obvious and is then useful for classification purposes. However, when it is not, it is not worth worrying about for, as was elegantly pointed out[7] in 1945, it has little or no relationship to the electronic structure of a molecule.

Subsequent to our submission of the article on the analysis of the EPR spectra of cobalt-dioxygen adducts, several molecular orbital calculations have appeared.[8,9] These have agreed with our estimates of the extent of electron transfer into the bound dioxygen from the EPR analysis. In the case of the cobalt(II) complexes, the calculations indicate that the metal orbital involved in the spin pairing interaction is d_{z^2}. Molecular orbital calculations on the iron(II) porphyrin-dioxygen adduct are also consistent with our spin pairing model and with little electron transfer into O_2. To quote from an article on a PPP, $x\alpha$ calculation,[9a] "The superoxo formulation (Fe^{+3}·O_2^- . . .) . . . may be of some heuristic value. However, it seems to us to be inappropriate since it has little basis in terms of the electronic structure of FeO_2." Contrary to our speculation about the involvement of the iron d_{z^2} orbital in the bonding interaction of dioxygen with iron(II), the MO calculations indicate that the essential spin-pairing interaction involves the iron d_{xz} and d_{yz} orbitals. The situation is too complex to predict confidently which orbitals will be involved in binding O_2 to chromium(II) and manganese(II), but the spin-pairing model with fractional electron transfer is expected to pertain.

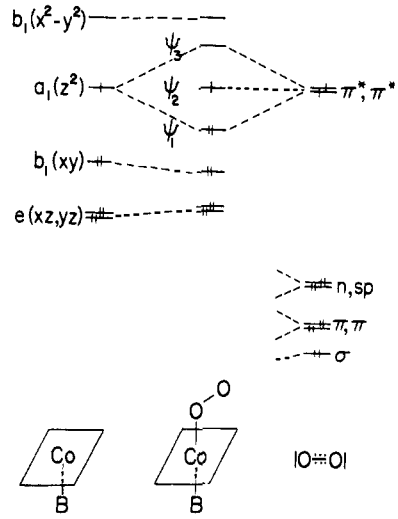

Figure 1. A restricted molecular orbital description of the essential spin pairing interaction involved in dioxygen binding to a cobalt(II)-base adduct.

With this information about the electronic nature of the dioxygen adducts available, the next question involves the influence that the axial base has on the metal-O_2 bond strength. Coordination by strong bases raises the energy of d_{z^2} increasing the mismatch in the energy of the metal and O_2 π^* orbital. This should lead to less effective bonding. However, additional stabilization for the system will result from the metal electron occupying the low-energy ψ_1^* bonding molecular orbital (see Figure 1). Experiments are needed to determine which effect is dominant.

We report in this study a determination of the enthalpies of axial base coordination to cobalt protoporphyrin IX dimethyl ester, CoPPIXDME, and a study of the enthalpy of dioxygen adduct formation as a function of axial base variation. Even though the coordination of dioxygen involves a pairing of electron spins as compared to electron pair donation, we show that the enthalpies of dioxygen adduct formation can be confidently predicted for a wide series of different axial bases by using our previously reported E and C equation.[10]

Experimental Section

I. Purification of Materials. Pyridine, py, was refluxed for 6–10 h over potassium hydroxide and then distilled at atmospheric pressure under dry nitrogen.

Hexamethylphosphoramide, HMPA, was stirred for 5 h over anhydrous BaO, followed by refluxing for 5 h at reduced pressure. It was then distilled at reduced pressure under dry nitrogen and the middle fraction collected.

N,N-Dimethylacetamide, DMA, was purified by stirring and refluxing over calcium hydride. It was then distilled under dry nitrogen at reduced pressure. The middle fraction is collected.

N,N-Dimethylformamide, DMF, was purified by stirring and refluxing over calcium hydride. It was then distilled under dry nitrogen at reduced pressure and the middle fraction collected.

N-Methylimidazole, 1-MeIm, was stirred over potassium hydroxide for 10 h and then distilled under dry nitrogen at reduced pressure.

Piperidine, Pip, and tetrahydrothiophene, THTP, were purified by stirring over anhydrous BaO under dry nitrogen for 12 h. They were then distilled at atmospheric pressure, the middle fraction being collected.

After purification, all bases were outgassed by freeze–thaw methods

Table I. Visible Spectroscopy for Protoporphyrin IX Dimethyl Ester and Its Cobalt II Complex

		Protoporphyrin IX Dimethyl Ester				
		band V, Soret	band IV	band III	band II	band I
ref	solvent	λ_{max}, nm/ϵ	λ_{max}, nm/ϵ	λ_{max}, nm/ϵ	λ_{max}, nm/ϵ	λ_{max}, nm/ϵ
Falk[18]	CH_2Cl_2	407/171 000	505/14 150	541/11 600	575/7440	630/5380
Grinstein[16]	$CHCl_3$	407.3/*a*	507.4/*a*	541.3/*a*	575.0/*a*	631.1/*a*
this study	CH_2Cl_2	407/171 000	506/13 900	542/12 400	575/8100	630/5500
Sigma[b]	CH_2Cl_2	407/171 000	506/14 000	542/12 400	575/8100	630/5500

		Cobalt(II) Protoporphyrin IX Dimethyl Ester		
ref	solvent	Soret λ_{max}, nm/ϵ	α λ_{max}, nm/ϵ	β λ_{max}, nm/ϵ
Falk[18]	benzene	404/165 000	563/23 100	530/11 860
Ibers[35]	toluene	404/160 000	563/22 800	528/11 300
this study	toluene	404/161 000	563/22 800	528/11 180
	benzene	404/165 000	563/23 100	528/11 800

[a] Extinction coefficients not reported. Extinction coefficients decrease in order Soret (V) > IV > III > I > II. [b] Sample purchased from Sigma Chemical, St. Louis, Mo., guaranteed purity 99%.

on a vacuum line. They were then transferred to screw cap vials (amber vials were used for piperidine and tetrahydrothiophene) equipped with mininert caps[11] in an inert atmosphere glove box.

Toluene and benzene were refluxed over CaH_2 for 24 h and distilled under nitrogen. All solvent transfers were done by employing gas-tight syringes to minimize exposure to air.

It is essential to use pure cobalt porphyrin. Impure and partially oxidized samples gave an isosbestic point as the base concentration was varied, but resulted in equilibrium constants that were wavelength dependent. To prepare CoPPIXDME, it has been found to be more convenient to start with the intermediate haemin and proceed by a modification of reported procedures.[12-16] Twenty grams of haemin (Nutritional Biochemical, Inc.) was mixed with 40 mL of pyridine and 800 mL of chloro form. One liter of MeOH and 150 g of $FeSO_4$ were added. Gaseous HCl was passed rapidly into the solution until the absorption band due to haemin (about 630 nm) was replaced by those of the protoporphyrin dication (601, 585 nm) as signaled by the red-brown solution changing to a deep red-violet color. Two liters of distilled water was added. The ester was concentrated in the organic phase. The chloroform solution was washed with three 500-mL portions of distilled water. These washings of the $CHCl_3$ solution should be carried out as quickly as possible to prevent hydrolysis of the ester by aqueous HCl. The chloroform solution was dried conveniently by filtering through a triple thickness of folded Whatman no. 1 paper wet with freshly washed and dried chloroform and was then evaporated to dryness in a rotary evaporator.

