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Spectroscopic Studies of Lanthanide Ion Binding to Multidentate Ligands in Aqueous Solution. 2. *N,N'*-Bis(2-hydroxyphenyl)ethylenedinitrilo-*N,N'*-diacetate

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The title ligand, also called *N,N'*-ethylenebis(2-(*o*-hydroxyphenyl))glycine (or EHPG), contains two carboxylate, two amino, and two phenolate ligating groups and forms a fully hexadentate chelate with Tb^{3+} in aqueous solution by pH 8. Potentiometric titration measurements, luminescence intensity and lifetime studies, and near-ultraviolet absorption and emission excitation spectra are used to probe the formation and structure of the Tb(EHPG) complexes in solution as a function of pH. Near-ultraviolet excitation in the 290-nm region leads to a large enhancement of terbium luminescence upon coordination of the phenolate groups to the metal ion (in the pH range 4–6). This enhancement is attributed to a sensitization process involving (1) direct (radiative) phenolate excitation, (2) radiationless phenolate-to-Tb^{3+} energy transfer, and (3) emission from Tb^{3+}. Luminescence decay constants for Tb(EHPG) in H_2O/D_2O solvent mixtures were measured as a function of χ_{H_2O} (mole fraction of H_2O in the H_2O/D_2O mixture) to determine the number of water molecules bound to Tb^{3+} at various values of pH. The analysis of this data required allowance for the contributions of both N–H oscillators (on the EHPG ligand) and O–H oscillators (from the bound water molecules) to radiationless deactivation of the terbium $^5D_4$ emitting state. For the fully formed Tb(EHPG) complex at pH 8, the total coordination number of Tb^{3+} was found to be 8, with six sites occupied by EHPG ligating groups and two by water molecules.

Introduction

In the present study we investigate the coordination properties of the ligand *N,N'*-bis(2-hydroxyphenyl)ethylenedinitrilo-*N,N'*-diacetate to trivalent terbium ions (Tb^{3+}) in aqueous solution under variable pH conditions. This ligand, also called *N,N'*-ethylenebis(2-(*o*-hydroxyphenyl))glycine (or EHPG), has the structure shown in Figure 1. The EHPG molecule has six potential ligating groups—two carboxylate groups, two amino nitrogens, and two phenolate oxygens—and also possesses two chromophoric moieties (the aromatic phenolic groups) which may be excited by near-ultraviolet radiation. Lanthanide ion binding to EHPG is of particular interest as an analogue of lanthanide ion binding to proteins. The ligating groups of EHPG are similar to those which are expected to be found at the lanthanide ion binding sites of many proteins (especially those containing tyrosinate residues), and the possibility of sensitizing lanthanide ion luminescence via energy transfer from an excited aromatic chromophore in the terbium–EHPG complex affords modeling similar processes known to occur in terbium–protein complexes.[1,2] Near-ultraviolet excitation enhancement of terbium luminescence in Tb^{3+}–protein systems has proved useful in identifying aromatic side chain chromophores located in the vicinity of the Tb^{3+} ion binding sites.[1,2] Although the basis for this observed enhancement has not yet been fully characterized, it is assumed to follow a mechanism involving (1) direct radiative excitation of an aromatic side chain chromophore, (2) nonradiative energy transfer from the aromatic chromophore to a bound Tb^{3+} ion, and (3) luminescence from the sensitized Tb^{3+}.

Several types of spectroscopic measurements were used in the present study to probe the coordination of EHPG to Tb^{3+} as a function of solution pH and to investigate ligand-to-metal energy-transfer processes leading to enhanced Tb^{3+} emission. Near-ultraviolet absorption and luminescence excitation spectra were obtained to follow the extent and nature of Tb^{3+}–phenolate interactions under various pH conditions. Luminescence intensity spectra were obtained as a function of solution pH to follow changes in the crystal-field environment of the Tb^{3+} ion (as reflected by changes in splitting patterns and intensity distributions within emission bands). Luminescence decay measurements were carried out in H_2O/D_2O solvent mixtures as a function of χ_{H_2O} (mole fraction of H_2O in the H_2O/D_2O solvent mixture) to determine the number of water molecules coordinated to the Tb^{3+} ion at various values of solution pH. The combination of results obtained from these various spectroscopic measurements are used to characterize the nature of the terbium–EHPG complexes formed in solution under different pH conditions. Potentiometric titration data were also obtained for both the free ligand and the Tb^{3+}/EHPG system, and these results are compared with the spectroscopic results.

