Synthesis, Relaxivity and MRI Enhancement of Linear Oligo-Gd(III) Complexes with Poly(DTPA-ester) Ligands Derived from Amino Acids

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Six linear oligo-DTPA-ester Gd(III) complexes being used for potential MRI contrast agents were synthesized from amino acids and characterized. Their longitudinal relaxation rates were measured. One of them, the phenylalanine derivative, with high relaxivity, was chosen for the acute toxicity and T_1-weighted imaging test. The results indicated that there was no obvious toxicity for this new oligomeric Gd(III) complex, and it exhibits the highly enhanced MRI signal intensity and the increasing signal duration in the liver tissue compared to Gd-DTPA.

Keywords Magnetic resonance imaging (MRI), contrast agents, oligomeric Gd(III) complex, polyester derived from amino acids, relaxivity, enhancement

Introduction

Magnetic resonance imaging (MRI) has become an important diagnostic tool in modern medical diagnosis. This technique relies upon the detection of ^{1}H NMR signal of water molecule *in vivo*.[1-3] MRI instrument with the magnetic field gradients allows ^{1}H NMR signal to be acquired as a function of three dimensional space. Bacause most human soft tissues contain about the same amount of water, it is diffict to use water density to distinguish the types of tissues directly.[3,4] However, the longitudinal and transverse relaxation times (T_1 and T_2) of water protons happen to vary distinctly depending on the tissue type[2] and thus can be used as the basis of MRI contrast.

The values of T_1 and T_2 of water protons in tissues are in the range of tens milliseconds to seconds, which apparently will slow down the speed of MRI measurements. The duration of the experiments can be speeded up significantly using the contrast agent consisting of paramagnetic metals that can shorten the T_1 and T_2 of the proton in water molecule.[1-3] Gadolinium(III) complexes, for its high paramagnetic property, have received much attention.[3-5] It is estimated that over 30% of MRI diagnosis involve the administration of the gadolinium contrast agents.[4] The commonly used contrast agents are Gd-DTPA (DTPA = diethylenetriamine pentaacetic acid) and Gd-DOTA (DOTA = N, N', N'', N'''-tetraazacyclododecane tetraacetic acid).[4-6] However, as nonspecific extra-cellular contrast agents, Gd-DTPA and Gd-DOTA have limited applications.[5,7] The design and development of novel tissue-specific contrast agents with higher proton relaxivity is one of the most impotent topics in MRI research.[3-5] Macromolecular contrast media have been demonstrated to have longer intravascular retention

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times and higher proton T_1 relaxivity than small compounds,[3,8] but run the risk of having much longer dwell times in the body and less complete elimination, thus increases the possibility of cellular uptake, leading potentially to the release of byproducts including free gadolinium.[4] Oligomeric contrast agents may be worked to the satisfaction of both higher relaxivity and lower toxicity.

Additionally, it is well known that in the tissues and organs there are receptors with high affinity to some of the bio-molecules, such as amino acids or derivatives of amino acids.[9] The modification of the Gd(III) complexes with derivatives of amino acids may overcome some of the disadvantages of the common contrast agents. In this work, a series of oligomeric poly-DTPA-ester Gd(III) complexes containing derivatives of amino acids were prepared, and their longitudinal relaxation rates were measured. One of these polyester-Gd(III) complexes was chosen for the acute toxicity measurement and T_1-weighted imaging test.

Experimental

Reagents

All the chemicals used were of analytical reagent and all the solvents were purified, dried and distilled prior to use. Dianhydride of DTPA (DTPAA),[10] *L*-*N*, *N*-bis(2-hydroxyethyl) alanine, phenylalanine, valine, leucine, isoleucine and *N*,*N*-(2-hydroxyethyl) glycine (**1a—1f**)[11] were prepared according to the literature method.

