Lanthanide Ethylenediaminetetra-acetate Chelates as Aqueous Shift Reagents: Evidence for Effective Axial Symmetry in Bidentate Cytidine 5′-Monophosphate and Alanine Complexes

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Lanthanide-induced shift (l.i.s.) data are presented for cytidine 5′-monophosphate (cydmp) and L-alanine binding to a series of lanthanide–edta chelates at high pH (edta = ethylenediaminetetra-acetate). Slow exchange of these substrates between their bound and free environments precludes the use of the heavier Ln(edta) chelates at 25 °C. The cydmp proton l.i.s. ratios at 90 °C are independent of the Ln(edta) used, indicative of effective axial symmetry for this substrate. The alanine l.i.s. values contain large contact shift components which once removed yield corrected pseudo-contact shifts which also are adequately described by an effective axial symmetry model. The results indicate that chemically reasonable structures may be determined from l.i.s. data for substrates acting as bidentate chelators of the lanthanide, providing rapid exchange conditions are met and contact shift contributions are extracted from experimental l.i.s. values for nuclei near the co-ordination site(s). Therefore the fact that the substrate acts as a bidentate chelator does not necessarily exclude the possibility that the axially symmetric model for the l.i.s. is a satisfactory model.

LANTHANIDE ethylenediaminetetra-acetate (edta) chelates acting as n.m.r. aqueous shift reagents offer several advantages over the aquo-cations. These include a greater solubility over a wide pH range allowing a study of lanthanide–substrate structures above pH 7 and co-ordination sphere restrictions which limit the metal–substrate equilibria to 1 : 1 stoicheiometry.[1,2] The pseudo-contact shifts induced by these chelates are described by equation (1) where D' and D'' contain

$$\text{l.i.s.} = D' \frac{3\cos^2\theta - 1}{r^3} - D'' \frac{\sin^2\theta\cos2\phi}{r^3} \qquad (1)$$

ligand-field and magnetic constants characteristic of a lanthanide at any temperature and r, θ, and ϕ are the spherical co-ordinates of the nucleus under observation with the lanthanide at the origin.[3] Although the static Ln(edta) chelates do not display axial symmetry,[4] rapid exchange of a second weakly binding ligand between the bulk solution and the lanthanide co-ordination sphere can result in an averaged structure with ' effective ' axial symmetry whose shifts are adequately described by the first term in equation (1).[5] This thesis of course implies the edta chelate bound in the lower lanthanide hemisphere does not dictate the binding orientation of the second ligand into the upper hemisphere.

Reuben [6] has recently presented shift data for binding of certain Ln(edta) complexes with the bidentate chelates salicylaldehyde and *o*-nitrophenol, and argued that the resulting ternary chelates lack effective axial symmetry because they could not undergo internal rotation or stereochemical rearrangement.[5] These conclusions were based upon variations within the proton shift ratios of salicylaldehyde and *o*-nitrophenol interacting with edta chelates of Pr^{3+}, Nd^{3+}, and Eu^{3+}. Possible shift ratio deviations resulting from contact shift origins, although not fully dismissed, were not investigated. Our previously reported data with L-alanine at low pH illustrate that proton shifts are not necessarily void of contact contributions (even in aliphatic systems) and furthermore such shifts do not necessarily attentuate rapidly away from the ligating atom position.[7] We propose that the proton shifts in the aforementioned aromatic substrates contain large contact contributions and, if the appropriate corrections could be made, the resulting pseudo-contact shifts may obey the effective axial symmetry model. In this paper, we present lanthide-induced shift (l.i.s.) data for two structurally quite different molecules which co-ordinate to Ln(edta) in a bidentate manner *and* yield shifts which are adequately described by the effective axial symmetry model. First, proton l.i.s. data are presented for cytidine 5′-monophosphate (cydmp) binding to the edta chelates of the entire lanthanide cation series at pH 8 and next, proton and carbon l.i.s. data are presented for L-alanine binding to the edta chelates of Pr^{3+}, Nd^{3+}, Eu^{3+}, and Tb^{3+} at pH 10.

