Binding of the amino acids to the cobalt center of CoTMPyP or CoTCPP induces the amino acids to adopt a predominant conformational state that in most cases brings the side chain of the ligand in close proximity to the porphyrin ligands. This conformational state, which surprisingly appears to vary little among the series of amino acids, is quite different from that of the free amino acid, at least around the C_α–C_β bond and must be stabilized by ligand–ligand interactions. Among these, stacking and electrostatic interactions are major contributors. Quite recently we have extended this study to the complexes of amino acids with zinc(II) water-soluble porphyrins:[21] we were able to measure

the complexation constants for different types of amino acids and found large variations among these, which again could be rationalized in term of ligand–ligand interactions. The stacking interactions between aromatic amino–acids and the free-base H_2TMPyP are of sufficient magnitude to allow the existence of molecular complexes between these amino acids and the water-soluble porphyrin.

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Modeling the Heteroleptic Equilibria of Organized Molecular Systems by Using a Double-Titration Technique: A Novel Determination of the Fundamental Equilibrium Constants in a Ternary System Involving a Macrocyclic Cobalt(II) Complex, Dioxygen, and Competing Axial Ligands

Naidong Ye and Daryle H. Busch*

The equilibrium relationships in a ternary system containing (2,9,10,17,19,25,33,34-octamethyl-3,6,13,16,20,24,27,31-octaaza-pentacyclo[16.7.2.$2^{8,11}$.$2^{3,6}$.$2^{13,16}$]octatriaconta-1,8,10,17,19,24,26,31,33-nonaene-κ^4N)cobalt(II) chloride (abbreviated as [$Co^{II}MeVD$]Cl_2), 4-(hydroxymethyl)pyridine (4-$HOCH_2py$), and dioxygen in water have been examined. A double-titration method was devised by quantifying the concept of an *effective equilibrium constant* and resolving it into the underlying fundamental equilibrium constants. The simplicity and power of this method was shown by applying it to solve simultaneously all the equilibrium constants involved in this heteroleptic ternary system. The equilibrium constants are (oxygen binding to the solvated complex) $K_{O_2}^{Co} = 0.075$ Torr^{-1}, (axial ligand binding to the deoxygenated complex) $K_B^{Co} = 11.4$ M^{-1}, (axial ligand binding to the oxygen complex) $K_B^{CoO_2} = 1071$ M^{-1}, and (oxygen binding to the axially ligated complex) $K_{O_2}^{CoB} = 7$ Torr^{-1}. The advantages, limitations, and broad applications of the method are discussed.

Introduction

Historically, the equilibria of greatest interest to coordination chemistry were homoleptic multiple equilibria,[1-5] in which molecules of the same ligand successively replace water molecules from the coordination sphere of a metal ion. Attention has, of course, been given to heteroleptic equilibria (involving unlike ligands) but as a rather less common phenomenon.[3,5-9] Recently, multiple heteroleptic equilibria have been increasingly important[10-31] because of the development of the modern fields of bioinorganic chemistry, bioorganic chemistry, inclusion chemistry, and those subjects collectively recognized as supramolecular chemistry. Biomimicry and homogeneous catalysis should be replete with examples of such phenomena in which several different species must be organized prior to some rate-determining process. Here we present a general approach to the problem with the example of the binding of an axial base and an oxygen molecule to a metal ion that is coordinated to a macrocyclic ligand (Scheme I (Co = [$Co^{II}MeVD(H_2O)$]Cl_2, VD refers to the 16-membered cyclidene structure with two piperazine molecules as bridgeheads and a durene as the bridge); see Figure 1). We are further interested[32-39] in the complex problem in which all of these species are at equilibrium along with a fifth equilibrant, a guest molecule, in a system that mimics the so-called *ternary complex* of the monooxygenase enzyme cytochrome P-450.[40-44] In the P-450 ternary complex, the enzyme complexes with both O_2 and the substrate molecule (our guest molecule). In reality our model system is a pentad: metal ion, macrocycle/host, axial ligand, O_2, and substrate/guest. Because of the large formation constants of macrocyclic ligands, one may assume that equilibrium to be complete and treat the system as a quaternary one. It is clear

