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Solution Chemistry of Lanthanide Complexes. 5. Circularly Polarized Luminescence Studies on Tb(III) Mixed-Ligand Complexes Containing L-aspartic Acid

HARRY G. BRITTAIN [1]

Chemistry Department, Seton Hall University, South Orange, N. J. 07079, U.S.A.

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The complexation of L-aspartic acid by a series of mixed-ligand Tb(III) complexes has been studied by means of circularly polarized luminescence (CPL) spectroscopy. In several $\mathrm{Tb(TDL)_2(ASP)}$ *and Tb(MDL)(ASP) complexes (TDL = terdentate ligand, and MDL = multidentate ligand), it was established that the L-ASP ligand bound in a bidentate manner between the two carboxyl groups. However, the resulting CPL spectra were found to be vastly different in the two series of compounds, an effect whose origin probably lies in different ASP conformations in the mixed-ligand complexes. A systematic variation of the concentration of L-ASP enabled calculation of the formation constants associated with binding of this ligand by* $\mathrm{Tb(TDL)_2^-}$ *and* $\mathrm{Tb(MDL)^-}$*. The binding of L-ASP by the Tb(MDL) series is more facile than that of the* $\mathrm{Tb(TDL)_2}$ *series, but in all cases the formation constants are greatly reduced from the analogous value known for Tb(ASP).*

Introduction

Lanthanide ions have found increasing use as metal ion probes for spectroscopically inert Ca(II), and substitution of Ln(III) for Ca(II) can enable the performance of absorption, luminescence, magnetic resonance, and Mossbauer studies on systems of biological interest [2, 3]. In peptide and protein complexes, the Ca(II) ion is normally bound by asparate and glutamate residues [4], so it is crucial to full understanding of Ln(III) binding by these materials that the solution phase coordination chemistry be understood. An approach to the problem may be made by examining the simple amino acid complexes of lanthanide ions, and after these model compounds are detailed then extensions to the more complicated bioinorganic systems may be attempted.

Ln(III) complexes of aspartic acid have been studied by means of ^{1}H [5] and ^{13}C [6] nuclear magnetic resonance methods, and luminescence/absorption studies have also been carried out [7]. During the course of these investigations, it became established that aspartic acid normally bound the metal ions in a bidentate manner after ionization of the two carboxyl groups. No evidence was obtained (below pH 9) to indicate terdentate bonding by the aspartate ligand, thus indicating that the ammonium group remains unbound within the range of conditions studied. Formation constants for the Ln(III)/aspartate complexes are known [8, 9].

As would be expected, chiroptical studies on Ln(III)/aspartate complexes have provided the maximum degree of information on the solution phase chemistry of these compounds. Katzin and Gulyas reported circular dichroism studies on Pr(III)/aspartate complexes [10], although the high ratios of ligand/metal used in these studies leave doubt as to the exact nature of the solution phase complexes. Much better results were obtained at significantly lower complex concentrations by using circularly polarized luminescence (CPL) spectroscopy as the chiroptical method [11, 12]. In these studies, it was demonstrated that the CPL spectra of Tb(III) and Eu(III) complexes were extremely sensitive to solution pH and that the spectra became most intense at high pH values.

However, in a series of CD and potentiometric titration studies, Martin and coworkers [13] showed that in the high pH regions (and for the ratios of ligand/metal used in the chiroptical studies) that complex hydrolysis was appreciable, and that the observed alterations in CD and CPL sepctra might actually be due to changes in the polynuclear state of the complexes and not due to changes in the metal–ligand bonding. Measurements of intermolecular energy transfer [14] confirmed the polymeric nature of the aspartate complexes. However, by preparing mixed-ligand complexes containing bulky achiral ligands and chiral aspartate, Brittain was able to obtain CPL spectra on monomeric complexes [15]. The spectra were found to be essentially pH independent after formation of the complexes, as one would expect for a monomeric compound, and characteristic of bidentate aspartate bonding.

In the present work, we extend the mixed-ligand studies on aspartate complexes in order to examine

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the effect of the achiral ligand on the observed chiroptical spectra. The complexes used in the present work were all found to remain monomeric in solution, and contained achiral ligands which bind Ln(III) ions with great efficiency (thus not being able to be displaced by large amounts of aspartate). In all cases, the observed spectra reach limiting intensities (the absolute magnitude of which is determined by equilibrium considerations) after full formation of the complexes, and are thus able to be taken as characteristic for that particular complex system.

