Molecular Dynamics of Ion−Chelate Complexes Attached to Dendrimers

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Abstract: We studied the molecular dynamics of vanadyl−chelate complexes covalently attached to the surface of cascade polymers, dendrimers. The rotational correlation times of the ion−chelate complex were determined from computer simulations of their EPR spectra. The chelate 2-(4-isothiocyanatobenzyl)-6-methyldiethylenetriaminepentaacetic acid was covalently attached to ammonia core poly(amidoamine) (PAMAM) cascade polymers via a thiourea (TU) linkage, resulting in PAMAM−TU−DTPA cascade polymers. X-band EPR spectra of their vanadyl complexes were taken, and the **A** and **g** matrices were determined from the rigid limit spectra using the SIMPOW program. Spectra were fitted with modification of the slow-motional line-shape theory. Our results indicate that the rotational correlation times of the surface chelate increase with molecular weight and resemble those of "internal" segmental motions found in PAMAMs. For this macromolecular system, the rotational correlation times alone cannot account for differences in the relaxivity between high and moderate molecular weight species. These data are consistent with the hypothesis that the differences between linear-based and cascade polymer-based MRI contrast agents in the response of their relaxivity to molecular weight partially result from differing responses of their rotational correlation time to increases in molecular weight. A comparison of isotropic and anisotropic tumbling models indicates anisotropic tumbling of the ion−chelate complex at physiological temperatures, which is consistent with a model that incorporates segmental motions of the dendrimer side chains.

Introduction

Macromolecular MRI contrast agents offer the potential for measuring changes in regional cerebral blood volume associated with brain function or tumor grade,[1] in the blood pool of an organ,[2,3] and for targeting various diseases and/or organs.[4−7] Soluble macromolecular contrast agents consist of an ion−chelate complex attached, covalently or noncovalently, to proteins,[8] linear polymers,[9−13] linear copolymers,[14] or cascade polymers.[15−19] The attachment of an ion−chelate complex to the macromolecule results in an increase in the ion relaxivity,[8,18] called the proton relaxation enhancement (PRE). This increase in relaxivity is consistent with the Solomon−Bloembergen−Morgan theory.[20−22] This theory predicts that increasing the

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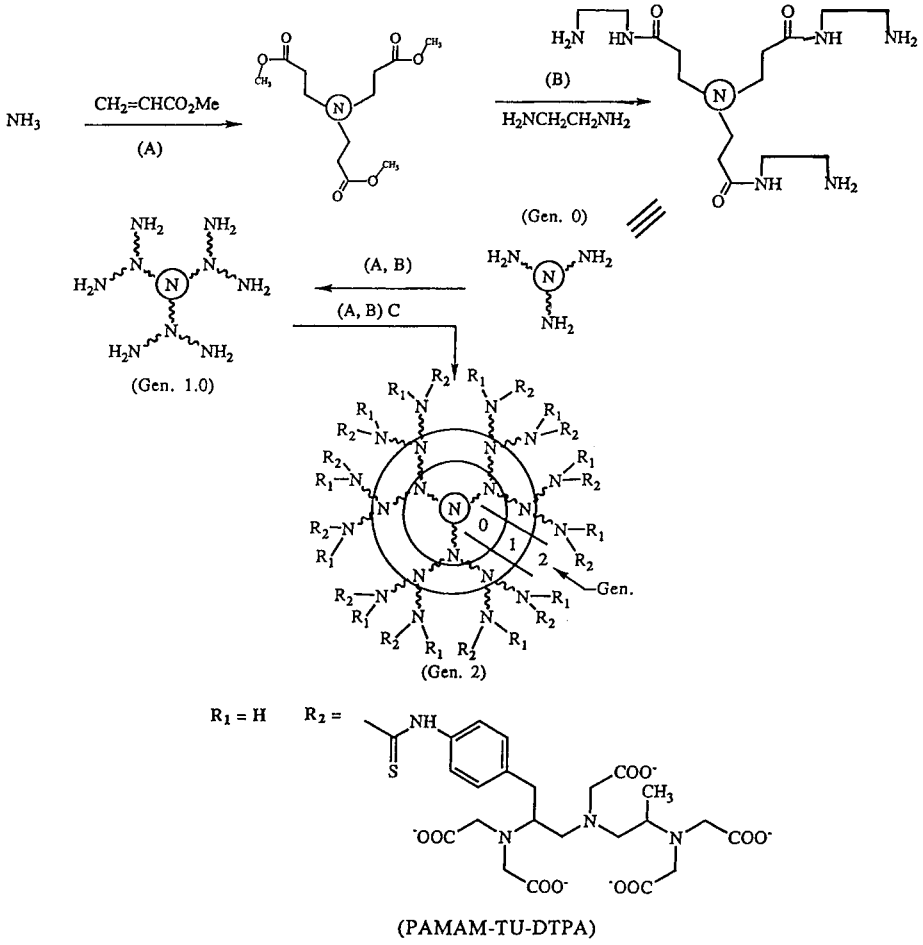
Scheme 1. General Synthetic Scheme for Ammonia Core Poly(amidoamine) Dendrimers and Their Derivatization With 2-(4-Isothiocyanatobenzyl)-6-methyldiethylenetriaminepentaacetic Acid



rotational correlation time of a paramagnetic ion with a relatively long electronic relaxation time will increase the ion relaxivity. Proton relaxation enhancements result from the fact that the molecular dynamics of such paramagnetic ions dominate the dipole−dipole interactions between the unpaired electrons for these ions and the protons of the water molecules in the inner coordination sphere of the ions.[23−26] This implies that changes in the rotational correlation time strongly influence the efficacy or relaxivity of MRI contrast agents composed of such ions complexed to low molecular weight metal chelates.

