Synthesis and Characterization of Conjugates of Poly(α-Amino Acids) and Manganese (III) Protoporphyrin IX as Relaxation Enhancement Agents for MRI*

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Synopsis

Polymers are finding increasing use in medicine, and conjugates of paramagnetic species and polymers are now being studied as relaxation enhancement agents to improve contrast in magnetic resonance imaging. To assess the potential of such agents, the enhancement of proton relaxation by paramagnetic species bound to macromolecules has been observed for four conjugates of amino acid polymers and manganese (III) protoporphyrin IX. The conjugates all differ in their abilities to enhance the relaxation of water protons in solution; values of relaxation rates range from 3.2 to 9.1 s^{-1} mM^{-1}. The rotational correlation times and volumes of the kinetic units within the macromolecules have been determined by fluorescence depolarization in an effort to understand the range of relaxation rates observed for the conjugates. The metalloporphyrins bound to the polymers all have very similar dynamic characteristics.

Many polymers have been synthesized during the last three decades for potential use in medicine.[1-6] These substances may be categorized as (1) biomaterials, as in artificial bones and replacement tissues, (2) devices for the controlled release of an active substance, (3) drugs themselves that are active in polymeric form or upon degradation *in vivo,* or (4) carriers for active bound molecules. The porphyrin-polymer conjugates discussed herein fall into the fourth class, for to a first approximation the polymers are simply carriers and the paramagnetic metalloporphyrins the "active" agents. "Activity" in this context does not correspond to traditional drug activity but rather to the role of the paramagnetic centers as agents that enhance the relaxation of water molecules and hence improve contrast in images generated in magnetic resonance imaging.

The polymers in this case, however, are not simply uninvolved carriers, for they influence the ability of the paramagnetic species to enhance the relaxation of the solvent protons. This report describes the synthesis of a series of polymer–metalloporphyrin adducts, the determination of the size of the adducts and of the rotational characteristics of the paramagnetic entities, and the evaluation of their ability to enhance the relaxation of water protons.

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EXPERIMENTAL

Materials

All reagents were of the highest purity grade. Double distilled water was used for all solutions, and other solvents (Fisher Scientific Co.) were used as received. Two general methods were developed to prepare the conjugates of manganese (III) chloride protoporphyrin IX disodium salt (MnPP) (Porphyrin Products) with polymeric starting materials (Sigma Chemical Co.). Method I is illustrated for the homopolymer poly (L-lysine) and is applicable for the lysine-containing polymers: poly (L-lysine) (PLL: MW 17,000, 23,000 and 27,000), poly(L-lysine-co-L-alanine) (PLA: 1 : 1 random copolymer, MW 37,000), and poly(L-lysine-co-phenylalanine) (PLP: 1 : 1 random copolymer, MW 40,000 and 46,000); method II is for poly(L-glutamic acid) (PGA, homopolymer, MW 46,000).

Method I: Preparation of Lysine Containing Adducts (Scheme 1)

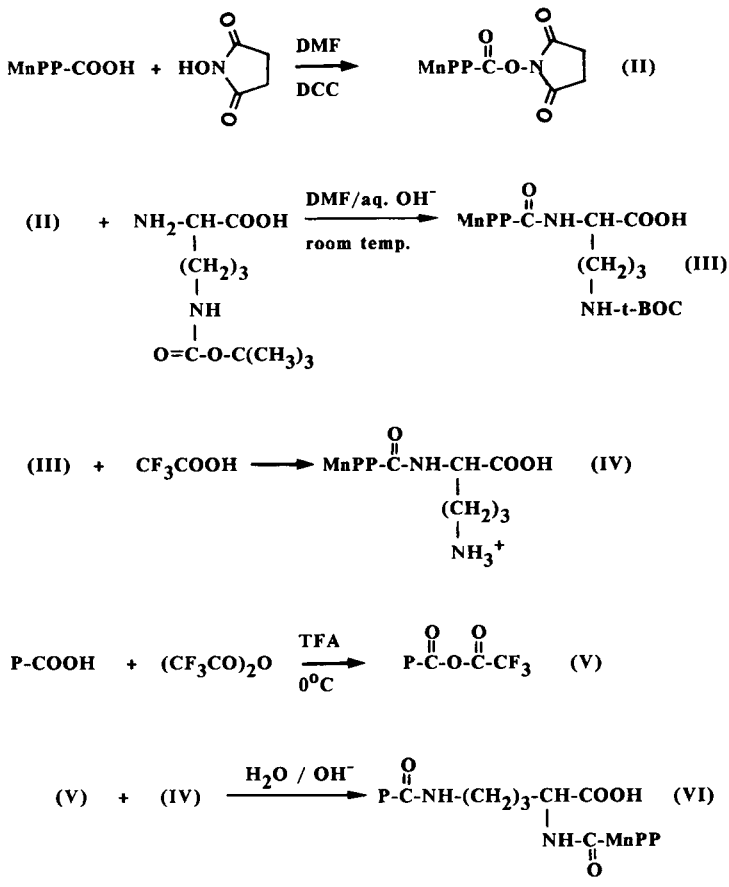
The metalloporphyrin (MnPP, 18 mg, 2.7×10^{-5} mol) was dissolved in *N,N*-dimethylformamide (3 mL) and the polymer (PLL, 17 mg, 1.2×10^{-7} mol) in a like amount of distilled water. The polymer solution was added in one portion to the metalloporphyrin solution and mixed well. The pH of the solution was adjusted to 5.5–6.0 by the addition of 0.01*N* aqueous sodium hydroxide. The DMF/water ratio was adjusted to approximately 1 : 1 (v/v) to prevent precipitation of the MnPP. Freshly weighed 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 18 mg, 9.4×10^{-5} mol) was added to the reaction mixture. The reaction was allowed to proceed at room temperature for 72 h with occasional stirring after which the mixture was centrifuged. The supernatant liquid was decanted from a dark, insoluble solid and concentrated in an Amicon Centricon-30 microconcentrator. The concentrate was washed two times with 1 : 1 (v/v) solution of DMF/water followed by several washes with water. The washed conjugate fractions were pooled and passed through a Sephadex LH-20-100 column equilibrated and eluted with 0.15 *M* sodium chloride solution. The polymer-metalloporphyrin conjugate (I in scheme 1) eluted as one dark band at the solvent front which was collected and stored at 4°C. Unbound metalloporphyrin was retained at the top of the column.