Experimental

Stock solutions of Tb(III) in H_2O were prepared by dissolving Tb_4O_7 in the stoichiometric amount of 70% $HClO_4$ with heating, neutralizing to pH 4.0 with NaOH, and then diluting to the desired volume with glass distilled H_2O. Achiral ligands used during the course of the work were pyridine-2,6-dicarboxylic acid (dipicolinic acid, or DPA), oxydiacetic acid (diglycolic acid, or ODA), 5-sulfosalicylic acid (SSA), iminodiacetic acid (IDA), ethylenediaminetetraacetic acid (EDTA), (2-hydroxyethyl)ethylenediaminetriacetic acid (HEDTA), *trans*-1,2-cyclohexanediaminetetraacetic acid (CDTA), ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA), and diethylenetriaminepentaacetic acid (DTPA). These ligands and L-aspartic acid were all obtained from Aldrich and were used as received.

D,L-phenylsuccinnic acid was obtained from Aldrich, and resolved according to the method of Wren and William [5]. Only the D-isomer was isolated in our determination, and in acetone solution we determined that $[\alpha]_D = +171°$. This value for the specific rotation agrees well with the quoted value [15] of +173°. Although the absolute configuration of this material appears not to be known, it is known that it is the R-isomer of n-alkyl succinnic acids which yields positive optical rotation [16].

Two classes of Tb(III) complex stock solutions were prepared, and each contained a final metal ion concentration of 15 m*M*. With the aminopolycarboxylate ligands, a 1:1 solution of Tb(EDTA), Tb(HEDTA), Tb(CDTA), Tb(EGTA), and Tb(DTPA) was made up by mixing stoichiometric amounts of more concentrated stock solutions. The coordination environment of these hexadentate ligands was duplicated for the terdentate ligands by preparing 1:2 mole ratio solutions in these systems, thus obtaining $Tb(DPA)_2$, $Tb(ODA)_2$, $Tb(IDA)_2$, and $Tb(SSA)_2$. The final ionic strength of each solution was adjusted to 0.1 with $NaClO_4$. Varying amounts of L-aspartic acid (ASP) were weighed out initially in a series of cuvettes, and then dissolved in the suitable Tb(III) complex stock solution. Mole ratios of Tb(III)/ASP ranging from 1:1 to 1:25 (and in some instances even higher) were used to fully evaluate the equilibria associated with the complex systems.

All luminescence spectra were obtained on a medium-resolution CPL spectrometer constructed in our laboratory [17]. All samples were excited by the 365 nm output of a 200 W Hg-Xe arc lamp, except when DPA was present as the achiral ligand (then an excitation wavelength of 295 nm was used). The UV excitation was selected by a combination of a 0.1 meter grating monchromator and suitable glass filters. An emission bandpass of 10 A was found to fully resolve all the spectral features, as the work was carried out in fluid aqueous solution at room temperature.

pH adjustment of the solutions was carried out by adding microliter amounts of standard NaOH or $HClO_4$ to the fluorescence cuvette, with the pH being read on an Orion 701A pH meter. The pH could be obtained by inserting a glass microcombination electrode directly into the cuvette, and this electrode was calibrated daily with a series of standard buffer solutions.

Results and Discussion

The mixed-ligand complexes used in the present study all contain ligands known to bind lanthanide ions with great efficiency, and the ratios of ligand/metal were chosen so that at least six coordination positions on the metal ion were occupied by donor atoms of the achiral ligands. For the terdentate ligands (DPA, IDA, SSA, or ODA), a ligand/metal ratio of 2:1 was employed, and here the initial equilibria can be written as:

$$Tb^{3+} + 2TDL^{2-} \rightleftharpoons Tb(TDL)_2^- \qquad (1)$$

In eqn. (1), TDL represents any of the terdentate ligands. The formation constants associated with these complexes are all known: log $\beta_2 = 9.98$ for ODA, log $\beta_2 = 12.24$ for IDA, log $\beta_2 = 12.86$ for SSA, and log $\beta_2 = 16.01$ for DPA [18].

