

Lanthanoid Endohedral Metallofullerenols for MRI Contrast Agents

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Received July 3, 2002 ; E-mail: noris@cc.nagoya-u.ac.jp

Abstract: Water-soluble multi-hydroxyl lanthanoid (La, Ce, Gd, Dy, and Er) endohedral metallofullerenes (metallofullerenols, $M@C_{82}(OH)_n$) have been synthesized and characterized for the use of magnetic resonance imaging (MRI) contrast agents. The observed longitudinal and transverse relaxivities for water protons, r_1 and r_2 , of the metallofullerenols are in the range 0.8–73 and 1.2–80 ($\text{sec}^{-1}\text{mM}^{-1}$), respectively, which are significantly higher than those of the corresponding lanthanoid-DTPA chelate complexes. Among these Gd-metallofullerenols, $Gd@C_{82}(OH)_n$ has exhibited the highest r_1 and r_2 values in consistent with our previous results. The observed large r_1 of the current metallofullerenols can mainly be ascribed to the dipole–dipole relaxation together with a substantial decrease of the overall molecular rotational motion. The large r_2 , except for the Gd-metallofullerenols, have been attributed to the so-called Curie spin relaxation. The MRI phantom studies are also performed and are consistent with these results. The metallofullerenols will be an ideal model for future MRI contrast agents with higher proton relaxivities.

1. Introduction

Endohedral metallofullerenes (fullerenes with metal atom(s) encapsulated) are a novel form of fullerene-related materials and have been studied extensively during the past decade in terms of electronic, structural, and solid-state properties.¹ One of the most important and novel electronic properties of the metallofullerenes is the so-called intrafullerene electron transfer from the encaged metal atom(s) to fullerene cages.^{1–3} For example, mono-metallofullerenes, $M@C_{82}$ (M: metal atoms), have been shown to have electronic structures formally expressed as $M^{3+}@C_{82}^{3-}$ ^{2,3} and $M^{2+}@C_{82}^{2-}$ ^{4,5} as a result of the electron transfer.

Although these fundamental structural and electronic properties of endohedral metallofullerenes have been well investigated,¹ only a few studies^{6–8} have been reported for chemical functionalization of the metallofullerenes. In our previous study,⁷ we have shown that an application of water-soluble metallo-

fullerenes to magnetic resonance imaging (MRI) contrast agents is one of the most intriguing aspects of the metallofullerene study.^{1–10}

Gadolinium chelate compounds are so far the most popular MRI contrast agents and have actually been used as commercial contrast agents.^{11–14} Such Gd chelate complexes have been used for several protocols to assess abnormalities such as brain tumors and hepatic carcinoma. Although MRI provides a superb imaging resolution, it oftentimes suffers from a limited sensitivity. It is thus necessary to accumulate more targeting probes or to enforce its potential image-enhancing ability for molecular/cellular diagnosis by MRI.^{15–18} Under these circumstances, novel materials having stronger proton relaxivity and higher MR signal enhancement at significantly lower concentration are required for the next generation.

Recently, we have found that water-soluble polyhydroxylated gadolinium metallofullerenols, $Gd@C_{82}(OH)_n$, have a very

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strong ability of reducing water proton relaxation times, T_1 and T_2 (the ability of the reagent is often expressed as relaxivities r_1 and r_2) both in vivo and in vitro.⁷ The observed r_1 values of $\text{Gd@C}_{82}(\text{OH})_n$ were more than 20 times as higher as that of the commercial MRI contrast agent such as Gd-DTPA (Magnevist).⁷

Similar but lower relaxivities have also been reported by Wilson and co-workers^{19,20} and Zhang et al.²¹ The $\text{Gd@C}_{82}(\text{OH})_n$ metallofullerenols have also been characterized by FT-IR, elemental analyses, and electron energy loss spectroscopy (EELS)²² in terms of the structural and electronic properties.

Here, we present a systematic investigation of a series of lanthanoid metallofullerenols, $\text{M@C}_{82}(\text{OH})_n$ ($\text{M} = \text{La}, \text{Ce}, \text{Gd}, \text{Dy}, \text{and Er}$), to obtain information on the proton relaxation mechanism of these novel forms of MRI contrast agents. We have found that both the r_1 and r_2 of these metallofullerenols are much higher than those of the corresponding free ions and lanthanoid-DTPA compounds.

II. Experimental Section

Materials. Carbon disulfide, pyridine, toluene, NaOH, methanol, diethylenetriaminepentaacetic acid (DTPA), $\text{Gd(III)Cl}_3 \cdot 6\text{H}_2\text{O}$, $\text{Ce(III)Cl}_3 \cdot 6\text{H}_2\text{O}$, $\text{La(III)Cl}_3 \cdot 7\text{H}_2\text{O}$, and $\text{Er(III)Cl}_3 \cdot 6\text{H}_2\text{O}$ were purchased from Wako Chemicals, and tetrabutylammonium hydroxide and 15-crown-5 were purchased from Aldrich. A cellulose dialyzer tube (cut off M. W. = 500) was purchased from Spectrum Laboratories, Inc.