Scheme I
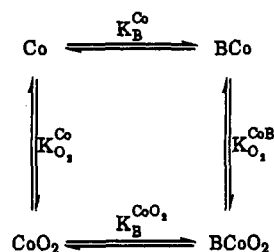
and systematic to describe such a system by a cube of separate equilibria as shown, in projection, in Scheme II. Note that each

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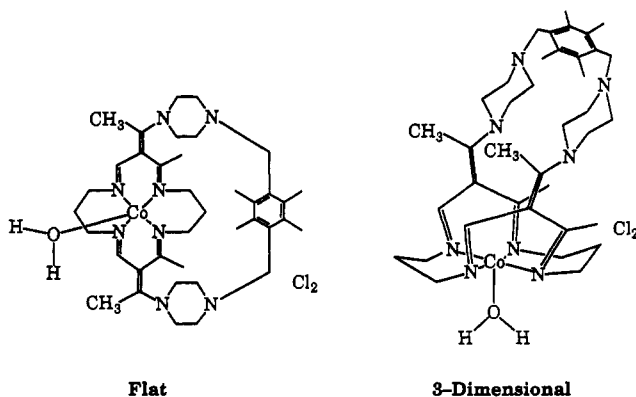
Figure 1. Structure of the vaulted cyclidene complex [Co^{II}MeVD-(H$_2$O)]Cl$_2$. VD refers to the 16-membered cyclidene structure with two piperazines as bridgeheads and durene as the bridge. The four-coordinate form without the axial water molecule is abbreviated as [Co^{II}MeVD]Cl$_2$.

Scheme II
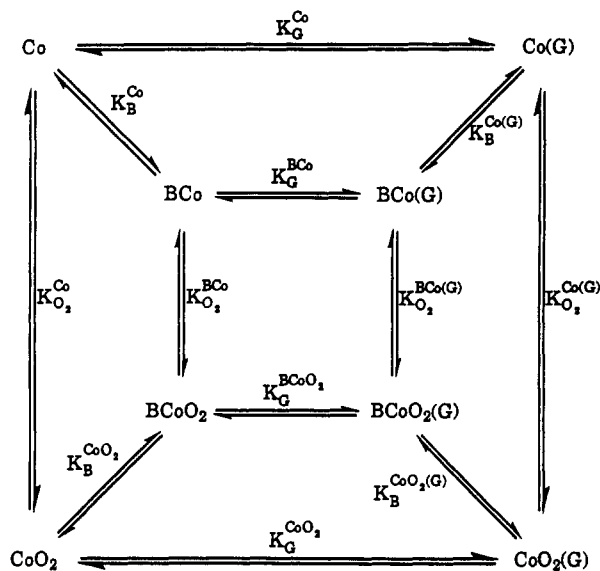
face of the cube fully describes the equilibria for a ternary subsystem of those generating the cube. In this report we treat the

ternary subsystem composed of the heteroleptic combining set: cobalt macrocyclic complex, O$_2$, and axial ligand (Scheme I).

The effects of an axial base on the dioxygen affinity of a transition-metal complex have been studied by many researchers.[45-50] It is generally accepted that the dioxygen affinity of a transition-metal complex is directly related to the electron density of the metal center, which can be modified by axial bases with different electron-donating abilities. Attention has focused on the effects of axial bases with varied σ- and π-ligating abilities.[46-50] Less effort has been directed toward the effects on dioxygen affinity due to various extents of ligation in mixed-axial-ligand systems. The latter is the major concern in this study. The competition for the sixth coordinate site in porphyrins between a second molecule of axial base and dioxygen has complicated attempts to measure dioxygen affinities.[51] Basolo et al.[47] approached this problem by invoking the concept of apparent dioxygen affinity. Assuming saturation of the first axial base equilibrium, they plotted the apparent $P_{1/2}$ vs the axial base concentration; the fundamental dioxygen affinity was obtained from the y intercept, and the binding constant of the second axial base was obtained by dividing the slope of the plot by the y intercept. This approach can be thought of as a special case of double titration (vide infra). Goldsby et al.[45] have suggested a multiple equilibrium model to account for the variations in dioxygen affinity of a solution containing an Fe(II) lacunar complex and two axial bases: chloride ion and 1-methylimidazole. Again, the concept of *effective binding constant* was employed to reflect the dioxygen affinity of this mixed-axial-base system, and the *effective binding constant* is found to be dependent on the concentrations of both axial bases. Now, this dependency is quantified: a *double titration method* has been devised and used to resolve the individual equilibrium constants in a Co(II) complex, axial base, and dioxygen ternary system.

