Proton and Carbon Lanthanide-Induced Shifts in Aqueous Alanine. Evidence for Structural Changes along the Lanthanide Series

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Abstract: Proton and carbon lanthanide-induced shifts are reported for aqueous alanine at pH 3. A comparison of the measured shifts with theoretical values provides evidence for changes in the coordination behavior of alanine between Tb^{3+} and Dy^{3+}. An apparent stability constant ($K_1 = 0.7 \pm 0.1\ M^{-1}$) was measured for eight lanthanide ions and found insensitive to changes in alanine coordination geometry. The measured shifts are separated into their contact and pseudocontact components and the resulting corrected pseudocontact shift ratios are compared with values calculated using the axial symmetry model to deduce the dynamic alanine structures. A monodentate coordination geometry is proposed for the ions $Pr^{3+} \rightarrow Tb^{3+}$ and a bidentate carboxyl coordination structure is evident for the ions $Dy^{3+} \rightarrow Yb^{3+}$. The carbon shifts are dominated by polarization of unpaired spin away from the ligating oxygen(s) and mediated by a direct delocalization of spin resulting in a greater unpaired spin density at C_α than C_0. Although the proton shifts are primarily pseudocontact in origin, the separation method reveals a change in sign and increased magnitude of spin density at the protons on changing from monodentate to bidentate coordination. This behavior may parallel known hydration sphere alterations along the lanthanide series.

The trivalent lanthanide ions have proven useful as aqueous NMR shift reagents for examining structures of small biological molecules in solution.[1-5] These ions bind to phosphate, carboxylate, hydroxyl, and peptide carbonyl oxygens in what is typically considered an electrostatic type of attraction and induce paramagnetic shifts in magnetically active nuclei near these ligands. Under favorable circumstances, these shifts may be used to determine the dynamic solution conformation of the metal-substrate complexes. The advantage to using the lanthanide-induced shifts (LIS) to deduce solution conformations over the typical relaxation probes,[6,7] i.e., Mn^{2+}, arises from not having to rely upon a rigorous determination of the correlation times dominating relaxation in the latter experiment. The LIS technique requires only a proven pseudocontact origin to the measured shifts and a metal-substrate complex having axial or effective axial symmetry.

The shifts induced in substrate nuclear resonances upon binding to a lanthanide ion result from a sum of the dipolar (pseudocontact) and scalar (contact) interactions. A dipolar shift results whenever the metal spin felt by a particular nucleus fails to average to zero while scalar shifts result from a delocalization of spin from the metal to the nucleus. Although any magnetically active nuclei may be probed using the LIS technique, only protons are often found devoid of significant contact effects. Those nuclei which comprise the molecular framework of the substrate or lie near the ligating center, i.e., carbon-13 and phosphorus-31, will in general display shifts which are a mixture of pseudocontact and contact effects.[8,9] These combined shifts must of course be separated into the dipolar and scalar contributions before information concerning the solution structures may be derived. This technique has been used to probe the aqueous structure of the lanthanide-alanine complex at pH 3. The shifts are quite sensitive to minor structural changes along the metal series and appear valuable for demonstrating alterations in the lanthanide ion hydration sphere.

Experimental Section

Lanthanide solutions were prepared as the chloride salts by dissolution of the appropriate Ln_2O_3 into a D_2O-DCl mixture followed by standarization against ethylenediaminetetraacetate using xylenol orange as indicator. The lanthanide oxides with a purity of greater than 99.9% were a gift from Molydenum Corp. pH measurements were made with a Sargent-Welch NX pH meter equipped with a glass combination electrode. All solutions were adjusted to pH 3 after mixing the appropriate combination of lanthanide salt, alanine, and tetramethylammonium chloride internal standard. The pH values are actual meter readings and have not been corrected for a deuterium isotope effect (pD = pH + 0.4).

The proton-decoupled carbon spectra were recorded on a Varian XL-100-15 Fourier transform spectrometer interfaced to a Nova 1210 computer. The instrument was locked on D_2O and all shifts were measured from internal tetramethylammonium ion. The reported LIS represent the shifts induced by the paramagnetic metal ions referenced to the diamagnetic lanthanum and lutetium complexes. Small aliquots were removed from each sample for recording proton spectra on a JEOL C60-HL spectrometer. All spectra were recorded at 24 °C.

A computer program, PDIGM,[10] supplied by R. E. Davis, The University of Texas at Austin, was used to assess the best agreement between calculated and observed LIS at each metal position from normalized standard deviations (R factors). The program was modified to allow a search for the best principal symmetry axis of each complex (see below). The lanthanide position which gave the best agreement between observed and calculated shifts was recorded and the resulting alanine-lanthanide complex was displayed with a microfilm or drum plot using the ORTEP[11] molecular graphics package.

