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Kinetics of the Formation of $[Ln(DOTAM)]^{3+}$ Complexes

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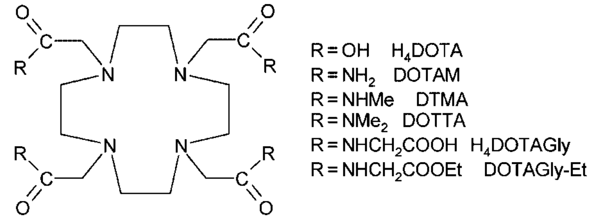
The kinetics of the formation of lanthanide(III) complexes of the neutral ligand DOTAM differ considerably from those of DOTA and its derivatives, which contain negatively charged pendant groups. Formation of the complexes $[Ln(DOTAM)]^{3+}$ occurs upon direct encounter of the Ln^{3+} ions and the fully deprotonated ligand in the pH range 4.7–5.8. The formation of a protonated intermediate, which is characteristic of the complexation of DOTA and its derivatives, was not detected. General base catalysis is not valid in the formation reactions of $[Ln(DOTAM)]^{3+}$, which also indicates the absence of protonated intermediates. The rates of the proton-exchange reactions for the ligand species H_2DOTAM^{2+} and $HDOTAM^+$ have been studied by ^1H NMR spectroscopy. Proton exchange was found to be a general base-catalysed process, therefore it cannot play an important role in the formation of these complexes. The second-order rate constants, k_L, which characterize the formation of the complexes $[Ln(DOTAM)]^{3+}$, are lower than the analogous rate constants reported for the Ln^{3+} complexes of open-chain multidentate ligands by three to four orders of magnitude. These low k_L values can be interpreted by assuming an early rate-determining step (probably the second or third Ln^{3+}–amide bond formation) in the reaction sequence that leads to the $[Ln(DOTAM)]^{3+}$ complex.

Introduction

Lanthanide(III) complexes of the different tetraamide derivatives of DOTA (Scheme 1) have attracted considerable interest in recent years because of their promising applications in different fields of biological chemistry. Positively charged complexes {e.g. $[La(DOTAM)]^{3+}$}, for example, promote or catalyse rapid RNA cleavage and hydrolysis of some biopolymers (e.g. phosphate esters),[1–3] and complexes of Eu^{3+} and Yb^{3+} with DOTAGly, DOTAGly-Et and other DOTA tetraamide derivatives, have been proposed as chemical exchange saturation transfer (CEST) agents in magnetic resonance imaging.[4–8] Another possible field of application for the tetraamide derivatives is the development of luminescent sensors.[9,10]

The structure of the lanthanide(III) (Ln^{3+}) complexes formed with the DOTA tetraamide derivatives was found to be similar to that of $[Ln(DOTA)(H_2O)]^-$,[11] with the Ln^{3+} ion occupying a position in the coordination cage formed by the four ring nitrogen atoms and four amide oxygen atoms, which form a square antiprism. A water molecule is coordinated to the Ln^{3+} ion in a capping position.[1,2,12–16]



Scheme 1. Molecular structures of the ligands discussed.

The solution structure of the Ln^{3+}–DOTA tetraamide complexes has been studied by ^1H, ^{13}C and ^{17}O NMR spectroscopy, and the lifetime of the inner-sphere water was found to be significantly higher than that of the complexes $[Ln(DOTA)(H_2O)]^-$.[12,16–19] The stability constants ($\log K_{ML}$) of the Ln^{III} complexes formed with the DOTA tetraamide derivatives are about 10–15 orders of magnitude lower than the $\log K_{ML}$ values of the DOTA complexes.[2,15,20,21] However, the rates of the Cu^{2+}- and proton-assisted dissociation of the complexes $[Eu(DOTAM)]^{3+}$, $[Eu(DTMA)]^{3+}$ and $[Eu^{III}DOTTA]^{3+}$ are significantly lower than that of $[Eu(DOTA)]^-$.[1,12] The fairly large stability constants and the unusually high kinetic inertness of the complexes formed with the neutral DOTA tetraamide ligands is quite unexpected since Ln^{3+} ions prefer ligands containing oxygen donor atoms with a negative charge. The moderate stability and high kinetic inertness of these complexes is probably the result of the good match between the size of the Ln^{3+} ions and the preformed cavity of the neutral

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ligands. The high thermodynamic and kinetic stabilities displayed by the Ln^{3+} complexes of $DOTA^{4-}$ and its negatively charged derivatives can be accounted for not only in terms of the size matching between the Ln^{3+} ions and the ligands but also the important role played by the electrostatic interactions. Thus, the study of the behavior of the DOTA tetraamide ligand DOTAM may give some new information about the kinetics and mechanisms of the reactions taking place between Ln^{3+} ions and neutral macrocyclic ligands possessing a preformed cavity.