We recently developed a new class of macromolecular MRI contrast agents based on the ammonia core poly(amidoamine) (PAMAM) cascade polymers known as dendrimers.[15−18] Cascade polymers differ from linear polymers in many ways. Cascade polymers have much lower polydispersities.[27] They have higher numbers of reactive functional groups per unit mass and volume.[27] The rotational correlation times of their backbone carbons increase with increasing molecular weight.[28] Meltzer et al.[28] reported two classes of carbon atoms based on the responses of their rotational correlation times to changes in molecular weight. They define these as "internal" and "terminal" carbons. The rotational correlation times of the internal carbons increased by more than 30 times, while those of the terminal carbons merely doubled, when the molecular weight increased from 360 to 87 300. These changes in rotational correlation times associated with increasing molecular weight differ from the results obtained with linear polymers,[29−32] and in theory may partially account for the dependence of the relaxivity on molecular weight that we observed for cascade polymer-based MRI contrast agents.[15−18]

Although increases in the rotational correlation times of the cascade polymer backbone carbons may explain the concomitant increase in relaxivity associated with increasing molecular weight, it remains unknown whether the rotational correlation times of the chelates attached to the dendrimer surface of these cascade polymer-based contrast agents also change with molecular weight, and if they do whether they resemble those of the internal or terminal carbons. We therefore studied the molecular dynamics of ion−chelate complexes attached to the surface of PAMAM−TU−DTPA contrast agents (see synthetic Scheme 1). We investigated whether the rotational correlation times of the complexes increased with increasing molecular weight and if they resembled those of the internal or terminal

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Table 1. **A** and **g** Factor Values for 2-(*p*-Nitrobenzyl)-6-methyldiethylenetriaminepentaacetic Acid, and PAMAM−TU−DTPA, $G = 2$ and $G = 6$, Complexed with Vanadyl

	A_{xx} (G)	A_{yy} (G)	A_{zz} (G)	g_{xx}	g_{yy}	g_{zz}
free chelate	−66.58	−61.41	−181.70	1.98	1.98	1.95
generation 2	−68.03	−62.22	−183.42	1.98	1.98	1.95
generation 6	−66.89	−61.00	−181.02	1.98	1.98	1.95

carbons. Our results indicate that the rotational correlation times of the surface chelate increase with molecular weight and that they resemble those of the internal carbons. We also present evidence for anisotropic motion of the chelates. These data are consistent with the hypothesis that the differences between linear polymer-based and cascade polymer-based MRI contrast agents in the response of their relaxivity to molecular weight partially results from differing responses of their rotational correlation times to increases in molecular weight. The particular linker attaching the chelate to the dendrimer also plays a crucial role in taking advantage of these differences.

Materials and Methods

Chemicals obtained from Sigma Chemical Co. (St. Louis, MO) included HEPES,[59] NaCl, TRIS, EDTA, NaAc, and boric acid. Ultrafiltration membranes XM50 and YM3 were obtained from AMICON (Danvers, MA). Transistor grade 70% nitric acid was purchased from Mallinckrodt (Paris, KY). We obtained $VOSO_4$ from Fisher Scientific.

Ammonia core poly(amidoamine) dendrimers were prepared at the Michigan Molecular Institute following the method described by Tomalia et al.[33,34] The chelate agent 2-(4-isothiocyanatobenzyl)-6-methyldiethylenetriaminepentaacetic acid was synthesized at the NIH by the methods of Brechbiel et al.[35] Conjugation of the isothiocyanate to the terminal amines of the dendrimer was carried out at the University of Illinois following the method described by Wiener et al.[18]

Vanadyl Complexation. Typically, vanadyl derivatives of the PAMAM−TU−DTPA dendrimers were prepared such that the chelate to ion ratio exceeded 6:1. A 200 ± 30 mM stock solution of $VOSO_4$, pH 2, was mixed with approximately 100 times its volume of a dendrimer solution containing a DTPA concentration, covalently bound to the dendrimer, of 13−26 mM in 50 mM HEPES, pH 6.46. The stock vanadyl solution was prepared the same day and stored under an argon atmosphere. The pH of the VO−dendrimer solution was adjusted to 5.7, the solution was vortexed, and the complexation reaction was allowed to continue for 1 h prior to adjusting the pH to 7.2. The sample was stored under argon, and used the same day. Although we routinely used fresh samples, we found that careful storage and handling of the samples, under argon and with refrigeration, resulted in reproducible spectra even after a month had passed.

In order to avoid spin−spin interactions, the VO concentration was maintained between 1.7 and 2.3 mM and the [DTPA]:[VO] ratio was kept greater than 6:1. This resulted in less than 0.15 VO group/nm^2 on the dendrimer surface. Spin−spin interactions between labels on separate dendrimers were tested for by using serial dilutions (2 by 1:2), and no interactions were detected. In addition, vanadyl solutions of up to 6 mM do not give Heisenberg spin−spin interactions. This concentration is far above our experimental concentration.

The total [V] was determined by inductively coupled argon plasma spectrophotometry using a Perkin-Elmer Model P2000. Samples were diluted 10-fold before analysis with a 1.75% nitric acid solution made from transistor grade 70% nitrice acid.

EPR Spectra. The sample, 300 μL, was transferred to a 1 × 6 cm quartz flatcell aligned parallel to the $\mathbf{B}_1$ field. Both 9 and 12 in. Varian magnets with a Varian 101 bridge were outfitted with modified consoles containing PC interfaces for data acquisition and field control at the X band.[36] Microwave power was attenuated to between 0.2 and 100 mW depending on the dissipative adsorption caused by the temperature-dependent dielectric constant of the sample as the temperature was varied from −140 to +50 °C.

Temperature control was achieved by gaseous nitrogen, precooled by liquid nitrogen, flowing through a modified temperature control unit.[37] The cavity was insulated on all sides with 1 in. styrofoam and wrapped in plastic to prevent water condensation. A glass chimney wrapped in a paper towel also averted condensation at low temperatures and high flow rates. The control temperature was calibrated at various positions, on and in the flatcell, for several nitrogen flow rates. Temperature variation between the top of the cell and the bottom of the cell was found to be small and negligible within the temperature range studied. The magnitude of the variation showed a temperature

