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Lanthanide(III)–1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid complexes in acidic medium: significant decrease in water exchange rate †

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UV-Vis and lanthanide-induced ^{17}O shift measurements on the complex $[Eu(DOTA)]^-$ (H_4DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) have shown that the inner co-ordination sphere of the Ln^{3+} ion is not affected on protonation which suggests that the proton is attached to a non-co-ordinated oxygen atom of a carboxylate group. Proton NMR measurements performed as a function of the H^+ concentration revealed that the protonation slightly accelerates the intramolecular dynamic processes: the enantiomerization for $[La(DOTA)]^-$ and the enantiomerization and interconversion between the major and minor isomer for $[Eu(DOTA)]^-$. Contrary to first glance expectations, the water exchange rate on $[Gd(DOTA)(H_2O)]^-$ decreases significantly with increasing extent of protonation, and at 1.0 M H^+ concentration is about ten times lower than in neutral media. In 1.0 M acidic solution the proton relaxivities were found to be higher than expected solely on the basis of the water exchange rates. This finding is interpreted with a faster proton exchange in acidic solutions which is the consequence of the catalytic effect of H^+ ions.

Introduction

In recent years there has been an active interest in studying lanthanide (Ln^{3+}) complexes of the ligand 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (H_4DOTA). This interest originates on the one hand from the unusual chemical properties of these complexes and, on the other hand, from their successful use in medical diagnosis and therapy. The $[Ln(DOTA)]^-$ complexes have extremely high thermodynamic stability,[1–3] and kinetic inertness.[4–9] Owing to these favourable properties, $[Gd(DOTA)]^-$ is a contrast enhancing agent used in clinical Magnetic Resonance Imaging (MRI), whereas $[^{90}Y(DOTA)]^-$ is a potential drug for cancer treatment.[10–14]

The high thermodynamic and kinetic stabilities result from the unusually rigid structure of the complexes, which itself is the consequence of the rigidity of the 12-membered macrocycle and of the perfect matching between Ln^{3+} ions and the cavity formed by the four nitrogen and four carboxylate oxygen donor atoms. This rigid structure leads to the formation of two isomers in solution as has been observed by ^1H and ^{13}C NMR spectroscopy.[15–19] The two isomers differ in the layout of the acetate groups, and have regular square antiprismatic (M isomer) and inverted antiprismatic structure, strongly distorted toward a regular prism (m isomer).[15] The interconversion of the two isomers occurs *via* the flip-flop motion of the acetate arms or *via* conformational changes of the ring ethylene groups, and can be followed by NMR. The rates of the two processes have been found to be different.[15–17]

The dissociation of the $[Ln(DOTA)]^-$ complexes occurs *via* a proton catalysed pathway, with the formation of protonated [HLn(DOTA)] complexes.[5,6,8] The proton assisted dissociation of $[Gd(DOTA)]^-$ is extremely slow at around pH 7 which makes it a very safe contrast agent. Owing to the catalytic effect of H^+ ions, the dissociation proceeds faster in acidic media, however even at pH 2 the half time of dissociation is about 1000 h (37 °C).[8] Therefore, the $[Gd(DOTA)]^-$ can be safely applied as an oral contrast agent to image the gastrointestinal tract.[9] Consequently, investigating the protonation of these chelates is important from the practical point of view. The objective of this work was twofold: (i) studying the structure and dynamics of the protonated complexes and intermediates, and (ii) determining their water and proton exchange rates. The structural investigation has been mainly done by means of pH-dependent UV-Vis, ^{17}O and ^1H NMR measurements. They also allowed for studying the protonation effect on the isomer interconversion and on the enantiomerization of the ligand, which is expected to be similar to the effect of a temperature increase.[15,16] Previous studies have shown that the diminution of the negative charge on gadolinium(III) complexes results in a significant decrease in the water exchange rate,[20,21] which is one of the parameters that determine the efficacy of an MRI contrast agent. Since $[Gd(DOTA)]^-$ is applied as a gastrointestinal agent, it is important to get information about its proton relaxivity and water and proton exchange rate under extreme acidic conditions, similar to those of the stomach. The very slow dissociation of the complex allowed us to perform NMRD (nuclear magnetic relaxation dispersion) and ^{17}O NMR measurements as a function of acidity and temperature which were used to obtain the water and proton exchange rates.

