An Investigation of Some Potential Uses of the Gadolinium(III) Ion as a Structural Probe

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The gadolinium(III) ion has a slow electron relaxation time and it is therefore possible in principle to study the nature of its ligands, including water, by a measurement of its e.s.r. spectrum and its effect on the nuclear-spin relaxation rate of protons in the co-ordination sphere. A large number of systems have been examined in a semiquantitative way in order to test such a use of Gd^{3+} as a probe of its own environment with a view to the possibility of structural studies in biological systems.

THE use of the Mn^{2+} ion in place of Ca^{2+} or Mg^{2+} in complexes, especially of macromolecules, is often recommended as the magnetic properties of Mn^{2+} allow, through a study of water-proton relaxation rate or the e.s.r. linewidth, an estimate of the hydration of the Mn^{2+} ion and thence an estimate of the hydration of Ca^{2+} or Mg^{2+} [1-5]

An alternative to Mn^{2+} in such a study is Gd^{3+} for it also has suitable electronic and magnetic properties, *e.g.* a long electron relaxation time, and binds to organic ligands in a manner rather similar to Ca^{2+}.[6-9] In this paper we describe a series of simple experiments designed to inspect the possibility of using either water-proton relaxation rates or e.s.r. line shapes of gadolinium(III) complexes in order to study the hydration of its complexes. The ligands used are very simple small molecules and it was our hope that these observations would indicate how similar work could be done with large biologically important macromolecules.

The essential relation between the paramagnetic contribution to the water-proton spin–lattice relaxation rates (T_{1p}^{-1}) in the hydration sphere of an isotropic S-state ion (Gd^{3+} has an ^8S ground state compared with the ^6S state of Mn^{2+}) and its hydration is given by equation (1),[10] where T_{1M}^{-1} and τ_M^{-1} are respectively

$$\frac{1}{T_{1p}} = \frac{x_M q}{T_{1M} + \tau_M} \quad (1)$$

the relaxation and exchange rates of the water protons from the q sites in the first co-ordination sphere of the metal ion, and x_M is the mole fraction of the metal ion relative to water itself. In fast-exchange conditions ($T_{1M} \gg \tau_M$) equation (1) reduces to (2). Assuming that

$$\frac{1}{T_{1p}} = \frac{x_M q}{T_{1M}} \quad (2)$$

T_{1M} is constant for the water in metal complexes formed

[1] A. S. Mildvan and M. Cohn, *Adv. Enzymol.*, 1970, **23**, 1.
[2] M. Cohn and J. Reuben, *Accounts Chem. Res.*, 1971, **4**, 214.
[3] G. H. Reed and M. Cohn, *J. Biol. Chem.*, 1972, **247**, 3073.
[4] G. H. Reed, J. S. Leigh, jun., and J. E. Pearson, *J. Chem. Phys.*, 1971, **55**, 3311.
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[6] R. J. P. Williams, *Quart. Rev.*, 1970, **24**, 331.
[7] R. A. Dwek, K. G. Morallee, E. Nieboer, R. E. Richards, R. J. P. Williams, and A. V. Xavier, *European J. Biochem.*, 1971, **21**, 204.
[8] J. Reuben, *Biochemistry*, 1971, **10**, 2834.
[9] J. Reuben, *J. Phys. Chem.*, 1971, **75**, 3164.
[10] T. J. Swift and R. E. Connick, *J. Chem. Phys.*, 1962, **37**, 307; Z. Luz and S. Meiboom, *ibid.*, 1964, **40**, 2686.

