Determination of the Residence Lifetimes of Water Molecules in the Inner Coordination Sphere of Paramagnetic Metalloporphyrins and Their Adducts with Poly(α-amino acids)†

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ABSTRACT: The residence lifetimes of water molecules in the inner coordination sphere of the metal ion have been investigated for the manganese complexes of tetrakis(*N*-methylpyrid-4-yl)porphyrin, protoporphyrin IX, and the adducts of manganese protoporphyrin IX with the homopolymers poly(L-glutamic acid) (PGA) and poly(L-lysine) (PLL) and the random copolymer poly(L-lysine-*co*-L-phenylalanine) (PLP). The ability of these metal species to enhance the relaxation of solvent protons was also determined in aqueous solutions. For the porphyrins not attached to polymers, a longer residence lifetime correlates with poorer relaxivity as expected. For the polymer-bound porphyrins, however, residence lifetimes fail to explain the relative relaxivities. This implicates cross relaxation as a significant mechanism in determining the ability of polymer-bound paramagnetic agents to enhance relaxation of solvent protons.

In the paper describing the first observation of the proton resonance of water, a paramagnetic species was added to the sample to enhance its spin–lattice relaxation time.[1] With the advent of magnetic resonance imaging (MRI), relaxation enhancement agents are again being introduced into samples, in this case living beings, to shorten the T_1 values of the water protons in tissues and brighten areas of clinical significance in the images.[2-5] Once introduced into the living system, small paramagnetic agents interact with omnipresent macromolecular solutes; these interactions effect the ability of the agents to enhance relaxation. Paramagnetic complexes bound to polymeric carriers are also being evaluated as potential enhancement agents,[6,7] and the covalent attachment of paramagnetic complexes to large molecules may also be expected to influence their enhancement of proton relaxation.

Because porphyrins are excellent ligands for most metal ions and because some porphyrins have reported site-specific biodistribution to sites of malignancies in vivo, it is logical to examine paramagnetic metalloporphyrins as possible contrast enhancement agents for MRI.[8-11] Furthermore, some porphyrin ligands contain suitable functional groups for attachment to polymeric carriers and hence may be useful for studies of polymer-bound species.

The literature is rich with reports assessing the relative contributions of various physical effects to proton relaxation enhancement by paramagnetic species.[12-15] This report describes the evaluation of one such effect, the residence lifetime of water molecules in the inner coordination sphere of the metal ion and its relationship to the relaxation of water protons in the bulk solvent in solutions containing polymer-bound paramagnetic species. Two paramagnetic metalloporphyrins and three covalently bound porphyrin polymer adducts have been studied. The comparison of the residence lifetimes with observed relaxivities indicates that effects other than those requiring the exchange of solvent in and out of the first coordination sphere of the metal are important in determining the extent of relaxation enhancement of the solvent protons. This result is significant for understanding the mechanisms of relaxation enhancement by polymeric agents as well as enhancement by small complexes that associate with macromolecular solutes in vivo and will assist the directed design of future enhancement agents by defining the features of paramagnetic species and polymers that contribute to maximum enhancement.

Experimental Section

The tosylate salt of the manganese(III) complex of tetrakis(*N*-methylpyrid-4-yl)porphyrin (MnT4NMPyP) and the disodium salt of manganese(III) protoporphyrin IX chloride (MnPP) were purchased from Porphyrin Products (Logan, UT). The conjugates of the poly(α-amino acids) and MnPP were prepared and characterized by methods previously described;[7] their characteristics are summarized on Table I, which also includes definitions of the acronyms used in this paper to name the conjugates. ^{17}O-enriched water (12 and 15.4 atom %) was purchased from ICON Services, Inc. (Summit, NJ), and MSD Isotopes (Montreal, CA), respectively. Water used in the preparation of samples was purified via a Millipore Milli-Q water purification system.

