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Use of Induced Circularly Polarized Luminescence (CPL) from Racemic D_3 Lanthanide Complexes to Determine the Absolute Configuration of Amino Acids

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The perturbation of the racemic equilibrium of luminescent D_3 lanthanide(III) complexes by added chiral agents, such as amino acids, may be a useful technique for determining the absolute configuration of the added species. It is shown in this work, however, that simple interpretation of the equilibrium shift of racemic tris-terdentate complexes of Tb(III) with 2,6-pyridine-dicarboxylate by amino acids, as measured by the sign of the resultant circularly polarized luminescence (CPL) in terms of specific structural characteristics is not possible. CPL results for a number of derivatized amino acids are also presented, and some insights into the nature of the chiral discriminatory forces that might be exploited in this kind of study are discussed.

KEY WORDS: Lanthanide; amino acid; chirality; circularly polarized luminescence.

INTRODUCTION

The determination of the absolute configuration of unknown chiral organic molecules is often difficult, and current techniques based upon NMR, intrinsic circular dichroism (CD), and chemical derivitization are sometimes unreliable. Furthermore, for small amounts of material, it is often not possible to prepare crystals suitable for X-ray structural determination. In this work, we describe a method of determining absolute configuration from the use of the perturbation of the racemic equilibrium of a highly coordinated lanthanide(III) complex followed by the measurement of the resultant circular polarization. This type of system appears to be amenable to this practical application, because small equilibrium shifts are easily measured due to the large chirality associated with a number of the $f \rightarrow f$ transitions involved.

Early attempts at exploiting the measurement of circularly polarized luminescence (CPL) from perturbed racemic solutions involving a lanthanide complex have been reviewed by Brittain [1,2]. Much of this early work was focused on the perturbation of the equilibrium between Λ- and Δ-[Tb(2,6-pyridine-dicarboxylate = $DPA)_3]^{3-}$. This nine-coordinate tris-terdentate complex is known to have almost exact D_3 symmetry, to be very stable in solution, and highly luminescent [3–5]. The perturbation of a racemic metal complex by an added chiral "agent" is often referred to as the "Pfeiffer-effect" [6–8]. It has been demonstrated that a variety of chiral species including tartrate substrates, amino acids, and sugar derivatives, may cause a perturbation of the D_3 equilibrium in aqueous solutions containing $[Tb(DPA)_3]^{3-}$ [9]. In our laboratory we have shown that this equilibrium shift was due to an outer-sphere interaction between the racemic lanthanide complex and the added chiral agent [10,11]. An important part of this analysis is the comparison of the CPL spectra from the Pfeiffer-perturbed system with the "pure" enantiomer generated in excess by a circularly polarized excitation beam [12]. These spectra were shown to be identical, confirming the fact that the measurement

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of CPL is due to an equilibrium shift and not the formation of a new species. These measurements have been extended to Eu(III), Dy(III), and Yb(III) complexes of DPA [10,13–15].

The use of CPL spectroscopy as a structural probe hinges on the ability to relate the created enantiomeric excess to some structural characteristic of the perturber molecule. An obvious target for this kind of analysis is the perturbation of the racemic equilibrium by L-amino acids. Under physiological pH conditions (6–7) these are perhaps more correctly written as their zwitterionic form. There has been a number of studies in which various amino acids have been added to racemic solutions of $[Tb(DPA)_3]^{3-}$ or $[Eu(DPA)_3]^{3-}$ [1,2,16,17]. Although the early work suggested that L-amino acids all gave the same sign for the CPL from selected transitions of the DPA complexes, we will show in this work that such a simple structural picture is unfortunately not correct. Just as in the development of other spectroscopic-based methods at determining chiral structure, the exploitation of the perturbation of racemic equilibria is based on very small differential effects. In the specific experiments involving neutral amino acids, the presumably outer sphere association is very weak, and the difference between the diastereomeric association constants is very small.

