In common with complex-formation reactions of metal ions, the reaction of iodine with iodide ions may be assumed to proceed *via* the formation of an outer-sphere complex. This is illustrated in reactions 17 and 18. The iodine molecule and the iodide ion are

$$I_2 + I_{aq}^- \underset{k_b}{\overset{k_a}{\rightleftharpoons}} I_2(OH_2)I^- \quad (17)$$

$$I_2(OH_2)I^- \xrightarrow{k_c} I_3^- + H_2O \quad (18)$$

separated by a water molecule in the outer-sphere complex $I_2(OH_2)I^-$. In terms of this interpretation the first step in the reaction is the diffusion-controlled formation of the outer-sphere complex, and the second step is the formation of triiodide ion. The rate constant for the formation of the outer-sphere complex may be estimated from eq 16 with r_1 equal to $(2.05 + 2r_w) \times 10^{-8}$ cm where $2r_w$ is the diameter of a water molecule (2.76×10^{-8} cm). This procedure gives $k_a = 2.4 \times 10^{10}\ M^{-1}\ sec^{-1}$. The value of the outer-sphere association constant may be calculated from eq 19[25]

$$K_0 = 4\pi Na^3/3(1000) \quad (19)$$

where a is the distance between the centers of the two reactants in the outer-sphere complex. Substitution of $a = (r_1 + r_2 + 2r_w)$ in eq 19 gives $K_0 = 1.0\ M^{-1}$. Finally, the steady-state approximation for the concentration of the outer-sphere complex leads to the following expression for the formation rate constant

$$k_1 = k_a k_c/(k_b + k_c) \quad (20)$$

Substitution in eq 20, and remembering that $K_0 = k_a/k_b$, yields $k_c = 8 \times 10^9\ sec^{-1}$. The rate constant k_c provides an estimate of the lower limit of the rate constant for water exchange on the iodide ion.[26] The value of $\geq 8 \times 10^9\ sec^{-1}$ for this process seems reasonable since the rate constant for water exchange on cesium ion is $5 \times 10^9\ sec^{-1}$ [27] and water exchange on this ion is almost certainly slower than water exchange on the larger, negatively charged, structure-breaking iodide ion.

The relaxation times measured in this work are almost two orders of magnitude faster than those previously measured by the temperature-jump method. As has been pointed out,[10] it may be possible to obtain heating times of 10^{-8} sec or less by using a cavity-dumped or mode-locked laser. Another advantage of the laser temperature-jump technique is that it may readily be used with a variety of sample cells and reaction media. These and other aspects of the laser temperature-jump technique are currently being actively explored.

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Thermodynamics of the Reversible Oxygenation of Amine Complexes of Cobalt(II) Protoporphyrin IX Dimethyl Ester in a Nonaqueous Medium

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Abstract: The equilibrium constant for the reversible binding of molecular oxygen to cobalt(II) protoporphyrin IX dimethyl ester in a nonaqueous medium, a simple model system for oxygen-carrying hemoproteins, has been measured in the temperature range −31 to −63°. Pyridine, 4-*tert*-butylpyridine, and 1-methylimidazole were used as ligands in the fifth coordination site. The measurements were carried out using visible spectroscopy. The standard enthalpy changes for O_2 binding to cobalt protoporphyrin IX dimethyl ester are −9.2 ± 1.0, −10.0 ± 0.5, and −11.5 ± 1.0 kcal/mol when pyridine, 4-*tert*-butylpyridine, and 1-methylimidazole are the ligands in the fifth coordination site respectively. The standard entropy changes are −53 ± 5, −57 ± 2, and −58 ± 4 eu, respectively, with a 1-Torr standard state. These data may be compared with those in the literature of −18.1 kcal/mol and −60 eu for myoglobin. Possible reasons for the marked differences in standard enthalpy between the model cobalt system and myoglobin are discussed.