In addition to its well-known application in determining successive formation constants of transition-metal complexes,[9,51-61]

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potentiometry has also been utilized to solve the problems of heteroleptic equilibria,[6-8,13-16,18,19,21-23,25-28,30] some of which involved dioxygen binding.[14-16,25,28] Potentiometry depends on adequate electrode behavior, and this is solvent and system dependent. The double-titration method described in this paper is not subject to the same limitations, but it is subject to other constraints and these will be discussed. These studies have been conducted by using the *vaulted cyclidene complex*, whose structure is shown in Figure 1.[33,38]

Experimental Section

Materials. The solvent used was doubly distilled and deionized water. 4-(Hydroxymethyl)pyridine (4-HOCH$_2$py) was recrystallized from toluene, and sodium chloride, from water/ethanol. 4-(Hydroxymethyl)-pyridine hydrochloride (4-HOCH$_2$py·HCl) was prepared by the addition of aqueous HCl to a solution of 4-HOCH$_2$py in water. [Co^{II}MeVD]Cl$_2$ (Figure 1) was synthesized and purified by the method of Takeuchi.[62] All materials were manipulated in an inert-atmosphere enclosure. Liquids were then degassed thoroughly. Samples were prepared by mixing 1.000 mL of 1.54 × 10^{-4} M [Co^{II}MeVD]Cl$_2$ solution, 1.000 mL of 0.24 M NaCl solution, and 1.000 mL of a solution made up of 0.30 M/0.06 M 4-HOCH$_2$py/4-HOCH$_2$py·HCl and 0.06 M NaCl solutions. Although the total amount of the 0.30 M/0.06 M 4-HOCH$_2$py/4-HOCH$_2$py·HCl solution and the 0.06 M NaCl solution was fixed at 1.000 mL, the relative amounts of the two solutions can be varied gradually with no change in ionic strength, which is 0.10 M in all experiments. The pH was maintained at 6.3 ± 0.2.

Physical Measurement. Visible spectral data were obtained by using a Varian 2300 spectrophotometer interfaced to an IBM personal computer. All K_{O_2} experiments were carried out at 0.0 °C by an automated version of a published procedure.[45,63] The $K_{O_2}^{eff}$ vs [B] data were fitted to eq 14 by using a nonlinear least-squares program.[64]

Results and Calculations

Expression for $K_{O_2}^{eff}$. In a simple system consisting of one unoxygenated and one oxygenated species (eq 1), the equilibrium constant between these two species is given by eq 2.

$$Co(soln) + O_2(g) \overset{K_{O_2}}{=} CoO_2(soln) \qquad (1)$$
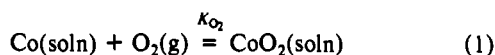
$$K_{O_2} = \frac{[CoO_2]}{[Co]P_{O_2}} \qquad (2)$$

Co is any dioxygen binding species; for the present case, Co stands for five-coordinate [Co^{II}MeVD(H$_2$O)]Cl$_2$ (Figure 1). The absorbance–P_{O_2} relationship is expressed by the Ketelaar equation:

$$A = A_0 + \frac{(\Delta\epsilon)C_0 K_{O_2}P_{O_2}}{1 + K_{O_2}P_{O_2}} \qquad (3)$$

where $\Delta\epsilon$ is the difference in the molar extinction coefficients of the oxygenated and the unoxygenated species, C_0 is the total concentration of the two species, and A_0 is the absorbency of the system when O$_2$ is absent. Usually, C_0 is known beforehand. If A_0 can be measured accurately, the absorbance–P_{O_2} relationship is described by two parameters, $\Delta\epsilon$ and K_{O_2}, otherwise, by three parameters, A_0, $\Delta\epsilon$, and K_{O_2}.

