cause of the large number of overlapping components in the spectrum.

Table VIII also includes the parameters which best describe the data on $Cs_2UO_2Cl_4$ which we reported in part I. It is clear that, apart from the equatorial field, both sets are closely comparable and that the description of the electronic structure in terms of a linear triatomic uranyl ion, weakly perturbed by the equatorial ligands, is a valid one. The only major difference between the two parameter sets is in $K_{\sigma\phi}$; the smaller value in $CsUO_2(NO_3)_3$ could be related to the presence of σ-bonding in the f_ϕ orbital set, which is absent in $Cs_2UO_2Cl_4$. It is also interesting but probably fortuitous that the radial parameter derived from a point charge potential expansion using actual ligand geometries,[12] $\langle B_6 \rangle = eq\langle r^6 \rangle / R^7$, where q is the charge on each coordinated atom in the equatorial plane, is essentially identical for both compounds.

The configurations responsible for the observed excited states are $\sigma\delta$ and $\sigma\phi$. It is the splitting of the f_ϕ orbitals by the trigonal field which dominates the equatorial field perturbation in $CsUO_2(NO_3)_3$. One of these is σ-antibonding with respect to the nitrate oxygens while the other is nonbonding. It is therefore easy to understand why the in-phase asymmetric stretching of the uranium bidentate oxygen bonds, ν_{10}, illustrated in Figure 7 is so effective at introducing intensity in conjunction with origin VI, producing the one strong OPA feature in Figure 3, because this excited state includes a large contribution from the $\sigma\phi$ configuration. Regarding the coordination sphere as a hexagon of oxygen atoms, ν_{10} shortens a bond in the $+x$ direction while lengthening a bond in the $-x$ direction, a perturbation that strongly mixes orbitals of *gerade* symmetry with the *ungerade* f-orbitals.

Conclusion

We have shown that TPA is a valuable adjunct to OPA for electronic excitations which are intrinsically parity conserving, even when the site symmetry of the chromophore is noncentric. The two types of experiment can be complementary in their selection rules and in their emphasis on vibronic structure. Here they are sufficient to establish an unequivocal assignment of the location and symmetry of eight excited states and to determine the location of a ninth (origin IV) to within about 20 cm^{-1}. The detail of the analysis makes it possible to identify an example of a transition which is magnetic dipole allowed as a result of the excitonic (or Davydov) coupling of electric dipole allowed single-center transitions. Magnetic dipole transition moments arising from coupled oscillators are more commonly manifest in the natural circular dichroism of chiral chelate complexes and helical polymers.

The quality of the data makes it possible to test the nature of the excited-state configurations in $CsUO_2(NO_3)_3$ semiquantitively through an empirical parametrization, and we conclude that the component configurations are closely similar to those which describe the excited states of $Cs_2UO_2Cl_4$.[3] These configurations are those in which a σ_u electron is promoted from the HOMO to a set of LUMO's composed of uranium f-orbitals in the energy order $f_\delta < f_\phi \ll f_\pi \ll f_\sigma$. We have recently reviewed the ability of MO calculations, at various levels of sophistication, to match these observations[16] and note that the occurrence of a σ_u HOMO is strongly influenced by the underlying pseudocore 6p-shell, whose participation in the bonding appears crucial in determining the stable trans geometry of the uranyl ion.[16,17] We have termed this phenomenon, which appears to have some general validity, the inverse trans influence.[16]

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Relaxation Enhancement of Water Protons by Manganese(III) Porphyrins: Influence of Porphyrin Aggregation

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The ability of eight manganese(III) porphyrins to enhance the longitudinal relaxation rate of water protons in aqueous solution has been studied by nuclear magnetic resonance dispersion (NMRD). In NMRD, the relaxation rates of solvent water protons are measured as a function of proton Larmor frequency from 0.01 to 50 MHz (field strengths from 0.23 mT to 1.17 T); a plot of relaxation rate versus frequency is called an NMRD profile. To enable comparison of the data, the observed rates are expressed as relaxivities by normalizing the rates to the concentration of manganese(III) porphyrin. Five porphyrins exhibited similar profiles characterized by high relaxivities, while the profiles for three porphyrins were different and demonstrated much lower relaxivities. Analysis of the NMRD profiles by relaxation theory yields correlation times, the values of which are sensitive to interactions between the hydrated manganese(III) porphyrin and its environment. The values of some correlation times for the three porphyrins with low relaxivities were inconsistent with those showing high relaxivities. Because the values of these correlation times indicate that interactions are occurring between manganese atoms and because differences in structure are apparent between the three porphyrins with lower relaxivities and the five with high relaxivities, it is hypothesized that the three porphyrins exist as aggregates in solution while the others exist as monomers. Addition of perdeuterated acetone to solutions of the porphyrins with low relaxivities caused them to attain relaxivities that are similar in magnitude to the others at all frequencies, while the addition of acetone to solutions of porphyrins with high relaxivities causes no significant change in their relaxivities. Because the addition of acetone is known to break up porphyrin aggregates, the assertion that aggregation is directly responsible for the low relaxivities in aqueous solution is strongly supported.

Introduction

The addition of a manganese(III) porphyrin to water causes an increase in the longitudinal relaxation rate of the water protons.[1,2] This increase in the relaxation rate normalized to the concentration of the porphyrin is called the relaxivity of the porphyrin. Investigators have shown that manganese porphyrins generally have what is considered a high relaxivity, typically about 9 mM^{-1} s^{-1} at 37 °C and 20 MHz.[3,4] This corresponds to about

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a 30-fold increase in the relaxation rate for a 1 mM manganese(III) porphyrin solution over that observed for pure water. However, the relaxivity of manganese(III) protoporphyrin IX [Mn(III)PP] has been reported to be 2 mM^{-1} s^{-1} under identical conditions.[5] Because manganese(III) porphyrins are currently being considered as potential contrast enhancement agents for magnetic resonance imaging,[6] it is of interest to determine if a low relaxivity is unique to Mn(III)PP or if it is a fundamental property of other manganese(III) porphyrins as well.

One common characteristic of porphyrins is their tendency to form aggregates in aqueous solution.[7] The formation of aggregates influences electronic properties of the porphyrins.[7,8] Consequently, the UV–visible,[9] fluorescence,[10] electron spin resonance,[9,11,12] and nuclear magnetic resonance spectra[8,9] are generally different for aggregated porphyrins than for monomeric ones. Aggregation may also influence the relaxivity of the manganese(III) porphyrins.

