Spectroscopic Characterization of some Rare Earth Complexes of Triethylenetetraaminehexaacetic Acid

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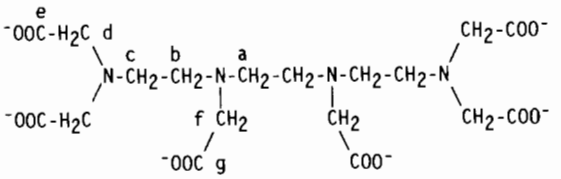
Abstract

The Eu^{3+} complexes formed with triethylenetetraaminehexaacetic acid (TTHA) were characterized in solution by laser-induced Eu^{3+} luminescence spectroscopy. Both 2:1 and 1:1 metal:ligand complexes were detected at pH 6.0 depending on the metal–ligand stoichiometry. These two species exhibit distinct excited-state lifetimes of 260 and 1210 μs, respectively. The 2:1 complex coordinates three or four water molecules at each Eu^{3+} ion while the 1:1 complex has no coordinated water molecules. The $^7F_0 \rightarrow {}^5D_0$ excitation spectra revealed multiple isomeric forms for both the 2:1 and 1:1 complexes. Spectra and excited-state lifetimes were taken as a function of pH for a 1:1 metal:ligand solution. As the pH increases, an equilibrium shift from the 2:1 species to the 1:1 species occurs. ^1H and ^{13}C spectra of the diamagnetic La^{3+}, Y^{3+} and Lu^{3+} complexes were recorded. The La^{3+} chelate of TTHA exhibits long-lived nitrogen and short-lived oxygen bonds, on the NMR time scale, while the Y^{3+} and Lu^{3+} adducts spectra are complicated due to fluctional processes.

Introduction

The development of NMR imaging as a diagnostic tool in medicine has prompted a great deal of interest in compounds which enhance tissue contrast [1]. In order to achieve enhancement, the imaging agent must efficiently relax nearby nuclei through a dipolar interaction. Paramagnetic transition metal and lanthanide ion complexes have received the majority of attention due to their favorable relaxation properties [1]. The only FDA approved imaging agent available, at this time, is the Gd^{3+} complex of diethylenetriaminepentaacetic acid. This complex has been well characterized in both the solution and the solid state [2–6]. Several other aminocarboxylate complexes of Gd^{3+} have been proposed as NMR imaging agents, including the complex formed with triethylenetetraaminehexaacetic acid (TTHA) (Scheme 1).



Scheme 1. Schematic representation of the free ligand $TTHA^{6-}$ with proton and carbon labels corresponding to those assigned to NMR chemical shifts in Tables 2 and 3.

The relaxation capabilities of the Gd^{3+} chelate of TTHA have previously been reported [1, 7–9], but little is known about the structural properties of this complex in solution. In order to understand thoroughly the potential of this compound as an NMR imaging agent, a knowledge of its solution complexation properties is essential. However, probing the Gd^{3+} ion environment directly with most spectroscopic techniques is not possible due to its f^7 ground state configuration. The ionic radii of Gd^{3+} and its periodic table neighbor, Eu^{3+}, are quite similar, suggesting that the complexes formed by these two metal ions should be nearly identical. By replacing Gd^{3+} with Eu^{3+}, the highly desirable spectroscopic properties of Eu^{3+} can be utilized. Eu^{3+} possesses non-degenerate ground ($^7F_0$) and first excited ($^5D_0$) states allowing neither level ($J = 0$) to be split by the ligand field. This results in a single electronic transition between these levels for each distinct Eu^{3+} environment [10].

The present study focuses on the complexes formed by the Eu^{3+} ion and the diamagnetic rare earth ions La^{3+}, Y^{3+} and Lu^{3+} with TTHA in aqueous solution. The Eu^{3+} complex was studied by exploiting the laser-induced luminescence and lifetime spectroscopic techniques which have been used extensively in this laboratory to probe the calcium binding sites in proteins and other biologically important systems [11]. The La^{3+}, Y^{3+} and Lu^{3+} complexes were probed using ^1H and ^{13}C NMR. The structural information obtained by combining these methods

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provides a reasonably accurate description of the solution structures of these rare earth ions with TTHA.

Experimental

Materials

TTHA (98%) in the free acid form, hydrated $EuCl_3$ (99.9%) and D_2O (99.8%) were purchased from the Aldrich Chemical Company. Rhodamine 590 and Coumarin 480 and 485 laser dyes were purchased from the Exciton Company while Rhodamine 610 was obtained from the Kodak Company. The H_2O used was deionized and doubly distilled while all the remaining reagents were the purest commercially available. The $EuCl_3$ stock solution used was standardized with EDTA using an arsenazo indicator [12].