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Table I. Equilibrium Constants for the Ternary System[a]

	calculation no.			
	1	2	3	4
$K_{O_2}^{Co}$	0.075 ± 0.006	0.075 ± 0.006	0.075 ± 0.006	
K_B^{Co}	11.4 ± 1.6	11.4 ± 1.6		11.2 ± 1.5
$K_B^{CoO_2}$	1071 ± 111		1078 ± 113	1072 ± 111
$K_{O_2}^{BCo}$		7.0 ± 0.7	7.0 ± 0.7	7.1 ± 0.8

[a] Three of the parameters defining the system are determined in any given calculation; as expected, the results are independent of the three parameters selected.

In water, [Co^{II}MeVD]Cl$_2$ is five-coordinate with a water molecule as its axial base (Figure 1). If a strong axial base (B), e.g., pyridine, is introduced into the system, equilibria depicted by Scheme I exist.

The equilibrium constants are defined as follows:

$$K_B^{Co} = \frac{[BCo]}{[Co][B]} \qquad (4)$$

$$K_{O_2}^{Co} = \frac{[CoO_2]}{[Co]P_{O_2}} \qquad (5)$$

$$K_B^{CoO_2} = \frac{[BCoO_2]}{[CoO_2][B]} \qquad (6)$$

$$K_{O_2}^{BCo} = \frac{[BCoO_2]}{[BCo]P_{O_2}} \qquad (7)$$

The following relationship is true:

$$K_B^{Co}K_{O_2}^{BCo} = K_{O_2}^{Co}K_B^{CoO_2} \qquad (8)$$

Clearly, only three of the equilibrium constants are independent and only three are required for the fitting of the data.

The absorbance–P_{O_2} relationship in this relatively complex system can be written in a form very similar to the Ketelaar equation:

$$A = A_0 + \frac{(\Delta\epsilon^{eff})C_0 K_{O_2}^{eff}P_{O_2}}{1 + K_{O_2}^{eff}P_{O_2}} \qquad (9)$$

where

$$\Delta\epsilon^{eff} = X/Z - W/Y$$

$$K_{O_2}^{eff} = Z/Y$$

W, X, Y, and Z are defined by rigorous derivation as follows:

$$W = \epsilon_{Co} + \epsilon_{BCo}K_B^{Co}[B] \qquad (10)$$

$$X = \epsilon_{CoO_2}K_{O_2}^{Co} + \epsilon_{BCoO_2}K_{O_2}^{Co}K_B^{CoO_2}[B] \qquad (11)$$

$$Y = 1 + K_B^{Co}[B] \qquad (12)$$

$$Z = K_{O_2}^{Co} + K_{O_2}^{Co}K_B^{CoO_2}[B] \qquad (13)$$

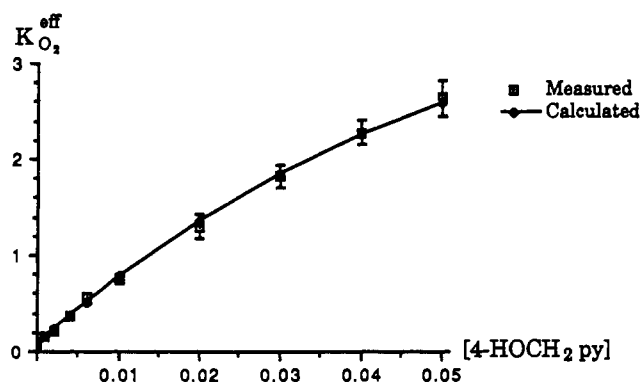where ϵ stands for molar extinction coefficient and its subscripts indicate species.

It should be emphasized that W, X, Y, and Z, and therefore, $K_{O_2}^{eff}$ and $\Delta\epsilon^{eff}$, are all constants in a given system with a fixed axial base concentration. $K_{O_2}^{eff}$ can be expressed as

$$K_{O_2}^{eff} = \frac{Z}{Y} = K_{O_2}^{Co}\left\{\frac{1 + K_B^{CoO_2}[B]}{1 + K_B^{Co}[B]}\right\} \qquad (14)$$

Significantly, eq 14 can also be obtained by assuming that $K_{O_2}^{eff}$ is the net dioxygen affinity of the mixture of deoxygenated forms (Co + BCo); this provides a quick way to write down the expression of $K_{O_2}^{eff}$.

