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Complexation of Amino Acids by Terbium(III) Ethylenediaminetetraacetate

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Received June 14, 1985

The solution-phase coordination chemistry associated with ternary Tb(EDTA) complexes with 27 amino acids has been studied by means of circularly polarized luminescence spectroscopy. Above pH 8, it was found that all aliphatic amino acids could bind Tb(III) in a bidentate manner employing the ionized carboxylate and ammonium groups. Two situations were detected for multifunctional amino acids. When the α-amino carboxyl group and another potential complexation site were close together, then chelation between the terminal functionality and the ionized carboxylate was found to be the dominant bonding mode. When that type of bonding would require the existence of a large chelate ring, then coordination took place solely at the α-amino carboxyl position. Systematic variation in the concentration of the amino acids permitted the computation of formation constants for the ternary Tb(EDTA)(AA) complexes.

Introduction

Since amino acids are the constituents from which proteins are built, the complexes formed by metal ions and amino acids have served as model systems for metalloproteins. Recently, the members of the lanthanide series have found extensive use as spectroscopic probes of metal ion binding sites in Ca(II)-binding proteins,[1,2] and particularly useful results have been obtained from studies of the intrinsic luminescence associated with Eu(III) and Tb(III) complexes.[3,4] Unfortunately, the interaction between lanthanide ions and amino acids are exceedingly weak. Below neutral pH, amino acids only interact via the ionized carboxyl group, and this interaction is weakened (relative to that of simple carboxylates) due to the presence of the positively charged amino groups. Raising the solution pH above neutral normally results in precipitation of the lanthanide hydroxide, since the amino acids bind far more weakly than the hydroxide ion. Consequently, essentially all studies of lanthanide ion interactions with simple amino acids have been carried out at low pH values,[5] and the observed effects are not necessarily related to those existing under physiological conditions.

One may eliminate precipitation of lanthanide hydroxides above neutral pH values, by working with mixed-ligand complexes. A suitable multidentate chelating agent is used to minimize complex hydrolysis reactions. As long as coordination positions are still available on the lanthanide ion, one may form ternary complexes. Besides the elimination of any solubility problem, one also greatly simplifies the complex stoichiometry. The complexation chemistry of a wide variety of lanthanide–ligand interactions has been studied through circularly polarized luminescence (CPL) studies of such ternary complexes.[6]

In an earlier work, it was shown that lanthanide ions could form chelates with α-amino acids at elevated pH values.[7] Lanthanide-induced-shift data have also been presented which indicate that alanine will chelate lanthanide ions at the α-amino carboxyl functionality after deprotonation of the ammonium group.[8] In the present work, we have used CPL spectroscopy to study the complexation phenomena associated with the interaction of simple amino acids with chelated Tb(III) complexes. Since the chirality of a Tb(III) species has been found to be exceedingly sensitive toward details of the metal–ligand bonding,[6] one would anticipate that CPL investigations of lanthanide amino acid complexes should enable determinations to be made regarding the nature of the interactions.

Experimental Section

All ligands were obtained from either Aldrich, Sigma, or Eastman and were used as received. Stock solutions of terbium perchlorate were prepared by dissolving the 99.9% oxide (Research Chemicals) in a stoichiometric amount of 70% $HClO_4$, neutralizing to pH 3.5 with NaOH, and then diluting to the desired volume. In all systems, ethylenediaminetetraacetic acid (EDTA) was used as the achiral ligand, and consequently all work concerns the interaction of amino acids with the Tb(EDTA) complex. Varying amounts of amino acid substrates were weighed directly in the fluorescence cuvette, and these materials were dissolved directly in the Tb(EDTA) complex solution. In this fashion, variation in the Tb(EDTA):amino acid ratio could be effected in a systematic manner. In solutions used for the CPL studies, the Tb(EDTA) concentration was 14 mM and the Tb(EDTA):amino acid ratios varied between 1:1 and 1:30. The pH of these solutions was varied between 3.0 and 11.0.

All CPL measurements were obtained in apparatus constructed in our laboratory. In all cases, a 1000-W Xe arc lamp was used as the excitation source, with an excitation wavelength of 365 nm (selected by a 0.1-m grating monochromator) being used throughout. Most CPL measurements were obtained within the $^5D_4 \rightarrow {}^7F_5$ Tb(III) band system. It is been established that of all the Tb(III) emission bands, this particular transition exhibits the highest degree of both total and circularly polarized emission.[9] The emission was analyzed by a 0.5-m grating monochromator at 4-Å resolution, and it was determined that further increases in resolving power did not yield a significant improvement in the spectral features.

Variation of the solution pH within the solutions was effected by the addition of microliter amounts of standard $HClO_4$ or NaOH directly to the fluorescence cuvette. The pH was obtained with a glass microcombination electrode, which could be inserted directly into the cuvette, and read on an Orion 701A pH meter. The system was calibrated daily with phosphate buffers and acetate buffers.

Results and Discussion

Addition of 1 equiv of EDTA to a Tb(III) solution results in complete formation of the 1:1 complex

$$Tb^{3+} + EDTA^{4-} \rightleftharpoons Tb(EDTA)^- \quad (1)$$

since the formation constant governing this reaction has been determined as $\log K = 17.92$.[10] Unlike transition-metal complexes, the lanthanide amino polycarboxylate compounds are known not to be coordinatively saturated. Horrocks and Sudnick have used spectroscopic techniques to determine the number of water molecules remaining bound by the lanthanide ion[11] and have determined that three coordinated waters still remain on the Tb(III) ion after the EDTA becomes fully bound.