The chelating properties of EHPG with a variety of transition metal ions and group 2A ions have been studied previously.[3,4] The results show that EHPG forms stable complexes with most of the metal ions investigated. This stability is attributed to the ability of the metal ions to induce deprotonation of the ligand binding groups at pH values appreciably lower than the pK_a values of the free ligand. This metal ion induced lowering of ligand pK_a's is also expected to occur for trivalent lanthanide ions such as Tb^{3+}.[5] Occurrence of a charge-transfer absorptive transition at ~245 nm in the EHPG complex of Eu^{3+} has been cited as evidence for Eu^{3+}–phenolate binding in aqueous solution.[6,7]

Experimental Section

$TbCl_3 \cdot 6H_2O$ was purchased from Alfa-Ventron and used without further purification. *N,N'*-Ethylenebis(2-(*o*-hydroxyphenyl))glycine (EHPG) was purchased from Pfaltz and Bauer. Because of its sensitivity to air oxidation, this ligand could not be used without prior purification. To a supersaturated solution of the ligand in 1 N HCl was added decolorizing carbon until a colorless filtrate remained. This clear solution was then titrated with 1 N NaOH, until at pH 4, a white precipitate of the purified ligand formed. The ligand was then dried in a vacuum desiccator. The Tb(EHPG) complex was formed by adding equimolar amounts of $TbCl_3$ and EHPG to a 0.01 N NaOH solution. The pH was adjusted by addition of the required amounts of HCl.

Potentiometric titrations were performed with a Radiometer automatic titrator, Model TTT-2, with AgCl/Ag combination electrodes. Absorption spectra were recorded on a Cary 14 spectrophotometer, and the excitation and emission spectra were recorded on an emission spectrophotometer constructed in this laboratory. For all the emission and excitation studies, the results were corrected for excitation source intensity with the aid of an optically dense solution of Rhodamine

(1) H. G. Brittain, F. S. Richardson, and R. B. Martin, *J. Am. Chem. Soc.*, **98**, 8255 (1976).
(2) R. B. Martin and F. S. Richardson, *Q. Rev. Biophys.*, **12**, 181 (1979).
(3) A. E. Frost, M. H. Freedman, S. J. Westerback, and A. E. Martell, *J. Am. Chem. Soc.*, **80**, 530 (1958).
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(6) R. K. Boggess and R. B. Martin, *J. Am. Chem. Soc.*, **97**, 3076 (1975).
(7) R. Prados, R. K. Boggess, R. B. Martin, and R. C. Woodworth, *Bioinorg. Chem.*, **4**, 135 (1975).

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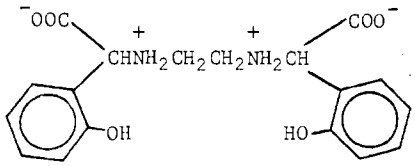

Figure 1. Structure of EHPG ligand.

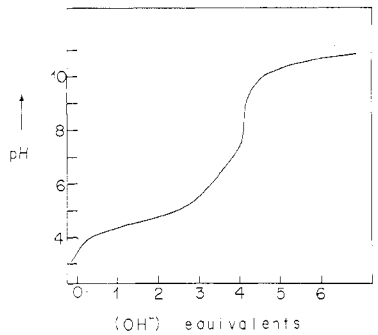

Figure 2. Proton titration curve for 1:1 $[Tb^{3+}]$:[EHPG] in aqueous solution.

6-G. All the intensities reported were obtained by measuring band areas with a planimeter.

Luminescence lifetime measurements were carried out by using a Molectron UV-14 pulsed N_2 laser, with a 400-kW peak pulse power, and a Molectron DL-II dye laser. The luminescence signal was passed through a Jobin-Yvon H-10 monochromator for stray light rejection and onto an RCA C7164R photomultiplier tube. The decay curves were plotted on a Hewlett-Packard 180 AR storage oscilloscope and photographed on Polaroid film type 46-L.

Results and Discussion

Potentiometric Titration Results. Figure 2 shows the potentiometric titration of 1:1 $[Tb^{3+}]$:[EHPG]. As the pH is increased above pH 4, 4 equiv of acid is titrated. The four protons removed in this process belong to the two ammonium and two phenolic groups of the EHPG ligand. In the free ligand, these protons are titrated at pH 6.3, 8.6, 10.2, and 11.7, respectively.[3,4] However, in the presence of Tb^{3+} these protons are titrated over the pH range 3–8, with a relatively sharp end point near pH 8. The decrease in the apparent pK_a values of these protons in the presence of Tb^{3+} provides strong evidence that the corresponding deprotonated ligating groups are bound to Tb^{3+}. The titration of all four protons within approximately 4 pH units for Tb^{3+}/EHPG vs. a range of 7 pH units for free EHPG is consistent with some cooperativity in the binding of the ligand to the metal ion. In the presence of Tb^{3+}, both carboxylate groups on the EHPG ligand are in their basic forms at pH <4.