by different ligands, then T_{1p}^{-1} is a direct comparative measure of q, the hydration number of the ion in the different complexes. It is assumed that the correlation time τ_c for the dipolar interaction of the metal-ion electron spin with the proton nuclear spin does not change appreciably from complex to complex and that the gadolinium(III)–water distance is not affected by complex formation. The equation neglects outer-sphere effects. Using all these assumptions it appears that the hydration number of a complex could be determined. This paper will make a qualitative inspection of this possibility. In these studies it is convenient to use the enhancement of the relaxation rate, ε^*, which is defined as in equation (3) where $(T_{1p}^*)^{-1}$ and T_{1p}^{-1} are

$$\varepsilon^* = (T_{1p}^*)^{-1}/T_{1p}^{-1} \quad (3)$$

the observed paramagnetic contributions to the relaxation rate in the presence and absence of the ligand, respectively. The observed enhancement is a sum of the contributions from the ligand-free and bound metal ions of concentration M_f and M_b, equation (4) where

$$\varepsilon^* = \frac{M_f}{M_t} + \frac{\varepsilon_b^* M_b}{M_t} \quad (4)$$

$M_t = M_f + M_b$ and ε_b^* is the enhancement of the bound form.

The essential equation for the e.s.r. linewidth of Gd^{3+} is that for the electronic transverse relaxation rate,[9] (5),

$$\frac{1}{T_{2s}} = \Delta^2 F(\omega_s, \tau) \quad (5)$$

where Δ^2 is the inner product of the zero-field splitting tensor (in $rad^2\ s^{-1}$) and $F(\omega_s,\tau)$ is the required relaxation matrix, for values of the correlation time, τ. Δ^2 will vary with the symmetry and strength of the ligand field and will express the dynamic process responsible for relaxation. This process can in principle be either the rotation of the complex (rotational modulation of static zero-field splitting) or symmetry fluctuations in the complex due to distortions induced by the impact of solvent molecules (modulation of the transient zero-field splitting).[11,12] Thus in principle it should be possible to find gross line broadening from a change in ligand-field symmetry or from a change in the fluctuating field of protons. The ligands can be chosen so that only water supplies protons near enough to interact with the gadolinium(III) spin so that it is the hydration of Gd^{3+} that is being inspected.

In outline we see that the measurement of both proton relaxation times and e.s.r. linewidths should describe much of the hydration states of gadolinium(III) complexes. While it is obvious that T_{2s}^{-1} and T_{1p}^{-1} could be correlated amongst different complexes, as both could vary with the number of protons in the hydration sphere, this is clearly not an essential relation.

[11] W. Bloembergen and L. O. Morgan, *J. Chem. Phys.*, 1961, **34**, 842.

EXPERIMENTAL

Gadolinium(III) oxide (99% purity) was obtained from Koch–Light. All the ligands, of analytical reagent purity, were obtained from commercial sources. Deuterium oxide (99.8%) was obtained from Norsk Hydro; DCl (38% by weight in D_2O, isotopic purity >99%) and Na[OD] (40% by weight in D_2O, isotopic purity >99%) were from CIBA. Gadolinium solutions were prepared by dissolving accurately weighed amounts of the oxide in 35% DCl by very gentle warming. The oxide had been previously held at 900 °C for 2 h to remove water and carbon dioxide. Solutions of $[Gd(edta)]^-$ (H_4edta = ethylenediaminetetra-acetic acid) were prepared by mixing stoicheiometric quantities of gadolinium(III) chlorides and solutions of the sodium salts of H_4edta. Complexes of Gd^{III} with various ligands were prepared by mixing appropriate quantities of gadolinium(III) or $[Gd(edta)]^-$ solutions with ligand solutions. Ligand titrations were carried out at pH 7.0.

