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Kinetics of Complex Formation by Macrocyclic Polyaza Polycarboxylate Ligands: Detection and Characterization of an Intermediate in the Eu^{3+}−dota System by Laser-Excited Luminescence

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Laser-excited luminescence spectroscopy of the ${}^7F_0 \rightarrow {}^5D_0$ transition of Eu^{3+} reveals rapidly formed intermediate species when this ion is mixed with dota (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate). This intermediate converts to the final complex $[Eu(dota)H_2O]^-$, with rate constants, k_{obs}, under conditions of saturating excess metal ion, which increase from 7.2×10^{-4} to 7.9×10^{-2} s^{-1} as the pH is raised from 3.78 to 5.81. The course of the reaction is followed by the increase in intensity of the excitation peak (579.77 nm) of the final product as it is formed. The intermediate (λ_{ex} = 579.20 nm) was determined to be the diprotonated species (EuH_2dota^+). The excited state luminescence lifetimes of this species measured in H_2O and D_2O solutions reveal 4.5 ± 0.5 coordinated water molecules, consistent with a proposed structure in which the Eu^{3+} ion is coordinated to four ligand carboxylate oxygens well away from the four ligand nitrogens of the macrocycle, two of which are protonated. Molecular mechanics calculations suggest that five water molecules complete the coordination sphere. A general mechanism for the formation of metal ion complexes of rigid macrocyclic polyaza polycarboxylate ligands is proposed in which the removal of a proton from a ring nitrogen is an important prerequisite to forming the activated complex which rearranges in the rate-determining step to the final product. Reaction of the intermediate with hydroxide ion in a rapid equilibrium step produces a monoprotonated intermediate in the case of dota and an unprotonated intermediate species in the cases of some tricarboxylate ligands previously studied. The stability constant of the intermediate (EuH_2dota^+) was determined to be 7.6×10^5 M^{-1} by directly monitoring the concentration of the intermediate as a function of pH and 1.8×10^6 M^{-1} from an analysis of the kinetic data. An upper limit for the rate constant, k, of the rate-determining rearrangement of the intermediate (EuHdota) to form the final product is 590 s^{-1}. It is concluded that protonation of ring nitrogen atoms of polyaza macrocyclic ligands greatly increases their rigidity, causing them to react with metal ions much more slowly than their acyclic counterparts.

Introduction

The complexation of metal ions in aqueous solution by multidentate amino carboxylate chelating agents has been of importance in inorganic chemistry, analytical chemistry, and biochemistry since the introduction of ethylenediaminetetraacetic acid, edta, early in the century.[1] There have been many studies of the complexation and dissociation kinetics of such systems.[2] Most complex formation reactions, particularly for acyclic ligands,[3−5] are extremely rapid, and there is little detailed information on intermediates, if any, which occur on the pathway to complex formation. For ligands involving 9- to 14-membered tri- or tetraaza macrocycles with three or four carboxylate-containing side chains, the rates of complexation with lanthanide (Ln^{3+}) and other ions have been observed to be relatively slow.[6−11] The existence of stable intermediates has been postulated on the basis of kinetic behavior (saturation kinetics)[7] and, in the cases of the Eu^{3+}− and Ce^{3+}−dota systems, have been detected by NMR[11] and absorption spectroscopy,[8] respectively (see Chart 1 for the structures of the ligands referred to in this paper).

Gd^{3+} complexes of macrocyclic ligands in this class, owing to their high thermodynamic stability, kinetic inertness, and other properties, are finding actual or potential chemical applications as magnetic resonance imaging (MRI) contrast agents.[11−14] For this reason, it is particularly important to have a complete understanding of the structures, kinetics, and thermodynamics of complexes of this type.

Laser excitation luminescence spectroscopy of the ${}^7F_0 \rightarrow {}^5D_0$ transition of Eu^{3+} is a well-established means for the detailed characterization of Eu^{3+} complexes.[15,16] Using this technique, we have directly observed a kinetic intermediate in the formation of $Eu(dota)^-$ from the metal ion and ligand and used excitation spectroscopy to follow the course of the reaction. Furthermore,

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(1) Garvan, F. L. In *Chelating Agents and Metal Chelates*; Dwyer, F. P., Mellor, D. P., Eds.; Academic Press, Inc.: New York, 1964; pp 283−333.

(2) Margerum, D. W.; Cayley, G. R.; Weatherburn, D. C.; Pagenkopf, G. R. In *Coordination Chemistry*; Martell, A. E., Ed.; American Chemical Society: Washington, D.C., 1978; Vol. 2; pp 1−220.

(3) Nyssen, G. A.; Margerum, D. W. *Inorg. Chem.* **1970**, *9*, 1814−1820.

(4) Laurenczy, G.; Brücher, E. *Inorg. Chim. Acta* **1984**, *95*, 5−9.

(5) Laurenczy, G.; Raddics, L.; Brücher, E. *Inorg. Chim. Acta* **1983**, *75*, 219−223.

(6) Brücher, E.; Cortes, S.; Chavez, F.; Sherry, A. D. *Inorg. Chem.* **1991**, *30*, 2092−2097.

(7) Kasprzyk, S. P.; Wilkins, R. G. *Inorg. Chem.* **1982**, *21*, 3349−3352.

(8) Brücher, E.; Laurenczy, G.; Makra, Z. *Inorg. Chim. Acta* **1987**, *139*, 141−142.

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

(10) Kumar, K.; Tweedle, M. F. *Inorg. Chem.* **1993**, *32*, 4193−4199.

(11) Wang, X.; Tianzhu, J.; Comblin, V.; Lopez-Mut, A.; Merciny, E.; Desreux, J. F. *Inorg. Chem.* **1992**, *31*, 1095−1099.

(12) Chacheris, W. P.; Quay, S. C.; Rocklage, S. M. *Magn. Reson. Imaging* **1990**, *8*, 467−481.

(13) Frey, S. T.; Chang, C. A.; Carvalho, J. F.; Varadarajan, A.; Schultze, L. M.; Pounds, K. L.; Horrocks, W. D., Jr. *Inorg. Chem.* **1994**, *33*, 2882−2889.

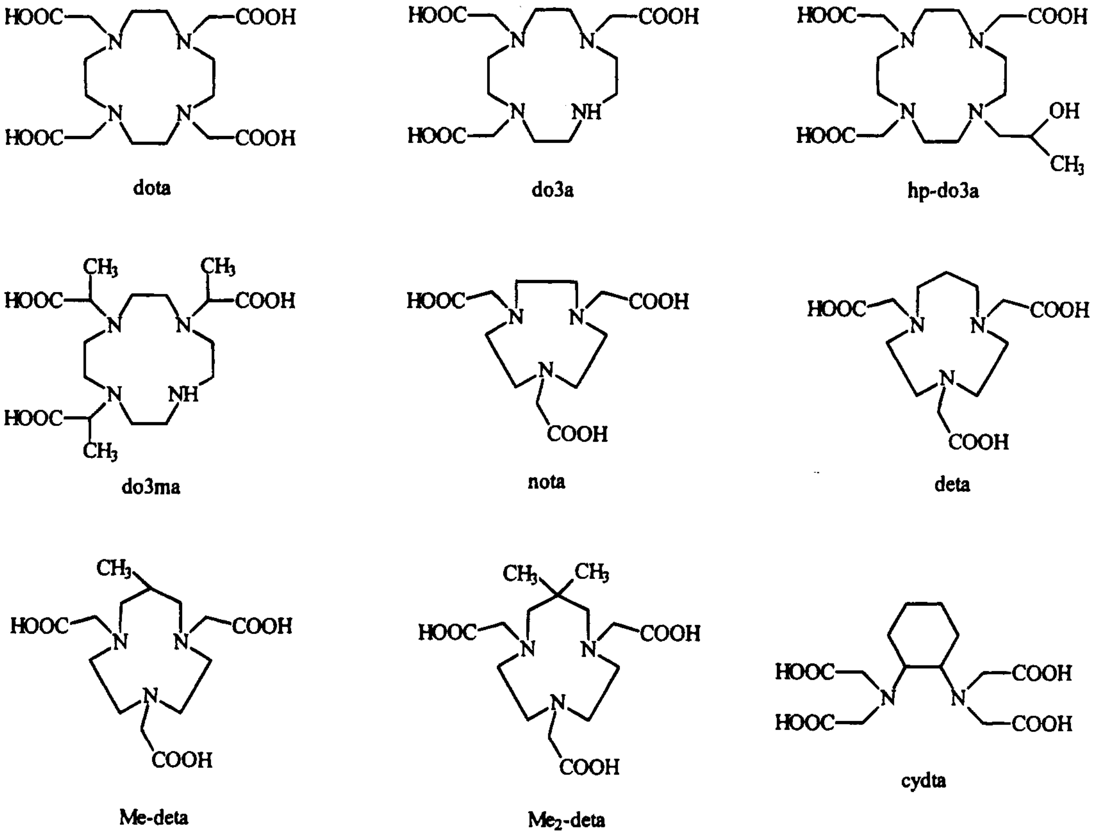
(14) Laufer, R. B. *Chem. Rev.* **1987**, *87*, 901−927.