There have been several other attempts at using the perturbation of the racemic equilibrium of lanthanide(III): DPA complexes as diagnostic of chiral structure. For example, Brittain has attempted to correlate the sign of the CPL signal to some element of the chiral structure of monosaccharide aldose sugars [18], but in a more quantitative study using modern instrumentation it has been shown that, in fact, no simple correlation is evident in the CPL results from racemic $[Tb(DPA)_3]^{3-}$ into which simple or complex sugars have been added [10].

Although there has been some progress in understanding the nature of the equilibrium shift induced by added chiral agents to racemic equilibria [10,19,20], no one has yet "designed" a system based on specific consideration of the possible intermolecular interactions in which differences in diastereomeric perturbations would be expected to be maximized. Various speculations concerning the importance of hydrogen bonding, coulombic forces, π-stacking, steric factors, hydrophobic effects, etc. have been considered, but only in attempts to understand empirical observations. To be useful in determination of unknown chirality, and to develop a useful tool for determining molecular chirality, a more rational process of designing racemic complexes, and chemical derivitization of target chiral molecules will probably be necessary. This is the focus of the work reported here.

EXPERIMENTAL

The CPL and total luminescence spectra were recorded on an instrument described previously [9], operating in a differential photon-counting mode. The net circular polarization in the luminescence is determined by employing a circular analyzer in the emitted beam. The circular analyzer is composed of an oscillating photoelastic polarization modulator attached to a high quality linear polarizer. The detector sees alternating signals corresponding to emitted left and right polarization, and the photo-pulses are counted by two separate digital counters corresponding to the intensity of left, I_L, and right, I_R, circularly polarized emitted light. It can be shown that the standard deviation, σ_d, in the measurement of the luminescence dissymmetry factor, g_{lum}, defined as follows

$$g_{lum} \equiv \frac{I_L - I_R}{\frac{1}{2}(I_L + I_R)} \tag{1}$$

may be calculated from the following equation

$$\sigma_d = \sqrt{\frac{2}{N}} \tag{2}$$

where N is the total number of photo-pulses counted.

The excitation source for all of the measurements reported here was the 450-watt xenon continuous wave arc lamp associated with a SPEX Fluorolog 2 spectrofluorimeter. The dispersion of the excitation and emission monochromators (SPEX 1681B) was 4 nm/mm. Emission slits were adjusted so as to ensure that the values reported for g_{lum} were not affected by wavelength resolution. CPL spectra for lanthanide (III) ions are often composed of alternating signed spectra.

The measurements were performed in aqueous solutions at 295 K. Stock solutions of Eu(III) and Tb(III) were prepared from the lanthanide chlorides (Aldrich), while the various amino acids were obtained from Acros or Aldrich, and used as received. 2,6-pyridinedicarboxylate was obtained from Aldrich and used without further purification. Aqueous stock solutions of the amino acids were prepared by dissolution in water. Solutions for luminescence and CPL measurements were prepared by mixing stock solutions of $LnCl_3$ (pH $\sim$ 3) and DPA (pH $\sim$ 9) in a 1:3.5 molar ratio, adding amino acid solution, and waiting for at least 24 h before use in order to allow them to reach conformational equilibrium. The concentration of lanthanide complexes and of amino acids were 0.01 mol/L and in the range 0.05–0.40 mol/L, respectively. The final pH of solutions was approximately 7.0.