Instrumentation. High-performance liquid chromatography (HPLC) separation of the lanthanoid endohedral metallofullerenes, M@C_{82} ($\text{M} = \text{La}, \text{Ce}, \text{Gd}, \text{Dy}, \text{and Er}$), were performed with UV (312 nm) detection on a 28×300 mm Buckyprep column (Nacalai Tesque) and a 21×500 mm Buckyclutcher column (Regis Chemical Co.) with toluene eluent. The purity of M@C_{82} was quantified by both laser-desorption/ionization (LD) TOF mass spectrometry (Shimadzu Kompact MALDI IV) and UV-vis-NIR absorption measurements (Shimadzu UV-3101PC).

Separation and Isolation of Metallofullerenes. Carbon soot containing endohedral metallofullerenes were produced by the direct current arc discharge method (500 A, 20–23 V) of metal/graphite composite rods ($15 \times 15 \times 300$ mm, 0.8 at. % of La, Ce, Gd, Dy, and Er, Toyo Tanso Co. Ltd.)¹ under a 17 l/min flow of helium at 60 Torr. The resulting soot were Soxhlet extracted with carbon disulfide for 60 h and the residue of the Soxhlet extraction were further extracted with pyridine by reflux for 3 h.

The separation of the various M@C_{82} from soot extract was achieved by the two-stage HPLC method.¹ Stage 1 is a preliminary HPLC process on the Buckyprep column. At this stage, we obtained fraction containing of $\text{M@C}_{82}(\text{I})$ and $\text{M@C}_{82}(\text{II})$, where (I) and (II) denote the structural isomer's numbering according to the increasing order of HPLC retention time on Buckyprep columns (cf. Figure 1a, b). In the second stage, $\text{M@C}_{82}(\text{I})$ and $\text{M@C}_{82}(\text{II})$ were isolated from various empty fullerenes on the Buckyclutcher column.

Syntheses of Metallofullerenols. Details of the synthesis of endohedral metallofullerenols have been reported elsewhere.^{7,22,23} Briefly, toluene solution of isolated $\text{M@C}_{82}(\text{I})$ was vigorously stirred with 50

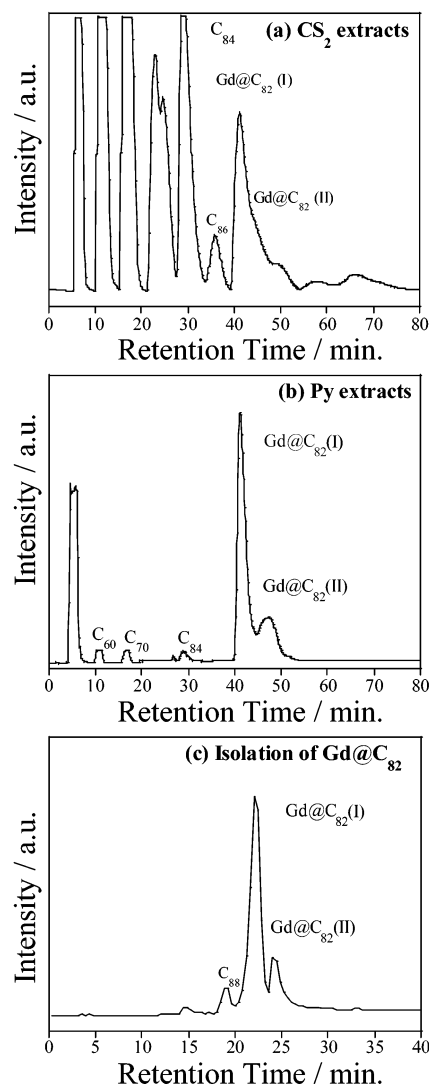


Figure 1. Isolation scheme of $\text{Gd@C}_{82}(\text{I})$ by the two stage HPLC method: (a) the first stage HPLC for CS_2 extracts using Buckyprep column at 30 mL/min flow with toluene 100% eluent; (b) the first stage HPLC for pyridine extracts using Buckyprep column at 30 mL/min with toluene 100% eluent. (c) the second stage HPLC using Buckyclutcher-I column at 10 mL/min with 100% toluene eluent. $\text{Gd@C}_{82}(\text{I})$ is easily separated from the C_{88} and $\text{Gd@C}_{82}(\text{II})$ fractions.

wt % of aqueous NaOH containing few drops of tetrabutylammonium hydroxide (40% in water) or 15-crown-5 as catalyst at room temperature. Isomer-free metallofullerenes $\text{M@C}_{82}(\text{I})$ ($\text{M} = \text{La}, \text{Ce}, \text{Gd}, \text{and Er}$) were used in the following fullerene synthesis except for a mixture of $\text{Dy@C}_{82}(\text{I})$ and $\text{Dy@C}_{82}(\text{II})$ isomers.²⁴

The reaction mixture was removed by decantation. Aqueous solution was precipitated with methanol, and the precipitation was filtrated and rinsed with methanol to ensure the removal of the catalyst and NaOH. The rinsed precipitates were dissolved in water. The resultant brown solution was put into a cellulose dialyzer tube (cut off M. W. = 500) in water for a week for the complete removal of residual catalyst and NaOH. Finally, purified metallofullerenols were obtained after filtration with a membrane filter (0.50 μm).