The formation of Ln^{3+} complexes with DOTA tetraamide ligands takes place very slowly in aqueous solutions and, because of these slow reactions, the complexes in the solid state are often prepared in non-aqueous solution.[1,12–14] The kinetics of formation of the tetraamide complexes $[Ln(DOTAGly)]^-$ are very similar to those of $[Ln(DOTA)]^-$, as we have reported previously.[21] This similarity is probably due to the presence of four negatively charged functional groups in both $DOTA^{4-}$ and $DOTAGly^{4-}$, which can coordinate to Ln^{3+} in the first step of the reaction and form mono- and diprotonated intermediates. The protons protonate the ring nitrogen atoms, while the Ln^{3+} ion is still outside of the coordination cage. The rate-determining step of the formation of $[Ln(DOTA)]^-$ and $[Ln(DOTAGly)]^-$ is the deprotonation of the monoprotonated intermediate in a first-order reaction.[21–26] Because they isolated the diprotonated compound $[Gd(H_2DOTAM)(H_2O)_4](ClO_4)_5$ in the solid state, Parker et al. have assumed that the formation of $Gd(DOTAM)^{3+}$ occurs by a similar pathway.[27] However, as our preliminary studies have indicated, the formation of Ln^{3+} complexes of the ligand DOTAM occurs by a completely different pathway as their rates of formation are directly proportional to the concentration of both Ln^{3+} and DOTAM. A detailed study of the rates of the reaction shown in Equation (1) between Ln^{3+} ions (Ln^{3+} = Ce^{3+}, Eu^{3+}, Gd^{3+}, Er^{3+} and Yb^{3+}) and DOTAM may provide new information about the role of the charged and uncharged functional groups in the reactions of a preorganized macrocyclic ligand.

$$Ln^{3+} + H_nL^{n+} \rightleftharpoons LnL^{3+} + nH^+ \quad (n = 1, 2) \tag{1}$$

Results and Discussion

Kinetics of Complex Formation

The rates of complex formation were studied in the pH range 4.7–5.8, where the ligand species H_2DOTAM^{2+} and $HDOTAM^+$ predominate [the protonation constants were found to be 6.05 and 9.07 by pH-metric titrations]. The protons presumably protonate two diagonal ring nitrogen atoms, as has been observed for H_2DOTA^{2-}.[28]

In the presence of an excess of Ln^{3+} ions, the rate of complex formation is given by Equation (2), where k_{obs} is a pseudo-first-order rate constant and $[L]_t$ and $[LnL]_t$ are the total concentrations of ligand and metal complex, respectively.

$$\frac{d[LnL]_t}{dt} = k_{obs}[L]_t \tag{2}$$

The k_{obs} values obtained for the formation reactions of $Ce(DOTAM)^{3+}$ and $Eu(DOTAM)^{3+}$ are shown in Figures 1 and 2, respectively.

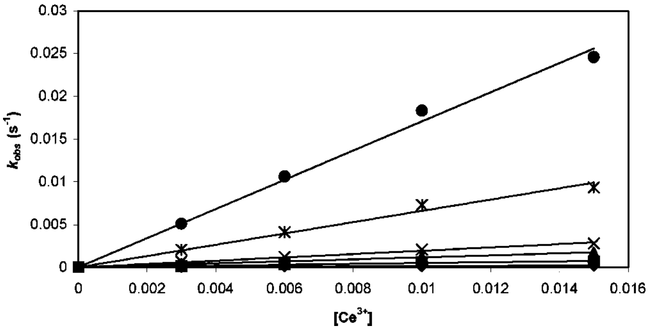


Figure 1. Rates of formation of $[Ce(DOTAM)]^{3+}$ at 25 °C in 1.0 M KCl. [DOTAM] = 3.0×10^{-4} M, [NMP] = 0.05 M. The pH values are 4.80 (◆), 5.01 (■), 5.16 (▲), 5.30 (x), 5.53 (∗) and 5.77 (●).

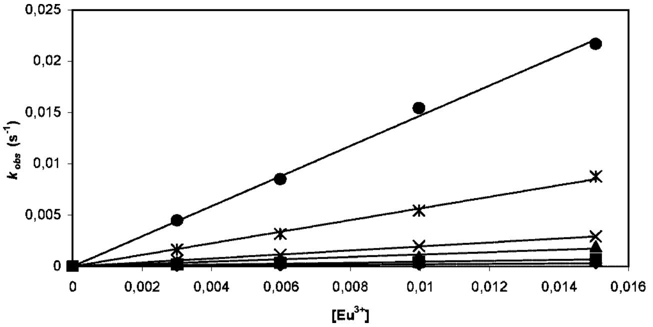


Figure 2. Rates of formation of $[Eu(DOTAM)]^{3+}$ at 25 °C in 1.0 M KCl. [DOTAM] = 3.0×10^{-4} M, [NMP] = 0.05 M. The pH values are 4.78 (◆), 5.01 (■), 5.17 (▲), 5.30 (×), 5.56 (∗), and 5.76 (●).

