Interactions Between Cyclodextrins and Tm^{III} Chelates of Polyazamacrocycles as Studied by NMR in Aqueous Solution

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The interactions between α-, β-, and γ-CD and the Tm^{III} chelates of the macrocyclic polyaminopolycarboxylates DOTA and NOTA were studied with the use of ^1H- and ^{13}C-NMR shift and relaxation rate measurements. Interactions were only observed between $Tm(DOTA)^-$ and γ-CD. The structure and the stability of the concerning supramolecular structures was elucidated by fitting of the NMR titration curves to a theoretical model. It appears that an inclusion compound is formed, where the hydrophobic macrocyclic part of the chelate sits in the γ-CD cavity. This inclusion compound binds a second $Tm(DOTA)^-$ molecule at the outside lower rim of the CD cone. The binding occurs probably via hydrogen bonds between non-chelated carboxylate oxygen atoms of the concerning $Tm(DOTA)^-$ and CH_2OH groups of the γ-CD molecule, which are in a favorable position due to opening of the γ-CD cone angle as a result of the inclusion of the first γ-CD.

Introduction

A number of stable lanthanide(III) complexes with polyazamacrocyclic ligands are utilized in biomedical diagnosis, for example as paramagnetic shift reagents for in vivo NMR,[1] and specially as contrast agents for Magnetic Resonance Imaging (MRI).[2−4] One of the currently applied contrast agents is $Gd(DOTA)^-$ (Dotarem®) (H_4DOTA = 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*′′,*N*′′′-tetraacetic acid) (For structures of the ligands see Figure 1).[5] The efficacy of MRI contrast agents is determined by their ability to enhance the solvent water proton relaxation rate. Among the strategies to improve this, optimisation of the inner-sphere relaxivity, through slowing down of the rotational dynamics, has proved to be quite useful.[2−4] This has been achieved by either covalent conjugation[2,6] or non-covalent binding[7] of the Gd^{III} complexes to high molecular weight aggregates or macromolecules, such as Human Serum Albumin (HSA).

Cyclodextrins (CDs) are cyclic oligosaccharides made up of six, seven, or eight α-1,4-linked D-glucopyranoside units (α-, β-, and γ-CD, respectively).[8,9] They have a cone-like shape with a hydrophobic cavity, the size of which depends on the number of glucopyranose units constituting the CD. Many NMR studies of the solution thermodynamic stability and structure of host-guest compounds of α-, β-, and γ-CDs with organic molecules have recently been published.[10−14] Several inclusion complexes of γ-CD with crown ethers, azamacrocycles and their complexes with metal cations such as Li^+, Ca^{2+}, and Ba^{2+}, have been characterized.[15−17] Inclusion compounds with cyclodextrins have drawn attention as enzyme model systems and found a wide range of applications, such as in drug delivery systems.[8,9,18] Formation of a stable inclusion compound between a contrast agent and a cyclodextrin would possibly increase its relaxivity and also alter its in vivo biodistribution. Aime et al. have studied inclusion compounds of β-benzyloxy-α-propionic substituted DOTA and DTPA-Gd^{III} complexes.[19] The benzyl groups of these compounds are included in the small CD cavity, which resulted in an increase of the relaxivity. Sherry et al. have observed the formation of weak inclusion compounds between γ-CD and $Tm(DOTP)^{5-}$ (H_8DOTP = 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*′′,*N*′′′-tetrakis(methylenephosphonic acid).[20]

The present paper reports an NMR study on the stability and structure of the inclusion compound(s) formed between γ-CD and $Tm(DOTA)^-$. The latter complex has a lower net charge than $Tm(DOTP)^{5-}$ and, therefore, is probably less hydrophilic. Tm^{III} was selected as Ln^{III} ion for these studies because this Ln^{III} ion induces both large chemical shifts and relaxation rate enhancements.[4] The geometry of the inclusion compounds was studied by measuring the cyclodextrin ^1H- and ^{13}C-NMR Lanthanide Induced Shifts (LIS) and spin-lattice (T_1) relaxation rate enhancements induced by the paramagnetic cation in the supramolecular system(s) formed. A one-step procedure is presented for the fitting of the measured LIS data to model the complexes formed. We also investigated the effect of a change in the size of the macrocyclic Tm^{III} complex [by using Tm(NOTA) complexes, H_3NOTA = 1,4,7-triazacyclononane-*N*,*N*′,*N*′′-tri-