Experimental

Sample preparation

The $LnCl_3$ solutions were prepared by dissolving lanthanide(III) oxides (99.9%, Fluka) in HCl, followed by evaporation of the acid excess. The concentration of $LnCl_3$ was determined by complexometry, using xylenol orange indicator. The compound

† *Supplementary data available*: relaxation rates, relaxivities, UV-Vis and ^1H NMR spectra. For direct electronic access see http://www.rsc.org/suppdata/dt/1999/2481/, otherwise available from BLDSC (No. SUP 57586, 5 pp.) or the RSC Library. See Instructions for Authors, 1999, Issue 1 (http://www.rsc.org/dalton).

H_4DOTA was prepared by a known procedure.[8] The concentration of DOTA was determined by pH-potentiometric titration in the absence and presence of an excess of Ca^{2+}. The complexes were prepared by mixing equivalent amounts of $LnCl_3$ and Na_4DOTA and heating the solution (80 °C) for about 4–5 h.

pH-potentiometry

The protonation constants of $[La(DOTA)]^-$ and $[Y(DOTA)]^-$ have been determined by titrating a 0.05 M $[Ln(DOTA)]^-$ solution (pH ≈ 5.5) with a HCl solution (1.04 M HCl + 0.96 M NaCl), at 5 °C. The NMR spectra have also been recorded at this low temperature, as they are simpler due to the slower dynamic processes. The ionic strength of the titrated solution was kept constant (2.0 M NaCl). The titration was carried out with a Radiometer PHM 85 pH-meter, an ABU80 autoburette and G202B glass and K401 calomel electrodes. For the calculation of the H^+ ion concentration from the measured pH values a 2 M NaCl solution was titrated with 0.103 M HCl + 1.90 M NaCl solution under similar conditions as all titrations, and the differences in the measured and calculated pH values were used later to correct all measured pH values.[22] The protonation constants have been calculated by the computer program PSEQUAD.[23]

UV-Vis spectrophotometry

The visible spectra of $[Eu(DOTA)]^-$ were recorded on a Perkin-Elmer Lambda 19 spectrometer, in thermostated cells with a 10 cm optical length (λ = 578–582 nm; T = 298 K), in the H^+ concentration range 1×10^{-5}–1.0 M, immediately after the acid was added to the complex solution.

NMR

The ^{1}H NMR spectra of $[Ln(DOTA)]^-$ (Ln = La, Eu or Y; c = 0.02 M) were recorded on a Bruker DPX 400 instrument at different H^+ concentrations (1×10^{-5}–1.0 M HCl). The ionic strength was constant, I = 2.0 M (HCl + NaCl). The sample solutions contained 10% D_2O and *tert*-butyl alcohol as internal standard.

The variable temperature ^{17}O NMR study on 0.03 mol^{-1} kg $[Gd(DOTA)(H_2O)]^-$ in 1.0 M $HClO_4$ solution was performed on a Bruker AC-200 spectrometer (4.7 T; 27.1 MHz). Transverse relaxation rates, $1/T_2$, have been obtained by the Carr–Purcell–Meiboom–Gill spin echo technique;[24] T_2 was measured repetitively several times after mixing the complex solution with the acid, and the first, constant values were accepted. In this way we made sure that the dissociation of the complex, taking place after a certain period of time and indicated by a decrease of the measured relaxation time, did not affect the T_2 data; 1 M $HClO_4$ was used as external reference. To improve sensitivity, ^{17}O-enriched water (10% $H_2{}^{17}O$, Yeda R&D Co.) was added to the solution to yield 2% ^{17}O enrichment. The absence of free Gd^{3+} ion was verified with xylenol orange indicator.[25] For all NMR measurements the temperature was measured by a substitution technique.[26] The europium induced shift measurements were performed on a Bruker AM-400 spectrometer at 25 °C, using ^{17}O enriched nitromethane as an internal chemical shift reference.[27]

NMRD

The $1/T_1$ NMRD profiles of the solvent protons were obtained at 5 °C on a Spinmaster FFC fast field cycling NMR relaxometer (Stelar), covering a continuum of magnetic fields from 7×10^{-4} to 0.47 T (corresponding to a proton Larmor frequency range 0.03–20 MHz).