In the course of our attempts to isolate crystals of a molecular oxygen complex of a cobalt porphyrin, it became necessary to make a detailed investigation of the conditions under which these metal porphyrins bind oxygen reversibly. We wish to report the first data on the thermodynamics of the reversible binding of molecular oxygen to a cobalt porphyrin. These measurements are further extensions of our studies on the binding of small molecules to transition metals and are of considerable biological interest as the metal porphyrin serves as a model system for the hemoproteins.

Although qualitative esr studies have been carried out on the ability of cobalt(II) porphyrins to carry

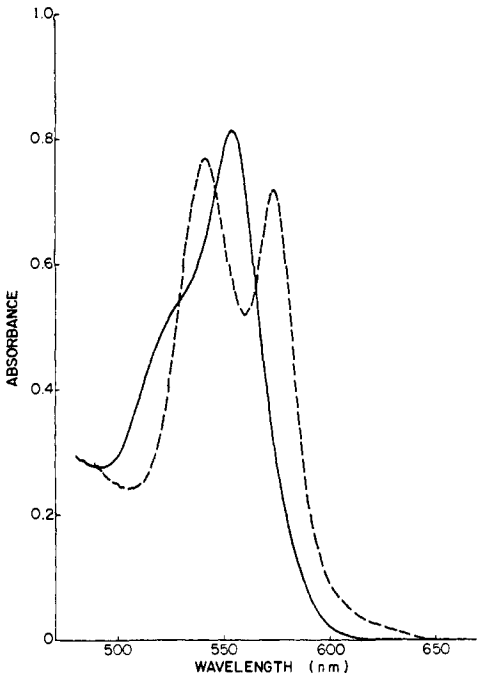

Figure 1. Visible spectra of CoP·B in toluene (———) at 25°; the same solution with 1 atm of O_2(g) at −63° (– – – –).

oxygen reversibly,[1] no quantitative thermodynamic data have yet been reported. Nearly all of the previous esr studies were carried out on frozen solutions at 77°K (liquid N_2), and so the temperature range over which the complexes bind O_2 reversibly was not known. We have found that the low-temperature visible spectra of cobalt(II) porphyrins in a toluene solution containing a nitrogen-donating base are distinctly different in the presence or absence of molecular oxygen (under anaerobic or aerobic conditions). With the aid of esr we have been able to show that these spectral changes are indeed caused by the following quantitative reaction.

$$\text{cobalt(II)–porphyrin–base} + O_2(g) \rightleftharpoons \text{cobalt(II)–porphyrin–base}\cdot O_2 \quad (1)$$

In this paper we discuss the thermodynamics of the binding of molecular oxygen to cobalt protoporphyrin IX dimethyl ester, using pyridine, 4-*tert*-butylpyridine, and 1-methylimidazole as ligands in the fifth coordination site in the temperature range −63 to −31°. All reactions were carried out in a nonpolar medium provided by toluene as the aprotic solvent. Thus the metal has a hydrophobic environment similar to that found around heme in myoglobin and hemoglobin.[2]

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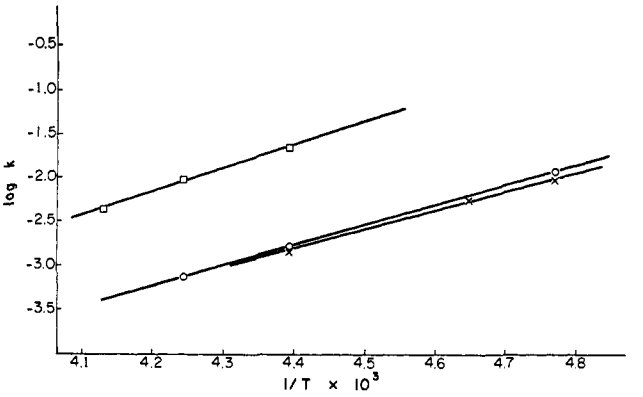

Figure 2. The van't Hoff plots for the binding of molecular oxygen to CoP·B for all three bases used. The ×, O, and □ represent the data obtained by using pyridine, 4-*tert*-butylpyridine, and 1-methylimidazole, respectively.