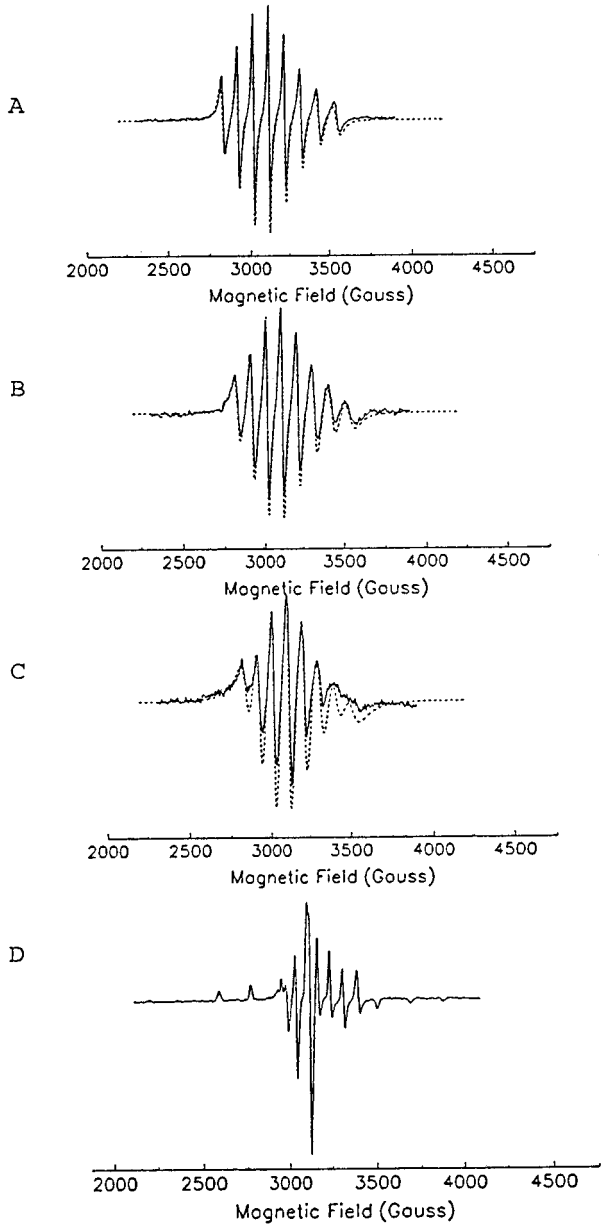

Figure 1. EPR spectra and their simulations with an isotropic tumbling model for 2-(*p*-nitrobenzyl)-6-methyldiethylenetriaminepentaacetic acid complexed with VO(II). Spectra were obtained at 35 (A), 20 (B), 5 (C), and −130 °C (D). The solid lines are the raw data, and the dashed lines are the computer simulations.

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dependence. The maximum variation was 2 °C at 37 °C, and the minimum variation was 0 °C at room temperature. All temperatures were corrected by the appropriate calibration factors.

A 100 kHz Zeeman modulation was employed with amplitude well below the line width of the sharpest spectral features. Typical values ranged from 0.8 to 5.0 G. Cumulative scan times of between 8 min and 1.5 h were used to obtain spectra with signal-to-noise ratios of at least 10:1. All spectra were taken as described above unless otherwise noted.

Computation. The best values for the **A** and **g** matrices were determined from the rigid-limit spectra with SIMPOW.[38] Both the isotropic and the anisotropic rotational correlation times were determined utilizing the nonlinear least squares fitting program FIT[39] based on the EPRLF engines developed by Schneider et al.[40] and later by Budil et al.[41] For the isotropic simulations, only the rotational diffusion constant was varied for each spectrum. However, the spectra simulated with the isotropic model did not always fit the experimental intensities well, indicating that the automatic simulations were not reliable. Therefore, we checked the results of the fitting procedure visually and refitted manually when necessary. For the anisotropic simulations, two diffusion constants were varied simultaneously: one for the overall rotation of the dendrimer ($\tau_\perp$) and one for the rotation about the spacer arm that connects the chelate to the dendrimer core ($\tau_{||}$). Because the principal *g* axis and the principal diffusion axis may not be coincident, a geometric factor, the diffusion tilt angle, which is the angle between the diffusion frame and the magnetic frame (Figure 6), was introduced into the simulation. We performed simulations at all tilt angles from 1° to 90°, and the results reported here are for the diffusion tilt angles that gave the best average fit to all of the spectra for a given dendrimer. A basis set pruning procedure with an error tolerance of less than 1×10^{-4}, following Vasavada et al.,[42] was carried out for each complex to determine the smallest basis set needed. All the computations were performed on an IBM RS/6000 320H. The powder patterns of all the dendrimers converged by −130 °C, indicating that all VO^{2+} environments are spectroscopically identical in the dendrimers at low temperatures.

Results

We previously modified the surface of ammonia core poly(amidoamine) dendrimers by covalently coupling 2-(4-isothiocyanatobenzyl)-6-methyldiethylenetriaminepentaacetic acid (reaction C in synthetic Scheme 1) to generation 2 and 6 PAMAMs. This resulted in generation 2 and generation 6 PAMAM−TU−DTPA derivatives with 11 and 170 surface chelates and molecular weights of 8508 and 139 000, respectively.[18]

Meltzer et al.[28] reported that PAMAM dendrimers possessed two types of segmental motions with vastly differing rotational correlation times. They called these motions internal and terminal. Since the rotational correlation time plays such an important role in determining the relaxivity of MRI contrast agents, we set out to determine the values of the rotational correlation times of the ion−chelate complexes attached to the cascade polymer-based MRI contrast agents that we developed,[18] and to identify the types of segmental motions that determine them.

We modified the technique developed by Bruno et al.[43] to study the molecular dynamics of metal chelates. The anisotropy of the **g** matrix of vanadyl makes this ion quite useful for the study of molecular dynamics with EPR. We complexed VO^{2+} with a derivative of the free chelate and with the surface chelates of the PAMAM−TU−DTPA, $G = 2$ and 6, dendrimers. Table 1 shows that all powder pattern parameters were well-converged by −130 °C, indicating that the vanadyl environments are spectroscopically identical in all dendrimers, at least at low temperatures. Since the rotational correlation times reported by Meltzer et al. are average times over all degrees of freedom, we started our comparisons with an isotropic tumbling model. In Figure 1 we present the spectra of VO^{2+} complexed to the free chelate and the corresponding simulations at −130, 5, 20, and 35 °C. These spectra cover the entire range of motions from rapid to slow tumbling and finally the rigid limit. The simulations of these spectra were quite good, with R^2 greater than 0.98. Simulations of the dynamic EPR spectra with an isotropic tumbling model for the generation 2 PAMAM−TU−DTPA (Figure 2) and generation 6 PAMAM−TU−DTPA (Figure 3) dendrimer derivatives complexed with vanadyl are also presented. These simulations with an isotropic model for