Data analysis

The least-squares fitting of the transverse ^{17}O relaxation rates was performed by the program Scientist®[46] for Windows™ by Micromath®, version 2.0. For the lineshape analysis of the ^{1}H spectra a Matlab® program, developed in our laboratory, has been used. All reported errors correspond to one standard deviation obtained by the statistical analysis.

Results and discussion

Protonation effect on the structure and dynamics of $[Ln(DOTA)]^-$ complexes

The properties of the protonated complexes, [HLn(DOTA)], can be safely studied at lower pH values, since the rate of the proton catalysed dissociation is extremely low. In a 1.0 M HCl solution the dissociation half time of $[Gd(DOTA)]^-$ is about 20 h,[6] while the experiments have been always carried out within 10–20 min after sample preparation (mixing the acid and the complex solutions).

The protonation constants determined by pH-potentiometric titration at 5 °C for $[M(DOTA)]^-$ (M = La or Y) are log K = 0.96 ± 0.04 and 0.95 ± 0.02, respectively. According to these data, at pH 1 approximately 50% of the complex is present in protonated, [HLn(DOTA)] form.

The protonations have been studied by ^{1}H NMR on two diamagnetic (La and Y) and one paramagnetic ($[Eu(DOTA)]^-$) complex. The complexes $[La(DOTA)]^-$ and $[Y(DOTA)]^-$ have been chosen because they are present practically only in one isomeric form, m and M, respectively, whereas for $[Eu(DOTA)]^-$ the ratio of the two isomers (m/M) under the conditions used is $K \approx 2$ at 298 K.[19] The ^{1}H NMR spectra of both complexes recorded in the H^+ ion concentration range 1×10^{-5}–1.0 M, indicate a significant change in the chemical shifts of the acetate methylene protons, while the shifts of the ring methylene protons are less affected on protonation. Since the changes are very similar for the two complexes we present only the spectra recorded for $[La(DOTA)]^-$ (Fig. 1). These findings could be interpreted in terms of the protonation of one carboxylate group which, in turn, might become non-coordinated. In such a case the free co-ordination site of the Ln^{3+} ion would be immediately occupied by a H_2O molecule. The entering of a second water molecule to the inner co-ordination sphere can easily be checked by a UV-Vis study on the europium(III) complex, since Eu^{III} has an absorption band which is very sensitive to changes in the inner co-ordination sphere. This $^7F_0 \longrightarrow {}^5D_0$ transition has previously been applied to distinguish between slightly different co-ordination environments, such as differently hydrated europium(III) chelates.[28,29] The visible spectra of $[Eu(DOTA)]^-$ recorded in the H^+ concentration range 1×10^{-5}–0.56 M are identical (at 1 M $[H^+]$ the band is broadened and slightly shifted). This observation suggests that the inner co-ordination sphere remains intact on protonation, *i.e.* the non-protonated and protonated complexes are both nine-co-ordinated and monohydrated.

This is further supported by ^{17}O NMR chemical shift measurements on the non-protonated and protonated form of $[Eu(DOTA)]^-$. This technique, well known and frequently used for dysprosium(III) complexes, is based on the predominantly contact character of the ^{17}O shift induced by the paramagnetic metal.[30] Dysprosium induces much larger shifts than Eu which represents a great advantage; however, using the europium(III) complex let us directly correlate the results to those obtained in the UV-Vis study. Addition of the europium(III) complex to water results in a shift of the water ^{17}O NMR signal to lower frequencies (Fig. 2). The chemical shifts reported are referred to water of pH 6.0 in the case of the non-protonated complex and to a $HClO_4$ solution of pH 1.0 in the case of the protonated complex. The exchange of water between the complex and the bulk is fast on the ^{17}O NMR timescale under the conditions applied. Consequently, the slope of a plot of the europium(III) induced shift *versus* the europium(III) concentration is proportional to the hydration number of the complex. The slopes

