interaction with partially accessible paramagnetic ions in macromolecules once a high resolution crystal structure is available.

Given the available theory for NMRD produced by anisotropic, high-spin Fe^{III}, only an estimate of τ_M for a coordinated solvent molecule is possible from the data. $(T_1)_{os}^{-1}$ for FeSOD can be estimated from the theory[18b] to be ≤ 0.5 mM^{-1} s^{-1}. Equation 2 then gives $\tau_M \leq 2 \times 10^{-5}$ s, implying a solvent exchange rate that is faster than turnover (25 000 s^{-1} in comparable conditions).[6] Thus the data reported here are consistent with innersphere electron-transfer steps in the catalytic mechanism of FeSOD. The on-rate for azide binding is 40 000 s^{-1} and also permits anion binding steps to be involved in turnover.[6,8]

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An Inclusion Complex Containing the Dioxygen Molecule and an Organic Guest Molecule Cohabiting within a Vaulted Cobalt(II) Cyclidene Host—A Rare Kind of Ternary Complex

Thomas J. Meade, Kenneth J. Takeuchi, and Daryle H. Busch*

Contribution from the Chemistry Department, The Ohio State University, Columbus, Ohio 43210. *Received May 14, 1986*

Abstract: A credible model of the ternary complex of cytochrome P450 has been demonstrated with use of NMR spin lattice relaxation determinations on guest protons in the presence of the dioxygen adduct of a vaulted cyclidene–cobalt(II) host. Hydrophobic forces produce regiospecific binding of the 1-butanol guest within the cavity of the vaulted cyclidene cobalt complex while the metal center is simultaneously coordinated to dioxygen. The ternary complex is formulated as [{vaulted cyclidene cobalt(II)}–dioxygen–guest]. The spin density localized on the coordinated dioxygen acts as the paramagnetic center, and results obtained at two spectrometer frequencies yield the same calculated distances. ESR and visible spectral measurements establish the stability and reversibility of the dioxygen adduct under the conditions used in the NMR studies. Distance determinations made with the deoxygenated form of the cobalt(II) complex are the same, within experimental error, as those found with the analogous copper(II) complexes of the ligands.

Given the enormous strides made recently in elaborating its active site chemistry, the static relationships and dynamic consequences of the ternary complex of cytochromes P450 should provide the basis for understanding the elegant oxidation chemistry orchestrated by that enzyme.[1] We present here the first credible model for this ternary complex. In the natural system, the binding of dioxygen to the heme iron atom is possible only after a substrate molecule is in place, a design feature that prevents activation of the enzyme in the absence of substrate.[2] The extreme reactivity of the activated site toward organic substances indicates that selectivity is achieved largely through the details of the substrate binding process. The crystal structure of the cytochrome-P450-cam from *pseudomonas putida* has confirmed this.[3] A hydrophobic region surrounds the coordination site at the heme group where the activation of dioxygen takes place. This enables the complex to bind organic substrates hydrophobically while simultaneously providing a site for the activation of dioxygen.

The elucidation of the interrelationships and possible synergisms that operate within such a ternary complex requires good models, and the family of cyclidene complexes developed in these laboratories[4-6] have now been applied to this purpose. The cyclidene ligand is especially favorable to the formation of dioxygen complexes by both iron(II)[7-9] and cobalt(II)[10,11] while the ease of synthesis and diversity of superstructures that can be appended to the cyclidene facilitate the study of inclusion complex formation with potential organic substrates.[12-15] Simultaneous substrate binding and dioxygen binding in a single synthetic complex are demonstrated for the first time here (Figure 1). Further, the spin density on the dioxygen species,[16,17] that is bound to cobalt(II), is exploited to disclose the regiospecific binding of the substrate.

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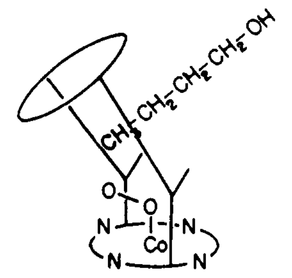
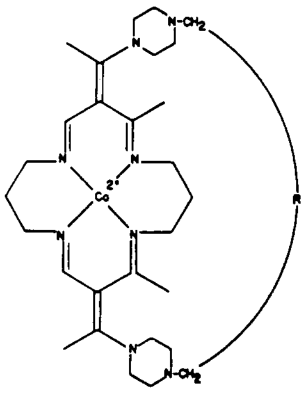


Figure 1. Conceptualization of the ternary complex containing dioxygen and an organic substrate simultaneously bound to a cobalt(II) cyclidene host.