In this report, nuclear magnetic resonance dispersion (NMRD), which measures the relaxation rates of water protons as a function of proton Larmor frequency from 0.01 to 50 MHz, is used to obtain relaxivities over a range of magnetic field strengths from 0.23 mT to 1.17 T for a series of eight manganese porphyrins. The analysis of an NMRD profile, a plot of the relaxation rate versus proton Larmor frequency, by relaxation theory[2,13] yields various correlation times for a porphyrin. The values of the correlation times are dependent upon the interactions of a hydrated manganese(III) porphyrin with its environment, and the effects of aggregation may be revealed in the values of the correlation times.

Experimental Section

The porphyrins used in this study are [5,10,15,20-tetrakis(4-sulfonatophenyl)-21H,23H-porphine]manganese(III) chloride [Mn(III)TPPS$_4$], [5,10,15,20-tetrakis(1-methyl-4-pyridyl)-21H,23H-porphine]manganese(III) pentachloride salt [Mn(III)T4NMPyP], 5,10,15,20-tetrakis[4-(trimethylammonio)phenyl]-21H,23H-porphine]-manganese(III) pentachloride salt [Mn(III)TMAP], [5,10,15,20-tetrakis(4-carboxyphenyl)-21H,23H-porphine]manganese(III) chloride [Mn(III)TCPP], [3,7,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionic acid-8,13-disulfonic acid]manganese(III) chloride [Mn(III)D24DSA], [7,12-diethyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,8-dipropionic acid]manganese(III) chloride [Mn(III)MSP], [8,13-divinyl-3,7,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionic acid]manganese(III) chloride [Mn(III)PP], and [3,8,13,18-tetramethyl-21H,23H-porphine-2,7,12,17-tetrapropionic acid]manganese(III) chloride [Mn(III)CP1]. The chloride salts of Mn(III)T4NMPyP and Mn(III)TMAP and of the free-acid forms of Mn(III)TPPS$_4$, Mn(III)TCPP, Mn(III)D24DSA, Mn(III)PP, Mn(III)MSP, and Mn(III)CP1 were purchased from Porphyrin Products (Logan, UT). All porphyrins were analyzed for the presence of residual MnCl$_2$.[14] If detected, this was removed by dissolving the sample in water adjusted to a pH of 11 by the addition of aqueous sodium hydroxide to precipitate the free manganese ion as manganese hydroxide. The pH of the solution was then adjusted to 7 by the addition of dilute aqueous HCl. Reagent grade acetone and HPLC grade water were purchased from Fisher Chemical Co. (Fair Lawn, NJ), and deuterated acetone (d_6-acetone, 99.5 atom % D) was purchased from Chemical Dynamics Corp. (South Plainfield, NJ); they were used without further treatment.

Aqueous solutions were prepared by dissolving a manganese porphyrin in HPLC grade water. Dilute sodium hydroxide was added to dissolve the porphyrins that were not soluble in the HPLC water itself. The final pH of the solution was 10 for Mn(III)TCPP, Mn(III)PP, and Mn(III)MSP and 8 for Mn(III)CP1. Samples that contained d_6-acetone were prepared by adding a given volume of d_6-acetone to 1 mL of an approximately 1 mM aqueous manganese(III) porphyrin solution. Concentrations of manganese in the stock solutions were determined on a Perkin-Elmer Model 373 atomic absorption spectrometer with a manganese lamp at 279.5 nm and a mixture of air–acetylene flame gases. A calibration curve was prepared from standard solutions and used to verify the manganese concentrations.

The longitudinal relaxation rates $(1/T_1)$ of solvent protons in aqueous solutions that were approximately 1 mM manganese(III) porphyrin were measured as a function of proton Larmor frequency on a field-cycling relaxometer at the IBM T. J. Watson Research Center, Yorktown Heights, NY.[15] The temperature was controlled to 27 (±0.1) °C. Fitting the data in the NMRD profiles to theory was accomplished using nonlinear least-squares Fortran programs executed on a Cyber 850 Mainframe computer. No corrections were made for outer-sphere relaxation because the estimated error resulting from its neglect is small; its contribution to the total relaxation rate is expected to be at most 5% at high fields.[2] The general format for the algorithm written to perform the least-squares analysis may be found in the paper on curve fitting by Maddams.[16] The fitting procedure requires that initial estimates of r, τ_R, τ_V, and τ_{S0} be entered into the program while the value of τ_M is fixed at 10 ns. Reasonable estimates of the parameters were taken from literature values for complexes similar to those under study.[2] The estimates for τ_R, τ_V, and τ_{S0} may range from $^1/_3$ to 3 times the best-fit values, and the program will still converge on a unique set of values. No lower limit for the initial value of r was found, but the upper limit could not be larger than 3.2 Å. Hence, the ranges for the parameters are large and an estimated value outside them would not be physically reasonable for hydrated manganese porphyrins. The resultant reproducibility of the best-fit values is estimated to be ±10%.

Optical spectra were recorded on a Perkin-Elmer Lambda 5 UV/vis spectrometer at a temperature of 25 °C. The concentration dependence of the absorbance of the Soret band (about 460 nm) was monitored because it is the most sensitive to aggregate formation.[7] The acetone-containing solutions were prepared by diluting the 1 mM aqueous solutions of porphyrins by a factor of 2 by the addition of reagent grade acetone.