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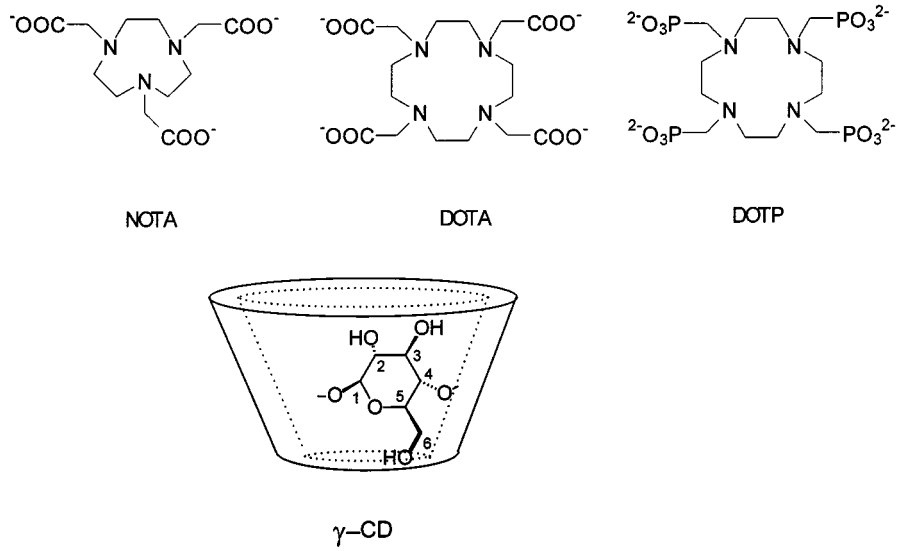

Figure 1. Structures of macrocyclic ligands and γ-CD

acetic acid] and of the cyclodextrin cavity size (by using α-, β-, and γ-CDs) on the stability of the inclusion compound(s) formed.

Results and Discussion

Referencing of NMR Chemical Shifts

Referencing of NMR chemical shifts in a study of the interaction between CDs and a paramagnetic complex is a non-trivial problem. Any internal reference used should not interfere with the equilibria between these species. ^{1}H-NMR spectra of γ-CD referenced with respect to the potential internal references *tert*-butyl alcohol, 1,4-dioxane, pentaerythritol, or tetramethylammonium bromide showed concentration dependence of the chemical shifts, which suggests an interaction between the CD and the "standard". To avoid these problems, we decided to use a solution of TMS in $CDCl_3$ in a coaxial tube inside the sample tube as external standard. Introduction of paramagnetic complexes into the outer compartment of the NMR samples causes large changes of bulk magnetic susceptibility (BMS), which are not felt in the inner tube containing the standard.[21] Therefore, corrections for these BMS effects are required. The BMS for a system with a coaxial cylindrical sample tube parallel to the main magnetic field is given by Equation (1), where Δ_χ is the paramagnetic contribution to the BMS in ppm, c is the concentration of the Tm^{III} complex in mol dm^{-3}, and T is the temperature.[21]

$$\Delta_\chi = 29\,997 \cdot c/T \quad (1)$$

Experimentally determined BMS values, determined with samples containing $Tm(DOTA)^-$ and *tert*-butyl alcohol as internal reference, were, within the experimental error, the same as those calculated with Equation (1). All chemical shifts mentioned below are corrected for BMS contributions.

Screening of the Interactions Between Macrocyclic Tm^{III} Complexes and α-, β-, and γ-Cyclodextrin

We started by titrating Tm(NOTA) or $Tm(DOTA)^-$ into 5 mM solutions of α-, β-, and γ-CD in D_2O at 25°C. Interactions were monitored by the ^{1}H resonances of the CD in question. Only upon addtion of $Tm(DOTA)^-$ to γ-CD, significant induced ^{1}H shifts were observed (after subtraction of the BMS contribution, see above). For all other combinations the induced shifts were less than 0.1 ppm when the concentration of the Tm^{III} chelate was increased up to a molar ratio Tm^{III} complex/CD (ρ) of 10. Only some line broadening was observed in these cases and the longitudinal ^{1}H relaxation rate enhancements were of the same order of magnitude as those observed for *tert*-butyl alcohol in a sample containing the same concentration of the Tm^{III} chelate. For example, ^{1}H relaxation rate enhancements ranging between 2.4 and 4.5 s^{-1} were measured for β-CD in the presence of $Tm(DOTA)^-$ at $\rho = 3$, whereas for *tert*-butyl alcohol under similar conditions an enhancement of 2.8 s^{-1} was observed. Therefore, it can be concluded that these relaxation rate enhancements are due to intermolecular effects, i.e. to effects resulting from diffusion of substrate molecules past the paramagnetic chelate complexes. Any direct binding of a Tm^{III} chelate to a CD molecule would lead to additional specific and substantial relaxation rate enhancements. Binding of the Tm^{III} chelates to the outer surface of the CD cones, therefore, does not play an important role.