(15) Horrocks, W. D., Jr.; Sudnick, D. R. *Acc. Chem. Res.* **1981**, *14*, 384−392.

(16) Horrocks, W. D., Jr. *Methods Enzymol.* **1993**, *226*, 495−538.

0020-1669/95/1334-3724$09.00/0

Chart 1. Structures of the Ligands



dota

do3a

hp-do3a

do3ma

nota

deta

Me-deta

Me_2-deta

cydta

for the first time, a rather complete characterization of such an intermediate has been achieved. Included in our findings are such details as the degree of protonation, formation constant, number of metal ion-coordinated water molecules, lower limit on its rate of dissociation, overall structure (with the aid of molecular mechanics calculations), and insight into the mechanism by which the intermediate converts into the final product.

A detailed mechanism for the complexation reaction is proposed that is consistent with our data and those of others on dota complex formation. With a minor modification, it is applicable to ligands containing only three carboxylate moieties, and data in the literature are reanalyzed using the new mechanism. Macrocycle ligand rigidity, as modified by the degree of protonation of the aza nitrogen atoms, is suggested to be of paramount importance in determining the complexation rates.

The methods described here may prove to be of some general utility in the study of slow Ln^{3+} complexation kinetics. We have made preliminary spectroscopic observations of intermediates in the formation of Eu^{3+} complexes with do3a- and dtpa-based macrocyclic ligands.

Experimental Section

Materials. Hydrated $EuCl_3$ and D_2O (99.8%) (Aldrich Chemical Co.), Mes (2-morpholinoethanesulfonic acid) (Sigma Chemical Co.), and Homopipes (homopiperazine-*N,N'*-bis(ethanesulfonic acid)) (Research Organic Inc.) were purchased from the sources indicated. dota (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) was a gift from Nycomed-Salutar Inc., Sunnyvale, CA. The water used was deionized and doubly distilled, and all remaining reagents were the purest commercially available. Europium chloride solutions were prepared at ~10 mM concentrations and standardized by an edta arsenazo titration. The concentration of the dota stock solution was determined *via* a titration of a ~2 μM solution with standardized Eu^{3+} at pH 6 on equilibrated samples using laser-excited Eu^{3+} luminescence to monitor complex formation.

Methods. A Continuum YG581 pulsed (10 Hz) Nd:YAG laser pumped tunable dye laser, Model TDL50, was used to obtain the excitation spectra and lifetime and intensity data. The details of this system are presented elsewhere.[17] The $^7F_0 \rightarrow {}^5D_0$ transition of Eu^{3+} ion (578–581 nm) was excited by using a mixture of Rhodamine 590 (Excition Co.) and 610 (Kodak Chemical Co.). The $^5D_0 \rightarrow {}^7F_2$ emission band at 614 nm was monitored in each case. An Orion pH meter (Model 720A) with an Orion glass electrode was used for all pH measurements. All spectroscopic measurements were carried out at 25.0 ± 0.1 °C. The commercially available Peakfit program (Jandel Scientific), which employs a nonlinear regression method, was used in the data analyses. In order to facilitate our discussion, we list the literature values of the dota protonation constants:[18] $\log(K_1^H) = 11.14$, $\log(K_2^H) = 9.69$, $\log(K_3^H) = 4.85$, $\log(K_4^H) = 3.96$; pD for the D_2O solutions was calculated as pH + 0.41.[19] The values of $K_w = [H^+][OH^-]$ and of $K_{D_2O} = [D^+][OD^-]$ used in the computations were $10^{-13.78}$ and $10^{-14.95}$, respectively.[20,21]

The reactions were studied by recording the $^7F_0 \rightarrow {}^5D_0$ excitation spectra over the 578.5–580.5 nm range or monitoring the intensity increase of the signal corresponding to the final $Eu(dota)^-$ complex at 579.77 nm or the intensity decrease of the signal of the intermediate at 579.20 nm, after mixing 1 mL samples of Eu^{3+} and dota, buffered at the same pH value. For quantitative kinetic measurements, the concentration of dota was varied between 0.2 and 2.0 μM, depending on the pH of the sample. In each run, Eu^{3+} was in excess in order to ensure pseudo-first-order reaction kinetic conditions. The buffers used were formate (pH <4.6), Homopipes (pH 4.6–5), and Mes (pH 5–7). The concentration of the buffers was 20 mM, and the ionic strength was adjusted to 0.10 M with KCl. No interaction of the buffers with the Eu^{3+} ion was observed.

Molecular Mechanics Calculations. These were carried out using

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(17) Frey, S. T. Ph.D. Thesis, The Pennsylvania State University, 1994.

(18) Clarke, E. T.; Martell, A. E. *Inorg. Chim. Acta* **1991**, *190*, 37–46.

(19) Covington, A. K.; Paabo, M.; Robinson, R. A.; Bates, R. G. *Anal. Chem.* **1968**, *40*, 700–706.

(20) Force, R. K.; Carr, J. D. *Anal. Chem.* **1974**, *46*, 2049–2052.

(21) Martell, A. E.; Motekaitis, R. J. *Determination and Use of Stability Constants*, 2nd ed.; VCH: New York, 1992.

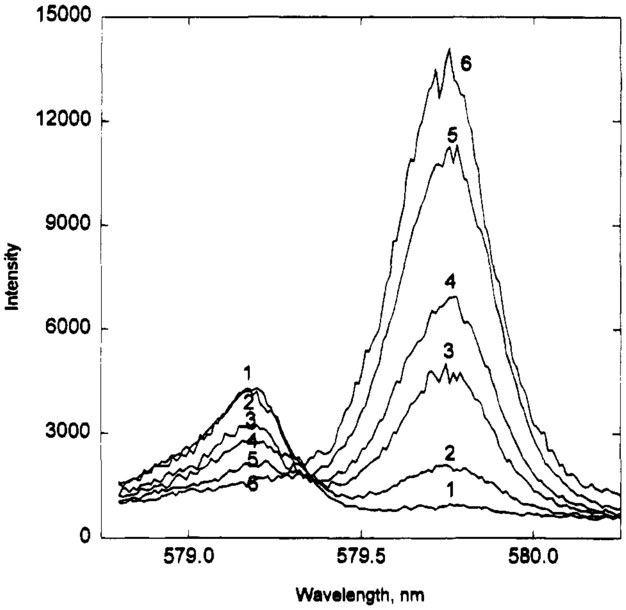

Figure 1. Excitation spectra of $^5D_0 \rightarrow {}^7F_0$ of the reacting mixtures recorded (0.01 nm/s) at (1) 40 s, (2) 6 min, (3) 27 min, (4) 45 min, and (5) 1 h and 39 min after the reaction was started and (6) at equilibrium. $[Eu^{3+}]_i = 100\ \mu M$; $[dota]_i = 10\ \mu M$; pD = 4.44; $\mu = 0.1$; 25 °C. (Subscript i indicates initial concentrations.)

QUANTA/CHARMm (Molecular Simulations) running on an IBM RISC 6000 graphics workstation. The procedures used were identical to those described by Frey *et al.*,[13] including the parameters given in Table 5 of that paper.

Results

Observation and Characterization of an Intermediate. The $^7F_0 \rightarrow {}^5D_0$ excitation spectra are highly revealing of the nature of Eu^{3+} complexes in solution.[15,16] It is expected that any rapidly formed, relatively long-lived intermediate in the reaction of Eu^{3+} and a ligand should be detectable using this spectroscopic technique. Since pervious work has found that complex formation between dota and Ln^{3+} is very slow,[8,22] it appeared that laser-excited Eu^{3+} luminescence spectroscopy might allow the detection of any such intermediate and aid in elucidating the reaction mechanism.