Results and Discussion

The longitudinal relaxation rate of water protons $(1/T_1)$ in the presence of manganese(III) porphyrins is described by the following equations:[2]

$$\frac{1}{T_1} = \frac{q[\text{Mn}]}{55.5} \frac{1}{T_{1M} + \tau_M} + \frac{1}{T_{1W}} \tag{1}$$

$$\frac{1}{T_{1M}} = \frac{B}{r^6}\left(\frac{7\tau_{C2}}{1 + \omega_S^2\tau_{C2}^2} + \frac{3\tau_{C1}}{1 + \omega_I^2\tau_{C1}^2}\right) \tag{2}$$

$$\frac{1}{\tau_{Ci}} = \frac{1}{\tau_{Si}} + \frac{1}{\tau_R} + \frac{1}{\tau_M} \quad i = 1, 2 \tag{3}$$

$$\frac{1}{\tau_{S1}} = \frac{1}{5\tau_{S0}}\left(\frac{1}{1 + \omega_S^2\tau_V^2} + \frac{4}{1 + 4\omega_S^2\tau_V^2}\right) \tag{4}$$

$$\frac{1}{\tau_{S2}} = \frac{1}{10\tau_{S0}}\left(3 + \frac{5}{1 + \omega_s^2\tau_V^2} + \frac{2}{1 + 4\omega_S^2\tau_V^2}\right) \tag{5}$$

In eq 1, q is the number of exchangeable water molecules in the inner coordination sphere and equals 2 for the Mn(III) porphyrins.[2] The molar concentration of the manganese(III) porphyrins is [Mn]; τ_M is the residence lifetime of a ligand in the inner coordination sphere, and $1/T_{1W}$ is the relaxation rate of water protons in the absence of any paramagnetic species. The ratio $q[\text{Mn}]/55.5$ is the mole fraction of water protons bound in the

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Table I. Results of a Least-Squares Comparison of Relaxation Theory with the NMRD Profiles of Manganese(III) Porphyrins in Aqueous Solutions at 27 °C[a]

	r, Å	τ_R, ps	τ_V, ps	τ_{S0}, ps
Mn(III)TMAP	2.35	81	43	16
Mn(III)TCPP	2.33	83	44	16
Mn(III)TPPS$_4$	2.26	66	38	14
Mn(III)T4NMPyP	2.29	65	32	15
Mn(III)D24DSA	2.23	56	31	14
Mn(III)PP	2.38	19	8	15
Mn(III)MSP	2.11	12	4	14
Mn(III)CP1	2.64	50	28	16
Mn(III)CP1 ($q = 1$)	2.35	50	28	16

[a] For all cases, τ_M was fixed at 10 ns and $q = 2$ unless otherwise specified.

inner coordination sphere of the manganese species.

For manganese(III) porphyrins in aqueous solution, τ_M is too short to contribute to eq 1 and too long to contribute to eq 3.[2,17] Consequently, it will be neglected in this analysis. In eq 2, $B = 2\gamma_I^2\gamma_S^2\hbar^2 S(S + 1)/15$, γ_I and γ_S are the nuclear and electronic gyromagnetic ratios respectively, S is the electron spin quantum number ($S = 2$ for manganese(III) porphyrins), ω_I and ω_S are the Larmor frequencies for the proton and the electron, respectively, and r is the distance between the protons of bound water molecules and the center of the manganese ion. This definition of r is not valid for manganese(III) porphyrins because the ground electronic state of the manganese ion is not an S-state; therefore Gauss' law no longer holds and the point dipole approximation breaks down. This breakdown causes the magnitudes of the relaxation rates of water protons in solutions of manganese(III) porphyrins to be higher than expected because of the anisotropy of the manganese electronic wave function in the direction of the protons of the bound water molecules.[2] The parameter τ_{Ci} is the dipolar correlation time; τ_R is the rotational correlation time of a hydrated manganese species; τ_{Si} ($i = 1, 2$) are the longitudinal and transverse relaxation times of the paramagnetic moments of the manganese; τ_V is the correlation time for modulation of the zero-field splitting; and τ_{S0} is a parameter which ensures that τ_{S1} and τ_{S2} are equal in the zero-field limit.

Aqueous Solutions. NMRD profiles for the eight manganese(III) porphyrins in aqueous solutions at 27 °C are shown in Figure 1. The solid curves through the data points result from a least-squares comparison of the data to eqs 1–5, where r, τ_R, τ_V, and τ_{S0} were treated as independent and unknown and τ_M was fixed at 10 ns.[2] The values for these correlation times as obtained in the least-squares analysis are reported in Table I. Figure 1 shows that the profiles can be divided into two groups: those with comparatively high relaxivities and similar profiles [Mn(III)-TPPS$_4$, Mn(III)T4NMPyP, Mn(III)TMAP, Mn(III)TCPP, Mn(III)D24DSA] and those with lower relaxivities [Mn(III)PP, Mn(III)MSP, Mn(III)CP1].

(a) Manganese(III) Porphyrins with High Relaxivities. The qualitative form of the NMRD profiles is characterized by an abrupt increase in the relaxivity above about 2 MHz and a high-field (frequency $\geq$ 10 MHz) relaxivity that is greater in magnitude than that of most other metal ions, such as Mn^{2+}, Fe^{3+}, and Cu^{2+}, and small chelates of these ions, such as Mn(II)EDTA, Mn(II)NOTA, Fe(III)EDTA, and Fe(III)TPPS$_4$.[2,18-20] The abrupt increase in relaxivity above 2 MHz is caused by τ_{Ci} being dominated by the magnetic field dependent τ_{Si} rather than τ_R. The value of τ_R is independent of field strength and dominates τ_{Ci} over all field strengths for most other ions and their metal chelates. The value of τ_{Si} increases at field strengths corresponding to frequencies of about 2 MHz because of a relatively large value
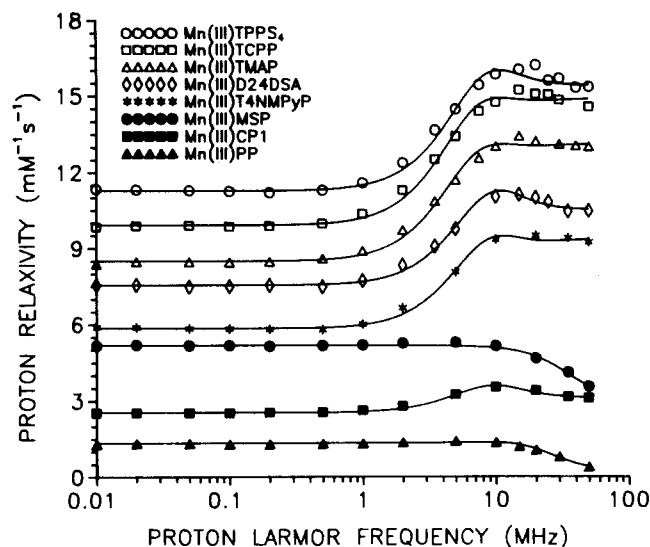

