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Localizing perturbations of the racemic equilibria involving dipicolinate derived lanthanide(III) complexes

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Abstract

Helical D₃ tris(4-amino-2,6-pyridine-dicarboxylate)terbium(III) and europium(III) complexes, which form a racemic equilibrium in aqueous solution, were prepared to study their secondary coordination sphere interactions with chiral amino acids. These interactions were probed using a combination of circularly polarized luminescence (CPL) and ¹³C NMR spectroscopy. Results indicate that regardless of the interaction between the chiral molecule and the complex, without an accessible hydrogen-bond donor on the associating molecule, perturbation of the racemic equilibrium cannot occur. A generalized conclusion is established which indicates the mechanism of chiral recognition by tris(dipicolinate)lanthanide(III) complexes is similar across a variety of analogous ligands.

Graphical abstract

Keywords

circularly polarized luminescence; Pfeiffer effect; supramolecular chemistry; D₃ lanthanide complexes; secondary coordination sphere

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Introduction

The secondary structure of multimolecular complexes is defined by a variety of intermolecular forces: charge-charge/dipole-dipole interactions, hydrogen-bonding, π - π interactions, etc. Beyond the incredibly important protein multiplexes of biology, multimolecular complexes of small molecules have significant applications in, among others, sensing,¹ catalysis,^{2,3} and supramolecular chemistry.⁴ Driven by their long excited state lifetimes, characteristic emission, and diverse chemistry, luminescent lanthanide, Ln(III), complexes have a long history of use as molecular probes.^{5,6} In particular, helical tris(dipicolinate) (dipicolinate = DPA) complexes of lanthanide cations with D_3 symmetry ($[\text{Ln}(\text{DPA})_3]^{3-}$) have been used to sense chirality of small molecules, where the racemic equilibrium between the Λ and Δ configurations of the complex is perturbed by the association of small chiral molecules in solution (figure 1).⁷⁻¹⁵ This perturbation of a racemic equilibrium has been termed the “Pfeiffer effect”, after its discoverer.^{6,16,17}

Chirality and chiral recognition is a critical concern in biology and chemistry, especially in pharmaceuticals and protein/ligand interactions in general. Different enantiomers of the same molecule can have drastically differential pharmacological properties, where beneficial activity has been observed for one enantiomer while its mirror image could have significant, deleterious effects.¹⁸ This has driven the need for a variety of simple screens of molecular structure and absolute configuration. Some of these techniques include HPLC,¹⁹⁻²² X-ray diffraction,²³⁻²⁶ NMR,²⁷⁻³⁰ and circular dichroism (CD),³⁰⁻³³ however each method has a variety of strengths and weaknesses with respect to sample requirements and the information obtained. Thus, multiple orthogonal methods are commonly required in order to verify results.

Circularly polarized luminescence (CPL) is the emission analog of CD and has a number of beneficial characteristics as a probe of chirality.^{5,13,30,34-36} First, the information obtained is highly specific and pertains only to the emissive, CPL active species. Second, when CPL active compounds have long excited state lifetimes, the data is free from significant background signal and time-resolved studies of the luminescence can reveal important information. And, finally, the circular polarization of photons emitted from a given species does not depend on that species' concentration. This means that, in principle, vanishingly small amounts of a chiral molecule could be required to obtain reliable CPL data.

As stated above, helical tris(DPA) complexes of lanthanide cations ($[\text{LnL}_3]^{n-}$, L = dipicolinate derivative) and their derivatives have been proposed as molecular probes of chirality.^{5,37,38} In solution, these coordination compounds form a racemic equilibrium between enantiomeric Λ and Δ ligand configurations (figure 1). Upon UV excitation of the coordinated ligands, each enantiomer emits circularly polarized light with equal intensity but opposite degrees of polarization.³⁹ With a racemic mixture, this polarization cancels and the detected luminescence is not circularly polarized; however, if the equilibrium is perturbed and there is an enantiomeric excess of the Λ and Δ form, the resulting luminescence is circularly polarized.

Simple chiral molecules have been shown to perturb the racemic equilibrium of these lanthanide(III) complexes, where outer-sphere diastereotopic multimolecular complexes are formed between each of the enantiomeric forms of the lanthanide “host” and the small chiral molecule “guest”.^{9,11} The differential interactions between each enantiomer of the complex and the chiral guest perturbs the ground state equilibrium between the Λ and Δ forms and induces a CPL signal from the resulting solution. This perturbation of a racemic equilibrium has been termed the “Pfeiffer effect”. It is also possible to generate a CPL signal from a racemic mixture by selectively quenching the excited state of one of the two enantiomers with a chiral quenching agent.^{5,7,39–42} Because an induced CPL signal is purely dependent on the interaction between the guest chiral molecule and the lanthanide probe, and the general specificity of lanthanide luminescence and CPL, this method represents an opportunity for chiral sensing within complex mixtures that would otherwise be unresolvable. However, the precise mechanism and requirements for outer-sphere chiral recognition by this class of lanthanide coordination compounds remains largely unexplored.^{40,43}