Although the residue may be recrystallized from chloroform–methanol at this point, better results are achieved by column chromatography. In preparing the column, 200 g of Woelm neutral alumina was deactivated to grade IV by addition of distilled water. The adsorbent was placed under benzene overnight. The residue was dissolved in a minimal amount of chloroform and excess benzene added. This was added to the column and a chloroform–benzene mixture (1:10 v/v) was used for the developing solvent. Only the desired product comes off the column. Porphyrin free acids, present in the preparation of the ester, remain as an immobile band at the top. The eluent was evaporated to dryness, dissolved in a minimum of chloroform, and briefly heated to boiling whereupon 1000 mL of methanol was added. The solution was then refrigerated for 2 days and filtered. The microcrystalline product was then dried at 50 °C under vacuum in a drying pistol, yield 11.2 g (63.8%). Anal. Calcd for $C_{36}H_{38}O_4N_4$: C, 73.19; H, 6.49; N, 9.49. Found: C, 72.89; H. 6.03; N, 9.30.

Protoporphyrin ester, prepared by this and similar methods, often contains considerable amounts of an impurity absorbing at about 600 nm. This can easily be removed by chromatography on columns of Al_2O_3, grade V, developed with benzene.[17] There were five bands in the visible spectrum of this purified porphyrin whose intensities were in the same order as those reported by Falk,[18] but different from those reported by Grinstein.[16] Although the same absorption maxima and the same extinction coefficient for the Soret band (band V) were obtained as those reported by Falk,[18] the other extinction coefficients were different. Our results are given in Table I. To check the purity of our sample, a guaranteed purity lot was obtained.[19] The visible spectrum of the purchased sample was identical with that of our compound.

Cobalt(II) was introduced into the porphyrin by the method previously reported.[20] The molar absorptivities in toluene at the band maxima, 563 nm (ϵ 22.8 × 10^3), 528 (11.1 × 10^3), 404 (161 × 10^3), were in good agreement with reported values. Anal. Calcd for $C_{36}H_{36}N_4O_4Co$: C, 66.77; H, 5.60; N, 8.65; Co, 9.10. Found: C, 66.53; H, 5.55; N, 8.36; Co. 9.19.

Owing to the oxygen sensitivity of many of the compounds and most of the solutions in these studies, all operations were carried out in oven-dried (140 °C) glassware in a dry nitrogen or argon atmosphere using Schlenk-type apparatus or in an inert atmosphere box.

II. Electron Spin Resonance. Electron spin resonance spectra were collected on a Varian Model E-9 spectrometer operating at ca. 9.1 GHz (X-band) and equipped with a Hewlett-Packard frequency counter and on a Varian Model E-15 spectrometer operating at ca. 35 GHz (Q-band). The field was calibrated on the X-band using a Varian weak pitch sample with $g = 2.0070$.[21] Cooling for low-temperature studies was provided by a stream of cold nitrogen gas or cold helium gas which provided a probe temperature of ca. 10 K.

Spectra were run in 4-mm o.d. quartz tubes fitted with either serum caps or Teflon needle valves. In all cases, the sample was placed in the tube as a solid and the appropriate base (if required) and solvent added under inert atmosphere in a nitrogen-filled drybox. The nonoxygenated samples were then studied. To oxygenate the samples, the serum caps were removed or Teflon-needle valves opened and repeated freeze–thaw cycles performed in the atmosphere. In all cases, a definite color change accompanies oxygenations.

III. Electronic Absorption Spectra. Electronic absorption spectra were recorded on a Cary 14 spectrophotometer. The wavelength scale was calibrated with a holmium oxide crystal[22] and the absorption scale was calibrated in the Soret region utilizing an aqueous solution made of 0.0400 g of K_2CrO_4 in 0.05 *m* KOH in a 1.000-cm cell at 25 °C.[23]

IV. Equilibrium Measurements. Equilibrium constant determinations for the reaction

$$\text{CoPPIXDME} + \text{base} \rightleftarrows \text{CoPPIXDME} \cdot \text{base}$$

were made by following the change in absorption in the visible and Soret bands with changes in base concentration and temperature. Because CoPPIXDME is unstable on standing at room temperature in the presence of base and O_2, special precautions were employed to eliminate O_2 in the spectral measurement. In a typical experiment, approximately 6 mL of a freshly made cobalt porphyrin solution (toluene solvent) is transferred to a bulb through a high-vacuum Teflon valve in an inert atmosphere glove box. The valve is closed, and the whole apparatus is taken out of the glove box and connected to a vacuum line through an o-ring. Four freeze–thaw outgassing cycles are performed, following which the cell is attached through an o-ring to a manifold equipped with a mercury bubbler, and the apparatus is filled with argon. This gives the cell a slightly positive pressure of argon atmosphere to prevent any leakage of air into the cell during use.

The Cary spectrophotometer is equipped with a temperature-controlling block through which water of constant temperature is flowing. This constant temperature is maintained to better than ±0.3

Table II. Wavelength Independence of the Equilibrium Constant for the Base Pyridine at 20 °C

λ, nm	404	490	537.5	560	563
K (σ)	2066 (152)	1924 (50)	2013 (50)	1970 (70)	1956 (22)

°C by a Braun Thermoboy constant-temperature bath. Temperature measurements utilize a calibrated YSI thermistor equipped with digital readout.

Base is added with a calibrated gas-tight 10-μL syringe (for tetrahydrothiophene 100 μL) directly into the sample through the serum cap. During the injection the cell is attached to a manifold under a positive argon pressure thus preventing leakage of O_2 into the cell from around the injection site. Before the base is injected, the calibrated syringe is flushed several times with argon.

Dilution effects from the base addition, although very small, are accounted for in the calculations. Changes in concentration of the solutions resulting from changes in the solvent (toluene) density with temperature are accounted for by using[24] $d_t = d_s + 10^{-3}\alpha(t - t_s) + 10^{-5}\beta(t - t_s)^2 + 10^{-9}\gamma(t - t_s)^3$ where, for toluene in the temperature range 0-99 °C, $d_s = 0.884\ 12$, $\alpha = -0.922\ 48$, $\beta = +0.0512$, $\gamma = 4.223$, and $t_s = 0$ °C.

The O_2 binding enthalpies are determined using a high-pressure cell[20] and calculated by the same procedures as reported previously.[20] The 1:1 adduct formation constants are also evaluated by reported procedures.[20,25] In order to determine the enthalpies of adduct formation along with reasonable estimates of the error limits, the calculated $\Delta\epsilon$ values at each temperature are used to calculate an equilibrium constant for each spectrum. The enthalpy is determined from a least-squares van't Hoff plot of all these points.