Figure 1. NMRD profiles of manganese(III) porphyrins in aqueous solutions at 27 °C. To display the data clearly on one graph, the relaxivities for several profiles have been offset. These amounts must be subtracted from or added to the following profiles to obtain the actual experimental relaxivities: Mn(III)TPPS$_4$, –5; Mn(III)TCPP; –4; Mn-(III)TMAP, –2; Mn(III)D24DSA, –1; Mn(III)PP, +2. The solid curves through the data derive from a least-squares comparison of the data to eqs 1–5 with τ_M fixed at 10 ns.

of τ_V, which has been attributed to the large radius and high ridigity of porphyrin ring system.[21] Because the value of τ_R is also related to the size and structure of a molecule,[2,22] attributing the value of τ_V to the size and structure of the porphyrin is supported by the trend of τ_V and τ_R values (Table I); porphyrins with longer values of τ_R also have longer values of τ_V. Generally, larger molecules will have longer values of τ_R.[22] That this assertion is reasonable is illustrated by the values of τ_R for these five porphyrins. The porphyrins with the large phenyl groups in the 5,10,15,20 positions, Mn(III)TPPS$_4$, Mn(III)TMAP, Mn(III)T-CPP, and Mn(III)T4NMPyP, have larger values of τ_R than Mn(III)D24DSA, which has smaller aliphatic substituents in the β-pyrrole positions.

The large value of the relaxivities for these manganese(III) porphyrins at magnetic field strengths corresponding to frequencies above 10 MHz results from the anisotropy of the electronic wave function of the manganese(III) ion that causes a breakdown in the point dipole approximation.[2] Because the wave function is elongated in the direction of the bound water molecules, the electron spin density of the manganese is closer to the protons of the bound water molecule and the mutual dipolar interaction is increased. The increased dipolar interaction is reflected in values of r that are smaller than those for complexes of ions that have spherically symmetric ground electronic states, such as Mn^{2+}, Fe^{3+}, and Gd^{3+}, for which the point dipole approximation is valid. The value of r for these S-state ions is typically 2.8 Å,[2,23,24] while the average value of r for the manganese(III) porphyrins is 2.29 Å. Because the value of r enters the theory as $1/r^6$, the r dependence of the dipolar interaction is a factor of 3.3 times greater for the manganese(III) porphyrins than for the S-state ions.

(b) Manganese(III) Porphyrins with Low Relaxivities. The NMRD profiles for Mn(III)PP, Mn(III)MSP, and Mn(III)CP1 are different from those for porphyrins with high relaxivities. In addition, the profile of Mn(III)CP1 may be distinguished from those of Mn(III)PP and Mn(III)MSP. Although the qualitative form of the profile for Mn(III)CP1 is similar to that for porphyrins

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with high relaxivities, the values of the relaxivities for Mn(III)CP1 are about 3 times smaller at all magnetic field strengths. The NMRD profiles for Mn(III)PP and Mn(III)MSP are both characterized by the absence of a significant increase in relaxivity at field strengths corresponding to frequencies of 2 MHz and by a sudden decrease in relaxivity at field strengths corresponding to frequencies greater than 10 MHz.

Table I shows that the differences in the NMRD profiles between two of the porphyrins with low relaxivities, Mn(III)PP and Mn(III)MSP, and the porphyrins with high relaxivities correspond to differences in the correlation times τ_V and τ_R. The profiles for Mn(III)PP and Mn(III)MSP show no rise around 10 MHz; this results in a value of τ_V calculated in the analysis that is very short in comparison to that of the other porphyrins. The decrease in relaxivities at field strengths corresponding to frequencies greater than 20 MHz results in the calculation of values of τ_R that are also unreasonably short compared to those of the other porphyrins, and indeed shorter than that of the Mn^{2+} ion itself (33 ps at 25 °C[19,24,25]). Because the size and structure of Mn(III)PP and Mn(III)MSP are similar to those of Mn(III)D24DSA, which has a high relaxivity, the low relaxivities and the values of τ_R and τ_V may not be accounted for on the basis of size differences among the ligands.

The NMRD profile for Mn(III)CP1, the third porphyrin with low relaxivity, is qualitatively similar to those of the porphyrins with high relaxivities, and its correlation times τ_R, τ_V, and τ_{S0} are similar to those for the high-relaxivity porphyrin Mn(III)D24DSA. However, the value of r for Mn(III)CP1 calculated from the analysis is much larger than that for any other porphyrin in the series because the relaxivities of Mn(III)CP1 at all field strengths are a factor of 3 lower than those of the porphyrins with high relaxivities.

Although the results of the analysis of the NMRD profiles indicate that the low relaxivities of Mn(III)PP and Mn(III)MSP may result from physical processes different from that for Mn(III)CP1, the same fundamental phenomenon may give rise to the low relaxivities of all three compounds: aggregation. The forms of the profiles of the three porphyrins with low relaxivities show less prominent or fewer features than those of the porphyrins with high relaxivities. Similar observations of decreasing relaxivity and decreasing dispersion have been made in systems in which the separation between paramagnetic ions has decreased to the point where metal ion–metal ion interactions occur.[26] Aggregation of the porphyrins would certainly give rise to systems in which Mn(III)–Mn(III) interactions could take place.

Among the porphyrins with high relaxivities, Mn(III)TPPS$_4$, Mn(III)T4NMPyP, and Mn(III)TMAP are known to be monomeric in aqueous solution.[27,28] The profiles of Mn(III)TCPP and Mn(III)D24DSA are similar to those three, providing strong evidence that they are monomeric as well. That Mn(III)TPPS$_4$, Mn(III)T4NMPyP, Mn(III)TMAP, and Mn(III)TCPP are monomeric is expected because of the large, charged phenyl groups in the meso positions of the porphyrin rings in these four macrocycles, which would prevent the close contact necessary for aggregation by charge repulsion and steric hindrance.[7,8,29] Although it is widely recognized that β-pyrrole substituted porphyrins tend to aggregate,[7] Mn(III)D24DSA also appears to be monomeric. Very little is known about the nature of the porphyrin–porphyrin interactions leading to aggregation,[30] and an inquiry into the monomeric behavior of Mn(III)D24DSA is beyond the scope of this report.