Results and Discussion

The lanthanide-induced pseudocontact shift observed for any nucleus in an effective axial ligand has been theoretically described by Bleaney[12] as

$$\Delta_{pc} = \frac{g^2\beta^2 J(J+1)(2J-1)(2J+3)D_z}{60kT^2}\,\frac{3\cos^2\theta - 1}{r_i^3} \qquad (1)$$

where r_i is the distance between nucleus i and the lanthanide ion, and θ_i is the angle between a vector connecting nucleus i with the metal ion and the principal symmetry axis of the complexed ion. The principal symmetry axis of the complexed ion is determined by the ligating water molecules *and* any added ligands which enter the coordination sphere. Thus, the principal symmetry axis is subject to change upon altering the ligation behavior of an added substrate.

When the entire lanthanide ion series form isostructural complexes with a given substrate, the pseudocontact shifts experienced by a nucleus in the substrate will be proportional to the first term in eq 1 containing the magnetic constants characteristic of each lanthanide ion and the ligand field term, D_z. These theoretical pseudocontact shift values, tabulated by Bleaney[12] for each lanthanide ion, are often compared with measured LIS's and offered as evidence for the presence or

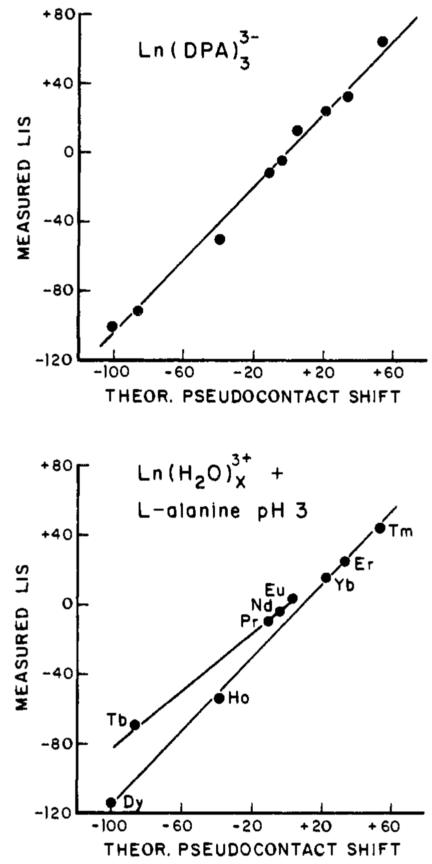

Figure 1. Measured lanthanide induced proton shifts in the (a) trisdipicolinic acid complexes (ref 14), and (b) alanine complexes at pH 3 vs. Bleaney's theoretical pseudocontact shift values.

absence of contact contributions.[3,8,13] Two such comparisons have been made in Figure 1. The linear correlation observed between the measured LIS's in the rigid chelate, dipicolinic acid,[14] and Bleaney's theoretical values is good evidence for an isostructural Ln(DPA)$_3^{3-}$ series having a relatively constant ligand field component, D_z. In contrast, when the LIS data for the methyl protons of alanine are plotted vs. the theoretical values, two linear regions seem to appear: one for the light lanthanides ($Pr^{3+} \rightarrow Tb^{3+}$) and another for the heavier lanthanides ($Dy^{3+} \rightarrow Yb^{3+}$). This observation could result from (a) a drastic change in lanthanide-alanine stability constants near the center of the metal series, (b) significant contact contributions to some of the shifts, (c) a change in the ligand field component, D_z, along the lanthanide-alanine complex series, or (d) an alteration in the average coordination geometry of alanine to the lanthanide resulting in a change in the principal symmetry axis. Although two lines have been generated in Figure 1 implying there are two distinct structural classes of lanthanide-alanine complexes given by cases c and d, we cannot for the moment dismiss a and b.

Under the conditions of the NMR experiment, alanine is exchanging rapidly between the lanthanide coordination sphere and the bulk solution and the resonance shifts will reflect an average of these environments. Assuming a single complex is formed at pH 3, a measured LIS will be proportional to the concentration of the bound alanine, [ML].[15]

$$\Delta_{\text{obsd}} = \frac{[\text{ML}]}{[\text{L}]_0}\Delta_0 \qquad (2)$$

Δ_{obsd} for a given nucleus is the measured chemical shift difference for alanine in the presence of a paramagnetic lanthanide ion vs. a diamagnetic lanthanide ion, Δ_0 is this same chemical shift difference for completely bound alanine, and

