diaqua species. In addition, at biological pH the diaqua species will mainly exist as aqua hydroxo or dihydroxo species,[4] which will drastically influence their substitution behavior. It follows that more systematic work is needed in terms of [Cl^-] and pH dependences to be able to extrapolate to biologically relevant conditions.

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Registry No. AMP, 61-19-8; IMP, 131-99-7; GMP, 85-32-5; Pd(H_4en)$(H_2O)_2^{2+}$, 23709-90-2; Pd(Me_4en)$(H_2O)_2^{2+}$, 106150-16-7; Pd(Et_4en)$(H_2O)_2^{2+}$, 137596-44-2; ribose 5'-monophosphate, 4300-28-1.

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A Kinetic Investigation of the Lanthanide DOTA Chelates. Stability and Rates of Formation and of Dissociation of a Macrocyclic Gadolinium(III) Polyaza Polycarboxylic MRI Contrast Agent

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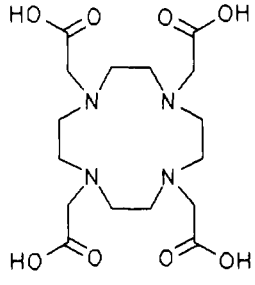
The complexation of the gadolinium(III) ion by DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracarboxylic acid) proceeds through the formation of an intermediate complex (stability constant $K^* = 6.9 \times 10^3$ M^{-1}) in which the metal ion is incompletely coordinated as shown by NMR spectroscopy. Dyes were used to measure the pH changes that take place during the complexation process. Between pH 4 and 6, the $HDOTA^{3-}$ form is the kinetically active species ($k_{f,HL} = 1.0 \times 10^6$ M^{-1} s^{-1}) despite its low concentration. The dissociation of $GdDOTA^-$ was investigated by scavenging the liberated $^{153}Gd^{3+}$ with an ion exchanger. The dissociation is exceedingly slow even in acidic solutions. It is catalyzed by the H^+ ions ($k_{d,H} = 8.4 \times 10^{-6}$ M^{-1} s^{-1}), and the acid-independent dissociation process can be neglected ($k_{d,0} < 5 \times 10^{-8}$ s^{-1}). The stability constant of $GdDOTA^-$ was computed from the rates of formation and of dissociation (log $K_{ML} = 22.1$). Contrary to earlier findings, this complex does not appear to be unusually stable. However, $GdDOTA^-$ should be a particularly safe MRI contrast agent because of its remarkable kinetic inertness.

Introduction

Nuclear magnetic resonance imaging (MRI) was originally considered a noninvasive radiological technique, but it now appears that contrast agents are often necessary to highlight lesions that otherwise could not have been detected.[1] The contrast of the NMR images depends both on the proton density and on the proton relaxation times T_1 and T_2. The water molecules contribute the most to the NMR signal, and a better contrast between different tissues can be achieved by the addition of various paramagnetic ions that drastically reduce the relaxation times of water. The ion Gd^{3+} appears to be the most useful paramagnetic species because of its high magnetic moment and its long electronic relaxation time.[2] However, this ion is too toxic to be used in vivo and it must be reacted with a chelating agent before being injected in the blood in order to facilitate its rapid excretion through the kidneys. Safe and effective contrast agents containing gadolinium should not dissociate in the body and thus should be highly stable and kinetically inert. High stability is achieved in the case of diethylenetriaminepentaacetic acid (DTPA), and $GdDTPA^{2-}$ is now a commonly used MRI contrast agent. However, it was anticipated that a better kinetic inertness would be achieved with macrocyclic tetracarboxylic ligands such as DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracarboxylic acid) and its derivatives. Indeed, the steric requirements of the tetraaza cavity of DOTA impart an unusual rigidity to its lanthanide chelates[3] and could be at the origin of the slow rate of formation and of dissociation of these complexes. Preliminary studies[4,5] indicate that $YDOTA^-$ and $GdDOTA^-$ undergo a negligible dissociation in serum at pH 7 over long periods of time. Moreover, a spectrophotometric analysis[6] of the kinetic properties of $CeDOTA^-$ also showed that the metal ion is released very slowly even in acidic media. This unusual kinetics contrasts significantly with the behavior of lanthanide complexes with linear chelating agents which are known to form and to dissociate rapidly. The rate and the mechanism of the formation and of the dissociation of $GdDOTA^-$ are of considerable interest to the design of effective MRI contrast agents[2] or radiolabeled monoclonal antibodies.[4] The primary goal of the present work was thus to study the kinetic properties of $GdDOTA^-$. An important second objective was to deduce the stability constant of $GdDOTA^-$ from kinetic data. Indeed, the very slow rates of complexation and of dissociation of the lanthanide ions by DOTA prevent the reliable measurement of the thermodynamic stability of the $LnDOTA^-$ chelates by classical methods such as potentiometry. It was thus necessary to resort to competition techniques using either a precipitating agent[7] or a dye,[8] but these approaches led to stability constants