In the pH range of this study (4–10), porphyrin aggregates typically consist of two or more porphyrins held together by van der Waals forces.[7,8] Two structures are commonly proposed for aggregates: a face-to-face structure where one porphyrin is stacked directly above the other[7] or a structure where the individual porphyrins are stacked in an offset fashion.[8] In both cases, the formation of aggregates would be expected to cause an increase in τ_R because of the increase in size over that of the monomer. The very small value of τ_R for Mn(III)PP and Mn(III)MSP cannot be rationalized on the basis of size and seems especially incongruous if aggregates are formed; this leads to the suspicion that the value of τ_R obtained in the least-squares analysis of the profiles of these two porphyrins is not the rotational correlation time. The value of τ_R for Mn(III)CP1 is within the range considered normal for monomeric porphyrins, so it too may be considered shorter than expected for aggregates, although it is clearly not as affected by aggregation as is τ_R for the other two porphyrins with low relaxivities. The effect of aggregation on τ_V cannot be predicted because the interactions that determine its magnitude are unclear, even in the case of monomeric porphyrins.[2,21]

The unreasonably short τ_R obtained for Mn(III)PP and Mn(III)MSP may be explained if other processes unaccounted for in this analysis are present having correlation times independent of magnetic field strength over the range of the NMRD profiles. Such processes can result from interactions between manganese ions in porphyrin aggregates. When aggregates form, the manganese ions are brought into close proximity. This causes electronic and magnetic perturbations on each interacting manganese atom that broaden the magnetic levels of the ions. It has been shown that electronic relaxation times are not significantly altered in homodinuclear species even if strong magnetic coupling occurs, but such effects can alter the interaction between the paramagnetic ions and the protons they relax.[31,32] If the structure of the Mn(III)CP1 aggregate is slightly different from that of the aggregates of Mn(III)PP and Mn(III)MSP, it is reasonable to assume that the perturbations are different and hence that the alterations in and effects on ion–proton interactions are different.

The unusually long value of r calculated for Mn(III)CP1 can also be explained as a consequence of aggregation. If Mn(III)CP1 aggregates to form face-to-face dimers, one can easily envision that coordinated water molecules either may be excluded from the region between the two porphyrins or may be held in that region in a manner to make exchange unlikely. In either case, the value of q would be less than 2 for each porphyrin in the aggregate. If a value of 2 were used in the analysis of a dimer of the type described, the value of r obtained would be unusually large. When q is assigned a value of 1 in the least-squares analysis of the profile of Mn(III)CP1, the value of r obtained is reduced to 2.35 Å, well within the range of r values found for monomeric manganese(III) porphyrins. Changing q from 2 to 1 is consistent with Mn(III)CP1 existing in solution as a face-to-face dimer in which each porphyrin bears only one exchangeable water molecule.

At this point, it is reasonable to suggest that aggregation is responsible for the low relaxivity of three manganese porphyrins and to speculate that Mn(III)CP1 exists as a face-to-face dimer, in which each porphyrin has one exchangeable water molecule, while Mn(III)PP and Mn(III)MPS have a different form, perhaps that of an offset dimer, in which each porphyrin retains two exchangeable water molecules. A complete analysis of the influence of homodinuclear magnetic coupling on the relaxation parameters of aggregated porphyrins is in progress. For this analysis more extensive equations are required than those shown herein to take into account the effects of metal–metal interactions.[31,32] Germane to this discussion are primarily the existence of aggregates of the paramagnetic manganese(III) porphyrins and secondly the influence of aggregation on observed solvent proton relaxation rates. The finer points of aggregate structure and the mechanisms contributing to the differences that give rise to two

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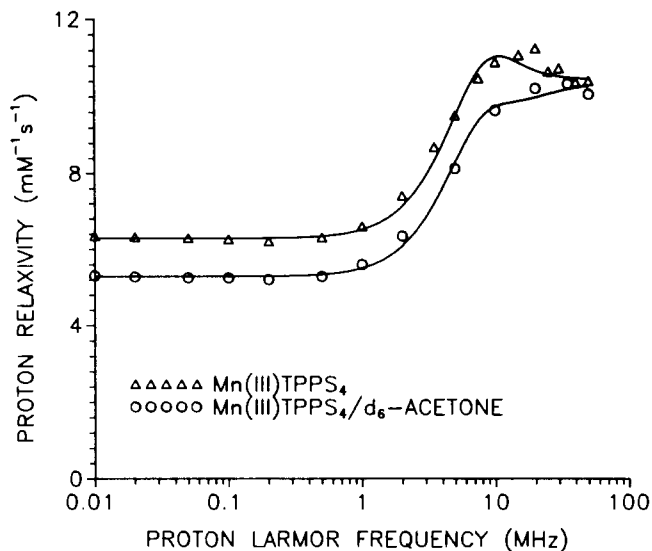(32) Banci, L.; Bertini, I.; Luchinat, C. *Struct. Bonding* **1990**, *72*, 113.

Figure 2. NMRD profiles for 1 mL of a 0.93 mM solution of Mn(III)TPPS$_4$ with 0 and 1 mL of added d_6-acetone at 27 °C.

Table II. Results of a Least-Squares Comparison of Relaxation Theory with the NMRD Profiles of Manganese(III) Porphyrins in Aqueous Solution Containing d_6-Acetone at 27 °C[a]

	V_A, mL	r, Å	τ_R, ps	τ_V, ps	τ_{SO}, ps
Mn(III)MSP	0	2.11	12	4	14
	0.1	2.26	24	12	16
	0.4	2.37	41	23	15
	0.7	2.28	39	26	13
	1.0	2.35	50	29	16
Mn(III)PP	0	2.38	19	8	15
	1.0	2.28	42	26	13
Mn(III)CP1	0	2.64	50	28	16
Mn(III)CP1 ($q = 1$)	0	2.35	50	28	16
	1.0	2.30	68	41	15
Mn(III)TPPS$_4$	0	2.26	66	38	14
	1.0	2.36	84	40	15

[a] For all cases, τ_M was fixed at 10 ns and $q = 2$ unless otherwise specified. The volume of d_6-acetone added to 1-mL aqueous solutions of manganese(III) porphyrins (approximately 1 mM) is indicated by V_A.
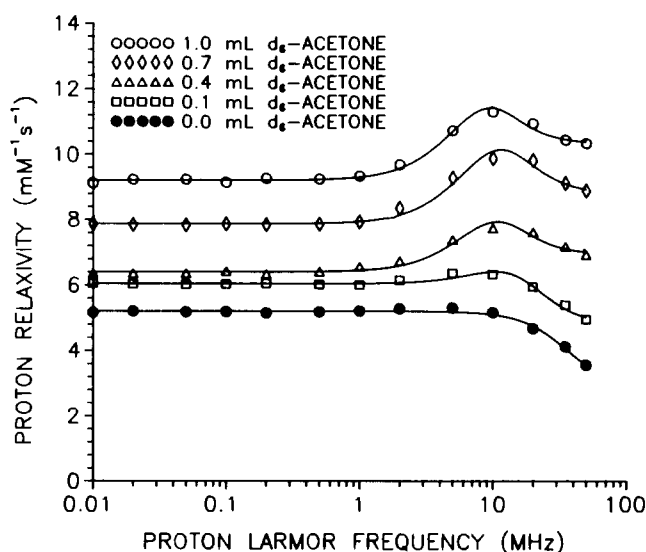
forms of profiles for the porphyrins with low relaxivities will be addressed elsewhere.