As seen in Figures 1 and 2, the k_{obs} values are directly proportional to the concentration of Ce^{3+} and Eu^{3+} over a broad concentration range. This observation differs considerably from the results obtained for the formation of DOTA and DOTA derivative complexes of Ln^{3+}, where similar k_{obs} vs. [Ln^{3+}] plots gave saturation curves, thus indicating the formation of a reaction intermediate.[21–26] The intermediates in the formation of $Ce(DOTA)^-$ and $Eu(DOTA)^-$ were detected by spectrophotometry and luminescence spectroscopy even at similar concentrations of Ln^{3+} and ligand.[22,24] The absence of any intermediate in the formation of Ln(DOTAM) is particularly interesting because the diprotonated complex $[Gd(H_2DOTAM)(H_2O)_4](ClO_4)_5$, which can be regarded as an intermediate, has recently been observed in the solid state by Parker et al.[27] The X-ray crystal structure of this complex shows that the Gd^{3+}ion is located outside the coordination cage of the diprotonated ligand, where four amide oxygen atoms and four water molecules are coordinated to the metal ion. The authors assume that the formation of the complex [Gd-

(DOTAM)]$^{3+}$ could occur by deprotonation of the intermediate assisted by water as a base.[27]

The formation of an intermediate does not imply that the reaction product is formed by transformation of the intermediate. Thus, the formation of an intermediate in the complexation of DOTA with some divalent metal ions has been detected by Kasprzyk and Wilkins, although they assumed that the intermediate was not on the reaction pathway (it acted as a "dead-end" complex) and that complex formation occurred according to a simple second-order reaction.[29]

The 4f–5d UV absorption bands of Ce^{3+} are suitable for detecting the formation of an intermediate in the reactions of Ce^{3+} with DOTA and its derivatives.[21,23] However, the formation of an intermediate in the reaction of Ce^{3+} with DOTAM could not be detected by spectrophotometry even if a 100-fold excess of Ce^{3+} was used (Figure 3). The absorption of the excess Ce^{3+} was compensated, but the spectrum obtained after 4 min is somewhat distorted, probably because of the imperfect compensation of one of the absorption bands of 0.05 M Ce^{3+}(aq), which appears at about 295 nm.

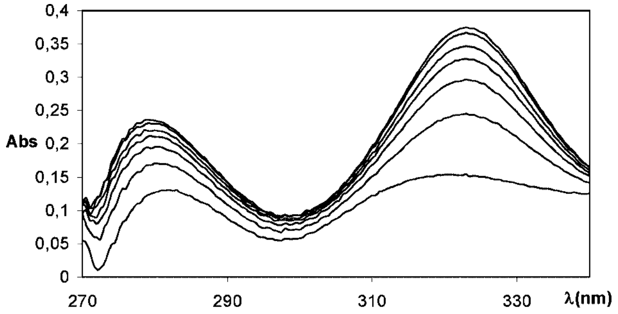


Figure 3. Absorption spectra of a 5 × 10^{-4} M solution of DOTAM containing 0.05 M Ce^{3+} at pH = 4.58 in the presence of 0.05 M NMP buffer 4, 8, 16, 20, 24, 28 and 32 min (in ascending order) after mixing the components (25 °C and 1.0 M KCl).

On the basis of the data presented in Figures 1 and 2, the pseudo-first-order rate constants can be expressed as $k_{\mathrm{obs}} = k_{\mathrm{f}}[\mathrm{Ln}^{3+}]$, and the rate of formation can be given as Equation (3).

$$\frac{\mathrm{d}[\mathrm{LnL}]_{\mathrm{t}}}{\mathrm{d}t} = k_{\mathrm{f}}[\mathrm{Ln}^{3+}][\mathrm{L}]_{\mathrm{t}} \tag{3}$$

These findings show that the complexes Ln(DOTAM)$^{3+}$ are formed by direct encounter between the Ln^{3+} ion and the ligand in a second-order reaction. In the pH range studied the ligand DOTAM is present in the form of HL^{+} and H$_2$L^{2+} species, and the concentration of the non-protonated species L is very low. However, it is expected that the neutral form of the ligand is the most reactive species because the Ln^{3+} ion and the positive charge of the protonated species HL^{+} and H$_2$L^{2+} hinder the reaction.[30] However, if we assume that the reaction can take place with all ligand species, the formation rate can be expressed as Equation (4).