DOTA

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(1) Runge, V. M. *Enhanced magnetic resonance imaging*; C. V. Mosby Co.: St. Louis, MO, 1989.
(2) Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901-927.
(3) Desreux, J. F. *Inorg. Chem.* **1980**, *19*, 1319-1324.
(4) (a) Moi, M. K.; Meares, C. F.; DeNardo, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 6266-6267. (b) Broan, C. J.; Cox, J. P. L.; Craig, A. S.; Kataky, R.; Parker, D.; Harrison, A.; Randall, A. M.; Feruson, G. *J. Chem. Soc., Perkin Trans. 2* **1991**, 87-99.
(5) Magerstadt, M.; Gansow, O. A.; Brechbiel, M. W.; Colcher, D.; Baltzer, L.; Knop, R. H.; Girton, M. E.; Naegele, M. *Magn. Reson. Med.* **1986**, *3*, 808-812.
(6) Brücher, E.; Laurenczy, G.; Makra, Z. S. *Inorg. Chim. Acta* **1987**, *139*, 141-142.
(7) Loncin, M. F.; Desreux, J. F.; Merciny, E. *Inorg. Chem.* **1986**, *25*, 2646-2648.

0020-1669/92/1331-1095$03.00/0

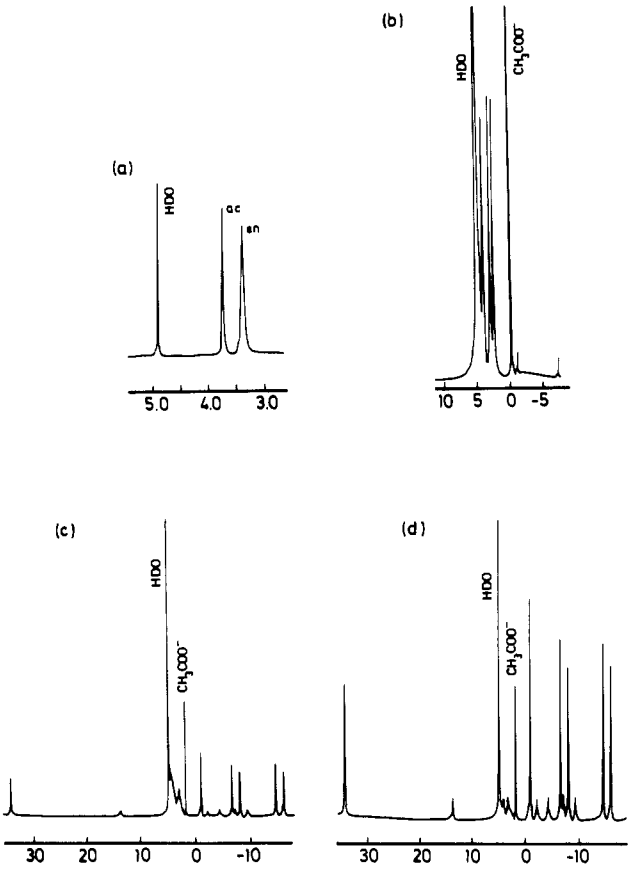

Figure 1. Analysis of the kinetics of formation of $EuDOTA^-$ by proton NMR spectroscopy at 400 MHz and probe temperature: (a) spectrum of DOTA; (b) spectrum taken immediately after mixing equimolecular solutions of DOTA and Eu^{3+} (pD 4.27); (c) spectrum of the same mixture after 6 min (pD 4.65); (d) spectrum of the same mixture after 20 min (pD 4.65). The NMR peak labeled CH_3COO^- represents the CH_2 group of the acetate ion used as buffer. It is shifted upfield in (b) because of partial complexation with the Eu^{3+} ion.

that differ by 3.5 orders of magnitude.

Experimental Section

The synthesis of DOTA was carried out as described earlier.[3] The purity of the ligand used in the kinetic experiments was checked by pH titration and by HPLC analysis; it was found to be higher than 99.5%. All other chemicals employed were of the highest analytical purity. The proton NMR spectra were recorded on a Bruker AM spectrometer at 400 MHz and at probe temperature. The radioactive tracer ^{153}Gd was obtained from the Radiochemical Center, Amersham, England, as a chloride salt in dilute hydrochloric acid. The γ activity of each solution was determined with an Intertechnique γ counter. All pH measurements were performed with a Radiometer 26 pH meter equipped with an Ingold U455 combined pH electrode. Acid concentrations were deduced from the pH by taking into account the activity coefficient of HCl in a 1 M NaCl solution (γ = 0.754). The protonation constants of DOTA were taken from refs 9 and 10.