Results and Discussion

Enthalpies of Adduct Formation for 1:1 Adducts. Upon the addition of base to a toluene solution of CoPPIXDME, the Soret band shifts to longer wavelength and decreases in intensity. In the visible region, the α band at 563 nm decreases in intensity and is blue shifted. The β band at 528 nm is not shifted, but, at least, in part as a consequence of more extensive overlap with the shifted α band, an increase in intensity is observed. Because of the limited solubility of CoPPIXDME in toluene, the Soret band was selected for our collection of thermodynamic data. However, prior to base variation study, the adduct formation equilibrium constant for the base pyridine was studied as a function of wavelength. The wavelength independence of the system is illustrated by the results in Table II.

For each base studied, spectral studies of varying base concentration was carried out on the Soret band at four temperatures. Excellent isosbestic points were obtained in all of the systems investigated. The raw data are presented in the microfilm edition and the calculated molar absorptivities as well as the equilibrium constants are summarized in Table III along with the marginal and conditional deviations.[26]

The CoPPIXDME system was selected for study with the hope that previous literature data[27] on the 1:1 adducts would simplify our investigation of the strength of dioxygen binding as a function of cobalt-base bond strength. The literature results are summarized in Table IV along with our measured enthalpies. We were interested in attempting to fit the literature data to our reported E and C equation[28]

$$-\Delta H = E_A E_B + C_A C_B \qquad (1)$$

Using the reported enthalpies, along with reported[10a] E_B and C_B values, the least-squares best fit of E_A and C_A is deter-

Table IV. Enthalpies of Adduct Formation (kcal mol^{-1}) for the 1:1 Base Adducts of CoPPIXDME

base	$-\Delta H°_{lit}$[a]	$-\Delta H°_{lit}$ (E and C)[b]	$-\Delta H°$[c]	$-\Delta H$ (E and C)
piperidine (Pip)	10.4	10.2	9.9 (0.2)	9.9
1-methylimidazole (1-MeIm)	10.7	9.6	9.6 (0.4)	9.4
pyridine (Py)	6.9	9.0	8.9 (0.2)	8.9
tetrahydrothiophene (THTP)			6.0 (0.2)	6.1
N,N-dimethylacetamide (DMA)			*f*	7.4[e]
N,N-dimethylformamide (DMF)	7.9	6.8		6.9
hexamethylphosphoramide (HMPA)			*f*	8.9[e]

[a] Data from ref 27. [b] Calculated by solving for E_A and C_A of eq 1 using the literature enthalpies. $E_A = 4.24$ and $C_A = 0.63$. Though the fit is bad because the data are poor, the E_A and C_A parameters are close to the correct ones for the errors are apparently random. [c] Enthalpies determined in this study. The standard deviation is presented in parentheses. [d] Calculated by solving for E_A and C_A of eq 1 using the data from this study; $E_A = 4.44$ (0.18) and $C_A = 0.58$ (0.02). The base C_B and E_B values employed are respectively Pip, 9.29, 1.01; 1-MeIm, 8.96, 0.934; py, 6.40, 1.17; THTP, 7.90, 0.341; DMA, 2.58, 1.32; DMF, 2.48, 1.23; HMPA, 3.55, 1.52. [e] Calculated for use in O_2 binding section. [f] The equilibrium constant for adduct formation was low for this system. Such large excesses of base were required for significant complexation that we were in effect working in a mixed solvent.

Table III. Equilibrium Constant Values for Base Binding to CoPPIXDME

base	K, L mol^{-1}	MSD[a] in K	CSD[b] in K	MSD/CSD[a]	$\Delta\epsilon \times 10^4$	CSD[b] $\Delta\epsilon$	temp, °C
py	1868	58	41	1.4	5.03	224	20.6
	1109	26	16	1.7	5.07	162	29.9
	725	25	12	2.0	5.12	228	39.0
	461	21	9	2.3	5.20	321	49.3
THTP	32	2.0	0.9	2.2	4.83	416	20.6
	23	0.6	0.2	3.0	4.75	183	29.9
	16	0.5	0.2	2.5	4.91	204	39.0
	13	0.8	0.2	4.0	4.87	361	49.3
Pip	4986	275	212	1.3	4.20	420	20.6
	2961	761	53	14.5	4.21	177	29.9
	1823	57	28	2.0	4.20	155	39.0
	1102	108	37	2.9	4.19	421	49.3
1-MeIm	6566	467	328	1.4	5.26	597	20.6
	3362	217	136	1.6	5.19	565	29.9
	2159	153	107	1.4	5.27	633	39.0
	1354	125	83	1.5	5.21	857	49.3

[a] Marginal standard deviation. [b] Conditional standard deviation.

Table V. Equilibrium Constants for Dioxygen Binding to CoPPIXDME·Base

base	K, atm^{-1}	CSD in K	MSD/CSD	$\Delta\epsilon$	CSD in $\Delta\epsilon$	temp, °C
DMA	0.0831	0.0038	2.3	9698	103	−26.7
	0.1425	0.0092	2.9	7625	179	−33.9
	0.5126	0.0223	2.2	5738	52.4	−43.1
THIP	0.0134	0.0005	9.6	5636	157	−22.8
	0.0300	0.0012	6.3	8910	188	−34.8
	0.0453	0.0071	4.1	8956	691	−41.1
	0.0645	0.0138	5.4	5841	693	−45.7
Pip	0.0786	0.0082	2.9	9647	332	−22.5
	0.1314	0.0276	2.5	8986	565	−28.8
	0.2927	0.0403	2.3	10 378	420	−40.8
HMPA	0.0273	0.0013	3.9	5100	103	−15.3
	0.2018	0.0055	3.2	8298	78.8	−40.6
	0.4335	0.0111	2.5	8707	60.6	−51.3
py	0.0335	0.0010	4.4	7947	106	−5.9
	0.1377	0.0033	2.5	7468	46.9	−29.6
	0.3881	0.0144	1.9	9558	69.6	−41.6
	0.7525	0.0222	1.6	8272	47.5	−52.6
MeIm[a]	0.114	0.0057	1.6	3138	40.7	2
	4.27	0.341	2.7	77 013	2540	−31.0
	6.37	0.773	2.4	78 141	3160	−37.4
	15.22	0.785	1.7	59 504	797	−45.0

[a] Data at 20 °C were measured in this laboratory. The lower temperature data and equilibrium constants were derived from the raw data of ref 36. In contrast to most of the data in this reference, these were found to satisfy our criteria for acceptance of the fit.

mined. The agreement between the reported experimental values and the enthalpies from this best fit can be seen by comparing the ΔH_{lit} values in Table IV with ΔH_{lit} (E and C). If the literature data were correct, one would have to conclude that the deviations of calculated and experimental enthalpies are so large that the CoPPIXDME system is an exception to the E and C model. This is a surprising conclusion because it has been previously reported[29] that the zinc(II) complex of tetraphenylporphine formed a series of adducts whose formation enthalpies fit the E and C equation very well. Upon redetermining the enthalpies of adduct formation with CoPPIXDME, the least-squares best fit of this enthalpy data yielded values of $E_A = 4.44$ and $C_A = 0.58$. Substitution of these values into eq 1, along with reported[10a] E_B and C_B values, leads to the series of enthalpies listed under $-\Delta H$ (E and C) that are in excellent agreement with the experimental values.