The $^7F_0 \rightarrow {}^5D_0$ excitation spectra, taken at various elapsed times after mixing dota and Eu^{3+}, are shown in Figure 1. Initially a band appears at 579.20 nm, the intensity of which decreases with elapsed time, concomitant with the growing-in of a second peak at 579.77 nm, characteristic of the final complex, the $Eu(dota)^-$ ion.[23] The band at 579.20 nm represents a rapidly formed reaction intermediate (EuH_xL) which slowly transforms to the final product.

A useful characterization of Eu^{3+}-containing species is the lifetime, τ, of the $^5D_0$ excited state.[15,16] The reciprocal of this quantity, τ^{-1}, represents the rate constant for deexcitation, including both radiative and nonradiative processes. Since the reaction intermediate represented by the $^5D_0 \rightarrow {}^7F_0$ excitation peak at 579.20 nm is relatively long lasting, its excited state lifetime can be measured at this wavelength. The lifetime of the intermediate was found to vary as a function of the ratio of dota to Eu^{3+} in the solution immediately after mixing. For $[Eu^{3+}]/[dota]$(at pH 4.94) = 10, 5.0, 1.0, 0.50, 0.20, 0.13, and 0.10, the τ values were found to be 109, 112, 167, 201, 212, 210, and 211 μs, respectively. The measured lifetime is independent of wavelength; moreover, the emission intensity is largest for excitation at 579.20 nm, even in the presence of excess $Eu^{3+}(aq)$ whose excitation maximum is at 578.8 nm. The observed lifetimes start out at a low value characteristic of free $Eu^{3+}(aq)$ ($\tau \sim 115\ \mu s$) and increase to about 210 μs. This behavior is consistent with a rapid exchange of Eu^{3+} between the free aqueous ion, $Eu^{3+}(aq)$, and Eu^{3+} in the (EuH_xdota) intermediate which has a lifetime of about 210 μs. This exchange is rapid with respect to the τ^{-1} values of either of the exchanging species. The following equations describe the processes which occur following the selective excitation of the intermediate to form the excited state species, $(EuH_xL)^*$:

$$(EuH_xL)^* \leftrightarrow Eu^{3+}(aq)^* + H_xL,\quad K_d \qquad (1)$$

$$(EuH_xL)^* \rightarrow (EuH_xL) + h\nu + \text{nonradiative loss},\quad \tau_1^{-1} \qquad (2)$$

$$Eu^{3+}(aq)^* \rightarrow Eu^{3+}(aq) + h\nu + \text{nonradiative loss},\quad \tau_2^{-1} \qquad (3)$$

where K_d is the equilibrium (dissociation) constant for the facile equilibrium between $Eu^{3+}(aq)$ and (EuH_xL), both the forward and reverse rates of which are much greater than the decay rates for the excited states of either species, τ_1^{-1} and τ_2^{-1}. The rate of chemical conversion of (EuH_xL) to the final product is very much smaller than either τ_1^{-1} or τ_2^{-1} and need not be considered in the above scheme. For instance, while τ_1^{-1} and τ_2^{-1} are about 4.8×10^3 and $9.1 \times 10^3\ s^{-1}$, respectively, the observed rate constant for formation of the final product at pH 5.8 (the fastest rate observed; *vide infra*) is $7.9 \times 10^{-2}\ s^{-1}$. For such rapid exchange between excited state species, the observed reciprocal excited state lifetime, τ_{obs}^{-1}, will be a weighted average of τ_1^{-1} and τ_2^{-1}. In this case[24]

$$\tau_{obs}^{-1} = \{[(EuH_xL)]/([(EuH_xL)] + [Eu^{3+}(aq)])\}\tau_1^{-1} + \{[Eu^{3+}]/([(EuH_xL)] + [Eu^{3+}(aq)])\}\tau_2^{-1} \qquad (4)$$

The equilibrium expressed by eq 1 is established for both the ground and excited state species. Moreover, since the excitation involves only the pairing of two f electrons in highly shielded orbitals, the position of this equilibrium will not be affected by electronic excitation.[24] Equation 4 predicts that as the ratio of dota to Eu^{3+} increases, the τ_{obs}^{-1} values will approach τ_1^{-1}, the excited state lifetime of the intermediate (EuH_xL). Moreover, the τ_{obs}^{-1} value will be independent of the wavelength at which it is measured. In accord with this prediction, the τ_{obs}^{-1} values are the same at 578.8 nm (the peak of $Eu^{3+}(aq)$) and at 579.2 nm. This behavior is reflected in the data indicated above and yields a τ_2 value of 210 μs for the intermediate.

Determination of the Number of Eu^{3+}-Coordinated Water Molecules, *q*. It was shown some time ago[25] that q can be estimated from eq 5, provided that lifetimes in both H_2O and

$$q = 1.05(\tau_{H_2O}^{-1} - \tau_{D_2O}^{-1}) \qquad (5)$$

D_2O solution are available. In D_2O solution the τ value corresponding to (EuD_xL) was found to be about 2.33 ms. Using this value and the H_2O results, we find that 4.5 ± 0.5 water molecules are coordinated to the Eu^{3+} ion in the intermediate. This implies that the ligand supplies 4 or 5 coordinating atoms to provide the usual total coordination number of 8 or 9. This finding is consistent with the initially

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(22) Desreux, J. F. *Inorg. Chem.* **1980**, *19*, 1319–1324.

(23) Albin, M.; Horrocks, W. D., Jr.; Liotta, F. J. *Chem. Phys. Lett.* **1982**, *85*, 61–64.

(24) Horrocks, W. D., Jr.; Arkle, V. K.; Liotta, F. J.; Sudnick, D. R. *J. Am. Chem. Soc.* **1983**, *105*, 3455–3459.

(25) Horrocks, W. D., Jr.; Sudnick, D. R. *J. Am. Chem. Soc.* **1979**, *101*, 334–340.

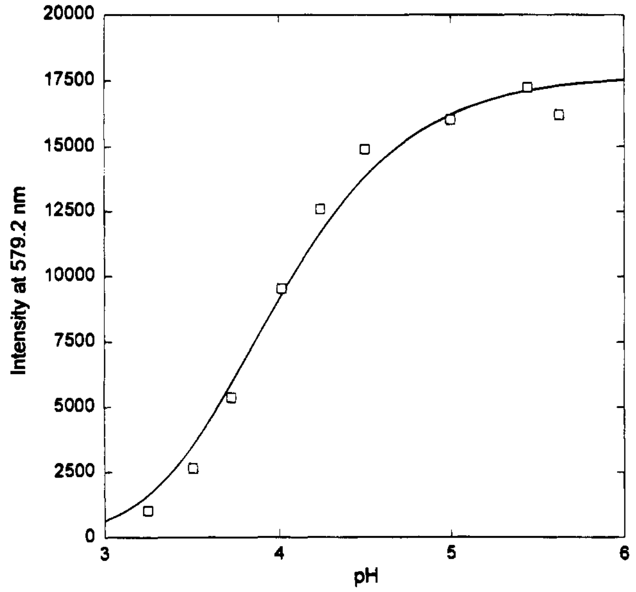

Figure 2. Plot of the intensity recorded at 579.2 nm immediately after mixing vs pH. $[Eu^{3+}]_i$ = $[dota]_i$ = 50 μM; 25 °C; $\mu = 0.1$. The data were fit by a nonlinear least-squares procedure to give the stability constant of the intermediate (EuH_2L^+), 7.6 × 10^5 M^{-1}. (Subscript i indicates initial concentrations.)

formed intermediate involving a tetradentate ligand coordinated through the four carboxylate groups as has been postulated by others.[8]

Determination of the Equilibrium Formation Constant of the Intermediate, (EuH_xL), and Its Degree of Protonation, *x*. A simple measurement of the pH immediately after mixing unbuffered solutions of Eu^{3+} and dota allows us to deduce the degree of protonation of the intermediate. At pH 6, where dota exists primarily (>90%) as the H_2dota^{2-} ion, mixing equimolar quantities of ligand and metal ion instantaneously produces the spectroscopically detected intermediate (peak at 579.20 nm, Figure 1); however, observation of the pH 1–2 min after mixing reveals only a small drop in pH (corresponding to less than 5% of the H^+ ions expected to be released upon formation of the final product, $Eu(dota)^-$). This is consistent with the formulation of the intermediate as (EuH_2dota^+) and with observations made by Brücher *et al.*[8] on the analogous Ce^{3+} system.