Kinetics of Formation. Indicators were used to monitor the small pH decrease that takes place when a lightly buffered solution of DOTA (0.2–0.5 mM) is mixed with a lightly buffered solution of an excess of $GdCl_3$ (0.5–10 mM) at the same pH. The pH variation caused by the formation of the Gd chelate was registered using bromocresol green (pH range 4.2–4.8, observation wavelength 615 nm) and chlorophenol red (between pH 5.4 and 5.8, at 575 nm). The pH changes were kept reasonably small (0.1–0.2 pH unit) with the following buffers: sodium acetate (pH <5) and 2-morpholinoethanesulfonic acid (pH range 5–6). Earlier measurements by Kasprzyk and Wilkins[11] indicate that these indicators do not interact with the ligand or the buffer. The ionic strength was adjusted to 1.00 with NaCl. The formation of $EuDOTA^-$ was followed by NMR spectroscopy at probe temperature immediately after mixing equal volumes of a 0.17 M solution of $EuCl_3$ with a 0.04 M solution of DOTA. Both solutions were prepared in D_2O and contained the same amount of buffer (0.16 M acetic acid/sodium acetate). The pD was adjusted to 4.65.

Kinetics of Dissociation. A 25-mL aliquot of a 1.11×10^{-3} M solution of $GdCl_3$ (2.78×10^{-5} mol) containing ^{153}Gd was added to 2.75×10^{-5} mol of DOTA dissolved in 110 mL of water. Both solutions were prepared at the same ionic strength (0.50 or 1.00 M NaCl) and at pH 5. The mixture was brought to 60 °C and was agitated at that temperature during 2 days. The complex was then considered as completely formed, and 0.5 g of a sulfonated ion exchanger Dowex 50W-X4 was added to the solution at room temperature in order to eliminate any free gadolinium. Because of the very slow kinetics of dissociation of $GdDOTA^-$ at pH 5, no free gadolinium is produced during this purification step. Aliquots (25 mL) of the filtered solution were added to 0.25–0.4 g of Dowex 50W-X4, and the acidity was brought to 0.02–0.23 M with concentrated HCl. Before each experiment, each batch of Dowex resin had been conditioned by equilibration with an aqueous solution of the appropriate HCl concentration and ionic strength. The mixtures were agitated at 25 °C, and small volumes of solutions were withdrawn at regular time intervals for γ counting. It was verified that, under our experimental conditions, the uncomplexed Gd^{3+} ion and the protonated $H_{4+n}DOTA^{n+}$ species were completely taken up by the cation exchanger. Moreover, it was checked that the presence of the ion exchanger had no effect on the kinetics of dissociation. Identical data were obtained if the kinetic runs were performed either with the ion exchanger added to the reaction mixture immediately after adjusting the acid content or with aliquots of the $GdDOTA^-$ solutions being taken at regular time intervals and added to small amounts of the Dowex resin. The rate of dissociation of $GdDOTA^-$ was deduced from the decrease of the γ radioactivity of the solutions with time due to the scavenging of free Gd^{3+} by the ion-exchange resin.

Results and Discussion

Kinetics of Formation. It has been suggested[12] that the complexation of a lanthanide ion M by a polyaza polycarboxylic ligand L takes place in two steps: first, the rapid formation of an intermediate species $(ML)^*$ in which the metal ion is incompletely coordinated and, afterwards, the slow rearrangement of this intermediate into the final complex ML. As suggested by Kasprzyk and Wilkins,[11] this kinetic scheme can be represented by eqs 1 and 2. The reaction path (1)–(2) cannot be distinguished from

$$M + L \rightleftarrows (ML)^* \quad \text{equilibrium constant } K^* \qquad (1)$$

$$(ML)^* \rightarrow ML \quad \text{kinetic constant } k^* \qquad (2)$$

$$M + L \rightarrow ML \quad \text{kinetic constant } k_f \qquad (3)$$

another complexation process in which the intermediate species $(ML)^*$ formed in eq 1 is unable to evolve into the final chelate ML, the latter being formed directly as shown in eq 3. The complexation of lanthanide ions is usually too fast to allow the direct observation of the formation of the intermediate species $(ML)^*$ and its subsequent rearrangement by classical spectroscopic methods. However, the formation of the $CeDOTA^-$ is remarkably slow and brings about very large shifts in the UV spectrum of the Ce^{3+} ion. Brücher et al.[6] were thus able to observe simultaneously the disappearance of the intermediate $(CeDOTA)^*$ and the formation of the final complex $CeDOTA^-$. The same authors were also able to show that the formation of the intermediate is not accompanied by the liberation of protons. These observations are most unusual in lanthanide chemistry, and NMR spectroscopy yields similar results. It is well known[13] that the paramagnetic lanthanide ions induce large dipolar shifts, and the relative magnitudes of these shifts have been used to elucidate the solution structure of the DOTA chelates.[3] In addition, the NMR spectra of paramagnetic chelates also provide mechanistic information on the complexation by DOTA. As shown in Figure 1, the two signals assigned to the CH_2 moieties of the acetate groups and of the tetraaza ring of DOTA appear as three peaks immediately

(8) Cacheris, W. P.; Nickle, S. K.; Sherry, A. D. *Inorg. Chem.* **1987**, *26*, 958–960.
(9) Desreux, J. F.; Merciny, E.; Loncin, M. F. *Inorg. Chem.* **1981**, *20*, 987–991.
(10) Delgado, R.; Frausto da Silva, J. J. R. *Talanta* **1982**, *29*, 815–822.
(11) Kasprzyk, S. P.; Wilkins, R. G. *Inorg. Chem.* **1982**, *21*, 3349–3352.
(12) Nyssen, G. A.; Margerum, D. W. *Inorg. Chem.* **1970**, *9*, 1814–1820.
(13) Inagaki, F.; Miyazama, F. *Prog. NMR Spectrosc.* **1981**, *14*, 67–111.

