

Proton Magnetic Resonance Study of the Equilibria between Lanthanide (3+) Ions and Amino Acids and the Nature of the Induced Shifts

B. T. PENNINGTON* AND J. R. CAVANAUGH†

*Eastern Regional Research Center, Agricultural Research Service, USDA,
Philadelphia, Pennsylvania 19118*

The equilibria between Pr^{3+} , Ho^{3+} , and Yb^{3+} and the ligands acetate, 4-aminobutyric acid (4-ABA), 3-aminobutyric acid (3-ABA), and L-valine were investigated at pH values near 6.0 over a wide range of ligand/ Ln^{3+} ratios. All ligands except L-valine were found to form 2:1 ligand/ Ln^{3+} complexes at high ligand/ Ln^{3+} ratios. From intramolecular shift ratios induced by a number of Ln^{3+} ions covering the entire rare earth series, it was concluded that the complexes of 4-ABA with Ce^{3+} , Pr^{3+} , and Nd^{3+} possess near-axial symmetry. Intramolecular shift ratios also indicate that Eu^{3+} may produce significant contact contributions in the α -proton shifts of 3-ABA and L-valine. Unequal shifts of the α protons of 3-ABA were observed in the presence of Pr^{3+} and Eu^{3+} . The non-equivalence of the α protons appears to be due to a preferred orientation in certain rotamers of the carboxylate group with respect to the amino group.

INTRODUCTION

The discovery and application of lanthanide shift reagents has had a substantial impact on the field of NMR spectroscopy (1, 2). However, because the common reagents such as $\text{Eu}(\text{fod})_3$ and $\text{Eu}(\text{dpm})_3$ react with water, their utility has largely been lost to aqueous systems. More recently (3-5) rare earth ions, in the form of the nitrate or chloride, have been shown to produce appreciable shifts (though consistently smaller than those produced by the complexes) for a variety of substances in aqueous solution.

However, a number of questions have been raised concerning the induced shifts of the rare earth ions (6-11), including the stoichiometry of the complexes formed in solution over a wide concentration range, the presence or absence of axial symmetry, possible contact contributions to the induced shifts, and the effect of conformational equilibria in solution. Our own interest in the NMR spectra of amino acids in solution (12) has prompted us to explore in depth the interactions of lanthanide ions with a number of amino acids.

Therefore, we have measured the induced proton shifts for a number of ligands in the presence of Pr^{3+} , Ho^{3+} , and Yb^{3+} ions in aqueous solution over a wide range of

* NRC-ARS Postdoctoral Research Associate. Present address: Navarro College, Corsicana, Tex. 75151.

† Author to whom inquiries should be addressed.

ligand/ Ln^{3+} concentration ratios and have analyzed the data in terms of one-step and two-step equilibria. From intramolecular shift ratios of these complexes, we were able to assess the presence or absence of axial symmetry in several favorable cases, possible contact contributions to the induced shifts of Eu^{3+} , certain details of solution geometry and in one instance effects on rotamer stability.

EXPERIMENTAL

The hydrated chlorides of the tripositive metal ions of Ce, Pr, Ho, Eu, Nd, Dy, Er, Gd, Yb, and La (Ventron, Alfa Products, 99.9%)¹ were dissolved in D_2O and evaporated to dryness under vacuum. After the second dissolution–evaporation was performed, the resulting solid in each case was dissolved in D_2O and filtered. The concentrations of the rare earth solutions were determined by titration with excess standardized EDTA which was back titrated in boric acid–NaOH buffered media at pH 8.5 using standardized Zn^{2+} solution and eriochrome black T as indicator (13). 4-ABA (Aldrich, 97%) and 3-ABA (Aldrich, 98%) were recrystallized twice from water and methanol. L- α -aminobutyric acid, L-valine (Mann Research Laboratories, Inc.), potassium chloride (Baker, A. R.), and sodium acetate (Baker, A. R.) were used without further purification.