All sample solutions were approximately 1×10^{-4} M in manganese at a pH of 5.5 in monobasic phosphate buffer with an ionic strength of 0.15 M. Strict control of pH is necessary as the ^{17}O line widths vary greatly as a function of pH above 6.5.[16] The concentration of manganese was determined by atomic absorption on a Perkin-Elmer Model 373 atomic absorption spectrometer with a manganese lamp at 279.5 nm and an air–acetylene flame gas mixture. Reference solutions containing no manganese porphyrin were prepared under identical conditions. No matrix effects were observed, as the presence of the polymer did not influence the accuracy of the manganese determination.

Measurements of spin–spin relaxation times (T_2) were performed on a JEOL FX-90Q multinuclear FT-NMR with tunable probe and an NM-PVT variable-temperature control accessory. Values of T_2 were obtained from the line width (full-width at half-height) of the ^{17}O water resonance. Determinations were made at temperatures varying from 0 ± 1 to 40 ± 1 °C. Spin–lattice relaxation times (T_1) for the calculation of relaxivities were determined at 20 MHz on an IBM PC-20 Minispec at a temperature of 37 ± 1 °C.

Results and Discussion

Paramagnetic substances introduce electron relaxation mechanisms which shorten relaxation times of nuclei

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Table I
Characteristics of Porphyrin–Polymer Adducts[a]

polymer	acronym for adduct	loading factor
poly(glutamic acid)	MnPP–PGA	1 MnPP/3 GA
poly(L-lysine)	MnPP–PLL	1 MnPP/7 L
poly(L-lysine-L-phenylalanine)	MnPP–PLP	1 MnPP/6 L

[a] Loading factors reflect the number of paramagnetic sites per amino acid residue on polymer; all porphyrin–polymer conjugates have molecular weights of greater than 300 000. The lysine-phenylalanine is a random copolymer with 1:1 ratio of lysine residue to phenylalanine residue.

excited in the NMR experiment. The theoretical treatment of the paramagnetic contribution to relaxation in the inner coordination sphere has been solved for symmetrical aquo complexes of paramagnetic ions.[12,17] The inner-sphere contributions to relaxivity for the aquo ions may be calculated from the Solomon–Bloembergen equations.[12,13] An important parameter in these equations is the dipolar correlation time, t_c, which is defined by the following equation

$$1/t_c = 1/t_r + 1/t_s + 1/t_m \quad (1)$$

where t_r is the rotational correlation time of a nuclear species, t_s is the electron spin relaxation time, and t_m is the residence lifetime of the nuclear species in the inner coordination sphere of the paramagnetic ion. The dominant parametric variable that determines the relaxation time of the species bound in the inner coordination sphere in eq 1 is usually t_s, but when one is concerned about the transmission of relaxation enhancement into the bulk, then the value of t_m becomes significant. If t_m is either very long or very short, no information will be transferred and no enhancement of relaxation in the bulk will be observed.

The relationship between the relaxation time of the water protons on molecules in the inner coordination sphere with those in the bulk solvent is given by

$$1/T_1 = P_M/(T_{1M} + t_m) \quad (2)$$

where T_1 is that part of the relaxation time of bulk water due to inner-sphere effects, T_{1M} is the spin–lattice relaxation time for water molecules bound in the inner coordination sphere, and P_M is the mole fraction of water molecules bound in the inner coordination sphere.[15] For the purposes of this evaluation, we assume that two axially coordinated water molecules undergo exchange. If chemical exchange occurs between water molecules in the inner coordination sphere and bulk water with a slow but significant rate on the NMR time scale, the resonances will be broadened in accordance with the following expression:[18]

$$1/T_{2M'} = 1/T_{2M} + 1/t_m \qquad 1/T_{2B'} = 1/T_{2B} + 1/t_b \quad (3)$$

Here $T_{2M'}$ and $T_{2B'}$ are the apparent transverse relaxation times of water in the inner coordination sphere and in bulk water; T_{2M} and T_{2B} are relaxation times of bound and bulk water in the absence of exchange; t_m and t_b are residence lifetimes of water in the inner coordination sphere and in the bulk water. Because of the low concentration of paramagnetic species in the samples in this study, no ^{17}O resonance signal due to bound water is observed. Consequently, t_m cannot be calculated directly from eq 3 but must be calculated by the following expression from the T_2 values obtained from the signal arising from ^{17}O bulk water