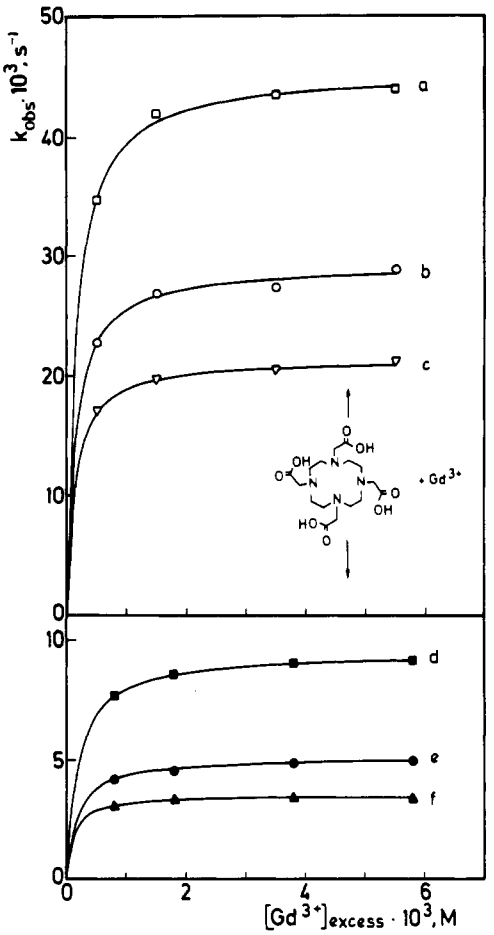

Figure 2. Formation kinetics of $GdDOTA^-$: plots of k_{obs} against excess $[Gd^{3+}]$ at various H^+ concentrations (25 °C, I = 1 M). The solid lines through the data points were calculated using the data listed in Table I. $[H^+]$, M: (a) 2.10×10^{-6}; (b) 3.33×10^{-6}; (c) 5.28×10^{-6}; (d) 1.33×10^{-5}; (e) 2.10×10^{-5}; (f) 3.31×10^{-5}.

after mixing solutions of Eu^{3+} and of the ligand. These peaks are shifted by about 1.0 ppm upfield or downfield from their original position, and they are progressively replaced by six resonances that are considerably more shifted and that were already found in the spectrum of the $EuDOTA^-$ complex.[3] The three peaks observed at the beginning of the complexation process are assigned to the intermediate species (EuDOTA)*. The small paramagnetic shifts exhibited by these peaks are in keeping with the stereochemical arrangement predicted for an intermediate complex (ML)*. Indeed, relatively small paramagnetic shifts are expected if DOTA is only partially coordinated to the Eu^{3+} ion when it forms a labile complex (EuDOTA)*, since these shifts depend on the distance between the metal ion and the protons under consideration.[13] However, the fact that three peaks are observed in the case of (EuDOTA)* instead of two for the free ligand indicates that the tetraaza cycle of this intermediate complex exhibits some form of rigidity.

The complexation of Gd^{3+} by DOTA cannot be investigated by NMR spectroscopy because of the small paramagnetic shifts and the large peak widths induced by this ion.[13] However, the pH changes that take place when Gd^{3+} reacts with the protonated forms of DOTA can be accurately measured with the help of dyes. This method has already been successfully applied by Kasprzyk and Wilkins[11] to an analysis of the kinetics of complexation of alkaline earth and transition metal ions by DOTA. In the present case, the kinetic investigation had to be carried out in a relatively small pH range (4.6–5.8) because the lanthanide ions start forming hydroxylated species above pH 6 and because the complexation was too slow below pH 4.5. As indicated in Figure 2, the dependence of k_{obs} on the excess Gd^{3+} concentration was curved. This "saturation kinetics" is in keeping with eqs 1–3 and is ascribed

Table I. Effect of Acidity on the Experimental Rate Data for the Formation of GdDOTA (25 °C, I = 1 M)

$[H^+]$, M	k^*, s^{-1}	K^*, M^{-1}	k_f, $M^{-1}\ s^{-1}$
3.31×10^{-5}	$(3.52 \pm 0.02) \times 10^{-3}$	$(9.2 \pm 0.9) \times 10^3$	32 ± 3^a
2.10×10^{-5}	$(5.11 \pm 0.06) \times 10^{-3}$	$(5.7 \pm 0.7) \times 10^3$	29 ± 4^a
1.33×10^{-5}	$(9.44 \pm 0.06) \times 10^{-3}$	$(5.3 \pm 0.3) \times 10^3$	50 ± 3^a
5.28×10^{-6}	$(2.14 \pm 0.02) \times 10^{-2}$	$(7.4 \pm 0.6) \times 10^3$	159 ± 13^b
3.33×10^{-6}	$(2.92 \pm 0.05) \times 10^{-2}$	$(7.0 \pm 1.1) \times 10^3$	205 ± 32^b
2.10×10^{-6}	$(4.55 \pm 0.04) \times 10^{-2}$	$(6.6 \pm 0.5) \times 10^3$	300 ± 24^b