Amino acid solution (300–1000 μl) was added to a 4-ml vial and enough NaOD (1–3 μl) was added to bring the pH meter readings to the range 7.0–7.5. A Chemtrix pH meter (type 40 AC) with accompanying combination glass electrode was used to measure the pH of the test solutions. The pH readings in this work are actual meter readings uncorrected for deuterium isotope effects ($\text{pH} = \text{PD} + 0.4$). After addition of the amino acid solution and a small amount of NaOD, aliquots of the slightly acidic ($\text{pH} = 4.0$ to 5.0) lanthanide ion solution (50–800 μl), KCl solution (to maintain ionic strength), and DC1 (to bring the pH into the desired range, ± 0.1 -pH unit) were then added. The transfers were made using Finnpiettes, and the final volumes were adjusted to 2.00 ± 0.01 ml using D_2O . In general, 15 to 20 test solutions were prepared for each amino acid studied with the amino acid concentration held constant near 0.15 M.

Proton NMR spectra were recorded at 90 MHz with a Bruker WH-90 Fourier transform spectrometer. In general, 5 to 25 scans were required for low noise spectra at an operating temperature of $25 \pm 1^\circ\text{C}$. Induced shift values are measured relative to the shifts of diamagnetic La^{3+} solutions with similar metal ion concentration, amino acid concentration, and pH. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS, Wilmad Glass Co.) was used as an internal reference.

RESULTS AND DISCUSSION

Ln^{3+} -Induced Shifts and Ligand Equilibria

Under the condition of rapid exchange between Ln^{3+} and its ligands in aqueous media, the observed shift is an average over all ligand molecules in solution. The shifts induced at the α protons by Pr^{3+} are shown in Fig. 1 for the ligands, acetate, 4-ABA, 3-ABA, and L-valine as a function of the ligand/ Ln^{3+} ratio, with the ligand concentration

¹The mention of commercial items is for the reader's convenience and does not constitute an endorsement by the Department of Agriculture.

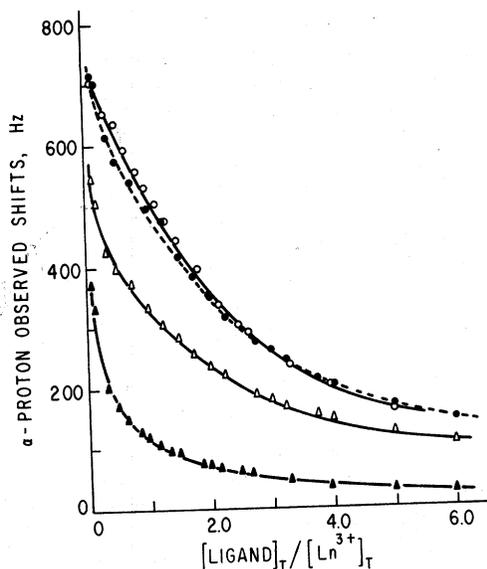


FIG. 1. The Pr^{3+} -induced shifts of acetate (○), 4-aminobutyric acid (●), 3-aminobutyric acid (△), and L-valine (▲) as a function of the ligand/ Ln^{3+} ratio. The ligand concentrations for acetate, 4-ABA, 3-ABA, and L-valine are 0.113, 0.165, 0.164, and 0.109 M, respectively. The solid lines represent the best computer fits: two-step equilibrium for acetate, 4-ABA, and 3-ABA; one-step equilibrium for valine.

held constant. These curves can be analyzed in terms of multiple-step equilibria, the first two of which are given below.

$$1:1 \quad \Delta v_{\text{obs}} = \frac{[\text{ML}]}{[\text{L}]_t} \cdot \Delta v_{\text{Lim1}}, \quad [1]$$

$$2:1 \quad \Delta v_{\text{obs}} = \frac{[\text{ML}]}{[\text{L}]_t} \Delta v_{\text{Lim1}} + \frac{2[\text{ML}_2]}{[\text{L}]_t} \Delta v_{\text{Lim2}}, \quad [2]$$

where Δv_{obs} is the observed shift, Δv_{Lim1} and Δv_{Lim2} are the limiting shifts of the 1:1 and 2:1 complexes, $[\text{ML}]$ and $[\text{ML}_2]$ are the concentrations of the respective complexes, and $[\text{L}]_t$ is the total ligand concentration. By means of these equations, the experimental data were analyzed using a computer best-fit approach. The results for the Pr^{3+} complexes are shown in Table 1.