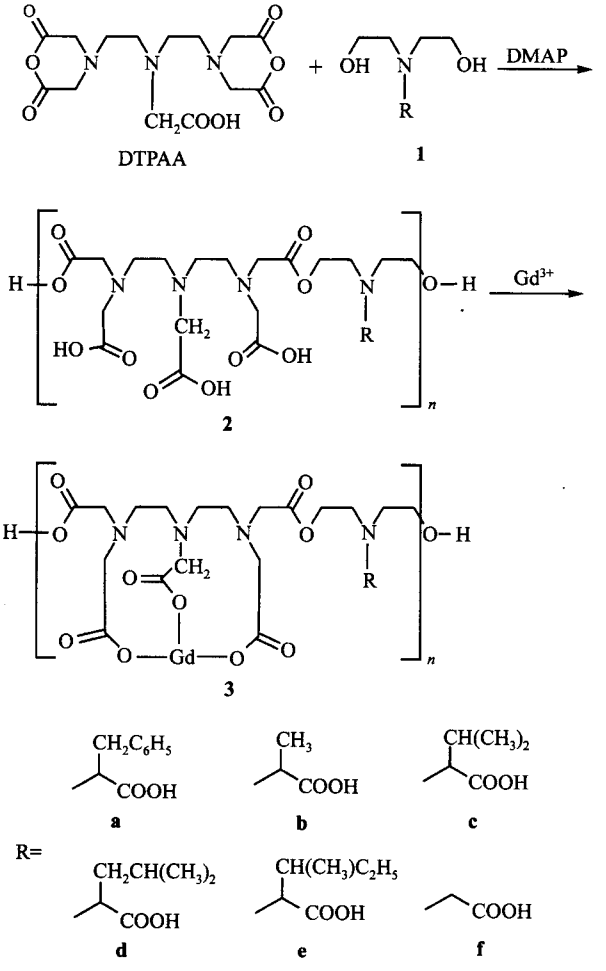
Synthesis of materials

For the convenience of discussion, the chemical structures and synthetic scheme of this polymers are shown as Scheme 1, and **2a** and **3a** are taken as examples.

Ligand: 10.0 mmol of **1a**, 15 mL of DMF and 5 mg of *N*,*N*-dimethylaminopyridine (DMAP) were mixed in flask. Under the protection of dry nitrogen, 10.0 mmol of DTPAA was added, the mixture was slowly warmed to 78℃ and stirred for 48 h. Then 15 mL of DMF was added. After cooling to *ca*. 50℃, the reaction mixture was dropped into the mixed solvents of ethanol and ether. The solid produced was resolved in DMF again, and precipitated in ethanol and ether. This purified procedure should be repeated 3—4 times, and white solid **2a** was obtained. The yield was 77.8%.

Complex: 2.0 mmol of **2a** was dissolved in 8 mL of water and then 2.0 mmol of $GdCl_3 \cdot 6H_2O$ was added. The mixture was warmed to 40℃ and stirred for 30 min, then dropped into ethanol. The precipitate was filtered and dried in vacuum, white powder product **3a** was obtained. The yield was 75.3%.

Scheme 1



Characterization

The number-average molecular weights ($\overline{M}_n$) were measured on a Knauer VPO spectrometer. IR spectra were measured on a Nicolet 170 SX IR spectrometer (KBr tablet). ^{1}H NMR spectra were recorded on a Jeol FX-90Q spectrometer. The elemental analyses were performed on a Carlo Erba 1106 analyzer. The metal content in each complex was determined by EDTA complexometric titration method after the complex nitrated with mixed acid of HNO_3(65%) and HCl (36%).

Relaxation measurement

The longitudinal relaxation times (T_1) of the protons of the solvent water in the solutions containing the polymeric Gd(III) complexes **3a—3f** were measured on a Varian-XL 200 (4.7T) NMR spectrometer using inverse recovery method at 21℃. The measurements used $[180° - \tau - 90° - AT - D]_n$ as pulse sequence where the value range of τ was from 10 ms to $\geq 5T_1$ and AT (acquisition time) + D (delay) were $\geq 5T_1$. The concentration of the complexes was in the range of millimole per liter. The 12 relaxation delays were used for each of the measurements with spectral width of 3000 Hz.

Acute toxicity

The compound **3a** was chosen for the acute toxicity test and for the comparison with Gd-DTPA according to the standard method.[12] The acute toxicity of **3a** and Gd-DTPA was tested by single intravenous (trail vein) injection of different volume of 0.2 mol/L solution at a rate of 2 mL/min to the mice (18—22 g, 10 mice each group, male and female) respectively. The animals were observed for 7—14 days after the injection. The results indicated that there was no obvious toxicity for the complex **3a** and Gd-DTPA (no mouse died).