^{a} [DOTA] = 2.0×10^{-4} M. ^{b} [DOTA] = 5.0×10^{-4} M.

to the formation of an intermediate complex (GdDOTA)*. Similar behavior has been reported[11] for the formation of the $CaDOTA^{2-}$ chelate and can be accounted for by eq 4 or 5 if the

$$k_{obs} = \frac{k^*K^*[M]}{1 + K^*[M]} \tag{4}$$

$$k_{obs} = \frac{k_f[M]}{1 + K^*[M]} \tag{5}$$

complexation proceeds according to either eqs 1 and 2 or eqs 1 and 3. These equations can be rearranged to give respectively

$$\frac{1}{k_{obs}} = \frac{1}{k^*K^*[M]} + \frac{1}{k^*} \tag{6}$$

and

$$\frac{1}{k_{obs}} = \frac{1}{k_f[M]} + \frac{K^*}{k_f} \tag{7}$$

Plots of $1/k_{obs}$ against $1/[M]$ lead to straight lines. The values of k^*, K^*, and the second-order rate constant $k_f = k^*K^*$ were deduced from eqs 4 and 5 and are collected in Table I. It should be noted here that a value of k_{obs} for $EuDOTA^-$ can be deduced form an analysis of the NMR data reported in Figure 1. Although the experimental conditions are not the same, the experimental value of k_{obs} for $EuDOTA^-$ at pD 4.65 ($k_{obs} = 1.6 \times 10^{-3}\ s^{-1}$) is in good agreement with the value calculated for $GdDOTA^-$ using the kinetic data collected in Table I ($k_{obs} = 3.5 \times 10^{-3}\ s^{-1}$). Three protonated forms of DOTA could contribute[9,10] to the kinetics of complexation of Gd^{3+} between pH 4.5 and 6: H_2L^{2-} is the major species in this pH range (from 53 to 96%), H_3L^- is an important contributor (from 31 to 3%), and HL^{3-} represents from 0.001 to 0.03% of the total ligand concentration. Such a situation is accommodated by a rate law of the form

$$d[ML]/dt = k_f[M][L]_{tot} = [M](k_{f,H_3L}[H_3L^-] + k_{f,H_2L}[H_2L^{2-}] + k_{f,HL}[HL^{3-}]) \tag{8}$$

and thus

$$k_f = k_{f,H_3L}\alpha_{H_3L} + k_{f,H_2L}\alpha_{H_2L} + k_{f,HL}\alpha_{HL} \tag{9}$$

with

$$\alpha_{H_nL} = \frac{\beta_n[H^+]^n}{1 + \beta_1[H^+] + \beta_2[H^+]^2 + \ldots + \beta_n[H^+]^n} \tag{10}$$

where β_n is the overall formation constant of the H_nL species

$$\beta_n = \frac{[H_nL^{(4-n)-}]}{[H^+]^n[L^{4-}]} \tag{11}$$

and where the second-order rate constants k_{f,H_nL} also represent $(k^*K^*)_{f,H_nL}$, since the mechanisms (1)–(2) and (1)–(3) account equally well for the kinetic data. If one species is markedly more reactive than all the others, eq 9 becomes[14]

$$\log k_f = \log k_{f,H_nL} + \log \alpha_{H_nL} \tag{12}$$

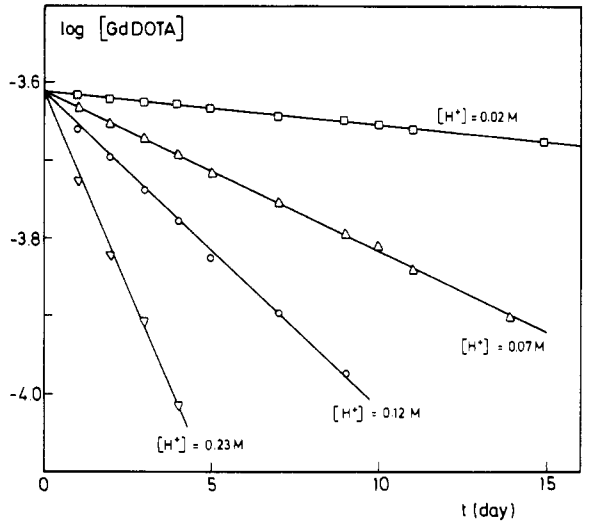

Figure 3. Dissociation kinetics of $GdDOTA^-$: concentration of $GdDOTA^-$ vs time as a function of acidity.