Syntheses of Lanthanoid-DTPA Complexes. $\text{Gd(III)Cl}_3 \cdot 6\text{H}_2\text{O}$, $\text{Ce(III)Cl}_3 \cdot 6\text{H}_2\text{O}$, $\text{La(III)Cl}_3 \cdot 7\text{H}_2\text{O}$, and $\text{Er(III)Cl}_3 \cdot 6\text{H}_2\text{O}$ were dissolved in distilled water. A 1 mol/L HNO_3 solution of $\text{Dy(III)(NO}_3)_3$ was used for preparing Dy-DTPA. 0.1 mmol/L and 1 mmol/L NaOH were added to the Gd, Ce, La, and Er solution and the Dy solution, respectively,

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until pH 7 was reached. These neutralized solutions were slowly added to 1.05 eq of DTPA aqueous solution, while maintaining pH 7 by addition of further 0.1 mmol/L NaOH.²⁵

Proton Relaxivity. The proton relaxivities, r_1 and r_2 (corresponding to $1/T_1$ and $1/T_2$ of solvent water, respectively) of the metallofullerenols, the lanthanoid-DTPA complexes, and the corresponding lanthanoid metal ions in aqueous solution were determined at pH 7 ± 1 at concentrations of 0.1, 0.5, and 1.0 mmol metal/L. The concentrations of La, Ce, Gd, Dy, and Er in the aqueous solution were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, SPS-3000, Seiko Electro Ind.) at 333.749, 418.660, 342.247, 353.170, and 337.271 nm, respectively.^{7,26} Lanthanoid standard solutions (1000 μg metal/mL in 1 mol/L of HNO_3) were used for the calibration of the measurements.

The relaxivities were determined at four different resonance frequencies: 20 MHz with NMS-minispec from Bruker at 0.47 T, 40.5 MHz with MAGNETOM Harmony from Siemens with a horizontal magnet of 1 T, 200 MHz with Unity INOVA from Varian with horizontal magnet at 4.7 T and 400 MHz with Unity INOVA from Varian at 9.4 T. The inversion–recovery method was used for measurements of T_1 at 20, 200, and 400 MHz. The spin–echo method with various repetition time (TR) was used at 40.5 MHz.

For T_2 measurements, the Carr–Pucell–Meiboom–Gill method was used normally at 20 and 400 MHz and in some cases at 200 MHz. Fast spin–echo was the main sequence used at 200 MHz. Spin–echo with various echo time (TE) was used for the measurements of T_2 at 40.5 MHz. The sample temperature was controlled by a built-in regulator at 20 and 400 MHz to better than ± 0.5 °C. In the case of horizontal magnet systems, temperature was controlled to ± 1 °C by a homemade system with a fluorescence thermometer AMOTH FX-8501 from Anritsu.

Spin State. The electron spin state of the endohedral Gd-metallofullerenols was determined by using the magnetic susceptibility of the solution. ^{19}F NMR signals from hexafluorobenzene in benzene solution (1/400, ratio of volume; sealed in a crank shaped capillary) measured in the sample solution in a 10 mm NMR tube at 23 °C with 500 MHz (Varian, INOVA) were analyzed according to the method described in Appendix A.

MRI. The NMR phantom images of lanthanoid ions, lanthanoid-DTPA complexes as well as the metallofullerenols solutions at concentrations of 0.1, 0.5 and 1.0 mmol metal/L were obtained at 40.5 MHz by the conventional spin–echo technique with TR/TE = 17–5000 ms/6 ms, FOV = 19×19 cm², slice thickness of 6 mm, and data point of 256×256 .

III. Results and Discussion

Electron Spin State. The valence state of Gd in the fullerenols, $\text{Gd}@C_{82}(\text{OH})_n$, has been known as Gd(III) by electron energy loss spectroscopy (EELS).²² The three electrons from the 6s and 5d orbitals are transferred to the fullerene cages which presumably has a doublet spin state, resulting in the electronic structure $\text{Gd}^{3+}@[C_{82}(\text{OH})_n]^{3-}$. To obtain information on the spin coupling between $4f^7$ electrons of Gd^{3+} and the transferred electrons on the cage, ^{19}F NMR measurements of the magnetic susceptibility were performed.