MR *imaging experiments*

MR image enhancement test of the contrast agents on rat livers was carried out on a Bruker Biospec 47/30 MRI machine (4.7T). Six Wistar rats weighted 190 ± 20 g were used as the testing models and were separated into two groups for enhanced imaging using the contrast agents of **3a** and Gd-DTPA, respectively. The rat was anaesthetized using 2% pentobarbital sodium solution minuets before taken into the MRI magnet. The axial images of 2-mm thick were acquired using a Bruker standard multi-slice multi-echo pulse sequence with TE (time of echo) = 13 ms, TR (time of repeat) = 500 ms, NA (number of averages) = 2. After the rats received injection of **3a** or Gd-DTPA (dose 0.11 mmol/kg) intravenously, the images on the same slices were acquired every 5 min for over one hour.

Results and discussion

Characterization

The results of VPO measurement indicated that the average degree of polymerization (n) of these polymers is around 4. IR spectra of **2a—2f** exhibited the characteristic absorption peaks or the ester bond $\nu_{c=o}$ 1745—1730 cm^{-1} and ν_{c-o} 1235—1220 cm^{-1}; in IR spectra of their gadolinium complexes, appeared strong absorption peaks $\nu_{c=o}$ 1620—1590 cm^{-1} and ν_{c-o} 1420—1390 cm^{-1}, which indicated the formation of complex. The data of ^1H NMR (in $CDCl_3$) and elemental analyses (EA) of ligands were as following:

2a ^1H NMR δ: 7.29—7.34 (m, 5H, C_6H_5), 4.32 (t, 4H, OCH_2), 2.67—3.68 (m, 25H, others); Anal. calcd for $C_{27}H_{38}N_4O_{12}\cdot H_2O$: C 51.59, H 6.41, N 8.91; found C 51.26, H 6.53, N 9.09.

2b ^1H NMR δ: 1.45 (d, 3H, CH_3), 4.32 (t, 4H, OCH_2), 2.79—3.89 (m, 23H, others); Anal. calcd for $C_{21}H_{34}N_4O_{12}\cdot 2H_2O$: C 44.21, H 6.71, N 9.82; found C 44.14, H 6.77, N 10.15.

2c ^1H NMR δ: 0.98 (d, 6H, $2CH_3$), 1.79—1.83 (m, 1H, CH), 4.32(t, 4H, OCH_2); 2.70—3.87 (m, 23H, others); Anal. calcd for $C_{23}H_{38}N_4O_{12}\cdot 2H_2O$: C 46.15, H 7.07, N 9.36; found C 45.98, H 6.93, N 9.84.

2d ^1H NMR δ: 0.93 (d, 6H, $2CH_3$), 1.72—1.80 (m, 3H, CH_2 and CH in R), 4.33—4.43 (t, 4H, OCH_2), 2.64—3.83 (m, 23H, others); Anal. calcd for $C_{24}H_{40}N_4O_{12}\cdot 2H_2O$: C 47.05, H 7.24, N 9.15; found C 46.94, H 7.20, N 9.81.

2e ^1H NMR δ: 0.91 (t, 3H, CH_3), 1.26—1.78 (m, 6H, $CH(CH_3)CH_2Me$), 4.33—4.43 (t, 4H, OCH_2), 2.65—3.84 (m, 23H, others); Anal. calcd for: $C_{24}H_{40}N_4O_{12}\cdot H_2O$: C 48.48, H 7.12, N 9.42; found C 47.87, H 7.11, N 9.89.

2f ^1H NMR δ: 4.30 (t, 4H, OCH_2), 2.78—3.90 (m, 24H others); Anal. calcd for $C_{20}H_{32}N_4O_{12}\cdot 2H_2O$: C 43.16, H 6.52, N 10.07; found C 42.92, H 6.61, N 9.90.

The data of elemental analyses (EA) and EDTA complexometric titration were as following:

3a Anal. calcd for $C_{27}H_{35}N_4O_{12}Gd\cdot 9H_2O$: C 34.98, H 5.76, N 6.04, Gd 16.96; found C 34.31, H 5.94, N 6.50, Gd 17.47.

3b Anal. calcd for $C_{21}H_{31}N_4O_{12}Gd\cdot 8H_2O$: C 30.28, H 5.69, N 6.73, Gd 18.88; found C 30.04, H 5.81, N 7.05, Gd 18.28.

3c Anal. calcd for $C_{23}H_{35}N_4O_{12}Gd\cdot 8H_2O$: C 32.09, H 6.27, N 6.59, Gd 18.26; found C 32.36,

H 6.34, N 7.03, Gd 18.72.