Previous work in our lab investigated the association of chiral amino acids to lanthanide complexes with triply anionic 4-hydroxy-2,6-pyridine dicarboxylate (CDA) ligands.⁴⁰ These studies indicated that hydrogen bond donor sites on guest chiral molecules were critical for recognition by $[\text{Ln}(\text{CDA})_3]^{6-}$ at the high pH values (i.e. > 12), which were required to stabilize this complex. These past studies have contributed to the fundamental understanding of the Pfeiffer effect. To further study the association of small molecules to $[\text{Ln}(\text{L})_3]^{n-}$ complexes and investigate the site of chiral recognition on this class of lanthanide probes, we have added a hydrogen bond donor to these probes by synthesizing 4-amino-2,6-pyridine dicarboxylate (ADPA), investigated its metallation by lanthanide cations, and studied the association of small chiral molecules to $[\text{Ln}(\text{ADPA})_3]^{3-}$. Somewhat surprisingly, the probe activity of $[\text{Ln}(\text{ADPA})_3]^{3-}$ is strikingly similar to that of $[\text{Ln}(\text{CDA})_3]^{6-}$. These results suggest that chiral recognition is similar across this range of $[\text{L}_3\text{Ln}]^{n-}$ compounds, regardless of the 4-substitution of the pyridine ring. Furthermore, data is presented that suggests multiple modes of small molecule binding to $[\text{LnL}_3]^{n-}$ complexes occurs and that these modes have differential effects on the racemic equilibrium and the resulting chiroptical properties.

Materials and Methods

Chemicals used were purchased from commercial suppliers and used without further purification. The Ln(III) content of stock solutions was determined by complexometric titrations with a standardized solution of EDTA in the presence of 0.1M ammonium acetate and aqueous arsenazo(III).⁴⁴ The synthesis of ADPA was performed with a minor deviation from a literature procedure.⁴⁵ Melting point measurements were performed on a Thermo Scientific Electrothermal MEL-TEMP 3.0. NMR studies were performed on a Varian Inova 400 MHz NMR spectrometer and chemical shifts were measured relative to residual solvent signals and are given with respect to TMS.

4-amino-2,6-pyridinedicarboxylic acid (H₂ADPA)

A literature procedure was used to produce the crude ADPA product. Analytically pure ADPA was produced by the following method: First, the crude was refluxed in acidified anhydrous methanol to convert the crude compound to dimethyl ADPA. Then, triethyl amine was added dropwise until solid dimethyl ADPA precipitated. The product was then filtered and washed with cold methanol. The dimethyl ADPA was purified by trituration in a 15:1 acetone:methanol mixture in order to yield analytically pure dimethyl ADPA after filtration and washing with the solvent mixture. The product was de-esterified by refluxing in 1 M NaOH, and precipitated with con. HCl and filtered. Spectroscopic data and melting points matched literature values.⁴⁵

The CPL instrument used for this work has been described previously.⁴⁰ In brief, it is equipped with a 1000 W xenon arc lamp from a Spex FluoroLog-2 spectrofluorometer, with excitation and emission monochromators of dispersions 4nm/mm (SPEX, 1681B). The maximum excitation wavelength was determined by running an excitation scan $\lambda_{em} = 545$ nm corresponding to the $^5D_4 \rightarrow ^7F_5$ transition. Then, the maximum emission wavelength was determined by running an emission scan at the maximum λ_{ex} . CPL is generally reported as a luminescence dissymmetry ratio, $g_{lum}(\lambda)$, as in equation 1, where I_L and I_R refer to the intensity of the left handed light and the right handed light, respectively. The standard deviation, δ , for the g_{lum} values is given simply by equation (2), where N is the number of photon-pulses counted. All g_{lum} values were recorded at the max λ_{ex} and λ_{em} values. CPL spectra were measured at the maximum λ_{ex} value, with λ_{em} ranging from 530.00 nm to 565.00 nm.

$$g_{lum} = \frac{\Delta I}{\frac{1}{2}I} = \frac{I_L - I_R}{\frac{1}{2}(I_L + I_R)} \quad (1)$$

$$\sigma = \sqrt{\frac{2}{N}} \quad (2)$$

$^5D_0 \leftarrow ^7F_0$ excitation measurements for $[Eu(ADPA)_x]^{n-}$ ($x = 1, 2, 3$) were measured using a Coherent-599 tunable dye laser (0.03 nm resolution) with a Coherent Innova Sabre TMS 15 as a pump source. The laser dye used in the measurements was rhodamine 6G dissolved in ethylene glycol. The calibration of the emission monochromator was performed by passing scattered light from a low-power He-Ne through the detection laser. Error in the dye-laser wavelength was assumed to correspond to the resolution of the emission monochromator (0.1 nm). The optical detection system consisted of a focusing lens, long pass filter, and 0.22 m monochromator. The emitted light was detected by a cooled EMI-9558B photomultiplier tube operating in photon-counting mode. All measurements were performed in a quartz cuvette with a path length of 1.0 cm.

All NMR perturbation studies, CPL, and laser spectroscopy measurements were done in aqueous solutions (NMR measurements being in D₂O). For the NMR studies, the concentration of $[Tb(ADPA)_3]^{3-}$ and $[AA]$ were 5 mM and 200 mM, respectively. For the CPL studies, the concentration of $[Tb(ADPA)_3]^{3-}$ and $[AA]$ were 1 mM and 40 mM,

respectively. For the direct excitation speciation studies, the concentration of $[\text{Eu}(\text{ADPA})_3]^{3-}$ was 1 mM with varying pH of approximately 1.0, 4.0, 7.0, 9.3, and 11.8. All $[\text{Ln}(\text{ADPA})_x]^{n-}$ samples were prepared by mixing Ln(III) with ADPA in a 1 to 5.5 ratio. The solid ADPA was dissolved by adding aqueous NaOH prior to the addition of aqueous Ln(III). Once the Ln(III) was added the amino acids were added into solution if needed, and the pH was carefully adjusted to the desired value using a minimal amount of concentrated NaOH or HCl (as needed). The system was allowed approximately 24 hours to equilibrate. If the pH changed at the end of this time period, the pH was adjusted once again. This process was repeated until the pH was stable.