The e.s.r. spectrum or proton relaxation time of a solution of a Gd^{3+} was first measured. Successive solutions of a metal–ligand complex were added to the first solution and

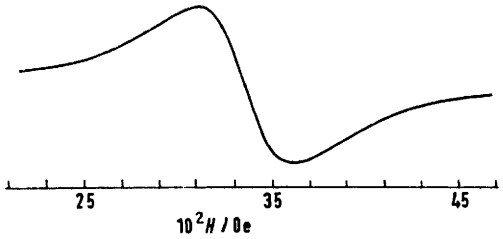

FIGURE 1 E.s.r. *X*-band spectrum of the gadolinium(III) aqua-ion at 25 °C

the information reobtained. The added solution contained the same metal concentration as the ligand-free solution and excess of ligand. In this way the metal concentration was kept constant and the ligand concentration increased throughout the titration. The spectrum of the gadolinium(III) ion was not affected by pH changes except where indicated. E.s.r. spectra of gadolinium(III) complexes in solution were recorded at 25 °C on a Jeolco Jespe-IX spectrometer operating at *X* band (9.8 GHz or 3 500 Oe). Aqueous thin-walled cells were standardised for optimum sensitivity with the least distortion of spectral line shape.

Longitudinal proton relaxation times (T_1) were measured at 2.67 and 20 MHz by a spin-echo technique employing a 180–90–180° pulse sequence. The samples (0.05 cm^3) at 20 °C were contained in Pyrex tubes.

RESULTS

Gadolinium(III) Complexes.—Figure 1 shows the experimental *X*-band e.s.r. spectrum (25 °C) for a dilute (5.4×10^{-3} mol dm^{-3}) aqueous solution of $GdCl_3$. It consists of one Lorentzian curve with linewidth ΔH = 493 Oe and g = 1.992, in agreement with the spectrum reported.[13] (ΔH is given by the peak-to-peak separation of the derivative of the absorption e.s.r. line. h is the height of the signal and was measured in cm at constant spectrometer settings. $\Delta H/h$ is then in Oe cm^{-1}, an arbitrary unit.)

[12] A. Hudson and J. W. E. Lewis, *Trans. Faraday Soc.*, 1970, **66**, 1297.

[13] B. M. Kosyrev, *Discuss. Faraday Soc.*, 1955, **19**, 135.

The effect of different inorganic ions (Table 1) and organic ligands (Tables 2 and 3), including multidentate carboxylates, on the e.s.r. spectrum of Gd^{III} in solution was investigated. The results were analysed so that the species present were known as indicated in the Tables. Although data were collected at many different concentrations of ligands only single data points are given in the Tables. The composition of the solutions was calculated from data in the literature.[14] In many cases the e.s.r. line maintained its Lorentzian shape, with a different linewidth [Figure 2(*a*) and (*b*)], and the *g* value changed only slightly

TABLE 1

Effect of inorganic ligands on the e.s.r. signal and the proton-relaxation enhancement parameter of Gd^{3+} in aqueous solutions; $[Gd^{III}] = 5.4 \times 10^{-3}$ mol dm^{-3}, pH 6.5, 298 K, amplitude $A = 450$ Oe. Measurements of proton relaxation were made at 20 MHz

Ligand L	$[Gd^{III}]$: [L] ratio	x in species $[GdL_x]$ [a]	ΔH/Oe	h/cm	ε*
H_2O		0	493	56	1.00
Cl^-	1 : 100	0—1 (0.3)	493	62	0.87
$[NO_3]^-$	1 : 100	1—2 (0.6)	290	84	0.57
$[NO_2]^-$	1 : 100	1—2 (0.5)	178	143	0.72
$[SO_4]^{2-}$	1 : 100	1—2 (0.8)	575	44	0.99
$[P_3O_{10}]^{5-}$ *	1 : 10	(2)	658	22	
$[P_2O_7]^{4-}$ *	1 : 10	(2)	822	11	1.34

[a] The range in which careful study was made is given followed by in parentheses the actual composition of the species for the ratio in column 2.