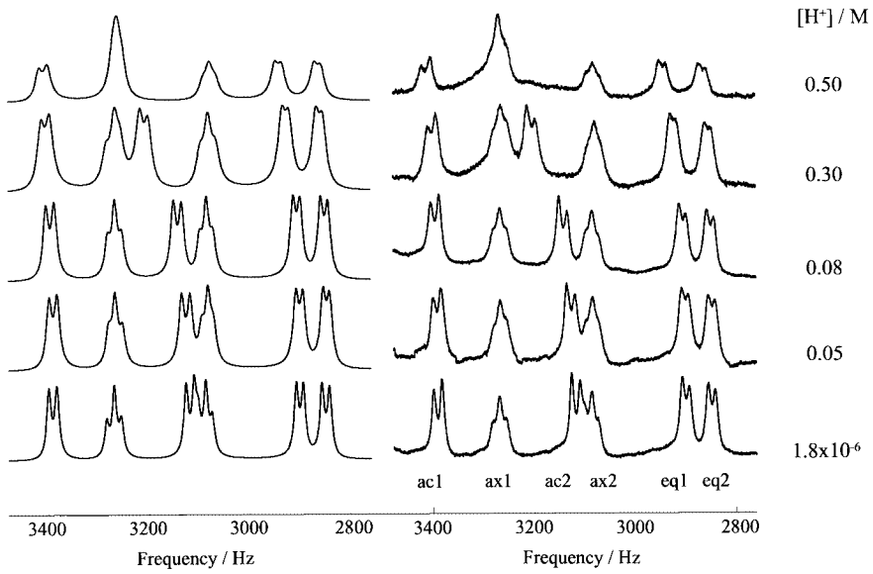

Fig. 1 Selected calculated (on the left) and experimental (on the right) 400 MHz ^{1}H NMR spectra of [La(DOTA)]$^-$ as a function of the H^+ concentration (c = 0.02 M; T = 278 K). In the assignment, ac, ax and eq stand for acetate, ring axial and ring equatorial protons, respectively. The peak of the *tert*-butyl alcohol reference is at 2374 Hz on this scale.

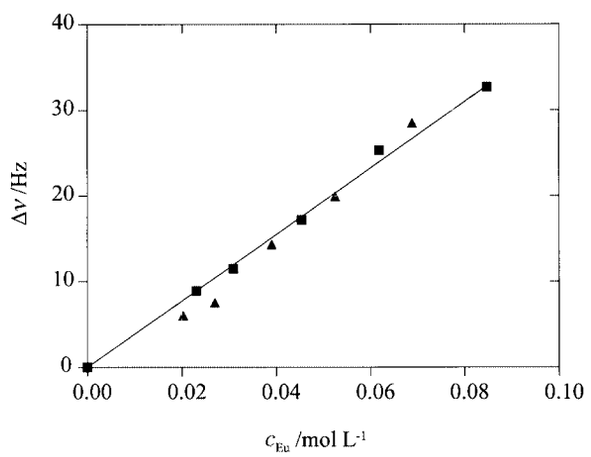

Fig. 2 Europium(III)-induced water ^{17}O shifts *versus* the europium(III) concentration for [Eu(DOTA)]$^-$ at pH 6.0 (■) and 1.0 (50% [HEu(DOTA)]) (▲); T = 298 K and B = 9.4 T.

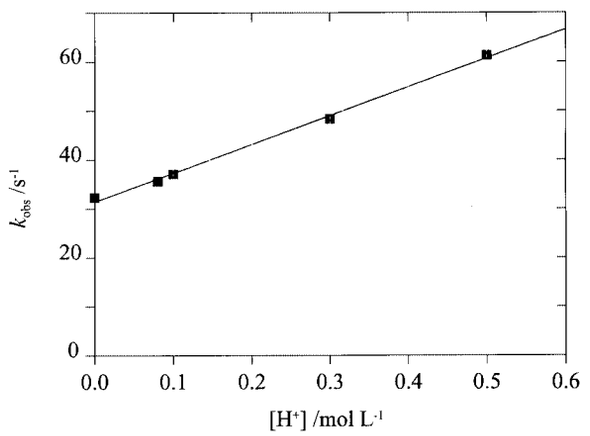

Fig. 3 Enantiomerization rates calculated for [La(DOTA)]$^-$ (0.02 M) as a function of the H^+ concentration. The straight line represents the least squares fit of the data points using eqn. (1).

obtained for [Eu(DOTA)]$^-$ at pH 6 (only non-protonated species) and 1 (50% protonated species) are identical. Therefore, in accordance with the UV-Vis measurements, we can conclude that the hydration number in the two complexes is identical.