Results and Discussion

Preparation of $[\text{Ln}(\text{ADPA})_3]^{3-}$ Complexes

Dipicolinate and its derivatives form stable mono, bis, and tris complexes of lanthanide metal cations.^{14,45} The tris complexes generally exhibit a helical, 9-coordinate, tri-capped trigonal prismatic geometry of D_3 symmetry. Two possible enantiomers, the Λ and Δ helices, of these complexes can form, and in solution they are in equilibrium (fig. 1). These compounds also emit circularly polarized light, where the polarization is defined by the helicity of the complex.^{5,34,35} When the mixture of enantiomers is racemic, the emitted light is not circularly polarized; however, perturbing the equilibrium between the Λ and Δ forms generates an excess of one enantiomer and the luminescence from this mixture is circularly polarized.^{31,44,46}

In order to determine the conditions necessary to form the CPL active tris complex $[\text{Ln}(\text{ADPA})_3]^{3-}$, we studied the coordination of ADPA^{2-} to Eu(III). The optimum Ln(III): ADPA^{2-} stoichiometry and pH was found by preparing solutions of 1:5.5 Eu(III): ADPA^{2-} at various pH (1.0, 4.0, 7.0, 9.3, and 11.8, note that ADPA can exist in three different protonation states depending on the pH and coordination by Ln(III): H_3ADPA^+ , H_2ADPA , H_1ADPA^- , and ADPA^{2-}).⁴⁵ The Eu(III) speciation in the solutions was determined by measuring direct excitation spectra of the $^5\text{D}_0 \leftarrow ^7\text{F}_0$ transition of Eu(III), which is sensitive to the coordination environment (fig. 2). As expected, the speciation gradually shifted across three or four distinct Eu(III)-containing species as the pH was increased. This would correspond to stepwise formation of the mono (peak max. = 579.8 nm), bis (peak max. = 580.3 nm when pH > 4.0), and tris (peak max. = 580.8 nm) complexes of Eu(III). Measurements at a different Eu(III): ADPA^{2-} ratio confirmed that the observed transitions were the result of varying ADPA^{2-} coordination and not deprotonation of coordinated water molecules; although, the excitation peak for the intermediate bis complex shifted between pH 4.0 and 7.0, which likely corresponds to deprotonation of coordinated H_1ADPA^- ligands, suggesting that ADPA is a pH-dependent probe.⁴⁷ A single, approximately Gaussian peak was observed at pH 11.8 and a 1:5.5 Eu: ADPA^{2-} ratio, which indicated that all of the Eu(III) speciates as the desired compound $[\text{Eu}(\text{ADPA})_3]^{3-}$ under these conditions. In order to ensure complete formation of the tris complex, all further experiments, with either Tb(III) or Eu(III) were performed with 5.5 equivalents of ADPA^{2-} , relative to Ln(III), and at pH 12.0–12.3.

CPL Studies of Association

Studies into the outer-sphere association of chiral molecules to $[\text{Ln}(\text{ADPA})_3]^{3-}$ were performed with $[\text{Tb}(\text{ADPA})_3]^{3-}$ because the CPL of the $^5\text{D}_4 \rightarrow ^7\text{F}_5$ transition of Tb(III) tends to be especially sensitive to the helicity of the complex,⁸ Tb(III) is known to cause a powerful NMR shift,⁴⁸ and the related dipicolinate derivatives have been well characterized.^{8–11,40} Lanthanide cations tend to have very similar coordination chemistry,⁵ so was assumed that 5.5 equivalents of ADPA^{2-} and a pH of at least 12.0 was sufficient to form the tris Tb(III) complex, as it was for Eu(III) (vide supra). Furthermore, the *tris* Tb(III) complexes of ADPA, CDA and DPA have been previously reported, including the stability constants for the formation of the *tris* Tb(III) complexes from additional ligand and the *bis* $[\text{TbL}_2]^{n-}$ species.⁴⁵

Introduction of an amino acid (AA) into a solution containing a racemic equilibrium of $[\text{LnL}_3]^{n-}$ (equation (3)) has been shown to create an enantiomeric excess of either the Λ and Δ complex. When L is a dipicolinate derivative, this perturbation has been proposed to be a ground state phenomenon^{9,11} driven by the formation of diastereotopic outer-sphere complexes, indicated by colons (:) in equations (4) and (5). The magnitude of this perturbation can be directly measured by CPL, and in some cases quantified, as in equation (6), where f is the magnitude of the g_{lum} for the pure Λ and Δ complex, and η is the enantiomeric excess of one of the two forms. For the sake of simplicity, only 1:1 complexes are considered, although we see no reason why multiple AA molecules cannot associate to each Ln(III) complex.