The potentiometric titration data suggests that EHPG binds quite strongly to Tb^{3+}. Evidence for strong binding is also provided by the observation that even at pH values as high as 13 there is no appreciable precipitation of $Tb(OH)_3$ (which readily precipitates from aqueous solutions of $TbCl_3$ at pH ~6.5).

Near-Ultraviolet Absorption and Luminescence Excitation Spectra. The solid curves in Figure 3 show the near-ultraviolet absorption spectra of Tb(EHPG) as a function of pH. The terbium absorption bands could not be monitored because their molar absorptivities were low (ϵ ~0.3 for $TbCl_3$ in H_2O) and because the maximum complex concentration attainable was 2 mM. The observed absorption bands are assigned to a $\pi \rightarrow \pi^*$ transition of the aromatic phenolic chromophore (corresponding to the L_b band of benzene) mixed with an $n \rightarrow \pi^*$ transition involving excitation of an oxygen nonbonding electron into the π system of the aromatic ring. The band appearing at low pH values at 277 nm (ϵ ~2000) is analogous to the 270 nm (ϵ ~1450) transition of phenol. Upon complexation to terbium, the band maximum is shifted to longer wavelength (292 nm, ϵ 4000) in analogy to the phenolate transition shift occurring upon deprotonation (287 nm, ϵ 2600). Comparison of the absorption spectrum of the terbium complex with that of the free ligand at different pH values[3] shows that the spectral changes due to the phenol deprotonation occur at pH values which are 5 pH units lower in the complex than in the free ligand. This difference supports the earlier statement that two of the protons titrated in the potentiometric titration belong to the phenolic groups of the EHPG ligand.

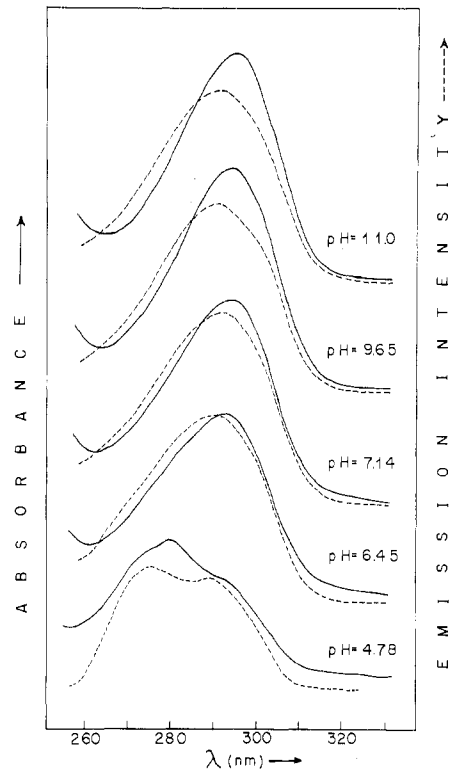

Figure 3. Absorption (—) and excitation (---) spectra for the 1:1 Tb(EHPG) complex at different pH values. The excitation spectra were obtained by monitoring the Tb^{3+} emission at 545 nm ($\Delta\lambda_{em}$ = 10 nm). The spectral slit width used in excitation was $\Delta\lambda_{ex}$ = 10 nm.

Exciting into the absorption region of the aromatic moiety of the EHPG ligand leads to sensitization of terbium emission. The excitation spectra of Tb(EHPG) obtained at different pH values are plotted together with the corresponding absorption spectra in Figure 3. At $\lambda > 260$ nm there is a close similarity and strong overlap between the excitation and absorption spectra observed for all the solutions studied. For the complex present at pH 4.78, this overlap suggests that both the protonated and deprotonated forms of the phenolic moiety are involved as energy donors in the sensitization process. Overlap of the excitation and absorption spectra does not extend to the region 260 nm > λ > 230 nm. Very little absorption in this region leads to terbium emission excitation.