The relative shift of ^{19}F NMR signals, $\Delta\nu$, between the parallel part and the perpendicular part of the capillary with respect to static magnetic field B_0 was used as a measure of magnetic susceptibilities of the solution. The details for deter-

mination of the molecular magnetic moment are given in Appendix A. The $\Delta\nu$ values at 23.0 °C obtained for Gd-fullerenols at 0.485 mmol/l and Gd-DTPA at 1 mmol/l were 39.0 ± 0.3 Hz ($\mu_{\text{eff}}/\mu_B = 7.9 \pm 0.3$) and 75.3 ± 1 Hz ($\mu_{\text{eff}}/\mu_B = 7.7 \pm 0.1$), respectively. The results are consistent with the spin state expected from a compound with $S = 7/2$ ($\mu_{\text{eff}}/\mu_B = 7.94$) or with an independent contribution from $S = 7/2$ and $1/2$ ($\mu_{\text{eff}}/\mu_B = 8.12$). Thus, we could exclude the spin states such as $S = 8/2$ ($\mu_{\text{eff}}/\mu_B = 8.94$) and $S = 6/2$ ($\mu_{\text{eff}}/\mu_B = 6.93$). The spin coupling between the $4f^7$ electrons of Gd^{3+} and the electrons in the cage should, therefore, be very small. The negligible coupling between $4f^7$ and cage electrons for $\text{Gd}@C_{82}(\text{OH})_n$ is also supported by the spectral analysis of X- and W-band EPR at 20 K,²⁷ where a sharp single line with $g = 2$ has been observed in X-band ambient temperature EPR spectrum of aqueous solution of $\text{Gd}@C_{82}(\text{OH})_n$.

Proton Relaxation Times. For paramagnetic aqueous solution, the longitudinal and transverse relaxation rates ($1/T_1$ and $1/T_2$, respectively) of the solvent proton spin are known to depend on the concentration of the paramagnetic species ($[\text{M}]$) as eq 1

$$(1/T_i)_{\text{obsd}} = (1/T_i)_d + r_i \times [\text{M}] \quad i = 1, 2 \quad (1)$$

where $(1/T_i)_{\text{obsd}}$ is the observed relaxation rate of water proton in the presence of paramagnetic species, and $(1/T_i)_d$ is the (diamagnetic) relaxation rate in the absence of the paramagnetic species. The relaxivity, r_i , commonly expressed in units of $\text{mM}^{-1}\text{sec}^{-1}$, reflects the relaxation enhancement ability of a paramagnetic compound. The proton relaxivities, r_1 and r_2 , of the metallofullerenols were determined at 20, 40.5, 200, and 400 MHz at 37 ± 1 °C. In general, the proton spin relaxation rate, $(1/T_i)_{\text{obsd}}$, can be expressed as a combination of interactions such as dipole–dipole interaction, contact interaction, and curie spin relaxation.^{14,28} Because the encapsulated lanthanoid ions in the fullerenols cages do not contact directly to water molecules, we can neglect the contact interaction.

Figures 2 (a) show r_1 and r_2 of the lanthanoid ions at 20, 40.5, 200, and 400 MHz, respectively. Obviously, the low magnetic moment ions, $\text{La}^{3+}(4f^0)$ and $\text{Ce}^{3+}(4f^1)$, have almost zero proton relaxivities, whereas high magnetic moment ions, $\text{Gd}^{3+}(4f^7)$, $\text{Dy}^{3+}(4f^9)$, and $\text{Er}^{3+}(4f^{11})$, exhibit higher proton relaxivities. Among these, Gd^{3+} ion with seven unpaired electrons in the half-filled $4f$ orbital exhibits the strongest relaxation capability of proton spins for nearby water molecules. The obtained values are in good agreement with the proton relaxation of paramagnetic solutions.²⁹

Similar tendencies were obtained for the lanthanoid-DTPA complexes, the conventional style of MRI contrast agents, at 20 MHz (cf. Table 1). The lanthanoid-chelate complexes have one direct coordination window to a water molecule that rapidly exchanges with the bulk water molecules surrounding the lanthanoid ions. This leads to an efficient nuclear spin relaxation of the solvent water molecules. Although Dy^{3+} and Er^{3+} possess higher magnetic moments than that of Gd^{3+} , the proton

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Table 1. Relaxivities of Lanthanoid Compounds at 0.47 T^a

Ln compds	r_i	La	Ce	Gd	Dy	Er
ions ^b	r_1	0.0	0.0	12	0.5	0.4
	r_2	0.0	0.0	14	0.6	0.5
M-DTPA ^b	r_1	0.0	0.0	4.4	0.1	0.1
	r_2	0.0	0.0	5.0	0.1	0.1
fullerenols	r_1	0.8	1.2	73	1.1	1.3
	r_2	1.2	1.6	80	1.9	1.5

^a The proton relaxivities r_1 and r_2 were determined at 20 MHz (Minispec, Bruker), 19 ± 1 °C and pH 7 ± 1 . ^b The corresponding relaxivities of pure ions and metal-DTPA complexes are shown for comparison.

relaxation of Dy³⁺ and Er³⁺ are known to be smaller than that of Gd³⁺.²⁹ The main reason of this is due to the difference in the electron spin relaxation times as described in Appendix B. In contrast, the observed relaxivities of metallofullerenols at 20, 40.5, 200, and 400 MHz show a novel tendency that even ions with low magnetic moments such as La³⁺ and Ce³⁺ have substantial proton relaxivities (cf. Figures 2b).