Comparisons of the E_A and C_A values for CoPPIXDME with those of zinc tetraphenylporphine indicates that in σ bond formation, the zinc complex is a slightly stronger acid than the cobalt complex toward all types of bases. (Both the E_A and C_A of zinc(II) are larger than those of CoPPIXDME.) This could in part be due to the fact that the cobalt system was investigated in toluene and the zinc complex in cyclohexane. Estimates[30,31] of the specific solvent interaction with CoPPIXDME might equate the acidity of the two complexes, but are not expected to reverse the order. If the difference found is in the inherent acidity, different inductive properties of the porphyrin ligand toward Co^{2+} than toward Zn^{2+} could be influencing the order that is determined.

Base adducts formed with the porphyrin $\alpha,\beta,\gamma,\delta$-tetra(*p*-methoxyphenyl)porphinatocobalt(II) and several donors have been studied.[32] The enthalpies of adduct formation for the donors pyridine, *N*-methylimidazole, and piperidine can be compared with those studied here. Enthalpies ($-\Delta H$) of 8.5, 11.4, and 6.8 kcal mol^{-1}, respectively, are reported compared to 8.9, 9.6, and 9.9 kcal mol^{-1} toward CoPPIXDME. The three literature enthalpies reported above plus the value of 7.3 kcal mol^{-1} reported for 4-methylpyridine cannot be fit to the E and C equation with any degree of accuracy.[33] The difference between pyridine and 1-MeIm is larger than the authors[32] expected and was attributed to the π-acceptor properties of 1-MeIm. In CoPPIXDME, the 1-MeIm enthalpy of adduct formation is predicted quantitatively from parameters that are derived for σ bonding systems so there is no evidence for π stabilization. One would not expect such a dramatic change in π back-bonding for the substituent change made in the porphyrin. Furthermore, we have not found any evidence for π-back-bonding stabilization of 1-MeIm in any of the Lewis acids studied to date, including some rhodium(I) systems.[33] Based on the above arguments, we conclude that there are substantial error limits on this literature data.

Table VI. $-\Delta H°$ (kcal mol^{-1}) for the Formation of Dioxygen Adducts of CoPPIXDME

	$-\Delta H°_{O_2}$			
base	$-\Delta H°_{1:1}$	obsd[b]	E, C, W[a]	lit.[35,36]
1-MeIm	9.6	10.0 (0.2)	9.8	11.8
piperidine	9.9		10.0	9.0
pyridine	8.9	8.0 (0.4)	8.9	9.2
hexamethylphosphoramide (HMPA)	8.9[c]	8.9 (0.2)	8.1	
N,*N*-dimethylacetamide (DMA)	7.4[c]	6.6 (0.7)	6.9	
N,*N*-dimethylformamide (DMF)	6.9[c]		6.5	11.0
tetrahydrothiophene (THTP)	6.0	7.6 (0.6)	7.4	

[a] Calculated from eq 3 with $\Delta E_A = 2.9$ and $\Delta C_A = 0.6$ and the C_B and E_B values given in Table IV. [b] Standard deviations are reported in parentheses. [c] Calculated in Table IV.

Binding of Dioxygen as a Function of Axial Base Variation. The equilibrium constants and molar absorptivities calculated for the various bases at different temperatures are reported in Table V along with their marginal and conditional deviations. The enthalpies of binding dioxygen to the 1:1 base adducts of CoPPIXDME, $-\Delta H_{O_2}$, are summarized in Table VI. The errors in the enthalpies at the 90% confidence level are listed in parentheses. These error limits are inversely related to the magnitude of the interaction. With the weaker binding systems, it is difficult, at accessible temperatures, to get the ex-

tensive conversion of the five-coordinate complex to the O_2 adduct that is needed[20] to define K and $\Delta\epsilon$ accurately.

The enthalpies reported[35,36] in the literature for these systems are listed in Table VI under $-\Delta H_{lit}$. Based on the large value of the equilibrium constant and enthalpy, fewer complications are expected to contribute to the literature result for the 1-MeIm system. Surprisingly, this system misses our determined enthalpy by even more than the very generous error of 1.2 kcal mol^{-1} that was eventually assigned[36] to the reported pyridine system. Comparison of the literature value for the enthalpy of binding of O_2 to the DMF adduct with that for the DMA adduct leads us to believe that there is at least a 3 kcal mol^{-1} error in this literature number.[27] We selected DMA for study instead of DMF because in other acid-base work[10] we experienced problems with the latter base presumably because of difficulty in purification. In order to determine that the DMF system was not different from DMA for some unforeseen reason, we determined the equilibrium constant for dioxygen binding to the DMF adduct at −28.4 °C. A value of 0.029 atm^{-1} resulted compared to a value of 0.11 atm^{-1} calculated for the DMA adduct at this temperature from our determined enthalpy and entropy.

Examination of the data in Table VI enables us to conclude that the order of enthalpies of dioxygen binding is

$$\text{1-MeIm} > \text{HMPA} > \text{DMA}$$

This is also the order of base binding strengths to CoPPIXDME. The pyridine and THTP dioxygen adducts and base binding enthalpies are less exothermic than the adduct of 1-MeIm, but cannot be distinguished[37] from HMPA and DMA.

We have attempted to measure the dioxygen binding enthalpy to the piperidine adduct. In spite of an expected large enthalpy, we were not able to obtain equilibrium constants and enthalpies that satisfied our reported criteria.[20] We feel that at the low temperature needed for O_2 binding, a 2:1 piperidine adduct is forming and, as a result, the equilibrium is more complex than simple addition of dioxygen to a five-coordinate cobalt complex.

There are many advantages that would be associated with the incorporation of enthalpies for dioxygen binding into the E and C model. For example, enthalpies of binding dioxygen to CoPPIXDME·base could be predicted for adducts of all 50 bases in the E and C correlation. However, it is not obvious that the enthalpy data for dioxygen binding should be amenable to the E and C model. The model was developed for Lewis acid-base interactions (electron pair donation) and dioxygen binding involves spin pairing. However, the following considerations demonstrate that the model is directly applicable.

First, add together the enthalpy of 1:1 adduct formation and that for dioxygen binding to produce a total enthalpy, $-\Delta H_T$.

$$\begin{array}{rr} \text{Co} + \text{B} \rightleftarrows \text{CoB} & -\Delta H_1 \\ \text{CoB} + \text{O}_2 \rightleftarrows \text{CoB} \cdot \text{O}_2 & -\Delta H_{\text{O}_2} \\ \hline \text{Co} + \text{B} + \text{O}_2 \rightleftarrows \text{CoB} \cdot \text{O}_2 & -\Delta H_T \end{array}$$

