Synthesis Neutral Rare Earth Complexes of Diethylenetriamine-*N*,*N*″-bis(acetyl-isoniazid)-*N*,*N*′,*N*″-triacetic Acid as Potential Contrast Enhancement Agents for Magnetic Resonance Imaging

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A novel ligand, diethylenetriamine-*N*,*N*″-bis(acetyl-isoniazid)-*N*,*N*′,*N*″-triacetic acid (H_3L) has been synthesized from diethylene triamine pentaacetic acid (DTPA) and isoniazid. Ligand and its five neutral rare earth (RE=La, Sm, Eu, Gd, Tb) complexes holding promise of magnetic resonance imaging (MRI) were characterized on the basis of elemental analysis, molar conductivity, ^{1}H-NMR spectrum, FAB-MS, TG-DTA analysis and IR spectrum. The relaxivity (R_1) of complexes and $Gd(DTPA)^{2-}$ used as a control were determined. The relaxivity of LaL, SmL, EuL, GdL, TbL and $Gd(DTPA)^{2-}$ were 0.14, 1.66, 3.14, 6.08, 2.79 and 4.34 $l \cdot mmol^{-1} \cdot s^{-1}$, respectively. The spin-lattice relaxivity of GdL was larger than that of $Gd(DTPA)^{2-}$. The relaxivity of GdL had also been investigated in human serum albumin (HSA) solution, the relaxivity of GdL was enhanced from 6.08 $l \cdot mmol^{-1} \cdot s^{-1}$ in water solution to 9.09 $l \cdot mmol^{-1} \cdot s^{-1}$ in HSA solution. In addition, thermodynamics stability constant of GdL complex was determined, the thermodynamic stability constant of GdL complex ($K_{GdL}=10^{20.84}$) was a few larger than that of $Gd(DTPA)^{2-}$ ($K_{Gd\text{-}DTPA}=10^{20.73}$). The results showed that complex of GdL may be a prospective MRI contrast agent with low osmotic pressure due to non-ion complex, high spin-lattice relaxivity, good stability and binding affinity for the serum protein.

Key words rare earth complex; relaxation property; human serum albumin; thermodynamics stability constant; magnetic resonance imaging (MRI) contrast agent

Magnetic resonance imaging (MRI) is at present well established as a safe and efficient imaging technique for the human body in clinical diagnosis.[1)] Therefore, studying MRI contrast agents is more important. Several types of paramagnetic metal ion-chelate complexes with various ligands have been proposed for use as contrast-enhancing agents in MRI.[1—3)] $Gd(DTPA)^{2-}$ was well-established contrast agent in clinical use for MRI due to its high relaxivity, high chemical stability and low toxicity.[3)] However, its passive and nonspecific distribution *in vivo* limits its utility in focal lesion detection and, moreover, its ionic characteristic leads to some side effects associated with its hyperosmolality at clinical dose.[1,4)] In attempts to decrease the side effects and improve the tissue- and/or organ-specificity, considering that isoniazid is a very useful medicament to treat tuberculosis and its excellent solubility in water that we designed and synthesized a novel ligand from DTPA and isoniazid to improve the tissue- and/or organ-specificity and enhance affinity to water. Its five neutral rare earth (RE=La, Sm, Eu, Gd, Tb) complexes holding promise of magnetic resonance imaging (MRI) were synthesized, and relaxivity of complexes and $Gd(DTPA)^{2-}$ used as a control were determined in water solution. The relaxivity of GdL has also been investigated in human serum albumin (HSA) solution. In addition, thermodynamics stability constant of GdL was determined. The results showed that complex of GdL was a prospective MRI contrast agent with low osmotic pressure due to non-ion complex, high spin-lattice relaxivity, good stability and binding affinity for the serum protein.

Experimental

Materials HSA was purchased from Sigma Chemical Co. and was used without any further purification. $RE_2(CO_3)_3$ was purchased from Shanghai Yuelong Chemical Works. DTPA, EDTA and isoniazid were purchased from Shanghai Reagent Factory. All other chemicals used were of analytical grade.