Double Titration Method. On the basis of eq 14, a double titration method was designed to resolve all the fundamental

Figure 2. Comparison of the $K_{O_2}^{eff}$ experimental data for [Co^{II}MeVD-(H$_2$O)]Cl$_2$ with the theoretical curve. $K_{O_2}^{eff}$ was measured at 0.0 °C at 1 atm in an aqueous solution containing 5.1 × 10^{-5} M [Co^{II}MeVD-(H$_2$O)]Cl$_2$ at pH = 6.3 ± 0.2; the ionic strength was 0.10 M. The theoretical curve was calculated according to eq 14 with the parameters in Table I. $K_{O_2}^{eff}$ is in Torr^{-1}, and [4-HOCH$_2$py], in M.

equilibrium constants in the ternary system. Since the absorbance–P_{O_2} relationship in a ternary (Co/B/O$_2$) system (eq 9) has the same form as the Ketelaar equation (eq 3), it is convenient to measure one $K_{O_2}^{eff}$ at one fixed [B] by titrating with dioxygen. Then [B] can be changed successively, and a series of $K_{O_2}^{eff}$ data can be obtained. This set of $K_{O_2}^{eff}$ vs [B] data can be fitted to eq 14 to resolve simultaneously all fundamental equilibrium constants. In this determination, both dioxygen and axial base are titrated; hence, it is called a *double titration*.

4-HOCH$_2$py was chosen as the axial base to avoid possible host–guest association.[32-39] The calculated values of the equilibrium constants obtained by fitting the experimental data to eq 14 are given in Table I. The theoretical curve is compared with the experimental data in Figure 2. Each point of the measured $K_{O_2}^{eff}$ data is the average of six observed values, on which the standard deviation is calculated. The distributions of Co, BCo, CoO$_2$, and BCoO$_2$ as a function of the axial base concentration are shown in Figure 3.

Discussion

Mutual Effects of Dioxygen Binding and Axial Base Binding. The dioxygen affinity of a transition-metal complex is directly related to the electron density of the metal center. As a result, complexes with different axial bases, or mixtures with various distributions of bound axial bases, will have different dioxygen affinities. If two or more ligands compete for a coordination site, ligation by any given ligand is not necessarily complete; e.g., Co and BCo coexist and the former cannot be ignored. The previous results and calculations show that this situation can be used to one's advantage. The relationship between an observed dioxygen affinity, which is actually a compromise between the dioxygen affinities of Co and BCo, and the fundamental equilibrium constants and the axial base concentration is described by eq 14. The mutual effects of dioxygen binding and axial base binding are also quantitatively expressed by eq 14, but they are best understood by examining Figure 2 and Table I. It can be concluded that dioxygen binding and axial base binding promote each other, as one would expect. Consequently, BCoO$_2$ will be the dominant species if both axial base and dioxygen are present in reasonable concentrations, for example, 0.01 M and 10 Torr, respectively, as shown in Figure 3. The concentration range within which the $K_{O_2}^{eff}$ value will reach saturation depends on the relative values of $K_B^{CoO_2}$ and K_B^{Co}. It is worth mentioning that any general conclusion concerning dioxygen binding is equally applicable to axial base binding because O$_2$ and B are mathematically interchangeable.