$$\frac{\mathrm{d}[\mathrm{LnL}]_{\mathrm{t}}}{\mathrm{d}t} = k_{\mathrm{L}}[\mathrm{Ln}^{3+}][\mathrm{L}] + k_{\mathrm{HL}}[\mathrm{Ln}^{3+}][\mathrm{HL}] + k_{\mathrm{H_2L}}[\mathrm{Ln}^{3+}][\mathrm{H_2L}] \tag{4}$$

On the basis of Equations (3) and (4), and taking into account the protonation constants of the ligand ($K_1^{\mathrm{H}} = [\mathrm{HL}]/[\mathrm{L}][\mathrm{H}^+]$ and $K_2^{\mathrm{H}} = [\mathrm{H_2L}]/[\mathrm{L}][\mathrm{H}^+]$), the second-order rate constant k_{f} can be calculated from Equation (5), which takes into account all the possible reaction pathways, although some experimental data indicate that the reaction occurs only by encounter of the Ln^{3+} ions and the neutral species L.

$$k_f = \frac{k_L + k_{HL}K_1^H[H^+] + k_{H_2L}K_1^H K_2^H[H^+]^2}{1 + K_1^H[H^+] + K_1^H K_2^H[H^+]^2} \tag{5}$$

The formation rates of the complexes [Ce(DOTAM)]$^{3+}$ and [Eu(DOTAM)]$^{3+}$ are directly proportional to the concentration of the neutral ligand L since the plots of k_{f} vs. [L] give straight lines. In this case, according to Equation (5), $k_{\mathrm{f}} = k_{\mathrm{L}}/\alpha_{\mathrm{L}}$, where $\alpha_{\mathrm{L}} = 1 + K_1^{\mathrm{H}}[\mathrm{H}^+] + K_1^{\mathrm{H}}K_2^{\mathrm{H}}\,[\mathrm{H}^+]^2$. A plot of $\log k_{\mathrm{f}}$ against $\log \alpha_{\mathrm{L}}$ gives a straight line with a slope of –1, which also indicates the predominance of the reaction between the neutral ligand L and the Ln^{3+} ions.[22,29]

The rates of formation were also studied for the complexes of Gd^{3+}, Er^{3+} and Yb^{3+} at different pH values and at two Ln^{3+}concentrations. The k_{obs} values obtained are presented in Table 1.

Table 1. Pseudo-first-order rate constants (k_{obs}, s^{-1}) obtained for the formation of Gd^{3+}, Er^{3+} and Yb^{3+} complexes of DOTAM ($[\mathrm{L}]_t = 3 \times 10^{-4}$ M, 1.0 M KCl, 25 °C).

		$10^3 \times k_{\mathrm{obs}}$ [s^{-1}]	
Ln^{3+}	pH	6 × 10^{-3} M	1.0 × 10^{-2} M
Gd^{3+}	5.01	0.58	1.11
	5.35	1.91	2.81
	5.46	3.45	6.99
	5.54	4.78	10.7
	5.68	9.55	22.5
Er^{3+}	5.20	0.13	0.26
	5.56	0.72	1.29
	5.71	1.77	2.63
	5.90	3.85	6.54
Yb^{3+}	5.19	0.15	0.25
	5.44	0.49	0.66
	5.54	1.22	1.98
	5.66	2.55	3.69

The rate constants k_{L} were calculated for the formation reactions by fitting the k_{f} data (calculated from the k_{obs} values) to the equation $k_{\mathrm{f}} = k_{\mathrm{L}}/\alpha_{\mathrm{L}}$. The obtained rate constants k_{L} are presented in Table 2. The k_{f} data were also fitted to Equation (5) when the k_{HL} values obtained for the formation reaction of [Ce(DOTAM)]$^{3+}$ and [Eu(DOTAM)]$^{3+}$ were low and had a negative sign, although the $k_{\mathrm{H_2L}}$values were also low and so the contribution of the third term of the numerator of Equation (5) to the k_{f} values is not significant.

Table 2. Rate constants k_L ($\text{M}^{-1}\,\text{s}^{-1}$) characterising the formation of $[\text{Ln(DOTAM)}]^{3+}$ complexes (25 °C, 1.0 M KCl).

Ln^{3+}	k_L ($\text{M}^{-1}\,\text{s}^{-1}$)
Ce^{3+}	$(7.7\pm0.3)\times10^3$
Eu^{3+}	$(2.7\pm0.1)\times10^4$
Gd^{3+}	$(4.0\pm0.5)\times10^4$
Er^{3+}	$(5.6\pm0.3)\times10^3$
Yb^{3+}	$(6.6\pm1.0)\times10^3$

The rate constants k_L, determined for the reactions of the Ln^{3+} ions with DOTAM, are about three to four orders of magnitude lower than those reported for the complexation reactions with monodentate or open-chain multidentate ligands.[31–33] The observed reaction rates are particularly low because of the extremely low concentration (ca. 10^{-8} M) of the deprotonated ligand L in the pH range investigated.