THEORY

Consistent with previous work [9], we assume here that the effect adding the chiral amino acids (AA^*) results in the preferential formation of one diastereomeric outer-sphere association complex. The three relevant equilibria are defined as follows

$$\Delta\text{-Ln(DPA)}_3^{3-} \rightleftarrows \Lambda\text{-Ln(DPA)}_3^{3-} (K_{\text{rac}} = 1) \tag{3}$$

$$\Delta\text{-Ln(DPA)}_3^{3-} + AA^* \rightleftarrows \Delta\text{-Ln(DPA)}_3^{3-} : AA^* (K_1) \tag{4}$$

$$\Lambda\text{-Ln(DPA)}_3^{3-} + AA^* \rightleftarrows \Lambda\text{-Ln(DPA)}_3^{3-} : AA^* (K_2) \tag{5}$$

where we have denoted the outer-sphere association complex by a colon (:). As shown previously [10], in the limit of large concentrations of AA^*, the concentration of free (i.e., unassociated) complex goes to zero, and the enantiomeric excess in the ground state, η, defined as follows

$$\eta = \frac{[\Lambda] - [\Delta]}{[\Lambda] + [\Delta]} \tag{6}$$

may be related to the diastereomeric association equilibrium constants as follows

$$\eta = \frac{K_2 - K_1}{K_2 + K_1} \tag{7}$$

In Eq. (5) the square brackets in this equation denote ground state concentrations. In the absence of other chiral effects, such as enantioselective excited state quenching, it may be assumed that enantiomeric excesses in the ground and excited states are equal.

In CPL spectroscopy it is common to report the observed circular polarization in the luminescence in terms of the so-called luminescence dissymmetry factor, g_{lum}, which is defined in Eq. (1). In the situation of interest here, it is easily seen that the g_{lum} measured for a perturbed racemic mixture may be written as

$$g_{\text{lum}}(\lambda) = \eta g_{\text{lum}}^{\Lambda}(\lambda) \tag{8}$$

where we have added the wavelength, λ, where the emission is being detected, and identified explicitly the luminescence dissymmetry of the Λ enantiomer. It should be noted that the luminescence dissymmetry factors for the two enantiomers have exactly equal but oppositely signed values, so that Eq. (7) has the appropriate limiting value for the pure enantiomeric compounds.

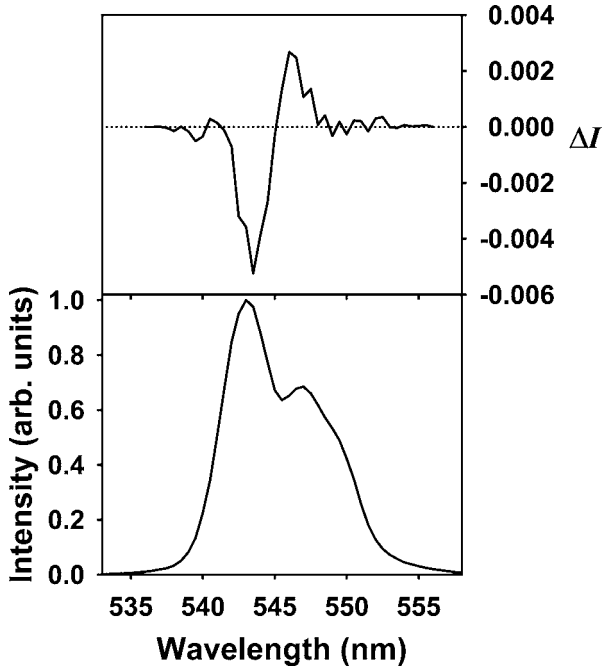


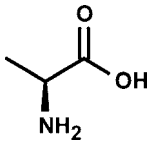
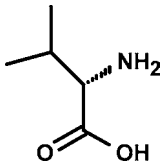
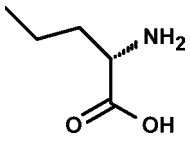
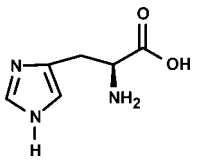
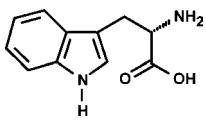
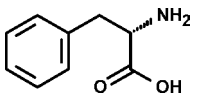
Fig. 1. Circularly polarized luminescence (upper curve) and total luminescence (lower curve) spectra for the $^5D_4 \rightarrow {}^7F_5$ transition of 0.010 mol/L $[Tb(DPA)_3]^{3-}$ after addition of 0.40 mol/L of L-norvaline in aqueous solution. Excitation wavelength was 293 nm.