3d Anal. calcd for $C_{24}H_{37}N_4O_{12}Gd \cdot 9H_2O$: C 32.28, H 6.21, N 6.27, Gd 17.61; found C 31.68, H 6.28, N 6.92, Gd 17.49.

3e Anal. calcd for $C_{24}H_{37}N_4O_{12}Gd \cdot 8H_2O$: C 32.95, H 6.06, N 6.41, Gd 17.97; found C 32.27, H 6.20, N 6.59, Gd 18.39.

3f Anal. calcd for $C_{20}H_{29}N_4O_{12}Gd \cdot 7H_2O$: C 30.00, H 5.41, N 7.00, Gd 19.64; found C 29.73, H 5.48, N 7.65, Gd 20.11.

Relaxivity

The longitudinal relaxation times (T_1) of the water proton in the aqueous solutions containing **3a—3f** were given in Table 1. For paramagnetic aqueous solution, in the absence of solute-solute interaction, the water proton relaxation rates are linearly dependent on the concentration of the paramagnetic media concentration, [M], as described in Eq. (1):

$$R_1 = ((1/T_1)_{obsd} - (1/T_1)_d)/[M] \quad (1)$$

where $(1/T_1)_{obsd}$ and $(1/T_1)_d$ are water proton relaxation rates in the presence and absence of paramagnetic media, respectively. Relaxivity (R_1) is defined as the slope of this dependence in the units of $L \cdot mmol^{-1} \cdot s^{-1}$, which represents the relaxation ability of the enhancement of a paramagnetic contrast agent. The T_1 of the solvent (distilled water) at 21℃ was measured to be 2.64 s, corresponding to 0.379 s^{-1} for $(1/T_1)_d$. Thus the values of R_1 for the Gd(III) complexes at 21℃ could be worked out and are listed in Table 1. The data indicates that the relaxivities of the macromolecular Gd(III) complexes **3a—3f** are higher than that of Gd-DTPA, the relatively a smaller complex.

MR imaging

In the MR imaging experiments, the signal to noise (S/N) ratios in the proton intensity image of the rat liver (parenchyma) were increased after the intravenous injection of **3a** and Gd-DTPA, respectively. The images before and after the injection of **3a** were shown in Fig. 1. The time course intensity changes were plotted in Fig. 2, where the data (Table 2) was the average from three rats and the error was within ± 5%. The first data point (0 min, Fig. 2) was taken from the images before injection of the agents and was used as reference to set the scale of the signals. It can be seen from Fig. 2 that the enhancements were increased steadily from 0 to 25 min after the injection and decreased after 40 min. The enhancements reached their maximum at 25 to 40 min, corresponding to the average values of 132% and 117% for **3a** and Gd-DTPA, respectively. At 50—60 min, the enhancements were still maintained at 119% and 114% for **3a** and Gd-DTPA, respectively. The statistic analysis using ANOVA method showed that there were remarkable difference in the period between 10 and 35 min ($P < 0.05$) (Table 2).

Table 1 Relaxation measurement results of Gd(III) complexes[a]

Gd(III) Complex	[M] (mmol/L)	$T_{1(obsd)}$ (ms)	R_1/Gd ($L \cdot mmol^{-1} \cdot s^{-1}$)	Enhancement[b] (%)
3a	1.24	109.6	7.05	142
3b	1.03	136.4	6.75	135
3c	1.35	108.2	6.57	132
3d	1.19	127.5	6.27	126
3e	1.28	109.3	6.85	137
3f	1.64	99.6	5.89	118
Gd-DTPA	1.25	151.2	4.99	100

[a] T_d = 2.64 s; Temperature 21℃; Frequency: 200 MHz. [b] Gd-DTPA was used as reference and its enhancement was set to 100%.