To obtain further evidence that protonated intermediates do not play any role in the formation of complexes $[\text{Ln(DOTAM)}]^{3+}$, we studied the effect of changing the concentration of the buffer, *N*-methylpiperazine (NMP), on the rates of formation of $[\text{Ce(DOTAM)}]^{3+}$ and $[\text{Eu(DOTAM)}]^{3+}$. If protonated intermediates were formed and the rate-determining step of the complexation were the deprotonation of the intermediates, then an increase in the concentration of a general base like NMP ($\text{p}K_1$ = 4.83) would cause an increase in the rate of complex formation.[26,27] The rate constants k_{obs} obtained for the formation of $[\text{Ce(DOTAM)}]^{3+}$ and $[\text{Eu(DOTAM)}]^{3+}$ do not show any change with an increase in the concentration of the buffer (Figure 4). These findings confirm that the formation of complexes $[\text{Ln(DOTAM)}]^{3+}$ occurs without the involvement of protonated intermediates.

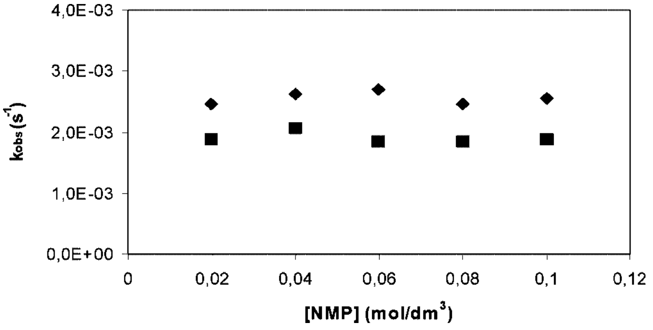


Figure 4. Rates of formation of $[\text{Ce(DOTAM)}]^{3+}$ (◆; pH = 5.32) and $[\text{Eu(DOTAM)}]^{3+}$ (■; pH = 5.2). The concentration of DOTAM and Ln^{3+} ions was 3×10^{-4} M and 6×10^{-3} M, respectively (25 °C and 1.0 M KCl).

Proton-Exchange Rates of the DOTAM Ligand

The ^{1}H NMR spectra of the DOTAM ligand (Figures 5 and 6) indicate that the rate of proton exchange in the species $\text{H}_2\text{DOTAM}^{2+}$ and HDOTAM^+ is relatively low, which could play some role in the slow formation of the $[\text{Ln(DOTAM)}]^{3+}$ complexes.

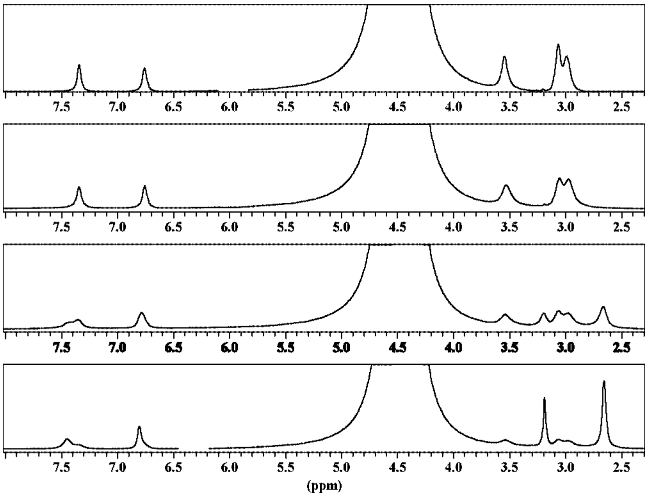


Figure 5. ^{1}H NMR spectra of the ligand DOTAM. The pH values from top to bottom are: 4.04, 5.03, 5.94 and 6.48.

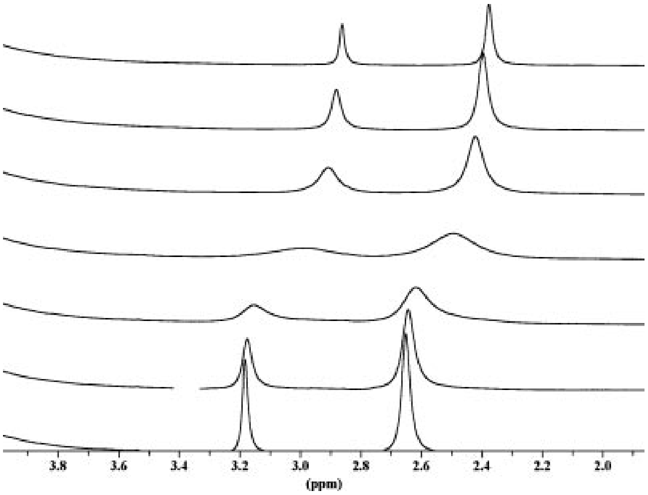


Figure 6. Variation of the ^{1}H NMR spectrum of DOTAM with pH. The pH values from top to bottom are: 7.45, 7.99, 8.53, 9.04, 9.55, 9.83 and 10.22.