Owing to the slowness of the reaction at pH values less than 5.5, the degree of conversion to the final product is negligible during the first 10–30 s after mixing. Thus it is possible to monitor, as a function of pH, the extent of intermediate formation immediately after mixing by measuring the emission intensity, *I*, for excitation at 579.2 nm. This intensity is related to the concentration of the intermediate by eq 6, where k' is a

$$I = k'\tau_{obs}[(EuH_2L^+)] \qquad (6)$$

proportionality constant. The variation of *I* with pH is shown in Figure 2, where the decrease at lower pH values reflects competition of H^+ ions with Eu^{3+} for the ligand. This behavior was modeled using a nonlinear regression technique wherein *I* is expressed as a function of the known protonation constants of dota, K_n^H, $n = 1-4$, the formation constant of the intermediate, K or K' (eq 7 or 8), and the constant k'. The protonation

$$Eu^{3+} + H_2L^{2-} \leftrightarrow (EuH_2L^+), \quad K = k_f/k_r \qquad (7)$$

$$Eu^{3+} + HL^{3-} \leftrightarrow (EuHL), \quad K' = k'_f/k'_r \qquad (8)$$

constants are held constant at their known values[18] while K or K' and k' are left as parameters to be fit. The fit for $K = 7.6 \times 10^5\ M^{-1}$ (eq 7) is shown in Figure 2. An equally acceptable fit (not shown) for a monoprotonated intermediate with K' (eq 8) equal to $1.1 \times 10^{12}\ M^{-1}$ may also be obtained. It should be noted, however, that complexes with formation constants in the latter range have always been found to be in the slow-exchange regime on the time scale of the $^5D_0$ excited state (*vide infra*). Further support for the fact that the diprotonated intermediate (EuH_2L^+) is the correct one comes from an analysis of eqs 7 and 8. The maximum possible formation rate constants for complexation of a Ln^{3+} ion (k_f and k'_f in eq 7 and eq 8) can be estimated from the outer-sphere association equilibrium constant, K_{os}, and the characteristic water exchange constant, $k^{Ln^{3+}-H_2O}$, for the loss of a coordinated water molecule from the first coordination sphere of the lanthanide ion.[2,4,26] For Eu^{3+}(aq), $k^{Eu^{3+}-H_2O}$ is about $6.5 \times 10^8\ s^{-1}$.[27] The estimated K_{os} values for a +3 ion associating with a −3 ion (HL^{3-}) or a −2 ion (H_2L^{2-}) are about 100 $M^{-1}$and 13 M^{-1}, respectively.[3] These lead to estimated values for k_f and k'_f of $8.4 \times 10^9\ M^{-1}\ s^{-1}$ and $6.5 \times 10^{10}\ M^{-1}\ s^{-1}$, respectively. Given these values and the experimentally determined K and K' values (eqs 7 and 8), one obtains the reverse rate constants: $k_r = 1.1 \times 10^4\ s^{-1}$ and $k'_r = 5.9 \times 10^{-2}\ s^{-1}$. Since both the forward and reverse rates must be greater than $9 \times 10^3\ s^{-1}$ (the deexcitation rate of the $^5D_0$ state of Eu^{3+}(aq), τ_2^{-1}, eq 3), only k_r satisfies this condition and is consistent with eq 7 and with (EuH_2L^+) being the intermediate.

These several lines of evidence establish the diprotonated intermediate (EuH_2L^+) as the one that forms immediately upon mixing. It likely involves coordination of Eu^{3+} to the four carboxylate groups well away from the plane of the four nitrogen atoms of dota, two of which are protonated and electrostatically repel the Eu^{3+} ion. On the basis of our experimental characterization of the intermediate (EuH_2L^+) (formation constant, no protons released immediately upon mixing, rapid ligand exchange, 4–5 coordinated water molecules) and molecular mechanics calculations we propose that the intermediate has the basic structure shown in Figure 3. The Eu^{3+} is coordinated to the four carboxylate groups on the side away from the plane of the macrocycle which has two protonated nitrogen atoms trans to one another. Our simulations indicate that, with these protons in place, it is impossible to achieve a stable structure with the Eu^{3+} ion in the "cage". The molecular mechanics were carried out by first simulating (EuH_2dota^+) *in vacuo* and then solvating the structure, at which point the Eu^{3+} ion picked up five waters of coordination, consistent with our experimental determination.

Molecular mechanical modeling was also done on the monoprotonated (EuHdota) species with the energy-minimized structure being almost identical to that for the diprotonated species. In this case, the single hydrogen on a ring nitrogen points inward. In none of the structures is more than one N–H--O(carboxylate) hydrogen bond formed. Others[10] have suggested a structure for the Eu^{3+}–dota intermediate with a ring nitrogen coordinated to the Eu^{3+} ion; however, in our calculations no stable structures with the Eu^{3+} ion in the ligand pocket or within coordination distance of an unprotonated ring nitrogen were found for either the mono- or the diprotonated species. Moreover, a structure involving a coordinated nitrogen would require fairly close proximity of the paramagnetic Eu^{3+} ion to the protons of the macrocyclic ring and would predict much larger paramagnetic (isotropic) NMR shifts than are in fact

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(26) Silber, H. B.; Farina, R. D.; Swinehart, J. H. *Inorg. Chem.* **1969**, *8*, 819–824.
(27) Fay, D. P.; Purdie, N. *J. Phys. Chem.* **1970**, *74*, 1160–1166.

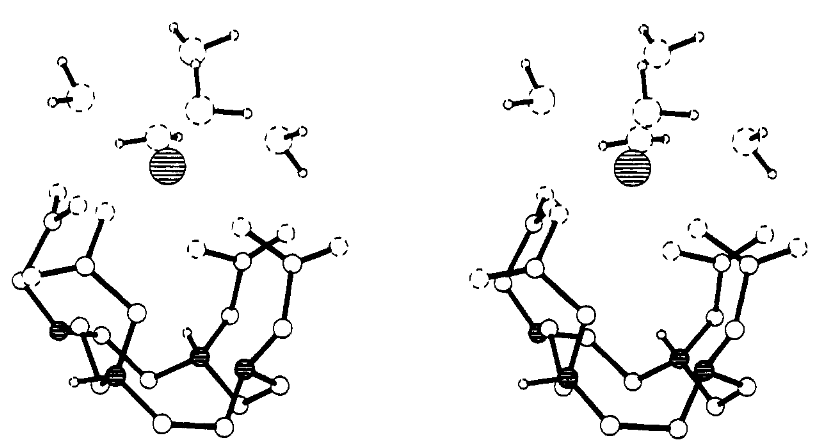

Figure 3. Stereoview of the intermediate (EuH_2dota^+) as deduced from molecular mechanics calculations, showing one protonated ring nitrogen N−H bond pointing into the cavity and one pointing out. Structures with either or both N−H bonds pointing in or both out had only slightly higher energies and may be contributing structures.

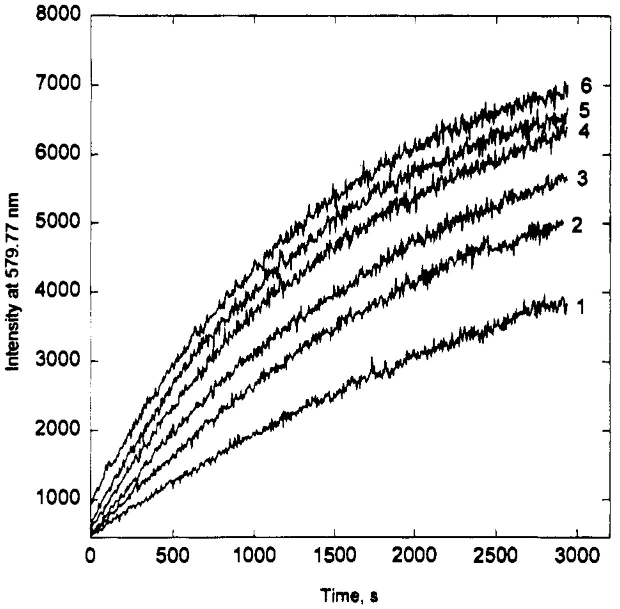

Figure 4. Plots of the intensity at 579.77 nm vs time in the Eu^{3+} complexation with 2 μM dota. pH = 3.78; 25 °C. [Eu^{3+}]: (1) 10 μM; (2) 20 μM; (3) 30 μM; (4) 60 μM; (5) 140 μM; (6) 400 μM.