Relaxation Mechanism. The present proton spin relaxation due to metallofullerenols within the framework of dipole or Curie spin seems to belong to the so-called “second sphere mechanism”. In the metallofullerenols, water molecules are not directly coordinated to the central metal ions. Because of the presence of hydrogen bonds together with the negatively charged surface of the metallofullerenols, motional freedom of the first hydration water molecules should be much less than those of bulk water molecules. In such a case, the most effective motion for the proton relaxation should be rotational motion.

The origins of the observed high r_1 and r_2 values of the metallofullerenols are closely related to a couple of novel features that the present metallofullerenols inherently possess: (1) the intra-metallofullerenols electron transfer, (2) large reorientational motion of the metallofullerenols in water solution, and (3) a large number of surrounding water molecules.

A. Electron Spins on the Metallofullerenol Cage. We have shown already that Gd³⁺ retains electron spin quantum number S equal or close to $7/2$ in Gd@C₈₂(OH)_{*n*} fullerenols by the susceptibility measurement. Furthermore, our preliminary EPR studies²⁷ on Gd@C₈₂(OH)_{*n*} in water show a clear paramagnetic signal at room temperature which is ascribed to long relaxation time of electron spins of the Gd³⁺ ion as well as the one on the fullerene cage.

The results are consistent with the long relaxation time of Gd 4*f*⁷ electron spins as known for Gd³⁺ ions. Consequently, the observed strong proton relaxivities of the Gd@C₈₂(OH)_{*n*} fullerenols can primarily be interpreted in terms of the dominant contribution from dipole–dipole interaction similar to the Gd ion and Gd-DTPA cases.³⁰

The presence of an electron spin on the fullerene cages thus play crucial roles in the observed high relaxivities. As described earlier, the M@C₈₂(OH)_{*n*} fullerenols have paramagnetic property due to the electron transfer from the engaged metal atom to the C₈₂ fullerene cage, and the formal electronic structures of the fullerenols can be expressed as M³⁺@[C₈₂(OH)_{*n*}]³⁻ similar to the intact M³⁺@C₈₂³⁻ metallofullerenes. The substantial r_1 and r_2 values of La-metallofullerenols (cf. Table 1) can partly be explained by the presence of the cage electron. All the

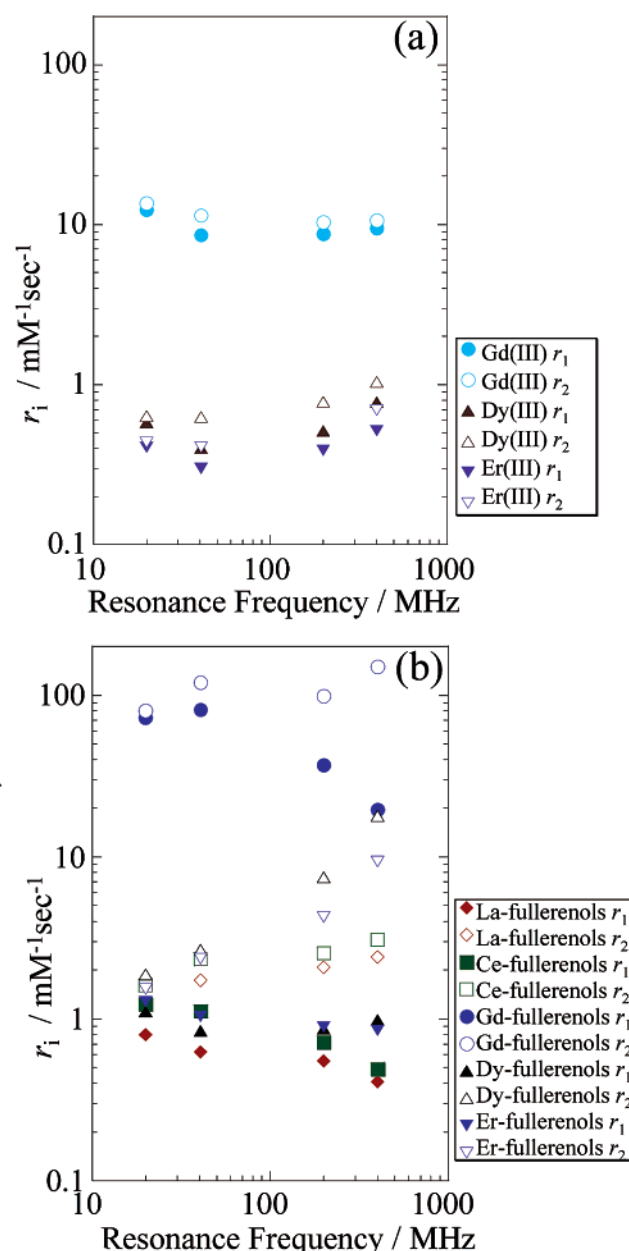


Figure 2. (a) Relaxivities of lanthanoid ions at 20, 40.5, 200, and 400 MHz (37 ± 1 °C, pH 7 ± 1). (b) Relaxivities of metallofullerenols at 20, 40.5, 200, and 400 MHz (37 ± 1 °C, pH 7 ± 1). Note that even La- and Ce-metallofullerenols exhibit proton relaxation ability (see text).

relaxivities of the other metallofullerenols must include common contribution from these cage electrons.