The sensitization of terbium emission in Tb(EHPG) by excitation in the 260–300 nm spectral region can be attributed to intramolecular energy transfer from the aromatic chromophores of EHPG to the terbium ion. With lowering of the pH and protonation of the phenolate ligating groups, the emission intensities of the terbium $^5D_4 \rightarrow {}^7F_J$ transitions are weakened considerably (by a factor of ~400), as shown in Figure 4. Thus the sensitization mechanism would appear to be more highly favored in those complex species involving direct Tb^{3+}–phenolate binding. The high pH part of the emission intensity vs. pH plot presents a plateau. This plateau region is indicative of the stability of the Tb(EHPG) complex against $Tb(OH)_3$ formation under the pH conditions studied.

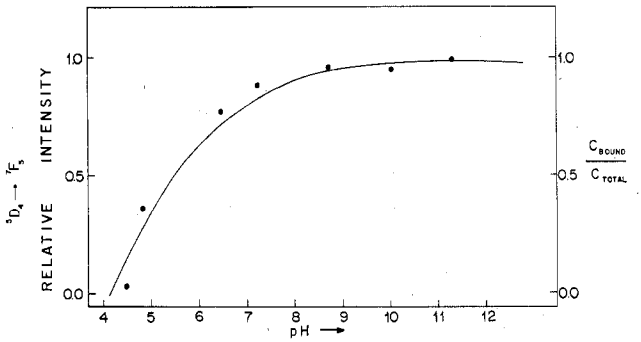

Figure 4. Left scale: Plot of Tb^{3+} $^5D_4 \rightarrow {}^7F_5$ emission intensity (—) vs. solution pH for the 1:1 Tb(EHPG) complex. Excitation wavelengths were varied to ensure constant sample absorbance over the pH range 4–12. Right scale: Plot of bound/total (free + bound) phenolate concentration ratios (experimental values plotted as ●) vs. solution pH for the 1:1 Tb(EHPG) complex as determined from the near-ultraviolet absorption spectra.

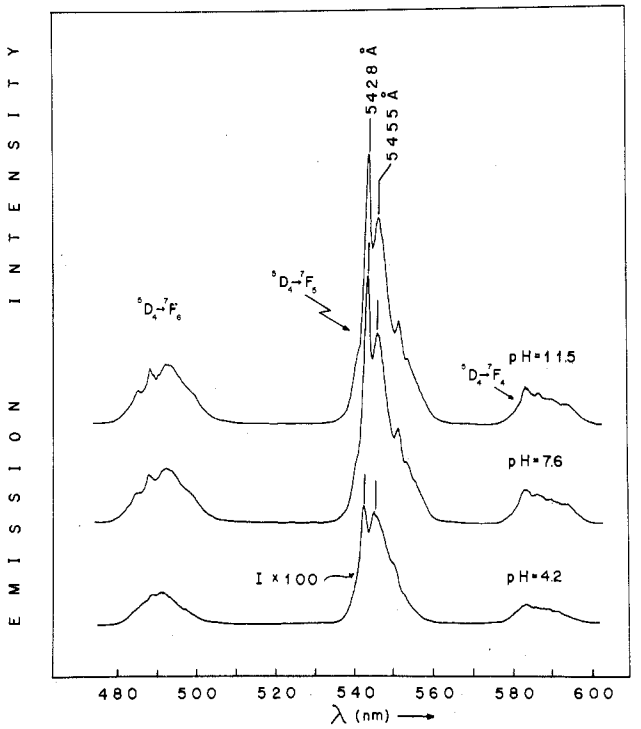

Figure 5. High-resolution emission spectra ($\Delta\lambda_{em}$ = 0.2 nm) for the 1:1 Tb(EHPG) complex at different pH values. Excitation was at 290 nm ($\Delta\lambda_{ex}$ = 10 nm) and $[Tb^{3+}]$ = [EHPG] = 10^{-4} M in each case.

Possible mechanisms for the energy transfer between the aromatic moieties of the ligand and the terbium ion will be discussed in a later section.

Terbium Luminescence Spectra. The emission spectra of Tb(EHPG) at different pH values are presented in Figure 5. The high-resolution spectra ($\Delta\lambda_{em}$ = 0.2 nm) show the terbium $^5D_4 \rightarrow {}^7F_6$, $^7F_5$, and $^7F_4$ emission bands. Each of these bands is comprised of a large number of crystal-field components, as of yet unassigned. All of the emission spectra were obtained by exciting into the aromatic moiety absorption band ca. 290 nm. The sensitization of terbium emission occurs by (a) absorption of the exciting radiation by the aromtic chromophore of the ligand phenolic groups, (b) nonradiative transfer of energy to the metal ion, and (c) nonradiative decay of the Tb^{3+} acceptor state to the Tb^{3+} emitting state ($^5D_4$).