EXPERIMENTAL

Materials and Methods.—Stock solutions of the Ln(edta) chelates were prepared in D_2O by dissolving ethylenediaminetetra-acetic acid (H_4edta) into a basic solution, adding a stoicheiometric amount of solid $LnCl_3 \cdot nH_2O$ (standardized using xylenol orange as an indicator), and adjusting the pH to the desired value. Cytidine 5′-monophosphate was obtained from Sigma and lyophilized from 99.8% D_2O before use. L-Alanine was used without purification.

Proton n.m.r. spectra were recorded on a JEOL JPS-100 Fourier-transform spectrometer operating at 100 MHz (cydmp data) or a JEOL C-60HL spectrometer operating at 60 MHz (L-alanine). Carbon n.m.r. spectra were recorded on a Bruker WP-60 Fourier-transform spectrometer at 15.1 MHz. 2,2,3,3-Tetradeuterio-3-trimethylsilylpropionate and Bu^tOH were used as internal shift standards for the cydmp and L-alanine data respectively. The pH values reported herein have not been corrected for the deuterium isotope effect.

RESULTS AND DISCUSSION

Cytidine 5′-Monophosphate.—The dynamic solution conformation of this mononucleotide has been examined previously using l.i.s. techniques with nine aqueous Ln^{3+} cations [8,9] at pH 2 and with two Ln(edta) chelates [9,10] (where Ln = Pr^{3+} and Eu^{3+}) at pH 7.5. These workers have shown that similar structural results are obtained using the aqueous Ln^{3+} cations *versus* using Pr(edta) or Eu(edta) as the shift reagent. In both cases, the measured l.i.s. show effective axial symmetry and the cydmp binds to the metal cation *via* bidentate co-ordination through the phosphate.

We have extended these studies on cydmp to the entire Ln(edta) chelate series in an effort to determine the general utility of these chelates as aqueous shift reagents. Table 1 gives the proton shift ratios determined for these systems using the different Ln(edta) chelates at 25 °C and at 90 °C. The conditions of fast exchange apply, and the shift ratios were obtained in the usual way,[1,9,10] from titration curves of 0.03 mol dm^{-3} cydmp with a variation of the concentration of the Ln(edta) chelates up to 0.05 mol dm^{-3}. The data show that for the first half of the series (Ce^{3+} to Eu^{3+}) the l.i.s. obey the effective axial model quite well as evidenced by the nearly constant shift ratios.[8] Furthermore, the shift ratios change only slightly as the temperature is increased to 90 °C. Exchange broadening of the proton resonances is however observed at 25 °C only for the latter half of the series (Tb^{3+} to Yb^{3+}). The association constants for cydmp binding to the Ln(edta) chelates at pH = 8.0 are smaller by a factor of three than cydmp binding to the aquo-ions,[8] but the fully bound shifts, $\Delta\omega_M$, are in the first case also larger by a factor of three. The reasons for the absence of a fast exchange condition $(\Delta\omega_M \cdot \tau_M \geqslant 1)$ in the l.i.s. of the heavier Ln(edta) complexes could be a combined effect of increased $\Delta\omega_M$ and τ_M values.

TABLE 1

Lanthanide-induced shift ratios in cytidine 5′-monophosphate at pH 8 using Ln(edta) chelates as shift reagents [a]

Lanthanide cation	θ_c/°C	Nucleus H^6	H^5	$H^{1'}$	$H^{2'}$	$H^{3'}$	$H^{4'}$
Ce	25	0.51	0.02	0.01	0.22	0.38	0.29
	90	0.45	−0.06	0.07	0.19	0.38	*b*
Pr	25	0.46	0.07	0.10	0.23	0.40	0.32
	90	0.43	0.03	0.07	0.21	0.36	0.31
Nd	25	0.43	0.07	0.08	0.20	0.35	0.28
	90	0.45	−0.03	0.10	0.20	*b*	0.28
Eu	25	0.46	−0.07	0.05	0.19	0.35	0.30
	90	0.41	−0.08	0.08	0.18	0.38	0.32
Tb	90	0.35	−0.06	0.05	0.14	0.34	0.18
Dy	90	0.38	−0.04	0.06	0.16	0.35	0.22
Ho	90	0.40	−0.05	0.05	0.18	0.36	0.23
Er	90	0.39	−0.08	0.05	*b*	0.30	*b*
Tm	90	0.33	−0.26	0.02	*b*	0.31	*b*
Yb	90	0.43	−0.06	0.06	*b*	0.38	*b*

[a] Relative to $H^{5'}$ = 1.00. [b] Not measured.