Acetone–Water Solutions. The addition of certain water-soluble organic solvents to aqueous solutions has been shown to break up aggregates of porphyrins[7,12] and other planar molecules[33-35] that are held together by van der Waals forces. Explanations of this phenomenon are given by several authors.[34-37] To verify the presence of aggregates in the solutions of porphyrins with low relaxivities, perdeuterated acetone was selected to break up the aggregates because it is a noncoordinating solvent for manganese(III) porphyrins[38] and, being fully deuterated, it does not interfere with the resonance signal of the water protons. Because acetone molecules do not compete with water molecules for coordination sites in the inner coordination sphere of the manganese(III) porphyrins, the addition of acetone does not dilute the solution from the point of view of all water molecules. The observed relaxation rate is proportional to the mole fraction of protons that is bound in the inner coordination sphere of the manganese, and this fraction does not change when acetone is added. Therefore, eq 1 still holds for the same concentration of the manganese(III) porphyrin prior to dilution with acetone, and the NMRD profiles can be analyzed quantitatively to study the effects of the addition of acetone.

Figure 2 shows the NMRD profile for Mn(III)TPPS$_4$ in aqueous and acetone–water solutions. Mn(III)TPPS$_4$ does not aggregate in aqueous solution and is therefore used as a control. The profiles for its solutions with and without added acetone are similar; the added acetone does not cause appreciable changes in relaxivity. For the relaxation parameters obtained in the least-squares analysis of the profiles (Table II), the most notable difference is that the value of τ_R for acetone–water solutions is 1.3 times greater than that for aqueous solutions. The acetone–water solution of Mn(III)TPPS$_4$ contained approximately 50% acetone by volume, and the viscosity of this solution was about 1.5 times greater than that of the acetone-free solution.[39] The magnitude of the increase in τ_R reflects the magnitude of the increase in viscosity. The values obtained for τ_V and τ_{SO} are about the same for both acetone-containing and acetone-free solutions;

Figure 3. NMRD profiles for a d_6-acetone titration of 1 mL of a 1.2 mM solution of Mn(III)MSP at 27 °C. These amounts must be subtracted from the following profiles to convert for the offset on the y axis used to display the data: 1.0 mL of d_6-acetone, 4; 0.7 mL of d_6-acetone, 3; 0.4 mL of d_6-acetone, 2; 0.1 mL of d_6-acetone, 1.

the value for r is slightly higher for the acetone-containing solution. Although acetone is classified as a noncoordinating solvent, a very small competion between water molecules and acetone molecules for coordination to the manganese could effectively dilute the concentration of Mn(III)TPPS$_4$ from the point of view of the water molecules; as a result of this dilution, the value of r will be slightly larger than expected. However, the differences in the r values do not complicate the analysis because this difference is small compared to the range of r values. The only significant effect of added acetone is to increase the value of τ_R by increasing viscosity.

Figure 3 shows the effects of an acetone titration on the relaxivity of an aqueous solution of Mn(III)MSP. As acetone is added, the form of the NMRD profile progressively approaches that typical of monomeric porphyrins with high relaxivities. The parameters obtained for each titration point are given in Table II; τ_R and τ_V approach values of monomeric manganese(III) porphyrins as the acetone content is increased. This is consistent with a decrease in the interactions between manganese ions brought about by a reduction in aggregation.

Figure 4 shows the NMRD profiles for aqueous and acetone–water solutions of Mn(III)PP and of Mn(III)CP1. The parameters obtained from a least-squares analysis of the profiles are given in Table II. Changes in the profiles occur for both solutions upon the addition of acetone that are similar to those observed for

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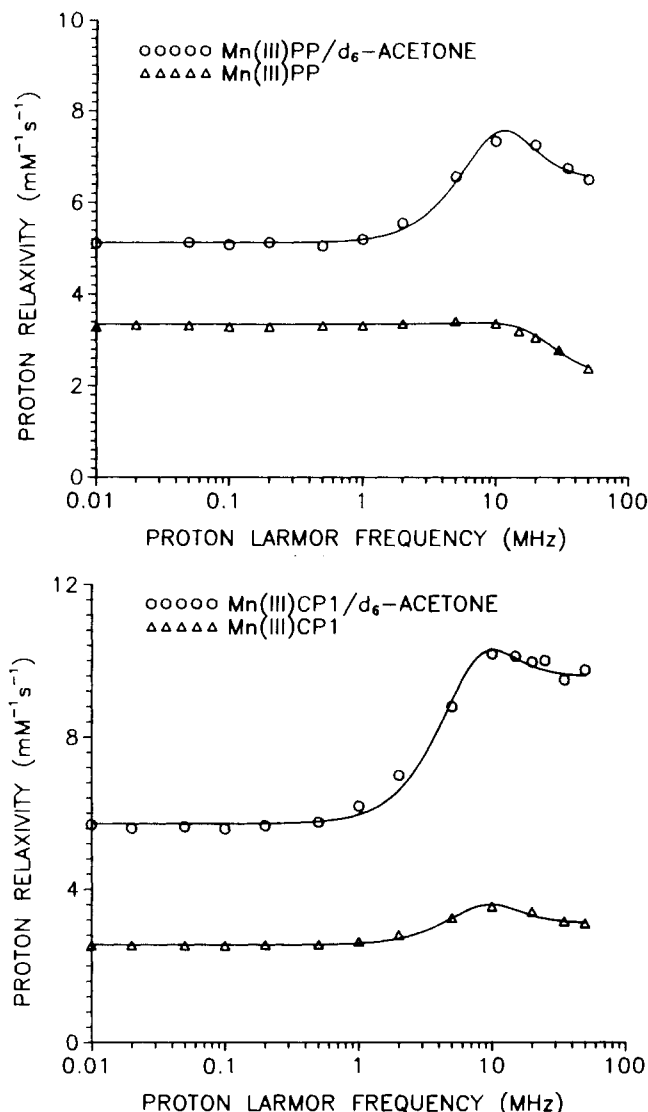
ooooo Mn(III)PP/d_6-ACETONE
ΔΔΔΔΔ Mn(III)PP

ooooo Mn(III)CP1/d_6-ACETONE
ΔΔΔΔΔ Mn(III)CP1

Figure 4. NMRD profiles for 1 mL of (top) 1.1 mM Mn(III)PP and (bottom) 1.2 mM Mn(III)CP1, with 0 and 1 mL of added d_6-acetone at 27 °C.