The internal diameters of the three CD cavities are: α-CD: 4.7−5.2 Å; β-CD: 6.0−6.4 Å; γ-CD: 7.5−8.3 Å, where the smaller value is for the ring 5-H and the larger value is for the ring 3-H.[8,22] The size of the Tm^{III} chelates was evaluated by measuring (using standard molecular modelling software) the maximum distance between two ring ethylenediamino protons or acetate arm protons oppositely placed in the chelate structure. For Tm(NOTA) these distances are 5.82 and 6.76 Å, respectively.[23] A comparison of the approximate sizes of the potential host cavities and

the chelate guest indicates that the α-CD and β-CD cavities are too small to accomodate Tm(NOTA), while the γ-CD cavity is already too large to interact with the bottom macrocyclic ring hydrophobic surface of that chelate. Similarly, molecular models show that $Tm(DOTA)^-$ (distances 7.5 and 8.3 Å) fits exactly into the cavity of γ-CD, but is too bulky for inclusion in α- or β-CD.

Thus, the observation of significant Tm^{III}-induced ^1H shifts corresponds with the ability of the Tm^{III} chelate to form a well-fitting inclusion compound with the CD. Apparently, a very fine geometry fitting is needed for a reasonable host-guest interaction, perhaps due to the need of the guest to expel the hydrogen-bonded network of water molecules from inside the host CD cavity. Similar phenomena have been reported for $Tm(DOTP)^{5-}$, which induces paramagnetic ^1H shifts in γ-CD nuclei, but not in β-CD nuclei.[20]

Interactions between $Tm(DOTA)^-$ and γ-CD; Tm^{III}-Induced NMR Shifts

The interaction between $Tm(DOTA)^-$ and γ-CD was studied in more detail. Upon titration of $Tm(DOTA)^-$ into a 25 mM γ-CD solution, all ^1H and ^{13}C resonances of the CD molecule, after correction for the BMS shift contribution (see above), show Tm^{III}-induced shifts, some to high and others to low frequency. The magnitude of each (corrected) LIS was sensitive to the amount of $Tm(DOTA)^-$ added, indicating fast exchange between free and bound γ-CD. Figure 2 illustrates the plots of experimental LIS values versus ρ for some ^1H and ^{13}C CD resonances. In a similar NMR titration performed with the diamagnetic $La(DOTA)^-$ no induced shifts were observed for the γ-CD resonances. Therefore, it can be concluded that diamagnetic contributions to the shifts induced by the Tm^{III} complex are negligible.

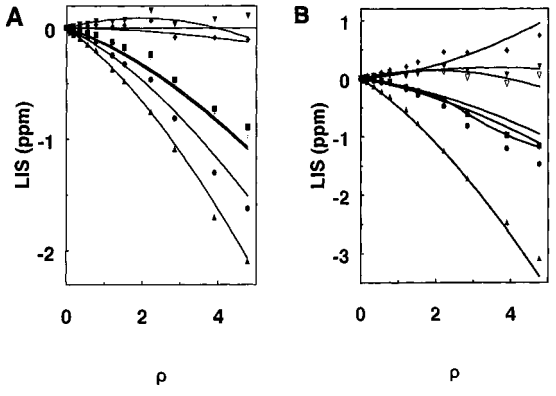

Figure 2. ^{13}C (A) and ^1H-NMR titration curves (B) for the addition of $Tm(DOTA)^-$ to a 25 mM solution of γ-CD in D_2O; the curves are calculated using the parameters given in Table 1 (see text); + C-1/1-H, ● C-2/2-H, ▲ C-3/3-H, ■ C-4/4-H, ◆ C-5/5-H, ▼ C-6/6-H, ▽ 6′-H; the experimental points of 2-H and 4-H coincide