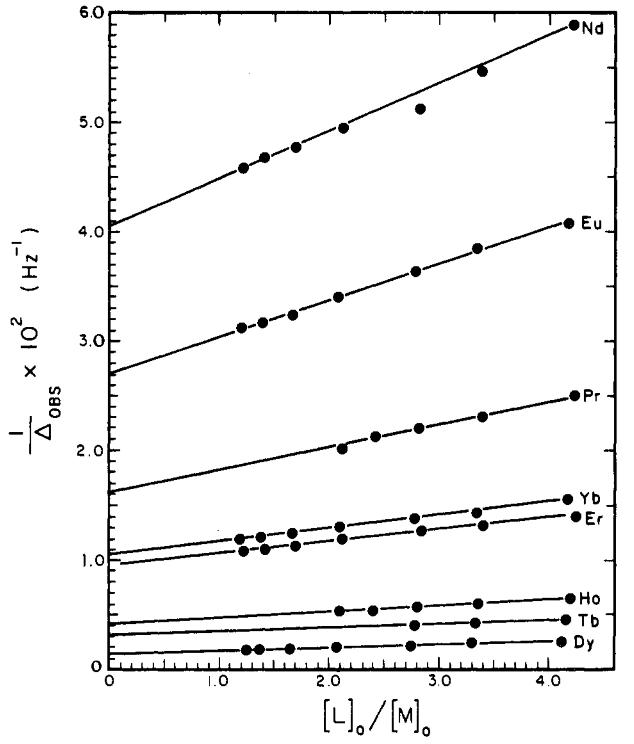

Figure 2. Double reciprocal plots of inverse lanthanide induced shifts vs. the alanine to metal concentration ratio. The lanthanide concentration was held constant at 0.2 M and the alanine varied over the indicated range. Other parameters include: pH 3, μ = 2.2 M, and T = 24 °C.

$[\text{L}]_0$ is the total alanine concentration. We may combine eq 2 with the stability constant expression to give

$$\frac{1}{\Delta_{\text{obsd}}} = \frac{1}{[\text{M}]_0 K_1 \Delta_0} + \frac{1}{\Delta_0}\frac{[\text{L}]}{[\text{M}]_0} \qquad (3)$$

where $[\text{M}]_0$ is the total paramagnetic metal concentration and [L] is the equilibrium concentration of uncomplexed alanine. K_1 is the apparent lanthanide-alanine stability constant at pH 3. At high alanine concentrations, [L] may be approximated by $[\text{L}]_0$ and eq 3 may be solved for Δ_0 and K_1 by plotting $\Delta_{\text{obsd}}^{-1}$ vs. $[\text{L}]_0/[\text{M}]_0$. Eight such plots are shown in Figure 2. The stability constants obtained from a least-squares analysis of these data were used to calculate the actual [L] concentrations and new plots were generated. This iteration was continued until the difference between K_{n+1} and K_n was <0.1. The resulting apparent stability constants (K_1 = 0.7 + 0.1 M^{-1} at μ = 2.2 M) were constant within experimental error for each of the eight lanthanides examined. These values may be compared with stability constants reported for Nd^{3+}-alanine at pH 4 (K = 6.5)[16] and various Ln^{3+}-glycine complexes at pH 3.64 (K = 3.4-5.0).[17] The lower pH used in this study reduces the zwitterion concentration of alanine and hence lowers the apparent lanthanide-alanine stability constant. As in the Ln^{3+}-glycine study,[17] we do not observe the formation of higher order ML$_n$ complexes over the concentrations examined in Figure 1. The data eliminate stability constant fluctuations as a source of the deviations observed in Figure 1.

A contact shift experienced by a nucleus may be described[18] by

$$\Delta_c = \frac{2\pi\beta A}{3kT\gamma_N} J(J+1) g_L(g_L - 1) \qquad (4)$$

where A is the scalar coupling constant, γ_N is the gyromagnetic ratio of the nucleus in question, and J and g_L are constants characteristic of a particular lanthanide ion. Golding and Halton[18] have outlined a second-order perturbation treatment

Table I. Lanthanide-Induced Shifts in Aqueous Alanine at pH 3

Lanthanide ion	LIS,[a] ppm ± 0.1				
	C_0	C_α	C_β	H_α	H_β
Pr^{3+}	+1.1	−8.3	−0.4	−1.3	−0.8
Nd^{3+}	+8.9	−10.3	+0.4	−0.4	−0.3
Eu^{3+}	−13.6	+20.9	−0.6	+0.5	+0.6
Gd^{3+}	−36	+52.3	−3.4	*b*	*b*
Tb^{3+}	−51.4	+33.8	−8.2	−9.5	−4.2
Dy^{3+}	−68.2	+13.3	−13.0	−15.5	−8.1
Ho^{3+}	−32.0	+14.3	−5.8	−7.0	−3.3
Er^{3+}	+5.9	+17.7	+1.2	+2.5	+1.5
Yb^{3+}	+9.0	+4.3	+1.7	+2.4	+1.3

[a] All LIS are measured with respect to the alanine resonances in the La^{3+} and Lu^{3+} complexes and are reported at a $C_L/C_M = 2$. A negative sign denotes a downfield shift. [b] Not observed due to extreme line broadening.

for estimation of the spin-expectation value at a nucleus, $\langle S_z \rangle$, which includes a mixing of lanthanide excited states. The calculated values agree favorably with ^{17}O and ^{15}N LIS data[19,20] in systems where contact effects dominate the measured shifts. Although Tb^{3+}, Dy^{3+}, and Ho^{3+} are included in that group of lanthanides expected to provide the greatest electron spin delocalization, the direction of the deviations from the lines in Figure 1 is not consistent with the similar signs of the scalar coupling constants expected for these ions.

