Solvent Effects on the Conformation of Nucleotides. Part 2.† Nuclear Magnetic Shift and Relaxation Effects induced by Lanthanide Ions on Adenosine 5′-Monophosphate in Water–Dimethyl Sulphoxide

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The shift and relaxation effects on the n.m.r. spectrum of adenosine 5′-monophosphate due to binding of lanthanide(III) cations in the mixed solvent D_2O–[$^2H_6$]dimethyl sulphoxide have been studied as a function of solvent composition, temperature, and addition of chaotropic agents. Relaxation time measurements show that all lanthanides form isostructural complexes with the mononucleotide. The dipolar shifts induced by the lanthanides are determined by an averaged susceptibility tensor, whose symmetry is determined by the structure and dynamics of the ion-solvation shell. This symmetry is closer to axial for lanthanides in the first half of the series than for those in the second half. A temperature increase and a decrease of [$^2H_6$]dimethyl sulphoxide mol fraction have similar effects on the symmetry of the dipolar shifts.

Lanthanide paramagnetic ions have been used extensively as probes in the study of complex molecules by n.m.r. spectroscopy as their binding to these molecules simplifies their spectra, helps assign reasonances, and gives detailed structural information.[1-5] In this respect lanthanide ions are more useful than other paramagnetic species such as transition-metal ions because in the case of lanthanide ions the dipolar mechanism of both shift and relaxation perturbations usually predominates over the contact mechanism.[1-3]

The dipolar relaxation induced by Gd^{III} is isotropic.[3] Therefore, in the absence of contact contributions the spin–lattice and spin–spin relaxation rates, $T_{1M}{}^{-1}$ and $T_{2M}{}^{-1}$, induced in different nuclei in a molecule bound to a lanthanide ion are given by equation (1) ($i = 1$ or 2), where r_j is the distance

$$\frac{1}{T_{iM}} \propto \frac{1}{r_j{}^6} \qquad (1)$$

between nucleus j and the paramagnetic ion. The relaxation due to any of the other paramagnetic lanthanide cations may also be overwhelmingly isotropic,[6-8] but this problem will be discussed later in more detail. Nuclear dipolar shifts,[9] induced by lanthanides other than Gd^{III}, are given by equation (2),

$$\Delta_d = DG + D'G' \qquad (2)$$

where the ligand-field parameters D and D' are constants for a given lanthanide and temperature;[9] they are related[3,10] to the principal values of the magnetic susceptibility tensor along the x, y, and z axes (χ_{xx}, χ_{yy}, and χ_{zz}) and are proportional to $\chi_{zz} - \frac{1}{2}(\chi_{xx} - \chi_{yy})$ and $\chi_{xx} - \chi_{yy}$, respectively. The parameters $G = (3\cos^2\theta_j - 1)/r_j{}^3$ and $G' = (\sin^2\theta_j\cos2\varphi_j)/r_j{}^3$ are two geometric functions which depend on the distance and spherical co-ordinates of the nucleus under observation. Therefore the dipolar shifts depend not only on a distance factor ($r_j{}^{3}$) but also on an angular factor defined in relation to the axis of the magnetic susceptibility tensor of the complex. In many cases this tensor has axial symmetry or becomes axially symmetric as a result of intramolecular motions or rearrangements.[11,12] Then, the term G' disappears and the pseudo-contact shift becomes proportional to G, a situation which simplifies the analysis of the shift data in terms of molecular structure.[2-4]

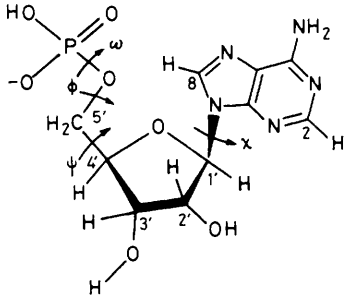

Figure 1. Chemical structure, torsion angle notation and numbering scheme of adenosine 5′-monophosphate

The conformation of 5′-mononucleotides in aqueous solution has previously been studied using lanthanide(III) aqueous ions as n.m.r. probes.[13,14] A preliminary conformational study of adenosine 5′-monophosphate (amp) (Figure 1) in dimethyl sulphoxide (dmso) using the lanthanide probe method showed that the preferred conformation of amp in dmso is different from that in water.[15] However it was later shown that the lanthanide-induced dipolar shifts upon which this conformational study was based incorrectly supposed axial symmetry,[16] therefore invalidating the conformational conclusion obtained.