Measurements Carbon, hydrogen, and nitrogen were analyzed on an Elemental Vario EL analyzer. The metal content of the complexes were determined by titration with EDTA. Melting points of the compounds were determined on an XT4-100x microscopic melting point apparatus (Beijing Electrooptical Instrument Factory, China). IR spectra were obtained in KBr discs on a Nicolet 5-DX spectrometer in the 4000—400 cm^{-1} region. ^{1}H-NMR spectra were recorded on a Varian VR 300-MHz spectrometer. Conductivity measurement was performed in DMF with a DDS-11A conductometer at 25 °C (Shanghai Jingke Instrument Factory, China).The thermal behavior was monitored on a PCT-2 differential thermal analyzer (Beijing Guangxue Instrument Factory, China). Mass spectrum was performed on a VG ZAB-HS (FAB) instrument.

Relaxation time of complexes were measured referring to literature[5)] by an inversion-recovery pulse sequence on a Bruker AC-80 NMR spectrophotometer, using the INVREC Au program at a 90° pulse width $t_p(90°)=2.8\,\mu s$.

Thermodynamics stability constant of Gd(III)L complex was measured referring to literature[6)] by potentiometric measurement (T=25 °C, 0.10 mol/l NaCl).

Preparation of Ligand The synthetic routes of ligand was shown in Fig. 1. Ligand was synthesized as following:

Diethylenetriamine pentaacetic bi-anhydride (DTPAA) has been prepared according to references.[7,8)]

2 mmol of isoniazid was dissolved in 25 ml of distilled water, 1 mmol of DTPAA and 5 ml of pyridine (Py) was placed in the aqueous solution, with stirring for 24 h at room temperature. H_2O and Py were removed under reduced pressure and the residue was diluted with H_2O and filtered. Then a drop of the solution of EtOH and Et_2O (v/v=1/2) added into filtrate, and a large amount of white precipitate separated out. Filter and was washed three times with a little EtOH. Finally, product was dried in a vacuum with P_4O_{10}. Recrystallization from H_2O–Et_2O–EtOH gain the final product. Yield: 86.2%. Melting point: 138—140 °C. ^{1}H-NMR (300 MHz, D_2O, 25 °C) δ: 3.15, 3.32 (each 4H, br s, $-NCH_2CH_2N-$), 3.56 (s, 10H, $-CO-CH_2-N$), 4.26 (s, 4H, –NH–NH–), 7.72 (d (J=7.6 Hz), 4H, Py-H), 8.74 (d (J=7.6 Hz), 4H, Py-H). FAB-MS: $[M+H]^+=632$.

Ligand was easily soluble in water, DMF and DMSO, slightly soluble in ethanol, insoluble in acetone, chloroform, and Et_2O, quite stable at room temperature and normal pressure. Elemental analysis, and empirical formula are shown in Table 1. Elemental analysis was in good agreement with the structure. Mass spectrum reveals that the molecular ion peak was in accor-

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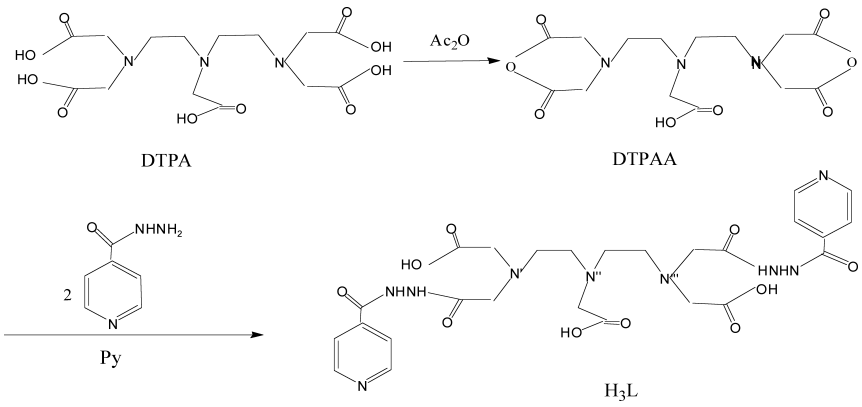