Two recent studies[5,21] have concluded that simple carboxylates and amino acids form two classes of lanthanide complexes: the light lanthanides forming bidentate structures and the heavier lanthanides, generally exemplified by Tm^{3+}, forming monodentate structures. These results agreed qualitatively with our predictions of a structural change from Figure 1 and led us to examine the ^{13}C shifts of alanine in an effort to quantitate these effects. Accordingly, the carbon LIS were gathered for this same series of metal ions over the concentration range established above. The combined ^{13}C and ^1H LIS data for a $C_L/C_M = 2$ are found in Table I.

The alternating signs of the carbon shifts away from the ligating atom(s) suggest that these shifts are dominated primarily by spin delocalization via a spin polarization mechanism.[22] An inspection of the Gd^{3+} shifts, which should be void of pseudocontact effects, reveals a larger negative spin density at C_α than the positive spin density at C_0. As the spin delocalization is expected to fall off monotonically through a series of bonds away from the ligating center,[23] the positive spin density at C_0 must be partially negated by a direct delocalization of negative spin density from the oxygen atom(s). The net effect of these two delocalization mechanisms is to cancel the spins at C_0 and C_β and enhance the spin at C_α. Now the question arises, can this delocalized spin be felt at the protons and if so by which mechanism do they arrive? If one continues the above arguments for the hydrogen nuclei, delocalization of spin by the two mechanisms could cancel at H_α and reinforce at H_β. Unfortunately, the Gd^{3+}-induced proton shifts cannot be observed because of extreme line broadening at the concentrations of paramagnetic ion required. The remaining proton LIS seem innocent enough with one exception; the Eu^{3+}-induced shift in the methyl proton resonance is *always* larger than that observed in the α-proton resonance. This strongly suggests these Eu^{3+}-induced proton shifts have a significant contact contribution and the contact shift at H_β is *larger* than that at H_α. Europium is expected, on the basis of both experimental[3,24] and theoretical[12,18] evidence, to present the largest ratio of contact/pseudocontact shifts across the lanthanide series and therefore it is not surprising that the remaining ions consistently induce a larger shift at H_α than at H_β. However, the Eu^{3+} clue provides a clear warning that the proton shifts contain significant, though obviously not dominant, contact contributions.

Several methods[8,13,24-26] have been proposed for separating contact and pseudocontact contributions to lanthanide-induced ^{13}C shifts. Some of the methods work only for rigid substrates where the geometric factors in the pseudocontact equation are easily predictable[24,25] while others rely upon simple factoring of contact contributions relative to those measured using gadolinium.[13] Unfortunately, each of the methods requires the assumption that all lanthanides bind to the substrate similarly resulting in a constant ligand field component (D_z in eq 1) and scalar coupling constant (A in eq 4). While the majority of the LIS data presented for the rigid, isostructural $Ln(acac)_3$ complexes and their substituted derivatives seem to comply with this requirement, the aqueous trivalent lanthanide cations often display structural changes along the series[27,28] as the ionic radii of the metals decrease and thus may not necessarily conform to the above rules. Dobson et al.[8] have proposed a general method for the separation of contact and pseudocontact contributions to LIS which relies upon measuring at least one nuclear shift which is not subject to contact effects, $\Delta_{total}{}^j = \Delta_{pc}{}^j$. This may be combined with the nuclear constants of the lanthanides to give eq 5, where $\Delta_{total}{}^i$ is the measured LIS for nucleus i which contains both pseudocontact and contact components and $\Delta_{pc}{}^i$ is the pseudocontact contribution to the shift.

$$\frac{\Delta_{total}{}^i}{\Delta_{total}{}^j} = \frac{\Delta_{pc}{}^i}{\Delta_{pc}{}^j} + \frac{(2\pi\beta/3kT\gamma_N)\cdot A\cdot J(J+1)g_L(g_L-1)}{\Delta_{pc}{}^j} \tag{5}$$