Experimental Section

Materials. Protoporphyrin IX dimethyl ester, grade 1, lot 49B-0140, was purchased from Sigma Chemical Co. and used without further purification. Co(II) protoporphyrin IX dimethyl ester (CoP) was prepared by the method described in Falk,[3] and the complex when stored under nitrogen was stable.

The toluene was refluxed over and distilled from CaH_2 prior to use and degassed three times by the freeze–thaw method. Pyridine was refluxed over and distilled from KOH and stored under N_2 over molecular sieves. 4-*tert*-Butylpyridine was treated similarly. 1-Methylimidazole (Aldrich Chemical Co.) was distilled at reduced pressure and stored at 0° under N_2 over molecular sieves. All bases were degassed three times by the freeze–thaw method prior to use.

Oxygen used for the preparation of the oxygen complexes was extra dry ultra pure grade (Matheson Co.).

Low temperatures were attained by surrounding the solution being studied with a cryostatic bath. These slush baths were prepared as described by Shriver.[4] The following compounds were used to attain the required temperatures: bromobenzene (−30.8°), anisole (−37.4°), chlorobenzene (−45°), chloral (−57.5°), and chloroform (−63.5°).

Preparation of Complexes. The following abbreviations will be used throughout this paper: cobalt protoporphyrin IX dimethyl ester, CoP; pyridine, py; 4-*tert*-butylpyridine, Bu^t-py; 1-methylimidazole, CH_3-Im; base, B, where base generally refers to any of the three nitrogen-donating ligands used; *N,N*-dimethylformamide, DMF; *N,N*-ethylenebis(acetylacetoniminato)cobalt(II), Co(acacen); myoglobin, Mb; coboglobin (the cobalt analog of myoglobin), CMb.

CoP·B·O_2. In a typical preparation 4×10^{-4} mmol of CoP in toluene was added to 60 ml of toluene in the cell which had been previously evacuated. Full precautions were taken to exclude oxygen from the system at this stage. Then $0.8–1.2 \times 10^{-3}$ mmol of base was added and the spectrum recorded. The cell was then cooled in the slush. The spectrum was recorded again at the slush temperature as a check on proper preparation. Then various pressures of O_2 were added to the solution, and the spectrum was recorded after each addition.

Cell. The cell is a standard low-temperature quartz cell with a 10-cm path length (Kontes-Martin Ltd.). It is constructed such that a slush bath can completely surround the solution in the path length without interfering with the beam.

Ligand-Binding Measurements. The electronic spectra were obtained on a Cary Model 14 recording spectrophotometer which was calibrated with a holmium oxide filter (λ 361.15 nm); scan speed used in all cases was 10 Å/sec.

Results

CoP·B has an absorption maximum at 555 nm. CoP·B·O_2 exhibits two peaks with absorption maxima at 540 and 573 nm (see Figure 1). The equilibrium

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Table I. Thermodynamic Changes in Reversible O_2 Binding to Cobalt(II) Protoporphyrin IX Dimethyl Ester·Base

Compd	Temp, °C	Log K_{eq}, mm^{-1}	$\Delta H°$,[a] kcal/mol	ΔS,[a] eu	ΔF,[b] kcal/mol	ΔS,[b] eu
CoP·py	−45	−2.84 ± 0.03			−0.04	
	−57.5	−2.25 ± 0.06	−9.2 ± 1.0	−53 ± 5	−0.62	−40
	−63.5	−2.09 ± 0.05			−0.76	
CoP·Bu^t-py	−37.5	−3.12 ± 0.03			+0.26	
	−45	−2.77 ± 0.02	−10.0 ± 0.5	−57 ± 2	−0.12	−44
	−63.5	−1.97 ± 0.03			−0.82	
CoP·CH_3-Im	−31	−2.36 ± 0.03			−0.58	
	−37.5	−2.04 ± 0.03	−11.5 ± 1.0	−58 ± 4	−0.91	−45
	−45	−1.70 ± 0.02			−1.23	