At 90 °C the conditions of fast exchange apply for the l.i.s. of the heavier Ln(edta) chelates and the corresponding shift ratios show that the axially symmetrical model becomes a satisfactory model. A notable exception is Tm^{3+}, a situation that has been observed previously for the aqueous ion.[8] This has previously been attributed to an increased conformational rigidity of the Tm^{3+} co-ordination sphere.[11]

Table 2 shows that the shift directions for the various Ln(edta) chelates agree with the theory of Bleaney [12] and that the agreement of their magnitudes with those predicted is not worse than that found for the aqueous ions.[8] This is of course not a proof of axial symmetry, but it indicates the constancy along the lanthanide series of the crystal-field parameters of both terms of equation (1).

L-*Alanine.*—The carbon and proton n.m.r. spectra of L-alanine solutions at pH 10 containing small quantities of Pr(edta), Nd(edta), Eu(edta), or Tb(edta) show resonances clearly shifted from the alanine–La(edta) reference positions indicative of a rapid exchange process. Exchange broadening of the carbon resonances precluded the use of Ln(edta) chelates from the latter half of the series. The association constant for alanine binding to these four chelates at pH 10 was 4.6 ± 0.8 dm^3 mol^{-1}, a factor of six larger than alanine binding to the aquo-ions [7] at pH 3. Although these stability con-

TABLE 2

Lanthanide-induced shifts in the $H^{5'}$ proton of cydmp [a]

Ln cation	Theory [b]	Experimental Aqueous ions [c] pH 2; 25 °C	Ln(edta); pH 8 25 °C	90 °C
Ce	−5.7	*d*	−2.1	−3.3
Pr	−10.0	−10.0	−10.0	−10.0
Nd	−3.8	−3.7	−4.3	−1.0
Eu	3.6	5.6	6.6	5.6
Tb	−78	−70	*e*	−43
Dy	−91	−140	*e*	−109
Ho	−35	−55	*e*	−29
Er	30	17	*e*	13
Tm	48	12	*e*	13
Yb	20	14	*e*	5.1

[a] All l.i.s. are relative to Pr = −10. [b] From ref. 12. [c] From ref. 8. [d] Not observed. [e] L.i.s. not in rapid exchange.

stants do not define the mode of co-ordination of L-alanine to the Ln(edta) chelates, Brittain[13] has recently used circularly polarized luminiscence to show that alanine changes from uni- to bi-dentate co-ordination in Tb(pydca)$_2$–alanine complexes above pH 8 (pydca = pyridine-2,6-dicarboxylate). We, therefore, assume for the moment that the primary mode of alanine co-ordination to the Ln(edta) chelates at pH 10 is bidentate. The heavier Ln(edta) chelates have a higher affinity for alanine at pH 10 as evidenced by proton resonance shifts and this may be partly responsible for the exchange broadening observed in the carbon resonances of L-alanine bound to these chelates. Increasing the temperature to 90 °C did not bring the carbon resonances for the heavier chelate–alanine mixtures into the rapid exchange regime. Thus, the l.i.s. could be directly compared only for those ions which rapidly exchange L-alanine between the bound and free environments.

The proton shift ratios presented in Table 3 clearly vary from one lanthanide chelate to another and this could be used to argue against effective axial symmetry for these bidentate structures. However, the carbon shifts are expected to have a large contact component and these shift ratios show the same trends as the proton shift ratios, expecially for Eu(edta) which is known to display the largest contact/pseudo-contact shift contribution. This suggests the observed variation in proton shift ratios may result from contact contributions to these shifts and is not necessarily due to a lack of axial symmetry.