A plot of $\Delta_{total}{}^i/\Delta_{total}{}^j$ vs. $J(J+1)g_L(g_L-1)/\Delta_{pc}{}^j$ gives an intercept equal to the true pseudocontact shift ratio of nuclei i and j, thereby allowing a separation of the contact contribution. This technique has been successfully used in two systems[8,29] where the reference nucleus, $\Delta_{total}{}^j$, is clearly far enough away from the ligating center to preclude contact effects. However, let us consider a case where the LIS measured for the nucleus j may contain an unexpected nonzero contact contribution (as in alanine). Under these conditions, the intercept only approaches the true pseudocontact shift ratio. A plot for the alanine methyl carbon ($\Delta_{total}{}^i$) to methyl proton ($\Delta_{total}{}^j$) shift ratios is shown in Figure 3. Again, it is clear the data fall into two classes of lanthanide complexes, one for $Pr^{3+} \rightarrow Tb^{3+}$ and another for $Dy^{3+} \rightarrow Yb^{3+}$. As these methyl proton shifts are known to contain contact components for at least some of the lanthanides, the absolute slopes and intercepts of these lines are not quantitatively interpretable. However, the considerable variation in the slope of the two lines would suggest the methyl carbon hyperfine coupling constant (A in eq 4) increases between Tb^{3+} and Dy^{3+}. An alternate method for evaluating the pseudocontact contributions to the shifts is clearly a prerequisite for mapping these two classes of lanthanide–alanine complexes.

All attempts to subtract out the contact contributions to the carbon shifts by scaling the remaining lanthanide shifts to the gadolinium shifts resulted in inconsistent pseudocontact shifts, some even reversing signs at neighboring nuclei. However, using a method first reported by Reilley et al.[26] in which both pseudocontact and contact scaling factors for each lanthanide are included in the calculation, excellent fits are obtained only if the data are allowed to fall into two data sets corresponding to the two groups of metals reflected in Figures 1 and 3. The relative standard deviation between all calculated and observed shifts using this method is ±0.12 ppm compared with nearly ±0.5 ppm if all lanthanides are forced to remain in a single class, i.e., if the hyperfine coupling constants and pseudocontact shift ratios are assumed independent of the lanthanide chosen.

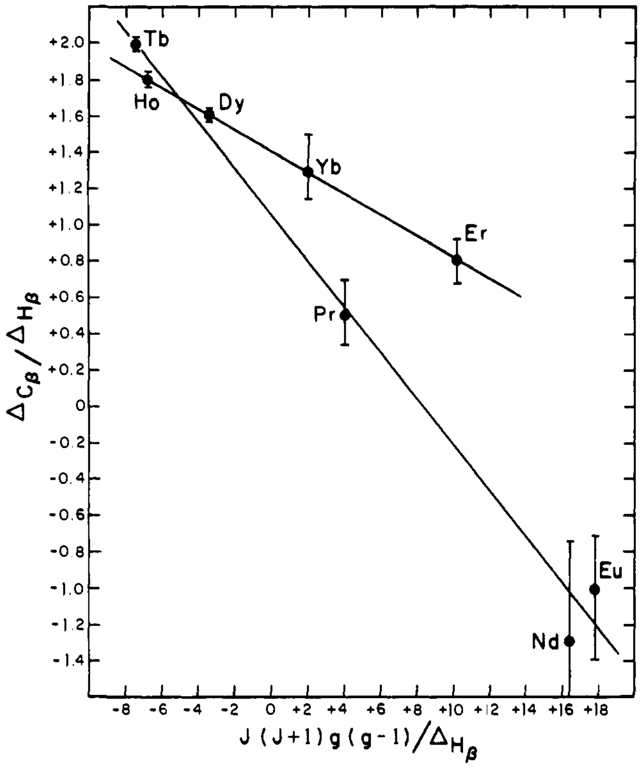

Figure 3. Observed alanine methyl carbon/methyl proton lanthanide induced shift ratios vs. the ratio of lanthanide magnetic constants/methyl proton shifts after ref 8.

Table II. Calculated Contact and Pseudocontact Contributions to the Alanine Proton Lanthanide-Induced Shifts

Lanthanide ion	Contact shifts, ppm[a] H_α	H_β	Pseudocontact shifts, ppm[a] H_α	H_β
Pr^{3+}	~0	−0.1	−1.2	−0.7
Nd^{3+}	~0	−0.2	−0.5	−0.3
Eu^{3+}	+0.1	+0.4	+0.4	+0.2
Tb^{3+}	+0.2	+1.0	−9.7	−5.2
Dy^{3+}	−2.9	−1.2	−12.5	−6.8
Ho^{3+}	−2.3	−0.9	−4.9	−2.6
Er^{3+}	−1.6	−0.6	+4.1	+2.2
Yb^{3+}	−0.3	−0.1	+2.8	+1.5

[a] A negative sign denotes a downfield shift.