Method II: Preparation of PGA Adduct (Scheme 2)

The metalloporphyrin (16 mg, 2.4×10^{-5} mol) was dissolved in 2 mL DMF and the solution heated to reflux. N-hydroxysuccinimide (8 mg, 3.0×10^{-5} mol) and 1,3-dicyclohexylcarbodiimide (DCC, 6 mg, 2.5×10^{-5} mol) were dissolved in 1 mL DMF and added to the refluxing MnPP solution. Reflux was continued for 2 h, after which the solution was cooled to room temperature with constant stirring. Progress of the reaction was monitored by TLC on silica

$$\text{MnPP-COOH} + \text{P-NH}_2 \xrightarrow[\text{EDC}]{\text{DMF/H}_2\text{O}} \text{P-NH-}\overset{\overset{\text{O}}{\|}}{\text{C}}\text{-MnPP} \qquad \text{(I)}$$

Scheme 1.



Scheme 2.

gel using pyridine : water : chloroform (10 : 5 : 2) eluant. In this system, the starting porphyrin migrates with an R_f of 0.65 with much tailing; the R_f of *N*-hydroxysuccinimide is 0.77. The reaction is judged complete when a spot assigned to the carboxysuccinimidyl porphyrin (**II** in scheme 2) appears with an R_f of 0.61 with no tailing and the spot at 0.77 has disappeared. (It should also be noted that the only other spot appearing on the plate is a small impurity in the starting metalloporphyrin with an R_f of 0.92; this spot does not change position during the course of the reaction.)

N-ϵ-*t*-butoxycarbonyl-L-lysine (*N*-*t*-BOC-lysine, 6.6 mg, 2.6×10^{-5} mol) was dissolved in 0.5 mL of 0.01*N* sodium hydroxide solution and was added to the solution containing product (**II**) from above and stirred. The reaction was allowed to proceed for 4 h, after which the volume was reduced to dryness under reduced pressure. TLC during the progress of the reaction in the same pyridine : water : chloroform system shows the appearance of a peak assigned to product (**III**) at $R_f = 0.77$, the gradual disappearance of the *t*-BOC-lysine peak at $R_f = 0.84$, and the continued presence of the impurity noted previously.

Aqueous trifluoroacetic acid (TFA, 2 mL, 90% v/v) was added to the residue and stirred at room temperature for 1.5 h to cleave the *t*-BOC residue and

deprotect the lysyl amine (**IV**). The volume was again reduced to dryness and the residue dissolved in a small amount of 0.01 N NaOH.

PGA (8 mg, 3.3×10^{-7} mol) was dissolved in 1 mL aqueous TFA and chilled in an ice bath; 10 drops of TFA–anhydride were added. The solution was retained in the ice bath and stirred occasionally for 25 min, after which the fluid was removed under reduced pressure to yield an off-white solid. This modified polymer (**V**) was dissolved in 1 mL of 0.01*N* NaOH solution and added to the solution of modified metalloporphyrin (**IV**), which was then stirred for 16 h at room temperature. The solution was concentrated and the polymer-porphyrin adduct purified by the same chromatographic protocol described in Method I.

Determination of Loading Factor

The ratio of the number of metalloporphyrins to the number of lysyl or glutamate residues was determined by a combination of flame ionization atomic absorption, used to determine the amount of manganese in a sample, and exhaustive acid digestion of the conjugate followed by subsequent amino acid analysis to determine the amount of monomer in a sample of completely degraded polymer.

Atomic Absorption

Flame ionization atomic absorption was carried out on a Perkin-Elmer Model 373 using a manganese lamp at 279.5 nm and a mixture of air-acetylene as flame gases. A calibration curve was generated for standard solutions; no matrix effects were observed. The solution of polymer–metalloporphyrin conjugate (0.2 mL) was diluted with 0.15 *M* sodium chloride solution to a total volume of 2.0 mL. All readings were taken against a 0.15 *M* NaCl reference.