Methods

The excitation and lifetime experiments were carried out using a Quantel series YG581C pulsed (10 Hz) Nd:YAG laser pumped tunable dye laser model TDL50 (~70 mJ). The remainder of the system is described in detail elsewhere [11, 13]. A 10 mM stock solution (15 mM piperazine buffer, pH 6) of TTHA was prepared. The metal–ligand solutions used were typically prepared in a 1:1 ratio at a concentration of 10 μM (pH 6) unless otherwise stated. The $^7F_0 \rightarrow {}^5D_0$ transition (580 nm) of Eu^{3+} was excited using a mixture of Rhodamine 590 and 610 laser dyes while monitoring the $^5D_0 \rightarrow {}^7F_2$ emission band at 614 nm. ^1H spectra were recorded on either a Bruker AM-300 or a Bruker WM-360 spectrometer at 300 or 360 MHz while ^{13}C spectra were recorded on a Bruker AM-300 spectrometer at 75.5 MHz. NMR samples were prepared in a 1:1 metal:ligand ratio at concentrations of 100 mM, in D_2O at pD 6.5.

Results and Discussion

Luminescence Studies

The stoichiometry of Eu^{3+} complexes can generally be determined by monitoring the intensity of the $^7F_0 \rightarrow {}^5D_0$ excitation spectrum through the course of a ligand → metal titration. By plotting the intensity of the $^7F_0 \rightarrow {}^5D_0$ excitation spectrum against the number of equivalents of ligand added, a binding curve can be generated. The binding curve, obtained for TTHA using this method, is non-linear between zero and one equivalent of added ligand. This non-linearity results from the fact that more than one exponential component contributes to the luminescence decay of the Eu^{3+} $^5D_0$ excited-state. The total luminescence decay curves obtained at each equivalent of ligand added, up to a 1:1 metal:ligand mixture, were resolved into their respective components. These components exhibit excited-state lifetimes of 260 and 1210 μs, which indicate that the two species present in solution undergo slow exchange on the $^5D_0$ excited-state time scale [14]. However, beyond a 1:1 ratio of added ligand to Eu^{3+} the decay curve corresponds to only a single exponential. These data indicate that the species present beyond a 1:1 metal:ligand ratio are in fast exchange on the $^5D_0$ excited-state time scale.

An alternative approach to obtaining stoichiometries of Eu^{3+} adducts, when the species present exhibit distinct excited-state lifetimes, is to accumulate known numbers of decay traces, at specific wavelengths, throughout the course of a metal:ligand titration. This method, known as Eu^{3+} time-resolved luminescence, allows the construction of a plot of the initial intensities (I_0) of individual exponential decay components vs. the number of equivalents of added ligand (Fig. 1) [15]. This technique provides interpretable titration curves for each species. These data indicate that the short lifetime is due to a 2:1 metal:ligand complex while the long lifetime arises from a 1:1 metal:ligand complex. The abrupt break at 1:1 is indicative of quantitative binding, which is indeed the case since lanthanide ions have been reported to bind TTHA quite strongly [16]. The observation of both 2:1 and 1:1 metal:ligand species is in accord with previous studies on solid state lanthanide ion TTHA precipitates [17].

Eu^{3+} $^7F_0 \rightarrow {}^5D_0$ excitation spectra were recorded at each addition of ligand throughout the course of a titration. These spectra (Fig. 2) consist of two well resolved bands at 579.58 and 580.15 nm. The peak positions of both bands remain constant over the course of a titration; however, the ratios of the intensities of the two bands change slightly. As ligand is added, the band at 580.15 nm increases in intensity

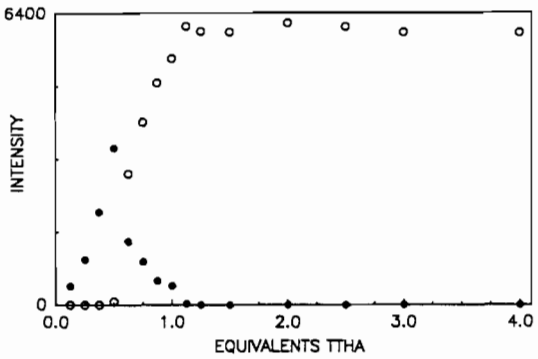

Fig. 1. Time-resolved initial intensities (I_0) for the 260 μs component (filled circles) and 1210 μs component (open circles) of luminescence emission (λ_{ex} = 579.6 nm, λ_{em} = 614 nm) as a function of added equivalents of TTHA. The filled circles and open circles correspond to the 2:1 and 1:1 Eu^{3+}:TTHA complexes, respectively.