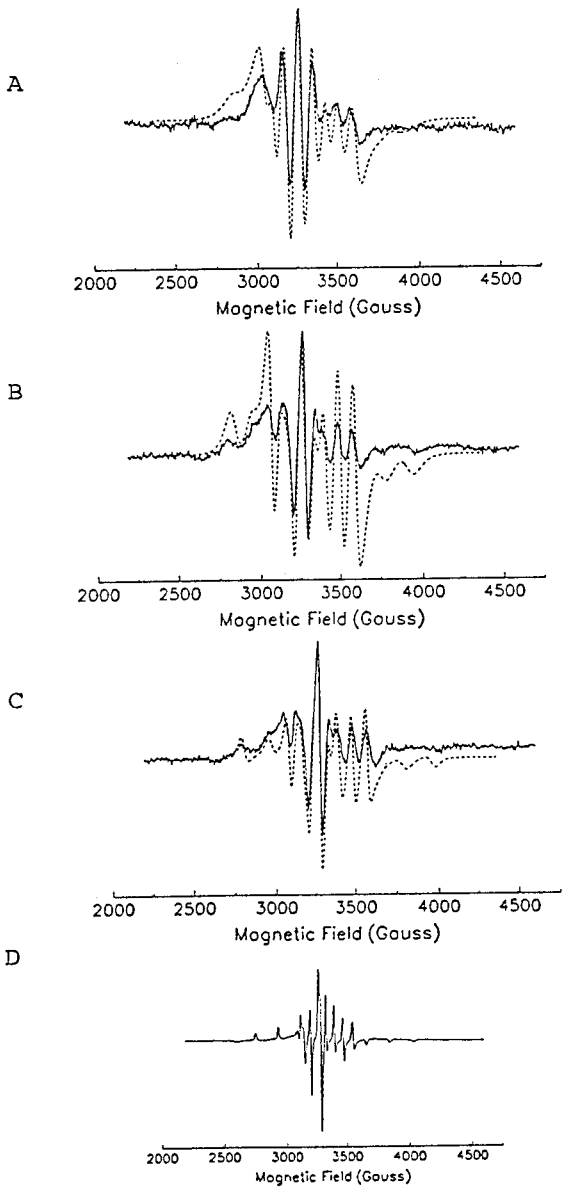

Figure 2. EPR spectra and their simulations with an isotropic tumbling model for PAMAM−TU−DTPA−VO (II), $G = 2$, complexes. Spectra, solid lines, were acquired at 35 (A), 20 (B), 15 (C), and −130 °C (D). Simulations, dashed lines, were obtained as described in the text.

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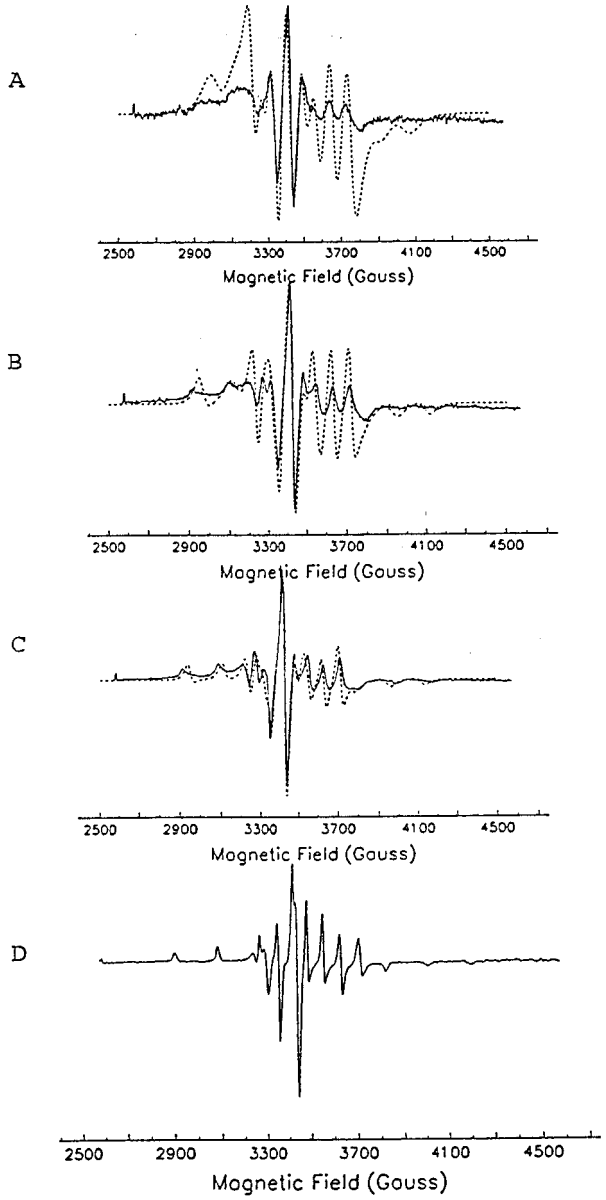

Figure 3. EPR spectra and their simulations with an isotropic tumbling model for PAMAM−TU−DTPA−VO(II), $G = 6$, complexes. Spectra, solid lines, were acquired at 35 (A), 20 (B), 5 (C), and −130 °C (D). Simulations, dashed lines, were obtained as described in the text.

the dendrimers could not fully reproduce all the features exhibited in the spectra. However, the most important factor in measuring the rotational correlation time is the goodness of fit of the principal line.[44,45] The simulations for the isotropic tumbling model depicted in Figures 2 and 3 fit the principal line quite well, and the rotational correlation times derived with the isotropic model are within 1.25 and 2.5 times those determined with the anisotropic model (see below). These spectra exhibit characteristic changes associated with slowly tumbling systems as the temperature decreases and the system approaches the rigid limit. Notice that the central lines start to dominate the spectra as the temperature decreases. Over the temperature range depicted in Figure 3, the molecules have motions in the region of slow tumbling for VO^{2+} complexes, $10^{-10} < \tau < 10^{-7}$ s.

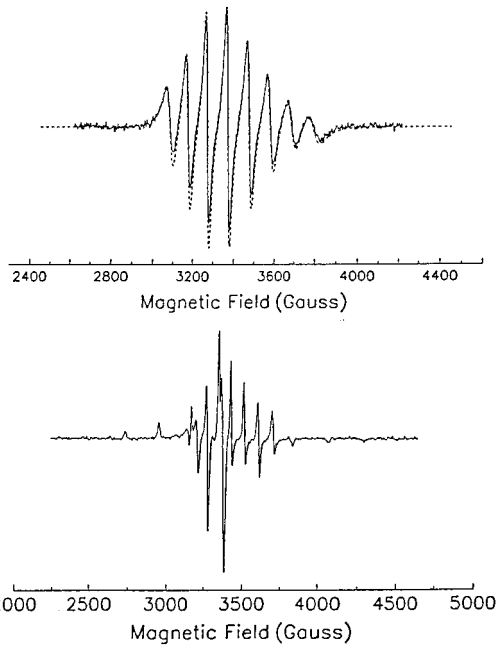

Figure 4. Vanadyl ion complexes with the DTPA derivative and not with hypothetical interior binding sites on the PAMAM−TU−DTPA derivatives. The 2-(*p*-nitrobenzyl)-6-methyldiethylenetriaminepentaacetic acid chelate was mixed with the *N,N*-dipropionato-PAMAM, $G = 6.5$, and vanadyl using the same chelate to ion ratio and chelate to dendrimer ratio described in the Materials and Methods, top. The spectra were taken at 20.6 °C. Note that in this experiment the chelate is not covalently bound to the dendrimer.