With the aminopolycarboxylate ligands, the achiral portion of the coordination sphere can be filled by the binding of only one multidentate ligand (represented as MDL):

$$Tb^{3+} + MDL^{4-} \rightleftharpoons Tb(MDL)^- \qquad (2)$$

The equilibria associated with eqn. (2) are also known to be very favorable: formation constants for the 1:1 metal/ligand complexes have been determined, with log $K_1 = 15.42$ for HEDTA, log $K_1 = 17.92$ for EDTA, log $K_1 = 17.80$ for EGTA, log $K_1 = 20.20$ for CDTA, and log $K_1 = 22.71$ for DTPA [18].

In a series of works involving mixed-ligand complexes of $Tb(DPA)_2$ with a series of chiral α-hydroxycarboxylic acids, we have shown that formation of ternary complexes is possible and that if the added substrate is chiral then strong optical activity can be observed in the f–f emission spectra [19–21]. In these works correlation of a systematic variation in the concentration of chiral ligand with observed CPL intensities permitted a computation of the association constant of $Tb(DPA)_2$ with the chiral hydroxycarboxylates. Earlier, we had noted that $Tb(DPA)_2$ also bound aspartic acid in a bidentate manner [17], although no equilibrium constants were evaluated at that time.

The luminescence spectrum of the Tb(III) ion shows no dependence on excitation wavelength, consists of a series of well-resolved emission bands in the visible region of the spectrum, and the wavelength of these bands is essentially unaffected by the nature of the ligands attached to the Tb(III) ion. All emissions originate in the $^5D_4$ excited state, and transitions to the $^7F_6$ (490 nm), $^7F_5$ (545 nm), $^7F_4$ (580 nm), and $^7F_3$ (620 nm) J-levels of the ground state are commonly observed. Of these, the 4–5 transition (we shall label the transitions by their J quantum numbers) is the most intense, and also exhibits the largest degree of CPL. While CPL spectra were obtained for all complexes in all bands, the strong intensity of the 4–5 transition insured that most attention would be focused on this band.

As is commonly the situation with luminescence measurements, the total luminescence (TL) and CPL signals were recorded in arbitrary units. If one defines the TL intensity as:

$$I = \frac{1}{2}(I_L + I_R) \tag{3}$$

and the CPL intensity as:

$$\Delta I = I_L = I_R \tag{4}$$

it then becomes possible to eliminate the unit dependence by taking the ratio of these quantities:

$$g_{lum} = \Delta I/I \tag{5}$$

In eqns. (3) and (4), I_L and I_R represent the emitted intensities of left- and right-circularly polarized light, respectively, and g_{lum} is known as the luminescence dissymmetry factor.

Tb(ASP) Studies

In order to fully evaluate the mixed-ligand complex results, it was determined that an understanding of the simpler 1:1 Tb/ASP system was first necessary. The formation of a 1:1 Tb/ASP complex is a favorable process, and it has been determined that log K_1 = 5.80 for this process [9]. The previous work on Tb/ASP complexes had been carried out at 5:1 ligand/metal ratios [12], but the subsequent energy transfer work demonstrated that the complexes were significantly associated in the pH regions where maximum CPL was observed [14]. As a result, dual energy transfer and CPL investigations were carried out on 1:1 Tb/ASP solutions.

The studies of intermolecular energy transfer were carried out as before [14], and were effected by titrating a 1:1 Tb/ASP solution at a given pH with microliter amounts of a 1:1 Eu/ASP solution of the same pH. The decrease in intensity of the Tb(III) 4-5 emission band was followed as a function of Eu(III) quencher concentration, and the data analyzed with the usual Stern-Volmer equation for luminescence quenching:

$$\frac{I_o - I}{I} = K_{sv}^{\phi}[Q] \tag{6}$$

In eqn. (6), I_o represents the intensity of Tb(III) emission in the absence of any Eu(III) quencher, I is the intensity of Tb(III) emission in the presence of Eu(III) having the concentration [Q], and K_{sv}^{ϕ} is the Stern-Volmer quenching constant. The magnitude of the Stern-Volmer constant can be taken as a measure of the efficiency of the energy transfer.

TABLE I. Stern-Volmer Quenching Constants for the Quenching of 1:1 Tb/ASP by 1:1 Eu/ASP as a Function of pH.[a]

pH	$K_{sv}^{\phi} \times 10^{-2}$
3.0	1.22
3.5	1.31
4.0	1.41
4.5	1.48
5.0	2.76
5.5	4.92
6.0	8.87
6.25	12.33
6.5	17.79

[a] All values are accurate to within $\pm 0.05 \times 10^2$, and have the units of L/mole. No data could be obtained above pH 6.5 due to the formation of a precipitate in the solution.