The results indicate that the new contrast agent **3a** shows a higher efficiency in the intensity enhancement on the rat liver image than Gd-DTPA. This is probably due to the binding of the plasma protein to the macromolecular complexes, and to the fact that **3a** contains benzyl group, which make it possess a amphiphilic character. Thus some fraction of this polyester Gd(III) complex is taken by liver cells and undergoes hepatibiliary excretion.[3]

Summary

Six linear oligo-DTPA-ester gadolinium (Gd) complexes derivatives of amino acids were prepared and characterized. The effects of the complexes on the water longitudinal relaxation rates were measured. The acute toxicity of phenylalanine derivative **3a** was tested on mice and there is no obvious toxicity observed. The signal intensity enhancements on MR images after injection of **3a**

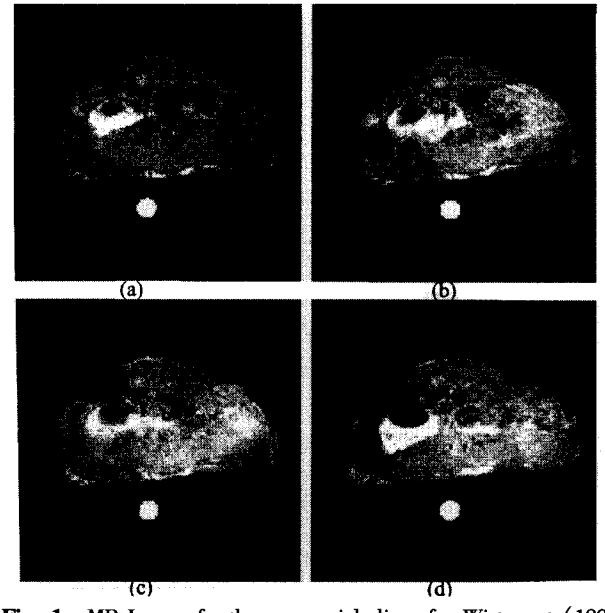


Fig. 1 MR Images for the same axial slice of a Wistar rat (180 g), before the injection (a), and 5 min (b), 30 min (c) and 60 min (d) after the injection of contrast agent of **3a**.

were quantitatively determined and compared with that of Gd-DTPA. The results show that the new polyester-Gd complex derived from phenylalanine gives rise to larger and longer enhancement on the MRI signal intensity in the liver tissue compared to Gd-DTPA.

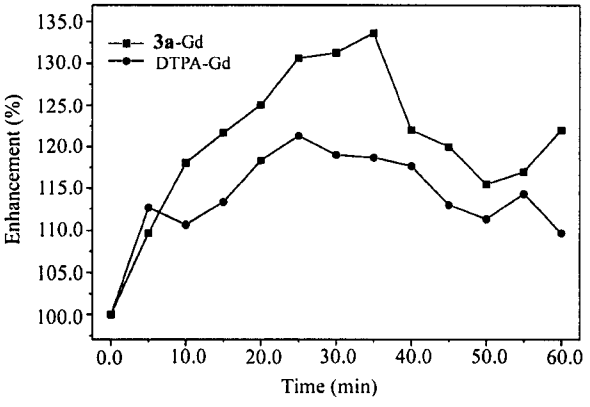


Fig. 2 Time course MRI signal intensity enhancement after the injection of **3a** (■) and Gd-DTPA (●).

Table 2 Average signal intensity (relative value, %) and statistic analysis results of the proton T_1-weighted imaging of the rat liver before and after the injection of **3a** and Gd-DTPA

Time (min)	$I_{Gd\text{-}DTPA}$ (X_A)	I_{3a} (X_B)	S_A ($S_{Gd\text{-}DTPA}$)	S_B (S_{3a})	$\overline{X}_B - \overline{X}_A$	$S_{\overline{X}_B - \overline{X}_A}$	t^a
0	100.0	100.0					
5	112.6	109.6	±2.16	±2.02	-3.0	1.70	1.76
10	110.5	117.7	±1.93	±3.10	7.2	2.11	3.41
15	113.6	122.0	±2.01	±2.29	8.4	1.76	4.77
20	118.2	125.2	±2.20	±1.71	7.0	1.61	4.35
25	121.0	130.7	±2.61	±2.93	9.7	2.25	4.31
30	119.1	131.2	±2.30	±2.59	12.1	2.00	6.05
35	118.6	133.7	±2.69	±1.89	15.1	1.90	7.95
40	117.6	122.1	±1.66	±2.52	4.5	1.73	2.60
45	113.1	119.5	±3.01	±2.13	6.4	2.12	3.03
50	111.4	115.9	±2.59	±2.01	4.5	1.89	2.38
55	114.0	117.1	±5.60	±1.40	3.1	3.33	0.93
60	110.8	122.0	—	—	—	—	—

[a] By analysis of variance (ANOVA) method; $t_{0.05(4)} = 2.776$.

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