The ultimate goal of these studies is the development of a new class of selective oxidation catalysts with the full capabilities of the family of cytochromes P450.



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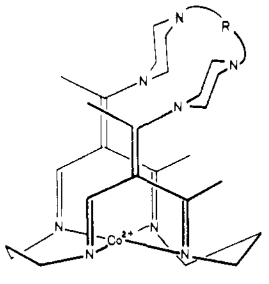

II

The structures of the vaulted cyclidene complexes are represented in projection and in perspective in structures I and II. Previous work with the copper(II) derivatives of the macrobicyclic cyclidene complexes has shown them to be capable of binding organic substrates regiospecifically.[13,18,19] Simultaneously, a coordination site remains vacant on the metal atom and, theoretically, it is available for the coordination of dioxygen. Substrate binding studies have been carried out in aqueous solutions with use of paramagnetic relaxation techniques, and the formation of these inclusion complexes has been attributed to hydrophobic forces. Much attention has focused on experimental justification[18,19] of the simplified dipolar form of the Solomon–Bloembergen equation (below) for the reduction of T_1 data to distance parameters,

$$r(\text{Å}) = C\left[pT_{1\text{p}}\left(\frac{3\tau_c}{1 + ((3.94 \times 10^{13})\nu_1^2\tau_c^2)} + \frac{7\tau_c}{1 + ((1.71 \times 10^{19})\nu_1^2\tau_c^2)}\right)\right]^{1/6}$$

where r(Å) is the distance from the paramagnetic center to the relaxing nucleus (protons on 1-butanol) and C is a coefficient which is a composite of many physical constants depending on the relaxing nuclei and the paramagnetic center.[23] ν_1 is the spectrometer frequency; τ_c is the correlation time for the process which modulates the dipolar interaction; p is the molar ratio of host to guest; and T_{1p} is defined as $1/T_{1p} = (1/T_1) - [1/(T_1)_o]$.

Table I. Relaxation Data and Calculated Distances for the Host $Co^{III}O_2^-$ Vaulted Anthracene Cyclidene and the Guest 2-Butanol at 274 K[c]

	$\overset{\alpha}{\text{HO–CH}_2}$–	$\overset{\beta}{\text{CH}_2}$–	$\overset{\gamma}{\text{CH}_2}$–	$\overset{\delta}{\text{CH}_3}$
	α	β	γ	δ
		ν = 300		
$(T_1)_o$,[a] s	1.55 ± 0.02	1.49 ± 0.04	1.72 ± 0.03	1.98 ± 0.07
T_1,[b] s	1.40 ± 0.08	1.31 ± 0.02	1.33 ± 0.09	1.09 ± 0.04
r, Å	7.3 ± 0.2	6.8 ± 0.1	6.3 ± 0.2	5.4 ± 0.1
		ν = 500		
$(T_1)_o$,[a] s	1.74 ± 0.06	1.67 ± 0.03	1.86 ± 0.09	2.14 ± 0.1
T_1[b], s	1.57 ± 0.05	1.41 ± 0.01	1.49 ± 0.1	1.19 ± 0.05
r, Å	7.3 ± 0.2	6.7 ± 0.1	6.4 ± 0.2	5.5 ± 0.1

[a] $(T_1)_o$ = T_1 of 1-butanol protons in D_2O with 100 Torr of O_2. [b] T_1 = T_1 of 1-butanol protons in D_2O with Co^{II} host and 100 Torr of O_2. [c] Host = $Co^{III}O_2^-$. R^1 = anthracene. Guest = 1-butanol ($p = 1.22 \times 10^{-3}$).