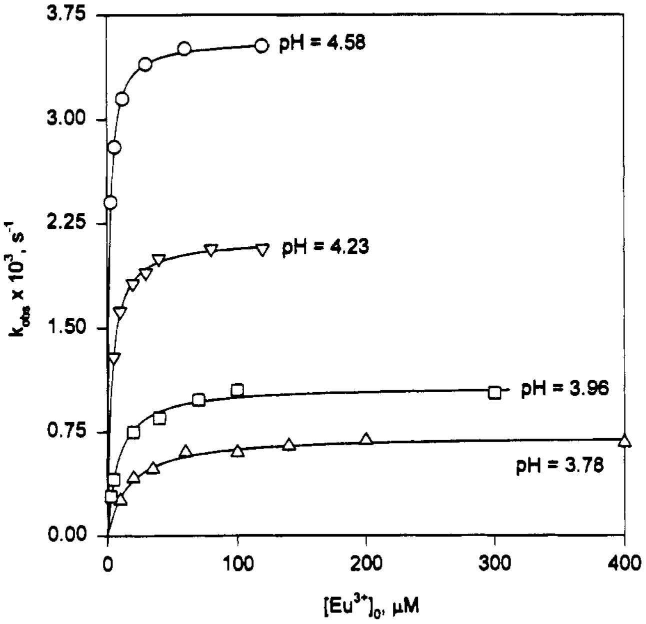

Figure 5. Plots of k_{obs} against excess $[Eu^{3+}]_0$ at various H^+ concentrations (25 °C, $\mu = 0.1$). The solid curves are the theoretical fits according to eq 19 as described in the text.

observed for the intermediate (≤2 ppm), compared to the final product (up to ~30 ppm).[11]

Kinetics of Formation of the Final Product, Eu(dota)⁻. The extent of the reaction was monitored by means of the increase in intensity of the peak at 579.77 nm in the $^7F_0 \rightarrow {}^5D_0$ excitation spectrum, which is characteristic of the final product (Figure 1). While not studied in detail, the intensity of the signal at 579.20 nm, corresponding to the intermediate (EuH_2dota^+), was observed to disappear at the same rate as the appearance of the product signal. The reaction was investigated as a function of pH (or pD) with an excess of Eu^{3+} present over dota to ensure pseudo-first-order kinetic conditions. Plots of the intensities at 579.77 nm vs time for various concentrations of excess Eu^{3+} are given in Figure 4. The observed rate constants (k_{obs}) were obtained by fitting the intensity as a function of time, $I(t)$, to a pseudo-first-order model:

$$I(t) = k''[L]_0(1 - \exp(-k_{obs}t)) \qquad (9)$$

where k'' is a proportionality constant and $[L]_0$ is the initial concentration of dota. The k_{obs} values so obtained are plotted in Figure 5 as a function of the initial Eu^{3+} ion concentration, $[Eu^{3+}]_0$, for several of the pH values investigated. The solid curves are the fits to eq 19, as described later. At higher $[Eu^{3+}]_0$ values, the k_{obs} values plateau for each pH implying the formation of an intermediate.[7] These "saturation" values are collected in Table 1, along with the results for D_2O solution and the kinetics data on the Gd^{3+}−dota system from Wang *et al.*[11] for comparison. The agreement with the latter values, which were obtained using an indicator method at much higher concentrations, is excellent.

Consistent with previous reports,[6,8−11] k_{obs} increases dramatically with increasing pH in the range 3.7−6. Plots of k_{obs} as a function of $1/[H^+]$ or $1/[D^+]$ are linear (Figure 6), although the rate constants are 17.7 times smaller in D_2O than in H_2O at the same temperature and D^+ or H^+ concentration. This apparent difference is entirely due to a difference in the ion products of H_2O and D_2O.[20,21] When this difference is taken into account, the same rate law is followed in both solutions

$$k_{obs} = k_0[OX^-], \quad X = H \text{ or } D \qquad (10)$$

and the average k_0 values in H_2O and in D_2O, calculated from the data in Table 1, are 7.2×10^6 M^{-1} s^{-1} (H_2O) and 6.7×10^6 M^{-1} s^{-1} (D_2O). The very close match of these k_0 values demonstrates that the different rate constants obtained when $[H^+]$

Table 1. Values of k_{obs}(max) Measured after "Saturation" for Reactions of Eu^{3+} or Gd^{3+} with dota at Various pH (or pD) Values at 25 °C and $\mu = 0.1$ (KCl)

Eu^{3+} (this work)				Gd^{3+} (Wang, et al.[a])	
pH	k_{obs}(max), s^{-1}	pD	k_{obs} (max) s^{-1}	pH	k_{obs} (max) s^{-1}
3.78	7.2×10^{-4}	4.00	1.0×10^{-4}	4.48	3.52×10^{-3}
3.96	1.1×10^{-3}	4.27	1.6×10^{-4}	4.68	5.11×10^{-3}
4.23	2.1×10^{-3}	4.58	2.5×10^{-4}	4.88	9.44×10^{-3}
4.44	2.7×10^{-3}	4.88	7.9×10^{-4}	5.28	2.14×10^{-2}
4.58	3.6×10^{-3}	5.17	1.1×10^{-3}	5.48	2.92×10^{-2}
4.69	4.8×10^{-3}			5.68	4.55×10^{-2}
4.79	5.7×10^{-3}				
4.86	7.5×10^{-3}				
5.15	1.4×10^{-2}				
5.23	2.1×10^{-2}				
5.41	3.1×10^{-2}				
5.64	4.8×10^{-2}				
5.81	7.9×10^{-2}				

[a] Reference 11; $\mu = 1$ (NaCl), 25 °C.

$= [D^+]$ is just due to the difference in base concentration (OH^- or OD^-) between the two solvents. This finding is strong evidence that OH^- is directly involved in the reaction.

We propose the following kinetic scheme to account for the formation of $Eu(dota)^-$, (L^{4-} = $dota^{4-}$):

$$Eu^{3+} + \left\{\begin{matrix} H_4L \\ H_3L^- \\ H_2L^{2-} \end{matrix}\right\} \rightleftharpoons (EuH_2L^+),\ K_1 \qquad (11)$$

$$(EuH_2L^+) + OH^- \rightleftharpoons (EuHL) + H_2O,\ K_2 \qquad (12)$$

$$(EuHL) \rightarrow EuL^- + H^+,\ k \qquad (13)$$

where (EuH_2L^+) is the long-lived intermediate whose protonation level was established as described earlier. K_1 is the (pH-dependent) *conditional* stability constant for the formation of (EuH_2L^+) which is related to the thermodynamic stability constant, K, for the reaction

$$Eu^{3+} + H_2L^{2-} \rightleftharpoons (EuH_2L^+),\ K \qquad (14)$$

by

$$K_1 = K\alpha_{H_2L} \qquad (15)$$

where

$$\alpha_{H_2L} = K_1^HK_2^H[H^+]^2(1 + K_1^H[H] + K_1^HK_2^H[H]^2 + K_1^HK_2^HK_3^H[H]^3 + K_1^HK_2^HK_3^HK_4^H[H]^4)^{-1} \qquad (16)$$

The first and second steps (eqs 11 and 12) represent rapidly established equilibria; however, owing to the values of K_1 and K_2 (*vide infra*) and the small initial concentrations of Eu^{3+}, dota, and OH^-, the monoprotonated intermediate is present in only very small concentrations (~10^{-9} M). It should be noted that the rate-determining final step (eq 13) is independent of OH^- concentration. This final step involves the rearrangement of (EuHL) from a configuration similar to that of (EuH_2L^+) to the final product by movement of the Eu^{3+} into the coordination "pocket" with concomitant expulsion of a proton. The value of K_2 is unknown; however, on the basis of the idea that the coordinated Eu^{3+} of EuH_2L^+ is relatively distant from the protonated nitrogen, one can use the second protonation constant of the free ligand as an approximation (upper limit) for the second protonation constant of the intermediate complex and thus obtain