It is known that Ln^{III} ions have only weak interactions with carbohydrates.[24] The Tm^{III} ion in $Tm(DOTA)^-$ is strongly chelated by the bulky macrocyclic ligand in an eight-dentate fashion[25] and, therefore, it may be assumed that additional coordination of a CD in the first coordination sphere of Tm^{III} does not occur. As a result, the contact contributions to the LIS can be neglected; the LIS are determined exclusively by the pseudo-contact mechanism.[4] Furthermore, the $Ln(DOTA)^-$ complexes have 4-fold symmetry and, consequently, the pseudo-contact contributions to the fully bound LIS values (LIS_{pc}) of each ligand nucleus is related to the structure of the chelate through Equation (2).[26] Here, the geometric term G depends on the spherical coordinates *r* and θ of the nucleus under study with respect to Ln^{III} at the origin and with the principal symmetry axis of the system as the *z* axis.

$$LIS_{pc} = D \cdot G = D \cdot \frac{3\cos^2\theta - 1}{r^3} \qquad (2)$$

The magnetic proportionality constant *D* is characteristic of the Ln^{III} ion and the chelate. Brittain and Desreux have reported that the *D* value for the major isomer of $Yb(DOTA)^-$ is 5082 ± 209 ppm·Å^3.[27] The ratio of the pseudocontact shifts of isostructural Yb^{III} and Tm^{III} complexes is known to be 53:22.[4][28] Therefore, the value of *D* of the major $Tm(DOTA)^-$ complex can be estimated to be 12243 ppm·A^3 (= 5082×53/22). From Equation (2) it follows that the magnitude and direction of the LIS is determined by the position of the nucleus relative to the paramagnetic center. The LIS is zero in spatial positions on a cone centered on the metal, with an axis coinciding with the magnetic axis of the chelate and with a cone angle of 54.7°, where the geometrical factor *G* and the shift change sign. We assume that the position of the principal axis and the value of *D* of adducts of the Tm^{III} chelates and γ-CD are the same as those in the free $Tm(DOTA)^-$ complexes, and also that binding to γ-CD does not affect the isomer equilibrium of $Tm(DOTA)^-$.[25]

The initial slopes of the titration curves of protons 1-H to 4-H, as well as the corresponding carbon atoms C-1 to C-4 are negative (shifts to lower frequencies, negative LIS values), whereas the atoms 5-H, 6-H, 6′-H, C-5, and C-6 have positive LIS values. This change in sign of the LIS between the C-4-H and C-5-H moieties of the γ-CD ring determines, through the change of sign of the geometrical term for the $Tm(DOTA)^-$ (see Equation 2), the depth at which the chelate sits inside the γ-CD cavity, and thus gives a qualitative picture about the relative position of the chelate and the cyclodextrin molecule (see Figure 3). This is quite similar to what has been previously found for $Tm(DOTP)^{5-}$/γ-CD.[20]

Surprisingly, almost all titration curves of Figure 2 show an increase of slope upon an increase of ρ, whereas for $Tm(DOTP)^{5-}$ the titration curves have been reported to to be linear up to ρ = 50.[20] Therefore, it can be concluded that besides the 1:1 adduct of $Tm(DOTA)^-$ and γ-CD [ML, M = $Tm(DOTA)^-$, L = γ-CD] another species is present, most likely M_2L. Furthermore, it can be noted from Figure 2 that, even at ρ = 5, the LIS values have not reached a

limiting value. This indicates that the association constant for the stability constants of the adducts are very low.

The most common procedure for the elucidation of solution structures of Ln^{III} complexes in the case of fast exchange between the free and bound substrate on the NMR time scale involves two steps.[4,29] First fully bound shifts of the ligand nuclei are evaluated from fitting titration curves or relative bound shifts are determined from the initial slopes of the titration curves. The bound shifts are then entered into a fitting to, for example, Equation (2) to give the molecular structure. In the present case, however the determination of bound shifts is difficult because of the curvature of the plots and because the limiting values of the shifts are not reached at high ρ-values. Therefore, we performed the two fitting procedures simultaneously. Since both the geometries of the host and the guest are rather rigid and well-known, structural constraints can be applied in the fitting procedure, which results indirectly in constraints on the calculated bound shifts. In a simultaneous fitting these constraints, on their turn, reduce the uncertainty in the resulting stability constants.