Table II. Relaxation Data[a] and Calculated Distances for the Host $[Co\{Me_2(piperazine)_2(9,10\text{-anthracene})[16]cyclidene\}]Cl_2$ and the Guest 1-Butanol[f]

	$\overset{\alpha}{\text{HO–CH}_2}$–	$\overset{\beta}{\text{CH}_2}$–	$\overset{\gamma}{\text{CH}_2}$–	$\overset{\delta}{\text{CH}_3}$
	α	β	γ	δ
		T = 304 K		
$(T_1)_o$,[d] s	6.18 ± 0.1	5.65 ± 0.13	6.19 ± 0.05	6.40 ± 0.03
T_1,[e] s	5.30 ± 0.05	4.59 ± 0.10	4.41 ± 0.09	3.50 ± 0.01
r, Å	8.4 ± 0.2	7.8 ± 0.2	7.2 ± 0.1	6.5 ± 0.1
		T = 274 K		
$(T_1)_o$,[d] s	2.11 ± 0.02	1.90 ± 0.05	2.20 ± 0.4	2.37 ± 0.1
T_1,[e] s	1.99 ± 0.03	1.75 ± 0.03	1.96 ± 0.1	1.71 ± 0.01
r, Å	8.3 ± 0.2	7.7 ± 0.2	7.4 ± 0.1	6.3 ± 0.1

[a] ν = 500 MHz. [b] [Host] = 6.69×10^{-5} M. [c] [Guest] = 5.48×10^{-2} M. [d] $(T_1)_o$ = T_1 of butanol protons in D_2O. [e] T_1 = T_1 of butanol protons in D_2O solution of Co^{II} host. [f] Host = vaulted complex.[b] R^1 = anthracene. Guest = 1-butanol.[c]

For the present studies, it was necessary to synthesize the cobalt(II) complex of the vaulted ligand[20] and demonstrate its efficacy in paramagnetic relaxation studies. The stability of the dioxygen adduct of the vaulted cyclidene ligand was then assessed and conditions defined wherein relaxation studies could be made with use of the paramagnetism of the dioxygen adduct itself. The positioning of a bound substrate was then determined in the presence of bound dioxygen; i.e., the ternary complex was defined in a regiospecific manner.

Results and Discussion

Results from the determination of relaxation rates for the protons of the guest 1-butanol, using the paramagnetism of the dioxygen adduct, are given in Table I. The spin density resides principally on the O_2 moiety in these adducts.[21,22] Data were taken at two spectrometer frequencies, 300 and 500 MHz. These data indicate that the distance from the mean spin density on the dioxygen to the methyl group of the 1-butanol is 5.5 Å while that to the protons α to the hydroxyl group is 7.3 Å. Thus, we immediately observe the same class of regioselectivity that was found in the absence of the dioxygen for both copper(II)[12,13,19] and cobalt(II) (see below) hosts. Clearly, the hydrophobic portion of the 1-butanol molecule penetrates into the vault of the host, and the hydrophilic functional group extends out into the solvent.

The distance parameters measured with the cobalt(II) host, in the absence of dioxygen (Table II), are approximately 1 Å greater than those measured for the dioxygen adduct (Table I). Since the bound dioxygen moiety extends up into the cavity (about 2.9 Å for the distant oxygen atom),[23] this difference is in the

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(18) The use of NMR relaxation techniques to determine geometric information about ligand or substrate binding has led to some controversy. We have gone to great lengths[19] to justify the application of this technique to the inclusion complexes of the vaulted cyclidene derivatives. These studies have specifically justified each of the assumptions that must be made to apply the technique. Further, we have demonstrated the specific hydrophobic nature of the host/guest complexing and the necessity for a substantial cavity in order to accommodate the guest.

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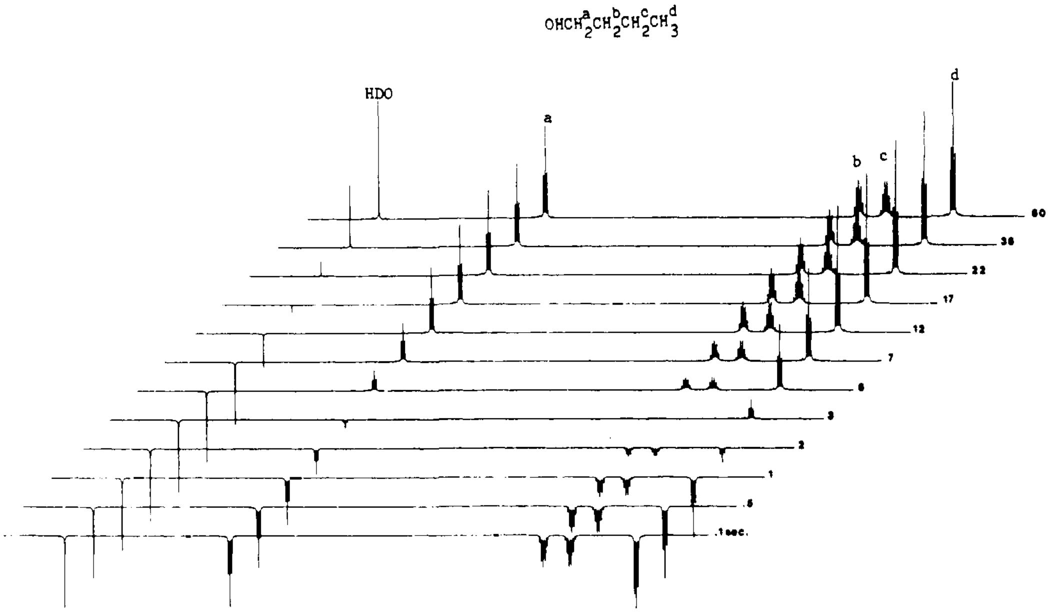