In the previous paper of this series[17] the conformation of amp in dmso and in water–dmso mixtures was established using the nuclear Overhauser effect (n.O.e.) and proton distance ratios obtained from relaxation effects induced by Gd^{III} ions. In this paper we use this conformational information to interpret the observed dipolar shifts[16] and study the effect of solvent structure on their symmetry. The results give useful information about the nature of susceptibility tensors of complexed lanthanide ions in solution.

Experimental

The lanthanide(III) chlorides were obtained from Koch-Light and from Research Organic/Inorganic Chemical Corporation, California, and deuteriated dmso ([$^2H_6$]dmso) from Ciba. The lanthanide(III) chloride solutions were prepared by dissolution of known weights of the oxides, supplied by Koch-Light (99.9% purity), in DCl and D_2O (99.8% from

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† Part 1 is ref. 17.

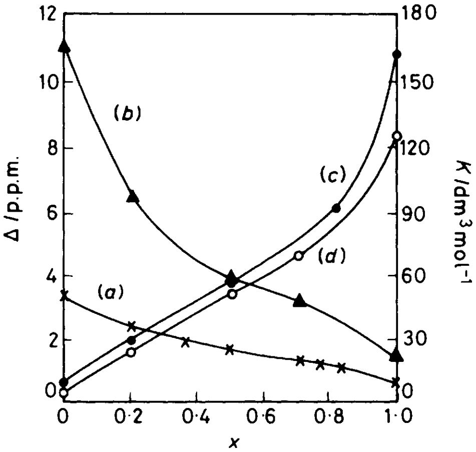

Figure 2. $H^{5'}$ fully bound shift (Δ) and association constant (K) of Ln^{III}–amp at room temperature as a function of x(dmso): (*a*) Δ(Eu); (*b*) Δ(Pr); (*c*) K(Eu); (*d*) K(Pr)

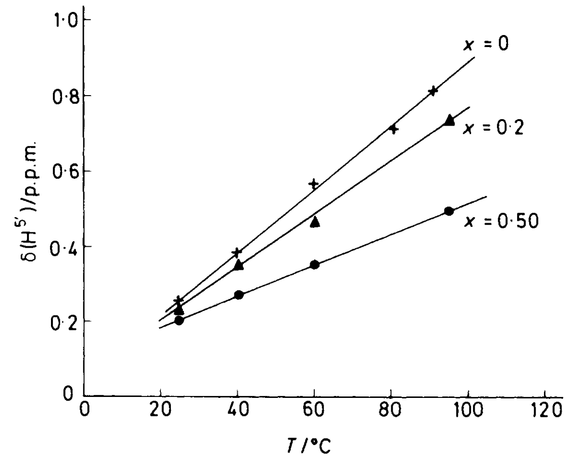

Figure 3. Temperature dependence of $H^{5'}$ shifts induced by Tm^{III} for various solvent compositions; $[Tm^{III}] = 2$ mmol dm^{-3}

Ciba). The anhydrous lanthanide(III) chlorides and anhydrous [$^2H_6$]dmso were prepared from the commercial products as described previously.[15] Adenosine 5′-monophosphate (sodium salt, from Sigma Chemicals) was lyophilized from H_2O at pH 1.8, dissolved in D_2O, in anhydrous [$^2H_6$]dmso, or in different D_2O–[$^2H_6$]dmso mixtures, in 0.03 mol dm^{-3} concentrations. The Ln^{III}–amp solutions were prepared by dissolving anhydrous lanthanide chlorides in an amp–anhydrous [$^2H_6$]dmso solution in appropriate proportions. pH Adjustments were made with DCl or NaOD using a Radiometer pH M63 digital pH meter equipped with a Radiometer CK 2321C electrode.

Proton n.m.r. spectra were recorded at 300 MHz using a Bruker CXP 300 spectrometer, or at 200 MHz using a Varian XL-200 spectrometer. All spectra were obtained in the Fourier-transform mode, using minimum digital resolution values of 0.25 Hz per point. Spin–spin relaxation times were obtained from linewidth measurements and spin–lattice relaxation times, T_1, were measured by application of a 180-τ-90° pulse sequence repeated at time intervals of longer than $5T_1$. Chemical-shift values were measured in p.p.m. downfield from the internal standard of tss (sodium 3-trimethylsilyl-[2,2,3,3-$^2H_4$]propionate). Computing was carried out on the Oxford University ICL 1906A computer.