B. Reorientational Motion of Metallofullerenols. The other important factor for the observed high r_1 values is related to a special feature of the metallofullerenols. The temperature dependence of r_1 obtained for Gd ion, Gd-, and La-metallofullerenols are shown in Figure 3. The r_1 of Gd ion decreases as the temperature increases whereas r_1 of Gd- and La-metallofullerenols exhibit much different behavior.

In terms of dipolar interaction, such behaviors are explained by the correlation time τ_R for the overall molecular motion when the main motion modulating the dipolar field for the proton is rotation or reorientational motion with respect to the static magnetic field B_0 (cf. eqs B1 and B2 in Appendix B).³¹ At high

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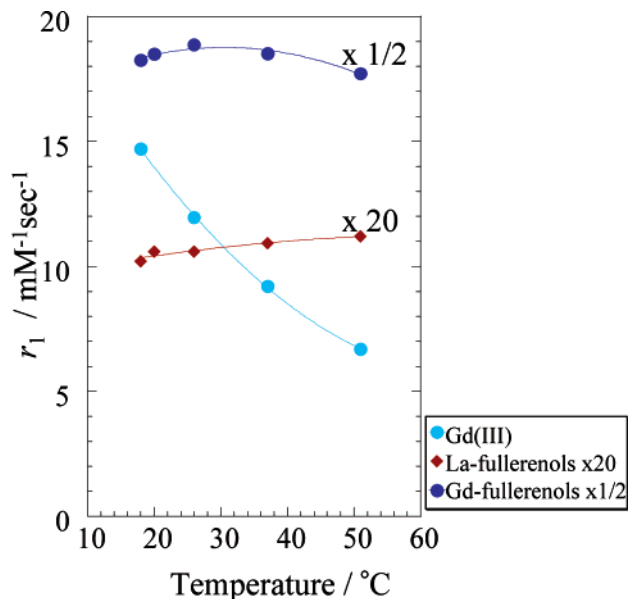


Figure 3. Temperature dependence of r_1 of Gd^{3+} ion, Gd-metallofullerenols ($\times 1/2$), and La-metallofullerenols ($\times 20$) at 200 MHz.

proton resonance frequencies, where the ω_S term is not important, $1/T_{1M}$ increases with τ_R until $\omega_1\tau_R$ approaches unity and decreases toward larger τ_R .

The present results can be explained by this dipolar interaction scheme. The reorientational motion of Gd ion in water solution is very rapid ($\tau_R = 41$ ps at 25 °C).³⁰ The observation that the Gd-metallofullerenols show a broad maximum in r_1 near 25 °C (cf. Figure 3) suggests $\omega_0\tau_R = 1$ at 200 MHz which, in turn, leads to $\tau_R = 0.8$ ns. This is much larger than the corresponding correlation time ($\tau_R = 41$ ps) of a Gd^{3+} –water molecule pair. The observed extremely large reorientational correlation time for the metallofullerenols increases substantially the proton relaxivities of the species.

Another well-known method for increasing molecular rotational motion (τ_R) is to attach the conventional chelates to a larger molecule, such as dendrimers, proteins, or biopolymers, which consequently increases the relaxivity of the chelate.³² The present experimental data strongly suggest that metallofullerenols have slower molecular rotational motion (τ_R) than expected correlation times from its molecular size. Metallofullerenols are highly hydrophilic and have many coordinating water molecules surrounded via hydrogen bonds. Although there is no experimental evidence on the dynamical structure of surrounding water molecules, the strong hydrophilic character of the metallofullerenols may increase effective hydrodynamic radius and thus induce substantial decrease in the overall molecular rotational motion (τ_R). Formation of aggregates also increases the hydrodynamic radius and induces slower molecular rotational motion (τ_R); Some of water-soluble fullerenes derivatives as well as C_{60} fullerenols are known to aggregate (or to form clusters) in aqueous solution.³³ Further experimental studies should be required for estimating the particle size of the aggregates (clusters) and for obtaining information on the dynamical