Figure 2. ^{1}H FT-NMR Spectrum and inversion recovery sequence (180–t–90) of 1-butanol (guest) and [Co{Me$_2$(piperazine)$_2$(9,10-anthracene)-[16]cyclidene}]Cl$_2$ (host) in D_2O at 304 K. The initial pulse has produced the inverted spectrum shown in the lower scan. Subsequent scans merely follow the recovery of the population of aligned spins.

expected direction. From molecular model studies (Chemgraph)[24] on the structures of the inclusion complexes, using data obtained on the copper(II) vaulted cyclidene hosts, we have concluded that the 1-butanol is positioned at the top of the cavity and is about as far from the metal as it can be while remaining within the host.[19] If the guest is assumed to remain in the same location (for the O_2 adduct), the center of action of the spin density on the dioxygen adduct is placed uncomfortably close to the cobalt atom. Consequently, it is indicated that the substrate is pushed slightly away from its previous average position when it must share the cavity with a dioxygen molecule.

The measurement of these relationships involves a number of special considerations. First of all, since O_2 is paramagnetic and gives measurable relaxation effects, both T_1 and $(T_1)_0$ were determined in the presence of the same partial pressure of O_2, 100 Torr. Because the oxygen carrier undergoes air oxidation at room temperature, the measurements were made at 274 K. At this temperature, the viscosity of water and the correlation time are substantially increased and this affects the relaxation times. This has been studied with use of the cobalt(II) complex. Also, it must be kept in mind that the delocalization of the spin density over the dioxygen moiety increases the uncertainty in the calculated distances.[25-27] However, the reproducibility of the experiment is evident from the results obtained at two spectrometer frequencies.

To verify that the cobalt(II) complex of the vaulted cyclidene ligand (deoxygenated form) is capable of effectively relaxing guest protons, data were obtained with this complex and the guest 1-butanol (Table II). At 304 K, the distances calculated are the same, within experimental error, as those obtained with the corresponding copper(II) complex.[19] As expected, the regiospecific binding of substrates by vaulted cyclidene hosts is not metal ion related but is responsive to geometric and solvational relationships. As Figure 2 shows, there can be little question about the assignments of the signals in the NMR spectrum of the 1-butanol in view of its simplicity and the familiar chemical shifts and splittings observed therein.

Because dioxygen affinity and dioxygen adduct sensitivity to autoxidation required studies on the ternary complex to be conducted at reduced temperatures, the cobalt(II) host–guest interaction was also characterized at 274 K. At this temperature, the distances calculated (Table II) are the same as those obtained at 304 K. The considerable reduction in T_1 values for both the standard and the host–guest complex is due, in part, to an increase in the correlation time, τ_c, and solvent broadening at reduced temperature.