QQQQQ Mn(III)CP1/ACETONE
□□□□□ Mn(III)CP1

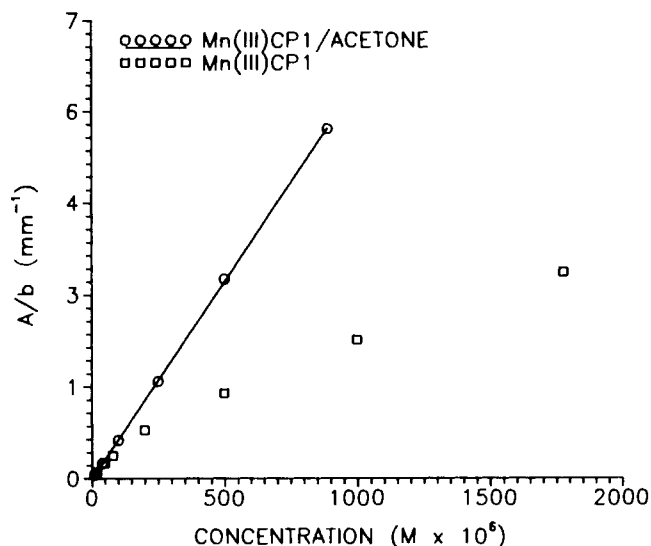**Figure 5.** Beer's law plots of Mn(III)CP1 in aqueous and acetone–water solutions. The temperature was 25 °C, and the pH of all solutions was 7.9.

Mn(III)MSP solutions. The profile and the relaxation parameters for Mn(III)PP are similar to those of Mn(III)MSP for solutions containing identical amounts of acetone. For Mn(III)CP1, the profile attains a magnitude close to that of the monomeric porphyrins, and the least-squares analysis gives a reasonable r value with the value of q set at 2. The results are also consistent with those of Mn(III)MSP solutions and indicate that aggregates are broken up by the acetone.

A comparison of the NMRD profiles for the 1:1 acetone–water solutions of Mn(III)TPPS$_4$, Mn(III)CP1, Mn(III)PP, and Mn(III)MSP shows that the relaxivities of Mn(III)TPPS$_4$ and Mn(III)CP1 are similar at higher magnetic field strengths, while those of Mn(III)PP and Mn(III)MSP are smaller by a factor of about $^2/_3$. The similarity between the relaxivities for Mn(III)-TPPS$_4$ and Mn(III)CP1 is consistent with the aggregates of Mn(III)CP1 being completely broken up. The differences between the profiles of Mn(III)TPPS$_4$ and Mn(III)CP1 occur mainly in their relaxivities at field strengths corresponding to frequencies greater than 10 MHz. The relaxivities of Mn(III)TPPS$_4$ increase with frequency after 10 MHz, while the relaxivities of Mn(III)CP1 decrease. This occurs because Mn(III)CP1 is smaller than Mn(III)TPPS$_4$ and has a smaller τ_R. Smaller values of τ_R and τ_V are obtained for Mn(III)PP and Mn(III)MSP in comparison to the other porphyrins, indicating that their aggregates are not completely dispersed by the acetone.

UV–Visible Spectroscopy. Only aggregation has consistently been shown to be responsible for deviations from Beer's law for

porphyrins.[7] Consequently, the assertions that Mn(III)PP, Mn-(III)MSP, and Mn(III)CP1 are aggregated in aqueous solution and that the addition of acetone to the solution breaks up the aggregates can be further supported by Beer's law studies.

Mn(III)TPPS$_4$ is again used as a control. Plots of absorbance versus concentration should be linear in accordance with Beer's law if no aggregation occurs. The experimental results show that Mn(III)TPPS$_4$ obeys Beer's law over concentrations ranging from 1 μM to 1 mM for aqueous solutions; no deviations from Beer's law were observed when acetone was added.

Aqueous solutions of Mn(III)CP1, Mn(III)PP, and Mn(III)-MSP all show deviations from Beer's law; therefore, these three porphyrins exist as aggregates. Aqueous solutions of Mn(III)CP1 (Figure 5) exhibit deviations from Beer's law in the concentration range between 0.1 and 0.5 mM, indicating that aggregation takes place in this range. Upon addition of acetone to the solutions, the plot of absorbance versus concentration becomes linear, which is consistent with only monomeric Mn(III)CP1 being present in the acetone–water solutions. Plots of absorbance versus concentration are similar for Mn(III)PP and Mn(III)MSP in aqueous solution. Deviations from Beer's law occur in the concentration range 1–50 μM, indicating that aggregation takes place within this lower range for Mn(III)PP and Mn(III)MSP. This is consistent with UV–visible results reported much earlier for Mn-(III)MSP.[40] Consequently, the data for Mn(III)MSP and Mn(III)MSP are not shown. Upon addition of acetone, the plots for Mn(III)PP and Mn(III)MSP become linear, indicating that the aggregates have been broken up.

Conclusions

Three manganese(III) porphyrins, Mn(III)PP, Mn(III)MSP, and Mn(III)CP1, exhibit relaxivities that are lower than those of the other five porphyrins studied. The analysis of the NMRD profiles suggests that Mn(III)PP, Mn(III)MSP, and Mn(III)CP1 exist as aggregates in solution, while porphyrins that have high relaxivities do not, and aggregation is responsible for their low relaxivities. The existence of aggregates is verified by experiments in which the addition of acetone, a known disruptor of porphyrin aggregates, to aqueous solutions of Mn(III)PP, Mn(III)MSP, and Mn(III)CP1 changes the form of their NMRD profiles as well as the values calculated from them, making them similar to those of the high-relaxivity porphyrins. In addition, Beer's law studies verify both the presence of aggregates and their disruption with acetone.