A feature of interest in the luminescence spectra of Tb(EHPG) shown in Figure 5 can be observed upon change in the pH of the solution. At high and intermediate pH values,

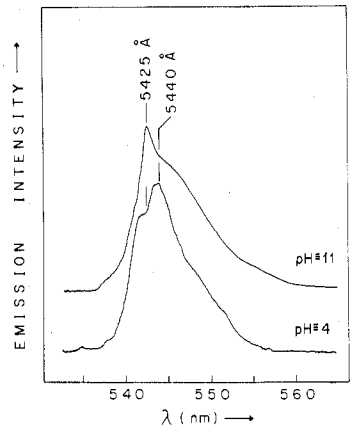

Figure 6. Emission for $TbCl_3$ in water at pH ~4 and at pH ~11. Excitation was at 488 nm (using an argon ion laser line), and the spectral slit width for the emission was $\Delta\lambda_{em}$ = 0.2 nm.

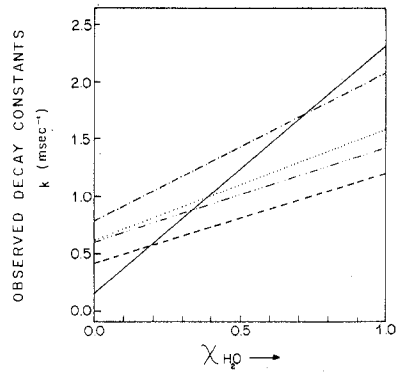

Figure 7. Plots of observed luminescence decay constants (k) vs. mole fraction of H_2O (χ_{H_2O}) in H_2O/D_2O mixtures for $TbCl_3$ (—) and for Tb(EHPG) at pH 4.1 (-·-·), pH 4.9 (···), pH 5.6 (-···-), and pH 7.0 and 8.1 (---).

the crystal-field components in the $^5D_4 \rightarrow {}^7F_5$ transition region remain unchanged. Upon protonation of ligand groups, beginning at pH 7, the second highest energy component, at 545.5 nm, gains relative intensity over the higher energy component, at 542.8 nm. At pH 4 the intensities of the two components are nearly equal. This alteration in relative intensities is also observed in the EHPG complex when terbium emission is excited directly with the 488.0-nm Ar^+ laser line (excitation in this case is directly into the terbium $^5D_4$ level). The alteration in relative emission intensities is also evident in an aqueous solution of $TbCl_3$ directly excited at 488 nm (Figure 6), where the most intense component is centered at 542.5 nm at pH 11 and at 544.0 nm at pH 4. An interpretation of the observed intensity changes in the crystal-field components of the Tb^{3+} emission spectra will be analyzed in a future publication.

Luminescence Lifetime Measurements. Lifetime measurements on terbium $^5D_4 \rightarrow {}^7F_5$ luminescence of Tb(EHPG) in H_2O/D_2O solvent mixtures were carried out as a function of χ_{H_2O} (mole fraction of H_2O in H_2O/D_2O solvent mixture) for several pH values. Plots of observed luminescence decay constants (k^{obsd}) vs. χ_{H_2O} are shown in Figure 7 for Tb(EHPG) in aqueous solution at five different pH values. At plot of k^{obsd} vs. χ_{H_2O} for $TbCl_3$ in water is also shown in Figure 7 (the solid line). The purpose of these measurements was to determine the "average" number of water molecules bound directly to the Tb^{3+} ion at a given pH. Having this number, which we shall call W, and knowing the total coordination number (CN) of the complex, we may deduce the number of EHPG ligating groups bound to the metal ion from l (number of bound ligating groups) = CN − W. Alternatively, knowing W and l,

we may deduce the total coordination number from CN = W + l.

The method we use to obtain values of W from k^{obsd} vs. χ_{H_2O} plots is similar to that first introduced by Horrocks and coworkers[8] and that used in previous studies by the present authors.[9] The method is based on an earlier observation by Kropp and Windsor[10] that the luminescence decay constant for Ln^{3+}(aquo) ions in H_2O/D_2O solvent mixtures varies linearly with the mole fraction of H_2O and that the O–H(D) oscillators of the bound H_2O (D_2O) molecules act independently in effecting radiationless deactivation of the Ln^{3+} emitting state. Furthermore, it was found that the efficiency of the H_2O (or D_2O) induced nonradiative deactivation is apparently independent of the nature and constitution of the remainder of the coordination sphere. Since the k^{obsd} for Ln^{3+}(aquo) ions in H_2O are significantly greater than the k^{obsd} for Ln^{3+}(aquo) ions in D_2O, then k^{obsd} for Ln^{3+}(aquo) ions in H_2O/D_2O mixtures should increase with χ_{H_2O}.

