Abstract The parameters governing the water proton relaxivity of the \([\text{Gd} \text{EGTA-BA-(CH}_2\text{)}_{12}]_n^+\) polymeric complex were determined through global analysis of \(^{17}\text{O} \) NMR, EPR and nuclear magnetic relaxation dispersion (NMRD) data. \([\text{EGTA-BA}^{2-} = 3,12\text{-bis(carbamoylmethyl)-6,9-dioxo-3,12-diaza-}
\text{tetradecanediolate(2-)}\). The Lipari-Szabo approach that distinguishes the global motion of the polymer \(\langle \tau_g \rangle\) from the local motion of the \text{Gd(III)}-water vector \(\langle \tau_l \rangle\) was necessary to describe the \(^1\text{H}\) and \(^{17}\text{O}\) longitudinal relaxation rates; therefore for the first time it was included in the global simultaneous analysis of the EPR, \(^{17}\text{O} \) NMR and NMRD data. The polymer consists on average of only five monomeric units, which limits the intramolecular hydrophobic interactions operating between the \((\text{CH}_2)_{12}\) groups. Hence the global rotational correlation time is not very high \(\tau_{g}^{298}=3880\pm750\text{ ps}\) compared to the corresponding DTPA-BA-based polymer (about 15 monomeric units), where \(\tau_{g}^{298}=6500\text{ ps}\). As a consequence, the relaxivity is limited by the rotation, which precludes the advantage obtained from the fast exchanging chelating unit \((k_{ex}^{298}=2.2\pm0.1\text{ x}10^6\text{ s}^{-1})\).

Keywords Magnetic resonance imaging \cdot Polymer \cdot Lipari-Szabo \cdot Gadolinium \cdot Contrast agent

Introduction

Nowadays, gadolinium(III) complexes are commonly used as magnetic resonance imaging (MRI) contrast agents [1, 2]. One of the challenges of these complexes is to attain the maximum relaxivity [enhancement of the water proton relaxation rate normalized to 1 mM of \text{Gd(III)} compound], first to reduce the injected dose and second to shorten the examination time. This can be achieved by slowing down the rotation of the complex by interactions with serum proteins [3, 4, 5], covalent binding to dendrimers [6, 7] or polymers [8, 9, 10, 11, 12]. The great advantage of these macromolecular compounds, as compared to the commercially available low molecular weight compounds, is their increased lifetime in the blood pool with less extravasation, which is favorable for their use as magnetic resonance angiography (MRA) contrast agents. However, the relaxivity cannot be directly predicted from the molecular weight: nonrigid attachment of the chelate to the macromolecule or internal flexibility are often limiting factors.

For instance, it has recently been shown that a poly(ethylene glycol) (PEG) chain was too flexible in water to reach a useful relaxivity [13]. On the other hand, alkyl-type spacers show promising features: owing to hydrophobic interactions with the solvent, they build intramolecular micelle-like structures that ensure the rigidity of the polymer [8, 14]. A promising relaxivity was observed for the \([\text{Gd(DTPA-BA-(CH}_2\text{)}_{12}]_n\) polymer \((\text{DTPA}=\text{diethylentriamine-}
\text{N,N',N''',N''''-pentaacetate})\). The analysis of \(^{17}\text{O} \) NMR, EPR and nuclear magnetic relaxation dispersion

Supplementary material Tables S1–S5 containing experimental \(^{17}\text{O} \) NMR, EPR and NMRD data and Fig. S1 showing calculated transverse electronic relaxation rates and \(^{17}\text{O} \) reduced longitudinal and transverse relaxation rates and chemical shifts, from the parameters obtained by the simultaneous fit of the \(^{17}\text{O} \) NMR and EPR data only, are available in electronic form on Springer Verlag’s server at http://dx.doi.org/10.1007/s007750000193
sion (NMRD) data have shown that, owing to slow rotation of the micelle-like structure of the polymer, the relaxivity is limited by the water exchange rate. Logically, a polymer of the same shape, but with a chelating unit whose water exchange is known to be faster, should provide a higher relaxivity. Hence, a polymer with EGTA-BA$^{2-}$ as a chelating unit was prepared [EGTA-BA$^{2-}$=3,12-bis(carbamoylmethyl)-6,9-dioxa-3,12-diazatetradecanediolate(2-)] (Scheme 1). We present here the analysis of the variable-field and -temperature $^{17}$O NMR, variable-temperature EPR and NMRD data in order to determine the parameters that govern the water proton relaxivity. We take advantage of this new polymer to improve our analysis by including the Lipari-Szabo approach [15, 16] in the simultaneous global fitting of the experimental data.