In order to determine whether the VO^{2+} is complexed to the surface chelate or to some hypothetical internal binding site, we combined the corresponding half-generation *N,N*-dipropionato-PAMAM with a derivative of the free chelate and added vanadyl at the same molar ratios as those used for the dendrimer system with covalently attached chelates, i.e., under the same experimental conditions as described in the Materials and Methods. The difference was that the chelate was not covalently attached. The EPR spectrum resembles that of the free metal−chelate complex in the absence of the dendrimer and not that of the dendrimer complexed with VO(II) (Figure 4). The rotational correlation times derived from the spectra of the free metal−chelate complex in the presence and absence of the dendrimer are identical, 1.38×10^{-10} s at 25 °C.

We obtained the rotational correlation times by fitting the spectra to the slow-motional line shape theory[43,44,46] based on the stochastic Liouville equation that includes nonsecular terms in the spin Hamiltonian, as given by Meirovitch et al.[47] The simulation is applicable for any $S = 1/2$ complexes, though the inclusion of nonsecular terms was necessary for vanadyl ion spectra because of the large hyperfine interaction in this ion relative to the electronic Zeeman interaction at the X band.[39] The isotropic rotational correlation times of the ion−chelate complexes for the free chelate and the $G = 2$ and $G = 6$ PAMAM−TU−DTPA derivatives increase as the temperature decreases, and they increase with increasing molecular weight when compared at the same temperature. At 20 °C the rotational correlation times of the free chelate and the $G = 2$ and $G = 6$ PAMAM−TU−DTPA derivatives are 1.5×10^{-10}, 9.3×10^{-10}, and 2.5×10^{-9} s, respectively (Table 2). In addition, over the temperature range studied, the ranges of correlation times for

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Table 2. Isotropic Rotational Correlation Times (ns) at 20, 25, and 35 °C

	20 °C	25 °C	35 °C
free chelate	0.15	0.14	0.12
$G = 2$ PAMAM−TU−DTPA	0.93	0.64	0.49
$G = 6$ PAMAM−TU−DTPA	2.5	2.1	0.64

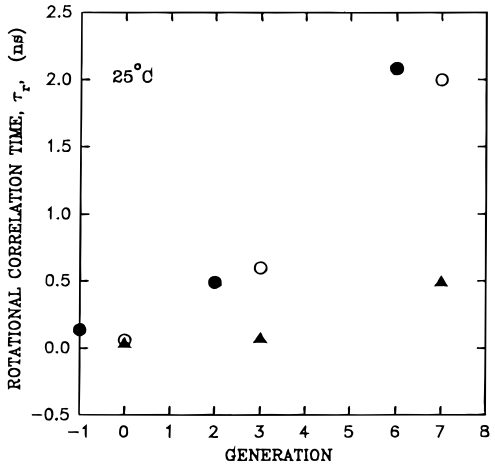

Figure 5. The rotational correlation time of the surface chelate corresponds to the internal segmental motions of amine-terminated ammonia core poly(amidoamine) dendrimers. The rotational correlation times of the VO(II) complexes, ●, are compared with the most internal, ○, and least internal, ▲, times determined by Meltzer et al.[28] Generation −1 refers to the free chelate 2-(*p*-nitrobenzyl)-6-methyldiethylenetriaminepentaacetic acid.

the two PAMAM−TU−DTPA derivatives overlap. Thus, the rotational correlation times are 9.3×10^{-10} s at 20 °C for the $G = 2$ and 6.4×10^{-10} s at 35 °C for the $G = 6$ PAMAM−TU−DTPA derivatives, respectively.

Meltzer et al.[28] used ^{13}C NMR to measure the rotational correlation times of the carbon atoms at 25 °C in the $G = 0$ through $G = 8$ PAMAM dendrimers, and identified two types of carbons on the basis of changes in their rotational correlation times with respect to increasing molecular weight. In Figure 5 we present the rotational correlation times of vanadyl ions complexed to the free chelate and the PAMAM−TU−DTPA cascade polymers compared to the rotational correlation times of the carbons in the corresponding amine-terminated PAMAMs. The rotational correlation times of the ion−chelate complexes correspond to those of the internal carbons and not the terminal carbons. Note that for this comparison we used an isotropic tumbling model to provide a consistent basis for comparison, although the EPR results show evidence of anisotropic rotation (see below).

The model used by Meltzer et al. measures an isotropic average of the rotational correlation times. An isotropic model gives only crude values of the rotational correlation times. An anisotropic model may fit the data better and provide more information on the chemistry of the system. We therefore fitted the data to such a model, described by an overall spherical rotation combined with a rotation about the axis of the arm branching out of the central core. The model also includes a diffusion angle tilt between the diffusion frame of the chelate and the magnetic frame (Figure 6). These simulations fit the data in the intermediate tumbling region better than does the isotropic model (Figures 7 and 8). The motions about the axis of the branch connecting the chelate to the central core, given by the correlation time $\tau_{||}$, are very rapid. The overall tumbling motions, given by the correlation time $\tau_{\perp}$, are slow (Tables 3 and 4).

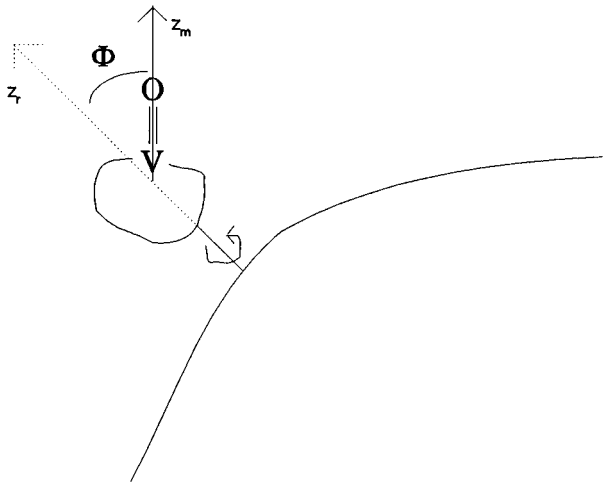

Figure 6. Anisotropic tumbling model used for simulations. The diffusion tilt angle, ϕ, is the angle between the magnetic Z_m axis (for the purpose of this illustration it is along the VO bond) and the diffusion Z_r axis (the axis parallel to the bond that connects the spacer arm of the ion−chelate complex to the dendrimer).