The lower value of relaxivity for Mn(III)CP1 is attributed to the formation of face-to-face dimers, which results in the number

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of exchangeable water molecules per porphyrin (q) being reduced from 2 to 1. In contrast, it is suggested that the aggregates of Mn(III)PP and Mn(III)MSP are arranged in an offset fashion, so that two exchangeable water molecules are still bound per porphyrin. In both types of aggregates, however, manganese–manganese interactions occur which influence the reliability of the τ_R and τ_V parameters derived from the least-squares analysis of the NMRD profiles. Regardless of the structure of the aggregates or the mechanism by which the relaxivity is affected, aggregation reduces the magnitude of the relaxivity of manganese(III) porphyrins in aqueous solutions. Furthermore, the addition of acetone to solutions of monomeric porphyrins results in no signficant change in the NMRD profiles; the relaxivities remain high at all magnetic field strengths. With the stipulation that the manganese(III) porphyrins exist as monomers, the results support Koenig's assertion that a high relaxivity is a general phenomenon of manganese(III) porphyrins.[2]

These results have important implications in the use of manganese(III) porphyrins as MRI contrast enhancement agents. Because the relaxivity of the aggregated manganese(III) porphyrins becomes high upon dispersion of the aggregates, any physical separation of these porphyrins in solution should result in the attainment of a high relaxivity. Separation of porphyrins can be achieved by covalently attaching the porphyrins to antibodies[6] or polymers.[41] This has been demonstrated in our lab-

oratory; the covalent attachment of Mn(III)PP to poly(α-amino acids)[41] resulted in a relaxivity of the Mn(III)PP conjugates that was greater than that of Mn(III)PP alone.

The binding of a metalloporphyrin to a macromolecule can also increase the tissue specificity[6] and water solubility[42] of the porphyrin as well as its relaxivity. The results indicate, however, that care must be taken when the number of bound metalloporphyrins per macromolecule is considered, because the highest possible loading factor will not necessarily result in the highest relaxivity.[26,43] If the loading factor becomes very high and interactions occur among the bound metalloporphyrins, a loss of relaxivity may be observed because the metalloporphyrins are no longer monomeric entities.

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Metal Substitutions at the Diiron Site of Hemerythrin. A Dicobalt(II) Derivative

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The invertebrate O_2-carrying protein hemerythrin contains a nonheme diiron active site. A simple method is described for high-yield preparation of hemerythrin in which cobalt is substituted for the nonheme iron. The cobalt-substituted hemerythrin contains nearly the same polypeptide helix content as the native protein and, like the native protein, is octameric, indicating that the protein subunit structure and intersubunit interactions have been preserved. Close to 2 Co/Hr subunit with no detectable iron is reproducibly obtained, suggesting that cobalt occupies the same coordination sites as iron in the native protein. Analysis of the EXAFS data provides proof of a dicobalt site (3.54-Å Co–Co distance) with histidine ligation. The requirement of correlated motion of the Co scatterers for observation of EXAFS implies the existence of one or more bridging ligands. Given the single distance (2.12 Å) found for the first coordination sphere donor atoms, and comparisons to synthetic complexes, a hydroxo bridge is suggested. The X-ray absorption as well as the UV/vis and near-IR absorption and CD spectra indicate that all of the cobalt in Co_2Hr is in the +II oxidation state, even in air, that the coordination spheres consist exclusively of N,O donor ligands, and that both five- and six-coordinate Co centers are present in significant proportions. Given the five- and six-coordinate iron centers with terminal histidine ligands in the (μ-hydroxo)bis(μ-carboxylato)diiron(II) site of deoxyHr, an isostructural dicobalt(II) site is strongly suggested in Co_2Hr. The absorption spectrum of the dicobalt(II) site in Co_2Hr is unaffected by variations in pH or by the presence of azide, O_2, or H_2O_2. Despite this lack of reactivity, the close structural analogies to native Hr and the stability of Co_2Hr are likely to facilitate studies of the relationship between protein folding and dimetal site assembly.

Hemerythrin (Hr)[1] is an O_2-carrying protein found in a few phyla of marine invertebrates. Despite its name, Hr contains no heme group but rather a nonheme diiron site that reversibly binds one molecule of O_2. Hr's are usually octameric ($M_r \sim 108\,000$) and composed of essentially identical subunits ($M_r \sim 13\,500$). The secondary, tertiary, and diiron site structures of these subunits, which have been established by X-ray crystallography,[2] are illustrated schematically in Chart I. The open coordination site on Fe2 binds O_2 according to eq 1. Several reviews have summarized the available data on spectroscopy, reactivity, and molecular structure/function relationships of Hr.[3–6]
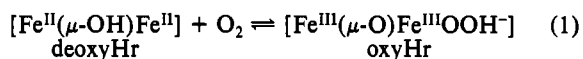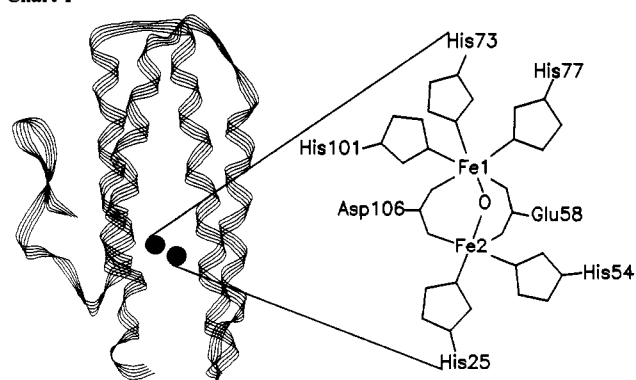
$$[Fe^{II}(\mu\text{-OH})Fe^{II}] + O_2 \rightleftharpoons [Fe^{III}(\mu\text{-O})Fe^{III}OOH^-] \quad (1)$$
$$\text{deoxyHr} \qquad\qquad\qquad \text{oxyHr}$$

Chart I

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[‡] University of Massachusetts.

Hr has been known to contain iron since 1890[7a] and was first proposed to contain a diiron functional site as long ago as 1955.[7b]