Table III. Experimental and Calculated Carbon-13 and Proton Pseudocontact Shift Ratios for Alanine at pH 3

	$Pr^{3+} \rightarrow Tb^{3+}$		$Dy^{3+} \rightarrow Yb^{3+}$	
Nucleus	(LIS ratio)$_{expt l}$[a]	(LIS ratio)$_{calcd}$[b]	(LIS ratio)$_{expt l}$[a]	(LIS ratio)$_{calcd}$[b]
C_O	2.71	2.75	7.29	7.26
C_α	3.78	3.39	1.74	2.09
C_β	0.95	1.54	1.40	1.20
H_α	1.86	2.49	1.84	1.46
H_β	1.00	1.00	1.00	0.94

[a] Corrected for contact contributions (see Results). [b] Calculated from eq 1 for the lanthanide position shown in Figure 4.

The calculated contact and pseudocontact contributions to the proton shifts are found in Table II. As predicted above for Eu^{3+}, each of the first four ions ($Pr^{3+} \rightarrow Tb^{3+}$) induce a larger contact shift in the methyl protons than in the α proton. This must reflect a cancellation of metal spin density at H_α and a summation of spin density at H_β by the two delocalization mechanisms. It is also quite clear that the ions $Dy^{3+} \rightarrow Yb^{3+}$ bind differently to alanine resulting in proportionately larger contact shifts with positive metal spin densities at both H_α and H_β. The reasons for the positive spin densities found at the protons are not clear at this time but may reflect the change in coordination geometry of alanine binding to these latter ions (see below). When the corrected pseudocontact shifts are replotted vs. Bleaney's theoretical values, two separate lines remain, indicative of two classes of lanthanide-alanine complex formation.

The corrected pseudocontact shift ratios for both light and heavy lanthanide-alanine complexes are tabulated in Table III. As these corrected ratios differ considerably for the two lanthanide groups, the two lines observed in Figure 1 must result from a change in the principal symmetry axis of the two groups of lanthanide complexes (a change in D_z only would have resulted in similar corrected pseudocontact shift ratios). These corrected ratios were then used to search for the best position of the lanthanide ion within each class of complexes by comparison with calculated shift ratios from eq 1 for each metal position. The best agreement between calculated and measured shifts for the heavy lanthanide ion ($Dy^{3+} \rightarrow Yb^{3+}$) data is observed with the metal bridging the two carboxyl oxygens, Ln-O bond lengths equal to 2.7 ± 0.2 Å, and the principal symmetry axis of the complex bisecting the OCO bond. If the principal symmetry axis of the complex is directed through atoms other than C_O, the agreement between calculated and observed shifts is immediately lost. The metal position depicted in the ORTEP structure is not a unique metal location but represents an average of a set of fits which locate the metal position within 0.2 Å from this average position. The agreement between the calculated and measured shifts for the best fit structure corresponds to a weighted standard deviation,[10] wR = 6.1%, where the weights are proportional to the inverse of each shift.[30] Our failure to find a set of solutions with zero R factors in the adjustment of six parameters (the three lanthanide coordinates, a scale factor, and the two direction cosines of the principal magnetic axis) with five experimental observations is inherent in the inadequacy of describing a dynamic solution structure by this simplified static model.

The best fit structure for the light lanthanide data in Table III corresponds to a metal position near the carboxyl oxygen opposite the molecule from the protonated α-amino group with a Ln-O bond length of 3.1 ± 0.3 Å. Again, this reflects the average location of the metal within a region that is less well defined than with the heavy lanthanide structure. The principal symmetry axis consistently minimized to a position within 15° of the lanthanide-oxygen bond. Attempts to allow a complete rotational averaging of the nuclear coordinates about the C_O-C_α bond result in poor agreement between calculated and observed structures. This suggests the alanine orients itself upon binding to the light lanthanides such that the repulsion between the trivalent lanthanide and monovalent amino charges is minimized. Assigning a lower weight to the H_α, H_β, and C_β LIS results in improved R factors which apparently reflects the even greater uncertainty in locating these nuclei within this less symmetrical structure.[30] The weighted R factor for the static structure observed in Figure 4 is 14.6% (0.5 weight for H_α, H_β, and C_β and 1.0 for C_O and C_α).

An obvious discrepancy exists between these calculated lanthanide-alanine structures and those determined earlier by Levine and Williams.[5] These authors state that the alanine methyl proton shifts are "known to be unaffected by contact interaction" and use these shifts as the reference shift for removing contact contributions to the carbon and α proton shifts. As outlined above, it is clear that the methyl proton shifts are *not* devoid of contact contributions and are therefore not useful in determining the corrected pseudocontact shift ratios. This failure to separate even minor contact contributions gives rise

Table IV. Lanthanide Induced Proton Shift Ratios before and after Correction for Contact Contributions

Lanthanide ion	Measured LIS ratio H_β/H_α	Corrected LIS ratio H_β/H_α
Pr^{3+}	0.62	0.58
Nd^{3+}	0.75	0.60
Eu^{3+}	1.20	0.50
Tb^{3+}	0.44	0.54
	0.75 ± 0.56	0.56 ± 0.08
Dy^{3+}	0.47	0.53
Ho^{3+}	0.52	0.54
Er^{3+}	0.60	0.54
Yb^{3+}	0.54	0.54
	0.53 ± 0.08	0.54 ± 0.01

to a set of considerably different pseudocontact shift ratios which leads to an alternate lanthanide ion binding position.