At pH ≈ 4, where the species $\text{H}_2\text{DOTAM}^{2+}$ predominates, there are three broad signals in the CH_2 region in a 1:1:1 ratio. Two of these are assignable to the ring protons (δ = 2.98 and 3.16 ppm), while the third belongs to the amide CH_2 group (δ = 3.54 ppm). The signals observed between δ = 6.6 and 7.5 ppm are due to the NH_2 protons of the amide group. The chemical shift of the ring protons is not affected by the pH. Interestingly, the ligand HDOTAM^+, which predominates at pH > 7, has only two peaks in the CH_2 region, probably due to some fluxional motion of the molecule that averages the two signals of the ring methylene protons. Figure 6 shows that both the chemical shifts and the line widths of these signals change considerably with increasing pH. It is likely that these spectral variations are related to the proton-exchange process that occurs for the species HDOTAM^+ and DOTAM. Analysis of the pH dependence of the proton chemical shifts allowed the determination of the $\log K_1^H$ value (9.01 ± 0.07), which is in good agreement with the value obtained by pH-metry.

Exchange between the monoprotonated and nonprotonated forms of DOTAM was analysed using the simplified analytical equation reported by Piette and Anderson.[34] The exchange rate constant (k_{ex}) can be determined from the nonexchange linewidth and the chemical shifts of the signals in the absence of exchange. These data were obtained from the spectra, and the first-order rate constants were calculated in the pH range 8–10.[34] The proton-exchange rate is linearly proportional to the OH^- concentration, as expressed by Equation (6), where the rate constant $k_0 = (1.2 \pm 0.3) \times 10^3\ s^{-1}$ refers to the rate of spontaneous deprotonation of $HDOTAM^+$, which probably takes place with the help of H_2O molecules.

$$k_{ex} = k_0 + k_1[OH^-] \tag{6}$$

The rate constant $k_1 = (5.3 \pm 0.5) \times 10^8\ M^{-1}\,s^{-1}$ corresponds to the rate of the OH^--assisted deprotonation of the species $HDOTAM^+$. The rate of proton exchange is relatively low, probably because the proton is attached to the ring nitrogen atom in the coordination cage. For the proton exchange to occur, the ligand must have a special conformation where it can donate the proton to an H_2O molecule or an OH^- ion. This conformation is formed by some fluxional motion of the ligand, although the experimental data are not good enough to be able to separate the contribution of fluxionality. The role of the OH^--assisted deprotonation is negligible in the pH range 4.7–5.8, and the spontaneous deprotonation of $HDOTAM^+$ is much faster than complex formation, which means that this deprotonation cannot control the rate of complexation.

The ^1H NMR signals of the DOTAM ligand are broad because of the slow proton exchange and fluxional processes. However, the widths of the signals decrease in the presence of a general base. Thus, an increase in the concentration of sodium acetate to 0.3 M at pH = 9.0 results in a decrease of 18 and 35 Hz in the linewidths of the ring and amide methylene protons, respectively. The two ring proton signals merge into one broad signal at pH = 4.1 when the concentration of sodium formate is increased to 0.25 M. These findings indicate that the proton-exchange reactions of the species H_2DOTAM^{2+} and $HDOTAM^+$ are general base catalysed processes. However, general base catalysis is not valid for the formation of $[Ln(DOTAM)]^{3+}$ (Figure 4), therefore we can conclude that the rate of proton exchange of the free ligand does not play a role in the slow complex formation.

Mechanism of Complex Formation

The kinetic data obtained for the formation of the $[Ln(DOTAM)]^{3+}$ complexes show that the complexation occurs through a direct reaction between the Ln^{3+} ions and the deprotonated ligand DOTAM that follows second-order kinetics. On this basis, since the only species formed is $[Ln(DOTAM)]^{3+}$, the rate of formation can be expressed on the basis of Equation (3) as Equation (7).