Discussion

Solomon−Bloembergen−Morgan (SBM) theory implies that increasing the rotational correlation time of low molecular weight MRI contrast agents, prepared from paramagnetic ions with long electronic relaxation times, is the single most important method for increasing the relaxivity of these agents.[20−26] This was interpreted to mean that increasing the molecular weight of the ion−chelate complex, by linking it to a macromolecule, would substantially increase the relaxivity. This idea motivated the initial preparation of macromolecular MRI contrast agents.[8] The ion relaxivity of agents that linked chelates to bovine serum albumin exceeded that of the free chelate by 4 times. Similar derivatizations of linear polymers such as polylysine[9−11] and dextran[13] resulted in macromolecular agents with 2−3 times the ion relaxivity. We reported that attaching a derivative of either DTPA or DOTA to a cascade polymer can increase the relaxivity 6-fold.[16−18,49−51] These results support the qualitative prediction that increasing the molecular weight results in an increase in relaxivity.

The results described above demonstrate the effect of attaching a low molecular weight ion−chelate complex to a macromolecule on the relaxivity. While this attachment was accompanied by an increase in relaxivity, the magnitude of this increase was smaller than that predicted by SBM theory for a rigid molecule of similar diameter. More recent studies on linear polymers indicate that the increase in relaxivity is independent of the macromolecule's molecular weight, so that attaching a chelate to the same polymer, but with different molecular weights greater than 10 000, has very little effect on the relaxivity.[8,10,11,13,52−55] In many cases the relaxivity decreased

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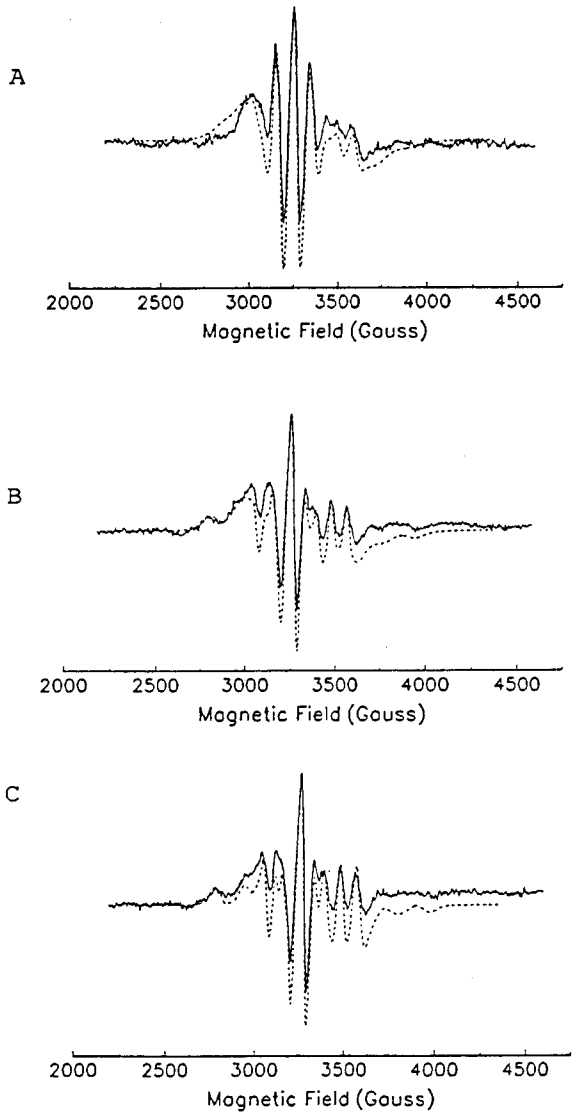

Figure 7. EPR spectra and their simulations with an anisotropic tumbling model for the PAMAM−TU−DTPA−VO(II), $G = 2$, complex. Spectra, solid lines, were acquired at 35 (A), 20 (B), 15 (C), and −130 °C (D). Simulations, dashed lines, were obtained as described in the text.

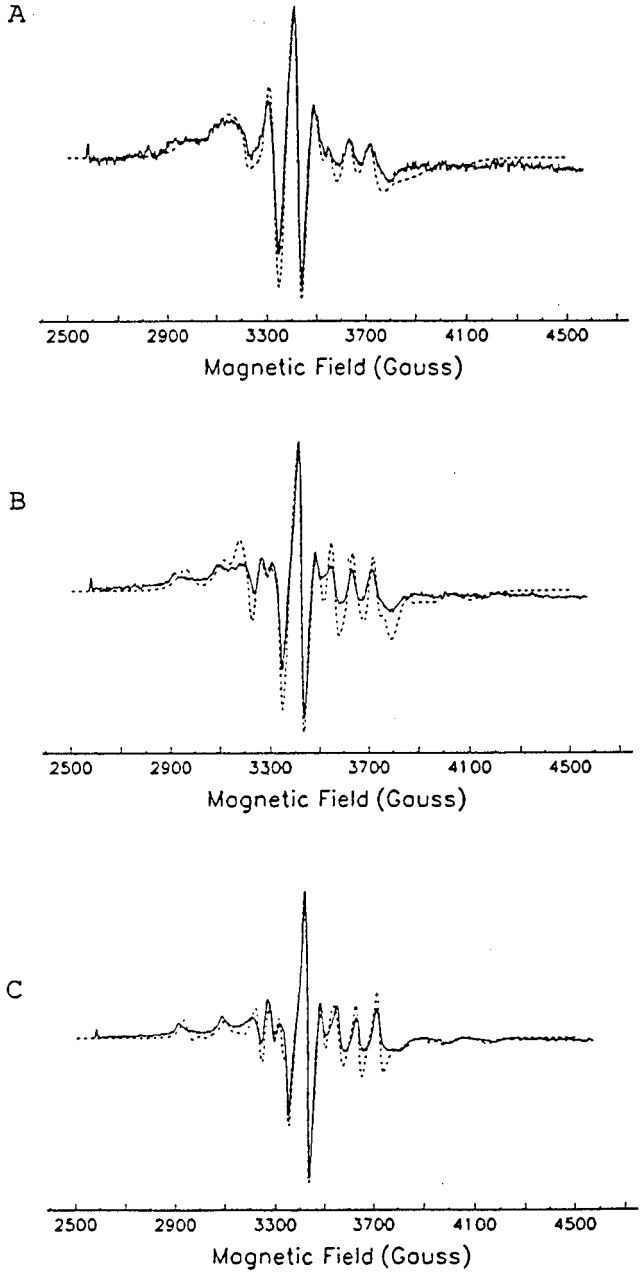

Figure 8. EPR spectra and their simulations with an anisotropic tumbling model for the PAMAM−TU−DTPA−VO(II), $G = 6$, complex. Spectra, solid lines, were acquired at 35 (A), 20 (B), 5 (C), and −130 °C (D). Simulations, dashed lines, were obtained as described in the text.