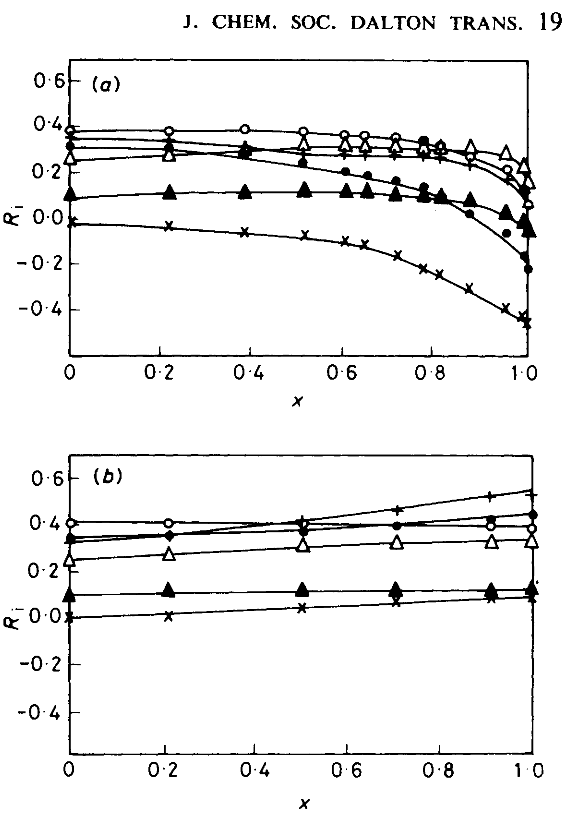

Figure 4. Ln^{III} shift ratios (R_i) at room temperature as a function of x(dmso): (*a*) Eu^{III} ratios; (*b*) Pr^{III} ratios; H^8 (●); H^2 (×); H^1 (▲); $H^{2'}$ (△); $H^{3'}$ (○); $H^{4'}$ (+)

Results

Proton n.m.r. titrations[6] with the different shift probes were carried out at constant amp concentration (30 mol) and at different mol fractions, x, of [$^2H_6$]dmso of the binary solvent D_2O–[$^2H_6$]dmso. However, full metal titrations over the entire range of solvent compositions were only possible for the early lanthanides, Pr^{III}, Nd^{III}, and Eu^{III}, as the later ones, especially for the higher values of x, cause large amounts of broadening and therefore had to be used in very low concentrations. From the titration curves with the earlier lanthanides, characteristic of formation of 1 : 1 metal–nucleotide complexes in fast exchange,[15,16] it is found in the usual way[6] that the fully bound shift for the $H^{5'}$ protons (Δ) decreases very markedly with an increase of x, whereas the association constant (K) of amp with Ln^{III} increases with x (Figure 2). For the later lanthanides the absolute shift for $H^{5'}$ at a constant lanthanide concentration was also found to decrease with x (see for example Figure 3). The shift ratios for the individual protons of amp, relative to $H^{5'}$, are shown in Table 1 as a function of the mixed-solvent composition and temperature for the different lanthanides (see also Figure 4 for the Pr^{III} and Eu^{III} shift ratios). For each lanthanide ion the shift ratios change with solvent composition and at the same solvent composition the ratios are not constant for different lanthanides. In pure D_2O ($x = 0$) these deviations are limited to Tm^{III}[6] but they increase with x for all lanthanides and at $x = 1$ even the earlier lanthanide shift ratios are greatly affected. The lanthanide Pr^{III} always shows the smallest deviations from the ' normal ' ratios (all except Tm^{III}) in D_2O. On the contrary, Tm^{III} and Er^{III} show the largest deviations.