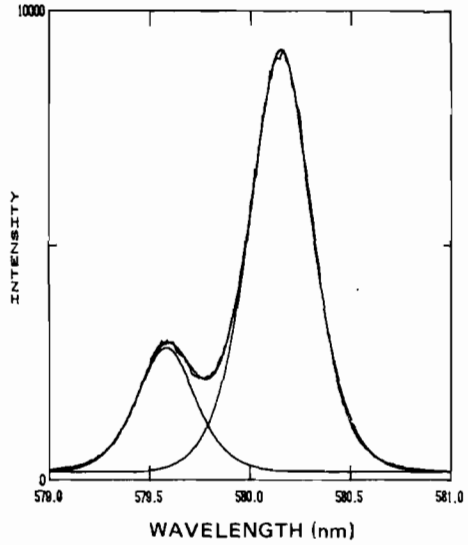

Fig. 2. Deconvoluted $^7F_0 \rightarrow {}^5D_0$ excitation spectrum of $[EuTTHA]^{3-}$ at pH 6.0 using Lorentzian-Gaussian type peaks.

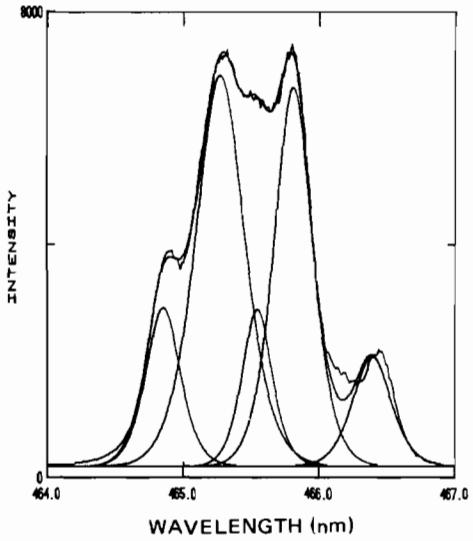

Fig. 3. Deconvoluted $^7F_0 \rightarrow {}^5D_1$ excitation spectrum of $[EuTTHA]^{3-}$ at pH 6.0 using Lorentzian-Gaussian type peaks.

relative to the one at 579.58 nm. This suggests that the 2:1 species exhibits two bands with peak positions identical to those of the 1:1 species. The observation of two bands when only the 2:1 species is present, at metal:ligand ratios greater than 2:1, and also two bands for metal:ligand ratios less than 1:1, indicates that two isomeric species are present in solution for both the 2:1 and 1:1 complexes. Single exponential decay curves are detected only when the metal:ligand ratios are greater than 2 or less than 1. These results are consistent with an exchange process which is fast on the $^5D_0$ excited-state time scale for the respective 2:1 and 1:1 isomers [14].

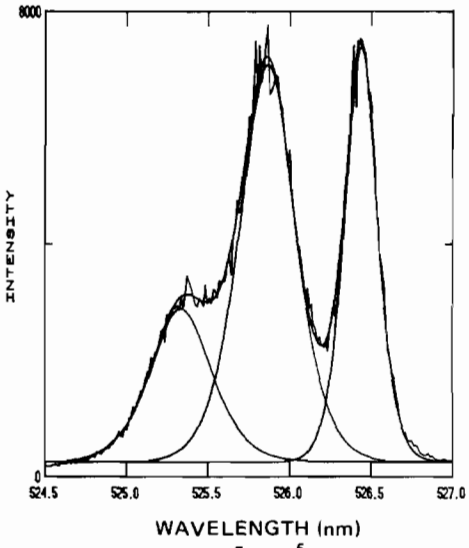

Fig. 4. Deconvoluted $^7F_0 \rightarrow {}^5D_2$ excitation spectrum of $[EuTTHA]^{3-}$ at pH 6.0 using Lorentzian-Gaussian type peaks.