In the Tb(EDTA) compound, the coordinated solvent is labile and may easily be replaced by more strongly binding ligands. This ability has led to the use of the Ln(EDTA) compounds as aqueous NMR shift reagents.[12] When the ternary ligand is an amino acid

(1) Williams, R. J. P. *Struct. Bonding (Berlin)* **1982**, *50*, 79.
(2) Martin, R. B. In "Calcium in Biology"; Spiro, T. G., Ed.; Wiley: New York, 1983. Chapter 6.
(3) Horrocks, W. DeW., Jr.; Sudnick, D. R. *Acc. Chem. Res.* **1981**, *14*, 384.
(4) Richardson, F. S. *Chem. Rev.* **1982**, *82*, 541.
(5) Reuben, J. In "Handbook on the Physics and Chemistry of Rare Earths"; Gschneider, K. A. Jr., Eyring, L., Eds.; North-Holland: Amsterdam, 1979; Vol. 4, Chapter 39.
(6) Brittain, H. G. *Coord. Chem. Rev.* **1983**, *48*, 243.
(7) Brittain, H. G. *J. Am. Chem. Soc.* **1980**, *102*, 3693.
(8) Sherry, A. D.; Stark, C. A.; Ascenso, J. R.; Geraldes, C. F. G. C. *J. Chem. Soc., Dalton Trans.* **1981**, 2078.
(9) Richardson, F. S. *Inorg. Chem.* **1980**, *19*, 2806.
(10) Martell, A. E.; Smith, R. M. "Critical Stability Constants"; Plenum Press: New York, 1974; Vol. 1.
(11) Horrocks, W. DeW., Jr.; Sudnick, D. R. *J. Am. Chem. Soc.* **1979**, *101*, 334.

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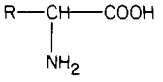
(represented generically as AA), the formation of ternary complexes would be given by

$$Tb(EDTA)^- + AA^- \rightleftharpoons Ln(EDTA)(AA)^{2-} \quad (2)$$

Since the amino acid substrates are inherently chiral, formation of the ternary complex will produce measurable optical activity. This optical activity can arise from either vicinal or conformational contributions, and the exact nature of the interaction will depend on the mode of coordination experienced by the amino acid ligand. When the amino acid binds to the Tb(III) ion in a monodentate fashion, then the Tb(III) will experience a sole vicinal effect. This chirality arises when a dissymmetric atom is bound somewhere near the Tb(III) ion and has been shown to be exceedingly weak in magnitude.[13] Should the amino acid form a chelate ring containing the dissymmetric carbon, then additional chirality due to conformational effects will exist. These effects (arising since the mirror images of the chelate ring are nonsuperimposable) have been found in many studies to be much stronger than simple vicinal effects, and the CPL spectra associated with conformational effects also exhibit drastically different line shapes.[6]

Thus, it may be concluded that the appearance of exceedingly weak, single-signed CPL within the $^5D_4 \rightarrow {}^7F_5$ Tb(III) emission band indicates the presence of sole vicinal effects,[13] and this situation would imply the presence of simple monodentate amino acid coordination. On the other hand, the observation of strong, multiple-signed CPL within the same Tb(III) band system indicates the existence of conformational effects.[6] These can only arise if the amino acid binds Tb(III) in a bidentate manner and if the dissymmetric carbon is part of the chelate ring. This condition could only arise after deprotonation of the ammonium group, and should strong CPL accompany this deprotonation, then the formation of an amino acid chelate ring is demonstrated.

A. Aliphatic Amino Acids. The first sequence of chiral amino acid ligands to be studied were the simple aliphatic amino acids. The particular substrates used were (*S*)-alanine (ALA), (*R*)-2-aminobutyric acid (ABA), (*S*)-norvaline (NVAL), (*S*)-valine (VAL), (*S*)-norleucine (NLEU), (*S*)-leucine (LEU), and (*S*)-isoleucine (ILEU).



R = CH_3	ALA
= CH_2-CH_3	ABA
= CH_2-CH_2-CH_3	NVAL
= $CH(CH_3)$-CH_3	VAL
= CH_2-CH_2-CH_2-CH_3	NLEU
= CH_2-$CH(CH_3)$-CH_3	LEU
= $CH(CH_3)$-CH_2-CH_3	ILEU

Essentially no optical activity could be detected in the Tb(III) emission bands below neutral pH, but above pH 8 reasonably strong CPL was measurable. Selected examples of the observed CPL spectra are shown in Figure 1. Replacement of the *S* enantiomer by the *R* enantiomer resulted in the generation of mirror-image CPL spectra, but the absolute magnitudes of the CPL were comparable.

The double-signed CPL spectra of Figure 1 (and the dissymmetry factors calculated for the bands) are indicative that the Tb(III) chirality is a result of conformational effects. Thus, the CPL data provide direct evidence that α-amino acids *are* capable of forming chelate rings with lanthanide ions.

Considerable insight into the complexation phenemona is available from the pH dependence of the CPL intensities. Essentially no CPL is observed below pH 7.5, but by pH 8.0 quite strong optical activity is noted. The CPL intensities (as measured by the dissymmetry factors) reach a limiting value by pH 9.0 and then generally remain constant until pH 10.0. Between pH 10.5 and 11.5, one observes a definite decrease in the degree of optical activity. The onset of the CPL is certainly associated with the deprotonation of the ammonium group. For most common amino

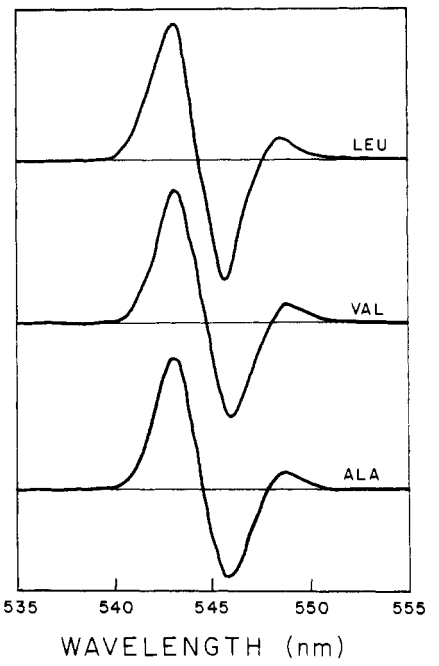


Figure 1. Circularly polarized luminescence spectra for the Tb(EDTA) complexes with (*S*)-alanine, (*S*)-valine, and (*S*)-leucine. The data were obtained at pH 10.5 for Tb:EDTA:AA ratios of 1:1:10.

acids, this deprotonation is characterized by a p*K* of approximately 9.5.[10] That the CPL is quite strong below pH 9 indicates that the metal ion can assist in the deprotonation step. Above pH 10.5, appreciable hydrolysis of the complex occurs, and the coordinated amino acid can be displaced. In an earlier work, it has been shown that hydrolysis of lanthanide amino polycarboxylate compounds takes place above pH 10, and these processes are characterized by the formation of oligomeric complexes.[14] The formation of these polymeric compounds would result in the expulsion of the amino acid ligand, if the formation constant of the $[Tb(EDTA)]_x$ compound exceeded that of the Tb(EDTA)(AA) complex.