Table 1 also shows that the proton shift ratios are in most cases very temperature dependent and at high temperatures approach the shift ratios for Pr^{III} in D_2O, but to different

Table 1. Adenosine 5′-monophosphate proton shift ratios (relative to $H^{5',5''}$) with different lanthanides at different mixed-solvent compositions and different temperatures

Ln	x	T/°C	H^8	H^2	$H^{1'}$	$H^{2'}$	$H^{3'}$	$H^{4'}$	$H^{5',5''}$
Pr	0	25	0.35	0.00	0.10	0.26	0.42	0.34	1.00
	0.5	25	0.40	0.05	0.13	0.33	0.42	0.42	1.00
	0.5	95	0.36	0.05	0.13	0.26	0.42	0.40	1.00
	1.0	25	0.46	0.12	0.16	0.36	0.41	0.56	1.00
Nd	0	25	0.29	−0.02	0.05	0.21	0.33	0.28	1.00
	0.5	25	0.55	0.14	0.17	0.38	0.41	0.48	1.00
	1.0	25	0.88	0.40	0.17	0.36	0.60	0.74	1.00
Eu	0	25	0.31	−0.02	0.09	0.25	0.38	0.33	1.00
	0.5	25	0.24	−0.10	0.11	0.30	0.38	0.28	1.00
	0.5	90	0.26	−0.05	0.12	0.30	0.40	0.28	1.00
	0.5	120	0.28	−0.03	0.13	0.30	0.40	0.30	1.00
	1.0	25	−0.23	−0.45	−0.05	0.12	0.05	0.05	1.00
	1.0	90	0.07	−0.12	0.03	0.15	0.28	0.28	1.00
	1.0	130	0.15	−0.08	0.05	0.18	0.33	0.30	1.00
	1.0	150	0.18	−0.05	0.08	0.20	0.38	0.32	1.00
Tb	0.5	25	0.64	−0.10	0.21	*a*	*a*	*a*	1.00
	0.5	60	0.26	−0.04	0.09	0.25	0.37	0.33	1.00
Dy	0.5	25	0.57	−0.08	0.17	*a*	*a*	*a*	1.00
	0.5	60	0.28	−0.05	0.10	0.25	*a*	*a*	1.00
Ho	0.5	25	0.81	−0.05	0.18	*a*	*a*	*a*	1.00
	0.5	60	0.33	−0.02	0.09	0.26	*a*	*a*	1.00
Er	0.5	25	0.05	−0.16	−0.02	*a*	*a*	*a*	1.00
	0.5	60	−0.11	−0.07	−0.07	*a*	*a*	*a*	1.00
	0.9	25	−0.35	−0.38	−0.15	*a*	*a*	*a*	1.00
	0.9	60	−0.32	−0.30	−0.17	0.10	0.18	*a*	1.00
Tm	0	25	−0.24	−0.52	−0.08	0.07	0.22	0.07	1.00
	0	95	0.08	−0.04	0.03	*a*	0.30	0.30	1.00
	0.2	25	−0.24	−0.61	−0.28	0.07	0.34	*a*	1.00
	0.5	95	0.21	−0.19	0.13	0.31	*a*	0.37	1.00
Yb	0.5	25	0.23	−0.07	0.07	*a*	*a*	0.36	1.00
	0.5	60	0.23	−0.09	0.08	*a*	*a*	0.36	1.00
	0.8	60	0.05	−0.28	0.03	0.24	0.37	*a*	1.00
Calculated (D_2O) [b]			0.33	−0.01	0.13	0.24	0.38	0.33	1.00
Calculated ([$^2H_6$]dmso) [b]			0.23	−0.03	0.13	0.24	0.38	0.33	1.00

[a] Not observed. [b] Calculated axial shift ratios for the conformational mixtures defined in the text.

extents, depending on the lanthanide and the [$^2H_6$]dmso mol fraction. Again, of all the lanthanides, Tm^{III} and Er^{III} show the largest anomalies. The temperature dependence of the shift ratios is a reflection of the anomalous temperature dependence of the proton shifts, especially of $H^{5'}$. For large values of x, the shifts induced on the $H^{5'}$ protons increase quite remarkably with temperature for most lanthanides and even at $x = 0$ for Tm^{III} (Figure 3). In pure D_2O ($x = 0$), at high temperature all the lanthanides give very similar shift ratios but at low temperature Tm^{III} gives values very much at variance with those of the other lanthanide aqueous ions. Moreover, a comparison of the relative magnitudes of the absolute shifts with the different lanthanides showed that at high temperature Tm^{III} approaches the expected theoretical value relative to the other lanthanides, but at low temperature it gives a very small value.[6] The ten-fold linear increase of the $H^{5'}$ shift induced by Tm^{III} when the temperature rises from 4 °C to 90 °C [Figure 5(*b*)] causes the proton shift ratios to approach the Pr^{III} ratios at high temperatures [Figure 5(*b*)]. Over the temperature range 40—90 °C the shift ratios are clearly the same as those of the other lanthanide cations but the shift magnitudes increase much more rapidly for Tm^{III} than for any other cation. The temperature dependence of the $H^{5'}$ shift for other cases can be quite complex: Figure 6 shows such a dependence for Eu^{III}-amp in [$^2H_6$]dmso ($x = 1$). The effect of typical denaturing agents, such as urea and SCN^-, on the amp proton shift ratios with Pr^{III} and Tm^{III} for $x = 0$ and at different temperatures was also investigated. Table 2 shows that, whereas the Pr^{III} ratios suffer no effect, the Tm^{III} ratios at a given temperature approach the Pr^{III} ratios upon addition of urea or SCN^-.