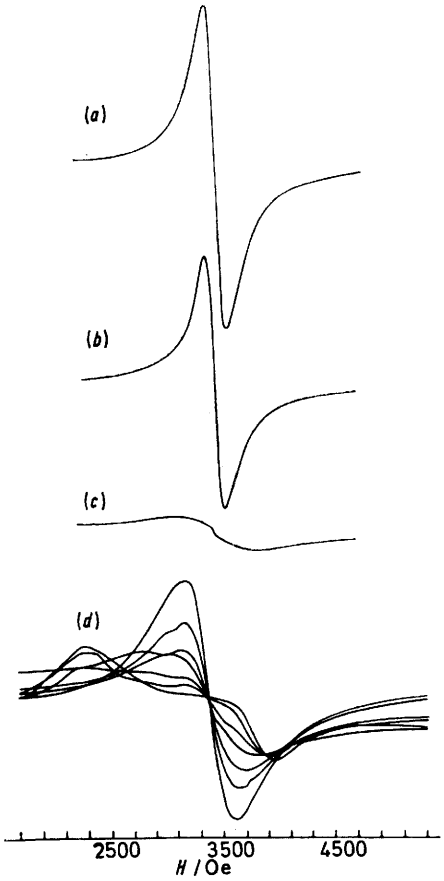

FIGURE 2 E.s.r. spectra of different gadolinium(III) complexes: (*a*) Gd^{3+}–$Na[P_2O_7]$ (1 : 10); (*b*) Gd^{3+}–$Na_2[H_2dnds]$ (1 : 10); (*c*) Gd^{3+}–$Na[CO_2Me]$ (1 : 100); (*d*) a typical titration of Gd^{3+} with increasing amounts of Na_2[pydca] in the mol ratios (from top to bottom curve) of 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 : 1. $[Gd^{III}] = 5.4 \times 10^{-3}$ mol dm^{-3}, pH 7.0, 25 °C

TABLE 2

E.s.r. parameters and proton-relaxation enhancement factors for fully formed gadolinium(III) complexes with organic ligands. $[Gd^{III}] = 5.4 \times 10^{-3}$ mol dm^{-3}, pH 7.0, 298 K, $A = 450$ Oe. Measurements of proton relaxation were made at 20 MHz

Ligand L	$[Gd^{III}]$: [L] ratio	x in species $[GdL_x]$	ΔH/Oe	h/cm	ε*
H_2O		0	493	56	1.00
adp *,[a]	1 : 10	2	822	11	1.01
atp *	1 : 10	2	1 370	13	1.68
Malate	1 : 1 or 1 : 20	3	493	21	0.67
Malonate *	1 : 100	2	822	13	0.34
Aspartate *	1 : 20	3	630	11	0.37
dbds *	1 : 10	3	986	13	0.55 [b]
dnds *	1 : 10	3	904	18	1.22
pydea *	1 : 10	3	1 726	26	0.21 [b]
nta	1 : 20	2	521	27	0.43
cdta [c]	1 : 10	1	630	26	0.71
apdpa [d]	1 : 10	1	601	42	0.49
dodta [e]	1 : 10	1	616	35	0.40
edta	1 : 1	1	315	50	0.53
hedta	1 : 1	1	301	116	0.50

[a] adp = Adenosine-5′-diphosphate. [b] $W_I = 2.67$ MHz. [c] cdta = *trans*-Cyclohexane-1,2-diamine-*NNN′N′*-tetra-acetate. [d] apdpa = 3-Azapentane-1,5-diamine-*NNN′N″N″*-penta-acetate. [e] dodta = 3,6-Dioxaoctane-1,8-diamine-*NNN′N′*-tetra-acetate.