The Pr^{3+} complexes with 3-ABA at low ligand/metal ion ratio yield separate resonances for the two α protons (see below). The chemical shift average was used in the above calculation. Since the ratio of the two chemical shifts is constant over the measurable range, the use of the average would not change the derived value of K but would affect the values of Δv_{Lim} .

For the Pr^{3+} complexes with acetate, 4-ABA, and 3-ABA, the one-step equilibrium gives a very poor fit over the entire concentration range. Only by using the two-step equilibrium can a reasonable fit be obtained between calculated and observed values. On the other hand, calculations on the Pr^{3+} -valine system yield equally good results for both the one-step and two-step cases. The computer fit can be visualized by the solid lines in Fig. 1.

TABLE 1
ONE- AND TWO-STEP EQUILIBRIUM DATA DERIVED FROM A COMPUTER BEST
FIT OF Pr^{3+} -INDUCED SHIFTS^a

Ligand	K_1 (L/M)	K_2 (L/M)	$\Delta\nu_{\text{Lim1}}$ (Hz)	$\Delta\nu_{\text{Lim2}}$ (Hz)	Goodness of fit ^b σ (Hz)
Acetate	60	10.2	708	640	7.5
	185	—	756	—	53.0
4-ABA ^c	20	9	735	638	7.0
	175	—	735	—	66.0
3-ABA	14	4	582	474	14.0
	110	—	531	—	52.0
L-Valine	2.4	0.23	373	951	14.2
	5.5	—	414	—	14.0

^a α -Proton shift data used in the calculation. The first line for each ligand entry is for the two-step equilibrium; the second line is for the one-step equilibrium.

^b Standard deviation of the real set of shift data from the set of data calculated from the best-fit parameters.

^c ABA is aminobutyric acid.

Therefore, the Pr^{3+} -valine equilibrium appears to be very adequately represented by the simple 1:1 complex. However, the other ligands are evidently forming the 2:1 complex at high ligand/ Ln^{3+} ratios.

Too much reliance on the precise values of the two-step equilibrium constants derived in Table 1 is unwarranted. In a multiple-parameter fit such as the two-step equilibrium, there is rarely a unique solution; often many different sets of parameters will fit equally well. As pointed out by other workers (14), the most accurately determined parameter is $\Delta\nu_{\text{Lim1}}$, which is almost independent of K_1 , but $\Delta\nu_{\text{Lim2}}$ is very sensitive to the values of $\Delta\nu_{\text{Lim1}}$, K_1 , and K_2 . However, there is good agreement between the equilibrium constants derived from the NMR data for the acetate- Pr^{3+} complex and the literature values determined by other methods (15, 16).

When the intramolecular ratios of the induced shifts are examined as a function of ligand/ Ln^{3+} ratio, it is found that they are essentially identical across the entire concentration range. Since these systems are undergoing a two-step equilibrium (with the exception of valine), the relative abundance of the 1:1 to the 2:1 complex varies as a function of the ligand/ Ln^{3+} ratio. Therefore, the induced shift ratio in the 1:1 and 2:1 complex must have nearly the same values and, consequently, the ligands in both species must be oriented very similarly with respect to the anisotropy of the metal ion in the complex.

Another trend brought out from the results is that the equilibrium constants (cf. Table 1) decrease in the order: acetate > 4-ABA > 3-ABA > L-valine. Because it has been shown that the Ln^{3+} complexation occurs at the carboxylate group at pH 7 and below, the decrease in the equilibrium constant reflects the closeness of the amino group to the

binding site. Since the amino group is protonated at the pH values employed, this effect appears to be due to the electrostatic repulsion of the charged amino group to the complexing Ln^{3+} ion.

Data were also developed for these ligands with the lanthanide ions Ho^{3+} and Yb^{3+} . Computer analysis yielded results similar to those displayed in Table 1 for the Pr^{3+} complex. No definitive differences in the binding constants for the three metal ions could be elucidated for acetate, 4-ABA, or 3-ABA complexes. However, the analysis of the one-step equilibrium for the L-valine complexes shows a systematic increase in the equilibrium constant in the order: $\text{Pr}^{3+} < \text{Ho}^{3+} < \text{Yb}^{3+}$ (5.5, 9.0, and 12.0 L/M, respectively). Since one can place more confidence in the computer results derived for the one-step equilibrium (a two-parameter fit), the trend is probably real and follows the decrease in atomic radius as the rare earth series is traversed from Pr to Yb.