The contact and pseudo-contact contributions to each shift were separated using techniques outlined previously[7,14] and the contact shifts are presented in Table 4 along with the corrected pseudo-contact shift ratios. As expected, the experimental carbon l.i.s. are dominated by contact interactions. The α-proton shifts also show a significant contact contribution with all four lanthanide cations but the β-proton shifts are nearly void of contact effects. The corrected pseudo-contact shifts are considerably different from the measured values for three of the four lanthanide cations. With the exception of the C_β l.i.s. (see conclusions), the Pr shift ratios were changed very little by the correction procedures even though the H_α and C_α shifts contained significant contact components. The Nd and Eu shift ratio changes were most dramatic because of the large contact/pseudo-contact shifts expected for these ions.[3]

The relaxation rates of the carbon nuclei of L-alanine in the presence of Gd(edta) (Table 3) clearly reflect the geometry of the bound L-alanine. The similar relaxation rates of C_0 and C_α dictate a bidentate ligation of alanine to the metal ion with nearly equal Ln–O and Ln–N bond lengths. These data together with the corrected pseudo-contact shift ratios from Table 4 were used in a computer search procedure which evaluates the standard deviation (R factors)[15] between the observed l.i.s. and those calculated from the axial symmetry model [first term in equation (1)] and the standard deviation between the measured relaxation rates and the calculated $r_{\alpha\,\mathrm{or}\,\beta}^{-6}/r_0^{-6}$ ratios for each lanthanide ion location. The principal symmetry axis vector from which θ is measured was included as a variable parameter in the fitting procedure. Minimum R factors were obtained only for lanthanide-ion locations between the nitrogen and oxygen ligating atoms as expected for a bidentate structure. The minimized R factors determined the exact position of the lanthanide with respect to the C_0, C_α, N plane but were somewhat insensitive to distances. The minimized

TABLE 3

Lanthide-induced shifts and relaxation rates in L-alanine at pH 10

Nucleus	L.i.s. ratios[a] Pr(edta)	Nd(edta)	Eu(edta)	Tb(edta)	T_{1P}^{-1} ratios[b] Gd(edta)
H_α	0.27	1.18	1.19	0.20	
H_β	0.11	0.38	0.04	0.11	
C_0	1.00	1.00	1.00	1.00	1.00
	(−2.73)	(−0.34)	(+0.68)	(−20.0)	(16.7)
C_α	0.43	0.00	−1.22	0.68	0.78
C_β	0.53	2.88	3.37	−0.10	0.14

[a] Relative to C_0 in solutions containing 0.07 mol dm^{-3} Ln(edta) and 0.4 mol dm^{-3} L-alanine at pH 10. The value in parentheses is the observed C_0 shift in p.p.m. relative to the La(edta)–alanine complex using an internal Bu^{t}OH reference. Downfield shifts are denoted as negative. [b] Relative to C_0 in solutions containing 2 mmol dm^{-3} Gd(edta) and 1.5 mol dm^{-3} L-alanine at pH 10. The value in parentheses is the paramagnetic contribution to the relaxation rate of C_0 using La(edta) as the diamagnetic standard. An estimated ±10% error in the measured T_1 values leads to an error of ±0.2 in the T_{1P}^{-1} ratios.

TABLE 4

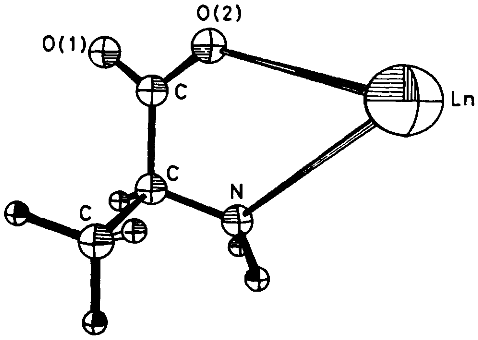
Contact shifts in L-alanine at pH 10 and the corrected pseudo-contact shift ratios

Nucleus	Contact shifts (p.p.m.) Pr(edta)	Nd(edta)	Eu(edta)	Tb(edta)	Corrected * pseudo-contact shift ratios
H_α	−0.15	−0.22	0.49	1.44	0.28
H_β	0.01	0.01	−0.02	−0.04	0.11
C_0	0.06	0.09	−0.20	−0.60	1.00
C_α	0.34	0.51	−1.12	−3.30	0.53
C_β	−0.64	−0.99	2.15	6.34	0.23

* Separated from the contact shift contribution using the methods outlined in refs. 7 and 14.

structure shown in the Figure suggests the five-membered chelate ring is essentially planar with Ln–O and Ln–N bond distances of 2.4 ± 0.2 Å.