In Table I, the results of the quenching studies are presented. In the past, we have shown many times that if the Stern-Volmer constant is less than 300 (approximately), then the energy transfer from Tb(III) to Eu(III) is essentially collisional in nature [14, 22–24] and that the complexes existing under these conditions are monomeric. Much higher Stern-Volmer constants are indicative of static quenching, which implies the presence of polynuclear species. According to the data of Table I, we can state that the 1:1 lanthanide complexes of L-aspartic acid are certainly monomeric below pH 5.0, and that they become increasingly associated above this pH value. Precipitation of insoluble species is noted above pH 6.5, and one may easily see that considerable association (probably through the formation of hydroxy bridges) exists in the 6.0–6.5 pH range.

The binding of L-aspartic acid by Tb(III) results in the generation of measurable optical activity within the 4–5 emission band (centered at 545 nm) of the Tb(III) ion, as has been shown before for Tb/ASP ratios of 1:5 [12] (it should be noted, however, that the wavelength scale in Fig. 2 of reference 12 is actually reversed). In Fig. 1, we present the TL (total luminescence) and CPL spectra obtained at a representative pH for 1:1 ratios of Tb/ASP. After accounting for the reversed wavelength scale of the earlier work, it is found that the CPL spectra corresponding to the two metal/ligand ratios are quite comparable in lineshape and magnitude. It may be noted that 99.9% of the Tb^{3+} ions are complexed by the ASP ligand (even at a 1:1 metal/ligand ratio) given the reported association constant of 6.31×10^5 [9].

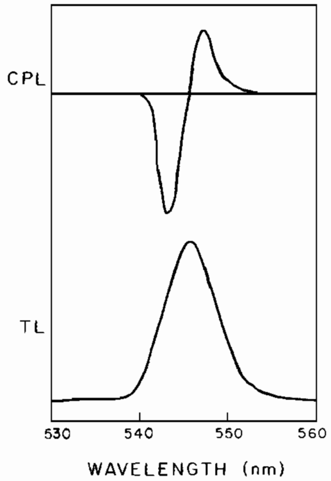

Fig. 1. TL (lower trace) and CPL (upper trace) spectra obtained within the $^5D_4 \rightarrow {}^7F_5$ band system of a 1:1 Tb/L-ASP solution at pH 5.5. The spectra are shown in arbitrary units.

Measurable CPL appears once a solution of pH of 4.5 is exceeded, and this feature of the metal/ligand interaction is consistent with the known ionization constants of the two carboxyl groups of the ASP ligand ($pK_1 = 1.93$ and $pK_2 = 3.70$ [18]). The CPL intensity (as measured by the luminescence dissymmetry factor) increases rapidly as the pH is raised from 4.5 to 5.5 and then remains essentially constant between pH 5.5 and 6.0. For the major CPL band located at 542 nm, it was determined that $g_{lum} = -3.1 \times 10^{-2}$.

However, raising the solution pH above 6.0 led to an increase in the degree of CPL within the 4–4 emission band, and to substantial modification of the CPL lineshape. At the same time, no change is seen in the TL lineshape, although some intensification is observed. In Fig. 2, the lineshape changes within the CPL spectra are illustrated, and a precipitate which formed in the solution above pH 6.5 prevented work from being carried out at higher pH values. The same lineshape changes were observed in the earlier work which employed 1:5 ratios of metal/ligand, but in those studies it was found that no precipitate formed (up to pH 11) and that the lineshape change took place at much higher pH [12].

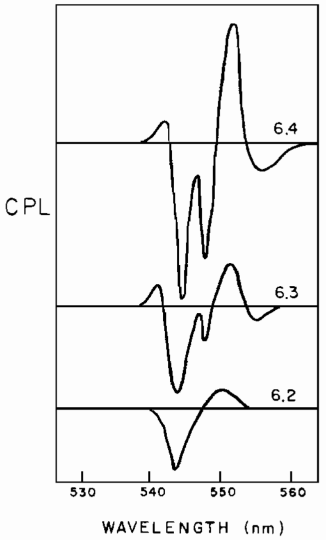

Fig. 2. Lineshape changes within the $^5D_4 \rightarrow {}^7F_5$ CPL spectra of 1:1 Tb/L-ASP observed above pH 6.0. The pH associated with each spectrum is given.