Amino Acid Analysis

Hydrochloric acid (0.5 mL, 6 N) was added to 0.1 mL of a solution of conjugate in a pyrex tube with a teflon lined screw cap. The sample tube was flushed with nitrogen gas, capped immediately, and retained at 100°C for 72 h. After cooling to room temperature, the liquid was removed under reduced pressure to yield a dry residue. A solution of ethanol/water (1 mL, 50 : 50 v/v) was added to the residue and shaken vigorously. The mixture was passed through a Millipore HV filter attached to a Plastipak syringe to yield a clear filtrate. Subsequent amino acid analysis was carried out on the filtrate according to literature procedure[7-8] using ninhydrin reagent. The absorbance of the Ruhemann's Purple formed was determined at a wavelength of 570 nm against a reference sample. Absorbance readings were taken within one hour of sample preparation and concentrations calculated by comparison to a standard curve prepared for each amino acid.

Estimation of Molecular Weights of Conjugates

Approximate molecular weights of the conjugates were determined both by gel filtration chromatography and ultrafiltration. A Spectrum liquid chromatography column (100 × 0.95 cm I.D.) equipped with a capillary system and a nylon filter for aqueous systems was packed to a bed height of 90 cm with Biogel

P-150 that had previously been swollen for 4 h in a hot 0.15 M sodium chloride solution. The column was operated at room temperature at a flow rate of 0.2–0.3 mL/min. Samples were eluted with 0.15 *M* NaCl solution. Aliquots of eluant were collected with an ISCO Cygnet fraction collector in a drop count mode and were monitored spectrophotometrically at a wavelength of 280 nm using a mercury lamp cassette with appropriate filters. Protein standards were used as references. The conjugates were also evaluated by ultrafiltration using membranes with molecular weight cutoffs of 30,000, 100,000, and 300,000.

NMR

Spin-lattice relaxation times (T_1) for water protons were determined for aqueous solutions of porphyrin–polymer adducts in 10 mm tubes on an IBM PC-20 Minispec multianalyzer operating at a field strength of 20 MHz at 40°C. Standard inversion–recovery pulse sequences were used to determine relaxation times. The efficiency of relaxation per μM of polymer and the relaxivities of the conjugates were calculated from graphs of relaxation rates in s^{-1} ($1/T_1$) versus the concentration of conjugate and of the paramagnetic species, respectively (Figs. 2 and 3).

Fluorescence Depolarization

Fluorescence data were obtained on an SLM Fluorimeter equipped with a xenon arc lamp. The monochromator in the exciting beam was set at 405 nm and a 10 nm bandwidth 600 nm filter was set in the emission beam. An SLM SPC-822 data processing module in the photon counting mode was used to determine the polarization values.

Because manganese porphyrins fluoresce very weakly, the polymer-metalloporphyrin conjugates were demetallated by treatment with concentrated sulfuric acid for 3–5 h at room temperature. The progress of the demetallation reaction was monitored by following changes in the Soret band regions of porphyrin absorption by UV-visible spectroscopy. No polymer degradation products were detected after this treatment. The viscosity of the sample solutions was varied by the addition of a glycerol solution; the viscosity of the resulting solution was calculated from literature values.[9-10]

The intensity of the emitted fluorescent radiation was measured at an angle of 90° from the direction of the exciting light after passing through a 600 nm filter. Measurements were made at 23°C as a function of the viscosity of the sample. Polarization data was analyzed by graphing degree of polarization [as $(1/P + 1/3)$] versus the ratio of absolute temperature to viscosity of the solution (as T/η) to yield standard Perrin plots [Fig. 1(a–d)], from which both rotational volume and the rotational correlation time of the kinetic unit can be determined.

RESULTS

All polymer conjugates underwent crosslinking during the synthetic procedures. The results of gel filtration chromatography indicated that all lysine containing polymers had molecular weights in excess of 150,000; the PGA-