Pseudocontact and Contact Contributions and Axial Symmetry

Lanthanide-induced shifts are generally interpreted as being predominantly pseudocontact in origin (17, 18). The pseudocontact shift induced by a paramagnetic ion with anisotropic magnetic susceptibility is given by the equation (19)

$$\frac{\Delta\nu}{\nu_0} = A \left(\frac{3 \cos^2 \theta_i - 1}{r_i^3} \right) + A' \left(\frac{\sin^2 \theta_i \cos 2\phi_i}{r_i^3} \right), \quad [3]$$

where $\Delta\nu$ is the observed pseudocontact shift, ν_0 is the probe frequency, A and A' are constants for each lanthanide complex at a given temperature, r_i is the distance between the Ln^{3+} ion and a specific nucleus, θ_i is the angle between the principal Z axis of the complexed ion and the distance vector r_i , and ϕ_i is the angle between the equatorial projection of r_i and the principal X axis. For an axially symmetric complex, the second term of Eq. [3] goes to zero and the pseudocontact shift of a particular nucleus is dependent upon only the angle θ_i and the distance r_i . As a practical necessity, the condition of axial symmetry must usually be met in order to obtain structural information about the ligand since Eq. [3] contains too many parameters to be evaluated in most cases.

For an axially symmetric complex with pseudocontact shifts, it is readily seen from the simplified form of Eq. [2] that the intramolecular shift ratios are independent of the identity of the Ln^{3+} ion. However, in a recent study (7), it was shown that for θ values of less than 40° the nonaxial contributions to the shifts are effectively suppressed, and it was concluded that the presence of axial symmetry can only be shown if the shift ratios produced by various Ln^{3+} ions do not deviate by more than a few percent. This is a stricter requirement than had been generally thought necessary previously.

The intramolecular shift ratios of 4-ABA, 3-ABA, and L-valine for various Ln^{3+} ions under similar conditions are listed in Table 2. While there is general agreement among the shift ratios for different metal ions with a given ligand, in most cases they vary by more than the few percent strictly required to show axial symmetry. The 4-ABA complexes with Ce, Pr, and Nd are exceptions; these shift ratios are sufficiently close to fall within the strict requirements for axial symmetry. The 3-ABA complexes with Ce, Pr, Er, and Yb and the L-valine complexes with Ce, Pr, and Nd, while not within the strict limits, come very close to meeting the requirements for axial symmetry.

TABLE 2
INTRAMOLECULAR SHIFT RATIOS^a OF 4-ABA,^b
3-ABA, AND L-VALINE FOR VARIOUS Ln³⁺ IONS AT A
LIGAND/Ln³⁺ RATIO OF 0.1

Ligand	Ln ³⁺	β/α	γ/α	γ/β
4-ABA	Ce	0.58	0.35	0.60
	Pr	0.58	0.35	0.61
	Nd	0.58	0.35	0.61
	Eu	0.59	0.38	0.66
	Dy	0.56	0.30	0.55
	Ho	0.53	0.28	0.52
	Er	0.55	0.28	0.52
	Yb	0.58	0.28	0.48
3-ABA	Ce	0.71	0.34	0.48
	Pr	0.67	0.35	0.52
	Eu	0.84	0.47	0.57
	Dy	0.63	0.31	0.49
	Ho	0.60	0.26	0.43
	Er	0.71	0.31	0.43
	Yb	0.70	0.26	0.37
L-Valine	Ce	0.77	0.36	0.46
	Pr	0.76	0.39	0.52
	Nd	0.78	0.36	0.46
	Eu	0.94	0.46	0.49
	Dy	0.64	0.33	0.52
	Ho	0.54	0.26	0.47
	Yb	0.56	0.24	0.43

^a Good to ± 0.02 or better in all cases. The values for Dy, Ho, Yb, and Pr are the most accurate because of their induced shift magnitudes.

^b ABA stands for aminobutyric acid.