TABLE 3

Effect of organic ligands on Gd^{3+}. E.s.r. signal and proton-relaxation enhancement factors when a mixture of complexes are present. $[Gd^{III}] = 5.4 \times 10^{-3}$ mol dm^{-3}, pH 7.0, 298 K, $A = 450$ Oe. Measurements of proton relaxation were made at 20 MHz

Ligand	$[Gd^{III}]$: [L] ratio	x in species $[GdL_x]$ [a]	ΔH/Oe (Increase)	h/cm (Decrease)	ε*
H_2O		0	493	65	1.00
amp [b]	1 : 10	0—1 (0.6)	548	33	0.74
Citrate	1 : 10 or 1 : 20	2, some 3	630	29	0.99
ida [c]	1 : 20	2 + 3	1 767 (Decrease)	16 (Decrease)	0.45
Maleate	1 : 100	1 + 2	288	44	0.84
Fumarate	1 : 20	1 + 2 + 3	219	37	0.35
Iso-phthalate	1 : 10	1 + 2 + 3	329	24	0.35
Glutamate	1 : 20	2 ± 3 (?)	260 (Decrease)	55 (Increase)	0.60
Acetate	1 : 4	0(0.3) + 1(0.4) + 2(0.18)	233	152	
	1 : 10	0(11) + + 1(0.4) + 2(0.21) + 3(0.08)	219	176	0.73
	1 : 100	2(0.2) + 3(0.4)	205	237	0.73

[a] See footnote to Table 1. [b] amp = Adenosine-5′-monophosphate. [c] ida = Iminodiacetate.

[14] T. Moeller, D. F. Martin, L. C. Thompson, R. Ferrus, G. R. Feistel, and W. J. Randall, *Chem. Rev.*, 1965, **65**, 1.

with complex formation. In other cases [Figure 2(*c*) and (*d*)] the e.s.r. line ceased to be Lorentzian, the distortion arising from incomplete averaging of static zero-field *splittings*. These cases are indicated with an asterisk in Tables 2 and 3.

The Tables also include the observed proton-relaxation enhancements ε^* [see equation (3)]. The data refer to the same conditions as those which apply to the e.s.r. spectral information in the Tables. Titrations were performed over a wide concentration range but the data in the Tables are chosen either as descriptive of a given species (shown) and/or a given solution stoicheiometry. In all the work we do not require highly precise information at this stage, for we are asking whether or not any general pattern is discernible following equations (2) and (5) (see Discussion section).

Mixed Complexes of $[Gd(edta)]^-$ *and* $[Gd(hedta)]$.—Mixed complexes of $[Gd(edta)]^-$ (1 : 1) and $[Gd(hedta)]$ (1 : 1) [H_3hedta = *N*-(2-hydroxyethyl)ethylenediamine-*NN'N'*-triacetic acid] with different ligands were also studied by e.s.r. Tables 4 and 5 list the linewidth ΔH (in Oe) for the fully formed mixed complexes with $[Gd(edta)]^-$ (see also Figure 3). The majority of the ligands used caused very extensive sharpening of the $[Gd(edta)]^-$ e.s.r. line, giving linewidths $\Delta H \ll 365$ Oe. The two exceptions were 2,3-dihydroxybenzene-1,4-disulphonate (dbds) and nitrilotriacetate (nta) which caused broadening and distortion of the line. Similar experiments were carried out with mixed complexes of [Gd(hedta)] (1 : 1) (see Table 7) with similar results. Again the g values changed only slightly.

TABLE 4

Effect of inorganic ligands on the e.s.r. signal and proton-relaxation enhancement parameters of $[Gd(edta)]^-$ in aqueous solutions. $[Gd(edta)^-] = 5.4 \times 10^{-3}$ mol dm^{-3}, 298 K, pH 10.0, $A = 63$ Oe

Ligand L	$[Gd(edta)^-]$: [L] ratio	x in species $[Gd(edta)L_x]$	ΔH/ Oe	h/cm	ε^*
H_2O			315	84	0.53
F^-	1 : 200	1—2 (2)	596	36	0.25
$[SO_4]^{2-}$	1 : 100	1	240	126	0.42
$[NO_3]^-$	1 : 100	1	240	119	0.42
$[NO_2]^-$	1 : 100	1	240	115	0.42
Cl^-	1 : 100	1	233	76	0.39
$[P_2O_7]^{4-}$	1 : 10	1	219	100	0.33
$[P_3O_{10}]^{5-}$	1 : 10	1	130	340	0.62