It is important to carefully consider the possibility of inner-sphere association of the perturbing AA molecules. In other words, whether direct displacement of an ADPA ligand can occur in the presence of 40 equivalents of AA. In previous studies of the $[\text{Eu}(\text{CDA})_3]^{6-}$ or $[\text{Eu}(\text{DPA})_3]^{3-}$ complexes,^{40,43} direct excitation and emission spectra conclusively demonstrated that the complexes are unaffected by the addition of large excesses of AA. The stability constants for formation of the $[\text{Tb}(\text{CDA})_3]^{n-}$ and $[\text{Tb}(\text{ADPA})_3]^{n-}$ at pH 8 are very similar (5.59 and 5.39, respectively). Given that inner-sphere coordination was not detected for the CDA complexes of Eu, the similar stability of the *tris* CDA and ADPA complexes of Tb, and the generally similar coordination chemistry of Eu and Tb, it is unlikely that the ADPA ligands are displaced by AA molecules during these analyses. Furthermore, previous work on the CPL activity of aqueous mixtures of Eu, DPA, and malic acid in 1:2:1.86 ratios indicated that $[\text{Eu}(\text{DPA})_2(\text{malate})]$ and related complexes are essentially CPL inactive compared to the *tris* complex $[\text{Eu}(\text{DPA})_3]^{3-}$.⁴⁹

$$|g_{lum}| = f * (|\eta|) = f * \left(\frac{|\frac{[\Delta]}{[\Delta]} - \frac{[\Lambda]}{[\Lambda]}|}{\frac{[\Delta]}{[\Delta]} + \frac{[\Lambda]}{[\Lambda]}} \right) = f * \left(\frac{|K_{\Delta} - K_{\Lambda}|}{K_{\Delta} + K_{\Lambda}} \right) \quad (6)$$

The association of proline to $[\text{LnL}_3]^{n-}$ (L = CDA or DPA) complexes has been well characterized,^{8,40} so we studied the effect of proline on the racemic equilibrium of $[\text{Ln}(\text{ADPA})_3]^{3-}$ in some detail prior to studying the association of other small chiral molecules. The addition of 40 equivalents of L-proline to a 1 mM solution of $[\text{Ln}(\text{ADPA})_3]^{3-}$ resulted in a significant and measurable CPL signal for the $^5\text{D}_4 \rightarrow ^7\text{F}_5$ transition (fig. 3), with a g_{lum} value of 11.5×10^{-3} ($\lambda_{ex} = 282.00$ nm, $\lambda_{em} = 542.00$ nm, table 1). To isolate the proline functionality responsible for chiral recognition, the association of various proline derivatives was examined. When 40 equivalents of L-proline methyl ester were added to a 1 mM solution of $[\text{Ln}(\text{ADPA})_3]^{3-}$, the induced g_{lum} value was reduced by approximately 40% to 7.1×10^{-3} ($\lambda_{ex} = 279.00$ nm, $\lambda_{em} = 542.00$ nm, table 1), and using 40 equivalents of L-proline amide decreased the g_{lum} value by approximately 70% to 3.7×10^{-3} ($\lambda_{ex} = 283.00$ nm, $\lambda_{em} = 542.40$ nm, table 1). It is important to note that some amount of the methyl ester was likely hydrolyzed under the basic conditions used for the analysis, and thus the data for the ester should only be viewed qualitatively. Identical studies with *N*-acyl-L-proline did not show a significant CPL signal, which indicates that amine functionality is key for chiral recognition by $[\text{Ln}(\text{ADPA})_3]^{3-}$ (fig. 3). This observation is identical to the reported activity of $[\text{Ln}(\text{CDA})_3]^{3-}$, where acetylating L-proline similarly suppressed chiroptical activity.⁴⁰ These experiments show that the N-H group of proline is necessary to disturb the racemic equilibrium of $[\text{Ln}(\text{ADPA})_3]^{3-}$, and that the proline carboxylate group plays a tunable, supporting role in the association mechanism.

The strong CPL signal induced by L-proline amide was utilized to illustrate the handedness of this association, where D- and L-proline amide induced essentially equal but opposite CPL signals (fig. 4). The chiral recognition of L-proline by the triply anionic $[\text{Ln}(\text{ADPA})_3]^{3-}$ is extremely similar to its recognition by hexa-anionic $[\text{Ln}(\text{CDA})_3]^{6-}$, which indicates that the association of proline is not significantly influenced by a reduction in overall charge or the substitution of an oxyanion for a neutral amino group on the ligand backbone. This suggests that chiral recognition is localized on the lanthanide complex, perhaps to the carboxylate groups of ADPA^{2-} , although the π^* system of DPA has been suggested as the site of binding (vide infra).⁸

A variety of amino acids were screened for their association to and recognition by $[\text{Tb}(\text{ADPA})_3]^{-3}$ (scheme 1), however none of these molecules had as discriminating an association as proline. There were essentially no measurable g_{lum} values when 40 equivalence of D-serine, L-arginine and L-cysteine were added to a 1 mM solution of the $[\text{Tb}(\text{ADPA})_3]^{-3}$ complex (table 1). This suggested that the additional H-bonding functionalities on these amino acids did not facilitate chiral discrimination by the lanthanide probe and perhaps have a deleterious effect, although the cyclic structure of proline could be critical to its recognition. Addition of a somewhat bulky, electron withdrawing group to the guanidinium moiety of L-arginine was also examined. Interestingly, this nitro-L-arginine compound did show enhanced recognition by the lanthanide probe. Although, at this time, it

is not clear why this is the case (vide infra). The CPL spectra of L-proline-NAc, L-proline-benzyl-ester, L-proline methyl ester, and nitro-L-arginine are displayed in fig. S1–S4, respectively. It is worth noting that the shape of the CPL spectra did not change across each of the amino acids studied. This confirms that the structure of the CPL active species does not change between different amino acids, and that the CPL of the *tris* complex is being specifically observed.