Systematic variation in the concentration of amino acid ligand resulted in CPL intensities that were dependent on the concentration of that ligand. At sufficiently high concentrations of amino acid, a limiting dissymmetry constant could be reached (within the pH 9.0–10.5 region). Knowledge of that limiting ratio and the concentration of all reactants permits the computation of the complex association constant corresponding to eq 2. The mole fraction of uncomplexed Tb(EDTA), X_i, is given by

$$X_i = (g_f - g_i)/g_f$$

where g_f = the limiting dissymmetry factor, and g_i is the dissymmetry factor at a given amino acid concentration. If Tb_a and AA_a represent the initial concentrations of the Tb(EDTA) complex and amino acid, respectively, then

$$[Tb(EDTA)] = (Tb_a)(X_i)$$
$$[Tb(EDTA)(AA)] = Tb_a - [Tb(EDTA)]$$
$$[AA] = AA_a - [Tb(EDTA)(AA)]$$

The complex formation constant corresponding to eq 2 is given by

$$K = \frac{[Tb(EDTA)(AA)]}{[Tb(EDTA)][AA]}$$

Formation constants calculated in this fashion have been collected in Table I.

The apparent formation constants all varied between 20 and 40 L/mol, indicative of fairly weak complexation. However, with large excesses of amino acid substrate, the complexation was observable in every case. For the most hydrophobic substrates,

(12) Elgavish, G. A.; Reuben, J. *J. Am. Chem. Soc.* **1976**, *98*, 4755.
(13) Brittain, H. G. *Inorg. Chim. Acta* **1981**, *53*, L7.
(14) Spaulding, L.; Brittain, H. G. *Inorg. Chem.* **1983**, *22*, 3486.

Table I. Apparent Formation Constants for the Interaction of Amino Acids with Tb(EDTA)[a]

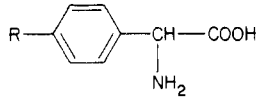
amino acid	K	amino acid	K
alanine	18.2	threonine	304
2-aminobutyric acid	29.9	homoserine	1245
norvaline	36.1	cysteine	1170
valine	26.1	methionine	36.2
norleucine	33.6	cystine	18.8
leucine	21.4	2,4-diaminobutyric acid	192
isoleucine	23.3		
phenylglycine	21.4	ornithine	20.2
(*p*-hydroxyphenyl)-glycine	24.4	lysine	27.1
		arginine	18.3
phenylalanine	16.6	asparagine	1220
tyrosine	1855	glutamine	41.9
aspartic acid	1340	citrulline	20.4
glutamic acid	66.9	histidine	1020
serine	373		

[a] All formation constants were obtained at pH 10.5 and thus correspond to the addition of the anionic ligand forms to the $Tb(EDTA)^-$ complex. Each value is significant to three figures.

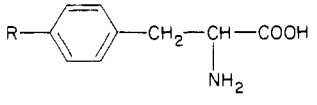
solubility constraints prevented the acquisition of data for the zwitterionic form of the amino acid. Association constants for the interaction of lanthanide ions with simple amino acids have been determined from NMR methods, and these are significantly smaller than those just reported. Tanner and Choppin used a solvent extraction method to determine that for Eu/glycine, K = 4.1 L/mol.[15] Sherry et al. studied the Nd/alanine complex and reported values of K = 6.5 L/mol (NMR method) and K = 4.4 L/mol (potentiometric titration method).[16] These studies were carried out at low pH values where the amino acid existed in the zwitterionic form. The higher magnitude of the formation constants determined by the CPL data may be taken as a reflection of the additional stability resulting from formation of the amino acid chelate ring structure.

It had been determined earlier that within this pH range, the association constant for the formation of polynuclear $[Tb(EDTA)]_x$ compounds was about 1000 L/mol.[14] This value is considerably larger than the association constants of the amino acids, and consequently one would not expect these substrates to be able to prevent the oligomerization of the Tb(EDTA) complexes. This observation provides further support that the loss of CPL at the highest pH values is due to formation of the oligomeric $[Tb(EDTA)]_x$ complexes and expulsion of the AA ligands.

B. Phenyl Amino Acids. Two amino acid substrates are available that contain a phenyl group as part of the aliphatic side chain, and each of these can be substituted with a hydroxyl group at the para position. One can thus obtain (*R*)-phenylglycine (PG), and (*R*)-(hydroxyphenyl)glycine (HPG), (*S*)-phenylalanine (PALA), and (*S*)-tyrosine (TYR).



R=H PG
=OH HPG



R=H PALA
=OH TYR

Solubility problems prevented extensive concentration studies from being performed, but data could be obtained above pH 10 in most cases. The CPL spectra obtained for Tb(EDTA)(PG), Tb(EDTA)(HPG), and Tb(EDTA)(PALA) were all found to be

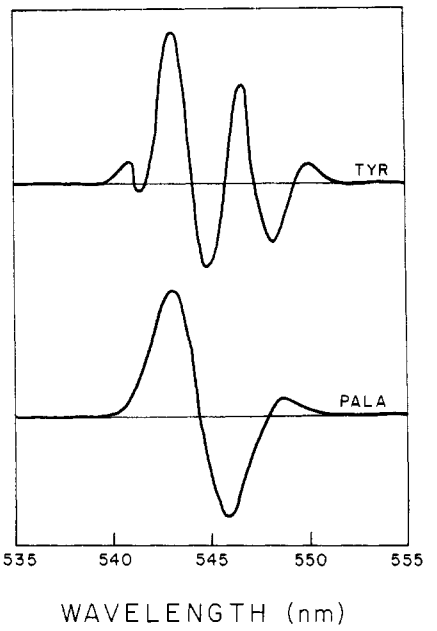


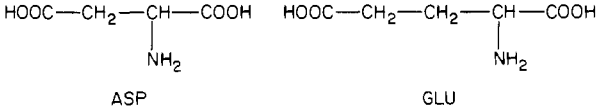
Figure 2. Circularly polarized luminescence spectra for the Tb(EDTA) complexes with (*S*)-phenylalanine and (*S*)-tyrosine. The data were obtained at pH 10.5 for Tb:EDTA:AA ratios of 1:1:10.