RESULTS AND DISCUSSION

In Fig. 1 we plot the total luminescence, I, and circularly polarized luminescence, ΔI, for an aqueous solution of $[Tb(DPA)_3]^{3-}$ into which the amino acid, L-norvaline has been added to a final concentration of 0.40 mol/L. The spectral region displayed corresponds to the $^5D_4 \rightarrow {}^7F_5$ transition of Tb(III). Both of these spectra are essentially identical to that measured for $[Tb(DPA)_3]^{3-}$ excited by circularly polarized light consistent with the assumption that the added amino acid does not significantly perturb the coordination of Tb(III) even at these high concentrations. The fine structure seen in Fig. 1 is due to the crystal field splitting of these complicated 4*f* electronic states.

In order to relate these measurements for different amino acids, we focus on the value of the luminescence dissymmetry factor at the peak (543 nm) of the total luminescence. In Table I we list values obtained for g_{lum}(543 nm) for a series of L-amino acids. The data presented are for an amino acid concentration of 0.05 mol/L which corresponds to a five-fold excess compared to the concentration of Tb(III) complex, except for L-alanine in which a 40-fold excess was necessary in order to obtain a reliable result. As mentioned previously, there is

Table I. Luminescence Dissymmetry Ratio Values (g_{lum}) in the Spectral Range of the $^5D_4 \rightarrow {}^7F_5$ Transition for 0.010 mol/L $[Tb(DPA)_3]^{3-}$ After Addition of 0.050–0.40 mol/L of Various L-amino Acids in Aqueous Solution

	Amino acid	[AA]/ $[Tb(DPA)_3]^{3-}$	g_{lum} (543 nm) σ_d = ±0.0003
	L-alanine	40	−0.00086
	L-valine	5	−0.00044
	L-norvaline	5	−0.00136
	L-histidine	5	+0.00039
	L-tryptophan	5	−0.00023
	L-phenylalanine	5	−0.00334

Note. Excitation wavelength was 293 nm.

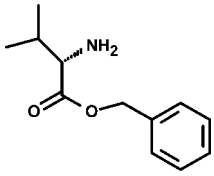
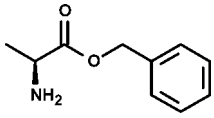
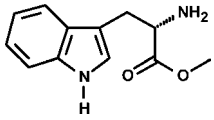
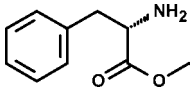
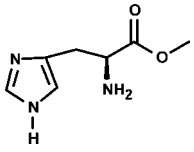
a sign variation that excludes a generalization concerning the L-amino acid structure and the direction of the perturbation.

In order to investigate whether or not a simple chemical derivitization would lead to a reliable spectra:structure correlation we performed CPL measurements on a series of modified amino acids. In Table II, for example, we show the results on three amino acid benzyl esters, and two methyl esters. In Table III we present results for three L-histidine derivatives. Examination of the results in these tables shows that, unfortunately, a simple scheme for determination of amino acid absolute configuration is not evident. The results in Tables II and III do indicate that addition of aromatic groups does seem to increase the perturbation of the racemic, probably due to $\pi - \pi$ interactions with the dipicolinate rings.

SUMMARY AND CONCLUSIONS

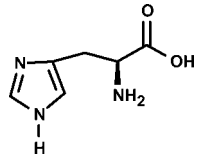
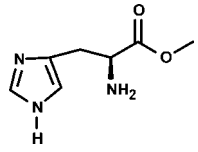
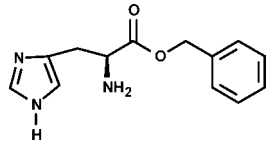
Determination of the absolute configuration of chiral molecules through their discriminatory interactions with dynamic racemic equilibria of lanthanide species and the measurement of CPL does show promise. Very small perturbations in the racemic equilibrium are easily and reliably detected, since the chirality as measured by g_{lum} for the magnetic-dipole transition of Tb(III) is very large. Although not presented in this work, complexes of Eu(III) and Dy(III) have also been studied and could be used.