These same authors have observed changing shift ratios for the heavy lanthanide ions upon binding to indole 3-acetate[21] and tryptophan[5] and use the thulium induced shifts to calculate metal-substate structures which purportedly represent the later half of the lanthanide series. Our results with alanine indicate contact effects make a greater contribution to the observed shifts with these ions and the reported discrepancies for thulium may reflect this difference. Our results also indicate that shift ratios may not necessarily be sensitive to contact contributions. This is demonstrated in the data of Table IV. With the exception of europium and possibly neodymium, the measured methyl to alpha proton LIS shift ratios in alanine are quite constant across the lanthanide series. Had we chosen only three or four of these ions in this study, we could have easily argued that the constancy in shift ratios demonstrates a lack of contact contributions to the measured shifts and therefore the data fit the effective axial symmetry pseudocontact model. However, even after large corrections for contact contributions are made to the heavy lanthanide shift data (see Table II), the average value of the corrected ratios is unchanged but the precision within each class is perceptibly improved. It is also clear that the proton LIS in alanine are quite insensitive to the two classes of structures formed.

Conclusion

Our results show that lanthanide induced shifts are quite sensitive to structural alterations in the metal coordination sphere. The apparent change from monodentate to bidentate alanine coordination between Tb^{3+} and Dy^{3+} may parallel a change in the metal primary hydration numbers in these alanine solutions. An abundance of thermodynamic evidence exists for a change in primary hydration number along the lanthanide series.[27,31,32] These changes are thought to result from an expulsion of at least one water molecule as the ionic radii decrease across the series. Reilley et al.[33] have separated contact and pseudocontact contributions to the lanthanide induced water proton shifts by the same methods used in this report and find that scalar hyperfine coupling constants and pseudocontact shifts vary in both sign and magnitude for the ions Pr^{3+} through Eu^{3+} vs. Gd^{3+} through Yb^{3+}. These effects may reflect a structural alteration in the coordinated water molecules or a change in hydration number between Eu^{3+} and Gd^{3+}. Our data also reveal a change in sign of the unpaired spin density, ρ, at the proton 1s orbitals of alanine but the change here occurs between Tb^{3+} and Dy^{3+}. The calculated spin densities vary from $\rho(H_\alpha) = -0.26 \times 10^{-6}$ and $\rho(H_\beta) = -1.29 \times 10^{-6}$ for the light lanthanide ions to $\rho(H_\alpha) = +4.10 \times 10^{-6}$ and $\rho(H_\beta) = +1.66 \times 10^{-6}$ for the heavy ions. There is evidence that counteranions may mediate hydration sphere changes. Grenthe et al.[34] have presented evidence for constant lanthanide ion hydration numbers in a perchlorate medium while induced shifts in lanthanide chelates have been used to detect structural changes in the bis(nitrilotriacetate) complexes occurring between Eu^{3+} and Tb^{3+} [35] and in the ethylenediaminetetraacetate chelates between Dy^{3+} and Ho^{3+}.[36]

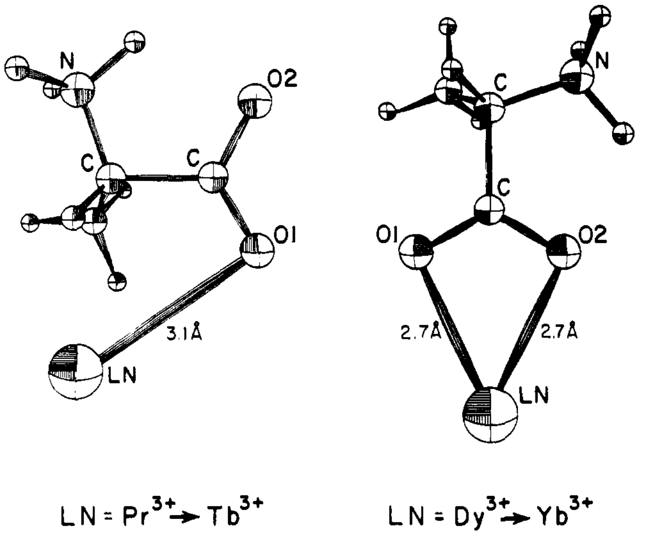

Figure 4. ORTEP structures depicting the two classes of lanthanide-alanine complexes at pH 3. These metal positions represent the best agreement between calculated and measured pseudocontact shifts.