For the Tb(EHPG) system in a H_2O/D_2O solvent mixture the observed luminescence decay constant may be expressed as

$$k^{obsd} = k_0 + k'\chi_{H_2O} \quad (1)$$

where k_0 is that part of the decay constant which is independent of the relative amounts of H_2O and D_2O present in the solvent mixture, and k' will be shown to be related to W (the number of bound water molecules). For $TbCl_3$ in a H_2O/D_2O mixture we may write

$$k^{obsd}(\text{aquo}) = k_0(\text{aquo}) + an_{OH}(\text{aquo})\chi_{H_2O} \quad (2)$$

where we have set $k'(\text{aquo}) = an_{OH}(\text{aquo})$, $n_{OH}(\text{aquo})$ is the number of deactivating O–H oscillators in the Tb^{3+} coordination sphere, and a is a proportionality constant. Assuming that each bound water molecule is comprised of two *independent* O–H oscillators, then $k'(\text{aquo}) = 2aW(\text{aquo})$, which is just determined as the slope of a k^{obsd}(aquo) vs. χ_{H_2O} plot. Taking W(aquo) = 9 (i.e., nine water molecules bound to Tb^{3+} in aqueous $TbCl_3$ solution),[8,9] we have

$$a = k'(\text{aquo})/18 \quad (3)$$

The EHPG ligand of Tb(EHPG) has two amino ligating groups, each of which contains a hydrogen atom which is easily exchangeable with a deuterium atom from D_2O solvent molecules. The relative numbers of N–H vs. N–D oscillators in the ligand environment of the Tb^{3+} ion will, therefore, vary with χ_{H_2O}. Since the effects of ligand N–H vs. N–D oscillators upon the observed decay constants may differ considerably, for the complex k' of eq 1 must be written as

$$k'(\text{complex}) = an_{OH} + bn_{NH} \quad (4)$$

where n_{NH} is the number of deactivating N–H oscillators in the ligand environment, which should equal the number of bound EHPG amino groups N (i.e., $n_{NH} = N$). The value of k'(complex) is determined as the slope of a k^{obsd}(complex) vs. χ_{H_2O} plot, and $n_{OH} = 2W$, where W is the number of bound water molecules in the Tb(EHPG) complex. The ratio of k'(complex) to k'(aquo) is an observable in our experiments and maybe expressed as

$$r = \frac{k'(\text{complex})}{k'(\text{aquo})} = \frac{2aW + bN}{2aW(\text{aquo})} \quad (5)$$

Again setting W(aquo) = 9, this equation becomes

$$r = \tfrac{1}{9}W + \tfrac{1}{18}(b/a)N \quad (6)$$

Table I. Luminescence Decay Results and Analysis of Tb(EHPG) Complexes

pH	r^a	P^b	N^c	CN^d	CN^e
4.1	0.60	0.0	0.6	7.8	7.1
4.9	0.46	0.7	1.6	8.0	7.8
5.6	0.40	1.1	1.9	8.0	8.0
7.0	0.35	1.7	2.0	8.2	8.2
8.1	0.32	1.9	2.0	8.2	8.2

[a] Ratio of slopes of observed luminescence decay constants vs. mole fraction H_2O in H_2O/D_2O mixtures for Tb(EHPG) complexes to aqueous Tb^{3+}. [b] Number of bound phenolate groups. [c] Number of bound amino groups. [d] Coordination number calculated according to eq 11 with $C = 2$ for all pHs >4. [e] Coordination number calculated according to eq 12.

The ratio b/a reflects the relative efficiencies of an N–H oscillator (on the EHPG ligand) vs. an O–H oscillator (on a bound water molecule) in promoting radiationless deactivation of the terbium $^5D_4$ emitting state. Rearranging eq 6, the number of bound water molecules in the complex is given by

$$W = 9r - \tfrac{1}{2}(b/a)N \quad (7)$$

Alternatively, setting CN = W + l, the total coordination number of the complex is given by

$$\text{CN} = 9r + l - \tfrac{1}{2}(b/a)N \quad (8)$$

where l = the number of EHPG ligating groups bound to the metal ion.