[a] Standard state of 1 Torr. [b] Standard state of 1 atm.

between O_2 and CoP·B was measured spectrally as a function of temperature and as a function of oxygen pressure. From the change in the spectrum in the visible region accompanying the addition of O_2 (typically, pressures were varied by 40–50 Torr on each addition of O_2), the fraction Y of CoP·B·O_2 present may be calculated. The equilibrium constant for the following reaction

$$\text{CoP·B} + O_2(g) \rightleftharpoons \text{CoP·B·}O_2 \qquad (2)$$

is obtained using the Hill equation

$$Y/(1 - Y) = [\text{CoP·B·}O_2]/[\text{CoP·B}] = KP_{O_2}{}^n$$

In all cases studied the data correspond to a value of $n = 1$ in the Hill equation. Figure 2 shows the van't Hoff plots for the binding of oxygen to CoP·B where B is pyridine, 4-*tert*-butylpyridine, and 1-methylimidazole, respectively.

The values of the thermodynamic quantities obtained by analysis of the van't Hoff plots are given in Table I. The errors given are the standard errors.

The linearity of the van't Hoff plots obtained for all three bases indicates a constant ΔC_p over the temperature range investigated and hence constant $\Delta H°$ values. The oxygen affinity is a function of the basicity of the axial ligand, CoP·CH_3-Im > CoP·Bu^t-py > CoP·py, in agreement with the results of Crumbliss and Basolo[5] on the cobalt–Schiff base complexes.

Since the solubility of $O_2(g)$ in toluene is not known in the temperature range used here, we confine our studies to the equilibrium expressed in eq 2 rather than to the following equilibrium

$$\text{CoP·B(soln)} + O_2(\text{soln}) \rightleftharpoons \text{CoP·B·}O_2(\text{soln})$$

The large negative entropy associated with the binding of $O_2(g)$ to CoP·B is consistent with the loss of translational entropy of $O_2(g)$ upon coordination to the metal porphyrin moiety. When $O_2(g)$ looses its translational degrees of freedom, the entropy decreases by 35 eu at −45°.[6] An even greater loss of entropy would occur if the $O_2(g)$ molecule lost its rotational degree of freedom upon coordination.

Reactions of the following type

$$CoL_5(\text{soln}) + L(\text{soln}) \rightleftharpoons CoL_6(\text{soln})$$

have been extensively investigated, and values for ΔS of about −20 eu for such a reaction appear to be reasonable. This estimated value and the ΔS_{trans} loss of $O_2(g)$ on coordination to CoP·B are sufficient to account for the large negative entropy.

The enthalpy ΔH for reaction 2 seems to be the important factor in determining the stability of the oxygen complexes formed. The larger the pK_a of the base the more negative the ΔH and the stronger the complex that is formed between CoP·B and O_2. The very small differences in ΔS as one varies the base are not sufficient to account for the large differences in K_{eq}.

Discussion

These oxygen carriers are closely related to the oxygen-carrying hemoproteins in the following ways: (1) the environment of the heme is hydrophobic as is the environment of the cobalt porphyrin in toluene; (2) the methylimidazole used in the fifth coordination site is analogous to the imidazole of a histidine residue found coordinated to heme in hemoproteins; (3) the bound oxygen has become spin paired upon coordination; (4) the visible spectra of CoP·B are similar to deoxyhemoglobin and deoxycoboglobin and the spectra of CoP·B·O_2 bear similar relationships to oxyhemoglobin and oxycoboglobin. Our systems form no oxygen complexes stable at room temperature and this represents an important contrast to the hemoproteins and to coboglobins.[1e]