The experimental LIS data for the titration of γ-CD with $Tm(DOTA)^-$ were fitted with a model taking into account the ML and M_2L adducts. It was assumed that the ML adduct is an inclusion compound in which the main symmetry axis of the host and the guest coincide. Any location of the second chelate M at the surface of the CD cone is less likely, because then averaging over the various equivalent positions in the eight sugar units would lead to averaging out of the angular term in Equation (2) and, therefore, to very small LIS values. Most likely, the second M is near one of the rims and its position probably coincides with the main symmetry axis of γ-CD. For this situation, equivalent nuclei of the various sugar units have equal θ values and averaging out of the induced shifts does not occur.

Reported crystal coordinates for γ-CD[30] were submitted to molecular mechanics with the MM+ force field, after docking of a model of the $Eu(DOTA)^-$ structure obtained from reported crystal coordinates[31] in the cavity. The geometry of the guest was fixed during these calculations. The resulting coordinates of the γ-CD moiety were used in the elucidation of the solution structure of ML and M_2L. From an initial guess of the location of the two Tm^{III} ions on the symmetry axis of γ-CD, the bound shifts of the γ-CD nuclei of ML and M_2L (Δ_{ML} and Δ_{M2L}) were calculated using Equation (2). The *D* value in Equation (2) was fixed at 12243 ppm·Å^3 (see above). It is assumed that the *D* values of the adducts ML and M_2L are the same as that of the parent compound M. The equilibrium constants of the species in question were defined as in Equations (3) and (4).

$$\beta_{ML} = \frac{[ML]}{[M][L]} \quad (3)$$

$$\beta_{M_2L} = \frac{[M_2L]}{[M]^2[L]} \quad (4)$$

From an initial guess of β_{ML} and β_{M2L}, Equations (3) and (4) and the mass balances, the concentrations of the various species were obtained by an iteration following a procedure outlined previously.[32] With these concentrations the fractions of L bound as ML and M_2L were calculated (f_{ML} and f_{M2L}, respectively). Then the induced shifts $\delta_{calcd.}$ at the various ρ values were calculated using Equation (5).

$$\delta_{calcd.} = f_{ML}\Delta_{ML} + f_{M2L}\Delta_{M2L} \quad (5)$$

The agreement between the calculated and experimental shifts was optimized using an iteration with the location of the two Tm^{III} centers on the symmetry axis of the γ-CD host, and β_{ML} and β_{M2L} as variables. An optimal fit was obtained for the parameters reported in Table 1. The corresponding bound shifts for ML and M_2L calculated with these parameters are included in Table 1. The Tm^{III} of the included $Tm(DOTA)^-$ chelate is located 2.7 Å above the plane formed by the anomeric oxygen atoms of the glucose units of the CD host. A similar position of Tm^{III} has been reported for $Tm(DOTP)^{5-}$ included in γ-CD.[20] The second Tm^{III} ion in the M_2L complex is found 5.1 Å below this plane, the second $Tm(DOTA)^-$ molecule is thus located at the lower rim of the host (see Figure 3). An inspection of molecular models of this complex shows that such a location is favorable for the formation of hydrogen bonds between the non-chelated carboxylate oxygens of the $Tm(DOTA)^-$ molecule and CH_2OH functions of the glucose units of γ-CD.

Table 1. Stabilities of 1:1 and 2:1 $Tm(DOTA)^-$: γ-CD complexes as obtained from Tm^{III}-induced shift measurements,[a] location of the Tm^{III} cations, and calculated bound shifts

	ML	M_2L
β [M^{-1}]	0.33 ± 0.03	3.4 ± 0.3
distance Tm^{III}−O-1 plane [Å][c]	2.67 ± 0.05	−5.14 ± 0.09
bound shifts [ppm]		
C-1	−14.5	−15.5
C-2	−22.4	−20.3
C-3	−32.6	−26.0
C-4	−16.3	−14.4
C-5	−0.3	−3.1
C-6	12.4	−13.1
1-H	−11.1	−15.5
2-H	−15.0	−16.3
3-H	−55.3	−41.2
4-H	−11.3	−15.3
5-H	6.1	19.7
6-H	19.5	−20.9
6′-H	14.1	−7.9

[a] Calculated with *D* = 12243 (see text). Agreement factor *R* = 0.16. − [b] M = $Tm(DOTA)^-$, L = γ-CD. − [c] Plane through the anomeric oxygen atoms of the glucose units of γ-CD, the direction towards the upper (wide) rim of γ-CD is denoted positive.