As the inner co-ordination sphere of [Ln(DOTA)]$^-$ does not change on protonation, the proton has to be attached to the non-co-ordinated oxygen atom of the carboxylate group. There are several examples for this phenomenon as evidenced by solid state structures,[31–33] although for open chain lanthanide-poly(amino-carboxylates) in general the protonation is accompanied by the formation of a non-co-ordinating CO_2H group.

The protonation of a non-co-ordinated carboxylate oxygen in acidic solutions is probably the consequence of the rigid structure of the [Ln(DOTA)]$^-$ complexes. As a result of this rigidity the ^{1}H NMR signals of the acetate methylene protons of [La(DOTA)]$^-$ appear as an AB multiplet (J = 16.3 Hz; the chemical shifts of the A and B protons are at δ 2.55 and 1.87, respectively).[4,34] The ring methylene protons form two triplets at δ 2.25 and 1.79 and two doublets at δ 1.31 and 1.18 referred to *tert*-butyl alcohol.[15] As indicative of a dynamic process, the signals broaden with increasing temperature. The rate constants, characterizing the "flip-flop" motion of the acetate groups and the conformational changes of the ring ethylene groups, have been previously calculated.[4,16,17]

Contrary to [Y(DOTA)]$^-$, where the increasing H^+ concentration (up to 0.5 M) has no effect on the ^{1}H linewidths, for [La(DOTA)]$^-$ the signals start to broaden at [H^+] > 0.1 M (Fig. 1). The broadening occurs on all acetate and ring methylene signals, indicating that protonation shortens the lifetime of the La^{3+}–^-O_2C bonds and accelerates the conformational change of the ring. This can be explained with the simple weakening of the La^{III}–O_2C bond, or more probably with a proton transfer from the CO_2H to the nitrogen atom followed by a parallel inversion of the nitrogen and the acetate and ethylene protons.

Since the major isomer (M) is present in a negligible quantity for the lanthanum(III) complex, the observed dynamic process is the exchange of enantiomeric pairs which involves concerted conformational changes of the ethylenic groups of the macrocyclic ring and rotation of the acetate arms. In order to obtain the rate of enantiomerization (k_{obs}) as a function of [H^+], we have performed a complete lineshape analysis for the [La(DOTA)]$^-$ ^{1}H NMR spectra. The experimental and fitted spectra are presented in Fig. 1. Increasing acidity accelerates the exchange of the enantiomeric pairs (Fig. 3) as depicted in eqn. (1). The least-squares fit of the k_{obs} data using eqn. (1)

$$k_{obs} = k_0 + k_H[H^+] \quad (1)$$

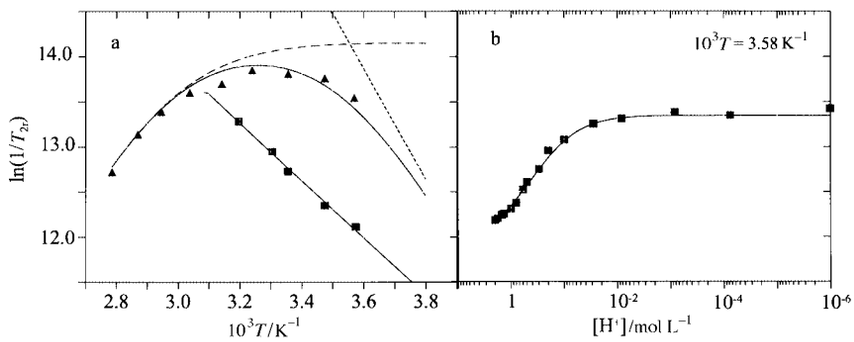

Fig. 4 (a) Temperature dependence of the reduced transverse ^{17}O relaxation rates measured for $[Gd(DOTA)]^-$ in neutral (▲) and for [HGd(DOTA)] in acidic (1.0 M) solution (■), at $B = 4.7$ T. The straight lines correspond to the least squares fit of the data points as described in the text. The long dashed line shows the relaxation rate of the co-ordinated water oxygen for $[Gd(DOTA)]^-$ ($\ln(1/T_{2m})$), whereas the short dashed line represents ln k_{ex}, as calculated on the basis of previously reported parameters[35] (k_{ex} and $1/T_{2m}$ are the two contributors to the measured reduced transverse relaxation rate). (b) Reduced transverse ^{17}O relaxation rates as a function of the H^+ concentration, at $T = 278$ K. The line corresponds to the fit that resulted in $k_{ex}^{278} = (1.7 \pm 0.1) \times 10^5\ s^{-1}$ for the monoprotonated [HGd(DOTA)] complex (see text).