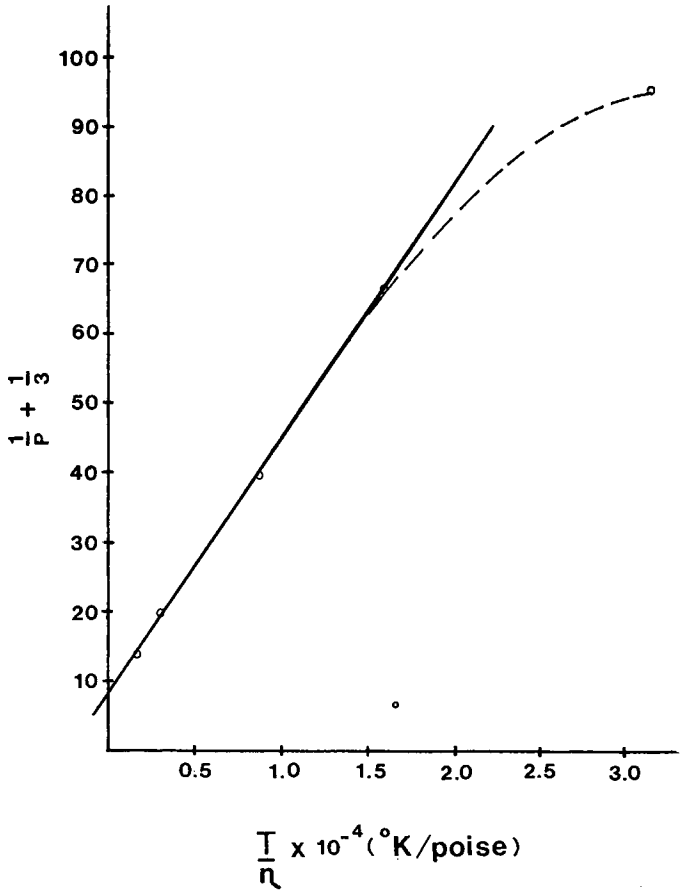


Fig. 1. Perrin plots of data from the fluorescence depolarization studies of the porphyrin–polymer adducts. For each solution, at least five alternate readings of fluorescence intensity at directions parallel and perpendicular to the exciting filter were made. This plot shows data for conjugate (a): MnPP–PLP.

adduct adsorbed to the resin and could not be determined by this method. Ultrafiltration experiments demonstrated that all conjugates had molecular weights above 300,000.

Table I summarizes typical loading factors characterizing the polymer-porphyrin adducts. Ratios are expressed as numbers of manganese porphyrins to lysyl or glutamate residues on the polymer. In the case of the random copolymers, the ratios reflect the number of porphyrins attached per potential reactive group on the polymer and not the strict porphyrin : amino acid monomer ratio.

Table I also contains the relaxivities, the slopes of the lines on graphs of relaxation rates vs. the concentration of paramagnetic species, which vary over a factor of three among the conjugates. Because the polymers have different numbers of metalloporphyrins bound to them, direct comparisons of relaxation rates among the adducts based on moles of polymer present cannot be made. Therefore, concentrations are expressed two ways: for the purpose of expressing the efficiency of relaxation per amount of polymer adduct present, as mM of

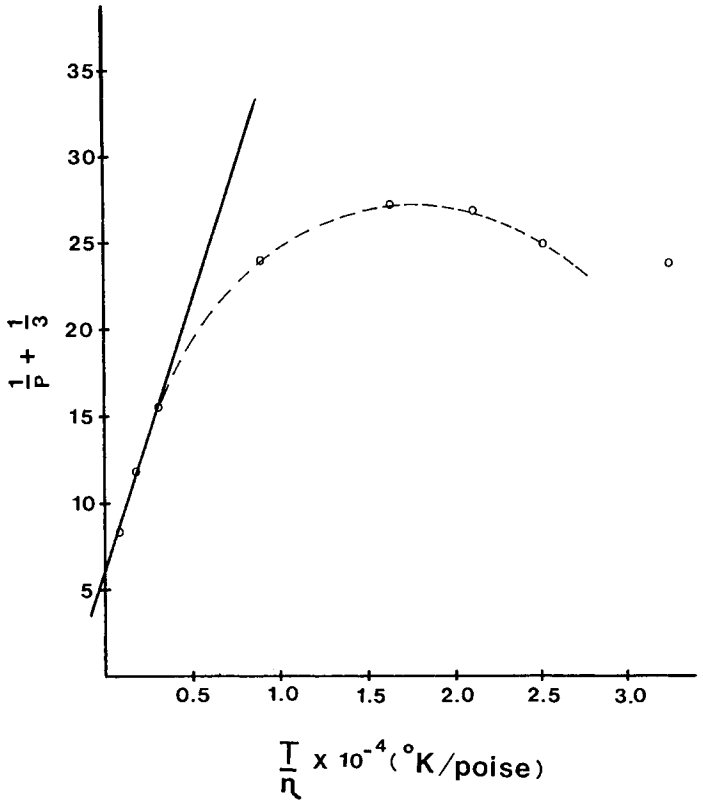


Fig. 1. (b) MnPP–PLP.

polymer (Fig. 2); for the purpose of relaxivity calculations, as mM of paramagnetic species present (Fig. 3).

The standard deviations reported for the relaxivities were obtained from best fit lines consisting of no fewer than three measurements at five different concentrations. For purposes of comparison, the relaxivity of the nonconjugated manganese (III) protoporphyrin complex was also determined at 20 MHz to be 2.63 s^{-1} mM^{-1}. Solutions of comparable quantities of the polymers (concentrations in the μM range) in water without paramagnetic agents present did not cause appreciable changes in the relaxation times of water protons.