Fig. 1. The Synthetic Routes of the Ligand

Table 1. Elemental Analyses and Molar Conductivity of Ligand and Complexes

Empirical formula	Yield (%)	Color	C%	N%	H%	Ln	Λ
			(Cal)%	(Cal)%	(Cal)%	(Cal)%	$S\cdot cm^2\cdot mol^{-1}$
H_3L	86.2	White	49.35	19.80	5.38		
$C_{26}H_{33}N_9O_{10}$			(49.44)	(19.96)	(5.27)		
$LaL\cdot 2H_2O$	97.6	Light yellow	38.91	15.79	4.35	17.2	12.6
$C_{26}H_{34}N_9O_{12}La$			(38.86)	(15.69)	(4.27)	(17.3)	
$SmL\cdot 2H_2O$	97.8	Light yellow	38.30	15.47	4.21	18.4	14.1
$C_{26}H_{34}N_9O_{12}Sm$			(38.32)	(15.41)	(4.23)	(18.3)	
$EuL\cdot 4H_2O$	98.0	Light yellow	36.50	14.63	4.51	17.5	17.0
$C_{26}H_{38}N_9O_{14}Eu$			(36.63)	(14.79)	(4.49)	(17.8)	
$GdL\cdot 5H_2O$	98.5	Light yellow	35.78	14.33	4.86	17.9	19.6
$C_{26}H_{40}N_9O_{15}Gd$			(35.65)	(14.39)	(4.60)	(18.0)	
$TbL\cdot 2H_2O$	98.1	Light yellow	37.95	15.22	4.10	19.2	11.1
$C_{26}H_{34}N_9O_{12}Tb$			(37.92)	(15.31)	(4.16)	(19.3)	

Λ: molar conductivities of the complexes; Ln: La, Sm, Eu, Gd, Tb.

dance with the given structure. ^{1}H-NMR and IR were conformed to the given structure.

Preparation of Complexes First, 1 mmol of H_3L was dissolved in 25 ml of distilled water to form a homogeneous solution which pH is approximately 3. Then an excess of $La_2(CO_3)_3$ was added to the system which was stirred on a water bath until the pH of the system is approximately 7. The excess $La_2(CO_3)_3$ was filtered off. Then the filtrate was concentrated on a water bath until it is nearly dry. The light yellow powder was then obtained after the mother liquor was removed. Finally, it was dried in vacuum with P_4O_{10}. Yield: 97.6%. Other complexes were synthesized by the same way. Yield: SmL=97.8%, EuL=98.0%, GdL=98.5%, TbL=98.1%.

Results and Discussion

The Composition and General Character of Complexes All complexes are easily soluble in water, *N*,*N*-dimethylformamide (DMF) and dimethylsulfoxide (DMSO), and slightly soluble in ethanol, insoluble in acetone, chloroform, and aether, quite stable at room temperature and normal pressure and not sensitive to light. Elemental analyses, and empirical formula are shown in Table 1. The elemental analyses show that the formulas of the complexes are $REL\cdot nH_2O$ (RE=La, Sm, Tb, n=2; RE=Eu, n=4; RE=Gd, n=5). Elemental analysis was in good agreement with the structures, respectively. The molar conductivities of the complexes are around 11.1—19.6 $S\cdot cm^2\cdot mol^{-1}$ in DMF (Table 1), which shows that complexes were non-electrolytic nature in DMF, which was composed of metal ions and ligand in a proportion of 1 : 1.[9)]

IR Spectra The main stretching frequencies of the IR spectra of the ligand and their complexes are tabulated in Table 2. It can be found that the characteristic absorption peaks of all complexes are similar. In the spectra of H_3L, the carboxylic ν(COOH) appears at 3500 cm^{-1}, the peak is absent in the complexes, but there is a broad peak at around 3218—3487 cm^{-1} in the complexes, which shows that there is H_2O in the complexes. A medium band at 1571—1582 cm^{-1} and a weak absorption at 537—544 cm^{-1} found only in the complexes, which shows that there is coordinating H_2O in the complexes.[10)] The carboxylic $\nu_{(C=O)}$ of free ligand is at 1707 cm^{-1}, which does not appear in the complexes. However, two new vibrations of CO_2^- for ν_{as} and ν_s appear at 1547 and 1395 cm^{-1}, the $\Delta\nu$ ($\nu_{as}-\nu_s$) of which is approximately equal to 152 cm^{-1}. The IR spectra obtained when the sodium salt of the ligands is used as a control show that its ν_{as} and ν_s are at 1550 and 1410 cm^{-1}, respectively, and $\Delta\nu$ ($\nu_{as}-\nu_s$) is 140 cm^{-1}, suggesting that carboxyl oxygen atom coordinated to metal ion with single-dentate.[11)] The vibration at 1226 cm^{-1} in the ligand was shifted to 1215 cm^{-1}, indicating the Gd-N band is really formed but weak.[10)] A new peak for the complex at 515 is assigned to $\nu_{M\text{-}O}$. This further confirms that the coordinate bond has formed between ligand and metal ion. The ligand provided three nitrogen atoms, five carboxyl oxygen atoms bonding to metal ion. At least one water molecule takes part in coordination to metal ion confirming thermal analyses.