Add to this equation the step

$$-[\text{Co} + \text{O}_2 \rightarrow \text{CoO}_2] \qquad +W$$

to yield

$$\text{CoO}_2 + \text{B} \rightleftarrows \text{CoBO}_2 \qquad -\Delta H_2$$

We can now write

$$-\Delta H_T = -\Delta H_2 - W = -\Delta H_1 - \Delta H_{\text{O}_2}$$

or

$$-\Delta H_{\text{O}_2} = -W + \Delta H_1 - \Delta H_2 \qquad (2)$$

Now ΔH_1 and ΔH_2 are clearly electron pair donation steps which can be treated by the E and C model. Accordingly, we can express[39] ΔH_1 and ΔH_2 in the form of eq 1 and rewrite eq 2 as

$$-\Delta H_{\text{O}_2} = -W - E_A^{(1)}E_B - C_A^{(1)}C_B + E_A^{(2)}E_B + C_A^{(2)}C_B$$

or

$$-\Delta H_{\text{O}_2} = -W + (\Delta E)E_B + (\Delta C)C_B \qquad (3)$$

where $\Delta E = E_A^{(2)} - E_A^{(1)}$ and $\Delta C = C_A^{(2)} - C_A^{(1)}$ with the superscript (2) referring to the hypothetical acid, O_2Co, and superscript (1) to the four-coordinate cobalt complex. In the CoPPIXDME system, $E_A^{(1)}$ and $C_A^{(1)}$ have been determined and presented in the previous section. Thus, if we treat eq 3 as a typical E, C, and W system,[38] we can solve a set of enthalpies for dioxygen binding directly for W, ΔE, and ΔC and use the known $E_A^{(1)}$ and $C_A^{(1)}$ to calculate $E_A^{(2)}$ and $C_A^{(2)}$. The data fit is indicated by comparing the experimental enthalpies with those listed under $-\Delta H$ (E, C, W) in Table VI. The parameters obtained are $\Delta E = 2.9$, $\Delta C = 0.6$, and $W = -1.5$ leading to $E_A^{(2)} = 7.3$ and $C_A^{(2)} = 1.2$. The W value obtained indicates an exothermic enthalpy of binding of O_2 to a four-coordinate complex of −1.5 kcal mol^{-1}. In view of the −30 to −40 eu determined for dioxygen binding to base adducts, an enthalpy of −1.5 kcal mol^{-1} is consistent with the fact that no O_2 adduct is observed in the absence of axial base binding over the temperature range studied. The increased $C_A^{(2)}$ and $E_A^{(2)}$ values for the CoO_2 fragment compared to those for CoPPIXDME is consistent with an increased acidity of the cobalt center as a result of the increased formal charge on cobalt resulting from dioxygen coordination.

The calculated W value is an upper limit for the metal-oxygen bond strength in the hypothetical Co-O_2 complex. Any constant solvation contribution to the measured enthalpies (for example, that associated with the coordinated dioxygen being solvated by toluene) would be included, i.e., the equilibrium being studied is

$$\text{CoPPIXDME} \cdot \text{B}_{(\text{soln})} + \text{O}_{2(\text{g})} \rightarrow \text{B} \cdot \text{CoPPIXDME} \cdot \text{O}_{2(\text{soln})}$$

For reactions of the type

$$\text{A}_{\text{soln}} + \text{B}_{\text{soln}} \rightleftarrows \text{AB}_{\text{soln}}$$

the solvation energy in benzene has been shown to be small.[31]

The determination of the enthalpy of dioxygen binding is a very difficult and time-consuming experiment. Equation 2 can now be used with reported C_B and E_B values to predict enthalpies in many cases more accurately than they can be measured. The experimental difficulties with this system are illustrated by the fact that the errors in the values reported in Table VI are larger than any of those for any system previously reported from this laboratory. Accordingly, having demonstrated that an E and C fit should apply, we would give more weight to the enthalpies predicted from the E and C values than those directly measured[41] for σ donor systems.

Some of the key conclusions of the earlier study can be reexamined in view of the fit, within experimental error, of our experimental enthalpies to the E and C model. It was concluded[35,41] that the π donating ability of a base was an important factor in determining the strength of dioxygen binding. In contrast to conclusions[32] on the 1:1 adducts where it is proposed that 1-MeIm is a π acceptor, 1-MeIm is proposed[35,41] to give rise to very large enthalpies of dioxygen binding because of its π-donating abilities. We find that with regard to both 1:1 adduct formation and dioxygen binding the enthalpies for the 1-methylimidazole systems are predicted quantitatively on the

basis of the σ donating,[10] E and C properties. Experimentally, there is no evidence that one need invoke π interactions of any sort (e.g., π interactions) to explain the behavior of 1-methylimidazole in this system. The large error in the enthalpy of dioxygen binding reported for the DMF adduct led these authors[35,41] to conclude incorrectly that DMF is a very good π-donating base.

One other significant conclusion can be drawn as a result of the E and C formulation of this problem. It has previously been shown[10b] that a linear plot of enthalpy of binding of a series of widely different types of bases (i.e., bases with varying C_B/E_B ratios) to acid I vs. the enthalpy for binding to acid II can result only when the C_A/E_A ratio of the two acids is about the same. With a C_A/E_A ratio of 0.13 for CoPPIXDME forming adducts with bases and a $\Delta C/\Delta E$ ratio of 0.21 for the enthalpy of dioxygen binding to the base adducts, a linear plot of $-\Delta H_{1:1}$ vs. $-\Delta H_{O_2}$ will not result if different types of bases are used. The large $\Delta C/\Delta E$ ratio of 0.21 for the enthalpy of dioxygen binding indicates that this reaction gains considerable stabilization from the increased tendency of 1-MeIm to undergo covalent bonding ($C_B/E_B = 9.6$) compared to pyridine ($C_B/E_B = 5.4$). This is probably the reason histidine is utilized as the axial base in the natural system as opposed to a pyridine (pyrimidine) type of donor.

Nature of the Cooperative Effects in Hemoglobin. The conclusions presented above have implications concerning the "restraint theory" of cooperativity in hemoglobin.[42] In the deoxy T state, the restraint theory proposes that the proximal histidine is held in place and restrains the five-coordinate deoxymetalloporphyrin from picking up O_2 and becoming six coordinate. This restraint opposes the movement of iron toward the plane. Weakened O_2 bonding results. According to this model, no strain is present in the deoxy form of native hemoglobin. The conformation change to the R state removes the restraint to O_2 binding and a higher affinity for O_2 results for the R form. Cooperativity is observed with carbon monoxide binding as well as with dioxygen and has also been reported[43] to occur in the dioxygen pickup by cobalt-substituted hemoglobin, CoHb.

We have found experimentally and the spin-pairing model of dioxygen binding predicts generally that with similar types of bases dioxygen binding will become weaker as the strength of the axial base bond becomes weaker. This relationship suggests an alternative description for the "restraint theory". It is possible that in the T form of hemoglobin the protein restrains the histidine from interacting with the iron as strongly as it might in the absence of this restraint. The iron is high spin and on the histidine side of the porphyrin plane. The restraint could bend the histidine, preventing direct overlap of the nitrogen with the iron d_{z^2} orbital, or simply prevent the two from as close an approach as desired. According to the enthalpy relationship between $\Delta H_{1:1}$ and ΔH_{O_2}, the oxygen affinity for this conformation is lowered relative to what it would be in the absence of this restraint. This effect causes both the metal-histidine and the metal-dioxygen bonds to be weaker in the T form than they would be in the absence of the restraint. Coordination of dioxygen to this form builds up potential energy in the system that could be released if the restraint were removed. If enough energy for the change cannot be obtained upon binding the first dioxygen, multiples of this potential energy arise on binding the second, etc., oxygens. Note that potential energy is to be gained from a stronger metal-base interaction as well as a stronger dioxygen interaction when each step occurs. Eventually, at some place in the four steps more than enough potential energy is available from removal of the restraint to effect the endothermic T to R protein transformation.