essentially superimposable with those of Tb(EDTA)(ALA), thus demonstrating that complexation at the α-amino carboxyl group was the sole coordination mode for these ligands. The complex formation constants were generally reduced with respect to the aliphatic amino acids, which would indicate that the steric bulk of the phenyl side chain was capable of interfering somewhat with the complexation process. The constants were also significantly smaller than that of the $[Tb(EDTA)]_x$ oligomer, and hence these substrates could not prevent formation of the polynuclear complex. Since the optical activity decreased drastically above pH 11, such behavior is in accord with the formation constant trends.

Quite different behavior was noted for the Tb(EDTA)(TYR) complex. A representative CPL spectrum for this compound is contrasted with that of Tb(EDTA)(PALA) in Figure 2. It is immediately clear that the nature of the Tb(III) chirality is completely different in the TYR complex. The CPL spectra persist without appreciable change up to pH 12, indicating that the oligomer formation is completely inhibited. Very small quantities of TYR ligand were required to reach a limiting dissymmetry, and from such studies a formation constant of 1855 L/mol was deduced.

Two observations permit an explanation for these observed trends. The hydroxyl group of tyrosine is certainly implicated in the bonding, and the extra methylene group in the aliphatic side chain (relative to HPG) is also important. A consideration of models reveals that no chelate ring can be formed that involves the ionized carboxyl and phenoxy groups. It is therefore concluded that the TYR ligand bridges two Tb(EDTA) complexes, using the ionized phenolate group on one end and the α-amino carboxyl group on the other. This possibility is reasonable within the pH range studied, since pK = 10.1[10] for the phenolate group. In the Tb(EDTA)(HPG) complex, the chain length of HPG is too short to permit such bridging, and steric interferences among the chelated EDTA ligands prevent such interactions.

C. Carboxy Amino Acids. The two carboxy amino acids, (*S*)-aspartic (ASP) and (*S*)-glutamic (GLU) acids, represent coordinating ligands for which chelation of lanthanide ions between the terminal carboxyl groups is possible. The lanthanide com-



ASP GLU

plexes of these ligands have been studied by means of NMR and luminescence spectroscopies,[17-19] and it is well established that

(15) Tanner, S. P.; Choppin, G. R. *Inorg. Chem.* **1968**, *7*, 2046.
(16) Sherry, A. D.; Yoshida, C.; Brinbaum, E. R.; Darnall, D. W. *J. Am. Chem. Soc.* **1973**, *95*, 3011.

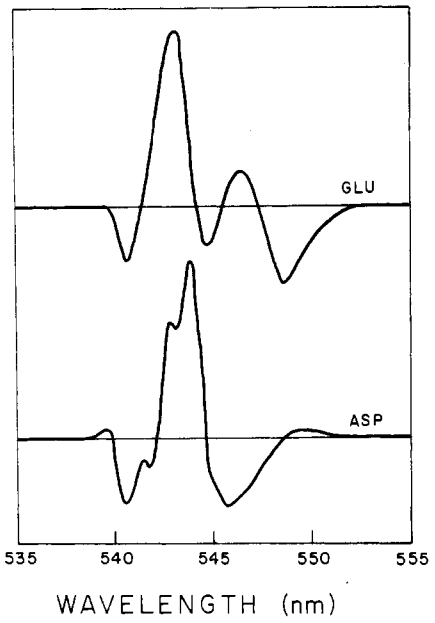


Figure 3. Circularly polarized luminescence spectra for the Tb(EDTA) complexes with (*S*)-aspartic acid and (*S*)-glutamic acid. The data were obtained at pH 10.5 for Tb:EDTA:AA ratios of 1:1:10.

ASP coordinates lanthanide ions solely between the ionized carboxylate groups. A detailed work has appeared treating the optical activity of mixed-ligand Tb(III) complexes with aspartate,[20] which confirmed that the ASP ligand would act as a bidentate chelate. No CPL spectra have been reported for the analogous complexes of glutamate. In Figure 3, the CPL spectra obtained for Tb(EDTA)(ASP) and Tb(EDTA)(GLU) are shown. Although the spectra are qualitatively similar, the dependences of CPL intensities with the substrate concentration and pH were found to be quite different.

For Tb(EDTA)(ASP), the CPL spectra were essentially invariant between pH 4 and 12. Quite low ASP concentrations were needed to reach limiting dissymmetry factors, and consequently these observations indicate that the association constant is quite high. In our previous work, it was reported that g_{lum}(limiting) = +0.0578 at the 544-nm CPL peak and that the ternary formation constant was 1340 L/mol.[20] This value is higher than the formation constant of the $[Tb(EDTA)]_x$ oligomer, and consequently one would predict that the coordinated ASP could not be displaced from the Tb(III) coordination sphere above pH 10.5 (where the oligomer formation becomes important). This result is exactly what was observed in the CPL spectra, which did not diminish in intensity even at pH 12.

For Tb(EDTA)(GLU), the behavior of the same parameters was found to differ. Essentially no CPL was observed below pH 8, but with sufficient substrate strong CPL would be observed above pH 8. This observation would imply that deprotonation of the ammonium group is essential for the observation of CPL in the Tb(EDTA)(GLU) compound. The major peak in the CPL spectra occurred at the same wavelength as the major positive CPL peak in the alanine-type CPL and not at the wavelength associated with the ASP derivative. The remainder of the CPL spectrum consists of a complicated series of peaks, which appear to represent the superimposition of another CPL line shape onto that reminiscent of alanine. Taken together, these results would indicate that coordination at the α-amino carboxyl group is essential for GLU complexation but that some additional bonding contribution with the γ-carboxyl group is also possible.