TABLE 5

E.s.r. signal and proton-relaxation enhancement parameters for fully formed complexes $[Gd(edta)L_x]$ ($x = 1$ except for H_2O) with organic ligands. $[Gd(edta)^-] = 5.4 \times 10^{-3}$ mol dm^{-3} (1 : 1), 298 K, pH 10.0, $A = 63$ Oe. Measurements of proton relaxation were made at 20 MHz

Ligand L	$[Gd(edta)^-]$: [L] ratio	ΔH/Oe	h/cm	ε^*
H_2O		315	84	0.53
adp	1 : 10	205	92	0.48
atp	1 : 10	107	446	0.36
Maleate	1 : 20	233	86	0.37
Fumarate	1 : 20	241	80	0.34
Isophthalate	1 : 10	178	65	0.36
Malate	1 : 20	105	1 100	0.27
Citrate	1 : 20	119	2 324	0.21
Aspartate	1 : 20	123	861	0.19
Glutamate	1 : 20	178	117	0.39
ida	1 : 10	130	450	0.19
edta	1 : 10	164	310	0.34
dnds acid	1 : 10	233	88	0.44
nta	1 : 10	452	31	0.19
dbds	1 : 10	685	17	0.22

DISCUSSION

This paper is largely concerned with a presentation of observed effects of ligands on the e.s.r. spectrum of Gd^{3+} in water and on the relaxation rates of solvent-water protons due to the presence of gadolinium(III)

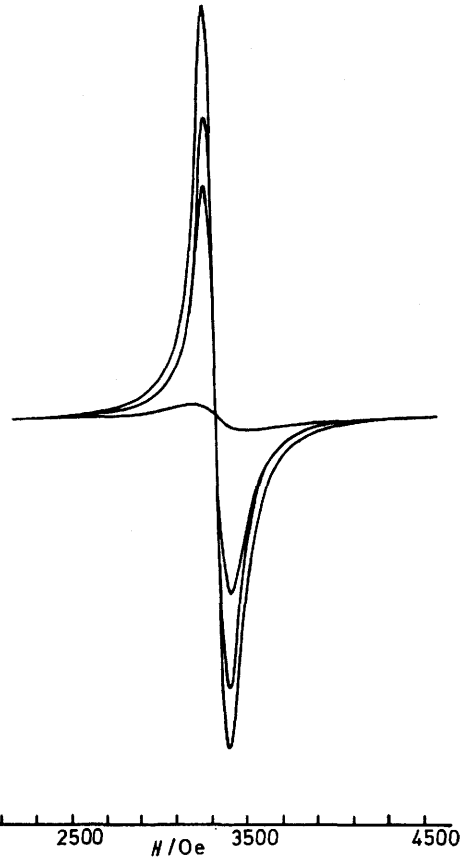

FIGURE 3 Formation of mixed complexes of $[Gd(edta)]^-$ with sodium citrate. $[Gd(edta)^-] = 5.4 \times 10^{-3}$ mol dm^{-3}, pH 10.0, 25 °C. The ratios of $[Gd(edta)^-]$: [citrate] are (from top to bottom curve) 0, 0.5, 1.0, and 2.0 : 1

complexes. Tables 1—3 give e.s.r. and proton-relaxation parameters. Their interpretation will follow an analysis of the equations described above. It is clear that ligands can either grossly broaden ($\Delta H/h \gg 10$ Oe cm^{-1}) the e.s.r. spectrum of $[Gd(OH_2)_x]^{3+}$, where $x = 8$—10 and $\Delta H/h = 10$ Oe cm^{-1} for the simple hydrated ion, or can narrow it ($\Delta H/h < 10$ Oe cm^{-1}), and that the proton relaxation rate can be decreased ($\varepsilon^* < 1.0$) or increased ($\varepsilon^* > 1.0$) by the binding of ligands.