Consequently, an attempt to locate the position and direction of the axially symmetric magnetic anisotropy was carried out by matching observed and predicted shift ratios in order to illustrate the kind of agreement that can be obtained in these systems. It was assumed that the metal ion was positioned along the axis bisecting the O—C—O bond of the carboxylate group, that the carboxylate group was freely rotating, and that the staggered rotamers obtained by internal rotation of the C _{α} —C _{β} bond were equally populated. The direction of the axis of magnetic anisotropy was varied from 0 to 90° relative to the plane of the carboxylate group in 10° increments, the Ln³⁺—oxygen bond distance varied over the distance 2.0 to 3.0 Å, and the calculated shift ratios averaged over the free rotation of the carboxylate group taken in 60° increments.

The best fit to the observed shift ratios was obtained with the principal magnetic axis lying in the carboxylate plane, the 0° orientation, with a bond distance of 2.3 to 2.5 Å. This configuration yielded shift ratios of 0.61, 0.39, and 0.64 corresponding to the observed ratios for Pr³⁺-induced shifts of 0.58, 0.35, and 0.60, respectively.

The shift ratios induced by Eu³⁺ deviate markedly from those of the other ions for 3-ABA and L-valine. As seen from Table 2, the β/α and γ/α ratios deviate 25–40% from

the same ratios induced by neighboring ions, and are indicative of substantial contact or nonaxial contributions. Since aberrant Eu^{3+} -induced shift ratios have been ascribed to contact interactions in other systems (20), and since the deviations here are seen primarily at the α proton, it is probable that contact contributions are present here also.

Nonequivalence of Induced Shifts and Rotamer Populations

The Pr^{3+} and Eu^{3+} complexes of 3-ABA at a high metal-to-ligand ration yield NMR spectra as shown in Fig. 2 that display a multiplicity of lines for the α -proton resonances

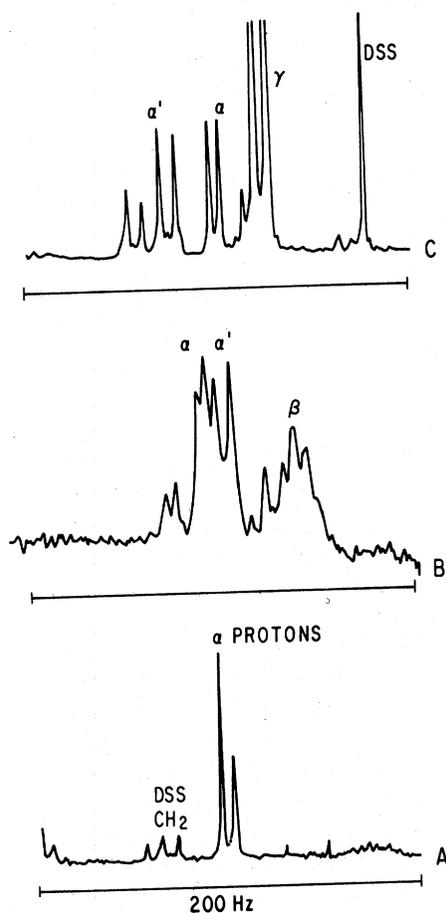


FIG. 2. The NMR spectra of the α protons of 3-aminobutyric acid in the presence of La^{3+} , Pr^{3+} , and Eu^{3+} . The conditions of metal ion concentration, ligand concentration (molar), and pH are: A (La^{3+}), 0.4, 0.04, 6.0; B (Pr^{3+}), 0.4, 0.04, 6.1; C (Eu^{3+}), 0.2, 0.15, 5.9. The spectra as shown are not in proper register. The mean chemical shifts for A, B, and C relative to DSS are 2.55, 7.39, and 0.98 ppm, respectively.

characteristic of nonequivalence. The α protons of the La^{3+} complex, on the other hand, do not exhibit any nonequivalence. In the Pr^{3+} and Eu^{3+} complexes, moreover, the ratios of the induced shifts for the two α -protons are not the same for both. The induced shifts for the two α -protons in the Pr^{3+} complex are in the ratio of 0.95/1 and in the

Eu^{3+} complex, 0.76/1. In both cases, the α proton with the smaller vicinal coupling constant is shifted the most.