These results suggest we should exercise caution in choosing aqueous lanthanides as shift reagents to map structures of water soluble substrates. Whenever possible, the LIS for substrate protons should be examined for the entire metal series and compared to Bleaney's theoretical shift values. Any deviations from this correlation may reflect contact contributions to the measured shifts or structural alterations in the substrate-lanthanide-aquo complexes along the series. It is not safe to assume a priori proton LIS are purely dipolar in origin. As revealed in this study, contact shifts do not necessarily attenuate rapidly away from ligating center in aliphatic substrates. The carbon shifts in alanine reflect a cancellation of spin density by spin polarization and direct delocalization mechanisms at nuclei near the metal and a reinforcement of spin density at distant nuclei. Contact shifts for the entire series should not be scaled to those measured with Gd^{3+} *unless* a constant substrate structure and hence a constant scalar hyperfine coupling constant is proven. Furthermore, lanthanide induced shift ratios may not be sensitive to contact shift contributions and should not be used as an argument for effective axial symmetry without proper examination for contact effects. As the Eu^{3+} shifts are most sensitive to contact contributions, any reproducible differences in shift ratios with this ion may reflect a contact contribution. With these precautions in mind, the LIS method will remain a valuable tool for examining dynamic metal-substrate structures in aqueous solution and may prove invaluable for tracing lanthanide hydration sphere alterations.

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A Thallium NMR Determination of Polyether Cation Selectivity Sequences and Their Solvent Dependences

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Abstract: An NMR method of measuring the stability constants relative to the thallous ion of the univalent cations Li^+, Na^+, K^+, Rb^+, Cs^+, NH_4^+, Ag^+, and Tl^+ with the macrocyclic polyethers 15-crown-5, 18-crown-6, dibenzo-18-crown-6, and dicyclohexo-18-crown-6 in methanol, dimethylformamide, and dimethyl sulfoxide has been developed. The NMR method provides a convenient means of measuring the effects of solvation on the ion selectivity sequences of ionophores. The precision of the stability constant ratios obtained by the NMR method is about ±6%. The stability constants determined in methanol agree with those determined by ion-selective potentiometry. The complex between the thallous ion and 18-crown-6 has 1:1 stoichiometry in a variety of solvents. The effect of the solvents on the binding constants is discussed in terms of solvation of the ions and the crown ether. The chemical shift of the bound thallous ion is diagnostic of the ether oxygen donor atoms of the macrocycle.

The macrocyclic polyethers synthesized by Pedersen[1] have aroused much interest because of the high stability of the complexes they form with the alkali and alkaline earth metal ions.[2-20] Complexation of alkali and alkaline earth metal ions by crown ethers has been studied by ultraviolet spectroscopy,[2] solvent extraction,[2] ion-selective electrode potentiometry,[3] calorimetric measurements,[4] ^1H[5] and alkali metal[6-9] NMR spectroscopy, and ultrasonic absorption methods.[10-12] Reviews covering various aspects of ion binding by crown ethers have recently appeared.[13-20]

Because the complexing ability of alkali metal ions by the crown ethers is highly selective, the crown ethers have been used as models for macrocyclic antibiotics believed to be responsible for alkali ion transport across membranes.[21-25] The ion-selectivity sequences are explained as a superposition of the effects shown in eq 1,[15]

$$-RT \ln K = \Delta G_{\text{bind}} - \Delta G_{\text{solv}}(M^+) - \Delta G_{\text{solv}}(L) - \Delta G_{\text{conf}}(L) - \Delta G_{\text{solv}}(ML^+) \qquad (1)$$

where K is the stability constant, i.e., the equilibrium constant for complexing, and the other terms are respectively the free energies of metal-ligand bonds, metal ion solvation, ligand solvation, ligand conformational changes, and solvation of the metal-ligand complex.

The stability constants should be strongly affected by the solvent because of the terms involving solvation of the ion and ligand. Previous measurements of the binding constants as a function of solvent have been restricted primarily to water and methanol because of the limitations in the methods of measurement. A knowledge of the direction and magnitude of the solvent effects is important for theoretical interpretations of ion selectivity and practical applications to synthesis and separations.

In this paper we develop, test, and apply a method of determining binding constants in a variety of solvents using the thallous ion NMR chemical shift. The thallous ion is a useful probe because its chemistry is very similar to that of the alkali metal ions,[26] it has spin $I = \frac{1}{2}$, and its chemical shift is extremely sensitive to its environment. For example, the solvent dependence of its chemical shift is over 2600 ppm[27] in contrast to a shift range of 8 ppm for ^7Li,[28-31] 30 ppm for ^{23}Na,[32-34] and 120 ppm for ^{133}Cs.[35] Because the chemical shift is often diagnostic of the environment of the thallous ion, the NMR