with increasing molecular weight within a family of polymers.[8,10,13] Vexler et al.[54] reported the relaxivities of polylysine derivatives with molecular weights of 36 000, 44 000, 139 000, and 480 000, at 0.25 T and 37 °C. They found that the relaxivity was independent of molecular weight, with values of 10.4, 11.9, 10.9, and 10.9 $(\text{mM s})^{-1}$, respectively. Desser et al.[55] reported on macromolecular agents prepared from the dianhydride of DTPA and α,ω-diaminopolyethylene glycols. They also found that the relaxivity at 20 MHz and 37 °C remained constant at 6 $(\text{mM s})^{-1}$ for 10 800, 13 600, 18 500, 21 900, 31 500, 39 600, and 83 400 derivatives.

A number of hypotheses have been proposed to reconcile these apparent anomalies with the predictions of SBM theory. Armitage et al.[13] suggested that intramolecular hydroxyl groups in the dextran derivatives could more easily wrap around and displace the inner sphere water molecules of the ion−chelate complex as the molecule becomes larger. Muller[56] presented an alternative hypothesis. He proposed that changes in the electronic relaxation time reduce the ion relaxivity. For contrast agents derived from linear polymeric backbones, in general, either the flexibility of the spacer arm linking the chelate to the

Table 3. Rotational Correlation Times (ns) for $G = 2$ PAMAM−TU−DTPA[a]

T (°C)	$\tau_\perp$	$\tau_{\|\|}$	τ_{av}	τ_{Riso}	Φ (deg)	R^2
15.8	29	0.29	2.9	3.0	72	0.94
18.3	27	0.28	2.8		72	0.95
20.9	21	0.27	2.4	0.93	72	0.95
23.3	12	0.26	1.8		72	0.95
25.4	10	0.24	1.5	0.64	72	0.93
28.6	5.1	0.20	1.0		72	0.91
31.2	4.2	0.19	0.85	0.44	72	0.91
36.7	3.0	0.15	0.67	0.40	72	0.92

[a] $\tau_{av} = (\tau_{||} * \tau_\perp)^{1/2}$, and τ_{Riso} is the rotational correlation time determined with the isotropic tumbling model. The symbol Φ is defined in Figure 6.

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Table 4. Rotational Correlation Times (ns) for $G = 6$ PAMAM−TU−DTPA[a]

T (°C)	$\tau_\perp$	$\tau_\parallel$	τ_{av}	τ_{Riso}	Φ (deg)	R^2
1.9				5.6		0.95[b]
8.0				3.9		0.93[b]
13.9	62	0.26	4.0	3.0	74	0.96
19.7	39	0.26	3.2	2.5	74	0.96
25.4	31	0.24	2.8	2.1	74	0.97
32.5	20	0.22	2.1	1.7	74	0.97
37.5	10	0.21	1.5	0.64	74	0.97
52.0	4.7	0.16	0.86	0.40	74	0.98

[a] $\tau_{av} = (\tau_\parallel * \tau_\perp)^{1/2}$, and τ_{Riso} is the rotational correlation time determined with the isotropic tumbling model. [b] At near freezing, the isotropic model and the anisotropic model give nearly identical simulated spectra. Therefore, only the isotropic results are reported. All the other R^2 values are for the anisotropic fits only.

polymer or the local segmental motions of the polymer may dominate the rotational correlation time. A number of reports establish that, for linear polymers, segmental motions dominate the rotational correlation time, and that for polymers larger than 10 000 these segmental motions become independent of molecular weight.[29−32]

We previously reported that the Gd(III) ion relaxivity of cascade polymer derivatives increased with increasing molecular weight.[15−18] We first showed that the relaxivity was a linear function of the molecular weight or generation of the *N*,*N*-dipropionato-PAMAM dendrimers.[15] We then showed that the increase with molecular weight was independent of the surface chelate.[18] The segmental motions of cascade polymers differ from those of linear polymers. As noted above, Meltzer et al.[28] studied the flexibility of poly(amidoamine) and poly(amidohydroxyl) cascade polymers, finding that the rotational correlation times of the internal carbons increase from 9×10^{-11} to 2.8×10^{-9} s as the molecular weight increases from 360 to 87 300 and the polymer generation goes from 0 to 7. Over this same range the rotational correlation times of the terminal carbons merely double, increasing from about 1.2×10^{-11} to 2.5×10^{-11} s.

If their interpretation is correct, then the difference in the magnitude of the changes in the rotational correlation time with increasing molecular weight has clear implications for how one should attach an ion−chelate complex to a dendrimer. If one wants to take full advantage of the more than 31-fold increase in the rotational correlation times of the internal segmental motions that accompanies the increase in molecular weight, then the first point of flexibility in the spacer arm connecting the chelate to the polymer must be buried in the relatively denser interior of the cascade polymer. Our results are consistent with such a location for the point of flexibility.

A comparison of our results, using either the isotropic model or the average of the anisotropic values, with the isotropic data of Meltzer shows that the rotational correlation times are either equal to or slower than those of the internal carbons. If their interpretation is correct, then the point of the linker flexibility in our system is located in the interior of the molecule. Alternatively, the agreement of the rotational correlation times may by coincidental.

Meltzer's data are also consistent with an alternative hypothesis. The increase in the apparent internal rotational correlation times associated with increasing molecular weight may result from the change in the overall tumbling time of the dendrimer. The method proposed by Schaffer and used by Meltzer has three parameters, *b*, *P*, and τ. The magnitude of the *P* value determines the relative contribution of the overall reorientational motions to the average rotational correlation time. This means that the change in the rotational correlation times of the internal sites may result from an increase in the overall tumbling time of the dendrimers that occurs as the molecular weight increases. That is, differences in the response of the isotropic rotational correlation times to increases in the molecular weight may result from differences in the contribution by the overall molecular motions as well as by the internal and terminal segmental motions to the overall rotational correlation time.

Regardless of the explanation, the responses of the rotational correlation times to increases in molecular weight for the cascade polymers differ from those of linear polymers. With linear polymers of molecular weight greater than 10 000 only the local segmental motions affect the rotational correlation times. The overall reorientational motions of the macromolecule barely contribute, and τ_r is independent of molecular weight. We show that, for cascade polymers, either the overall reorientational motions of the macromolecule contribute or the interior density of the dendrimer is important. Both these hypotheses differentiate cascade polymers from linear polymers, and suggest methods for improving the relaxivity of dendrimer-based contrast agents.