For the Eu^{3+} complex, the induced chemical shift difference is sufficiently large that the coupling constant information can be obtained over a wide range of metal ion concentrations. Consequently, a series of spectra were taken from 0.010 to 0.40 M in metal ion. These concentrations correspond to 0.14 to 0.85 fraction of ligand bound to metal as calculated from equilibrium data as discussed previously. The portion of the spectrum corresponding to the α and β protons was analyzed as an ABC spin system and the results yield the data presented in Fig. 3.

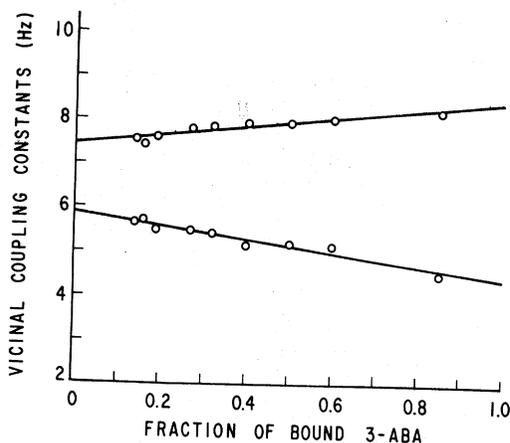
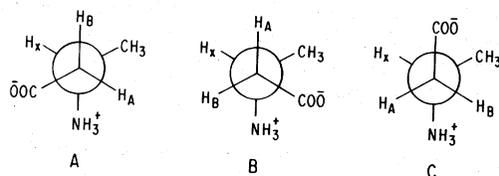


FIG. 3. The $\alpha\beta$ and $\alpha'\beta$ proton vicinal coupling constants of 3-ABA as a function of the fraction of ligand bound to Eu^{3+} . The 3-ABA concentration was held constant at 0.04 M while the Eu^{3+} concentration was varied from 0.010 to 0.40 M at $\text{pH } 6.0 \pm 0.1$.

The variation of the vicinal coupling constants is approximately linear with the extent of complexation. Consequently, values for the coupling constants for the uncomplexed and complexed species can be obtained by linear extrapolation using a least-squares fit of the data. These values are 5.87 and 7.43 Hz for the vicinal coupling constants for the uncomplexed ligand and 4.43 and 8.44 Hz for the complex.

The vicinal coupling constants so obtained provide a means of estimating the population of the internal rotational states. It has become well established that the rotational isomerization of these molecules can be represented as an equilibrium mixture of the rapidly rotating staggered rotamers as shown below.



If it is assumed that the values for the proton-proton *gauche* and *trans* coupling constants, J_g and J_t , respectively, are independent of the particular conformation, the

averaged vicinal coupling constants, J_{AX} and J_{BX} , are given by

$$J_{AX} = aJ_t + bJ_g + cJ_g = (1 - a)J_g + aJ_t, \quad [4]$$

$$J_{BX} = aJ_g + bJ_g + cJ_t = (1 - c)J_g + cJ_t, \quad [5]$$

where a , b , and c are the normalized populations of rotamers A, B, and C. If values for J_g and J_t were known, values for a , b , and c could be calculated from experimentally determined values for J_{AX} and J_{BX} . Values for J_g and J_t have been derived for α -amino acids (21) and applied to a number of systems (12).

There is an ambiguity in the assignment of J_{AX} and J_{BX} so that the relative magnitudes of a and c cannot be resolved from NMR coupling constant data alone. From electrostatic arguments, rotamer C would be predicted to have the largest population in the complexed form since it would reduce electrostatic repulsion between the positively charged amino group and the Ln^{3+} ion.

On the other hand, rotamer A for the uncomplexed species would be predicted to be more stable than C both from electrostatic considerations (maximum interaction between the positive amino group and negative carboxyl group) as well as from steric considerations (minimum interaction between the methyl and carboxylate groups). The variation of the averaged vicinal coupling constants with increasing complexation shows unequivocally that the relationship of the A and C populations must be the same in the complexed and uncomplexed forms. Otherwise, a crossover of the two vicinal coupling constants would occur. The fact that the larger coupling increases with increasing complexation strongly suggests that it corresponds to J_{BX} and that it is the C population that increases with complexation rather than the A population. With this assignment in mind, and with the values of J_g and J_t set equal to 2.60 and 13.56 Hz, respectively (21), the populations for the A, B, and C rotamers obtained are 0.30, 0.26, 0.44 and 0.17, 0.30, 0.53 for the uncomplexed and complexed species, respectively.