TABLE 1. Ligand field splitting of the $^5D_0$, $^5D_1$ and $^5D_2$ levels of the Eu^{3+} complex of TTHA at pH 6.0

	λ (nm)[a]	ε (cm^{-1})	σ (nm)
$[EuTTHA]^{3-}$			
$^7F_0 \rightarrow {}^5D_0$	579.58	17254	0.34
	580.15	17237	0.36
$^7F_0 \rightarrow {}^5D_1$	525.34	19035	0.44
	526.87	18980	0.40
	526.44	18996	0.23
$^7F_0 \rightarrow {}^5D_2$	464.85	21512	0.30
	465.27	21493	0.42
	465.55	21480	0.26
	465.81	21468	0.33
	466.39	21441	0.33

[a]The reported wavelength values are accurate to ±0.02 nm.

Excitation spectra of the $^7F_0 \rightarrow {}^5D_0$, $\rightarrow {}^5D_1$, and $\rightarrow {}^5D_2$ Eu^{3+} transitions were recorded and curve resolved into Lorentzian-Gaussian type peaks using a Marquardt non-linear regression algorithm [18]. Deconvoluted spectra of the 1:1 metal:ligand complex at pH 6.0 for each excited-state level are shown in Figs. 2–4, with spectral data given in Table 1. The wavelengths of both bands in the $^7F_0 \rightarrow {}^5D_0$ excitation spectrum of the $[EuTTHA]^{3-}$ complex are quite similar to those of $[EuEDTA]^-$, although the relative intensities are markedly different [19]. The spectral similarity between these two complexes is not surprising when one compares the ligand structures of TTHA and EDTA. Nevertheless, the excitation spectra recorded for the higher excited states of Eu^{3+} are markedly different from those of

EDTA [19]. The characteristic differences in the Stark splitting of each Eu^{3+} level compared to corresponding data for EDTA illustrates the sensitivity of the Eu^{3+} ion with respect to its ligand field. The unambiguous assignment of metal ion site-symmetry in this case is not possible due to the multiple species present concurrently in solution. However, the symmetry about the metal ion is likely low, probably less than C_{2v}.

Horrocks and Sudnick [20] have previously reported a method which utilizes the excited-state lifetimes of Eu^{3+} to determine the number of coordinated water molecules. The relationship established between the number of bound water molecules, q, and the inverse excited-state lifetimes, τ^{-1} (ms^{-1}), taken separately in H_2O and D_2O is given in eqn. (1).

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

The luminescence decay curve recorded for a 2:1 metal:ligand mixture in D_2O reveals excited-state component exponentials with lifetimes of 2350 and 1220 μs. Only the shorter lifetime component is observed in D_2O at a 1:1 metal:ligand ratio. These data indicate that the 2:1 species coordinates three or four water molecules at each Eu^{3+} ion while the 1:1 species is devoid of inner-sphere water molecules. NMR relaxation and luminescence lifetime studies on the Gd^{3+} and Tb^{3+} 1:1 adducts of TTHA, respectively, also indicate the coordination of zero water molecules [9].

A hexaprotic acid such as TTHA may potentially exhibit diverse complexation properties as a function of pH. This prompted us to explore the types of complexes formed and chelating abilities for a 1:1 mixture of TTHA with Eu^{3+} over the pH range 2–12. The wavelengths of the two bands in the $^7F_0 \rightarrow {}^5D_0$ excitation spectra of TTHA do not change with pH; however, the relative intensities and excited-state lifetimes are altered. At the lowest pH studied (2.3) the intensity of the band centered at 579.58 nm is greater than the band centered at 580.15 nm. Only a single excited-state lifetime of 260 μs was detected, indicating the presence of only the 2:1 species. For pH values between 3.0 and 6.0 the band centered at 580.15 nm gains intensity and surpasses that of the band centered at 579.58 nm. A concomitant increase in the excited-state lifetime also occurs over this pH range, indicating a shift in equilibrium toward the 1:1 species. At pH values above 6.0, the spectral intensity and excited-state lifetime remain constant, suggesting that only the 1:1 species exists. These data are also consistent with NMR relaxation studies for the Gd^{3+} adduct [9].

NMR Studies

Rare earth complexation of TTHA was further probed by recording ^1H and ^{13}C NMR spectra of the 1:1 metal:ligand adducts of the diamagnetic ions La^{3+}, Y^{3+} and Lu^{3+} at pH 6.0 (Tables 2 and 3). Day and Reilley [21, 22] have previously reported a method by which the metal–nitrogen and metal–oxygen bond labilities can be determined from the characteristic proton splitting patterns observed for certain EDTA complexes. The ^1H spectra of these rare earth complexes are expected to conform to one of these splitting patterns. The ^1H spectrum of the La^{3+} adduct recorded at 25 °C is well resolved and contains three AB splitting patterns due to acetate protons (Fig. 5). This type of ^1H splitting pattern is indicative of long-lived metal–nitrogen and short-lived metal–oxygen bonds, on the NMR time scale,