Table 3 shows the observed ratios of the Gd^{III}, Pr^{III}, and Tm^{III} induced variations of T_1 and T_2 relaxation rates (T_{1M}^{-1} and T_{2M}^{-1}) in various D_2O–[$^2H_6$]dmso solvent mixtures and at different temperatures. The Gd^{III} ratios clearly indicate a conformational change of the Ln^{III}-amp complex from D_2O to [$^2H_6$]dmso.[17]

Discussion

The lanthanide(III) aqueous ions in D_2O have been shown to bind selectively to the phosphate group of amp forming 1 : 1 complexes in fast exchange on the n.m.r. time-scale.[18] The induced paramagnetic proton shifts are of pseudo-contact origin. This situation also applies in [$^2H_6$]dmso and in D_2O–[$^2H_6$]dmso solvent mixtures [16] except for the fast exchange conditions. Intermediate exchange arises with the heavier lanthanides, which induce the largest shifts, for high metal-ion concentrations associated with a large increase of the stability constant for the Ln^{III}-amp complex when the solvent changes from D_2O to [$^2H_6$]dmso.

The observed solvent dependence of the stability constant is readily explained by the donor–acceptor properties of the two solvents: [19,20] complex stability in solution is enhanced by a decrease in both the donor number and acceptor number of the solvent, which measure its solvating power towards the

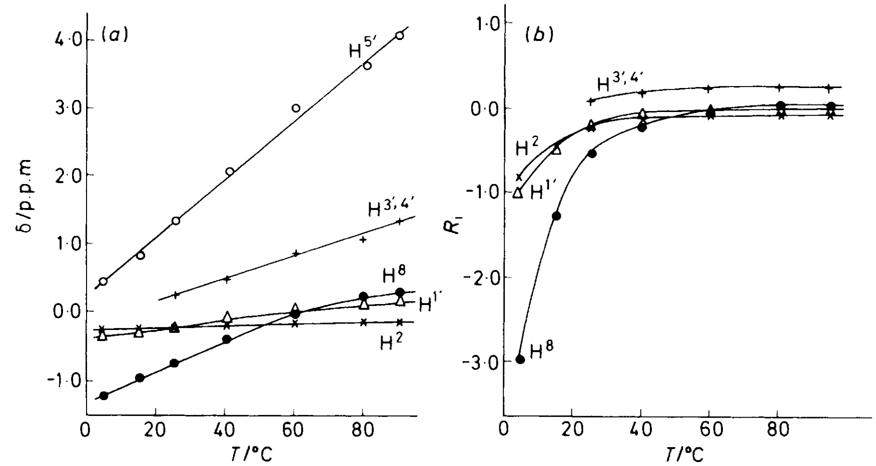

Figure 5. Temperature dependence of (*a*) proton shifts; (*b*) proton shift ratios of Tm^{III}-amp in D_2O; [Tm^{III}] = 50 mmol dm^{-3}

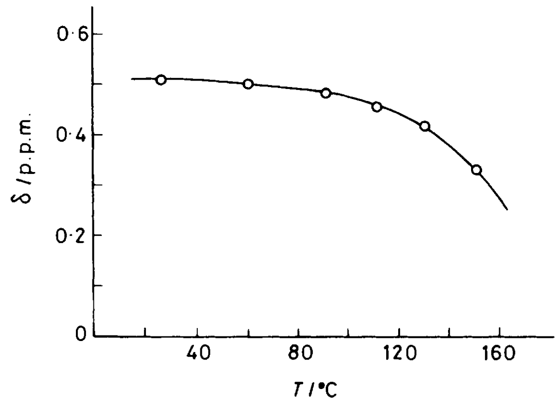

Figure 6. Temperature dependence of H$^{5'}$ shift induced by Eu^{III} in [$^{2}H_{6}$]dmso; [Eu^{III}] = 15 mmol dm^{-3}