$$1/t_b = 1/T_{2B'} - 1/T_{2B} = P_M/t_m \quad (4)$$

Table II
Activation Parameters, Residence Lifetimes, and Relaxivities for Metalloporphyrins and Their Polymer Conjugates[a]

species	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, cal/mol·K	t_m, ns	relaxivity, s^{-1} mM^{-1}
MnT4NMPyP	7.95	3.22	21.4	10.6 ± 0.3
MnPP	5.03	−8.15	47.4	2.6 ± 0.3
MnPP–PGA	7.15	−2.62	104	6.1 ± 0.8
MnPP–PLL	na	na	na	7.1 ± 0.6
MnPP–PLP	na	na	na	9.1 ± 0.4

[a] Residence lifetimes are reported for 25 °C, and relaxivities at 37 ± 0.5 °C.

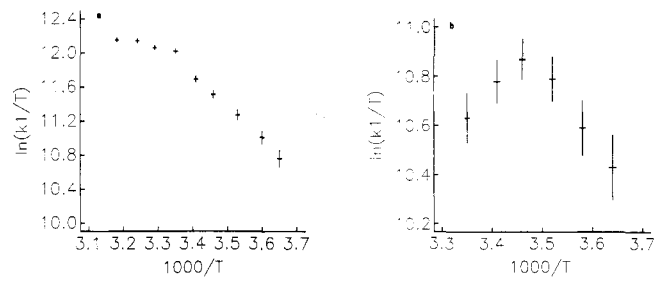

Figure 1. Plots of the natural log of the exchange rate as a function of temperature for metalloporphyrins: (a) Mn^{III}T4NMPyP; (b) Mn^{III}PP.

where $1/T_{2B}$ is determined from a reference solution, prepared identically to the sample but containing no paramagnetic species, and $1/T_{2B'}$ from the sample containing the paramagnetic species.

Because the chemical exchange is first order, the rate constant, k_1, implicit in eq 4 should follow the Eyring equation:

$$1/t_m = k_1 = (kT/h)\exp[-\Delta H^{\ddagger}/RT + \Delta S^{\ddagger}/R] \quad (5)$$

The residence lifetimes reported herein were calculated at 25 °C via this equation after the values of the activation parameters had been determined.

In a series of compounds, the agent exhibiting the greatest relaxation enhancement, or relaxivity, is defined as the agent having the largest slope in a linear plot of $1/T_1$ against concentration. The relaxivities for the species reported herein are given in Table II. It should be noted that an extension from the lifetimes measured at 90 MHz to the relaxivities obtained at 20 MHz is justified as the residence lifetime is independent of frequency.[19]

Plots of the natural log of the variation in exchange rate as a function of temperature for the nonconjugated porphyrins MnT4NMPyP and MnPP are shown in Figure 1. Activation parameters, summarized in Table II, were calculated with eq 5 from the region on these plots where rate constant increases linearly with increasing temperature.

Plots of the same parameters for the porphyrin–polymer conjugates are shown in Figure 2. For these systems, only the data from the MnPP–PGA adduct (Figure 2a) demonstrated a region of linear increase and allowed the calculation of activation parameters. Both MnPP–PLL and –PLP conjugates (parts b and c of Figure 2) showed no variation of the rate constant with respect to temperature. Consequently, it was impossible to assign any meaningful residence lifetimes for water molecules in the inner coordination sphere for either of these species. Because the paramagnetic species did not appreciably broaden the ^{17}O resonances in these two solutions, a large error in the value of the exchange rate in these samples results from the small difference between