Thus a plot of log k_f against log α_{H_nL} should lead to a straight line of slope 1.0. All calculations were carried out by employing the protonation constants of DOTA determined previously[9] in the same ionic medium as used here (ionic strength 1.00). Nonlinear relationships or slopes very different from unity were obtained for all the $H_nL^{(4-n)-}$ forms (with $n = 0$–3). The calculations carried out in the case of the HL^{2-} species yielded the slope that was the closest to unity (0.71), but it was however too different from 1.0 to assume that there was only one reactive species in our experimental conditions. Equation 9 thus had to be solved by a multiple-regression approach. Negative kinetic constants with large errors were systematically obtained for the L^{4-} and H_3L^- species when they were included in the calculations, but an excellent agreement was reached if HL^{3-} and H_2L^{2-} were the only forms taken into account. The second-order rate constants for the HL^{3-} and H_2L^{2-} species are

$$k_{f,HL} = (1.0 \pm 0.8) \times 10^6\ M^{-1}\ s^{-1}$$

$$k_{f,H_2L} = 35 \pm 14\ M^{-1}\ s^{-1}$$

Despite its very low concentration in the pH range investigated, the HL^{3-} form appears to be kinetically much more reactive than the diprotonated species H_2L^{2-} even though the latter is the major component in solution. The same conclusion was reached previously by Wilkins[11] in a investigation of the complexation of the alkaline earth and the transition metal ions by DOTA. The low reactivity of the H_2L^{2-} form was assigned to the large electrostatic repulsion caused by the two protonated nitrogen atoms in the macrocyclic ring of this species. Indeed, NMR[9] and X-ray[15] studies indicate that the first two protons that associate with macrocyclic tetraaza tetracarboxylic ligands are attached to two nitrogen atoms in the trans position. The two $N-H^+$ groups in the 12-membered ring of H_2DOTA^{2-} probably hinder the rearrangement of the intermediate complex ML* into the final chelate because of the electrostatic repulsion with the incoming metal ion. The steric requirements of the tetraaza ring of DOTA are also likely to lower the rate of formation of the complexes, since the rate constant $k_{f,HL}$ of DOTA is about 20 times lower than in the case of CDTA (1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid).[12] As expected, this rate constant is also 4 orders of magnitude lower than in the case of simple ligands such as murexide for which the water-substitution step is rate determining.[16]

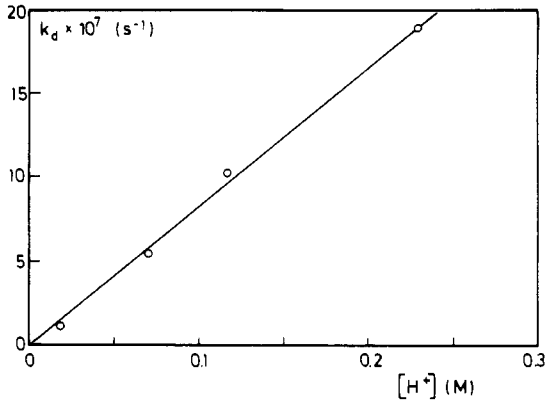

Figure 4. Dissociation rate of $GdDOTA^-$ vs $[H^+]$.

Kinetics of Dissociation. The very slow dissociation of $GdDOTA^-$ dictates that all measurements be carried out in fairly acidic media. Even under these conditions, the dissociation is a matter of days rather than seconds as observed for noncyclic polyamino polycarboxylic acids (Figure 3). As shown in Figure 4, the first-order rate constant for the dissociation k_d is linearly proportional to the proton concentration $[H^+]$. The dissociation reaction is thus consistent with the rate law

$$k_d = k_{d,0} + k_{d,H}[H^+] \qquad (13)$$

Within the limits of the experimental errors, the rate constant $k_{d,0}$ is null (error on $k_{d,0} = 5 \times 10^{-8}\ s^{-1}$) and $k_{d,H} = (8.4 \pm 0.4) \times 10^{-6}\ M^{-1}\ s^{-1}$. As far as our experience goes, it appears that $GdDOTA^-$ is the most inert lanthanide complex reported so far. This unusual kinetic inertness probably stems from the tight packing and the high rigidity of the DOTA chelates. EuDOTA is known[17] to adopt a square-antiprismatic conformation with all CH_2 groups fully staggered; this structure is rigid on the NMR time scale, and even the carboxylic groups appear to be nonlabile and to remain fully coordinated in solution.[3] The rate of inversion of the nitrogen atoms and the rate of dissociation of the metal–oxygen bonds is thus considerably reduced by the steric requirements of the DOTA ligand. The dissociation of $GdDOTA^-$ most probably proceeds through the rapid formation of a protonated species (HGdDOTA*) which undergoes a slow rearrangement into an intermediate complex featuring a protonated nitrogen atom. It is likely that the entering of a proton into the macrocyclic cage is very difficult because of the rigidity of the tetraaza ring. The acid-catalyzed reaction is thus much less effective than for the linear polyamino polyacetate complexes. The role of the stereochemical requirements of DOTA in determining the kinetic inertness of the lanthanide chelates is particularly notable in comparison with recently published analyses of the dissociation of the lanthanide complexes with 1,7-diaza-4,10,13-trioxacyclopentadecane-*N*,*N*′-diacetic acid[18] or with 1,4,7-triazacyclononane-*N*,*N*′,*N*″-triacetic acid (NOTA).[19] The former macrocycle is larger than DOTA, while the latter is smaller. Both are less rigid, and they feature less coordinating groups. The