cation and the ligand. Although [$^{2}H_{6}$]dmso has a higher donor number than D_2O, the decreased stability of the complex in D_2O is easily explained by the strong acceptor properties of D_2O relative to [$^{2}H_{6}$]dmso due to hydrogen-bond formation with the phosphate group of amp. Therefore in this case solvation of the anionic ligand is the dominant factor. The sharp increase of the association constant with x (Figure 2) is a reflection of preferential solvation of the lanthanides by [$^{2}H_{6}$]dmso in the solvent mixtures.[21,22]

The change of the shift ratios for each lanthanide with solvent composition (Figure 4) is partly due to a solvent-induced conformational change of amp, as shown by the solvent dependence of the Gd^{III} induced proton relaxation rate ratio (Table 3) and nuclear Overhauser effects.[16,17] The conformation of the ribose–phosphate backbone is not influenced by the solvent: the relaxation and n.O.e. data have been best described by a *g'g'* rotamer about the O$^{5'}$–P bond, 80% *gg*, 20% *gt* and *tg* rotamers about the C$^{5'}$–O$^{5'}$ bond and a mixture of 40% ^{3}E and 60% ^{2}E puckered conformations of the ribose ring.[17] The conformational change is therefore limited to the orientation of the adenine base about the glycosidic bond: within the approximations of the two-state model it can be described by a predominant *anti* conformation, (χ_N, χ_S) = (90°, 40°) in D_2O and a *syn–anti* equilibrium, (χ_N, χ_S) = (80°, 160°) in [$^{2}H_{6}$]dmso {χ_N and χ_S are the angles associated with the N[C(3'),*endo*] and S[C(2'),*endo*] furanose ring conformations}.[17] The bottom two lines of Table 1 show the calculated axial shift ratios of the conformational mixtures described above for the two solvents, which have been calculated using methods previously described.[15] The change of the axial shift ratios from D_2O to [$^{2}H_{6}$]dmso is limited to the adenine base protons.

There are various possible explanations for the large observed dependencies of the room-temperature proton shift ratios on the nature of the lanthanide ion and the mixed-solvent composition (Table 1): the structure of the Ln^{III} complexes could change from one part of the series to the next giving in all cases effectively axial shifts; the complexes could be isostructural and have effective axial symmetry with a direction of the effective principal magnetic axis differing from one lanthanide to the other; finally the isostructural complexes could have non-axial symmetry. These possibilities will now be discussed.

The hypothesis of a structural change along the lanthanide series has been put forward before in order to interpret the shift ratio anomalies observed with various carboxylate ligands. For instance, the ligand alanine was assumed to be monodentate for the lighter members of the series and bidentate for the heavier ones,[23] but the reverse trend was also suggested for lanthanide complexes of indol-3-yl acetate [24] and tryptophan.[25] These conclusions were however later shown to be premature and to arise from the inappropriate application of the axial symmetry model to the analysis of the shift data.[26-28] The isostructurality of the complexes can be tested using the Ln^{III} induced relaxation ratios, which give the distance ratios of the protons to the metal ion in the different complexes if isotropic relaxation applies [see equation (1)]. In spite of some theoretical controversy,[5,25,29] it now seems likely from experimental evidence [6-8,24-28,30] that relaxation induced by Ln^{III} (other than Gd^{III}) is isotropic and that the transverse, unlike the longitudinal, relaxation may not be purely dipolar but contain contributions from scalar or exchange mechanisms. The experimental longitudinal relaxation ratios for Ln^{III}-amp in D_2O and [$^{2}H_{6}$]dmso (Table 3) show that in each solvent the complexes are isostructural, and therefore the first hypothesis is not applicable.