More specifically, the effect of axial base binding on dioxygen affinity in this system is dramatic. The aqua axial base produces an affinity of 0.075 Torr^{-1} whereas that of the 4-HOCH$_2$py derivative is almost 2 orders of magnitude greater, 7.0 Torr^{-1}. This large value is expected in view of the large cavity in the vaulted cyclidene ligand, which allows the system to exhibit its full dioxygen affinity. This is in contrast to cyclidene complexes with
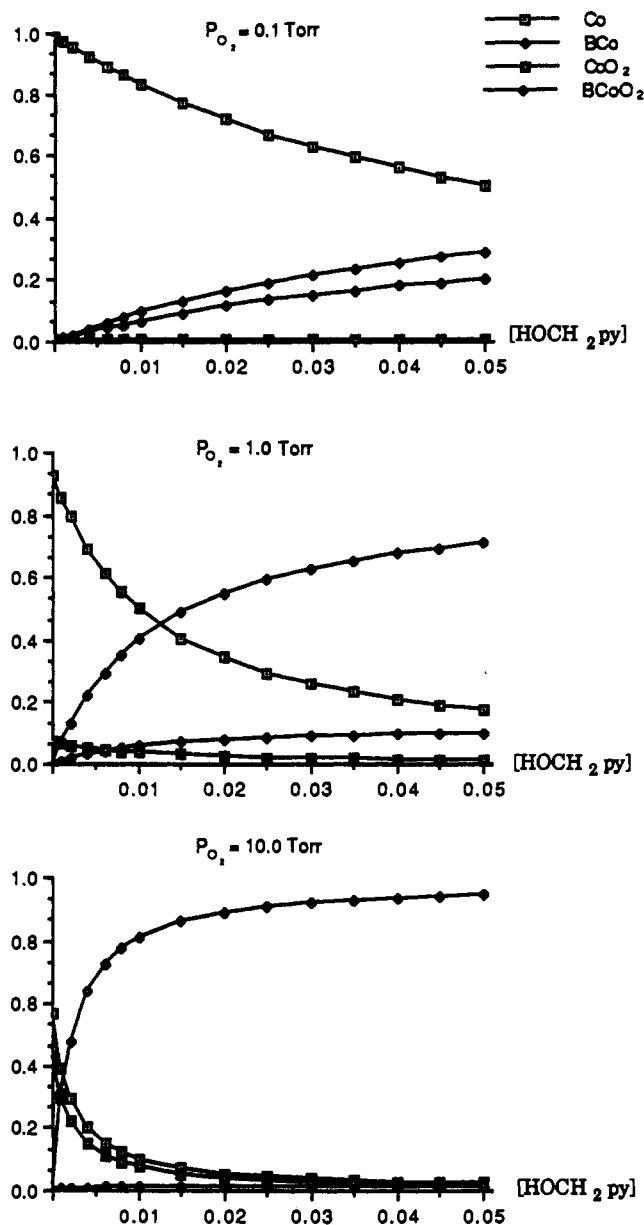
Figure 3. Distributions of the different forms of Co as a function of the axial base concentration. These curves were calculated by using the parameters in Table I. [4-HOCH$_2$py] is in M.

smaller cavities, where crowding of the bound O$_2$ reduces the dioxygen affinity.[46,65] The polarity of the solvent water is expected to further enhance this affinity.[46,48,66] Finally, the thorough analysis of the equilibria within this system makes an unusual quantity available—the actual binding constant of the axial base to the oxygen complex. Generally, independent determinations lead to the value for the axial base binding by the deoxy complex alone.
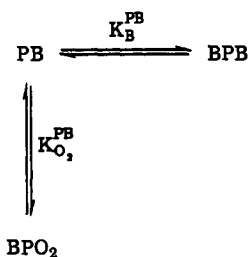
Advantages and Disadvantages of the Double-Titration Method. The beauty of the double-titration method for the evaluation of multiple heteroleptic equilibria is its simplicity and power. Its simplicity is achieved by taking advantage of the well-defined equilibrium model and by relating the measurements of, in this example, $K_{O_2}^{eff}$ values to the measurements of a K_{O_2} value in a simple (binary) system. The latter is especially important to the easy implementation of the double-titration method, since methods of measuring a K_{O_2} value in a simple system are well established and have become routine. The fact that the measured $K_{O_2}^{eff}$ is the composite dioxygen affinity of all the deoxygenated forms of the
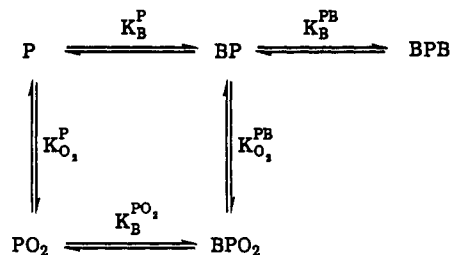
(65) Stevens, J. C.; Busch, D. H. *J. Am. Chem. Soc.* **1980**, *102*, 3285.
(66) Busch, D. H. *Trasfus. Sangue* **1988**, *33*, 57.