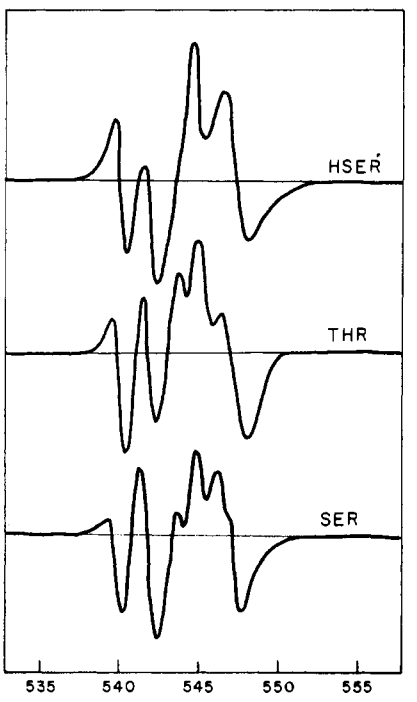
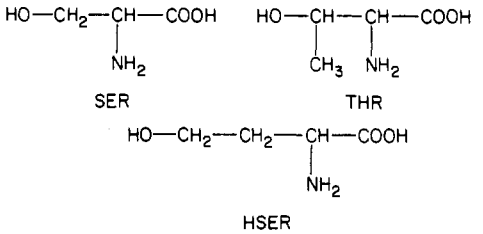


Figure 4. Circularly polarized luminescence spectra for the Tb(EDTA) complexes with (*S*)-serine, (*S*)-threonine, and (*S*)-homoserine. The data were obtained at pH 10.5 for Tb:EDTA:AA ratios for 1:1:10.

Any extra involvement of a ligating group should enhance the stability of the GLU complex relative to those of the alanine-type ligands. This prediction was verified through calculation of the formation constant for the GLU complex (Table I), which was found to be at least twice that characteristic of the aliphatic amino acid ligands. The magnitude of the GLU constant was also very much smaller than that of the ASP complex, indicating that the dicarboxylate contribution was much less. This effect is certainly due to the fact that the GLU must form a larger chelate ring than ASP and that ring structure is certainly less stable.

D. Amino Acids Containing Hydroxyl or Sulfur Functionalities. A series of potentially multidentate chelating amino acids may be obtained through the introduction of oxygen or sulfur heteroatoms onto the backbone of the aliphatic amino acids. The addition of a hydroxyl group produces (*S*)-serine (SER), (*S*)-threonine (THR), or (*S*)-homoserine (HSER). Very little work



has concerned the lanthanide complexes of these amino acids, other than the NMR work of Sherry et al.[16] At low pH values, the interaction of SER with Nd(III) was very weak (K = 12.6 L/mol) and the interaction of Nd(III) with THR was found to be even weaker (K = 7.6 L/mol). In a previous work, it was noted that both SER and THR were capable of forming chelate rings in Tb(III) mixed-ligand complexes containing dipicolinic acid.[7]

Upon binding to Tb(EDTA), all three hydroxy amino acids were found to lead to the generation of strong optical activity within the Tb(III) emission bands. As may be seen in Figure 4, these CPL patterns were all extremely complicated. The lack of any similarity to the spectra obtained with alanine-type ligands indicates that sole coordination at the α-amino carboxyl group is not the predominant binding mode. The lack of similarity between the spectra of Figure 4 and those obtained with known

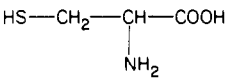
(17) Legendziewicz, J.; Kozlowski, H.; Burzala, E. *Inorg. Nucl. Chem. Lett.* **1978**, *14*, 409.
(18) Legendziewicz, J.; Huskowska, E.; Kozlowski, H.; Jezowska-Trzebiatowska, B. *Inorg. Nucl. Chem. Lett.* **1981**, *17*, 57.
(19) Legendziewicz, J.; Huskowska, E.; Strek, W.; Jezowska-Trzebiatowska, B. *J. Lumin.* **1981**, *24/25*, 819.
(20) Brittain, H. G. *Inorg. Chim. Acta* **1983**, *70*, 91.

terdentate ligands indicates that the major complexation takes place between the carboxyl and hydroxyl groups.

The pH dependence of the observed Tb(III) CPL spectra differed somewhat with the amino acid used. The optical activity of the Tb(EDTA)(SER) and Tb(EDTA)(THR) compounds was found to appear around neutral pH, maximized around pH 10, and then rapidly decreased at pH values above pH 10.5. These observations indicate relatively weak binding of the SER and THR ligands, since these can be expelled from the Tb(III) coordination sphere at the elevated pH values for which the $[Tb(EDTA)]_x$ oligomers form. On the other hand, the CPL associated with Tb(EDTA)(HSER) did not decrease significantly even at pH 12. This behavior is indicative of very strong binding and that the complex formation constant must exceed that of the $[Tb(EDTA)]_x$ oligomer. These predictions are in exact accord with the computed results, which may be found in Table I. The binding constant for the HSER ligand is considerably larger than those of the SER or THR ligands, and larger than the value known for the $[Tb(EDTA)]_x$ oligomer.[14]

It is highly unlikely that deprotonation of the hydroxyl group has taken place in any of these complexes, since the pK of this group is expected to be extremely high. One would conclude that the hydroxyl group actually hydrogen bonds to a water molecule that still remains coordinated by the Tb(EDTA) complex. The low stability of the SER and THR complexes (relative to that of the HSER complex) reflects the smaller ring sizes that result when these compounds form chelate rings. The ring size of the HSER ligand is apparently quite favorable and lends a large degree of stability to the complexation process.

The substitution of a sulfhydryl group generates (*S*)-cysteine (CYS), which may be considered as being analogous to serine.