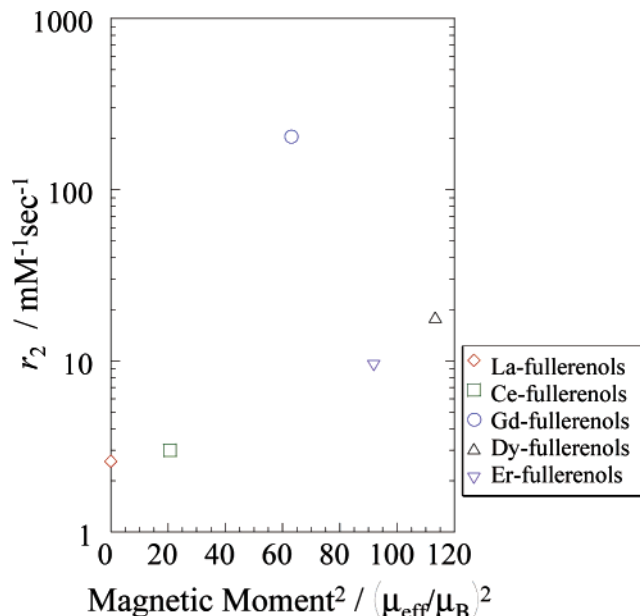


Figure 4. Magnetic moment dependence of r_2 (the effect of $1/T_2$) for metallofullerenols at 400 MHz (37 ± 1 °C and $\text{pH } 7 \pm 1$). The r_2 values of La-, Ce-, Dy-, and Er-metallofullerenols have linear relationship with respect to the square of calculated magnetic moments of free trivalent ions (see text).

behavior of metallofullerenols in aqueous solution and the accompanying effect on the relaxivities.

T_2 -Enhancing MRI Contrast Agents. The r_2 values of the present metallofullerenols increase as the magnetic field B_0 increases (cf. Figure 2), whereas the r_1 values are almost constant with respect to B_0 . The salient dependence of resonance frequency on r_2 should be a result of the so-called Curie nuclear spin relaxation terms³⁴

$$(1/T_{1M}) = [(2/5)(\mu_0/4\pi)^2 \omega_1^2 \mu_B^4 g^4 J^2 (J+1)^2 / r^6 (3kT)^2] [3\tau_c / (1 + \omega_1^2 \tau_c^2)] \quad (2a)$$

$$(1/T_{2M}) = [(1/5)(\mu_0/4\pi)^2 \omega_1^2 \mu_B^4 g^4 J^2 (J+1)^2 / r^6 (3kT)^2] [4\tau_c + 3\tau_c / (1 + \omega_1^2 \tau_c^2)] \quad (2b)$$

where $(\tau_c)^{-1} = (\tau_R)^{-1} + (\tau_M)^{-1}$, $\mu_B^2 g^2 J(J+1)$ the square of effective magnetic moment μ_{eff} , τ_R the correlation time for reorientational motion, and τ_M the exchange lifetime.

The r_2 values of the metallofullerenols at 400 MHz show a clear increase with the square of effective magnetic moments evaluated for the ground-state free trivalent ions (cf. Figure 4).³⁵ The result is consistent with the negligible coupling of 4f electron spins of Ce^{3+} , Dy^{3+} , and Er^{3+} ions in the fullerene cages as in the Gd-metallofullerenols. The longer correlation time for reorientational motion (τ_R) of metallofullerenols significantly enhances r_2 of Curie spin relaxation as in the case of Dy bound to dendrimers³⁶ and albumin³⁷ especially at high

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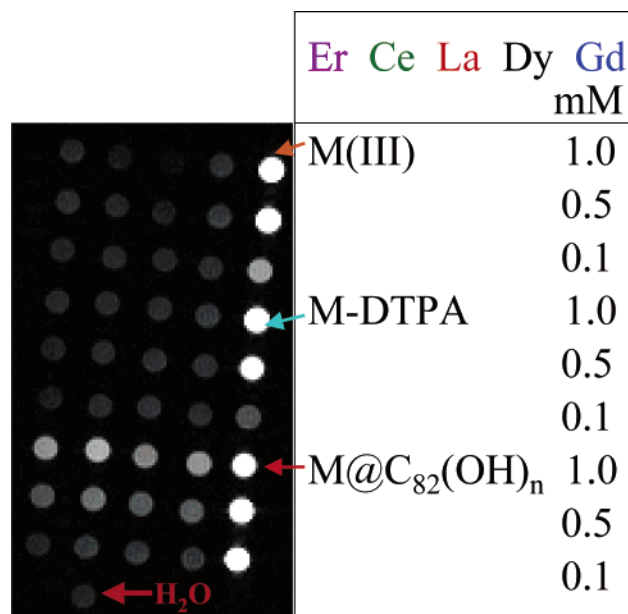


Figure 5. Phantom NMR images of various metallofullerenols (together with those of lanthanoid ions and lanthanoid-DTPA complexes) solutions at 1.0, 0.5, and 0.1 mmol metal/L. All MRI images were obtained by 1 T (MAGNETOM Harmony, Siemens), 1 spin-echo with TR/TE = 200 ms/6 ms, FOV = 19 × 19 cm², slice thickness of 6 mm, 256 × 256 points, and at 20 °C.

magnetic field.^{38,39} The fact that Gd-, Dy-, and Er-metallofullerenols have such high r_2 values strongly suggests that these can be used as T_2 -enhancing MRI contrast agents at higher magnetic field.

Phantom MRI Studies. The present high values of r_1 prompted us to carry out in vitro MRI phantom study of the metallofullerenols on the basis that the sufficient MRI signal enhancement should be obtained at lower Gd concentration as r_1 increases. The measurement was Gd concentration as r_1 increases. The measurement was performed by the T_1 -weighted spin-echo method (40.5 MHz under T_1 weighted condition with TR/TE = 200/6 ms) and was evaluated according to the following relation⁴⁰

$$\text{signal intensity} \propto \rho (1 - e^{-\text{TR}/T_1}) e^{-\text{TE}/T_2} \quad (3)$$

where ρ is proton density, TR pulse repetition time, TE echo time, T_1 longitudinal relaxation time, and T_2 transverse relaxation time.