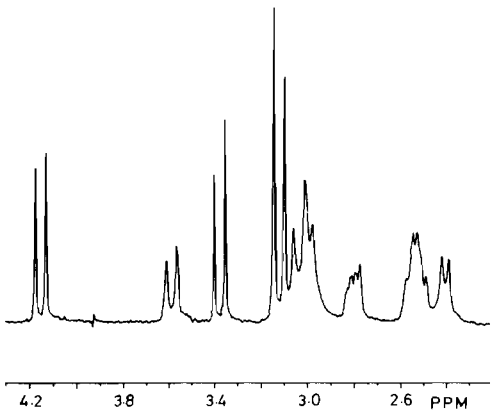

Fig. 5. ^1H NMR spectrum of $[LaTTHA]^{3-}$ at 20 °C and pH 6.0.

TABLE 2. ^1H and ^{13}C NMR chemical shift data of the free ligand TTHA and the $[LaTTHA]^{3-}$ complex[a]

	^1H		^{13}C
TTHA			
(a)	3.11 (s, 4)		50.62
(b)	3.21 (t, 4; J = 6 Hz)		51.57
(c)	3.30 (t, 4; J = 6 Hz)		51.89
(f)	3.46 (s, 4)		56.49
(d)	3.70 (s, 8)		57.69
(e)			172.07
(g)			175.25
$[LaTTHA]^{3-}$			
(a)	3.01, 2.98, 2.43, 2.39	(AB, 4)	54.23
(b)	2.55	(m, 4)	57.22
(c)	2.80	(m, 4)	58.95
(d) or (f)	3.62, 3.56, 3.07, 3.01	(AB, 4)	61.67
(d) or (f)	3.41, 3.35, 3.15, 3.10	(AB, 4)	62.80
(d) or (f)	4.18, 4.13, 3.15, 3.10	(AB, 4)	63.73
(e) or (g)			181.01
(e) or (g)			181.30
(e) or (g)			181.59

[a] ^1H and ^{13}C NMR spectra are referenced relative to Me_4Si (δ = 0 ppm) with assignments based on the labeling scheme of Scheme 1.

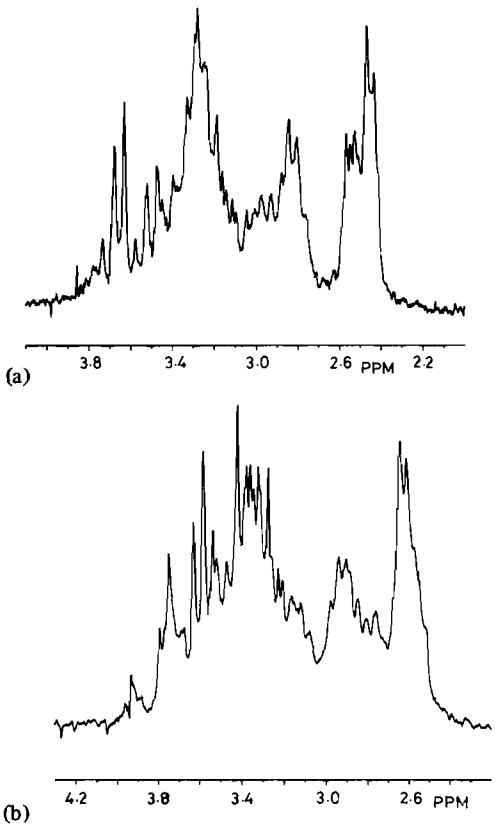

Fig. 6. ^1H NMR spectra of: (a) $[YTTHA]^{3-}$, (b) $[LuTTHA]^{3-}$ at 20 °C and pH 6.0.

for each amine–acetate ligating pair (Table 2). The observation of three AB patterns suggests three distinct sets of acetate protons (Structure 1). The ^{13}C NMR spectrum contains three carboxylate carbon resonances, all shifted downfield from the free ligand resonances, three acetate carbon resonances and three ethylenic carbon resonances. These data indicate that each carboxylate and amine ligating group of TTHA interacts directly with the La^{3+} ion.