Since the bulk of the molecules change their quaternary structure and expel diphosphoglycerate (DPG^-) as the third oxygen is taken up,[44] the energetics for the protein transformation which includes many complex processes whose net is endothermic can be divided over the three iron-imidazole and three iron-dioxygen interactions. Depending upon the net exothermic and endothermic contribution, the magnitude of the enhanced dioxygen binding could be large or small for this third step. However, the oxygen affinity would be expected to be very large for the R form of the protein in the fourth step. In this view, understanding the mechanism of cooperativity involves understanding the mechanism and energetics for interconverting the tertiary structure of the protein. The complications associated with understanding the energetics of protein transformations have been elegantly presented in a review article by Weber.[45]

It is to be emphasized that the "restraint" and "modified restraint" mechanisms are not mutually exclusive. Steric effects which prevent a strong-base cobalt interaction and, as a result, substantially reduce dioxygen binding, model the "modified restraint" proposal. Such a system[46] involves the 1,2-diMeIm adduct of the cobalt(II) picket fence porphyrin. A reduced equilibrium constant for binding 1,2-diMeIm to the porphyrin and a decreased affinity for dioxygen binding is observed when compared to the 1-MeIm adduct. The binding of carbon monoxide to a sterically hindered 2-methylimidazole adduct of an iron(II) porphyrin is also reported[47] to be substantially reduced from that observed for the 1-MeIm adduct.

To model the restraint mechanism, one needs to find an adduct in which the metal-base interaction is not substantially weakened by a steric effect, but the steric effect becomes operative when dioxygen binding occurs. The data in Tables IV and VI indicate that the 1:1 base adducts have enthalpies that are predicted by the E and C equation and hence steric effects are absent. Most of these bases have dioxygen enthalpies that fit the E and C equation and thus have no steric problems. This relationship would break down in the restraint model, for steric effects would set in on O_2 binding. The pyridine adduct data in Tables IV and VI come closest to modeling the restraint mechanism. When steric effects are encountered, the measured enthalpy is less than that predicted by the E and C equation. No steric effects exist in the 1:1 pyridine-CoPPIXDME adduct, but the measured dioxygen binding enthalpy is lower than predicted by 0.9 kcal mol^{-1}. However, differences here are just barely within an expected experimental error of ± 0.2 kcal mol^{-1} in the data fit and ± 0.7 kcal mol^{-1} in the measured enthalpy. The quinuclidine adduct of CoPPIXDME may be a better model for the restraint mechanism. The 1:1 adduct forms readily and nearly complete complexation occurs at low concentrations of base. However, even at 1500 psi of O_2 pressure at -50 °C, not enough oxygen complex formed to permit a determination of the equilibrium constant.

It is interesting to examine the decreased extent of cooperativity found in CoHb relative to Hb in terms of deciding between the "restraint" and "modified restraint" models. A closer position of the cobalt than iron relative to the mean plane of the porphyrin is predicted in these systems from spin state considerations.[2,48] Decreased cooperativity is predicted in the restraint model because less strain is induced by dioxygen coordination. In the modfied restraint model, less distortion of cobalt from the plane would imply less effective interaction of the cobalt with the histidine suggesting a greater restraint in the T form of CoHb than Hb. However, in the modified restraint model, the potential energy gained and stored upon oxygen complexation would depend upon the relative cobalt-base and iron-base bond energies as well as the sensitivity of the dioxygen-iron bond strengths to coordinated axial base strength, i.e., the ΔC and ΔE of iron compared to cobalt. Even with these data differences in DPG^{4-} binding and in tertiary structure interactions for the various forms of the cobalt and

Table VII. Spin Hamiltonian Parameters for CoPPIXDME-Base-O_2

solvent	base	$A_{\parallel}(Q)$	$A_{\perp}(A)$	$A_{iso}(A)$	A_{aniso}	α'^2	ET
toluene	1-MeIm	16.23	7.3	10.25	5.98	0.286	0.43
	THTP	16.6	10.6	12.44	4.16	0.212	0.58
	Pip	15.9	7.1	10.03	5.87	0.282	0.44
	HMPA	15.3	7.9	10.35	4.95	0.244	0.51
	DMA	15.6	8.6	10.90	4.70	0.234	0.53
	py	16.0	8.6	10.57	5.43	0.264	0.47
toluene/CH_2Cl_2 (3/2 v/v)	THTP	18.0	9.6	12.4	5.6	0.270	0.46
	DMA	16.5	8.1	10.9	5.6	0.270	0.46
	MeIm	16.7	8.3	11.1	5.6	0.270	0.46
toluene/heptane/base (3/3/1 v/v/v)	THTP	15.5	10.5	12.3	3.2	0.172	0.66
	py	15.7	8.4	10.8	4.9	0.242	0.52
	Me-Im	17.1	6.9	10.3	6.8	0.320	0.36

[a] Cobalt hyperfine; values in 10^{-4} cm^{-1}; the EPR does not give the sign of these quantities but the spin-polarization model and the calculation of the electron transfer values requires a negative number.

iron systems must also be considered before the causes of the differences are resolved.

An x-ray diffraction investigation of the coordinated dioxygen would probably be of little help in distinguishing the two models. In a recent study, it was shown that very substantial differences in dioxygen binding by a series of iridium complexes do not lead to large enough changes in the O-O distance to be detected in a single-crystal x-ray diffraction investigation.[49] In the Hb system, the difference in free energy of binding the fourth and first O_2 is about 3.6 kcal mol^{-1}.[50] Studies[51] with stripped hemoglobin indicate that 1.2 kcal mol^{-1} of this difference is due to inhibition of O_2 binding in the T form by DPG.$^{4-}$ This leaves 2.4 kcal mol^{-1} to be distributed over the eight axially coordinated ligands and protein contacts. It is conceivable that information regarding the problem could be obtained from structural details of the iron-proximal histidine interaction.

EPR Investigations. The cobalt hyperfine coupling constant arises mainly from spin polarization of the bonding molecular orbital, ψ_1, which is mainly composed of d_{z^2}, 4s, and oxygen atomic orbitals:

$$\psi_1 = \alpha'(d_{z^2}) + \gamma(4s) + \beta(p_0)$$

The d_{z^2} coefficient α' is given from a McConnell-type interpretation of the anisotropic hyperfine coupling constant as indicated with the equation

$$A_{aniso} = Q_{Co\text{-}O}\rho_0\alpha'^2 \quad (4)$$

where Q is the polarization constant and ρ_0 the spin density on the oxygen directly bound to cobalt. In a previous article, we[1] indicated the assumptions employed to determine Q, leading to a value of -6.09×10^{-3} cm^{-1}. A value of ρ_0 of 0.4 was employed in view of the results from the ^{17}O anisotropic hyperfine coupling constants. We also estimated a dipolar contribution from the electron density on the terminal oxygen of 1×10^{-4} cm^{-1} which was subtracted from the directly measured A_{aniso}. The corrected A_{aniso} is used in eq 4, which can thus be solved for an α'^2. The calculated value of α'^2 can be converted into an electron transfer, ET, into the bound dioxygen from a zero overlap, Mulliken population type analysis with[1]

$$ET = 2(1 - \alpha'^2) - 1 \quad (5)$$

This assumes a constant $3d_{z^2}$ to 4s ratio or a small constant 4s contribution to ψ_1. There are also a great many assumptions involved in the use of eq 4 and the results should be viewed as semiquantitative. Interpretations of values that differ by less than 0.1-0.2 are not warranted unless a careful analysis of all the assumptions is shown not to affect the conclusion.