The simplest case to consider first is that in which all the water is removed from the inner sphere of the Gd^{3+} ion by ligand binding. In Table 2 this is known to be the case for $[Gd(pydca)_3]^{3-}$ (pydca = pyridine-2,6-dicarboxylate) for the ligands form a nine-co-ordinate

structure.[15] It is seen that there is now a very broad e.s.r. line, and that the proton relaxation rate is much reduced presumably to a value due to outer-sphere effects. Most other strong organic chelating agents have similar effects on the e.s.r. spectrum, increasing $\Delta H/h$ and relaxation rates and decreasing ε^* (Tables 1—3) but to a lesser degree, suggesting that the shielding of the Gd^{3+} from water prevents relaxation of its electron spin and that water relaxation rates depend on the number of water molecules bound quantitatively.

Unfortunately, however, these generalisations are not found to apply in all cases and some strong multidentate chelating agents give narrow lines (hedta) or large ε^* values, *e.g.* complexes of adenosine-5′-triphosphate (atp) and 1,8-dihydroxynaphthalene-3,6-disulphonate (dnds). Taking the e.s.r. line shape first, symmetry factors [see equation (5)] do not seem to be dominant in these cases since in Tables 1 and 2 several ligands which form strong 1 : 1 or 1 : 2 complexes and which must be of low symmetry, *e.g.* $[P_3O_{10}]^{5-}$ and $\{[atp]^{4-}\}_2$, give broad lines but others, *e.g.* edta, give narrow lines. Only in the case of the pydca complex was it possible to see that the line was broken into a series of closely related lines [Figure 2(*d*)], clearly suggesting a lowering of symmetry from the hydrate.

The effect of binding of small inorganic ligands (Table 1) is very much the same as that of small organic ligands (Table 3). Despite the fact that the weakest inorganic ligands, *e.g.* Cl^- or $[NO_3]^-$, bind poorly and asymmetrically to Gd^{3+} under the conditions shown, they sharpen the e.s.r. line (Table 1) and we are forced to conclude that the combination of the number of water molecules bound to Gd^{3+} and their symmetry do not determine the line shape in any simple way.

Turning to the comparison of e.s.r. line shape and water relaxation rates, we find no general correlation with the e.s.r. line shape (Tables 1—3) and no obvious correlation of relaxation rates with ligand binding strengths or with expected degree of hydration of $[GdL_x]$. However, it is clear that one group of ligands, the polyphosphates, are quite different from the other ligands both in Tables 1 and 2. With these contradictory data in mind we turned to the examination of complexes with much more limited degrees of hydration.

In Table 4 data are given for the complexes $[Gd^{III}(edta)L]$ (L = an inorganic ligand). It is known that the $[Gd(edta)]^-$ complex has three or possibly four water molecules of hydration.[16] Although the ligand is tightly bound there is the possibility that one of the $CH_2CO_2^-$ arms can move away from the metal on binding of an additional ligand.[17,18] The data for the complexes show quite unexpected features. Addition of most ligands increases the sharpness of the e.s.r. line while it decreases ε^*. Two ligands, F^- and $[P_3O_{10}]^{5-}$, are quite exceptional, but in different ways. Again in Tables 5 and 6 all the organic ligands sharpen the $[Gd(edta)]^-$ e.s.r. line and reduce ε^*. Some of the values of ε^* are very close to that for $[Gd(pydca)_3]^{3-}$, *i.e.* apparently there is no water in the co-ordination sphere (when there is only outer-sphere relaxation), but the e.s.r. linewidths of these complexes have been affected in an opposite manner to that of $[Gd(pydca)_3]^{3-}$ when compared with Gd^{3+} itself. It is difficult to see how symmetry factors could be important in these low symmetries.