The evaluation of the conjugates by fluorescence depolarization defines two related parameters, summarized on Table II. The rotational volume (V) and the rotational relaxation time (ρ) of the kinetic unit have been calculated from eqn 1[9]:

$$(1/P + 1/3) = (1/P_0 + 1/3)(1 + RTt_0/V\eta) \text{ and } RT/V\eta = 3/\rho \quad (1)$$

where P is the degree of polarization, P_0 the limiting degree of polarization, R the gas constant, t_0 the fluorescence lifetime of the fluorescent species, V the rotational volume of the kinetic unit, η the viscosity of the solution, and ρ the rotational relaxation time of the kinetic unit. If t_0 and V are constant, the

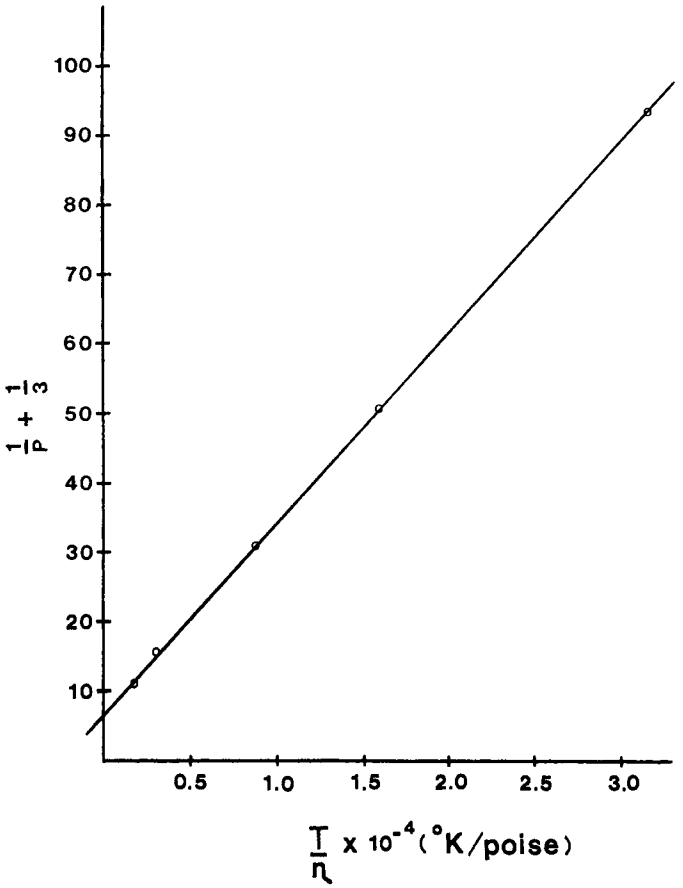


Fig. 1. (c) MnPP-PLA.

Perrin law of isotropic depolarization by Brownian motion predicts a linear relationship between $(1/P + 1/3)$ and T/η. The intercept of a graph of $(1/P + 1/3)$ vs. T/η is P_0, the limiting degree of polarization, and the slope is the value for V from which ρ can be calculated.

The value of V for the conjugates ranges from 181 to 239 cm^3/mol. By molecular modeling using data in the literature,[11] the volume of protoporphyrin IX was approximated to be 300 $Å^3$ or 180 cm^3/mol. The rotational correlation time of each kinetic unit within the conjugates is approximately 2×10^{-10} s, and for all practical purposes this does not vary among the adducts.

DISCUSSION

Synthesis

The high molecular weight of the conjugates indicates that crosslinking occurs among the chains during the formation of the adducts. The MnPP attached to the polymers is bifunctional, and the presence of two carboxylic acid side chains

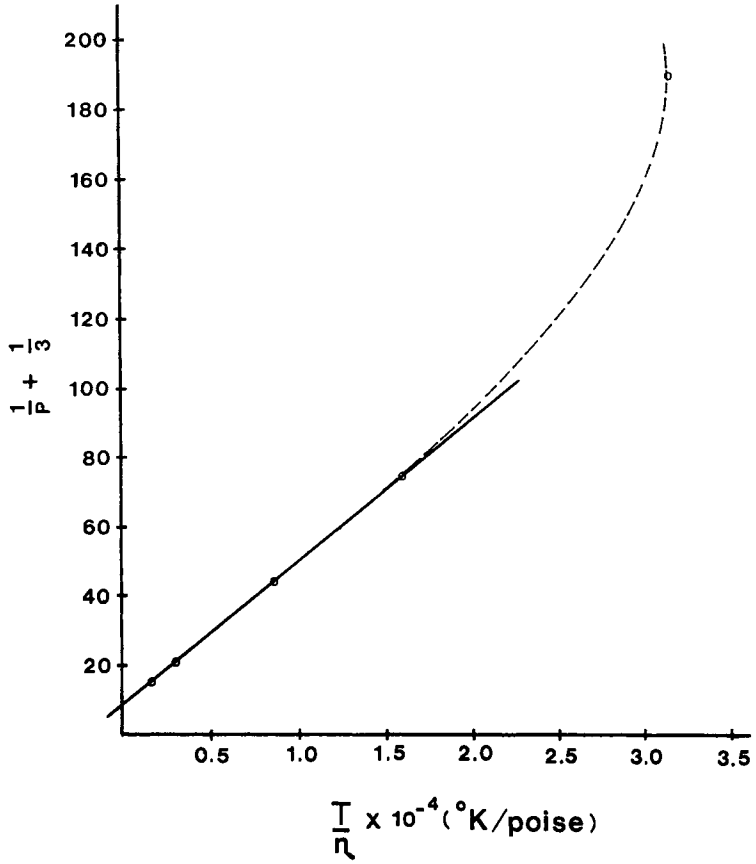


Fig. 1. (d) MnPP–PGA.