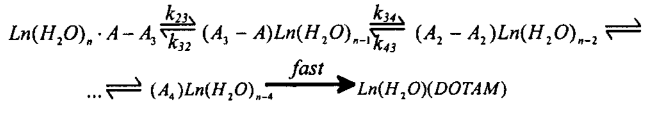
$$\frac{d[LnL]}{dt} = k_L[Ln^{3+}][L] \tag{7}$$

A low reactivity of the protonated ligands is known even for the reactions of open-chain aminocarboxylate ligands, where the ratio of the rate constants k_L/k_{HL} is often as large as 10^5–10^6.[30] In the case of the macrocyclic DOTA and its derivatives, the metal ion cannot enter directly into the coordination cage formed by the four ring nitrogen atoms and four oxygen donors, thus maintaining the four negatively charged pendant arms. This is due to the electrostatic repulsion caused by the presence of the protonated nitrogen atoms. Complex formation requires that this proton is shifted to another donor atom, such as the pendant carboxylate group in the case of DOTA. In the complexation of DOTAM, the proton cannot be transferred to the carboxamide moiety because it can be protonated only in very strongly acidic solutions. On this basis, the formation of complexes of DOTAM does not take place via a protonated intermediate, thus it must occur only with the participation of the fully deprotonated ligand DOTAM.

The rate constants k_L reported in Table 2, are three to four orders of magnitude lower than the second-order rate constants determined for the formation of Ln^{3+} complexes with murexide, oxalate or anthranilate ligands. The rate-determining step for these reactions was assumed to be the second bond formation, in other words the chelation step.[31–33]

In metal complex formation with macrocyclic ligands, formation of the first metal–ligand bond is followed by several subsequent steps in which new metal–ligand bonds are formed sequentially as the coordinated water molecules are released. Unfortunately, it is very difficult to determine which is the rate-determining step in such multi-step processes.

The very slow formation of the complexes $[Ln(DOTAM)]^{3+}$ may be tentatively interpreted in terms of the knowledge obtained for the reactions of multidentate ligands.[30] The first step of the complexation is the formation of an outer-sphere complex between the DOTAM ligand and the $[Ln(H_2O)_n]^{3+}$ ion ($n = 9$ for the ions between La^{3+} and Nd^{3+}, $n = 8$ for the ions from Gd^{3+} to Lu^{3+}; n is between 8 and 9 for Sm^{3+} and Eu^{3+}).[33,35] By defining the tetraamide ligand as A_4, this outer-sphere complex can be formulated as $Ln(H_2O)_n{\cdot}A\text{-}A_3$, which is characterized by the stability constant K_{os}. The subsequent steps of the formation of $[Ln(DOTAM)]^{3+}$ complexes are shown in Scheme 2, where k_{jk} are first-order rate constants.



Scheme 2. The subsequent steps of the formation of $[Ln(DOTAM)]^{3+}$ complexes.

If the second bond formation were the rate-controlling step, then the expression for the second-order rate constant k_L would be as shown in Equation (8).[30]

$$k_L = \frac{k_{23}k_{34}K_{os}}{k_{32} + k_{34}} \quad (8)$$

Since dissociation of the intermediate $(A_3\text{-}A)Ln(H_2O)_{n-1}$ is probably much faster than the formation of $(A_2\text{-}A_2)\text{-}Ln(H_2O)_{n-2}$, that is $k_{32} >> k_{34}$ and $k_{23} \approx k_{H_2O}$ (k_{H_2O} is the water exchange rate), Equation (8) can be simplified to Equation (9).

$$k_L = k_{H_2O}K_{os}\frac{k_{34}}{k_{32}} \quad (9)$$

To obtain k_L values of around 10^3–10^4, the K_{os} and k_{34}/k_{32} values must be low, since the value of k_{H_2O} is about 10^7–10^8 s^{-1}.[31–33] The value of K_{os} for an Ln^{3+} ion and a neutral ligand can be estimated to be 0.3 M on the basis of the Fuoss equation.[36,37] The ratio k_{34}/k_{32} must be low, and if these values were about 10^{-3} for the different Ln^{3+} ions then the k_L values would be approximately 10^3–10^4, as was found experimentally. These considerations indicate that the rate-determining step for the formation of $[Ln(DOTAM)]^{3+}$ complexes must be quite early in the sequence shown in Scheme 2 (second or third bond formation) because the ratios analogous to k_{34}/k_{32} are presumably lower for the later intermediates in the sequence.