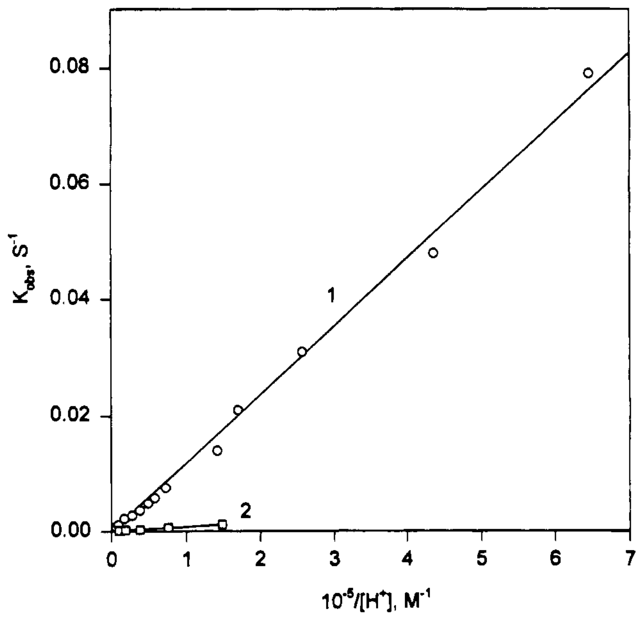

Figure 6. Effect of pH on k_{obs} measured after "saturation". Plots of (1) k_{obs} vs vs $[H^+]$ and (2) k_{obs} vs $1/[D^+]$. The solid lines represent the theoretical fits based on $k_{obs}=k/[H^+]$. The k values obtained from the fittings are 1.2×10^{-7} s^{-1} (H_2O) and 6.8×10^{-9} s^{-1} (D_2O).

Table 2. The Parameters kK_2 and Conditional Stability Constants K_1 of the Reaction Intermediate (EuH_2L^+) at Various pH Values

pH	kK_2, M^{-1} s^{-1}	K_1, M^{-1}
3.78	$(7.2 \pm 0.1) \times 10^6$	$(6.2 \pm 0.7) \times 10^4$
3.96	$(7.1 \pm 0.2) \times 10^6$	$(1.2 \pm 0.2) \times 10^5$
4.23	$(7.6 \pm 0.5) \times 10^6$	$(3.0 \pm 0.1) \times 10^5$
4.58	$(5.7 \pm 0.3) \times 10^6$	$(6.5 \pm 0.3) \times 10^5$

$$K_2 \approx 1/(K_2^HK_w) \approx 10^{4.09} \qquad (17)$$

as a lower-limit estimate for K_2.

On the basis of this scheme (eqs 11−13), the following rate constant expression is derived:

$$k_{obs} = kK_1K_2[OH^-][Ln^{3+}]/(1 + (1 + K_2[OH^-])K_1[Ln^{3+}]) \qquad (18)$$

Since $K_2[OH^-] \approx 10^{4.09}$ $[OH^-] \ll 1$ for the pH values (3−6) examined here, eq 18 can be simplified to

$$k_{obs} = kK_1K_2[OH^-][Ln^{3+}]/(1 + K_1[Ln^{3+}]) \qquad (19)$$

Under "saturation" conditions, $K_1[Ln^{3+}] \gg 1$, eq 19 simplifies to

$$k_{obs} = kK_2[OH^-] \qquad (20)$$

The values of kK_2 and K_1 at each pH investigated were obtained by fitting the k_{obs} values to eq 19 (Figure 5). The results are collected in Table 2.

The conditional stability constants, K_1, are, as expected, quite pH dependent. This strong pH dependence was not reported in conjunction with earlier studies of macrocyclic polyaza carboxylate ligands, including dota.[7,9−11] This may reflect a limitation of the indicator method.[7,9,11] Using our more sensitive technique, the dramatic decrease in k_{obs} at $[Eu^{3+}]_0$ values below 20 μM is readily observed (Figure 5), and it is only from data in this region that information about K_1 is obtainable. The

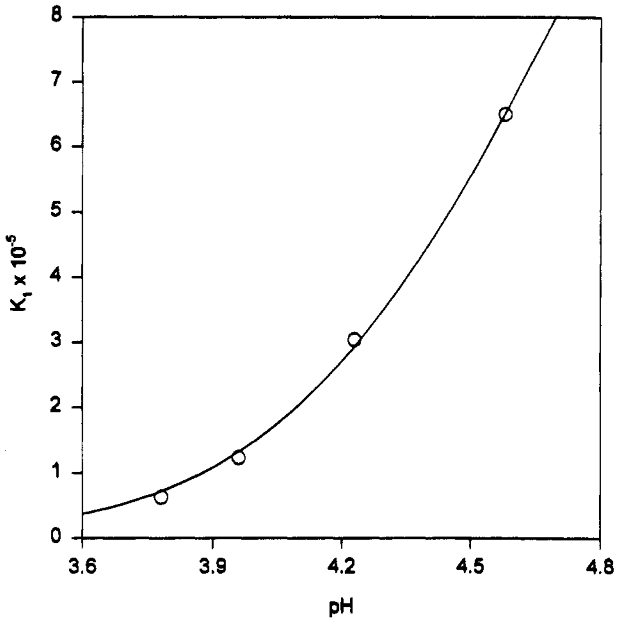

Figure 7. pH dependence of the conditional stability constant (K'_1, eq 11) of the intermediate (EuH_2dota^+). Fitting of the data (eq 15) yields $K = 1.8 \times 10^6$, the thermodynamic formation constant, eq 14.

present method is applicable to the systems in which the concentrations of the reactants are as low as 10^{-7} M and over a large range of pH values. The pH indicator method, however, is generally limited to concentrations of reactants above 10^{-4} M and to a smaller range of pH values.[7,9,11]

The thermodynamic stability constant of (EuH_2L^+), K (eq 14), was determined from the kinetic data (Table 2) to be 1.8×10^6 M^{-1} by fitting the observed values of K_1 vs pH to eq 15 (Figure 7). This is reasonably close to the value of 7.6×10^5 M^{-1} estimated from the pH–intensity titration at 579.20 nm (Figure 2). The rate constant, k, is calculated to be 590 s^{-1} if the K_2^H value of $dota^{4-}$ ($10^{9.69}$ M^{-1}) is taken as the value for the second protonation constant of (EuL^-).

Discussion

Mechanism of the Reaction. A hydroxide-assisted mechanism for the formation of Ln^{3+} ion complexes was proposed by Brücher and Sherry.[9] A monoprotonated intermediate was proposed for nota[9] as well as dota[10] and cydta[3] and has been assumed by most authors[6,9,10] to account for pH effects on the rates of these ligation reactions. The usual mechanism (but not the one proposed here for dota) involves a rapidly formed intermediate (LnHL) followed by a slow reaction of (LnHL) with OH^- to form the final product, LnL, in the rate-determining step as indicated in eqs 21 and 22. Furthermore, Kumar and Tweedle,[10] in the case of Gd^{3+} complexes of tricarboxylate derivatives of dota, proposed an intermediate involving a ring nitrogen coordinated to Gd^{3+} in which the transfer of a proton from a ring nitrogen to an attacking OH^- and the formation of an additional Gd^{3+}–N bond are concerted. The proton in the (LnHL) intermediate is postulated to be bound to a noncoordinated nitrogen atom. While electrostatic repulsion between the metal ion and the protonated nitrogen is not favorable for metal–nitrogen coordination, it not important enough to inhibit or to slow significantly metal ion coordination in flexible, acyclic ligands such as edta[4] or cydta.[3] Thus, deprotonation of the ligand is important for reasons other than just for eliminating this electrostatic inhibition to coordination.

$$\mathrm{M + HL \leftrightarrow (MHL)}, \quad K \qquad (21)$$

$$\mathrm{MHL + OH^- \rightarrow ML + H_2O}, \quad k_{\mathrm{OH}} \qquad (22)$$

In our view, removal of a proton from a ring nitrogen has a role more significant than just decreasing electrostatic repulsion and making a coordination site available to the metal ion. Deprotonation for a free-amine-like ligand is expected to dramatically increase the flexibility of the macrocycle and increase its ability to accommodate the structural changes which necessarily occur upon going from the protonated intermediate to the final coordination configuration. Support for this view comes from the well-known tendency of protonation or quaternization of an amine nitrogen to inhibit nitrogen inversion.[28] An extensive discussion of the stereodynamics of cyclic organonitrogen compounds can be found in a recent monograph, particularly in a chapter by Delpuech.[28] Base-catalyzed interconversions in transition metal complexes of acyclic[29–33] and macrocyclic[34,35] amine ligands also provide evidence for the importance of nitrogen protonation to rates of configurational interconversion.