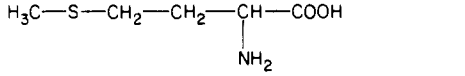


CYS

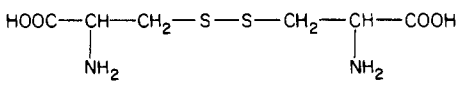
The sulfhydryl group can be deprotonated at relatively low pH values, since its pK = 8.2.[10] Sulfur functionalities are considered as being "soft" bases and generally are assumed not to be capable of binding to "hard" lanthanide ions in aqueous solution.

In fact, formation of the Tb(EDTA)(CYS) ternary complex was found to be quite favorable above pH 8. The CPL spectra shown in Figure 5 were found to be essentially pH invariant up to pH 12, and this trend is indicative of quite strong chelation processes. The CPL appears upon deprotonation of the sulfhydryl group, implying that the chelate ring involves the ionized carboxyl and sulfhydryl groups. The large formation constant calculated for the Tb(EDTA)(CYS) compound (Table I) is capable of preventing formation of the $[Tb(EDTA)]_x$ oligomer. It is significant to note that aside from differences in relative peak heights, the CPL spectra of Tb(EDTA)(CYS) and Tb(EDTA)(ASP) are actually quite similar. This observation would imply that these chelate systems are somewhat similar in their conformational properties.

In either (*S*)-methionine (MET) or (*S*,*S*)-cystine (CYT) no ionizable sulfhydryl group exists, and any chelation (other than that taking place at the α-amino carboxyl grouping) would require interaction with the sulfur lone-pair electrons. Examination of



MET



CYT

the CPL spectra of Figure 5 reveals that only alanine-type complexation takes place. Formation constants for the Tb(EDTA)(MET) and Tb(EDTA)(CYT) compounds are found in Table I, and these are also indicative of weak complexation at the α-amino carboxyl group.

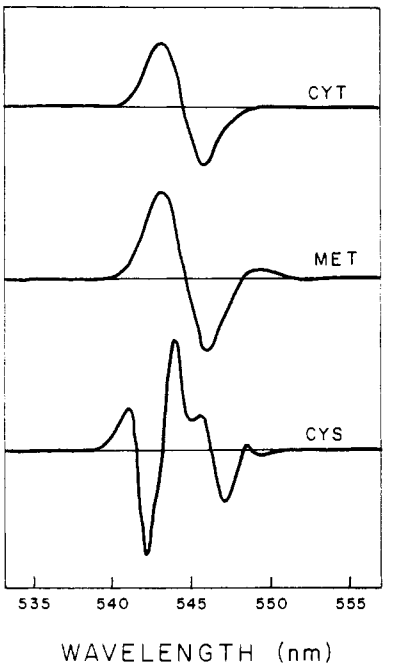
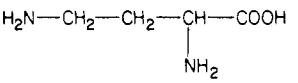
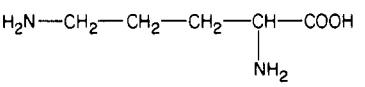


Figure 5. Circularly polarized luminescence spectra for the Tb(EDTA) complexes with (*S*)-cysteine, (*S*)-methionine, and (*S*)-cystine. The data were obtained at pH 10.5 for Tb:EDTA:AA ratios of 1:1:10.

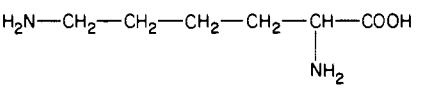
E. Diamino Carboxylic Acids. A series of amino acids exist that contain a terminal amine group. The particular systems studied include (*S*)-diaminobutyric acid (DABA), (*S*)-ornithine (ORN), (*S*)-lysine (LYS), and (*S*)-arginine (ARG).



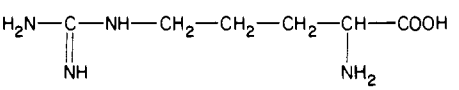
DABA



ORN



LYS



ARG

It was found that the CPL spectra of Tb(EDTA)(ORN), Tb(EDTA)(LYS), and Tb(EDTA)(ARG) were all essentially the same as those observed for the aliphatic amino acids. These results indicate that complexation takes place entirely at the α-amino carboxyl grouping and that the terminal amine functionality does not become involved in the bonding. The complexation phenemona were also characteristically weak, as the formation constants shown in Table I demonstrate. It is clear that the terminal amine cannot become involved in the bonding since the resulting chelate ring would be too large to stable.

Quite different CPL spectra were obtained for Tb(EDTA)(DABA), as may be seen in Figure 6. Strong CPL was observed above pH 8.0, which indicates that ionization at the terminal amine group is required for the complexation process. The CPL spectra were found to be considerably different from those obtained with the aliphatic amino acids but closely resembled the spectra observed for Tb(EDTA)(ASP) and Tb(EDTA)(CYS). This observation provides strong evidence that the DABA ligand forms a chelate ring with the terminal amine group (after its deprotonation) and the ionized carboxyl group. The strength of the complexation was found to be significantly higher for the DABA

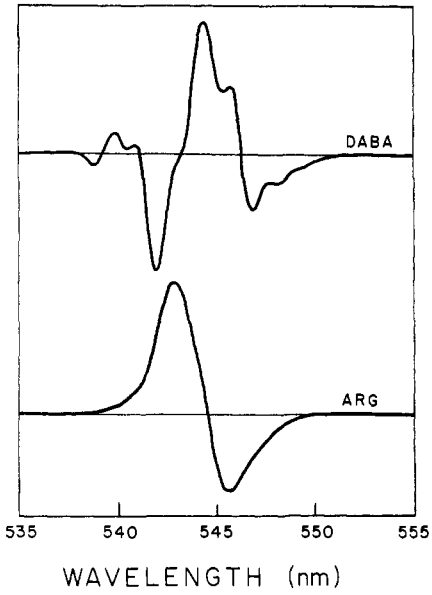
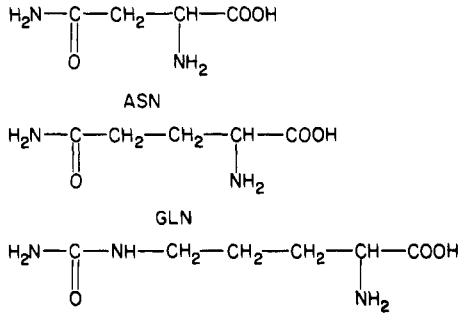


Figure 6. Circularly polarized luminescence spectra for the Tb(EDTA) complexes with (*S*)-diaminobutyric acid and (*S*)-arginine. The data were obtained at pH 10.5 for Tb:EDTA:AA ratios of 1:1:10.

ligand than for ORN, LYS, or ARG (see Table I) but far weaker than the complexation phenomona observed for ASP or CYS. These observations indicate that although chelation is possible by using a deprotonated amine group, complexation modes that involve other functionalities are much stronger. The CPL intensities decrease drastically above pH 11, indicating that the chelate ring is not sufficiently stable so as to be able to prevent formation of the $[Tb(EDTA)]_x$ oligomers.