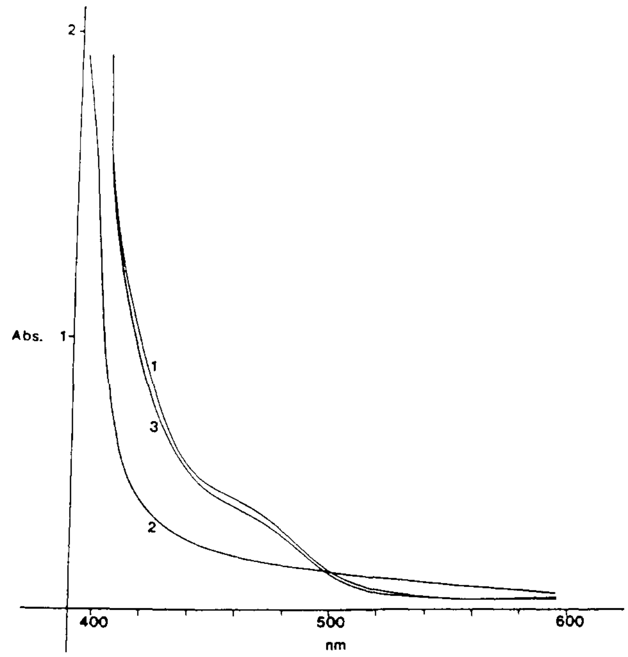

Figure 3. Visible spectra of [Co{Me$_2$(piperazine)$_2$(9,10-anthracene)-[16]cyclidene}]Cl$_2$ and its dioxygen adduct in aqueous solution at 274 K: (1) Spectrum before exposure to dioxygen; (2) after exposure to 760 Torr of dioxygen; (3) after bubbling N_2 through the same solution for 33 min.

In order to use the dioxygen adduct to demonstrate the formation of the ternary complex, it was necessary to determine conditions under which the dioxygen binding is reversible for the necessary time period. Figure 3 shows the visible spectral changes observed when [Co{Me$_2$(piperazine)$_2$(9,10-anthracene)[16]-cyclidene}]Cl$_2$ is exposed to dioxygen in aqueous solution at 274 K. Trace 1 was measured on the cobalt(II) species before exposure to dioxygen. Trace 2 corresponds to the spectrum of the complex after bubbling 760 Torr of dioxygen through the solution for 60 s. Finally, trace 3 shows the spectrum of the complex after

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bubbling nitrogen through the solution for 33 min. This shows that the removal of the dioxygen from the fully oxygenated solution results in almost complete return of the spectrum of the nonoxygenated cobalt complex. Essentially, the same results have been obtained by using ESR spectra to monitor the oxygenation of the vaulted cyclidene complex of cobalt(II) in water at 274 K and in acetonitrile as a frozen glass. In water, the ESR spectrum of the cobalt(II) complex displays an axial signal with $g_{\|} = 2.33$ and $g_{\perp} = 2.04$ and is replaced by that of a dioxygen adduct when the solution of the complex is exposed to 100 Torr of O_2 for 60 s with values of $g_{\perp} = 2.09$ and $g_{\|} = 2.02$. These ESR spectra were run at 233 K. The sample was allowed to warm to 273 K and held there for 30 min and the spectrum was then rerun at 233 K. The spectrum showed that the dioxygen adduct was still present. These results confirm the reversible dioxygen binding ability of the cobalt(II) vaulted compound in aqueous solution at 274 K and show that the complex is stable enough, under these conditions, to permit the study of inclusion complex formation with use of the dioxygen adduct.

It may be concluded that the dioxygen adduct of the vaulted cyclidene complex with cobalt(II) does indeed act as a host, accepting the 1-butanol guest into its partially occupied cavity, in a regioselective way. The binding is hydrophobic in character and, apparently, the presence of the dioxygen in the cavity causes the guest to be immersed less deeply into the host. The 1-butyl group extends into the hydrophobic void and the hydroxyl group remains solvated by the water surrounding the inclusion complex. The mode of binding of the guest parallels that for substrates in the ternary complexes of cytochrome P450, while the well-established "Pauling-type" of dioxygen adduct formed by cobalt closely mimics O_2 binding by heme groups.[16] Thus, the ternary complex demonstrated herein represents a meaningful model for the ternary complex of the enzyme.

Confidence in the experimental conclusions presented here comes from the following: (1) the T_1 data for both the dioxygen adduct and the deoxy–cobalt(II) host give reproducible distances over some variation in conditions of measurements; (2) the reversibility and durability of the dioxygen adduct under the conditions of study of the inclusion phenomenon have been well demonstrated; and (3) in previous studies much attention has been given to the limitations of the techniques used in these studies.[19]

Experimental Section

Reagents and Materials. All reagents used in the synthesis and subsequent study of cobalt(II) cyclidene complexes were of reagent grade and were used without further purification unless otherwise noted.