Although nitrogen inversion has been proposed to be involved in the formation reactions of Gd^{3+} with dota derivatives[10] and in the dissociation reactions of lanthanide complexes of linear and macrocyclic polyamino carboxylates,[5,6,9,11] the precise details of this inversion are not known. The intermediates in the complexation reactions of Ln^{3+} ions with macrocyclic ligands have been suggested to be monoprotonated or diprotonated species (only for dota) under the usual pH conditions (pH = 3–7) investigated, but the formation rates for both cases are only first order in [OH^-], implying that the departure of a single proton from a nitrogen can relax the intermediate stiffened by the proton for either monoprotonated or diprotonated intermediates. The rigidity of such macrocycles makes it reasonable to assume the existence of a relatively stable activated complex (independent of pH) with a configuration similar to the structure of the intermediate observed by us for dota. Thus, the actually observed intermediate species depends on both the activated complex properties and the pH conditions. This assumption leads to the prediction that the complexation rate for macrocyclic ligands will reach a limit which is smaller than those of flexible acyclic chelating agents even when both are fully deprotonated under high-pH conditions.

The diprotonated intermediate is also likely to occur for dota complexation of dipositive ions.[7] From the viewpoint of the structural stability of the intermediate, the coordination of four carboxylate oxygen atoms of dota with the metal ion in (MH_2L) comprises a fairly symmetric and stable structure; the tetracarboxylate coordination plane is above the nitrogen plane, in a configuration similar to the [3,3,3,3] conformation of the free ligand[10,36] (Figure 3). The present mechanism is in excellent agreement with the experimental results of Kasprzyk and Wilkins[7] for dota reacting with Ca^{2+}, Sr^{2+}, Ni^{2+}, Zi^{2+}, Ba^{2+}, and Mg^{2+}. A relationship between the maximum k_{obs} at a

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(28) Delpuech, J.-J. In *Cyclic Organonitrogen Stereodynamics*; Lambert, J. B., Takeuchi, Y., Eds.; VCH: New York, 1992.
(29) Buckingham, D. A.; Marzilli, L. G.; Sargeson, A. M. *J. Am. Chem. Soc.* **1967**, *89*, 825–830.
(30) Buckingham, D. A.; Marzilli, L. G.; Sargeson, A. M. *J. Am. Chem. Soc.* **1967**, *89*, 3428–3433.
(31) Buckingham, D. A.; Marzilli, L. G.; Sargeson, A. M. *Inorg. Chem.* **1969**, *8*, 1595–1604.
(32) Searle, G. H.; Keene, F. R. *Inorg. Chem.* **1972**, *11*, 1006–1011.
(33) Wilkins, R. G. *The Study of Kinetics and Mechanism of Reactions of Transition Metal Complexes*; Allyn and Bacon: Boston, 1974; p 354.
(34) Lee, C.-S.; Chung, C.-S. *Inorg. Chem.* **1984**, *23*, 639–644.
(35) Lee, C.-S.; Wang, G.-I.; Chung, C.-S. *J. Chem. Soc., Dalton Trans.* **1984**, 109–114.
(36) Dale, J. *Isr. J. Chem.* **1980**, *20*, 3–11.

specific pH value and the concentration of H^+ may be derived from eq 18 for "saturation" conditions:

$$k_{obs}(\max) = kK_2[OH^-]/(1 + K_2[OH^-]) = k/(1 + K_2^H[H^+]) \tag{23}$$

According to the literature,[7] k_{obs}(max) times K_1 equals to the second-order rate constant, k^*:

$$k^* = k_{obs}K_1 = kK_1/(1 + K_2^H[H^+]) \tag{24}$$

Equation 24 predicts that a plot of log(k^*) vs log(1+ $K_2^H[H^+]$) should be a straight line with slope of −1.0. Interestingly, a relationship identical to eq 24 was shown by Kasprzyk and Wilkins[7] to be consistent with their experimental data.

Reanalysis of the Results of Previous Studies. In order to reanalyze previous work[6,9,10] on macrocyclic ligands containing three carboxylate groups where monoprotonated intermediates were confirmed, a mechanism similar to that proposed above for dota is suggested. It is described by the following reactions:

$$Ln^{3+} + HL \leftrightarrow (LnHL),\quad K_1 \tag{25}$$

$$(LnHL) + OH^- \leftrightarrow (LnL) + H_2O,\quad K_2 \tag{26}$$

$$(LnL) \rightarrow LnL,\quad k \tag{27}$$

where K_1 and K_2 are equilibrium constants of eq 25 and eq 26, respectively, and k is the rate constant of rate-determining step. Equation 25 describes the fast equilibrium formation of the intermediate with a protonated, noncoordinated ring nitrogen. The partial coordination of the ligand in the intermediate (LnHL) will cause the complex to be somewhat more rigid than the free ligand. It is postulated that deprotonation of (LnHL) produces an activated complex, (LnL), which maintains the coordination of the Ln^{3+} ion to the carboxylate groups as in (LnHL), with a similar configuration. Equation 27 represents the rearrangement of the intermediate (LnL) to the final complex LnL. The rate constant, k, depends largely on the magnitude of the ring strain and the inversion barrier of the nitrogen atoms during the rearrangement of the intermediate. On the basis of this scheme (eqs 25−27), the rate constant expression is derived as

$$k_{obs} = kK_1K_2[OH^-][Ln^{3+}]/(1 + (1 + K_2[OH^-])K_1[Ln^{3+}]) \tag{28}$$

Equation 28 can be simplified as follows under the usual pH conditions (pH 3−7) where $K_2[OH^-] \ll 1$:

$$k_{obs} = kK_1K_2[OH^-][Ln^{3+}]/(1 + K_1[Ln^{3+}]) \tag{29}$$

After "saturation" with Ln^{3+} ions ($K_1[Ln^{3+}] \gg 1$) eq 29 simplifies to

$$k_{obs} = kK_2[OH^-] \tag{30}$$

Equations 29 and 30 predict that plots of k_{obs} vs $[OH^-]$ yield straight lines with intercepts of zero. This conclusion is consistent with the previously reported data.[6,9,10] It should be noted that in other analyses k_{obs} was given by

$$k_{obs} = k_{H_2O} + k_{OH}[OH^-] \tag{31}$$

However k_{H_2O}, the rate constant for a non-hydroxide-assisted reaction, is found to be almost negligible in each case and is ignored here.

The present kinetic scheme predicts (eq 28) that, with increasing pH, the reaction rate constant will approach the value of the rearrangement rate constant of the intermediate, k, as a limit when L^{n-} becomes the predominant species of ligand in solution.

The value of k characterizes the rigidity of the ligand or, more accurately, that of the intermediate. The activation free energy ($\Delta G^\ddagger$) for this first-order reorganization process can be calculated from k values by using the Eyring equation[37]

$$k = (k_BT/h)e^{-\Delta G^\ddagger/RT} \tag{32}$$

where k_B is the Boltzmann constant and h is Planck's constant. This equation may be recast in the form

$$\Delta G^\ddagger = 4.57T(10.32 - \log(k)/T) \tag{33}$$

where $\Delta G^\ddagger$ is given in units of cal mol^{-1}. The results for a variety of macrocyclic polyamino carboxylate ligands and metal ions are displayed in Table 3, together with the original kinetic parameters. dota has a particularly high activation energy. This has been rationalized in terms of the inflexibility of the high-symmetric intermediate. Comparing the relative values of the rearrangement rate constant k (or $\Delta G^\ddagger$) in Table 3, one may conclude that rigidity decreases in the order dota > do3ma > hp-do3a ~ do3a > nota and Me_2deta > Me-deta ~ deta. This order reflects, among other things, the contribution of the substituents to the rigidity of the ligands.