A sequence of amino acids is also available in which the terminal functionality contains an amide group. The substrates examined were (*S*)-asparagine (AsN), (*S*)-glutamine (GLN), and (*S*)-citrulline (CIT).



The chiroptical data obtained on the amide compounds was found to be quite similar to those of the simpler diamino carboxylic acids. The CPL spectra of Tb(EDTA)(GLN) and Tb(EDTA)(CIT) were identical with those of the aliphatic amino acid substrates, and the complexes were destroyed at high pH in the analogous manner. These observations are indicative that simple α-amino carboxyl complexation is operative with these ligands and that any chelate ring involving the carboxyl and terminal amide group would be too large to be stable. As indicated in Table I, the formation constants for the ternary complexes are also in the range anticipated for such interactions.

As may be seen in Figure 7, the CPL spectrum of Tb(EDTA)(ASN) is quite different from those of either Tb(EDTA)(GLN) or Tb(EDTA)(CIT), and actually is superimposable with that of Tb(EDTA)(ASP). In addition, the CPL spectra persist without change up to pH 12, indicating that the ternary formation constant of the Tb(EDTA)(ASN) compound exceeds that of the $[Tb(EDTA)]_x$ oligomer. These observations all indicate that the ASG ligand binds Tb(III) by using the ionized carboxyl and amide groups. The magnitude of the Tb-

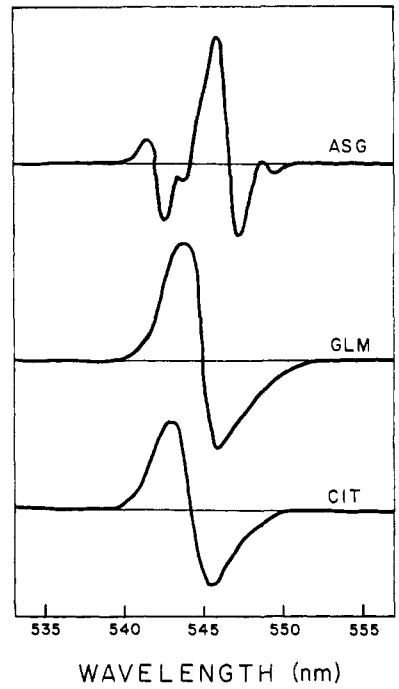


Figure 7. Circularly polarized luminescence spectra for the Tb(EDTA) complexes with (*S*)-citrulline, (*S*)-glutamine, and (*S*)-asparagine. The data were obtained at pH 10.5 for Tb:EDTA:AA ratios of 1:1:10.

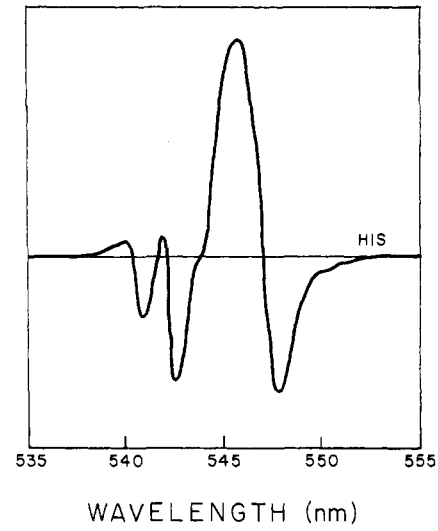
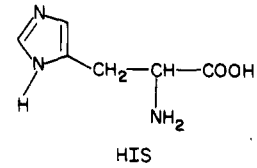


Figure 8. Circularly polarized luminescence spectra for the Tb(EDTA) complexes with (*S*)-histidine. The data were obtained at pH 10.5 for a Tb:EDTA:HIS ratio of 1:1:10.

(EDTA)(ASN) formation constant (1221 L/mol) is considerably higher than that of Tb(EDTA)(DABA) (192 L/mol), indicating that the amide group is more capable of interacting with a lanthanide ion than is an amine group.

F. Histidine. The final amino acid substrate to be studied was (*S*)-histidine (HIS), where the additional functionality was that of imidazole. Since the p*K* of the imizazolium hydrogen is known



to be 6.0,[10] one might anticipate that bonding to Tb(III) might take place.

In fact, CPL spectra were observed for Tb(EDTA)(HIS) at pH 6, and persisted without change up to pH 11. An example of the CPL line shape is shown in Figure 8. Aside from the small

negative peak observed at highest energy, the spectrum is exceedingly similar to those obtained with ASP, CYS, DABA, or ASG and is certainly not like those of the aliphatic amino acids. This result would indicate that the bonding interactions involve the ionized carboxylate and imidazole functional groups. Between pH 11 and 12, the CPL spectra decreased by approximately 50%, but were not observed to disappear completely. This observation indicates that the HIS ligand is only able to partially prevent the formation of the $[Tb(EDTA)]_x$ oligomers. In that case, the ternary formation constant of the Tb(EDTA)(HIS) complexes must be comparable to that of the $[Tb(EDTA)]_x$ oligomer, and this has been found to be the case (see Table I).