The calculated locations of the Tm^{III} centers are not very sensitive to the choice of the value of *D* in Equation (2). If the value of this parameter was fixed at 7434, a value derived from that reported for the minor isomer of $Yb(DOTA)^-$,[27] positions 2.6 Å above and 5.2 Å below the plane of the anomeric glucose oxygen atoms were obtained.

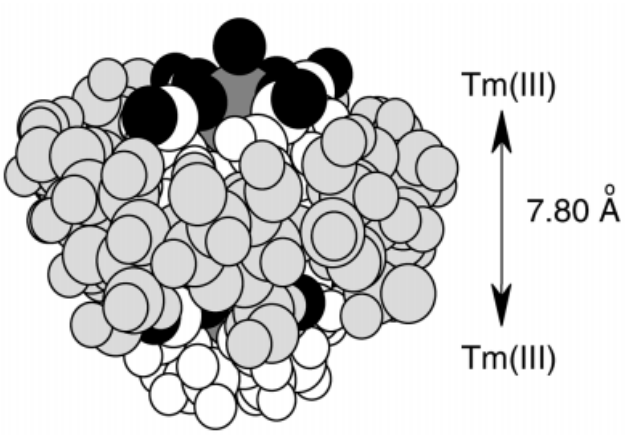

Figure 3. Schematic representation of the M_2L complex [M = $Tm(DOTA)^-$, L = γ-CD]

The calculated formation constants were, however, somewhat higher ($\beta_{ML} = 0.51\ M^{-1}$; $\beta_{M2L} = 6.6\ M^{-2}$).

Iterations with a model including a second 1:1 adduct with M bound at the outside of the cone at the lower rim rather than inside the CD cavity, did not lead to a better agreement between the calculated and observed titration curves. Furthermore, the resulting stability constant for the concerning complex was relatively small ($\beta = 0.10 \pm 0.05\ M^{-1}$). This is in line with the observation that α- and β-CD, which are not able to include $Tm(DOTA)^-$, also do not show interaction with this chelate at the outside surface of their cones. Furthermore, it should be noted that the stepwise formation constant of M_2L is larger than the formation constant of ML. Apparently, inclusion of the first $Tm(DOTA)^-$ chelate leads to a situation that is favorable for binding of the second one. It is known that inclusion of bulky guests into CDs results in adaption of the cone-angle of the CD host.[17] We assume that the inclusion of $Tm(DOTA)^-$ in γ-CD brings the CH_2OH functions of the CD in a position that is more favorable for the formation of multiple hydrogen bonds with a second $Tm(DOTA)^-$ molecule. This also explains, why an M_2L complex was not observed with $Tm(DOTP)^{5-}$,[20] as the distance between the oxygen atoms of the phosphonate functions is too large for the formation of hydrogen bonds with CH_2OH functions. Also the high negative charge of the Tm(DOTP) chelate (about −3 at pH = 7) causes it to hinder the binding of the second one at the lower rim through electrostatic repulsion.

Interactions between $Tm(DOTA)^-$ and γ-CD; Tm^{III}-Induced Relaxation Rate Enhancements

The longitudinal ^1H relaxation rate enhancements of γ- and β-CD upon addition of $Tm(DOTA)^-$ at ρ = 3 are given in Table 2. The ^{13}C relaxation rate enhancements are at least an order of magnitude smaller and are not taken into consideration. Various relaxation enhancing mechanisms contribute to the total effect. Two contributions resulting from the binding of the $Tm(DOTA)^-$ to the CD molecule should be envisaged: (i) a dipolar one ($1/T_{1,p}$) caused by through space interactions due to the random fluctuations of the electronic field of Tm^{III}[33,34] and (ii) the Curie contribution ($1/T_{1,Curie}$), which arises from the interaction of the nuclear spin with the thermal average of the electronic spins[35−38] (Equation 6).