The energy transfer results can be examined together with the CPL results in order to provide an explanation for the observed CPL trends. The basic lineshape shown in Fig. 1 is associated with a monomeric, 1:1 Tb/ASP complex, and the new CPL lineshapes which develop at higher pH are indicative of polynuclear complex formation. It would appear that the use of higher ratios of ligand/metal can prevent (to some extent) the formation of associated species.

Tb(TDL)$_2$(ASP) Studies

As had been shown in the earlier work involving $Tb(DPA)_2^-$ complexes [17], the metal ion in a bis(terdentate ligand)Tb(III) complex contains solvent molecules which can be replaced by ionized L-aspartic acid:

$$Tb(TDL)_2^- + ASP^{2-} \rightleftharpoons Tb(TDL)_2(ASP)^{3-} \qquad (7)$$

The hydration and structure of the bis(DPA)Ln(III) complexes have been studied by means of NMR spectroscopy [25], and the monomeric nature of the DPA complexes has also been demonstrated with measurements of intermolecular energy transfer [26].

The CPL spectra which result within the 4–5 Tb^{3+} emission band when $Tb(TDL)_2$ complexes bind L-aspartic acid are essentially identical in every way with the spectra shown in Fig. 1 for the Tb(ASP) complex. This result indicates that in the $Tb(TDL)_2^-$ complexes, the nature of the achiral ligand does not play a major role in determining the nature of the optical activity experienced by the Tb(III) ion. In these systems, the achiral ligands are performing their desired functions of preventing polynuclear association of the Tb(III) complexes and restricting the Tb/ASP ratio in the complexes to 1:1 (in spite of the concentration of added ASP ligand). It was also found that no precipitates formed in the solutions up to at least pH 10, and no changes in CPL lineshape were noted at any pH.

However, while the CPL lineshape remained essentially invariant up to pH 9.0, the CPL intensity (as measured by the dissymmetry factors) was found to be a function of both the L-aspartic acid concentration and of the nature of the achiral ligand used in the mixed-ligand complex. Both dependencies are illustrated in Fig. 3. At sufficiently high concentrations of L-ASP, all of the curves eventually leveled off at a limiting value for the dissymmetry factor (these limiting values are located in Table II). Except for the SSA ligand, all the limiting dissymmetry factors are quite close in magnitude to that obtained for the 1:1 Tb(ASP) complex. The SSA ligand does permit some polynuclear complex association, and its lineshape can vary somewhat from the basic form illustrated in Fig. 1 [27].

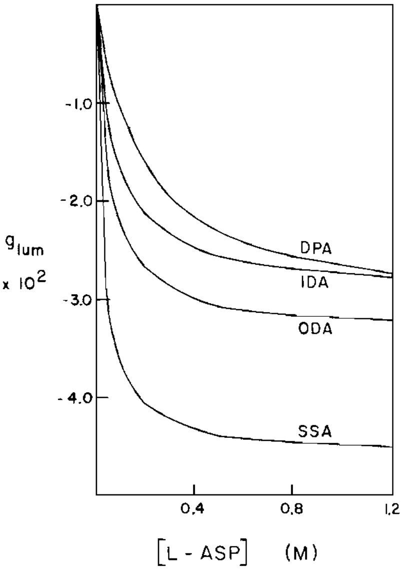

Fig. 3. Dependence of the luminescence dissymmetry factor (obtained at 542 nm) of the $Tb(TDL)_2(ASP)$ complexes upon concentration of added L-aspartic acid. The identity of the TDL ligand is given with its corresponding curve.

It is quite clear that the dissymmetry factor obtained at intermediate L-ASP concentrations is proportional to the amount of $Tb(TDL)_2(ASP)$ complex formed. It is not difficult to show that the mole fraction of $Tb(TDL)_2$ remaining in the equilibrium mixture is given by:

$$X_i = \frac{g_{fin} - g_i}{g_{fin}} \qquad (8)$$

where g_{fin} = limiting dissymmetry factor, g_i = dissymmetry factor at a given L-ASP concentration, and X_i = the mole fraction of uncomplexed $Tb(TDL)_2$ existing at that L-ASP concentration. Knowledge of the initial concentrations of $Tb(TDL)_2$ (symbolized as Tb_a) and L-ASP (symbolized as ASP_a) permits a calculation of the concentration of all species present at equilibrium:

$$[Tb(TDL)_2] = (Tb_a)(X_i) \qquad (9)$$

$$[Tb(TDL)_2(ASP)] = Tb_a - [Tb(TDL)_2] \qquad (10)$$

$$[ASP] = ASP_a - [Tb(TDL)_2(ASP)] \qquad (11)$$

One then computes the formation constant corresponding to eqn. (7) from:

$$K_1 = \frac{[Tb(TDL)_2(ASP)]}{[Tb(TDL)_2]\,[ASP]} \qquad (12)$$

Formation constants calculated in this manner have been collected in Table II with the limiting dissymmetry factors.

TABLE II. Limiting Dissymmetry Factors and Association Constant for the Mixed-Ligand $Tb(TDL)_2$(L-ASP) Complexes.[a]

Ligand	$g_{lum} \times 10^2$	K_1
Pyridine-2,6-dicarboxylic acid	−3.15	5.56
Oxydiacetic acid	−3.36	21.37
Iminodiacetic acid	−2.93	14.50
5-Sulfosalicylic acid	−4.61	41.89

[a] All dissymmetry factors were obtained at the negative CPL maximum located at 542 nm. The association constants are accurate to approximately ± 0.06.

The formation constants in Table II are seen to be greatly reduced from the analagous constant known for K_1 of Tb(ASP) [9]. This decrease in binding ability for L-ASP may arise from three possible sources: (a) decreased positive charge on the Tb(III) ion which results from binding the negative TDL ligands, (b) a decreased number of coordination sites on the metal ion available for binding of the L-ASP ligand, and (c) crowding of the L-ASP site by the TDL ligands which partially blocks the entrance of the L-ASP ligand into the inner coordination sphere of the Tb(III) ion.

Tb(MDL)(ASP) Studies

That the lanthanide complexes of aminopolycarboxylate ligands contain solvent molecules which may be displaced by an entering ligand has been demonstrated many times; for example, lanthanide complexes of EDTA have been used as aqueous shift reagents in NMR spectroscopy [28]. In the course of the present work, it has been determined that L-aspartic acid is capable of binding to the Tb(III) ion, thus forming the mixed-ligand complex:

$$\mathrm{Tb(MDL)^-} + \mathrm{ASP^{2-}} \rightleftharpoons \mathrm{Tb(MDL)(ASP)^{2-}} \qquad (9)$$

In preliminary work, it has been shown that the lanthanide aminopolycarboxylate complexes are monomeric up to pH 9 [29].

It was anticipated that the CPL spectra of the Tb(MDL)(ASP) complexes would closely parallel the spectra obtained with the $\mathrm{Tb(TDL)_2(ASP)}$ complexes, but this was not found to be the case. As may be seen in Fig. 4, the spectra associated with the Tb(MDL)(ASP) complexes are absolutely non-equivalent with those of the previous series. The component exhibiting maximum CPL is no longer the same, and the sign is also opposite. These results strongly suggest that the configurational/conformational chirality experienced by the Tb(III) ion is substantially different in the two series of compounds.

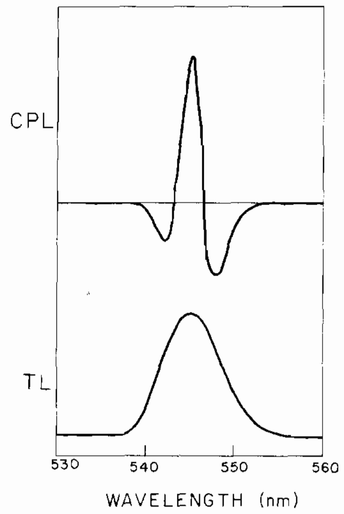

Fig. 4. TL (lower trace) and CPL (upper trace) spectra obtained within the $^5D_4 \rightarrow {}^7F_5$ band system of Tb(EDTA)-(ASP) at pH 6.0.