Scheme III

$$PB \underset{}{\overset{K_B^{PB}}{\rightleftharpoons}} BPB$$

$$\Big\updownarrow K_{O_2}^{PB}$$

$$BPO_2$$

Scheme IV

$$P \underset{}{\overset{K_B^{P}}{\rightleftharpoons}} BP \underset{}{\overset{K_B^{PB}}{\rightleftharpoons}} BPB$$

$$\Big\updownarrow K_{O_2}^{P} \qquad \Big\updownarrow K_{O_2}^{PB}$$

$$PO_2 \underset{}{\overset{K_B^{PO_2}}{\rightleftharpoons}} BPO_2$$

complex in the system makes it simple to write down the expression of the $K_{O_2}^{eff}$ of one system. To implement a double-titration method is simply to process the systematically acquired data according to eq 14, or its equivalent, which is merely a multiple parameter fitting problem. Despite its simplicity, the double titration is powerful. The entire set of equilibria represented in the square in Scheme I was solved by a series of K_{O_2} measurements. The information obtained from this method is sufficient to understand all the equilibrium relationships in that ternary system. The power of the double-titration method derives from its ability to use the conveniently measurable effective equilibrium constants to probe the underlying equilibrium relationships and to resolve the fundamental equilibrium constants of the complex system. In the absence of the double titration, or some equally powerful method, it would be extremely difficult, if not impossible, to obtain $K_B^{CoO_2}$ or, for some cases, $K_{O_2}^{BCo}$. K_B^{Co} is somewhat easier to obtain experimentally, compared with $K_{O_2}^{BCo}$ or $K_B^{CoO_2}$. However, experiments are prone to dioxygen interference, and the media during measurement have to be varied substantially, since K_B^{Co} values for pyridine in water are small, e.g., 3.4 M^{-1} for pyridine at 20 °C[62] and 11 M^{-1} for 4-HOCH$_2$py at 0.0 °C (Table I).

The power of the double-titration method is also found in its generality. Although it was applied here to the $Co/B/O_2$ ternary system, it should be applicable to many kinds of equilibrium studies on heteroleptic systems with three or more components. For example, the problem of binding a second mole of axial base to the metal ion of a porphyrin complex in competition with dioxygen can be solved very easily by the double-titration method (Scheme III (P = metal–porphyrin complex)). Experimentally, one needed only to determine a set of $K_{O_2}^{eff}$ values at different axial base concentrations and fit the data to eq 15 (assuming that the first

$$K_{O_2}^{eff} = K_{O_2}^{PB} \left\{ \frac{1}{1 + K_B^{PB}[B]} \right\} \tag{15}$$

axial base equilibrium is saturated). This case is special because the data can be treated linearly. If measurable amounts of the four-coordinate complex are present, and that is commonly the case for complexes of tetradentate ligands like porphyrins and cyclidenes, then Scheme IV should be used. The condition under which $K_{O_2}^{eff}$ in eq 16 has a maximum is that $K_B^{PO_2} > K_B^{P}$. This is

$$K_{O_2}^{eff} = K_{O_2}^{P} \left\{ \frac{1 + K_B^{PO_2}[B]}{1 + K_B^{P}[B] + K_B^{P}K_B^{BP}[B][B]} \right\} \tag{16}$$

always the case for complexes of tetradentate ligands according to our previous conclusion that dioxygen binding and axial base binding promote each other. Therefore, nonlinearity is expected for the function and Scheme III could give misleading results if applied to the dioxygen complexes and axial base binding of complexes of tetradentate ligands.

Like a potentiometric method, the double-titration method also has its limitations. This method is not as general as one might like. It requires at least three distinct chemical components; therefore, it cannot treat homoleptic ML_n ($n \geq 2$) systems. Furthermore, it requires a central component, although this is usually the case in coordination chemistry. Before this method can be employed, the equilibrium model must be determined; i.e., this is not a method for speciation. A determination by the double-titration method also requires more experimental effort than a similar set of potentiometric determinations.