resulted in $k_0^{278} = 32 \pm 1\ s^{-1}$ and $k_H^{278} = 56 \pm 4\ M^{-1}\ s^{-1}$. Based on bandshape analysis of ^{13}C NMR spectra, Desreux[4] reported $k_0^{278} = 23\ s^{-1}$ for the enantiomerization rate of the non-protonated $[La(DOTA)]^-$, which is comparable to our k_0^{278} value. As the relative ratio of k_0 and k_H shows, the effect of protonation on the enantiomerization rate is relatively small for the lanthanum(III) complex. In the case of $[Y(DOTA)]^-$ (only M isomer) the spectra show almost no line broadening on protonation which indicates that the enantiomerization is hardly affected. This is again a proof of the strong interaction between the Ln^{3+} ion and the non-protonated oxygen of the CO_2H group.

The ^1H NMR spectra of $[Eu(DOTA)]^-$ (equilibrium of m and M isomers) obtained at variable H^+ concentrations (3×10^{-7}–5×10^{-1} M) show the proton assisted acceleration of the dynamic processes (isomer interconversion and enantiomerization; spectra are in SUP 57586). The broadening of the acetate and ring methylene proton signals is approximately the same; however, due to the complexity of the spectrum, no quantitative analysis has been performed to determine the exact rates. Similarly to $[La(DOTA)]^-$, the protonation effect on the dynamics is relatively small as compared to the effect of increasing temperature, *e.g.* at 0.5 M H^+ concentration (278 K) the spectrum is similar to that recorded at 305 K (pH 7.0). The ratio of the peak integrals for the two isomers remains constant on protonation. It clearly shows that the proportion of the two isomers is not affected thus protonation does not favor one isomer to the other.

Protonation effect on the water exchange of $[Gd(DOTA)(H_2O)]^-$

The reduced ^{17}O transverse relaxation rates, $1/T_{2r}$, obtained for a $[Gd(DOTA)(H_2O)]^-$ solution in 1.0 M $HClO_4$ are shown in Fig. 4(a) and compared to those previously measured at neutral pH; $1/T_{2r} = [(1/T_2) - (1/T_{2,ref})]/P_m$.[35] The protonation results in a great decrease in the $1/T_{2r}$ values as well as in a shift of the changeover from the slow to the fast exchange region. Contrary to $[Gd(DOTA)(H_2O)]^-$, the protonated complex is in the slow exchange region throughout the temperature range studied (0–40 °C), *i.e.* the reduced transverse relaxation rate increases with temperature. This clearly indicates that the water exchange is much slower on [HGd(DOTA)(H_2O)] than on $[Gd(DOTA)(H_2O)]^-$. A quantitative analysis of the $1/T_{2r}$ values, which directly correspond to the water exchange rate, let us calculate the rate and activation enthalpy of the water exchange, which are $k_{ex}^{298} = (3.7 \pm 0.2) \times 10^5\ s^{-1}$ and $\Delta H^{\ddagger} = 25.3 \pm 2.5\ kJ\ mol^{-1}$, respectively.

We have measured the transverse relaxation rate and chemical shift for a $[Gd(DOTA)(H_2O)]^-$ solution at 278 K as a function of the H^+ concentration. The reduced chemical shifts referred to a $HClO_4$ solution of the same $[H^+]$ as the sample are identical which is another evidence of the invariant hydration number. The $1/T_{2r}$ data plotted *versus* $[H^+]$ give a "titration curve" (Fig. 4b). Although at very high H^+ concentration it was no longer possible to measure $1/T_2$ due to the rapid dissociation, a protonation constant of $\log K_{HGdL} = 0.7 \pm 0.1$ could be calculated. In addition, we can determine the water exchange rate on the protonated form by separating the contributions of the protonated and non-protonated complex to the overall relaxation rate enhancement at each $[H^+]$, based on the protonation constant. Since at this temperature the non-protonated form is not completely in the slow exchange region, its contribution was evaluated by using the full Swift–Connick equations[36] and parameters determined previously.[35] The calculations made in the $[H^+]$ range 0–0.5 or 0–1.0 M equally result in $k_{ex}^{278} = (1.7 \pm 0.1) \times 10^5\ s^{-1}$, which corresponds well to the value obtained in the variable temperature study at 1.0 M H^+ concentration ($k_{ex}^{278} = 1.6 \times 10^5\ s^{-1}$). The inclusion of $1/T_{2r}$ values measured at extremely high H^+ concentration (above 1.0 M) results in a smaller k_{ex}, which indicates that, beside [HGd(DOTA)(H_2O)], a diprotonated complex is also present. Correspondingly, the identical k_{ex}^{278} values obtained from the variable $[H^+]$ and variable temperature study ($[H^+] = 1.0$ M) show that at this H^+ concentration the formation of diprotonated complexes can be neglected.