¹³C-NMR Studies of Association

The ¹³C-NMR shift activity of the [Tb(ADPA)₃]^{3−} complex was utilized to localize the regions of the amino acids responsible for association with the lanthanide complex. The ¹³C chemical shifts of 0.2 M amino acid solution (40 equivalents) were measured in the presence and absence of 5 mM [Tb(ADPA)₃]^{3−} at pH 12.0–12.3. The extent of interaction was determined by subtracting the chemical shift in the presence of lanthanide (δ_{assoc}) from the chemical shift in the absence of lanthanide (δ_{free}) in order to determine the difference (δ) as in equation 7 with explicit values displayed in table S1. A large δ value is expected for a strong interaction due to the shielding and deshielding effects between the complex and amino acid. The results of these studies for a selection of amino acids are shown in fig. 5. For comparison, previously reported values using the related [Tb(CDA)₃]^{6−} complex (fig. 1) are shown in red.⁴⁰

$$\Delta\delta = \delta_{free} - \delta_{assoc} \quad (7)$$

The ¹³C NMR data for proline is in agreement with the g_{lum} data discussed above. A significant δ value (0.900 ppm) is found for the carbonyl carbon, verifying that it plays a significant role in the interaction. Substitution for the methyl ester, L-Pro-COOMe, leads to a slight depression of the δ values, however the relative values between each C atom are approximately maintained. Further, when the amino group is acylated (L-Pro-NAc), there is no evidence for significant interaction. This is a strong confirmation that the carboxylate group of an amino acid plays a significant, but unnecessary role in interacting with the Ln(III) complex and that having an H-bond donor is required for strong association.

In contrast, the ¹³C NMR data for L-Arg highlights the potential for significant interaction without chiral recognition (note that the pK_a of the guanidine group (pK_a = 12.5) of L-Arg means that it is in equilibrium with the protonated guanidinium at the pH used, 12.0–12.3. See scheme 1). The large δ value (−1.258 ppm) for the guanidinium/guanidine carbon of L-Arg indicates that it is forming an outer-sphere complex with [Tb(ADPA)₃]^{3−}, however the g_{lum} data shows essentially no perturbation of the racemic equilibrium (table 1). We suspect that the lack of discrimination is related to the additional N-H groups present on L-Arg, relative to L-Pro, which compete with the amino group for association to [Tb(ADPA)₃]^{3−}. Because the binding guanidinium group is distant from the chiral center and spaced by a flexible chain of methylene units, the resulting diastereotopic multi-molecular complexes are essentially identical in energy. In an attempt to block the association of the guanidinium N-H bonds, we also measured the g_{lum} and δ values for *N*-nitro-L-arginine under identical conditions (see table 1 and fig. 5). The results of this experiment were mixed: the g_{lum} increased to the measurable value of 1.2×10^{-3} ; however,

the δ values indicated a *stronger* association of the guanidine group. The precise reason for this observation is not clear, but we suspect it is related to the fact that Nitro-L-Arg must have a much lower pKa value for the guanidinium group and is likely fully deprotonated at the measured pH.

Comparing the measured δ values for $[\text{Tb}(\text{ADPA})_3]^{3-}$ with those reported⁴⁰ for $[\text{Tb}(\text{CDA})_3]^{6-}$ yields significant insight; however, it should be noted that the δ values for CDA were gathered under different conditions (1.2 M AA, 40 mM $[\text{Tb}(\text{CDA})_3]^{6-}$, relative to 0.2 M AA and 5 mM $[\text{Tb}(\text{ADPA})_3]^{3-}$, due to low solubility of ADPA^{2-} and its complexes of Tb(III)), so only very qualitative conclusions can be drawn. For $[\text{Tb}(\text{CDA})_3]^{6-}$, almost no δ was measured for the carbonyl carbon of proline and all association was attributed to the N-H group. In the case of $[\text{Tb}(\text{ADPA})_3]^{3-}$, the measured δ values indicated that the carboxylate played a key role. This comparison shows that the outer-sphere complexation of $[\text{Tb}(\text{ADPA})_3]^{3-}$ by L-proline is very different than that of $[\text{Tb}(\text{CDA})_3]^{6-}$, as expected from the drastic change in overall charge and functionality, although the *chiral recognition* by each complex is very similar. One possibility, as discussed above, is that there are multiple modes of small molecule binding to $[\text{LnL}_3]^{3-}$ complexes, where each mode effects the Λ/Δ equilibrium to varying degrees. In this model, the δ values measured report the weighted average of all possible association modes, whereas the g_{lum} values are only sensitive to modes of association that perturb the Λ/Δ equilibrium. $[\text{Tb}(\text{CDA})_3]^{6-}$ and $[\text{Tb}(\text{ADPA})_3]^{3-}$ give nearly identical values (in magnitude) for δ for L-Arg. This is not necessarily surprising given the putative strong binding of the guanidine group to $[\text{Tb}(\text{L})_3]^{3-}$, although studies under identical conditions would be required to verify this.