(14) Equation 12 is a more general form of eq 10 in ref 11

$$\log k_f = \log k_{f,HL} - \log (1 + K_{H_2L}[H^+])$$

where K_{H_2L} is the protonation constant of the H_2DOTA^{2-} form.[9] This equation is obtained after some simplifications, and it can be written as

$$k_f = k_{f,HL}/K_{H_2L}[H^+]$$

provided the kinetic measurements are carried out below pH 7. The rate constant k_f is thus inversely proportional to the proton concentration $[H^+]$. This relationship has been taken as an indication that the rearrangement of the intermediate complex (GdDOTA)* into the final chelate is catalyzed by the OH^- anion.[19] Although this hypothesis cannot be excluded, we have adopted a more general approach in the present work, and the potential role of the OH^- ion was not taken into account.

(15) Spirlet, M. R.; Rebizant, J.; Barthélemy, P. P.; Desreux, J. F. *J. Chem. Soc., Dalton Trans.* **1991**, 2477–2481.

(16) Margerum, D. W.; Cayley, G. R.; Weatherburn, D. C.; Pagenkopf, G. K. In *Coordination Chemistry, Vol. 2*; ACS Monograph 174; Martell, A. E., Washington, D. C., Eds.; American Chemical Society: Washington, D.C., 1978; pp 1–220.

(17) Spirlet, M.-R.; Rebizant, J.; Desreux, J. F.; Loncin, M. F. *Inorg. Chem.* **1984**, *23*, 359–363.

(18) Sekhar, V. C.; Chang, C. A. *Inorg. Chem.* **1986**, *25*, 2061–2065.

(19) Brücher, E.; Sherry, A. D. *Inorg. Chem.* **1990**, *29*, 1555–1559.

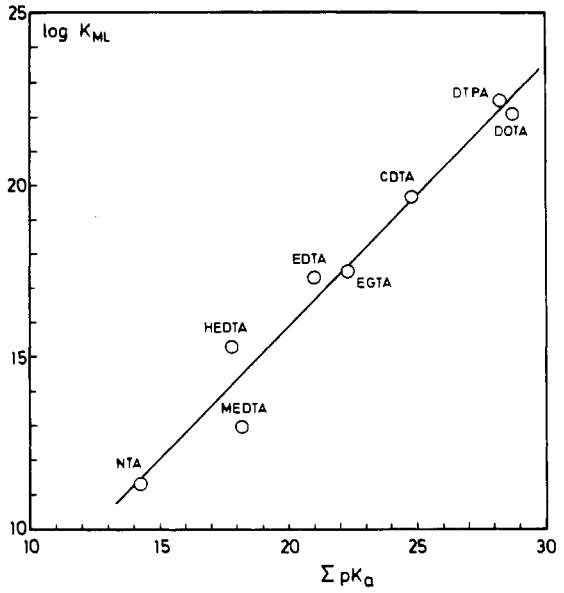

Figure 5. Correlation between the stability constant log K_{ML} of various Gd^{3+} chelates and the sum of the pK_a values of the ligands. The abbreviated names of the ligands and the values of their stability constants are taken from ref 24.

acid-catalyzed dissociations of their lanthanide complexes are approximately 7 and 4 orders of magnitude faster than in the case of GdDOTA$^-$. However, it should be noted that a growing number of kinetically inert lanthanide compounds will probably be reported in the future. Besides various DOTA derivatives,[4,20] the (2.2.1) cryptates[21] and macrocyclic complexes prepared by template synthesis in the presence of a lanthanide ion[22] also exhibit an unusual kinetic inertness toward substitution reactions in water. In our hands, these complexes were found to be kinetically less inert than the GdDOTA$^-$ chelate.

Thermodynamic Stability. The stability constant K_{ML} of GdDOTA$^-$

$$K_{ML} = \frac{[GdDOTA^-]}{[Gd^{3+}][DOTA^{4-}]} \quad (14)$$

can be deduced from the rates of the forward and backward reactions[22]

$$Gd^{3+} + HDOTA^{3-} \underset{k_{d,H}}{\overset{k_{f,HL}}{\rightleftharpoons}} GdDOTA^- + H^+$$