Materials and methods

Polymer preparation

**Synthesis of EGTA-BA [bis-anhydride of ethylenebis(oxyethylenetriitol)tetraacetic acid]**

A 250 mL round-bottom flask was charged with 57.05 g (0.150 mol) of EGTA [ethylenebis(oxyethylenetriitol)tetraacetic acid] (Aldrich), 66.54 mL (0.750 mol) of acetic anhydride (Aldrich), and 8.09 mL (0.100 mol) of anhydrous pyridine (Aldrich) and stirred overnight at 60°C under dry argon. The solids were filtered off while the reaction mixture was still hot. The residual solid was washed with about 30 mL of acetic anhydride, filtered, and the filtrate combined with the filtrate from above. Subsequently the filtrate was treated with about 175 mL of anhydrous tetrahydrofuran and sonicated in an ultrasonic bath for several minutes. It was then filtered through a GF/F glass filter and stripped on a rotary evaporator under oil-pump vacuum at room temperature for 3 days. The resulting tan oil comprised 14.54 g. Its $^1$H NMR spectrum is consistent in peak multiplicity and peak ratio with that expected for the desired product, and this intermediate was used directly in the subsequent reaction, described below.

**Synthesis of the 1:1 linear copolymer of EGTA-BA and 1,12-diaminododecane**

A 100 mL round-bottom flask was charged with 2.50 g (0.00726 mol) of EGTA-BA from above, 29.0 mL of anhydrous DMSO (Aldrich), 1.498 g (0.00748 mol) of 1,12-diaminododecane, and 3.14 mL of (0.0225 mol) of triethylamine. The reaction mixture was heated under dry argon at 60°C overnight. It was then treated with 150 mL of distilled water, filtered through a GF/F glass filter, and placed in a 200 mL Amicon stirred-cell dialfiltration apparatus fitted with an Amicon YM-1 dialfiltration membrane. The clear, tan solution was dialfiltrated with a distilled water feed at constant volume for 28 h, during which time a total of six turnovers of dialfiltration was accomplished. The retentate was then freeze dried, yielding 2.45 g of a tan, sponge, polymeric product. Size exclusion HPLC with both UV and RI detection indicated the resulting polymer had a fairly broad molecular weight, ranging from about 3000 to about 15,000 compared to PEG standards. A portion of this polymer was subsequently converted to the gadolinium complex as described below.

**Conversion of the copolymer of EGTA-BA and 1,12-diaminododecane to its Gd$^{3+}$ complex**

A total of 0.544 g of the 1:1 linear copolymer of EGTA-BA and 1,12-diaminododecane (calculated to contain 0.001 “molar units” of the copolymer), as prepared above, was dissolved in 20 mL of distilled water, forming a clear, colorless solution. In a separate container a solution of 0.334 g (0.001 mol) of gadolinium acetate in 10 mL of water was prepared. The clear, colorless gadolinium acetate solution was slowly dripped into the stirred polymer solution, causing no precipitation. The resulting solution was then treated with an additional solution of 0.066 g of gadolinium acetate in 2 mL of distilled water to provide a 20% excess of gadolinium to ensure that all sites were complexed with gadolinium. The resulting clear, colorless solution was transferred to a small Amicon stirred-cell dialfiltration unit fitted with a YM-3 (nominal 3000 MW cutoff) and purified by dialfiltration at constant volume overnight. After a total of 16 turnovers of dialfiltration the clear, colorless retentate was removed and freeze dried. The resulting product was a white, fluffy, polymeric solid comprising 0.40 g. Elemental analysis indicated the polymer contained 43.69 (43.44) % C, 6.81 (6.90) % H, 7.26 (7.32) % N, and 20.51 (20.22) % Gd.

The molecular weight of the polymer was measured by size-exclusion HPLC against PEG standards. It had a weight-average molecular weight of 5.1 kD (corresponding to 6.7 monomeric units), a number-average molecular weight of 2.6 kD (corresponding to 3.4 monomeric units), and a polydispersity of 1.9.

**Sample preparation**

The solutions were prepared by dissolution of the solid polymer in water and adjustment of the pH to 5. For the $^{17}$O NMR and EPR measurements the solutions contained 2% of $^{17}$O-enriched water (Yeda, Israel). The gadolinium concentration of the solutions was as follows: $C_{Gd}=78.0$ mmol kg$^{-1}$ for EPR, $C_{Gd}=49.2$ mmol kg$^{-1}$ for $^{17}$O NMR, and $C_{Gd}=5.2$ mmol dm$^{-3}$ for NMRD. The absence of free gadolinium was checked by the xylenol orange test [17].

**$^{17}$O NMR measurements**

Variable-temperature $^{17}$O NMR measurements were performed at three different magnetic fields using Bruker AMX2-600 (14.1 T, 81.3 MHz) and AM-400 (9.4 T, 54.2 MHz) spectrometers and a WP-60 electromagnet (1.4 T, 81 MHz) adapted for use with an AC-200 console. The samples were sealed in glass