Thermal Analyses Some data of thermal analyses are

Table 2. Some Main IR Data of the Ligand and Its Complexes

	Compound					
	H_3L	$LaL\cdot 2H_2O$	$SmL\cdot 2H_2O$	$EuL\cdot 4H_2O$	$GdL\cdot 5H_2O$	$TbL\cdot 2H_2O$
$\Delta\nu_{OH}$	3500b	3228—3467b	3225—3455b	3220—3444b	3218—3487b	3230—3427b
ν_{NH}	3186w	3185m	3186	3189m	3184m	3186m
$\nu_{C=O}$	1707s					
δ_{HOH}		1571s	1580	1582s	1574s	1577s
$\nu_{(HOH)}$		535w	540	545w	532w	541w
ν_{C-N}	1226w	1218m	1215	1217w	1215	1215m
$\nu_{as}(CO_2^-)$		1547m	1551	1552m	1550m	1561m
$\nu_s(CO_2^-)$		1395m	1412	1410m	1398m	1420m
ν(M–O)		521w	520	515w	515w	518w

s=strong; m=medium; w=weak; b=broad.

Table 3. Thermal Analyses Data of the Compounds

Compound	Dehy. (°C)	mp (°C)	H_2O Loss (Calcd) %	Decomposition (°C)		
				t_1	t_2	t_3
H_3L		140		296		
$LaL\cdot 2H_2O$	117		4.45 (4.48)	365	451	477
$SmL\cdot 2H_2O$	120		4.40 (4.42)	364	455	474
$EuL\cdot 4H_2O$	115		8.31 (8.44)	362	456	470
$GdL\cdot 5H_2O$	121		9.99 (10.28)	369	450	471
$TbL\cdot 2H_2O$	114		4.19 (4.37)	368	454	466

Dehy.: Dehydration temperature.

Table 4. Relaxivity of the Complexes

Compound	[M]/ mmol·l^{-1}	t_0/s	T_1/s	$(1/T_1)_p$/s^{-1}	R_1/ mmol·l·s^{-1}
H_2O+D_2O		5.40	0.128		
$LaL\cdot 2H_2O$	0.093	4.90	0.141	0.013	0.14
$SmL\cdot 2H_2O$	0.097	2.40	0.289	0.161	1.66
$EuL\cdot 4H_2O$	0.097	1.60	0.433	0.305	3.14
$GdL\cdot 5H_2O$	0.099	0.95	0.730	0.602	6.08
$TbL\cdot 2H_2O$	0.092	1.80	0.385	0.257	2.79
Gd-DTPA	0.148	0.90	0.770	0.642	4.34

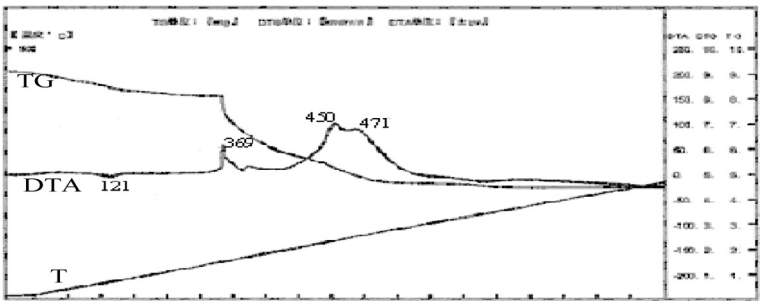

Fig. 2. The TG-DTA Spectra of GdL

listed in Table 3. The differential thermal analysis (DTA) curves of ligand has an endothermic peak at 140 °C, but there is no weight lost on the corresponding thermogravimetry (TG) curves, showing that this is a phase transition process, which is the same as the melting point of the ligand. The complexes have an endothermic peak between 115 and 121 °C. The corresponding TG curves show that the weight loss is equal to two-five water molecules. The results are in accordance with the composition of the complexes as determined by elemental analyses. Three exothermic peaks appear around 362—477 °C. The initial temperature of decomposition is greater than 362 °C, which indicates that the thermal stability of the complexes is higher than that of the free ligand and which decomposed at 296 °C. A typical TG-DTA spectra of GdL was shown in Fig. 2.