In Table II the thermodynamic data available to date for myoglobin, Co(acacen)·DMF, and Co(acacen)·py are tabulated for comparison. It is noteworthy that the entropy changes associated with oxygen uptake by CoP·B are equal within experimental error to those reported for sperm whale myoglobin.[7,8] The enthalpies, however, differ by approximately 7 kcal/mol. These two systems differ in two important respects: the myoglobin contains Fe, rather than Co, and its bound O_2 molecule is protected by the globin protein, rather than being exposed to toluene. It is impossible at this time to state whether one or the other or both of these differences are responsible for the large change in enthalpy in going from CoP·B·O_2 to oxymyoglobin and concomitantly for the increased stability of oxymyoglobin. Measurements in progress on CMb[9] should lead to a better understanding of the role of the protein and the metal atom in stabilizing reversible molecular oxygen carriers.

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Table II. Comparison of the Thermodynamic Changes Associated with Reversible Oxygen Binding

Ref	Compd	Ligand	$\Delta F°$,[a] kcal/mol	$\Delta H°$,[a] kcal/mol	$\Delta S°$,[a] eu
Keyes, *et al.*[7]	G-25 Mb[b]	O_2	−0.1	−18.1	−60
Amiconi, *et al.*[10]	Co^{II}(acacen)·py[c]	O_2	+0.7	−15.1	−54[d]
Crumbliss and Basolo[5]	Co^{II}(acacen)·DMF[e]	O_2	+0.9		
Stynes and Ibers[g]	CoP·CH_3-Im[f]	O_2	+1.8	−11.5	−58

[a] Standard state is 1 Torr. [b] pH 8.5; 0.001 *M* THAM buffer; $T = 27°$. [c] Neat pyridine solution; $T = 20°$. [d] See ref 10; Amiconi, *et al.*, have determined the enthalpy and entropy changes for oxygen binding of Co^{II}(acacen) in pyridine and report $\Delta F° = -6.45$ kcal/mol, $\Delta H° = -15.0$ kcal/mol, and $\Delta S° = -29$ eu, for an oxygen standard state of 1 *M* in pyridine. These calculations are based on the assumption that the solubility of O_2(g) in pyridine remains constant over the temperature range used, 15–31.5°. Since ΔH_{soln} of O_2(g) + py $\rightleftharpoons$ O_2(soln) is not known, this assumption may not be valid and could lead to serious errors in the calculations. Therefore, we have recalculated the results from the data given in the original paper, excluding the solubility of O_2(g) in pyridine and using a 1-Torr standard state. In that paper also, Amiconi, *et al.*, cite unpublished results of Amiconi, Antonini, Brunori, and Magnusson for oxygen binding to whale myoglobin as $\Delta F° = -8.0$ kcal/mol, $\Delta H° = -14.8$ kcal/mol, $\Delta S° = -23$ eu based on a 1 *M* standard state for oxygen. Since the full details of how these calculations were made are not known, we are unable to convert them for comparative purposes to a 1-Torr standard state. [e] DMF solution; $T = -10°$. [f] Toluene solution, $T = -45°$. [g] This work.

The cobalt–Schiff base complexes bind molecular oxygen at higher temperatures than cobalt porphyrins.[5,10–12] This indicates that the nature of the in-plane ligands is extremely important in determining the stability of these molecular oxygen complexes. In CoP the cobalt atom is coordinated to four nitrogen atoms which are part of a highly conjugated tetrapyrrole system, whereas in the cobalt–Schiff base complexes the cobalt atom is coordinated to two oxygen and two nitrogen atoms which in turn are part of only a partially conjugated system. Thus, the dinegative charge of the ligand is less delocalized in the Schiff base complexes and hence there is more electron density on the metal. It has been well established that as one increases the electron density on the metal atom the strength of the M–O_2 bond increases;[5,13] thus one would expect a stronger cobalt–oxygen bond in the Schiff base complexes than in porphyrins. Thus one needs a sufficiently electron-rich metal atom which can bind molecular O_2 reversibly without ensuing oxidation.

Further extensions of the present work to different bases, solvent systems, and metal porphyrins will enable us to establish more fully the contributing factors in stabilizing reversible oxygen carriers.

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