may enable it to crosslink polymer chains and cause the formation of high molecular weight adducts. Crosslinking can also occur by reaction of the terminal carboxylic acid group in one polymer with an amine in a second. When a struc-

TABLE I
Characterization of Porphyrin–Polymer Conjugates With Respect to Loading Factor and Relative Relaxation Enhancement of Water Protons Measured at 20 MHz

Polymer	MnPP : reactive monomer unit	Relaxivity (s^{-1} mM^{-1} MnPP)	Concn (mM)[a] (MnPP)	Concn (mM)[b] (polymer)
PLL	1 : 5–1 : 7	7.1 ± 0.6	0.38	0.052
PLP	1 : 5–1 : 6	9.1 ± 0.4	0.30	0.039
PLA	1 : 7–1 : 9	3.2 ± 0.3	0.96	0.075
PGA	1 : 2–1 : 3	6.1 ± 0.8	0.46	0.0053

[a] Concentration of paramagnetic species necessary to provide 10-fold enhancement of relaxation above that of pure water.

[b] Concentration of polymer conjugate necessary to provide a 10-fold enhancement of relaxation above that of pure water.

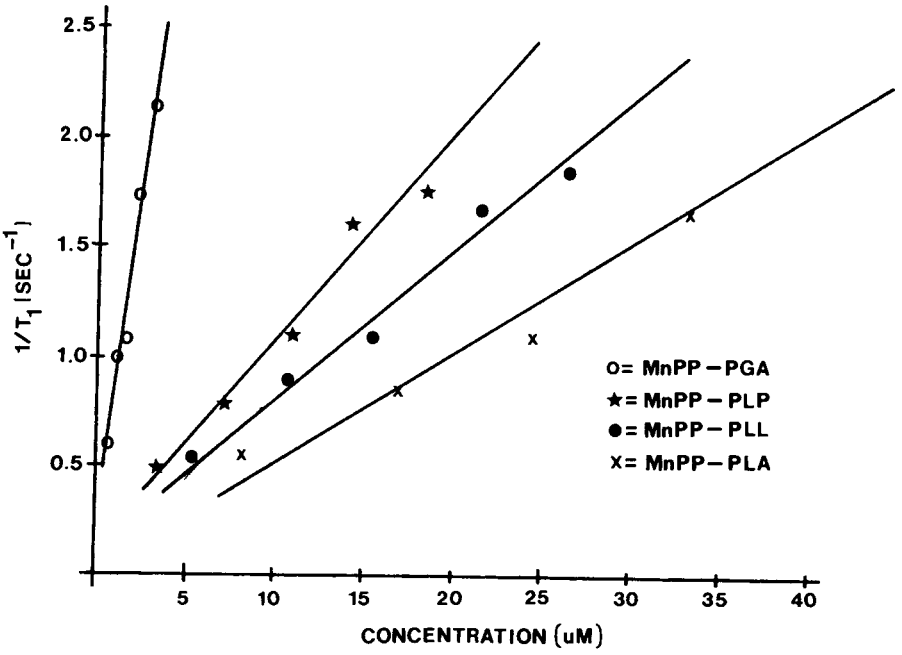


Fig. 2. Proton relaxation rates in aqueous solution as a function of concentration of polymer.

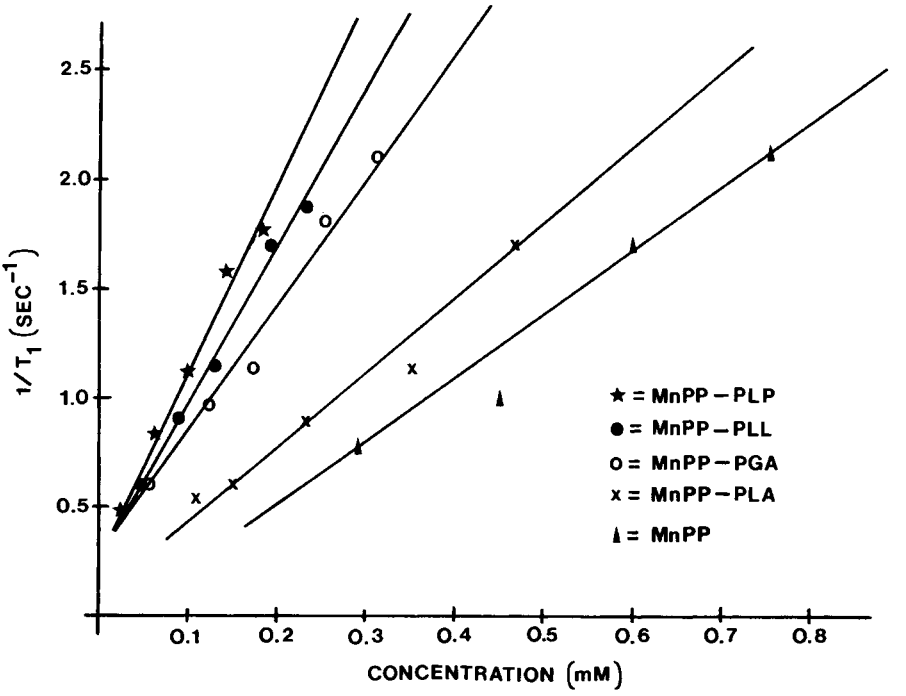


Fig. 3. Proton relaxation rates in aqueous solution as a function of concentration of metalloporphyrin.