Implicit in this development are a number of assumptions including the constancy of J_g and J_t between complexed and uncomplexed forms. Nor are these values for the β -amino acids known with great certainty. Consequently, the numbers obtained should be viewed as estimates. Nevertheless, these results show that the relative rotamer populations of 3-ABA change with Eu^{3+} complexation and that all rotamers of both the acid and complex have significant populations.

The induced chemical shift differences between the two α protons for the Eu^{3+} -3-ABA complex can also be followed over a wide variation of metal ion concentration. The result is that the ratio of the one α -proton shift to the other remains constant at 0.76 over the measured range. Consequently, the effects at the α protons are proportional and evidently arise directly from the presence of the Eu^{3+} metal ion and not indirectly as a result of changes in rotamer population.

The fact that the two α protons have significantly different induced shifts is rather remarkable, especially in view of their symmetry with regard to the carboxylate group. If there is free rotation about the α -carbon-carboxylate-carbon bond, there does not appear to be any mechanism that could account for the differences in induced shift, whether they are contact or pseudocontact in origin. For pseudocontact shifts, even with nonaxial terms, the induced shift averaged over the carboxylate group rotation would be the same for both α protons. The same is true for contact shifts which are

dependent upon the dihedral angle of the proton with the metal–ligand bond (22). Therefore, the carboxylate group must be restricted in its internal rotation.

A possible source for its restricted rotation may be found in its interaction with the charged amino group. In those conformations in which the amino group is *gauche* to the carboxylate group, it is possible that the carboxylate oxygen lines up with the charged amino group in such a way that the carboxylate–metal bond direction no longer bisects the H–C–H bond angle of the α protons and creates a nonequivalence at the two α proton positions.

Given the restricted rotation of the carboxylate group, it is conceivable that contact or pseudocontact terms (including nonaxial contributions) or both are responsible for the induced nonequivalence at the α protons. However, the induced shift differences of the α protons of the 3-ABA complexes with most of the other Ln^{3+} ions are estimated not to exceed that seen for the Pr^{3+} complex and in any event do not approach that for the Eu^{3+} complex. Since the α -proton shifts of 3-ABA and L-valine produced by Eu^{3+} have been indicated previously to have a possible contact contribution, the nonequivalence at the α protons might also be traced in part to a contact interaction.

Nonequivalent induced shifts have been reported for the β protons of serine (23) and, in the present study, nonequivalent shifts have been observed for the γ -methyl groups of valine in the presence of Pr^{3+} and Eu^{3+} . In all these cases, however, the nonequivalence is one bond further removed from that seen for the α protons in 3-ABA and can be easily accounted for by unequally populated rotamers (24). What is unique about the case of the 3-ABA complex is that the induced nonequivalence is seen at the α protons.

CONCLUSIONS

The interactions of lanthanide ions with amino acids in aqueous solution produce measurable effects in the NMR spectra which can provide a means of assessing solution geometry. Such an assessment, however, is dependent on a number of factors, including the presence or absence of contact contributions to the observed shifts, nonaxial symmetry in the complex, multiple equilibria, and molecular internal rotation. In many cases it may be extremely difficult, if not impossible, to separate the myriad factors. For instance, there may not be a single complex present that is axially symmetric, but the shifts averaged over all rotamers and complex geometries may only appear to arise from axial symmetry.

Several of the lanthanide ions also bring about observed changes in the vicinal coupling constants of the ligand upon complexation. Under the usual assumptions, these effects can be utilized to estimate changes in the rotamer populations. There are even areas where the presence of the metal ion in the complex produces a measurable asymmetry in the chemical shifts of the α protons.

Therefore, deriving structural information from the shifts induced by rare earth ions in aqueous solution on flexible molecules is a hazardous undertaking at best. We are currently involved in the study of planar carboxylate ligands devoid of rotamer states as a means of comparing a known rigid ligand structure to the Ln^{3+} -induced shifts and relaxation data. Hopefully, the information obtained from this approach will be useful in establishing more reliable methodology for internally rotating systems.

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