For an estimate of the value of b/a, an independent set of k^{obsd} vs. χ_{H_2O} experiments were carried out for Tb^{3+} complexes with iminodiacetic acid (IDA) and *N*-methyliminodiacetic acid (MIDA) at various pH values. The IDA and MIDA ligands differ only by the presence of a methyl substituent on the imino nitrogen in MIDA, where the IDA ligand only has a hydrogen atom. Each IDA ligand has, therefore, a single N–H oscillator, whereas MIDA has no N–H oscillators. The details of the studies on these systems are reported in a separate communication.[11] However, a comparison of the luminescence decay properties for Tb(IDA) and Tb(MIDA) in H_2O/D_2O mixtures yielded a value of ~0.7 for b/a. That is, an N–H oscillator was found to be approximately 70% as efficient as an O–H oscillator in radiationlessly deactivating the $^5D_4$ emitting state of the Tb^{3+} ion. It may be assumed that the relative efficiencies of an N–D oscillator and an O–D oscillator are also ~0.7.

Assuming a value of 0.7 for (b/a), eq 7 and 8 may be rewritten as

$$W = 9r - 0.35N \quad (9)$$

and

$$\text{CN} = 9r + l - 0.35N \quad (10)$$

Letting $l = C + P + N$, where C is the number of bound carboxylate groups and P is the number of bound phenolate groups, eq 10 may be reexpressed as

$$\text{CN} = 9r + C + P + 0.65N \quad (11)$$

The value of r can be determined from the k^{obsd} vs. χ_{H_2O} data of Figure 7. Values of r obtained at five different pH values are listed in the second column of Table I. The values of P and N are pH dependent and may be determined by considering the emission intensity curve in Figure 4 and the potentiometric titration curve in Figure 2. Values of P and N so determined appear in the third and fourth columns of Table I. Now if we assume that $C = 2$ for all pHs >4 (both carboxylate groups are bound), then the total coordination num-

(8) (a) W. D. Horrocks, Jr., G. F. Schmidt, D. R. Sudnick, C. Kittrell, and R. A. Bernheim, *J. Am. Chem. Soc.*, **99**, 2378 (1977); (b) W. D. Horrocks, Jr., and D. R. Sudnick, *ibid.*, **101**, 334 (1979).

(9) S. Salama and F. S. Richardson, *Inorg. Chem.*, preceding paper in this issue.

(10) J. L. Kropp and M. W. Windsor, *J. Phys. Chem.*, **71**, 477 (1967).

(11) S. Salama and F. S. Richardson, *J. Phys. Chem.*, in press.

bers may be calculated from eq 11 to yield the values listed in the fifth column of Table I.

For the Tb(EHPG) complex from pH 4 to 8, the calculated coordination number in Table I is a remarkably constant 8.0 ± 0.2. Thus coordination of oxygens from two carboxylate groups, already ligated at pH 4, is sufficient to reduce the terbium coordination number from 9 in the aqueous complex to 8 in the EHPG complexes. Coordination of amino and phenolate groups as the pH is increased to 8 does not result in a further decrease in coordination number. At pH 8, where EHPG is hexadentate, two water molecules complete the coordination sphere about terbium. A corollary of the constant coordination number 8 listed in Table I for the Tb(EHPG) complexes is the assignment of coordination number 9 to aqueous Tb^{3+}. Assignment of the lesser number 8 to the aqueous metal ion would yield unreasonably low values for the EHPG complex at low pH values.

An alternative analysis to that given above is suggested by the possibility that only one carboxylate group of the EHPG ligand is bound at pH <4. For EHPG to bind in a bidentate fashion to Tb^{3+} through two Tb^{3+}–carboxylate linkages requires formation of an 11-membered chelate ring with quite bulky bridging groups. Formation of such a chelate ring would be highly unlikely unless the Tb^{3+}–carboxylate binding constants were uncharacteristically very large in the Tb^{3+}/EHPG system. If we assume instead that EHPG binds unidentate to Tb^{3+} at pH <4 via one Tb^{3+}–carboxylate linkage and that the second carboxylate group only binds simultaneously with amino group binding over the pH range 4–6, then eq 11 may be modified to

$$CN = 9r + 1 + P + 1.15N \qquad (12)$$

where C in eq 11 has been set equal to $1 + 0.5N$. The values of CN calculated according to eq 12 are listed in the last column of Table I.