The water exchange on the protonated complex is about one order of magnitude slower than on $[Gd(DOTA)(H_2O)]^-$ ($k_{ex}^{298} = 4.8 \times 10^6\ s^{-1}$).[36] In the interpretation of this value we can consider the following effects. The decreased negative charge always results in decreased water exchange rates, as has been widely demonstrated.[21,37] However, in the case of this protonated complex, the diminution of the exchange rate due to the charge effect could be compensated by another factor. Namely, by the transitional formation of a non-co-ordinating CO_2H group, which is very probable, although it could not be detected experimentally (the permanent presence of a second water molecule was excluded). The free co-ordination site created transitionally in this way could facilitate the water exchange, hence compensate the rate decrease caused by the diminished negative charge. However, the significantly smaller value of k_{ex} for the protonated complex as compared to that of $[Gd(DOTA)(H_2O)]^-$ shows that this hypothesis is not valid, or at least the charge effect largely dominates. Unfortunately, no variable pressure study could be performed to determine the activation volume, since these measurements take a relatively long time during which the complex would dissociate in this strong acidic medium.

The water exchange rate on the two isomeric forms of the complex can significantly differ as it has recently been demonstrated for the europium(III) complex of a DOTA amide deriv-

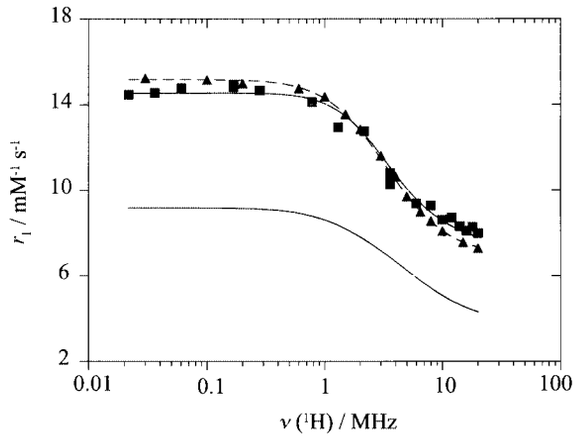

Fig. 5 Proton relaxivities for $[Gd(DOTA)]^-$ in neutral (▲) and for [HGd(DOTA)] in acidic (1.0 M) solution (■), at $T = 278$ K. The straight line represents the least-squares fit of the data points (■); the dashed line is a simulation with previously obtained parameters for $[Gd(DOTA)]^-$.[41] The same outer sphere contribution was assumed in both cases, given by the bottom line.

ative.[38] Consequently, the exchange rates reported in the literature for $[Gd(DOTA)(H_2O)]^-$ and here for [HGd(DOTA)-(H_2O)] are average values weighted by the molar fraction of the isomers, and they cannot be decomposed to obtain the real rates on each species present in the solution. The ^{1}H NMR spectra of the europium(III) complex have shown that the isomer ratio remains constant on protonation which has to be true for $[HGd(DOTA)(H_2O)]$ as well. Therefore, the protonation induced decrease of the water exchange rate can by no means be attributed to a shift in the isomer equilibrium towards the isomer with the slower exchange rate.