This complicated picture for chiral sensing by $[\text{LnL}_3]^{3-}$ is supported by comparing the current study to work performed in other laboratories. It should be noted that the behavior of $[\text{Tb}(\text{ADPA})_3]^{3-}$ and $[\text{Tb}(\text{CDA})_3]^{6-}$ with L-Pro is in stark contrast with the reported^{8,9} association of $[\text{Tb}(\text{DPA})_3]^{3-}$ with L-His. L-His has been shown to significantly perturb the Λ/Δ equilibrium of $[\text{Tb}(\text{DPA})_3]^{3-}$, where the aromatic imidazole was key to binding relative to other amino acid derivatives.⁸ In contrast to our studies on less acidic N-H functionalities, acetylating the α -amino group of L-His did not destroy the induced optical activity. In fact, the N-acetyl-L-histidine had a higher induced CPL signal relative to underivatized L-His at high pH values where the imidazole ring would be deprotonated. Furthermore, the methyl ester of L-His significantly enhanced chiral recognition by $[\text{Tb}(\text{DPA})_3]^{3-}$ and increased the induced CPL signal at all measured pH.⁹ Considering the reported work with L-His,^{8,9} our previous results,⁴⁰ and the current study, it appears that the association of small chiral molecules to $[\text{LnL}_3]^{3-}$ compounds does not follow a particular pattern, even considering the relatively small chemical diversity of the subset of amino acids that have been studied in detail. These observations support the notion that there are multiple modes of small molecule binding, with varying chiral discrimination, occurring in solution and that the behavior of each system is difficult to predict.

Conclusion

This work presents a complicated but consistent picture for the binding of small molecules to $[\text{Ln}(\text{L})_3]^{n-}$ complexes. We have shown that, within a subset of amino acids, chiral recognition by $[\text{Tb}(\text{ADPA})_3]^{3-}$ is surprisingly similar to that of $[\text{Tb}(\text{CDA})_3]^{6-}$ despite that the overall charge and functionality is significantly different. In contrast, ^{13}C -NMR results suggest that the general association of small molecules to $[\text{Tb}(\text{ADPA})_3]^{3-}$ is significantly different than to $[\text{Tb}(\text{CDA})_3]^{6-}$. That chiral recognition is so similar for ADPA^{2-} and CDA^{3-} complexes of $\text{Tb}(\text{III})$, suggests that – while the association of small molecules to these complexes differs – the associations responsible for inducing optical activity are relatively similar.

It has been suggested that molecules associating to $[\text{LnL}_3]^{n-}$ complexes interact with the π^* orbitals of the pyridine rings,⁸ in analogy to phenanthroline complexes of transition metals;⁴⁶ however, we are not convinced that these interactions govern this effect. Consider that when the carboxylate groups of dipicolinate are transformed into chiral amide groups, the resulting complexes of lanthanides self-assemble in solution as enantiopure Λ or complexes.^{44,50} Furthermore, the similar chiral recognition of small molecules across the range of $[\text{Ln}(\text{L})_3]^{n-}$ complexes reported herein suggests that the carboxylate groups of dipicolinate derivatives play a significant role in the stereochemistry of the resulting complex. Associations of these overall negatively charged groups to positively charged chiral functionalities leads to induced shifts in the racemic Λ/Δ equilibrium. This localization of the interactions specific to guiding the secondary structure of $[\text{LnL}_3]^{n-}$ complexes is key to understanding these molecular probes and designing further sensitivity and specificity in chiral recognition by this class of CPL active compounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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REFERENCES AND NOTES

1. Janata, J. Principles of Chemical Sensors. Dordrecht: Springer; 2009.
2. Shaw WJ. The Outer-Coordination Sphere: Incorporating Amino Acids and Peptides as Ligands for Homogeneous Catalysts to Mimic Enzyme Function. *Catal Rev.* 2012; 54:489–550.
3. Bennett SD, Core BA, Blake MP, Pope SJA, Mountford P, Ward BD. Chiral lanthanide complexes: coordination chemistry, spectroscopy, and catalysis. *Dalton Trans.* 2014; 43:5871–5885. [PubMed: 24577185]
4. Gale PA. Supramolecular chemistry anniversary. *Chem Soc Rev.* 2007; 36:141–142. [PubMed: 17264916]
5. Muller G. Luminescent chiral lanthanide(III) complexes as potential molecular probes. *Dalton Trans.* 2009:9692–9707. [PubMed: 19885510]