The results of EPR investigations of the adducts investigated in this study are listed in Table VII. There is a relative insensitivity of the calculated electron transfers to the axial base in these systems. The spin pairing model predicts that there should be an increase in the extent of electron transfer into the dioxygen as the cobalt-axial base bond strength increases. However, the EPR spectral changes in this system are not large enough to give ET transfer values that we are willing to interpret as arising from this effect. Though the numbers obtained from the spectra are good to a few percent, the calculated values of the electron transfer are all within the 0.1-0.2 range in which the differences could be dominated by factors other than α'^2. The values calculated for 1-MeIm and THTP from spectra measured in toluene also suggest that third-row donors should not be compared with second-row donors in deducing electron transfer values. If one investigates the influence of the different approximations involved in obtaining a constant Q, most tend to increase the electron transfer as the base becomes stronger. Thus, the apparent reversal for these two donors is all the more puzzling. A rationalization can be presented. Note that the difference between A_{iso} for the sulfur donor and that for other donors is large compared to all other pairs. Weak donors will compete less effectively for the cobalt 4s orbital in the bonding interaction than strong donors. Accordingly, with the weak donors more of the 4s orbital will be available for bonding to the oxygen and contributing to ψ_1. Spin polarization of ψ_1 will thus give rise to a larger A_{iso} as a result of more 4s character. Accordingly, A_{aniso}, which is the difference in $A_{\parallel}$ and A_{iso}, will decrease, the value of α'^2 calculated by eq 4 will give too low an estimate of the metal character in ψ_1, and the ET calculated will be too large. This effect is apparently exaggerated in comparing second- and third-row donors, but one notes that the weaker the oxygen binding donor (as manifested by $-\Delta H_{O_2}$) the larger A_{iso} for almost all of the systems studied in toluene or toluene-heptane, base glasses (Table VII). With axial base variation in this system, the changes in actual electron transfer are small enough that the EPR changes are dominated by other factors. Accordingly, in this system the EPR spectra do not provide information on the actual trends in electron transfer. Further support for the semiquantitative nature of the ET values (and the caution that should be exercised in the interpretation of small differences) arises from the variations found in this quantity with solvent change.

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Supplementary Material Available: Raw absorbance and concentration data for all equilibrium constants reported in this article (8 pages). Ordering information is given on any current masthead page.