We next considered the possibility that the time-averaged symmetry of the complexes is the same for the different ions but that the direction of the principal axis changes from one ion to another. Although such a variation could explain small changes of the shift ratios with x (for $x \ll 1$) or for the same x within the earlier lanthanides, no direction of an axial symmetry can explain the shift ratios with the later lanthanides, especially for large values of x. For instance, a conformational

Table 2. Effect of added 5 mol dm^{-3} denaturing agents upon proton shift ratios (relative to H$^{5',5''}$) of amp induced by Pr^{III} and Tm^{III} in D_2O

Ln	Denaturing agent	T/°C	H^8	H^2	H^1	H$^{2'}$	H$^{3'}$	H$^{4'}$	H$^{5',5''}$
Pr	—	30	0.35	0.00	0.10	0.26	0.42	0.34	1.00
	Urea	30	0.32	0.00	0.08	0.27	0.40	0.32	1.00
	SCN$^-$	30	0.26	0.02	0.08	0.29	0.40	0.34	1.00
Tm	—	30	−0.24	−0.52	−0.08	*	*	*	1.00
	—	90	0.08	−0.07	0.01	*	*	*	1.00
	Urea	30	−0.03	−0.23	−0.04	*	*	*	1.00
	Urea	90	0.18	−0.03	0.06	*	*	*	1.00
	SCN$^-$	30	−0.08	−0.29	−0.06	*	*	*	1.00
	SCN$^-$	90	0.14	−0.03	0.03	*	*	*	1.00

* Not observed.

Table 3. Proton relaxation ratios (R_l) relative to H$^{5',5''}$ ($\equiv$ 100) of amp in D_2O–[$^2H_6$]dmso with various lanthanide(III) cations

R_l [a]	x([$^2H_6$]dmso)	Ln^{III}	T/°C	H^8	H^2	H$^{1'}$	H$^{2'}$	H$^{3'}$	H$^{4'}$	H$^{5',5''}$
T_{1M}	0	Gd	20	48	4	6	7	14	19	100
	0	Gd	80	45	4	6	9	15	20	100
	0	Pr	20	40	6	9	14	20	24	100
	0	Pr	80	56	5	6	15	19	24	100
	0	Tm	20	60	8	3	*b*	*b*	*b*	100
	0	Tm	80	40	7	3	*b*	*b*	22	100
	1.0	Gd	20	8	3	6	12	18	23	100
	1.0	Gd	80	10	3	7	14	21	26	100
	1.0	Pr	20	10	2	5	13	20	24	100
	1.0	Pr	80	11	2	6	12	20	26	100
	1.0	Tm	20	18	6	6	*b*	*b*	*b*	100
	1.0	Tm	80	13	5	5	*b*	*b*	*b*	100
T_{2M}	0	Gd	20	49	1	6	*b*	*b*	*b*	100
	0.2	Gd	20	28	5	6	*b*	*b*	*b*	100
	0.5	Gd	20	14	4	6	*b*	*b*	*b*	100
	0.8	Gd	20	6	3	6	*b*	*b*	*b*	100
	1.0	Gd	20	6	3	6	*b*	*b*	*b*	100

[a] Spin–lattice (T_{1M}) or spin–spin (T_{2M}) relaxation ratios as indicated; with experimental errors of 2% and 4% respectively. These ratios are the differences between $\dot{T}_i^{-1}$ (i = 1 or 2) for the unbound ligand and for the ligand bound to Ln^{III}. [b] Not observed.

search could not find any position of the metal ion or direction of the symmetry axis consistent with a description of the Tm^{III} shift ratios for $x = 0$ in terms of axial symmetry. Such a conclusion has been obtained before for a different system.[30]

Therefore, we conclude that the apparent magnetic susceptibility tensor has an averaged symmetry.[5,30] For the earlier lanthanides, *e.g.* Pr^{III} in D_2O, this averaging is extensive and results in an effective axial symmetry with the principal axis oriented along the metal–phosphorus atom direction. For the later lanthanides, *e.g.* Tm^{III} in D_2O, or for all the lanthanides with x([$^2H_6$]dmso) $\lesssim$ 1, which give shift ratios quite different from those with Pr^{III}, the extent of averaging is smaller. This situation results in an axially asymmetric averaged susceptibility tensor.