6. Zinna F, Di Bari L. Lanthanide circularly polarized luminescence: bases and applications. *Chirality*. 2015; 27:1–13. [PubMed: 25318867]
7. Meskers SCJ, Dekkers HPJM. Enantioselective quenching of luminescence: molecular recognition of chiral lanthanide complexes by biomolecules in solution. *J Phys Chem A*. 2001; 105:4589–4599.
8. Brittain HG. Optical activity induced in terbium(III) tris(pyridine-2,6-dicarboxylate) through association with certain chiral amino acids. *Inorg Chem*. 1981; 20:3007–3013.
9. Brittain HG, Rispoli L. Studies of the pfeiffer effect induced in tris(pyridine-2,6-dicarboxylate)Tb(III) by histidine derivatives. *Polyhedron*. 1984; 3:1087–1092.
10. Madaras JS, Brittain HG. Induced optical activity in the terbium(III) complex of pyridine-2,6-dicarboxylic acid through association with resolved tris(ethylenediamine)chromium(III). *Inorg Chem*. 1980; 19:3841–3842.
11. Madaras JS, Brittain HG. Induced optical activity in the Tb(III) complex of pyridine-2,6-dicarboxylic acid through outer-sphere coordination with L-ascorbic acid. *Inorganica Chim Acta*. 1980; 42:109–113.
12. Muller G, Schmidt B, Jiricek J, Hopfgartner G, Riehl JP, Bünzli J-CG. Lanthanide triple helical complexes with a chiral ligand derived from 2,6-pyridinedicarboxylic acid. *J Chem Soc Dalton Trans*. 2001; 19:2655–2662.
13. Riehl JP, Richardson FS. Circularly polarized luminescence spectroscopy. *Chem Rev*. 1986; 86:1–16.
14. Muller G, Riehl JP, Schenk KJ, Hopfgartner G, Piguet C, Bünzli J-CG. Structural, photophysical and chiro-optical properties of lanthanide complexes with a bis(benzimidazole)pyridine-based chiral ligand. *Eur J Inorg Chem*. 2003; 22:4065–4072.
15. Muller FC, Muller G, Riehl JP. Emission detected circular dichroism from long-lived excited states: application to chiral Eu(III) systems. *Chirality*. 2007; 19:826–832. [PubMed: 17786909]
16. Pfeiffer P, Quehl K. Aktivierung von komplexsalzen in wäßriger lösung (II. Mitteil.). *Berichte der Dtsch Chem Gesellschaft (A B Ser)*. 1932; 65:560–565.
17. Kirschner S. The Pfeiffer effect, outer-sphere complexation, and the absolute configuration of coordination compounds. *J Indian Chem Society*. 1974; L1:28–31.
18. Ariëns EJ. Stereochemistry, a basis for sophisticated nonsense in pharmacokinetics and clinical pharmacology. *Eur J Clin Pharmacol*. 1984; 26:663–668. [PubMed: 6092093]
19. Zhai Z, Shi Y, Wang T. Development and validation of HPLC methods for enantioseparation of mirtazapine enantiomers at analytical and semipreparative scale using polysaccharide chiral stationary phases. *Anal Chim Acta*. 2005; 550:123–129.
20. Zhang Y, Wu D, Wang-Iverson D, Tymiak A. Enantioselective chromatography in drug discovery. *Drug Discov Today*. 2005; 10:571–577. [PubMed: 15837600]
21. Okamoto Y, Ikai T. Chiral HPLC for efficient resolution of enantiomers. *Chem Soc Rev*. 2008; 37:2593–2608. [PubMed: 19020674]
22. Ye J, Yu W, Chen G, Shen Z, Zeng S. Enantiomeric separation of 2-arylpropionic acid nonsteroidal anti-inflammatory drugs by HPLC with hydroxypropyl-beta-cyclodextrin as chiral mobile phase additive. *Biomed Chromatogr*. 2010; 24:799–807. [PubMed: 20017213]
23. Albright A, White J. Determination of absolute configuration using single crystal X-ray diffraction. *Methods Mol Biol*. 2013; 1055:149–162. [PubMed: 23963909]
24. Stout, GH.; Jensen, LH. *X-ray Structure Determination*. New York: Wiley; 1989.
25. Flack H, Bernardinelli G. The use of X-ray crystallography to determine absolute configuration. *Chirality*. 2008; 20:681–690. [PubMed: 17924422]
26. Chen X, Huang Z, Chen S-Y, Li K, Yu X-Q, Pu L. Enantioselective gel collapsing: a new means of visual chiral sensing. *J Am Chem Soc*. 2010; 132:7297–7299. [PubMed: 20446686]
27. Jaki B, Franzblau S, Pauli GF. An NMR method towards the routine chiral determination of natural products. *Phytochem Anal*. 2004; 15:213–219. [PubMed: 15311839]
28. Dale JA, Mosher HS. Nuclear magnetic resonance enantiomer reagents. Configurational correlations via nuclear magnetic resonance chemical shifts of diastereomeric mandelate, O-methylmandelate, and a-methoxy-a trifluoromethylphenylacetate (MTPA) Esters. *J Am Chem Soc*. 1973; 95:512–519.