For comparison with the $[Gd(edta)]^-$ complexes, Table 7 gives data on $[Gd^{III}(hedta)L]$ complexes. The

TABLE 6

E.s.r. signal and proton-relaxation enhancement parameters for $[Gd(edta)]^-$ when a mixture of complexes is present; $[Gd(edta)^-] = 5.4 \times 10^{-3}$ mol dm^{-3}, 298 K, pH 10.0, $A = 62$ Oe. Measurements of proton relaxation were made at 20 MHz

Ligand L	$[Gd(edta)^-]$: [L] ratio	x in species $[Gd(edta)L_x]$	ΔH/Oe	h/cm	ε^*
H_2O			315	84	0.53
amp	1 : 10	Some 1	315 (Decrease)	84 (Increase)	0.49
Acetate	1 : 100	1—2	240	121	0.39

TABLE 7

E.s.r. signal and proton-relaxation enhancement parameters for $[Gd(edta)L_x]$ ($x = 1$ except for H_2O) with different ligands. $[Gd(edta)^-] = 5.4 \times 10^{-3}$ mol dm^{-3}, $[Gd(edta)^-] : [L] = 1 : 10$ (except for L = H_2O), pH 10.0, 298 K, $A = 45$ Oe. Measurements of proton relaxation were made at 20 MHz

Ligand L	ΔH(Oe)	h/cm	ε^*
H_2O	335	116	0.50
$[P_3O_{10}]^{5-}$	130	361	0.25
hedta	158	196	0.32
atp	164	202	0.40
Malate	137	284	0.28
Aspartate	123	397	0.20
Citrate	130	361	0.22
ida	116	874	0.18

data show very similar trends to those for $[Gd^{III}(edta)L]^-$ complexes in Tables 4—6.

It is obvious from all these data that the physical parameters of the gadolinium(III) complexes are being influenced by very many factors. Using $[Gd(pydca)_3]^{3-}$ as a basis for comparison, which we take to be an example of outer-sphere hydration only, we can examine first other complexes with ε^* values of *ca.* 0.25. The best examples are the complexes of (hedta)L and (edta)L, where L = aspartate or citrate (for both hedta and edta series), $[P_3O_{10}]^{5-}$ (for hedta), and dbds, nta, and F^- but not $[P_3O_{10}]^{5-}$ (for edta). These complexes have ε^* values <0.30. If it is true that there is no inner-sphere hydration in any of these complexes they represent an odd assortment of liganded species. None of the other complexes have such low ε^* values and we suspect that they all have residual hydration. We are

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[16] J. L. Hoard, B. Lee, and M. D. Lind, *J. Amer. Chem. Soc.*, 1965, **87**, 1612.
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not able to assess this hydration, however, for it is clear that ε^* reflects not only the number of water molecules but other factors as well since all complex formation must reduce the amount of water in the first co-ordination sphere but it does not reduce ε^*. Ligands could catalyse H^+ or water exchange or they could affect the electron relaxation time. We have assumed throughout of course that τ_c, the molecular tumbling time, does not change significantly from one complex ion to another. It would be very surprising indeed if this is not the case, for the situation here where all the molecules are of very similar size is very unlike that described by us previously where binding of Gd^{3+} to a large protein was involved.[19] Again some of the changes in ε^* seen in the Tables are in the wrong sense for this to be the case. Thus ε^* is greatly increased by the binding of polyphosphate ligands (Tables 1—3) while it is decreased by the binding of many other ligands of similar molecular size. We conclude that changes other than those of τ_c and hydration can grossly alter ε^*.

We conclude too that it would be dangerous indeed to use e.s.r. spectra of gadolinium(III) complexes to discuss their chemical nature. Thus Gd^{3+} is not a useful structure probe of biological sites although it could be used as a good reporter group. The very sharp e.s.r. signals in some of the complexes will make admirable probes of binding, for example [Gd^{III}(atp)], whether it be of this complex ion, of free Gd^{3+}, or of free atp.

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