An extremely strong signal enhancement was observed for Gd compounds whereas another compounds at the same concentration showed only a slight enhancement of MRI signals as compared with pure water (Figure 5). As clearly seen, the MRI signal intensities of all of the present metallofullerenols are much stronger than those of the corresponding metal ions and DTPA complexes. These phantom MRI results were consistent with the high r_1 and r_2 values at 20 MHz in Table 1. The observation that very high Gd concentrations result in decrease of the signal intensity is consistent with the very short T_2 of Gd-metallofullerenols.

IV. Conclusions

We have synthesized, for the first time, multi-hydroxylmetalofullerene derivatives (metallofullerenols) having La, Ce, Gd, Dy, and Er lanthanoid metal atoms inside. The La-, Ce-, Gd-, Dy-, and Er-metallofullerenols have r_1 values in the range 0.8–73 (sec⁻¹mM⁻¹) at 0.47 T. In contrast, the corresponding lanthanoid-DTPA complexes and intact ions have zero or extremely low r_1 . The strong relaxivities of the current metallofullerenols are mainly due to the dipole–dipole relaxation together with a substantial decrease of the overall molecular rotational motion (τ_R). The Curie spin relaxation contributes to r_2 of the metallofullerenols except for Gd-metallofullerenols. Gd-, Dy-, and Er-metallofullerenols can be used as T_2 -enhancing MRI contrast agents at higher magnetic fields. The in vitro MRI phantom studies were also performed and were consistent with these results.

Appendix A

The determination of magnetic susceptibility in solution by NMR is based on the principle that the signal from the capillary perpendicular to B_0 is shifted by $(2\pi/3)(\chi_{\text{capillary}} - \chi_{\text{solution}})$, whereas the one from the parallel part is unaffected.⁴¹ The observed difference $\Delta\nu$ is the measure of susceptibility. Because a common capillary (thus common $\chi_{\text{capillary}}$) was used for the sample solution and solvent, the $\Delta\nu$ values should correspond to those of the solute. Pure water was also measured as the susceptibility standard to calibrate the ¹⁹F sample and nonideal crank shape of the capillary.

Magnetic moment of the solute can be obtained from the molar susceptibility χ_{mol} in equation (A1), where α is the diamagnetic part of the susceptibility, μ_0 the permeability of vacuum, μ_B Bohr magneton, and μ_{eff} the effective magnetic moment of the molecule

$$\chi_{\text{mol}} = N_A[\alpha + \mu_0\mu_{\text{eff}}^2/3kT] \quad (\text{A1})$$

$$(\mu_{\text{eff}}/\mu_B)^2 = 4S(S + 1) \text{ for the case of spin only.} \quad (\text{A2})$$

Appendix B

The dominating term in paramagnetic relaxation for a spherically symmetric ion like Gd³⁺ is the dipolar interaction between electron spin and proton spin as in equation (B1 and B2). Long relaxation time of electron spin (τ_{Si}) paramagnetic ions such as Cr³⁺, Mn²⁺, Eu²⁺, and Gd³⁺ have large proton relaxivities originated from dipole–dipole relaxation^{14,28,30,31}

$$(1/T_{1M}) = [B/r^6][7\tau_{c2}/(1 + \omega_S^2\tau_{c2}^2) + 3\tau_{c1}/(1 + \omega_I^2\tau_{c1}^2)] \quad (\text{B1})$$

$$(1/T_{2M}) = [B/r^6][6.5\tau_{c2}/(1 + \omega_S^2\tau_{c2}^2) + 1.5\tau_{c1}/(1 + \omega_I^2\tau_{c1}^2) + 2\tau_{c1}] \quad (\text{B2})$$

Here, $(\tau_{\text{ci}})^{-1} = (\tau_R)^{-1} + (\tau_M)^{-1} + (\tau_{\text{Si}})^{-1}$, $B = (\mu_0/4\pi)^2\gamma_I^2\gamma_S^2h^2S(S + 1)/30\pi^2$, τ_R correlation time for reorientational motion, τ_M exchange lifetime, τ_{Si} relaxation times of electron spin, ω_S resonance frequency of electron spin, ω_I resonance frequency of proton, r the distance between electron spin and proton, and S spin quantum number of the metal ion.

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Acknowledgment. H.S. thanks the JSPS for Future program on New Carbon Nano-Materials and Special Coordination Funds of the Ministry of Education, Culture, Sports, Science and Technology for the financial support of the present study. H.K. thanks the Japan Society for the Promotion of Science for a Research Fellowship for Young Scientists.

Supporting Information Available: Laser-desorption TOF mass spectra, UV–vis–NIR electronic absorption spectra and ^{19}F NMR spectra of the metallofullerenols. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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