29. Luy B. Distinction of enantiomers by NMR spectroscopy using chiral orienting media. *J Indian Inst Sci.* 2010; 90:119–132.
30. Tsukube H, Shinoda S. Lanthanide Complexes in Molecular Recognition and Chirality Sensing of Biological Substrates. *Chem Rev.* 2002; 102:2389–2404. [PubMed: 12059273]
31. Berova, N. *Comprehensive Chiroptical Spectroscopy*. Chichester: Wiley; 2012.
32. Berova, N.; Nakanishi, K.; Woody, R. *Circular Dichroism: Principles and Applications*. New York: Wiley; 2000.
33. Polavarapu PL. Renaissance in chiroptical spectroscopic methods for molecular structure determination. *Chem Rec.* 2007; 7:125–136. [PubMed: 17394174]
34. Riehl, JP.; Muller, G. *Comprehensive Chiroptical Spectroscopy*. Hoboken: Wiley; 2012. p. 65-90.
35. Riehl JP, Muller G. Circularly Polarized Luminescence Spectroscopy from Lanthanide Systems. *Handb Phys Chem Rare Earths.* 2004; 34:289–357.
36. Gunnlaugsson T, Bradberry SJ, Savyasachi AJ, Peacock R. Quantifying the formation of chiral luminescent lanthanide assemblies in aqueous medium through chiroptical spectroscopy and generation of luminescent hydrogels. *Faraday Discuss.* 2015; 185:413–431. [PubMed: 26404059]
37. George MR, Golden CA, Grossel MC, Curry RJ. Modified dipicolinic acid ligands for sensitization of europium(III) luminescence. *Inorg Chem.* 2006; 45:1739–1744. [PubMed: 16471988]
38. De Bettencourt-Dias A, Barber PS, Viswanathan S. Aromatic N-donor ligands as chelators and sensitizers of lanthanide ion emission. *Coord. Chem. Rev.* 2014; 273–274:165–200.
39. Hopkins TA, Metcalf DH, Richardson FS. Intermolecular chiral recognition processes probed by chiroptical luminescence. *Chirality.* 2008; 20:511–523. [PubMed: 17963202]
40. Moussa A, Pham C, Bommireddy S, Muller G. Importance of hydrogen-bonding sites in the chiral recognition mechanism between racemic D3 terbium (III) complexes and amino acids. *Chirality.* 2009; 21:497–506. [PubMed: 18698640]
41. Schipper PE. Contributions to the pfeiffer effect. *Inorganica Chim Acta.* 1975; 12:199–204.
42. Yan F, Brittain HG. Pfeiffer effect optical activity developed in lanthanide tris(pyridine-2,6-dicarboxylate) complexes by chiral tartrate substrates. *Polyhedron.* 1982; 1:195–199.
43. Muller G, Riehl JP. Use of induced circularly polarized luminescence (CPL) from racemic D3 lanthanide complexes to determine the absolute configuration of amino acids. *J Fluorescence.* 2005; 15:553–558.
44. Hua KT, Xu J, Quiroz EE, Lopez S, Ingram AJ, Johnson VA, Tisch AR, Bettencourt-Dias A, Straus DA, Muller G. Structural and photophysical properties of visible- and near-IR-emitting tris lanthanide(III) complexes formed with the enantiomers of N,N'-bis(1-phenylethyl)-2,6-pyridinedicarboxamide. *Inorg Chem.* 2012; 51:647–660. [PubMed: 22148725]
45. Lamture JB, Zhou ZH, Kumar AS, Wensel TG. Luminescence properties of Terbium(III) complexes with 4-substituted dipicolinic acid analogs. *Inorg Chem.* 1995; 34:864–869.
46. Kirschner S, Ahmad N, Munir C, Pollock RJ. The Pfeiffer effect, outer-sphere complexation, and the absolute configuration of dissymmetric coordination compounds. *Pure Appl Chem.* 1979; 51:913–924.
47. Smith, DG. Durham thesis. Durham University; 2012. Intracellular Responsive Lanthanide Probes. Available at Durham E-Theses Online: <http://etheses.dur.ac.uk/3591/>
48. Di Bari L, Salvadori P. Solution structure of chiral lanthanide complexes. *Coord Chem Rev.* 2005; 249:2854–2879.
49. Hilmes GL, Riehl JP. Circularly Polarized Luminescence Spectroscopy of Mixed-ligand Complexes of Europium(III) with 2,6-Pyridine-dicarboxylic acid and L-malic acid. *Inorganic Chimica Acta.* 1987; 129:123–125.
50. Bonsall SD, Houcheime M, Straus DA, Muller G. Optical isomers of N,N'-bis(1-phenylethyl)-2,6-pyridinedicarboxamide coordinated to europium(III) ions as reliable circularly polarized luminescence calibration standards. *Chem Commun (Camb).* 2007:3676–3678. [PubMed: 17728891]

FIGURE 1.
The racemic equilibrium of $[\text{LnL}_3]^{n-}$ complexes.

FIGURE 2.

Direct excitation spectra of the ${}^5D_0 \leftarrow {}^7F_0$ transition at various pH. Conditions: 1 mM Eu(III), 5.5 equivalence ADPA²⁻. $\lambda_{em} = 615$ nm. The intensities of each spectrum were normalized.

FIGURE 3.

CPL (top half) and total luminescence (lower half) spectra for the $^5D_4 \rightarrow ^7F_5$ transition of 1 mM $[\text{Tb}(\text{ADPA})_3]^{3-}$ with L-proline (black line) and *N*-acetyl-L-proline (grey line) in a 1:40 equivalence of complex to amino acid. Measurements were taken at pH 12.0–12.3 at room temperature.

FIGURE 4.

CPL (top half) and total luminescence (lower half) spectra for the $^5D_4 \rightarrow ^7F_5$ transition of 1 mM $[Tb(ADPA)_3]^{-3}$ with L-proline amide (solid line) and D-proline amide (dotted line) in a 1:40 equivalence of complex to amino acid. Measurements were taken at pH 12.0–12.3 at room temperature.

FIGURE 5.

δ values for amino acids and derivatives studied by ^{13}C -NMR. Values in blue are for ADPA ligand ($[\text{AA}] = 0.2 \text{ M}$, $[\text{Ln}] = 5 \text{ mM}$, $\text{Ln:ADPA} = 1:5.5$, $\text{pH } 12.0\text{--}12.3$), values in red are for a CDA ligand and were measured under different conditions ($[\text{AA}] = 1.2 \text{ M}$, $[\text{Ln}] = 40 \text{ mM}$, $\text{Ln:CDA} = 1:5.5$, $\text{pH } 12.0\text{--}12.3$). Structures are shown at the protonation state expected at pH 12, where L-Arg is a mixture.

Table 1

Luminescence dissymmetry values (g_{lum}) of $[\text{Tb}(\text{ADPA})_3]^{3-}$ with amino acids

Amino Acid	g_{lum}^a
L-proline	+0.0115
L-proline methyl ester	+0.0071
L-proline amide	+0.0037
N-acetyl-L-proline	−0.0003
L-arginine	+0.0003
No-nitro-L-arginine	+0.0018
D-serine	+0.0000
L-cysteine	+0.0003

^aConditions: 1 mM $[(\text{ADPA})_3\text{Tb}]^{3-}$ with 40 equivalents of amino acid. Monitored at spectral range of the transition $^5\text{D}_4 \rightarrow ^7\text{F}_5$ at room temperature and pH 12.0–12.3. Measured at $\lambda_{\text{ex}} = 279.00\text{--}287.00$ nm and $\lambda_{\text{em}} = 542.00\text{--}542.40$ nm.