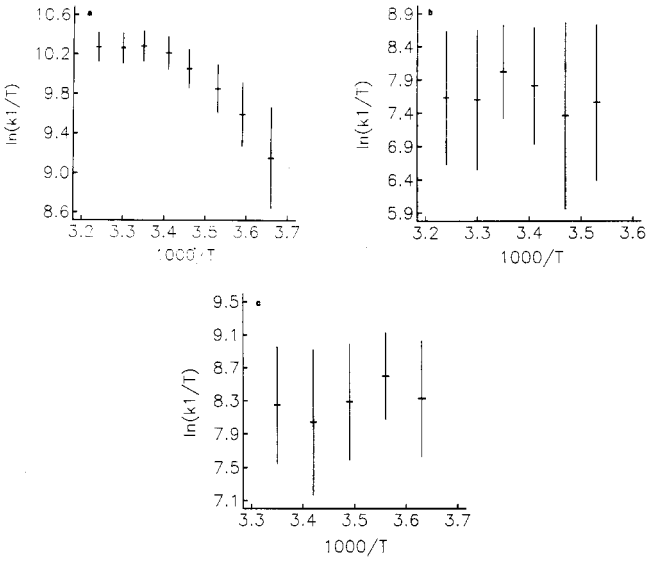

Figure 2. Plots of the natural log of the exchange rate as a function of temperature for polymer-bound $Mn^{III}PP$: (a) poly(L-glutamic acid) conjugate; (b) poly(L-lysine) conjugate, and (c) poly(L-lysine-*co*-L-phenylalanine) conjugate.

the ^{17}O line widths in the sample solution and the reference.

Regarding the porphyrins not attached to polymers, the relaxivity of MnT4NMPyP is about 4 times greater than that of MnPP, whereas the residence lifetime of water on MnT4NMPyP is 2 times shorter than on MnPP. Although it is possible, it would be premature to state that the residence lifetimes are totally responsible for the difference in relaxivities observed for the two species as defined in eq 2. Under conditions where $T_{1M} \ll t_m$, the differences in residence lifetimes would certainly account for the differences in relaxivities. However, when T_{1M} is on the order of or greater than t_m, contributions from both terms become important in determining differences in relaxivities after which T_{1M} becomes dominant. This implies that differences in other terms in eq 1 become increasingly significant because t_c is contained in the expression for T_{1M}.[19] Comparing the values of t_r and particularly t_s for MnT4NMPyP and MnPP is not straightforward. The description of the electron–nuclear dipolar interaction for MnPP may be complicated due to static zero-field splitting of the electronic spin levels because MnPP is asymmetric.[20] It is not possible to estimate at this point the magnitude of the effect of differences in T_{1M} values on the relaxivities for these two species.

For the polymer-bound manganese porphyrins, residence lifetimes completely fail to explain the relative relaxivities. The MnPP–PGA adduct exhibits greater relaxation enhancement than MnPP (Table II), which is contrary to the effect of residence lifetime on relaxivity as indicated by eq 2 for systems where $T_{1M} \ll t_m$. Furthermore, the adducts MnPP–PLP and MnPP–PLL have higher relaxivities than either MnPP or MnPP–PGA, although no meaningful residence lifetime for water in the inner coordination sphere could be established for either conjugate. The exchange rate of water molecules is either too fast or too slow on the NMR time scale to be measured.

Since we are unable to observe water bound in the inner coordination sphere of a metal ion, we rely on chemical exchange, and hence on the residence lifetime, to carry the relaxation information to the observable bulk water. Regardless of the dominant relaxation mechanism or whether the exchange is too rapid or too slow to be measured, the key interpretation drawn from these data is that the relaxation enhancement observed for MnPP–PLL and MnPP–PLP is not carried into the bulk from water bound in the inner spere via a detectable exchange process. Therefore, another phenomenon must be responsible for the transfer of the relaxation enhancement to the bulk water protons.

One reasonable mechanism to account for the transfer of enhancement in the case of porphyrin–polymer adducts is spin diffusion, in which protons near the paramagnetic ion relax rapidly and serve as a relaxation sink for the bulk water protons.[21,22] That cross relaxation occurs between water protons and the protons on diamagnetic polymers has been clearly established.[23–26] The effects of cross relaxation on T_1 values of paramagnetic proteins have also been analyzed in terms of its influence on the interpretation of structural data from experiments with shift reagents.[27–29] The comparison of residence lifetimes in the porphyrin–polymer adducts with relaxivities gives likely indication of the importance of cross relaxation in the enhancement of relaxation by paramagnetic species in systems containing polymeric solutes.