TABLE II
Results of Fluorescence Polarization Studies to Define the Dynamics of the Porphyrin–Polymer Conjugates

Polymer	Dynamics		
	P_o	V^a ($cm^3\ mol^{-1}$)	ρ^b (s)
PLL	0.129	209	2.38×10^{-10}
PLP	0.176	181	2.00×10^{-10}
PLA	0.152	239	2.72×10^{-10}
PGA	0.124	192	2.19×10^{-10}

[a] Rotational volume of the kinetic unit.
[b] Rotational correlation time of the kinetic unit.

turally related monofunctional porphyrin was coupled to the polymer to avoid crosslinking, the resultant conjugate was insoluble in aqueous medium.

The loading factors are surprisingly consistent among the lysine containing polymers: between one porphyrin in every five lysyl residues to one in every nine. On the lysine homopolymer, the 1 : 5 ratio indicates that the binding of one porphyrin to a lysyl residue may inhibit the binding of another porphyrin nearby on the chain. The lack of variation of load for the random copolymers may point to the existence of short clusters of lysine monomers between like regions composed of the second monomer. The next reactive site after a porphyrin binds could still be five to nine lysines away, as opposed to being five to nine monomer units away.

The loading factor achieved on PGA is much higher than for any of the lysine containing polymers. A factor contributing to this occurrence is the length of the spacer group separating the porphyrin ring from the polymer backbone. In the three lysine systems, the coupling results in an eight atom spacer (five from the lysine chain itself, three from the porphyrin). After functionalizing the porphyrin and attaching it to the PGA, the tether is thirteen atoms long (three from the glutamate residue, seven from the lysine linker, and three from the porphyrin). The additional distance from the backbone to the metal complex decreases the influence of an attached porphyrin on an incoming one and a higher loading factor can be achieved.

NMR

All conjugates enhanced the relaxation of water protons in aqueous solutions. The supporting data for this statement may be analyzed several ways. First, plots of the concentration of porphyrin–polymer adducts vs. relaxation rates (Fig. 2) are linear. From these graphs, the concentration of conjugate necessary to cause a 10-fold enhancement in proton relaxation rate can be calculated and used as a measure of the effectiveness of the conjugates. Because the amount of paramagnetic agent present varies from polymer to polymer in solutions of equivalent conjugate concentration, it is not surprising that the conjugate with the highest loading factor (PGA) is the most efficient relaxation agent based

on the amount of polymer present. The efficiency of each member of the three lysine systems decreases with decreasing loading factor.

Secondly, the concentrations have also been normalized and expressed in terms of the molar amount of paramagnetic metalloporphyrin present in solution (Fig. 3). This is appropriate and necessary for the calculation of meaningful relaxivities. Relaxivities range from a low of approximately 3.2 s^{-1} mM^{-1} for the PLA adduct to almost threefold higher, 9.1 s^{-1} mM^{-1}, for MnPP–PLP. All adducts display higher relaxivities than the nonconjugated MnPP (2.6 s^{-1} mM^{-1}).

Well-established theories may be consulted to explain the variation among the adducts in terms of their ability to enhance solvent proton relaxation. The theoretical treatment of relaxation enhancement by paramagnetic species was developed over forty years ago for symmetrical aquo complexes, and contributions to relaxivity for aquo ions may be calculated from the Solomon–Bloembergen equations.[12–14] For a series of porphyrin–polymer adducts in which the same metalloporphyrin is present in all systems, many terms in this equation will be constant from conjugate to conjugate. The discussion of differential relaxation enhancement may therefore be reduced to examining differences in three parameters—t_c, the dipolar correlation time, p, the probability that a proton is in the hydration sphere of the paramagnetic ion, and N, the molar concentration of the paramagnetic species.

Of these three, the normally dominant parameter in the Solomon-Bloembergen equations for solutions of constant N is the dipolar correlation time t_c, defined in eqn (2) as

$$1/t_c = 1/t_r + 1/t_s + 1/t_m \tag{2}$$

where t_r is the rotational correlation time, t_s the electron-spin relaxation time, and t_m the residence lifetime of the water molecule in the inner coordination sphere of the paramagnetic ion. In principle, all of these times can contribute to the value of t_c and thereby influence enhancement; as t_c increases, relaxation rates increase. In practice, t_c will be determined by the term with the shortest time. This is usually t_r or t_s, or perhaps t_r and t_s together, if they are of the same relative magnitude.

For the small aquated manganous ion, t_c is dominated by t_r, which is much shorter than the other parameters (ca. 10^{-11} [15,16]). For manganese (III) porphyrins, t_s is much shorter than that for the Mn^{+2} aquo ion (10^{-11} vs. 10^{-8} [16–18]) and is of the same relative magnitude as t_r for the complex; both contribute to the value of t_c.[19] One would expect t_m, typically in the 10^{-8} s range for aquo ions and porphyrin complexes[20,21] to be slower than t_s for any Mn^{+3} species and hence not to contribute to the dipolar correlation time. Indeed, other work in our laboratory[20] has shown that the trend in the relaxivities for the porphyrin–polymer conjugates does not depend upon residence lifetimes of water in the coordination spheres. Although all conjugates enhance relaxation, a meaningful residence lifetime could be established only for MnPP–PGA; the metalloporphyrins in the PLL and PLA conjugates do not seem to be exchanging water. Therefore, the rotational correlation time for the conjugated porphyrins must be determined by a combination of t_s and t_r.