Identical lineshapes were obtained when L-ASP was bound by Tb(EDTA), Tb(HEDTA), and Tb(CDTA), although the absolute magnitudes of the CPL were found to differ with the nature of the polyaminocarboxylate ligand. The dissymmetry factors followed trends similar to those illustrated in Fig. 3; the g_{lum} factors rose rapidly with increasing L-ASP concentration, but eventually leveled off at sufficiently high concentrations of L-ASP. However, as may be seen in Fig. 5, the concentration of L-ASP required to reach the limiting dissymmetry factor value is much lower for the Tb(MDL) complexes than for the $\mathrm{Tb(TDL)_2}$ complexes.

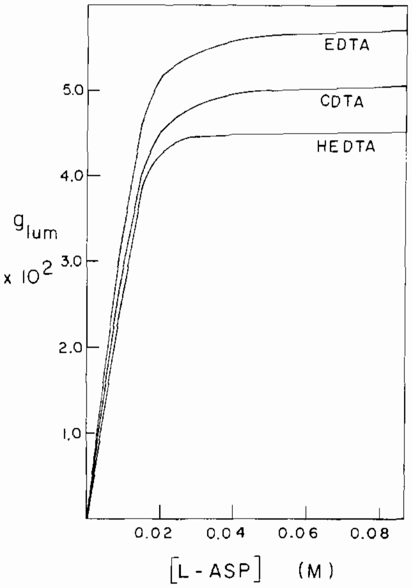

Fig. 5. Dependence of the luminescence dissymmetry factor (obtained at 544 nm) of the Tb(MDL)(ASP) complexes upon concentration of added L-aspartic acid. The identity of the MDL ligand is given with its corresponding curve.

TABLE III. Limiting Dissymmetry Factors and Association Constant for the Aminopolycarboxylate Tb(III) Complexes with L-Aspartic Acid.[a]

Ligand	$g_{\mathrm{lum}} \times 10^2$	K_1
Ethylenediaminetetraacetic acid	+5.78	1340
(2-Hydroxyethyl)ethylene-diaminetriacetic acid	+4.52	3422
trans-1,2-Cyclohexane-diaminetetraacetic acid	+5.13	1087

[a] All dissymmetry factors were obtained at the positive CPL maximum located at 544 nm. The association constants are accurate to approximately ±10.

These experimental trends suggest that the bonding between the Tb(MDL) complex and L-ASP is stronger than the affinity of $\mathrm{Tb(TDL)_2}$ for L-ASP. Such predictions are bourne out by the computed association constants collected for the Tb(MDL)-(ASP) complexes in Table III (limiting dissymmetry factors are also provided in this table). While the values of K_1 are still much smaller than K_1 of the Tb(ASP) complex, they average almost 100 times as great as those obtained for the $\mathrm{Tb(TDL)_2(ASP)}$ complexes. We attribute this difference as being due to a greater accessibility of the Tb(III) ion in the MDL complexes: in this situation, the achiral ligand is forced to occupy six coordination positions and the L-ASP site can be well-defined. However, in the $\mathrm{Tb(TDL)_2}$ complexes, the two terdentate ligands exhibit a great deal of fluxional behavior, and are constantly scrambling positions in the metal ion coordination sphere [25]. As a result, the L-ASP site may be blocked for significant portions of time, thus resulting in decreased affinities for the chiral ligand.

We also find that the Tb(HEDTA) complex has a much greater affinity for L-ASP than does either Tb(EDTA) or Tb(CDTA). A series of studies has shown that HEDTA binds in a pentadentate (or hexadentate, but including a bridging water molecule) manner to heavy lanthanide ions [30, 31]. Thus, a Tb(HEDTA) complex would present an even more favorable situation for binding of a L-ASP ligand, and this possibility would be manifest in the larger stability constant.

Further studies of this L-ASP binding site were carried out by attempted formation of mixed-ligand complexes with Tb(EGTA) and Tb(DTPA). Both ligands are more sterically demanding than any of the aminopolycarboxylates which have been studied thus far; DTPA is known to bind lanthanide ions in a heptadentate manner [32], and EGTA contains two ether linkages in the ethylenediamine backbone which greatly extends its coordinative 'bite' around a metal ion. In no case could any CPL be obtained within the Tb(III) emission, even after the addition of 40-fold excesses of L-ASP. These results demonstrate that crowding of the L-ASP site can prevent formation of a mixed-ligand complex, and that the site in the other Tb(MDL) complexes may actually contain a degree of steric constraint. Certainly, one should not attempt to use the lanthanide complexes of EGTA or DTPA as aqueous shift reagents.