It has been noted in the literature that relaxation information may be transmitted to the bulk solvent by means other than the physical exchange of solvent molecules coordinated to the inner sphere of a metal ion with the molecules in the bulk. Outer-sphere effects also contribute to the observed relaxation of solvent protons.[30–31] Outer-sphere relaxation is best described by Pfeifer in equations that relate the electron–nuclear dipolar interaction of the paramagnetic ion with the water molecules outside the coordination sphere.[32] These equations include the effects of electron spin relaxation as the water molecules sample the paramagnetic center by means of translational diffusion. However, for this relaxation mechanism to be effective, the paramagnetic center must be available to the bulk water protons within a distance of a few angstroms.[30] If the apparent absence of water exchange in the lysine-containing polymers is due to an inaccessibility of the paramagnetic center to solvent, whether this is caused by direct coordination of ω-amine groups on the lysine side chains to axial sites on the manganese or by the physical sequestering of the porphyrin within the polymer, outer-sphere relaxation will not be effective.

This study has shown that the greatest relaxivity is associated with the conjugates in which water has no measurable exchange rate with the paramagnetic center. Even if outer-sphere relaxation occurs in this situation, it generally contributes very little to the overall relaxivity[14] and should not account for the variations noted here. These two facts point to the significance of cross relaxation in determining the ability of polymer-bound paramagnetic agents to enhance solvent relaxation. The possible role of cross relaxation in enhancing the T_1 of solvent protons illustrates the importance of polymer carriers of paramagnetic contrast agents as participants in the overall enhancement process observed in living tissues. Obviously, the interactions of small paramagnetic agents with biotic macromolecules will also influence the ability of those agents to enhance proton relaxation in tissues by the same mechanism.

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Registry No. MnT4NMPyP, 123962-95-8; MnPP, 123933-64-2; H_2O, 7732-18-5.

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(*p*-Methoxybenzyl)- and (α-Methylbenzyl)-*p*-cyanopyridinium Hexafluoroantimonates: Activated Latent Thermal Catalysts

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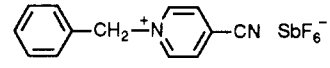
ABSTRACT: (*p*-Methoxybenzyl)- and (α-methylbenzyl)-*p*-cyanopyridinium hexafluoroantimonates (**2b**, **2c**) were synthesized by the reaction of *p*-cyanopyridine and corresponding benzyl halides followed by exchange of the counteranion with SbF_6^-. Both salts showed much higher catalytic activities than benzyl-*p*-cyanopyridinium hexafluoroantimonate (**2a**) in the cationic polymerization of glycidyl phenyl ether (1). The thermally latent effectiveness of these catalysts, in promoting efficient polymerization of 1 at 100-120 °C but not below 60 °C, was demonstrated.

Introduction

External stimulation-induced polymerization is of special interest in control of the initiation of polymerization. Photoinduced cationic polymerization for epoxy resins with triaryl sulfonium salts has been studied by Crivello and his co-workers.[1] It has been shown by Pappas et al. and Endo et al. that sulfonium salts can be excellent latent thermal catalysts in the curing and polymerization of epoxy resins,[2] spiro ortho carbonates,[3] bicyclo ortho esters,[4] and styrene.[5] We have extended the idea of latent catalysts to quaternary ammonium salts and recently found that quaternary ammonium salts such as benzyl-*p*-cyanopyridinium hexafluoroantimonate (**2a**) serve as thermally latent cationic catalysts in the polymerization of a cyclic ether [1-phenyl-4-ethyl-2,6,7-trioxabicyclo[2.2.2]octane][5] and a vinyl monomer (styrene)[6] as well. The merit of these salts is their chemical stability and ease of handling owing to their low hygroscopicity. Although **2a** is the most active catalyst among various



2a

quaternary ammonium salts, we have examined, so far, further activation of **2a** is required because of its rather high initiating temperature (>120 °C). Meanwhile, for the availability and the practical use of ammonium salts as latent catalysts, it is also important to synthesize a variety of quaternary ammonium salts with a variety of activities based on their molecular design.

For this purpose, we have devised some chemical modifications of **2a** by introducing electron-releasing substituents onto the benzene ring and benzylic carbon. In this

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