Table 2. Contributions to the Tm^{III}-induced longitudinal relaxation rate enhancements of a 0.023 M solution of γ-CD nuclei in D_2O at ρ = 2.8[a]

	$1/T_{1,p} + 1/T_{1,Curie}$ $[s^{-1}]$[a]	$1/T_{1,inter}$ $[s^{-1}]$[b]	$1/T_{1,calcd.}$ $[s^{-1}]$[c]	$1/T_{1,exp}$ $[s^{-1}]$
1-H	0.51	5.79	6.30	5.23
2-H	0.41	4.95	5.36	7.25
3-H	4.39	7.15	11.54	12.44
4-H	0.64	4.16	4.80	4.59
5-H	4.05	4.41	8.46	5.01
6-H/6′-H	3.03	3.85	6.88	4.90

[a] Calculated with Equations 6−9 (see text). − [b] Estimated from relaxation rate measurements on a 0.013 M solution of β-CD in D_2O at ρ = 3.0. A correction for the difference in the $Tm(DOTA)^-$ concentrations between the two samples was applied. − [c] Sum of $1/T_{1,p}$, $1/T_{1,Curie}$, and $1/T_{1,inter}$.

$$\frac{1}{T_{1,b}} = \frac{1}{T_{1,p}} + \frac{1}{T_{1,Curie}} \tag{6}$$

Tm^{III} has a very short electronic relaxation time ($T_{1e} \approx 10^{-13}$ s). If zero-field splitting (ZFS) effects are neglected,[38] $1/T_{1,p}$ can be given by the simplified Solomon-Bloembergen Equation (7).[33,34]

$$\frac{1}{T_{1,p}} = \frac{4}{3}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 \mu_{eff}^2 \beta^2}{r^6} T_{1e} \tag{7}$$

Here, $\mu_0/4\pi$ is the magnetic permeability of a vacuum, γ_I is the magnetogyric ratio of the nucleus under study, μ_{eff} is the effective magnetic moment of Tm^{III}, β is the Bohr magneton, and *r* is the distance between the nucleus under study and Tm^{III}. The Curie spin contribution can be expressed by Equation (8) where H_0 is the magnetic field strength, *k* is the Boltzmann constant, *T* is the temperature, τ_R is the rotational tumbling time of the complex and ω_I is the Larmor frequency of the nucleus under study.[35,36]

$$\frac{1}{T_{1,Curie}} = \frac{6}{5}\left(\frac{\mu_0}{4\pi}\right)^2 \left(\frac{\gamma_I^2 H_0^2 \mu_{eff}^4 \beta^4}{(3kT)^2 r^6}\right)\left(\frac{\tau_R}{1+\omega_I^2 \tau_R^2}\right) \tag{8}$$

Merbach et al. have reported a value of 77 ps for the rotational correlation time (τ_R) of $Gd(DOTA)^-$ at 298 K[39] and Aime et al. have reported values of 72 and 80 ps for the Gd^{III} and Lu^{III} complexes of DOTA, respectively.[40] The rotational correlation times are, according to the Debye-Stokes-Einstein equation, proportional to a^{-3} for spherical complexes, where *a* is the molecular diameter. On this basis, we have used 80, 268, and 339 ps as estimates for the τ_R values of M, ML, and M_2L, respectively. Values of T_{1e} of lanthanides other than Gd^{III} are usually not very

dependent on the structure of bound ligands. The T_{1e} values reported by Aime et al. for DOTA chelates of Tb^{III}, Dy^{III}, and Ho^{III}[40] are close to those tabulated for the aquo complexes.[41] It should be noted, however, that T_{1e} values obtained with data processing including ZFS effects are about 2−3 times higher,[38] although the same nuclear relaxation rates were calculated as with a procedure in which ZFS effects were neglected. Therefore, we have used T_{1e} = 0.369 ps for $Tm(DOTA)^-$ and its complexes with γ-CD. With the use of these parameters and Equations (5−8) the $1/T_{1,b}$ values for ML, and M_2L were calculated. The exchange of γ-CD between ML, M_2L, and L is fast on the NMR time scale and, therefore, the relaxation rate enhancement due to binding of $Tm(DOTA)^-$ to γ-CD can be estimated with Equation (9).

$$\frac{1}{T_{1,calc}} = \frac{f_{ML}}{T_{1,ML}} + \frac{f_{M_2L}}{T_{1,M_2L}} \qquad (9)$$

The relaxation rates have no angular dependence, and therefore, in contrast to the LIS values the effects of random diffusion controlled encounters between the paramagnetic complexes and the γ-CD moieties do not average out. These intermolecular contributions ($1/T_{1,inter}$) were estimated from the longitudinal relaxation rate enhancements of β-CD upon addition of $Tm(DOTA)^-$ (see Table 2), since inclusion does not occur there. It can be seen that this mechanism plays a major role in the present case. After inclusion of $1/T_{1,inter}$ the order of magnitude of the resulting calculated relaxation rates is the same as that of the experimental ones and the large relative value of the relaxation rate of 3-H is reproduced. The agreement between the calculated and experimental values is reasonable, considering the many assumptions that have been made in the course of these calculations.