References and Notes

(1) B. S. Tovrog, D. J. Kitko, and R. S. Drago, *J. Am. Chem. Soc.*, **98**, 5144 (1976).
(2) B. M. Hoffman, D. L. Diemente, and F. Basolo, *J. Am. Chem. Soc.*, **92**, 55 (1970); F. Basolo, B. M. Hoffman, and J. A. Ibers, *Acc. Chem. Res.*, **8**, 384 (1975).
(3) (a) The superoxide model views the coordination of dioxygen as resulting from a nearly complete transfer of an electron into O_2 producing the strong coordinating ligand O_2^-. The superoxide ion then undergoes an essentially ionic interaction (90% ionic character or more) with a cobalt center whose oxidation state has been increased by one, i.e., $Co^{III}O_2^-$. Support for the superoxide description came from an interpretation of the EPR of the O_2 complex and a reduced frequency for the O–O stretching vibration of the bound O_2 compared to dioxygen. To quote the authors,[2] "Nearly complete electron transfer from cobalt to oxygen will also account for the low value of α^2 in the adducts". Here α is the d_{yz} cobalt coefficient and complete transfer gives O_2^-. The EPR has been reanalyzed and the point made[1] that the observed infrared frequency decrease for bound O_2 cannot be interpreted in terms of charge transfer into O_2 unless one knows the vibration frequency for a nearly neutral oxygen bound to cobalt(II) and that for a superoxide ion bound to cobalt(III). (b) The smaller cobalt hyperfine associated with stronger ligands on cobalt is explained[2] in the superoxide model by: "Electron pair donation by the lone pair reduces the actual charge which develops on the cobalt and that the greater the pair donation to cobalt from the base, the more unpaired spin density is transferred from cobalt to oxygen". This is a direct transfer of positive spin density. The spin pairing model claims that, as the base strength becomes greater, the smaller the cobalt contribution to ψ_1 and the smaller the amount of negative spin density on cobalt.
(4) For 0.9 and greater electron transfer, the spin pairing and superoxide ion ($Co^{III}O_2^-$) descriptions are comparable except for the sign of the spin density on cobalt. For 0.5 of an electron transfer or less, not only does it make little sense to transfer a full electron to O_2 and then transfer 50% or more back, but the nature of the molecular orbital containing the unpaired electron is very different in the two models.
(5) J. P. Collman, *Acc. Chem. Res.*, **10**, 265 (1977). On p 269, this article states that "the unpaired electron in the Co–dioxygen complexes is widely assigned to an oxygen π orbital,[67] suggesting that these compounds are best described as Co(III)-superoxide complexes". Reference 67 of this paper attributes a dissenting opinion to the authors of ref 1. It is clearly stated in ref 1 that "the unpaired electron resides in a molecular orbital which was mainly composed of oxygen p-orbitals". The complex does not contain a plane of symmetry; so it cannot be a π orbital. A cursory examination of Figure 3 of our ref 1 clearly shows that this molecular orbital arose from the π^* orbital of O_2. Furthermore, we offer a convincing argument that the oxygen character of this orbital has nothing to do with whether the bound dioxygen is superoxide, neutral dioxygen, or even cationic dioxygen.
(6) The suggested experiment for oxidation state classification in ref 5, footnote 68, has arisen from a misunderstanding of our position. It should be noted that the symbols $Co^{III}O_2^-$ and $Co^{III}O_2(-I)$ are not the same. $Co^{III}O_2^-$ infers the 90% or more electron transfer originally proposed[2] in the EPR study. $Co^{III}O_2(-1)$ is an oxidation state description. This is the same distinction chemists make between Na^+Cl^- and HCl.
(7) C. A. VanderWerf, H. H. Sisler, and A. W. Davidson, *J. Chem. Educ.*, **22**, 450 (1945).
(8) P. Fantucci and V. Valenti, *J. Am. Chem. Soc.*, **98**, 3832 (1976).
(9) (a) B. H. Huynh, D. A. Case, and M. Karplus, *J. Am. Chem. Soc.*, **99**, 6103 (1977); (b) R. V. Kirchner and G. H. Loew, *ibid.*, **99**, 4639 (1977); (c) A. Redieu, M.-M. Rohmer, M. Bernard, and A. Veillard, *ibid.*, **98**, 3717 (1976).
(10) (a) R. S. Drago, *Struct. Bonding (Berlin)* **15**, 73 (1973), and references cited therein; (b) R. S. Drago, G. C. Vogel, and T. E. Needham, *J. Am. Chem. Soc.*, **93**, 6014 (1971).
(11) "Mininert Valves", Supelco, Inc., Supelco Park, Bellefonte, Pa.
(12) W. S. Caughey, J. O. Alber, W. Y. Fujimoto, and J. L. York, *J. Org. Chem.*, **31**, 2631 (1966).
(13) H. Fischer and K. O. Deilmann, *Hoppe-Seyler's Z. Physiol. Chem.*, **280**, 186 (1944).
(14) V. G. Ramsey, "Biochemical Preparations", Vol. 3, Wiley, New York, N.Y., 1953, p 39.
(15) H. Fischer, A. Treibs, and K. Zeile, *Hoppe-Seyler's Z. Physiol. Chem.*, **193**, 138 (1931).
(16) M. Grinstein, S. Schwartz, and C. J. Watson, *J. Biol. Chem.*, **157**, 323 (1945).
(17) J. E. Falk, "Porphyrins and Metalloporphyrins", American Elsevier, New York, N.Y., 1964, p 206.
(18) Reference 17, p 167.
(19) Sample purchased from Sigma Chemical, St. Louis, Mo.
(20) T. J. Beugelsdijk and R. S. Drago, *J. Am. Chem. Soc.*, **97**, 6466 (1975).
(21) Varian Associates standard sample no. 904450-01, Palo Alto, Calif.
(22) C. K. Jurgenson and J. S. Brenen, *Mol. Phys.*, **6**, 629 (1963).
(23) A. J. Gordon and R. A. Ford, "The Chemist's Companion", Wiley, New York, N.Y., 1972.
(24) National Research Council, "International Critical Tables of Numerical Data, Physics, Chemistry and Technology", McGraw-Hill, New York, N.Y., 1926.
(25) R. M. Guidry and R. S. Drago, *J. Am. Chem. Soc.*, **95**, 6645 (1973).
(26) See F. L. Slejko, R. S. Drago, and D. G. Brown, *J. Am. Chem. Soc.*, **94**, 9210 (1972), for the significance of these terms.
(27) D. V. Stynes, H. C. Stynes, B. R. James, and J. A. Ibers, *J. Am. Chem. Soc.*, **95**, 1796 (1973).
(28) Reference 10 and ref 31 contain the most recent set of parameters for use in the *E* and *C* equation.
(29) G. C. Vogel and J. R. Stahlbush, *Inorg. Chem.*, **18**, 950 (1977).
(30) Using the E_A and C_A values for the cobalt complex from this study with E_B and C_B values for benzene[39] leads to a predicted enthalpy of specific interaction of benzene with the CoPPIXDME of 1.2 kcal mol^{-1} of adduct. This would constitute an upper limit to the contribution to the measured enthalpy in benzene solvent, for CoPPIXDME may not be fully complexed.
(31) R. S. Drago, L. B. Parr, and C. S. Chamberlain, *J. Am. Chem. Soc.*, 99, 3203 (1977).
(32) F. A. Walker, *J. Am. Chem. Soc.*, **95**, 1150 (1973).
(33) The calculated and experimental (in parentheses) values for the best fit follow: pyridine, 7.6 (8.5); 4-methylpyridine, 7.9 (7.3); piperidine, 9.4 (6.8); *N*-methylimidazole, 9.0 (11.4).
(34) M. P. Li and R. S. Drago, *J. Am. Chem. Soc.*, **98**, 5129 (1976).
(35) D. V. Stynes, H. C. Stynes, J. A. Ibers and B. R. James, *J. Am. Chem. Soc.*, **95**, 1142 (1973).
(36) J. A. Ibers, D. V. Stynes, H. C. Stynes, and B. R. James, *J. Am. Chem. Soc.*, **96**, 1358 (1974), and references cited therein.
(37) With as few points as one has on a van't Hoff plot, statistical procedures for determining the error do not apply rigorously and often tend to produce large values.
(38) R. M. Guidry and R. S. Drago, *J. Am. Chem. Soc.*, **95**, 759 (1973). This reference shows that if in a series of enthalpies there is some process which makes a constant energy contribution, *W*, to all the data, then this data will not fit eq 1. Instead, it will fit $-\Delta H + W = E_A E_B + C_A C_B$. In this case, the constant energy is the Co–O_2 bond strength in the hypothetical Co–O_2 acid, using bases with known E_B and C_B values. The *E*, *C*, and *W* equation is solved for the three unknowns, E_A, C_A, and *W*.
(39) At this step, we can either solve for $-\Delta H_2 = -\Delta H_T + W$ and then get ΔH_{O_2} from ΔH_1 and ΔH_T or, as suggested by C. Chamberlain, Ph.D. Thesis, University of Illinois, 1977, solve directly for ΔH_{O_2} as shown here.
(40) If the model applies and the errors in a large enough data set are random, the *E* and *C* equation will predict enthalpies more accurately than they can be measured. Note in Table IV that even with errors of 2 kcal mol^{-1} in the literature values, the *E* and *C* predicted enthalpies from this data ($-\Delta H_{lit}$ (*E* and *C*) are within a few tenths of our measured numbers. This argument implies that the literature enthalpy for the dioxygen adduct of pyridine is closer to the correct value than ours. However, examination of the raw data[35] indicates that this agreement is fortuitous and the authors were not as fortunate with either the dioxygen enthalpies of the *N*-MeIm or DMF adducts or with most of the 1:1 adducts that were reinvestigated (Tables IV and VI).
(41) F. Basolo, B. M. Hoffman, and J. A. Ibers, *Acc. Chem. Res.*, **8**, 384 (1975).
(42) M. F. Perutz, *Br. Med. Bull.*, **32**, 195 (1976), and references cited therein.
(43) K. Imai, T. Yonetani, and M. Ikeda-Saito, *J. Mol. Biol.*, **109**, 83 (1977), and references cited therein.
(44) R. MacQuarrie and Q. H. Gibson, *J. Biol. Chem.*, **247**, 5686 (1972).
(45) G. Weber, *Adv. Protein Chem.*, **29**, 1 (1975).
(46) Private communication in the form of a preprint of an article by J. P. Collman, J. I. Brauman, K. M. Doxsee, T. R. Halbert, S. E. Hayes, and K. S. Suslick.
(47) M. Rougee and D. Brault, *Biochem. Biophys. Res. Commun.*, **55**, 1364 (1974); *Biochemistry*, **14**, 4100 (1975), and references cited therein.
(48) W. R. Scheidt, P. N. Dwyer, and P. Madera, *J. Am. Chem. Soc.*, **96**, 4815 (1974); R. G. Little and J. A. Ibers, *ibid.*, **96**, 4452 (1974).
(49) M. J. Nolte, E. Singleton, and M. Laing, *J. Am. Chem. Soc.*, **97**, 6396 (1975).
(50) This value is dependent upon the pH as well as the presence of electrolytes, CO_2, and other materials in solution.
(51) I. Tyuma, K. Shimizu, and K. Imai, *Biochem. Biophys. Res. Commun.*, **43**, 423 (1971); **44**, 682 (1971).