Table II. Luminescence Dissymmetry Ratio Values (g_{lum}) in the Spectral Range of the $^5D_4 \rightarrow {}^7F_5$ Transition for 0.010 mol/L $[Tb(DPA)_3]^{3-}$ After Addition of 0.050–0.40 mol/L of Various L-amino Acid Benzyl and Methyl Esters in Aqueous Solution

	Amino acid	[AA]/ $[Tb(DPA)_3]^{3-}$	g_{lum} (543 nm) σ_d = ± 0.0003
	L-valine benzyl ester	5 (pH = 6.2)	−0.00047
	L-alanine benzyl ester	5	+0.00355
	L-tryptophan methyl ester	35 (pH = 3.8)*	−0.02185
	L-phenylalanine methyl ester	40	−0.01847
	L-histidine methyl ester	40	+0.02258

Note. Excitation wavelength was 293 nm.
*Not soluble at pH 7.0.

Table III. Luminescence Dissymmetry Ratio Values (g_{lum}) in the Spectral Range of the $^5D_4 \rightarrow {}^7F_5$ Transition for 0.010 mol/L $[Tb(DPA)_3]^{3-}$ After Addition of 0.050 mol/L of Three L-histidine Derivatives in Aqueous Solution

	Amino acid	[AA]/ $[Tb(DPA)_3]^{3-}$	g_{lum} (543 nm) σ_d = ±0.0003
	L-histidine	5	+0.00039
	L-histidine methyl ester	5	+0.00373
	L-histidine benzyl ester	5	+0.00850

Note. Excitation wavelength was 293 nm.

The ion of choice for this type of study is Tb(III), since it is generally the most luminescent of the lanthanide(III) ions.

As the results of this work and earlier studies have shown, however, simple spectra-structure relationships that can be reliably implemented are not as yet available. Part of the difficulty in this area is due to the fact that the nomenclatures that have been developed in order to unambiguously define the structure of a chiral molecule, are not really suitable to relate to the net discriminatory interactions that are important here. For example, the R/S notation for chiral centers is based upon the mass of attached substituents, but the important discriminatory interactions, such as hydrogen bonding or π-stacking, may be completely unrelated to mass. The L and D nomenclature of amino acids are more likely to lead to useful relationships, since this notation is based upon comparative functional groups. Even with this notational advantage, however, we have shown that one can not assign an L or D structure of an amino acid or amino acid derivative based upon the sign of the CPL from a perturbed racemic solution of $Tb(DPA)_3^{3-}$.

From the measurements described here, some insight has been obtained concerning the factors necessary to make this identification technique successfully. Certainly the effect can be made larger when the amino acid contains an aromatic group. It is expected that this aromatic species is to some extent interacting in a chirally selective manner with the pyridine part of the DPA ligand. It should be noted that attempts to grow crystals suitable for X-ray structure determination of lanthanide:DPA complexes containing added chiral species have to date not been successful.

In our research groups we have begun to probe in a more consistent way the possible differential interactions in order to design a racemic luminescent lanthanide probe that would more directly interact with chiral amino acids and other small chiral molecules. In this regard we are synthesizing racemic complexes with more extended structures, and complexes containing chiral substituents. The use of chiral substituents on the lanthanide ligands no longer yields racemic mixtures, but very preliminary studies indicate that an enhancement of the *diastereomeric* interactions may lead to significant increases in the perturbation of the pseudo-racemic-D_3 equilibrium associated with the central Ln(III) coordination.

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