The formation rate constants k_L show a maximum at Gd^{3+} in the series of lanthanides. Similar phenomena have often been observed for the formation of lanthanide complexes of mono- and multidentate ligands.[32,33] Since the water exchange rates of the Ln^{3+}(aq) ions decrease from Gd^{3+} to Yb^{3+}, and probably also to the lighter elements, interpretation of the trend of the complexation rates is based on the assumption that the water exchange rates may influence the rates of complex formation, as expressed by Equation (9).[31–33]

Conclusions

The complexation properties of DOTAM, which contains four uncharged acetamide functional groups, differ considerably from those of the parent ligand DOTA, which possesses four negatively charged pendant groups. The mechanisms of formation of $[Ln(DOTAM)]^{3+}$ and $[Ln(DOTA)]^-$ complexes are therefore also different. Thus, during the formation of the latter complexes, the acetate groups of the diprotonated ligand coordinate to the Ln^{3+} ion to form a stable intermediate, which then undergoes transfer of the ring NH protons to the donor atoms of the pendant groups followed by entry of the metal ion into the macrocyclic cage. The complexes with DOTAM cannot be formed via protonated intermediates of this type, however, because the ring protons cannot be transferred to the amide oxygen atom. The formation of $[Ln(DOTAM)]^{3+}$ complexes therefore does not involve the formation of protonated intermediates.

The kinetic data indicate that the formation of $[Ln(DOTAM)]^{3+}$ complexes occurs in a direct reaction between the fully deprotonated DOTAM ligand and the Ln^{3+} ion in a second-order reaction. Although the proton-exchange reactions of the protonated species H_2DOTAM^{2+} and $HDOTAM^+$ are relatively slow on the NMR timescale, they have no effect on the rate of complex formation.

The second-order rate constants k_L, which characterize the formation of the $[Ln(DOTAM)]^{3+}$ complexes, are three to four orders of magnitude lower than the rate constants reported for the reactions of open-chain multidentate ligands. However, the trend of the k_L values in the series of lanthanides is somewhat similar to that of the rates of complexation with some monodentate and multidentate ligands.

The formation of $[Ln(DOTAM)]^{3+}$ complexes occurs by successive replacement of the coordinated water molecules of the $Ln(H_2O)_n{}^{3+}$ ion. The low k_L values observed can be interpreted by assuming that the rate-determining Ln^{3+}–amide bond formation probably occurs quite early in the reaction sequence, probably being in the second or third step of the complex formation.

The preformed coordination cage of the ligand plays an important role in determining the moderate stability constants and extremely low proton-assisted dissociation rates of the Ln^{3+} complexes formed with the neutral ligand DOTAM. The neutrality of the acetamide pendant groups of the ligand has a major influence on the kinetics and mechanisms of the formation reactions; however, the rates are also influenced by the water exchange rates of the Ln^{3+}(aq) ions.

Experimental Section

The chemicals used in the experiments were of the highest analytical grade. The $CeCl_3$ solution was prepared from $CeCl_3·7H_2O$ (Aldrich, 99.9%). For the preparation of the other $LnCl_3$ solutions, Ln_2O_3 (Fluka, 99.9%) was dissolved in 6.0 M HCl and the excess of acid evaporated. The concentration of the $LnCl_3$ solutions was determined by complexometric titration with standardized Na_2H_2EDTA and xylenol orange as indicator. The ligand DOTAM was prepared as described previously.[1] The concentration of the solution of DOTAM was determined by pH-metric titrations in the presence and absence of a large excess of $CaCl_2$. The protonation constants of DOTAM were also determined by pH-metry at 25 °C in 1.0 M KCl. The method proposed by Irving et al. was used to calculate $[H^+]$ from the measured pH values.[38] A Radiometer PHM93 pH meter, an ABU 80 autoburette and a Metrohm 6.0234.100 combined electrode were used for the pH measurements and titration. The pH-meter was calibrated with potassium hydrogen phthalate (pH = 4.005) and borax (pH = 9.180) buffers. The protonation constants were calculated with the program PSEQUAD.[39] The rates of formation of $[Ce(DOTAM)]^{3+}$ and $[Eu(DOTAM)]^{3+}$ were studied by spectrophotometry (Cary E1 spectrophotometer) at 320 and 250 nm, respectively. To study the formation of the Gd^{3+}, Er^{3+} and Y^{3+} complexes, the release of H^+ from the ligand was monitored by spectrophotometry at 616 nm in weakly buffered solutions with bromocresol green as indicator.[29] The decrease in pH was about 0.07–0.1 pH units in the presence of *N*-methylpiperazine (NMP) buffer. The formation of complexes was studied in the presence of a 5- to 50-fold Ln^{3+} excess in order to maintain pseudo-first-order conditions. The concentration of

DOTAM was varied between 1×10^{-4} and 5×10^{-4} M. The pseudo-first-order rate constants were calculated by fitting the absorbance values to Equation (10), where A_0, A_e and A_t are the absorbance values at the start, at equilibrium and at time t of the reaction.

The rates of formation [Equation (1)] were studied in the pH range 4.7–5.8 (25 °C, 1.0 M KCl). The ^{1}H NMR spectra were recorded with a Bruker Avance 600 spectrometer in H_2O solution with a capillary containing D_2O as lock and reference.

$$A_t = (A_0 - A_e)e^{-k_{obs}t} + A_e \qquad (10)$$

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