It is worth noting that for the $[Gd(DTMA)(H_2O)]^{3+}$ complex no change in the water exchange rate has been detected by ^{17}O NMR in a highly acidic medium (DTMA = tetrakis(methylamide of DOTA).[39] This finding, contrary to our results on $[Gd(DOTA)(H_2O)]^-$, could be explained by the fact that the positively charged DTMA complex is not protonated under these conditions, although a pK_a of 3.4 has been claimed for it.[40]

Proton relaxivity

The frequency dependence of proton relaxivities, measured by NMRD, contains a great deal of information for MRI contrast agents. The proton relaxivity has two contributions; one arises from the relative diffusional motions of unbound water molecules and the Gd^{3+} ion (outer sphere term), whereas the other is the relaxivity of the proton on the exchangeable water molecule bound in the inner co-ordination sphere (inner sphere term). This latter depends on correlation times involving rotation, water exchange and electronic relaxation, and for monohydrated gadolinium(III) complexes contributes about 50% to the overall relaxation enhancement.[41]

Fig. 5 shows the NMRD profiles recorded at 5 °C for $[Gd(DOTA)(H_2O)]^-$ in 1.0 M $HClO_4$ and at neutral pH. Whereas the low field relaxivities are slightly lower for protonated form, above 5 MHz proton Larmor frequency there is a small relaxivity increase. Since the two complexes most probably have similar rotational correlation times, these differences can be explained by a higher proton exchange rate and a slight difference in the electron spin relaxation. The acceleration of the proton exchange in acidic or basic solution is a natural phenomenon and has recently been demonstrated by proton relaxivity measurements for several gadolinium(III) complexes.[39,42] The prototropic exchange can be significantly faster than the whole water exchange even without the significant equilibrium protonation of the complex.

We have fitted the NMRD data in Fig. 5 using the common Solomon–Bloembergen theory for inner sphere relaxation,[43,44] and the Freed model for the outer sphere term.[45] We have fixed all parameters to those previously obtained for the non-protonated $[Gd(DOTA)(H_2O)]^-$[41] except for the proton exchange rate and the electronic relaxation parameters (the correlation time for the modulation of the zero-field splitting (τ_V) and the mean-square ZFS energy (Δ^2)) which were adjusted. The fitted curve is shown in Fig. 5 and the resulting parameters are $k_{ex}^{H,278} = (1.4 \pm 0.3) \times 10^6$ s^{-1}, $\tau_V^{278} = 27 \pm 5$ ps; $\Delta^2 = (0.14 \pm 0.03) \times 10^{20}$ s^{-2}, whereas the rotational correlation time τ_R^{278} was fixed to 123 ps.

The $k_{ex}^{H,278}$ can be expressed as the sum of the water exchange rate (k_{ex}) and a term describing the catalytic effect of H^+,[39] eqn. (2). The proton exchange *via* the exchange of entire

$$k_{ex}^{H} = k_{ex} + k_{ex,H}[H^+] \quad (2)$$

water molecules (k_{ex}, measured by ^{17}O NMR) contributes only about 10% to the overall proton exchange as obtained from NMRD. The value of $k_{ex,H}$ is 1.2×10^6 M^{-1} s^{-1}, which is comparable to that reported by Aime *et al.*[39] for $[Gd(DTMA)(H_2O)]^{3+}$ ($k_{ex,H} = 2.4 \times 10^6$ M^{-1} s^{-1}).

Conclusion

The extremely slow dissociation of the protonated $[HLn(DOTA)(H_2O)]$ species allows for studying their structure and dynamics even at highly acidic media (log $K_{HLnL} \approx 1$). UV-Vis and lanthanide-induced ^{17}O shift measurements on the europium(III) chelate have shown that on protonation the complex remains nine-co-ordinated and monohydrated, suggesting that the proton is attached to the non-co-ordinated oxygen of the carboxylate group.

The ^{1}H NMR spectra of $[Ln(DOTA)]^-$ complexes recorded as a function of the H^+ concentration indicate a moderate proton assisted acceleration of the dynamic processes (enantiomerization for $[La(DOTA)]^-$ and the enantiomerization and interconversion between the major and minor isomer for $[Eu(DOTA)]^-$). In all cases the chemical shifts of the acetate protons are much more affected than those of the ring ethylene protons.

The water exchange rate on $[Gd(DOTA)(H_2O)]^-$ decreases one order of magnitude between neutral pH and 1.0 M acidity. However, at high H^+ concentration, fast proton exchange compensates the slow water exchange; the exchange *via* entire water molecules contributes about 10% to the overall proton exchange as obtained from an NMRD study.

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