Conclusions.—The l.i.s. data presented here for cydmp at pH 8 and L-alanine at pH 10 show that the Ln(edta) chelates do serve as useful aqueous shift reagents under a variety of solution conditions. The primary difficulty seems to be for substrates to undergo slower exchange between the available lanthanide co-ordination positions in Ln(edta) and bulk solution than is normally found with the aqueous cations. This is especially noticeable for substrates binding to the smaller, heavier Ln(edta) chelates. However, as shown above for cydmp, the problems associated with slow exchange may in some cases be overcome by increasing the temperature. The shift ratios for cydmp protons previously found have been interpreted in terms of a nucleotide conformation with the phosphate binding as a bidentate ligand to the lanthanide cation. In spite of exchange problems at low temperature for the heavier lanthanide complexes, the present results at 90 °C confirm that, although the edta protons in the Ln(edta) chelates do not display axial shifts,[1] other bidentate ligands binding to them may show axial symmetry. The exception repeatedly found for Tm^{3+}, related to an increased rigidity of its co-ordination sphere, only tends to confirm the mechanism proposed for achieving effective axial symmetry.[5]



ORTEP structure of the bidentate L-alanine–lanthanide complex derived from the effective axial symmetry model after correcting the l.i.s. for contact contributions

The chemically reasonable structure derived for L-alanine bound to the Ln(edta) chelates in a bidentate manner is an especially stringent test of the effective axial symmetry model. The proton and carbon l.i.s. may be easily purified of contact shift contributions without assuming axial symmetry when data from several Ln(edta) chelates are available and the resulting pseudocontact shifts are adequately described by the first term in equation (1). The tests for axial symmetry proposed by Reuben and Elgavish [16] have also been applied to the alanine data. Excellent fits to their equations (4) and (5) (correlation coefficients ⩾0.99) were found for those l.i.s. containing minor contact contributions (C_0 and H_β) and large contact contributions (C_α, H_α, and C_β) respectively. This analysis confirms that the measured pseudocontact shifts may be fit using the axial symmetry model. Hence, the bidentate alanine has achieved effective axial symmetry. Perhaps a rapid rotational averaging of the chelated edta in the lower Ln^{3+} co-ordination hemisphere (about the C_2 symmetry axis) above pH 6 [17] results in an averaging of the χ_x and χ_y susceptibility components and insures effective axial symmetry in the more weakly bound upper hemisphere ligand. Alternatively, when intermolecular exchange of the ligand is fast on the n.m.r. time scale, the process of internal rotation of the bidentate ligand, which could also be accomplished by intermolecular exchange, has no *a priori* reason to be slow.

The contact shifts induced in L-alanine at pH 10 by the Ln(edta) chelates show some interesting trends. First, the spin densities at C_0 and C_α consistently display the same sign, indicative of spin delocalization *via* both oxygen and nitrogen ligating atoms. The greater spin density found at C_α than at C_0 may reflect a greater degree of covalency in the Ln–N bond than in the Ln–O bond. Alternatively, these differences may simply reflect variations in spin seen at these atoms as a result of direct delocalization *versus* spin polarization mechanisms.[7] One would expect, *a priori*, a cancellation of spin density by the two mechanisms at C_0, C_α, and H_β and a summation at H_α and C_β. These predictions are supported by the data: the contact shift contribution at H_α is larger than H_β and larger at C_β than at C_0 or C_α. Thus, as reported previously,[7] contact shifts do not necessarily attenuate monotonically away from the ligating atom(s) and caution should be exercised in making this assumption.

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