Gathered in Table 3 are various kinetic parameters and derived or estimated quantities for metal ion complexation of dota and related macrocyclic ligands. Our measurements yield the product kK_2 (eqs 12, 13, and 20 or eqs 26, 27, and 30) which is equivalent to k_{OH} in the notation of others.[6,8−10] In order to obtain a value for the constant, k, of the rate-determining rearrangement of the intermediate, it is necessary to know the value of K_2, the equilibrium constant for the reaction of OH^- with the diprotonated intermediate $(LnH_2L)^+$ in the case of dota (eq 12) or the monoprotonated intermediate (LnHL) (eq 26) in the case of the other ligands. These values are unknown but can be estimated with the assumption that the protonation constants for the complex intermediate are the same for the uncomplexed ligands (eq 17). K_2^H is used in the case of the tetraacid, dota, and K_1^H in the case of the remaining ligands, which are triacids. Owing to the presence of the positively charged metal ion in the intermediate, it is highly probable that the protonation constant of the intermediate will be smaller than the constant for the ligand alone. This makes the estimated K_2 values lower limits and the derived k values, upper limits. Although the absolute k values are uncertain, comparisons for the same ligand or within a closely related series of ligands should be valid. The $\Delta G^\ddagger$ values derived from the k values via eq 33 should be considered only to have relative significance.

First, considering the dota systems, as noted earlier the results for Eu^{3+}− and Gd^{3+}−dota are very close, as expected for adjacent ions in the periodic table. The Ce^{3+} system has a k value smaller by a factor of about 7 than Eu^{3+}. It may be no coincidence that the stability constant of Ce^{3+}−dota is smaller by a factor of about 6 than that of Eu^{3+}−dota.[38] The smaller rate constant in the case of the larger Ce^{3+} ion is consistent with the size effect noted by Kasprzyk and Wilkins,[7] who found

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[37] Espenson, J. H. *Chemical Kinetics and Reaction Mechanisms*; McGraw-Hill Book Co.: New York, 1981.

[38] Wu, S. L. Unpublished results.

Table 3. Kinetic Parameters for Various Macrocyclic Polyaza Carboxylate Ligands Including Estimates of the Lower Limits of the Activation Free Energies ($\Delta G^{\ddagger}$) and the Upper Limit of the Rate Constants (k) of the Rate-Determining Step (25 °C)

ligand	metal ion	intermediate	$\log K_n^{\mathrm{H}}$	(a) kK_2 or (b) k_{OH}, $\mathrm{M^{-1}\,s^{-1}}$	k,[a] $\mathrm{s^{-1}}$	$\Delta G^{\ddagger}$,[b] kcal/mol	ref
dota	Eu^{3+}	EuH_2L	9.69 ($n = 2$)	(a) 7.2×10^{6}	5.9×10^{2}	13.7	this work
dota	Gd^{3+}	GdH_2L	9.69 ($n = 2$)	(b) 5.9×10^{6}	4.8×10^{2}	13.8	10
dota	Ce^{3+}	CeH_2L	9.69 ($n = 2$)	(b) 1.0×10^{6}[c]	8.3×10^{1}	14.8	8
dota	Ca^{2+}	CaH_2L	9.69 ($n = 2$)		3.5×10^{2}[d]	14.0[d]	7
do3a	Gd^{3+}	GdHL	11.59 ($n = 1$)	(b) 2.1×10^{7}	1.4×10^{5}	10.4	10
do3ma	Gd^{3+}	GdHL	13.38 ($n = 1$)	(b) 7.2×10^{4}	2.9×10^{4}	11.3	10
hp-do3a	Gd^{3+}	GdHL	11.96 ($n = 1$)	(b) 1.2×10^{7}	1.9×10^{5}	10.2	10
nota	Gd^{3+}	GdHL	11.61 ($n = 1$)	(b) 7.1×10^{7}	4.8×10^{5}	9.69	10
deta	Gd^{3+}	GdHL	14.8 ($n = 1$)	(b) 8.2×10^{5}	8.6×10^{6}	7.98	6
Me-deta	Gd^{3+}	GdHL	15.3 ($n = 1$)	(b) 7.3×10^{5}	2.4×10^{7}	7.37	6
Me_2-deta	Gd^{3+}	GdHL	13.9 ($n = 1$)	(b) 3.8×10^{4}	5.0×10^{4}	11.0	6

[a] Calculated from kK_2 assuming $K_2 = (K_n^{\mathrm{H}}K_{\mathrm{w}})^{-1}$. [b] Calculated from eq 33 using upper-limit k values; thus these are lower limits. [c] The k_{OH} value was calculated from $k_{\mathrm{OH}}K_{\mathrm{w}} = 1.7 \times 10^{-8}$ M $\mathrm{s^{-1}}$. [d] First, we calculated the stability constant of the intermediate (CaH_2L) as $K_1 = 2246$ from fitting the conditional stability constants (K) of the intermediate to $K = K_1\alpha_{\mathrm{H_2L}} + K'\alpha_{\mathrm{HL}}$, where $K_1 = [CaH_2L]/([Ca^{2+}][H_2L^{2-}])$ and $K' = [CaHL^-]/([Ca^{2+}][HL^{3-}])$. Since at the high-pH conditions the concentration of HL^{3-} is too large to be neglected, the k value was obtained from $kK_1 = 7.9 \times 10^{5}\ \mathrm{M^{-1}\,s^{-1}}$.

Sr^{2+} and Ba^{2+} to complex with dota much more slowly than does the smaller Ca^{2+} ion. The kinetic results for Ca^{2+}, included in Table 3, result from an analysis somewhat different from that used for the Ln^{3+} systems, but the rates determined and the derived $\Delta G^{\ddagger}$ values are quite similar. This suggests that size effects may be more important than differences in charge.

The remaining ligands listed in Table 3 all involve a monoprotonated intermediate (LnHL) and since the relevant K_1^{H} values are all higher than the K_2^{H} value for dota, the derived K_2 values are smaller. The K_2k products for these systems range from larger to smaller than those of the dota systems studied. The derived k values, on the other hand, are all larger than those for the dota systems, implying that these intermediates are less rigid and that the rate-determining rearrangement of the initially-formed intermediate is more facile.

In their study of do3a, hp-do3a, and do3ma, which included earlier data available on dota and nota, Kumar and Tweedle[10] noted a linear correlation between the $\Delta G^{\ddagger}$ values (derived by applying the Eyring relationship to the second-order rate constants, k_{OH}) and ligand strain energy calculations of Fossheim and co-workers.[39,40] The $\Delta G^{\ddagger}$ values derived by us from the first-order constants, k, also correlate monotonically with the strain energy calculations, suggesting that the rigidity of the ligand, and hence of the intermediate, is a major factor in determining the rate of complex formation. Kumar and Tweedle[10] found, however, that the $\Delta G^{\ddagger}$ value for do3ma was much higher than those for all of the other ligands, including dota, which seems unreasonable (molecular mechanics strain energy calculations are unavailable for this ligand). In our estimation of $\Delta G^{\ddagger}$ (Table 3), the do3ma value (10.2 kcal/mol) is only slightly higher than the values for the other tetraaza tricarboxylate macrocycles and is smaller than the dota value (13.8 kcal/mol), as is expected.

Conclusions. A kinetic intermediate (EuH_2dota^+) in the formation of $[Eu(dota)(H_2O)]^-$ has been detected by luminescence excitation spectroscopy. The intermediate is diprotonated at two ring nitrogens and involves the coordination of the Eu^{3+} to the four carboxylate groups and probably five water molecules (Figure 3). This intermediate converts slowly (k_{obs} increases from 7.2×10^{-4} to $7.9 \times 10^{-2}\ \mathrm{s^{-1}}$ as the pH is raised from 3.78 to 5.81) to the final product by a base-catalyzed mechanism. An equilibrium deprotonation by OH^- to form a very small quantity of a second intermediate, (EuHdota), is the first step in this mechanism and accounts for the finding that the rate is proportional to the OH^- ion concentration. This is followed by the final rate-determining step involving a concerted rearrangement to the final complex as the Eu^{3+} ion moves into the tetraaza coordination "pocket" and the second proton is expelled. Protonation of aza macrocycles greatly increases their rigidity and leads to slower complex formation than found for nonmacrocyclic ligands. The mechanism proposed here applies to tricarboxyate macrocyclic ligands previously studied, except that in those cases a monoprotonated intermediate (LnHL) is involved and no proton is expelled in the final rate-determining rearrangement.

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(39) Fossheim, R.; Dahl, S. G. *Acta Chem. Scand.* **1990**, *44*, 498–706.

(40) Fossheim, R.; Dugstadt, H.; Dahl, S. G. *J. Med. Chem.* **1991**, *34*, 819–826.