This decreased averaging, due to slower motions within the metal-ion solvation shell, has been observed for various complexes in aqueous solution,[25,31] and is probably related to an increased stereochemical rigidity of the lanthanide co-ordination sphere for the ions in the second half of the series around Tm^{III}. This rigidity is further increased in D_2O–[$^2H_6$]dmso due to the mixed solvation of the metal ion and/or to a co-operative orientation of water and dmso molecules through hydrogen bonding around the co-ordination shell.[16]

This explanation is supported by the following observations. (1) For each solvent composition, the shift ratios for all Ln^{III} ions tend at higher temperatures towards the values for the first members of the series, which agree more closely with the calculated axial dipolar shifts (Table 1). (2) The effect of typical denaturing agents, such as urea and SCN$^-$, on the shift ratios, causing them to approach the axial symmetry values (Table 2), could be due to a decreased rigidity of the metal-ion solvation shell caused by disruption of existing hydrogen-bond networks around it.[25] (3) The relative deviations from axial symmetry of the observed shifts for the different lanthanides at room temperature (see the shift ratios of Table 1) strongly correlate with the deviations from axial symmetry of the magnetic susceptibility tensor of lanthanide complexes in the crystal state, as measured by the magnitude of $(\chi_{xx} - \chi_{yy})$ relative to $[\chi_{zz} - \frac{1}{2}(\chi_{xx} + \chi_{yy})]$. In fact, magnetic susceptibility measurements obtained in single crystals of bis(4-methylpyridine)tris(2,2,6,6-tetramethylheptane-3,5-dionato)lanthanide complexes[10,32] show that the largest asymmetries are found for lanthanides in the second half of the series, notably Tm^{III} followed by Er^{III} and the smallest for Pr^{III}, followed by Eu^{III}. This correlation suggests that the difference of the effective symmetry of susceptibility in solution from axiality depends on its asymmetry in the static system.

The anomalous dependence of dipolar shifts on solvent composition (Figure 2) and temperature (Figures 3, 5, and 6) reflects the effect of these parameters on the extent of averaging processes on the non-axial term $D'G'$ of equation (2). In this way, the observed decrease of the H$^{5'}$ fully bound shift with increased x([$^2H_6$]dmso) (Figure 2) is a reflection of the increased value of the non-axial term $D'G'$ relative to the axial term DG in equation (2), the two terms having opposite signs.

In the same way, the anomalous temperature dependence of the proton shifts, *e.g.* the $H^{5'}$ shift with Eu^{III} in $[^2H_6]$dmso (Figure 6) and of all protons with Tm^{III} in D_2O [Figure 5(*a*)], can be attributed to the decrease of the non-axial term $D'G'$ with temperature, due to thermal averaging, which is superimposed on the normal T^{-2} dependence of the pseudo-contact shift.[9] In the case of Eu^{III}, the $H^{5'}$ shift decreases, but not with T^{-2} because, as $D'/D \approx 1.2$ at room temperature,[32] $|DG| \gg |D'G'|$. On the contrary, $D'/D \approx 18$ for Tm^{III} and $|D'G'|$ is at room temperature comparable to $|DG|$ for most protons. Therefore, if DG and $D'G'$ have opposite signs and DG is positive (except for H^2), when the temperature is increased $D'G'$ is averaged to zero and all the shifts become more positive [Figure 5(*a*)]. With this model for effective axial symmetry it is now understandable why the shifts induced by Tm^{III} on the protons of amp have such a large temperature dependence, which is different for the different nuclei. Indeed, the deviations from axial symmetry, which depend on the value of $D'G'$ relative to DG, are different for each nucleus because the G and G' factors depend on the position of the nuclei relative to the principal axis of the magnetic susceptibility tensor. The protons $H^{5'}$ and H^8, which have the largest temperature dependence of their shifts, should also have the largest $D'G'$ factors. The large rhombicity found for these two nuclei is also responsible for the large variation of the H^8 shift ratio with temperature [Figure 5(*b*)].

Conclusions

This work clearly points out that the structure and dynamics of the solvation shell of the lanthanide complexes, which cause only minor differences in ligand–metal geometry, can cause very large effects on n.m.r. parameters like dipolar shifts by drastically changing the average magnetic symmetry of the complexes. The lanthanides of the second half of the series induce larger shifts and are more rigid than those of the first half. Therefore motional averaging of the shifts is more difficult. The increased stereochemical rigidity is a maximum at Tm^{III}.[31] This effect is not just a consequence of changes in ionic radius, but also of the magnitude of the ligand-field splittings of these ions, which are largest at Tm^{III}.[5,33]

Finally, the conclusion that in the case of non-axial symmetry different nuclei may approach effective axial symmetry in different experimental conditions from each other [34,35] is a consequence not only of their different mobilities on the surface of the lanthanide ion, but also of their different geometric positions relative to the paramagnetic centre.

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