Relaxivity of the Complexes in Water Solution The enhancement value of the relaxation rate of the complex for water protons is calculated by the equation.[5)]

$$(1/T_1)_p=(1/T_1)_o-(1/T_1)_d$$

$$R_1=(1/T_1)_p/[M]$$

Where $(1/T_1)_o$ is the observed solvent relaxation rate in the presence of a paramagnetic species, $(1/T_1)_d$ is the solvent relaxation rate in the absence of a paramagnetic species, and $(1/T_1)_p$ represents the additional paramagnetic contribution. [M] is the concentration of paramagnetic metal ion.

The relaxivity mainly consists of two components: the inner-sphere and outer-sphere relaxivity. The high relaxivity is favourable of tissue imaging. The relaxivity (R_1) of complexes and $Gd(DTPA)^{2-}$ used as a control were given in Table 4. The results showed that the spin-lattice relaxivity of GdL was larger than that of $Gd(DTPA)^{2-}$. Measurement of relaxation time of GdL was shown in Fig. 3.

Thermodynamic Stability Constant of the GdL Complex The normal chelate of thermodynamic stability constants is expressed as in equation[6)]:

$$K=[ML]/[M^{n+}][L^{n-}]$$

Where M^{n+} represents the free, unhydrolysed aqua-metal

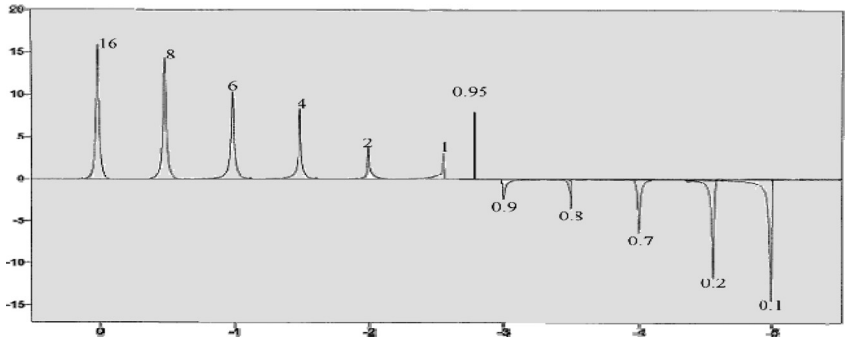

Fig. 3. Measurement of Relaxation Time of GdL

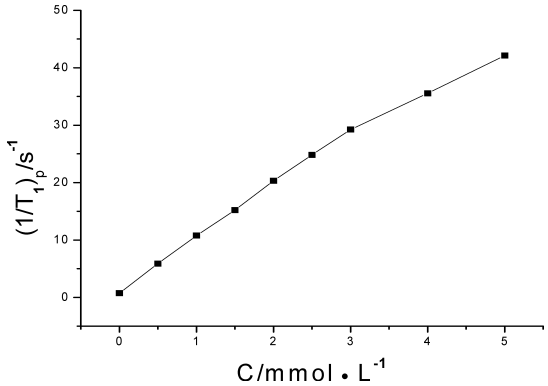

Fig. 4. The Longitudinal Relaxation Rates of Water Protons in 0.6 mmol HSA Solution on Increasing the Concentration of GdL (0—5 mmol/l)

ion, L^{n-} the uncomplexed, totally deprotonated from the ligand and ML is the normal unprotonated and unhydrolysed complex. Thermodynamic stability constant of GdL ($K_{GdL}=10^{20.84}$) was a few larger than that of $Gd(DTPA)^{2-}$ ($K_{Gd\text{-}DTPA}=10^{20.73}$).

Relaxivity of the GdL Complex in HSA Solution HSA is one the most important plasma proteins involved in drag delivery in the body. The albumin concentration in human plasma is close to 0.6 mmol/l.[12] For the application in MRI, it may be useful to study the relaxation rates of the complex at the physiological concentration of HSA.

In Fig. 4 we report the behaviour of the longitudinal relaxation rates of water protons on increasing the concentration of GdL (0—5 mmol/l) in 0.6 mmol/l HSA solution. The relaxivity (R_1) of Gd(III)L of complex was $9.091\cdot mmol^{-1}\cdot s^{-1}$ which was larger than that of in water solution ($R_1=6.081\cdot mmol^{-1}\cdot s^{-1}$). The results showed that Gd(III)L of complex displays a binding affinity for the serum protein.

Conclusions

Taken together, neutral Gd(III) complex derivative from diethylene triamine pentaacetic acid and isoniazid may be a prospective MRI contrast agent with low osmotic pressure due to non-ion complex, high spin-lattice relaxivity, good stability and binding affinity for the serum protein.

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