Synthesis of the Vaulted Cobalt(II) Cyclidene Complex. (2,17,19,25-Tetramethyl-3,6,13,16,20,24,27,31-octaazaheptacyclo[20.7.7.6^{8,15}.2$^{3.6}$.2^{17,20}.0^{9,14}.0$^{37.42}$]hexatetraconta-1,8,10,17,19,24,26,31,33,35,37,39,41-tridecaene-κ^4N)cobalt(II) Hexafluorophosphate, [Co{9,10-anthracene(CH_2-piperazine)$_2$[16]cyclideneN$_4$}](Cl)$_2$. The vaulted ligand was prepared by a known template synthesis with use of its nickel complex.[14] The free ligand salt was obtained by a standard technique developed for the closely related lacunar cyclidene ligands.[6] The cobalt(II) complex was prepared under nitrogen in a good glovebox by adding a hot methanolic solution containing cobalt(II) acetate 4-hydrate and sodium acetate 3-hydrate to a hot methanolic solution of the free ligand salt. The addition of a hot methanolic solution of tetrabutylammonium hexafluorophosphate was followed by formation of an orange microcrystalline precipitate, yield 80%. The bis-PF_6 salt was converted to the water-soluble dichloride by a previously published method,[14] yield 35%. Anal. Calcd for $CoC_{42}H_{54}N_8Cl_2\cdot 2H_2O\cdot CH_3CN$: C, 60.19; H, 7.22; N, 14.36; Cl, 8.10; Co, 6.71. Found: C, 60.30; H, 7.71; N, 14.13; Cl, 8.52; Co, 7.12.

Physical Measurements. Electronic spectra were obtained with use of a Varian 2300 spectrophotometer. EPR spectra were obtained on a Varian E-112 spectrometer with an X-band frequency of 9.3 GHz with g values quoted relative to external dpph ($g = 2.0036$). Samples in water were run at −40 °C in quartz tubes. The proton NMR spectra of the guest species, in the presence and absence of the paramagnetic cobalt(II) and CoO_2 hosts, were acquired at two different frequencies with use of Bruker WM300 and Bruker AM500 spectrometers. Samples were allowed to equilibrate at 274 and 304 K by using a variable temperature control unit. Five millimeter outside diameter Wilmad Royal Imperial sample tubes, 528-PP, 7 in. in length, were used to contain the guest and host–guest sample solutions. Aldrich Gold Label 99.96% hydrogen-2 deuterium oxide, low in paramagnetic impurities, was used as the solvent in all experiments. A 10 μL Hamilton syringe was used to prepare the host–guest solutions. The organic guest molecules used during inclusion complexation experiments were of spectrophotometric grade, Gold Label quality, purchased from Aldrich Chemical Co, and were used without further purification.

Experimental Precautions. (1) All glassware used in the study of the host–guest phenomena were soaked in 0.5 M EDTA for 24 h prior to use. (2) The deuterium oxide was low in paramagnetic impurities and degassed by freeze–pump–thaw techniques on a vacuum line at 10^{-5} Torr. Guest solutions were also degassed in this manner. (3) All samples for the study of the intramolecular association phenomena were prepared in an inert atmosphere glovebox and sealed.

Procedure for Host–Guest Studies. (1) A stock solution of the host compound was prepared by accurately weighing an amount of the chloride salt of the host species and dissolving the complex in a 2 mL volumetric flask containing deuterium oxide (typically 1.5×10^{-2} M). (2) A 10-L syringe was used to deliver an aliquot of the stock host solution into a 2 mL volumetric flask containing D_2O and an accurately measured amount of the guest solution. The referenced solution was prepared in an identical manner, excluding the paramagnetic host complex. (3) Aliquots were then taken from each of the two 2 mL volumetric flasks and placed into the two 5 mm NMR sample tubes. (4) Each sample was removed from the drybox and placed in an ice bath at 0 °C and allowed to cool for 15 min. One hundred Torr of O_2 was then bubbled through the solutions (both reference and sample) for 1 min while the temperature was kept at 0 °C. (5) Measurement of T_{1p} was achieved by two sets of standard inversion–recovery experiments obtained at each of two different frequencies. At least 12 different values of the variable delay parameter were used to determine accurately the relaxation times. The D_1 relay value = $5T_1$ = 30 s. The program used to calculate the T_1 values was from the Bruker library of software programs, and the errors indicated in the table are standard deviations of the fit.

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