Conclusions

During the course of the present work, it has become established that amino acids may complex lanthanide ions by using many combinations of ligand functionalities. The formation of a chelate ring involving the α-amino carboxyl grouping is a relatively weak process but is certainly possible when metal ion solubility problems can be overcome. Other chelate ring systems can be formed (involving the ionized carboxylate and some other functionality) as long as the resulting ring is sufficiently small. These other ring systems are generally found to be considerably more robust than is the α-amino carboxyl ring system and will dominate the complexation chemistry whenever possible. When the formation of a chelate ring would require a large ring size, then complexation will take place solely at the α-amino carboxyl group and the additional functionality will play no role in the Tb(III) bonding.

It is quite clear from the present work that the solution-phase coordination chemistry of lanthanide complexes with amino acids is considerably more varied than hitherto suspected and certainly warrants further investigation.

Acknowledgment. This work was supported by the Camille and Henry Dreyfus Foundation, through a Teacher-Scholar award to H.G.B.

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Effect of a Single Ortho Substituent on the Rate of Dimerization of Iron(III) Tetraphenylporphyrin Hydroxides

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Received May 28, 1985

The rates of conversion of iron(III) tetraphenylporphyrin hydroxides to the corresponding μ-oxo-bridged dimers have been studied for a series of porphyrins with a substituent attached by an ether linkage to the ortho position of one phenyl ring. The substituents were alkyl groups (ethyl and nonyl) and *tert*-butyl amides with one, two, or four CH_2 groups between the ether oxygen and the amide carbon. In these monosubstituted porphyrins the hydroxide ligand can be on the same side of the porphyrin plane as the ortho substituent (cis isomer) or on the opposite side of the plane (trans isomer). The rates of formation of the μ-oxo-bridged dimer in CCl_4 solution at room temperature were obtained by ^1H NMR. The data were consistent with a two-step mechanism. In the first step hydroxide dissociates from an iron porphyrin, either cis or trans. That porphyrin then reacts with a trans isomer to form the μ-oxo-bridged dimer. The rate of dissociation of hydroxide from the cis isomers was 7-70 times slower than from the trans isomers. As the number of CH_2 groups between the ether oxygen and the amide increased, the population of the cis isomer increased.

Introduction

In organic solvents many iron(III) porphyrin hydroxides (Fe-POH)[1] readily form μ-oxo-bridged dimers.[2] However, bulky substituents on iron(III) tetraphenylporphyrins inhibit dimerization and permit isolation of iron(III) porphyrin hydroxides.[3-11] One bulky ortho substituent on each side of the porphyrin plane was sufficient to prevent dimerization of an iron(III) porphyrin hydroxide.[12] We have recently shown that the rate of dimerization of FeTTPOH at room temperature in CCl_4 solution can be monitored by ^1H NMR.[13] The data were consistent with the two-step mechanism

$$\mathrm{FeTTPOH} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{FeTTP^+ + OH^-} \qquad (1)$$

$$\mathrm{FeTTP^+ + FeTTPOH} \xrightarrow{k_2} \mathrm{(FeTTP)_2O + H^+} \qquad (2)$$

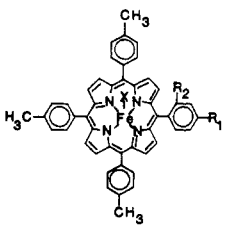
which gives the rate law

$$-\frac{\Delta[\mathrm{FeTTPOH}]}{\Delta t} = \frac{2k_1[\mathrm{FeTTPOH}]^2}{k'[\mathrm{OH^-}] + [\mathrm{FeTTPOH}]} \qquad (3)$$

where $k' = k_{-1}/k_2$ times the partition coefficient for the distribution of OH^- between water and CCl_4. For FeTTPOH $k_1 = (1.5 \pm 0.2) \times 10^{-4}$ s^{-1} and $k' = (0.2 \pm 0.15) \times 10^{-3}$.

In the preparation of a series of spin-labeled iron(III) tetraphenylporphyrins[14] it became evident that for some substituents on the ortho position of the phenyl ring a single substituent substantially decreased the rate of dimerization of the iron porphyrin hydroxide. We have therefore examined the rates of dimerization for five ortho-substituted porphyrins containing ether

Chart I



R_1	R_2	abbrev.
CH_3	H	Fe(TTP)X
H	OEt	Fe(TTP-OC_2)X
H	$O(CH_2)_8CH_3$	Fe(TTP-OC_9)X
H	$OCH_2\overset{\overset{H}{|}}{\underset{\underset{O}{\|}}{C}}N$-t-Bu	Fe(TTP-OC_1A)X
H	$O(CH_2)_2\overset{\overset{H}{|}}{\underset{\underset{O}{\|}}{C}}N$-t-Bu	Fe(TTP-OC_2A)X
H	$O(CH_2)_4\overset{\overset{H}{|}}{\underset{\underset{O}{\|}}{C}}N$-t-Bu	Fe(TTP-OC_4A)X

(1) Abbreviations used: P = porphyrin dianion; other abbreviations are defined by the structural formulas in Chart I.
(2) White, W. I. In "The Porphyrins"; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. V, p 318. Sams, J. R.; Tsin, T. B. Ibid., Vol. IV, p 452. Buchler, J. W. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 407. O'Keefe, D. H.; Barlow, C. H.; Smythe, G. A.; Fuchsman, W. H.; Moss. T. H.; Lilienthal, H. R.; Caughey, W. S. *Bioinorg. Chem.* **1975**, *5*, 125.
(3) Cense, J.-M.; Le Quan, R.-M. *Tetrahedron Lett.* **1979**, 3725.
(4) (a) Gunter, M. J.; Mander, L. N.; Murray, K. S. *J. Chem. Soc., Chem. Commun.* **1981**, 799. (b) Gunter, M. J.; McLaughlin, G. M.; Berry, K. J.; Murray, K. S.; Irving, M.; Clark. P. E. *Inorg. Chem.* **1984**, *23*, 283.
(5) Groves, J. T.; Haushalter, R. C.; Nakamura, M.; Nemo, T. E.; Evans, B. J. *J. Am. Chem. Soc.* **1981**, *103*, 2884.
(6) Gunter, M. J.; Mander, L. N.; Murray, K. S.; Clark, P. E. *J. Am. Chem. Soc.* **1981**, *103*, 6784.

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0020-1669/85/1324-3698$01.50/0