Another feature of the data presented in Figure 7 which deserves some comment is the spread of k^{obsd} values at $\chi_{H_2O} = 0$. The left-hand ($\chi_{H_2O} = 0$) intercepts of Figure 7 show that the k_0 of eq 1 is not a constant but varies with solution pH and the type of complex species present. The decay constant k_0 includes all radiative and nonradiative contributions to k^{obsd} which are independent of differences between N–H vs. N–D and O–H vs. O–D oscillators in the ligand environment of the Tb^{3+} ion. It is expected, therefore, that k_0 should be very sensitive to the symmetry and detailed physical nature of the crystal field created about the metal ion by the ligand donor groups. A quantitative analysis of these observed k_0 values is beyond the scope of the present study.

Phenolate-to-Terbium Energy Transfer. The final point in the discussion of our results concerns sensitization of terbium emission via energy transfer from the aromatic chromophores of the EHPG ligand. The data plotted in Figure 4 clearly demonstrate the strong pH dependence of terbium emission intensity in the Tb(EHPG) complex. The simplest interpretation of this data in structural terms is that the emission intensity (and, therefore, the efficiency of aromatic chromophore-to-terbium energy transfer) closely follows the binding of the phenolate donor moieties to Tb^{3+}. The extent of direct Tb^{3+}–phenolate binding increase with increasing pH, and the terbium emission intensity also increases with increasing pH. This implies that the efficiency of the sensitization process (or energy-transfer mechanism) is greatly enhanced when the phenolate donor moieties are bound to the terbium ion. There are a number of ways this can be understood in terms of a simple Förster[12] –Dexter[13] resonance energy-transfer model based on Coulombic, nonexchange interactions between the donor (phenolate) and acceptor (Tb^{3+}) species. With the assumption that the donor–acceptor interaction mechanism is dipole–dipole, the efficiency of energy transfer according to this model is proportional to R_{da}^{-6} (where R_{da} is the separation distance between the donor and acceptor species), to the relative orientations of the interacting transition dipoles, to the luminescence quantum yield of the donor species (Φ_d) in the absence of energy transfer, and to a spectral overlap factor defined by

$$J = \left[\int F_d(\bar{\nu})\epsilon_a(\bar{\nu})\, d\bar{\nu}\right] \Big/ \bar{\nu}^4 \qquad (13)$$

where $\epsilon_a(\bar{\nu})$ is the molar extinction coefficient of the acceptor species, $F_d(\bar{\nu})$ is a normalized spectral distribution function describing donor luminescence, and $\bar{\nu}$ is the frequency variable expressed in wavenumbers.

It is not clear how Φ_d and J will be altered by deprotonation of the phenolic group and by Tb^{3+}–phenolate binding. However, it *does* seem likely that the transition dipole induced in the phenolate group by photoexcitation in the 270–300 nm region will lie closer to the Tb^{3+} acceptor when the phenolate group is bound vs. when it is not bound. This decrease in R_{da} upon Tb^{3+}–phenolate binding should, then, lead to enhanced energy transfer and terbium emission sensitization. Tb^{3+}–phenolate binding would also tend to "fix" the relative orientations of the interacting donor and acceptor transition dipoles.

Enhanced emission sensitization via phenolate-to-Tb^{3+} energy transfer when the phenolate group is directly bound to Tb^{3+} can also be rationalized in terms of an exchange mechanism. According to this mechanism, the efficiency of energy transfer is in large part governed by an "overlap transition density" involving donor and acceptor orbitals on the respective donor and acceptor species in the energy-transfer couple. This "overlap transition density" is generally believed to exhibit an exponential dependence upon R_{da}. Clearly the efficiency of energy transfer according to this model should be greater for the case where Tb^{3+} and the phenolate groups are bound, compared to the case where they remain unbound.

Conclusions

In this study we have used a combination of spectroscopic measurements and potentiometric titration data to characterize the coordination properties of the ligand EHPG with Tb^{3+} in aqueous solution under various pH conditions. Emission lifetime and emission excitation/intensity measurements proved to be especially valuable in following the formation of the Tb(EHPG) complex and in determining, qualitatively, the structural features of the complex at various pH values. The absorption, emission, and potentiometric titration data led to self-consistent interpretations of structure, and the luminescence lifetime results permitted determination of total coordination numbers for the Tb(EHPG) complex. Furthermore, the interpretation of the near-ultraviolet excitation spectra in terms of phenolate binding and phenolate-to-Tb^{3+} energy transfer would appear to be secure, although no definite conclusions can be drawn regarding the mechanism of the energy-transfer process.

It must be emphasized that the spectroscopic measurements performed in this study were designed to probe the formation and structural characteristics of *bound* complexes. No conclusions can be drawn from the data regarding binding constants or the details of complex equilibria.

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