Three possibilities exist for the difference in CPL lineshape observed in the Tb(TDL)$_2$(ASP) and Tb(MDL)(ASP) series: (a) the bonding mode of the L-ASP ligand is different in the two systems, (b) the overall configuration of the metal complex differs in the two systems, and (c) the conformation of the L-ASP ligand differs in the two series. Case (a) would require that L-ASP bind in a bidentate manner through the two carboxylates in one system, and bind between one carboxylate and the deprotonated ammonium group in the other. Examination of our earlier work involving monodentate binding of chiral ligands by Tb(DPA)$_2$ complexes [20] shows that we cannot invoke monodentate binding by L-ASP as a cause for the difference in CPL spectra. Given the evidence which is already in the literature [5–7], binding of the ammonium group represents a highly unlikely possibility. However, the mixed-ligand complexes of Tb(ODA)$_2$ and Tb(EDTA) with resolved phenylsuccinnic acid were prepared, and the CPL spectra obtained for each. This chiral ligand binds very weakly to the Tb(III) ion in each mixed complex, but examination of the spectra in Fig. 6 reveals that the same basic CPL lineshapes are generated in each ligand system. We therefore conclude that L-ASP binds to the Tb(III) ion in a bidentate manner in both the Tb(TDL)$_2$ and Tb(MDL) complex systems via the two ionized carboxyl groups.

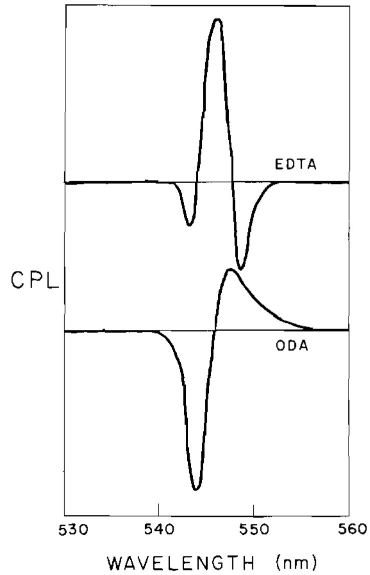

Fig. 6. CPL spectra obtained for the S-phenylsuccinnic acid complexes of Tb(EDTA)$^-$ (upper trace) and Tb(ODA)$_2^-$ (lower trace). Both spectra were obtained at pH 5.75, and at a phenylsuccinnate/Tb(III) ratio of approximately 30.

With case (a) eliminated, we now focus on the distinction between cases (b) and (c) as plausible explanations for the differing CPL spectra. It is difficult to totally distinguish between the two situations, but we believe that case (c) better explains the trends as they have been presented. A fluxional complex (such as Tb(TDL)$_2$) would readily re-adjust the placement of its component ligands to allow binding of the L-ASP in the conformation most preferred in solution. The close identity of CPL spectra obtained with the Tb(ASP) and Tb(TDL)$_2$(ASP) complexes supports this proposal. We believe the trends just discussed for the Tb(MDL)(ASP) complexes indicates some steric constraint on the L-ASP binding site, and no fluxional behavior exists which can adjust the nature of the L-ASP 'pocket' to suit the incoming chiral ligand (indeed, the metal–nitrogen bonds have been shown to be long-lived on the NMR time scale for the EDTA complexes [31]). We therefore believe that the L-ASP ligand adopts a slightly different conformation to bind to the Tb(MDL) complexes, and this different conformation induces a new chirality at the Tb(III) ion. Case (b) would require that some mechanism exist whereby the incoming L-ASP binds at the metal ion, and then partially resolves the resulting complex. At the present time, a rationale for such a mechanism is not known.

Conclusions

Evidence has been presented which demonstrates that the chirality experienced by a lanthanide ion (as measured by CPL spectroscopy) in a mixed-ligand complex can be profoundly affected by the nature of the achiral portion of the metal ion coordination sphere. Completely different spectra are possible, even though the sole chiral ligand present in the complexes contains the same sense of dissymmetry. The binding of the chiral ligand appears to be a function of the steric nature of the site, and of the ability of the incoming ligand to find solvent-occupied positions on the metal ion suitable for binding. While we have shown that L-ASP invariably binds lanthanide ions between the two carboxyl groups, the conformation of this ligand can be affected by the rest of the mixed-ligand complex. We are currently pursuing these studies further to learn more about ligand stereochemistry in mixed-ligand complexes.

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