The ^1H spectra of the Y^{3+} and Lu^{3+} adducts are not well resolved compared to the ^1H spectrum of La^{3+} (Fig. 6(a) and (b)). These spectra are complicated by fluctional processes making unambiguous assignments difficult. The ^{13}C spectra of these two complexes are also indicative of fluctional processes, since at least nine resonances are observed in the carboxylate carbon region for the Y^{3+} complex and at least ten resonances are present for the Lu^{3+} complex (Table 3). The remaining portions of these spectra contain more resonances than can be accounted for if no fluctional processes were occurring. These data suggest that as the metal ion size decreases, the steric bulk of the TTHA ligand intercedes causing incomplete metal–ligand binding. This allows either multiple 1:1 species to exist in which the ligand is involved in an unwrapping/

TABLE 3. ^{13}C NMR chemical shift data for the carboxylate carbon region of the $[YTTHA]^{3-}$ and $[LuTTHA]^{3-}$ complexes[a]

	^{13}C	
	$[YTTHA]^{3-}$	$[LuTTHA]^{3-}$
(e) or (g)	177.46	178.06
(e) or (g)	180.27	179.89
(e) or (g)	180.43	180.61
(e) or (g)	180.54	180.78
(e) or (g)	180.61	180.98
(e) or (g)	180.92	181.25
(e) or (g)	180.99	181.39
(e) or (g)	181.13	181.48
(e) or (g)	181.58	181.82
(e) or (g)		182.17

[a] ^{13}C NMR spectra are referenced relative to Me_4Si ($\delta = 0$ ppm) with assignments being based on the labeling scheme of Scheme 1.

wrapping process or the coexistence of 2:1 and 1:1 metal:ligand species in solution.

Conclusions

The luminescence data obtained for the Eu^{3+} ion indicate the formation of both 2:1 and 1:1 metal: ligand complexes. The $^7F_0 \rightarrow {}^5D_0$ spectral characteristics of the 2:1 species are very similar to those observed for EDTA suggesting that these two complexes exhibit similar coordination geometries. Hence, the Eu^{3+} ions in the 2:1 complex presumably coordinate at opposite ends of the TTHA ligand, binding two amine nitrogen atoms, three carboxylate oxygen atoms, and three or four water molecules giving each Eu^{3+} ion a total coordination number of eight or nine (Fig. 7(a)). The $^7F_0 \rightarrow {}^5D_0$ spectral characteristics of the 1:1 species are very similar to those observed for EDTA; however, the $^7F_0 \rightarrow {}^5D_1$ and $^7F_0 \rightarrow {}^5D_2$ excitation spectra are markedly different from those of EDTA. The TTHA ligand in the 1:1 complex appears to completely 'wrap' itself around the Eu^{3+} ion excluding all the water molecules from the inner-coordination sphere. This results in the coordination of all four amine nitrogen atoms and all six carboxylate oxygen atoms, providing the metal ion with a coordination number of ten (Fig. 7(b)). Coordination numbers of eight or greater are not uncommon for rare earth complexes [23, 24]. The apparent fluctional processes observed in the ^1H and ^{13}C spectra of the smaller rare earth ions are likely due to a ligand unwrapping/wrapping equilibrium process. This unwrapping process is not apparent for the larger La^{3+} ion. Therefore, as the metal ion size decreases across the lanthanide series the steric

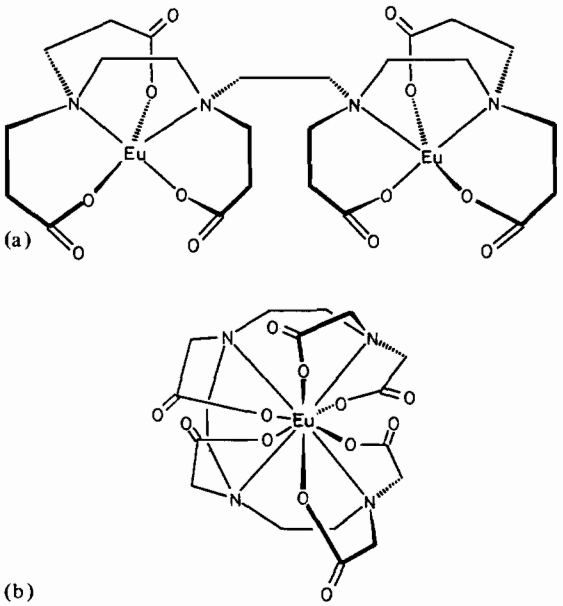

Fig. 7. Schematic representations of the possible solution structures of the Eu^{3+}–TTHA complexes: (a) 2:1 metal: ligand complex, (b) 1:1 metal:ligand complex.

constraints of this large chelating ligand, contribute to the number and types of species present in solution.

Acknowledgements

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