since

$$K_{ML} = \frac{k_{f,HL}K_{HL}}{k_{d,H}} \quad (15)$$

where K_{HL} is the first protonation constant of DOTA that has been determined[9] in the same ionic medium as the one used in the present work (log K_{HL} = 11.08). The calculations lead to log K_{ML} = 22.1 ± 0.1. This value is considerably lower than the stability constants reported previously[7,8] for the lanthanide DOTA chelates. However, this value is in keeping with a linear correlation between the log K_{ML} values of lanthanide complexes with linear polyamino polyacetic ligands and the $\sum pK_a$ of these ligands as shown in Figure 5. This correlation was suggested by Choppin et al.;[23] it holds, provided no ligand features a steric hindrance for the formation of complexes. The log K_{ML} vs $\sum pK_a$ relationship can thus be used to determine if a polyamino polyacetic ligand exhibits an unusual complexation behavior toward the lanthanides. The data used in the correlation depicted in Figure 5 are "critical stability constants" collected by Martell and Smith.[24] The $\sum pK_a$ values that are considered are the sum of the protonation constants leading to the obtention of the fully protonated electrically neutral form of each ligand. We used the data obtained for a 0.1 ionic strength with NH_4^+ (or, if not available, with Na^+) as the background ion. As shown in Figure 5, the stability constant of GdDOTA$^-$ compares well with the stability of the gadolinium chelates of noncyclic polyamino polyacetic ligands. In contrast to an earlier report,[7] the peculiar structure of the DOTA ligand does not seem to result in an usually high thermodynamic stability of its GdDOTA$^-$ complex. This conclusion remains valid even though our value of the stability constant K_{ML} could be subjected to some fairly large errors because we have systematically adopted the simplest approach to the analysis of the kinetics of formation and of dissociation of GdDOTA$^-$. Our kinetic models could thus be oversimplified. For instance, we neglected the partial protonation of GdDOTA$^-$ in strongly acidic medium or a catalysis by the acetate ions. Furthermore, the kinetics of formation and of dissociation had to be investigated under very different pH conditions. Finally, it should be noted here that Cacheris et al. have reported[8] a correlation identical to the relationship presented in Figure 5 although their value of K_{ML} is nearly 3 orders of magnitude higher than the stability constant calculated above. When computing $\sum pK_a$ in the case of DOTA, these authors have apparently used all the protonation constants reported for this ligand,[9,10] including those for the negatively charged forms H_5DOTA^- and H_6DOTA^{2-}, while these species do not seem to have been taken into account for the other ligands. Finally, we stress again that the correlation shown in Figure 5 is only approximate and should not be used as a test for the accuracy of a stability constant K_{ML}: this correlation only indicates that the complexing properties of a ligand are in keeping with the behavior of analogous chelates.

Conclusion

The thermodynamic stability of GdDOTA$^-$ is not unusual despite the cyclic structure of the ligand DOTA, but the rates of formation and of dissociation of this chelate are very low. The kinetic properties of analogues of GdDOTA$^-$ are being investigated at present, and it seems that all tetraaza macrocyclic chelates exhibit similar behaviors. The very slow dissociation of GdDOTA$^-$ is particularly interesting in view of the use of this complex as a MRI contrast agent. When compared to $GdDTPA^{2-}$, GdDOTA$^-$ presents the major advantage of dissociating much more slowly even in acidic media. With a half-life of 85 days at pH 2 and of more than 200 days at pH 5 (if the error on $k_{d,0}$ is taken as the maximum value of the spontaneous rate of dissociation), GdDOTA$^-$ is indeed kinetically remarkably inert. This chelate should thus be a safer contrast agent for NMR imaging even if its thermodynamic stability is not higher than in the case of $GdDTPA^{2-}$. Finally, it should be noted that the ionic size of the lanthanides seems to have a drastic effect on the kinetic phenomena. Brücher et al.[6] reported a $k_{d,H}$ value for CeDOTA$^-$ which is 2 orders of magnitude higher than the value we obtained for GdDOTA$^-$. This is in keeping with an earlier report[25] that shows that the lanthanides can be separated into two groups by chromatography on a cationic ion exchanger in acidic medium because of the faster kinetics of dissociation of the (La–Eu)DOTA$^-$ chelates.

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(20) Tweedle, M. F.; Eaton, S. M.; Eckelman, W. C.; Gaughan, G. T.; Hagan, J. J.; Wedeking, P. W.; Yost, F. J. *Invest. Radiol.* **1988**, *23*, Suppl. 1, S236–S239.
(21) Yee, E. L.; Gansow, O. A.; Weaver, M. J. *J. Am. Chem. Soc.* **1980**, *102*, 2278–2285.
(22) Smith, P. H.; Brainard, J. R.; Morris, D. E.; Jarniven, G. D.; Ryan, R. R. *J. Am. Chem. Soc.* **1989**, *111*, 7437–7443.
(23) Gritmon, T. F.; Goedken, M. P.; Choppin, G. R. *J. Inorg. Nucl. Chem.* **1977**, *39*, 2021–2023.
(24) Smith, R. M.; Martell, A. E. *Critical Stability Constants*; Plenum Press: New York, 1989; Vol. 6.
(25) Merciny, E.; Desreux, J. F.; Fuger, J. *Anal. Chim. Acta* **1986**, *189*, 301–311.