Because the same metal complex is present in all conjugates, t_s should be constant across the set. Small changes in t_s may be induced by variations in the coordination environment caused by interactions with side chains on the polymer which alter the anisotropy of the wave function of the metal ion, but these small changes cannot account for the three-fold difference in relaxation rate observed across the set of conjugates. Based on these considerations, t_r may initially be expected to be the chief parametric variable that determines the differences between the conjugates studied.

Fluorescence Depolarization and Dynamics

Regarding t_r, the Solomon–Bloembergen equations assume that a paramagnetic species bound to a macromolecule rotates isotropically and that only one rotational correlation time need be considered, the overall tumbling time of the macromolecule. Rotational times for polymers in the 300,000 molecular weight range are slow; values of 10^{-7} or longer are typical.[9] For porphyrins covalently attached to polymers, however, other possibilities for internal rotation exist that could contribute to t_r.[17] If motions of segments of the macromolecule and, in this case, of the bound complex and its tether occur, they will most likely be considerably faster than the t_r of the macromolecule. The values of the rotational correlation times of the kinetic units in the conjugates (ca. 2×10^{-10} s) are in the range of expected values for small molecules. The t_r values determined in these fluorescence depolarization experiments reflect local rotation of the metalloporphyrin and part of its tether, intrachain rotation, or both, and are clearly of a magnitude that could contribute to t_c.

Examination of the rotational volumes (V) of the kinetic units determined from Perrin plots enables a more exact description of the moiety characterized by the t_r value measured. The volumes determined for the conjugates range from 181 to 239 $cm^3\ mol^{-1}$. These are only slightly larger than the volume calculated for MnPP alone; hence, the kinetic unit must include the porphyrin and a portion of the tether attaching it to the polymer backbone. The kinetic unit observed in the PLP adduct must be just the porphyrin itself; it must contain the porphyrin plus a small increment of the tether in PGA, slightly more tether in PLL, and the greatest participation of the chain in the kinetic unit is in PLA.

Only the MnPP–PLA conjugate resulted in a Perrin plot that was linear over the entire range of viscosities evaluated [Fig. 1(c)]. This linearity indicates that both the kinetic unit and the rotational relaxation time remain the same throughout the entire range of viscosities studied.[9,22] The concave curvature toward the T/η axis that is slight in the region of low viscosity for MnPP–PLL [Fig. 1(a)] and considerable over a larger range of viscosities for MnPP–PLP [Fig. 1(b)] could be caused by the presence of free porphyrin in the solution,[22] but chromatographic evidence indicates no porphyrin has been cleaved from the polymer. Concave curvature also is known to occur when more than one rotational relaxation time is present and is observable when the times differ by more than fivefold.[22] The initial slope at small T/η values describes the shorter relaxation time of the porphyrin rotational unit itself; the curvature reflects the mobility of a larger kinetic unit within the polymer chain, in this case having a relaxation time of 1×10^{-9} or longer.

The fluorescence depolarization data for MnPP–PGA [Fig. 1(d)] demonstrated linear behavior for high viscosities and convex curvature at low viscosities. Generally in studies of fluorescence depolarization as a function of temperature, such curvature is associated with an increased amplitude of internal rotations with increasing temperature[9,22] resulting from a "loosening" of the tertiary structure into smaller subunits capable of faster rotations. The same interpretation should apply whether the increasing T/η ratio is achieved by increasing the temperature or decreasing the viscosity of the medium. Since the kinetic volume of the rotational unit for the porphyrin in MnPP–PGA is already close to that of the free porphyrin itself, the more rapidly rotating unit must be an essentially free porphyrin unhindered by any component of the tether. This faster rotation may occur in this conjugate due to the longer 13-atom spacer capable of more rotational freedom in comparison to the other conjugates with only 8-atom spacers.

CONCLUSIONS

Metalloporphyrins can be coupled to polymers to yield conjugates with reproducible loading factors. In evaluating the potential of these adducts to enhance the relaxation of water protons, all conjugates proved better enhancement agents than the unconjugated porphyrin.

The degree of enhancement provided by the polymeric agents is a function of the loading factor, but when concentrations are normalized to express concentration of paramagnetic species as opposed to concentration of polymer, differences in ability to enhance proton relaxation are still apparent. Classical considerations of the rotational correlation time as an influential factor in the determination of relaxation enhancement by paramagnetic species have shown that all the porphyrins have very similar dynamic characteristics after attachment to the polymer. The evaluation of residence lifetimes of water molecules in the coordination sphere of the metal in another study recently reported[25] also demonstrates that water exchange or the lack thereof with the paramagnetic site is not a factor in determining enhancement. We are continuing evaluations of these conjugates to establish the origins of their differential abilities to enhance solvent proton relaxation.

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