The results depicted in Figure 4 indicate that, under the experimental conditions used, the VO(II) binds to the DPTA chelate and not to some hypothetical interior binding site of the cascade polymer itself. Thus, the rotational correlation times derived from the data in Figure 3 and 4 are those of the surface chelate covalently attached to the dendrimer and complexed with VO(II).

Regardless of the model used, our results shown in Figure 5 and Tables 3 and 4 demonstrate that the rotational correlation time of the ion−chelate complex of the PAMAM−TU−DTPA derivative increases with increasing molecular weight. The free chelate exhibits an isotropic rotational correlation time at 20 °C of 1.5×10^{-10} s which compares with 9.3×10^{-10} and 2.5×10^{-9} s for the $G = 2$ and $G = 6$ derivatives, respectively. This increase in the rotational correlation time is approximately the same as that reported for the internal carbons of the parent PAMAM. In addition, the rotational correlation times of the ion−chelate complexes on the surface of the PAMAM−TU−DTPA derivatives match those of the parent PAMAM of the generation immediately above the PAMAM used as a backbone for the polychelate. This is consistent with the fact that each generation adds a seven-atom linear branch chain, and that the spacer arm attaching the chelate to the polymer is also seven atoms long. The rotational correlation time of the $G = 6$ derivative at 20 °C calculated with the isotropic model is less than 25% smaller than that determined with the anisotropic model (Table 4). The average value determined with the anisotropic model for the $G = 2$ derivative is much larger than that determined with the isotropic model.

Our results support the conclusion that the segmental motions which dominate the rotational correlation times of ion−chelate complexes attached to the surfaces of the PAMAM−TU−DTPA derivatives are in the interior of the molecule. They are also consistent with the hypothesis that the overall reorientation of the macromolecule contributes to the average rotational correlation time. Either hypothesis would result in the observed increase in the rotational correlation time associated with increasing molecular weight. Both hypotheses may partially explain the previously-observed increase in relaxivity associated with increasing molecular weight of PAMAM−TU−DTPA derivatives,[15−18] and the differences between the responses of the relaxivities of cascade polymer- and linear polymer-based MRI contrast agents to increasing molecular weight.

If Meltzer et al. are correct, then the observance of internal and terminal segmental motions also implies that the spacer arm connecting the chelate to the polymer should play an important

role. Thus, not all linkers are the same. those spacer arms that have internal-like segmental motions will allow higher potential relaxivities than those which have terminal segmental motions. In addition, different dendrimer families should have different relaxivities for the same size polymer and the same spacer arm. If, as Meltzer suggests, the interior density of the polymer determines the rotational correlation time of the internal carbons, then the poly(ethylenimine) or poly(propylenimine) families of dendrimers, which have much denser interiors than PAMAMs, should have longer rotational correlation times of the internal carbons. We therefore predict that the low molecular weight derivatives based on poly(ethylenimine) or poly(propylenimine) dendrimers should have higher relaxivities than those derived from poly(amidoamine) or poly(amidohydroxyl) dendrimers. We are currently testing this hypothesis. If the reorientation of the cascade polymer contributes to the average rotational correlation time, then the dendrimer shape will play an important role. Dendrimers with ellipsoidal, flattened, or elongated shapes should have higher relaxivities than spherical dendrimers of the same molecular weight.

While the increase in the relaxivity of cascade polymer-based MRI contrast agents associated with increasing rotational correlation time supports the qualitative predictions of the Solomon−Bloembergen−Morgan theory, changes in the electronic relaxation time and the coordination lifetime of the inner sphere water molecule may also contribute to the effects of increasing the molecular weight on the relaxivity of these reagents. The isotropic rotational correlation time of the $G = 2$ derivative at 20 °C is larger than that of the $G = 6$ derivative at 37 °C, 9.3×10^{-10} vs 6.4×10^{-10} s, respectively. Thus, if the rotational correlation time dominates the relaxivity of the PAMAM−TU−DTPA system, then the relaxivities of the $G = 2$ derivative should be larger than those of the $G = 6$ derivative at these two temperatures. This is true regardless of the tumbling model used. The $\tau_{\perp}$ for the $G = 2$ derivative is 21 ns at 20 °C compared with 10 ns for the $G = 6$ derivative at 37 °C. Similarly, the τ_{av} is 2.4 ns for the $G = 2$ derivative at 20 °C compared with 1.5 ns for the $G = 6$ derivative at 37 °C (Tables 3 and 4). We previously showed that the Gd(III) ion relaxivity of the $G = 6$ derivative at 35 °C exceeded that of the $G = 2$ derivative at 20 °C by more than 50%.[18] The relaxivity of the $G = 6$ derivative showed the temperature dependence opposite that of the $G = 2$ derivative.[49−51] This implies that either the electronic relaxation time or the residence time of the water molecules residing in the inner coordination sphere, or both, contribute significantly to the Gd(III) ion relaxivity of the PAMAM−TU−DTPA derivatives, and that attaching the ion−chelate complex to macromolecules of different sizes differentially alters the electronic relaxation time, the water residence time, or both. Evidence exists that attaching an ion−chelate complex, either covalently or noncovalently, to a macromolecule increases the inner sphere residence time of the water molecules coordinated to the ions.[57,58] Alternatively, the density of the ion−chelate complexes may affect the electronic relaxation time by altering the zero field splitting.

In conclusion we have shown that the rotational correlation times of ion−chelate complexes attached to PAMAM−TU−DTPA polymetal chelates resemble those of the internal carbons of the corresponding parent PAMAM. We have found that an anisotropic model describes the motionally modulated line shapes better than an isotropic model. While the isotropic rotational model does not fully describe the motions of these complex dendrimers, and the overall molecular reorientation plays a significant role in determining the observed rotational correlation time, it is still advantageous to employ the isotropic model for comparison to others' work. Our results also showed that, for this macromolecular system, the rotational correlation time alone cannot account for differences in the relaxivity that were previously reported. This work also suggests that, for lower molecular weight derivatives, cascade polymers with denser interiors, with ellipsoidal, flattened, or elongated shapes, or with rigid side chains should maker better MRI contrast agents.

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(59) Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; TRIS, 2-amino-2-(hydroxymethyl)-1,3-propanediol; NaAc, sodium acetate; PAMAM, poly(amidoamine).