Conclusion

$Tm(DOTA)^-$ forms a weak inclusion complex with γ-CD. The hydrophilic carboxylate groups remain outside the cavity. The resulting inclusion compound is able to bind a second $Tm(DOTA)^-$ chelate at the outer surface of the CD molecule at the lower rim. The binding occurs probably by hydrogen bonding between the carboxylate oxygen atoms of $Tm(DOTA)^-$ and the CH_2OH functions of the glucose units of γ-CD. Similar complexes are not formed with α- or β-CD or with the less bulky chelate $Tm(NOTA)^-$. Apparently, a good spatial fit of the guest in the cavity of the CD is required for the formation of an inclusion compound. The stability of the inclusion compound is even lower than that of the previously reported inclusion compound of $Tm(DOTP)^{5-}$ and γ-CD.[20] For that compound, however, binding of a second chelate molecule was not observed. Because of the low stability constants of the inclusion compounds, it can be concluded that inclusion of $Gd(DOTA)^-$ into γ-CD is not a useful approach to increase its efficacy as contrast agent for MRI.

Experimental Section

General Remarks: $TmCl_3 \cdot 6\ H_2O$ was purchased from Aldrich, and α-, β-, and γ-cyclodextrin from Fluka. All reagents were used without further purification. The macrocyclic ligands H_4DOTA and H_3NOTA were synthesized according to known procedures.[42−44] The respective Tm^{III} complexes [$Tm(DOTA)^-$ and Tm(NOTA)] were prepared by adding stoichiometric amounts of $TmCl_3 \cdot 6\ H_2O$ to a ligand solution, while maintaining the pH at about 6. The solution was kept at 80°C for about 6 h to ensure complete complex formation, and was tested for free lanthanide ions using xylenol orange and arsenazo III as indicators.[45,46] Salts were removed by nanofiltration through a Toray "Romembra" UTC-60 membrane under 20 bar nitrogen pressure. The final solutions were freeze-dried. The Tm^{III} content of the products was determined from the induced Bulk Magnetic Susceptibility (BMS) shift.[47,48] The latter was measured in a 5-mm sample tube equipped with a 2-mm coaxial inner cell (New Era Enterprises, Vineland, NJ, USA). The inner cell was filled with a 1% solution of TMS in $CDCl_3$ and the outer compartment with a 1% solution of *tert*-butyl alcohol in D_2O. The BMS was determined from the increase of the separation between the TMS and *tert*-butyl alcohol signals upon addition of the Tm^{III} complex.

The NMR titrations were performed by adding weighted amounts of the Tm^{III} complexes to 5 mM or 25 mM CD solutions in D_2O at pH = 6−7, using 10-mm NMR tubes. A 2-mm coaxial tube containing a solution of TMS in $CDCl_3$ was used as external reference for ^{1}H- and ^{13}C-NMR shifts.

All pH measurements were made at room temperature using a Corning 125 pH meter and a calibrated microcombination electrode purchased from Aldrich Chemical Co. Adjustments of the pH of the solutions were made with DCl and NaOD solutions in D_2O (all supplied by Merck), and no correction was made for the deuterium isotope effect.

NMR spectra were obtained with Varian VXR-400S or Unity 500 spectrometers. Spin-lattice relaxation rates were measured using the inversion recovery method. The ^{1}H- and ^{13}C-NMR spectra of γ-CD were assigned by two-dimensional homonuclear (COSY) and heteronuclear (HETCOR) correlated spectra, and agreed with the literature.[10−15,20] Molecular mechanics was performed with the use of the HyperChem program (version 3, MM+ force field, HyperCube Inc., Gainsville, FL, USA).Computer fitting of the LIS data was carried out with a homemade computer program using the Micromath Scientist version 2.0 (Salt Lake City, UT, USA) program. The speciations used in this program were evaluated following procedures outlined previously.[32] The equation file used in this program is available from the authors upon request.

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