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A ^{31}P and ^{23}Na NMR and Terbium(III) Luminescence Study of Bistriphosphato–Lanthanide(III) Complexes Including the Cation Shift Reagent $[Dy(PPP)_2]^{7-}$

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Abstract

Measurement of the ^{31}P NMR spectra of the cation shift reagent, dysprosium bistriphosphate, $[Dy(PPP)_2]^{7-}$, shows that bound and free ligand are in slow exchange at pH 6.75. As the pH is decreased the exchange rate increases but the central phosphate group is still coordinated at pH 2.1. The ^{31}P spectra were measured for eleven other lanthanide bistriphosphate complexes and the contact and dipolar contributions to the shift were separated, enabling geometrical information to be obtained from the latter.

The association constants for Na^+ and limiting shift were obtained from the ^{23}Na spectra in the presence of six $[Ln(PPP)_2]^{7-}$ complexes, the dipolar mechanism dominates, but there is a small contact shift. The observed shift decreases with pH, depending approximately on a group with pK_a 6.3, one of the phosphate residues.

The hydration of $[Tb(PPP)_2]^{7-}$, determined from measurements of the luminescence lifetimes in H_2O and D_2O, was found to increase from 2 to 4 as the pH decreased from 9 to 3, a rough parallel with the decrease in ^{23}Na shift is noted. These results, together with the geometrical information from the dipolar shifts, allow an 8 coordinate structure to be proposed for the dysprosium bistriphosphate complex which possesses a pseudo-axial binding site for Na^+, accounting for the large shift and other observations.

Introduction

Dysprosium bistriphosphate, $[Dy(PPP)_2]^{7-}$, has been used as a shift reagent for ^{23}Na and ^{39}K NMR in a wide range of biological preparations from yeast cells [1], plant cells [2], frog skin [3] and amphibian oocytes [4], to human red cells [5], and has also been employed in studies of the model membrane system, phosphatidylcholine liposomes [6]. The shift reagent allows the measurement of intra-cellular cation concentrations and *trans*-membrane exchange rates by causing separate NMR signals to be observed from the intra- and extra-cellular cation [7]. The large external cation peak is shifted well away from the less intense peak due to the internal cation when relatively low concentrations (1:100, shift reagent: $[Na^+]$) are employed.

Although $[Dy(PPP)_2]^{7-}$ has been employed quite widely, there have been few reports on the chemical properties of the complex. Two structures have been suggested [8, 9] for the sodium complex, and it has been suspected [10] that there is a contact contribution to the ^{23}Na chemical shift. It is also known that the ^{23}Na shift is strongly pH dependent [8]. We have elucidated the origin of these effects by combining ^{31}P and ^{23}Na NMR studies of up to ten lanthanide bistripolyphosphate complexes so that the contact and dipolar shifts could be separated. The extent of the coordination of water has been elucidated by measuring the luminescence lifetimes of solutions of the analogous Tb^{3+} complex in H_2O and D_2O. Coupling this information with the geometric data from the dipolar shift terms enables a model to be proposed which can account for the experimental observations.

Experimental

Sodium triphosphate was obtained from BDH Ltd, and purified by four recrystallisations from ethanol [11]. Purity was established from ^{31}P NMR and infrared spectroscopy [12]. Solutions were used on the day of preparation. Lanthanide solutions were prepared from the oxides or chlorides (Johnson Matthey Ltd), in the latter case the concentration was determined by titration [13].

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^{31}P NMR spectra were obtained on a Jeol FX100 spectrometer operating at 40.50 MHz and a Bruker CXP300 spectrometer operating at 121.47 MHz. ^{23}Na spectra were obtained on the CXP300 operating at 79.37 MHz. ^{31}P chemical shifts were referenced to 85% phosphoric acid as external standard, and for ^{23}Na 0.1 M sodium chloride was used as external standard. In all cases the high frequency positive convention was used. Shimming was carried out by observing the proton or sodium signals from the samples and adjusting the shims for the maximum length of free induction decay. Deuterium locking was not used.

Luminescence measurements were made on a Perkin Elmer LS5 instrument with a PE 3600 data station.

Results and Discussion

A 121 MHz ^{31}P NMR spectrum of an aqueous solution of dysprosium chloride and sodium triphosphate, mole ratio 1:4, at pH 6.75 is shown in Fig. 1. Four peaks are clearly visible, those at −7.6 ppm and −21.5 ppm are assigned to the terminal and central phosphorus atoms respectively of the uncomplexed triphosphate. The peaks at 134.1 and 231.3 ppm are assigned to the terminal, P(t), and central, P(c), phosphorus atoms of the complexed ligand on the basis of their relative intensities. As pH is progressively lowered the peaks from free and bound ligand broaden and shift towards each other, the chemical shift difference between the high frequency peaks also increases, the peak assigned to bound P(t) is more affected by pH than that assigned to bound P(c). At pH 3.86 there are only two peaks remaining, these are at 27.9 and 164 ppm. The 164 ppm peak is very broad and is no longer observable at lower pH. The general pattern of behaviour observed in Fig. 1 is consistent with the well known [14] effects of exchange on line shape. Here it is interpreted as the result of a decrease in pH increasing the exchange rate between bound and free ligand. The presence of the central and terminal groups is, however, not uniform. The P(t) signals coalesce at higher pH than the P(c) signals.

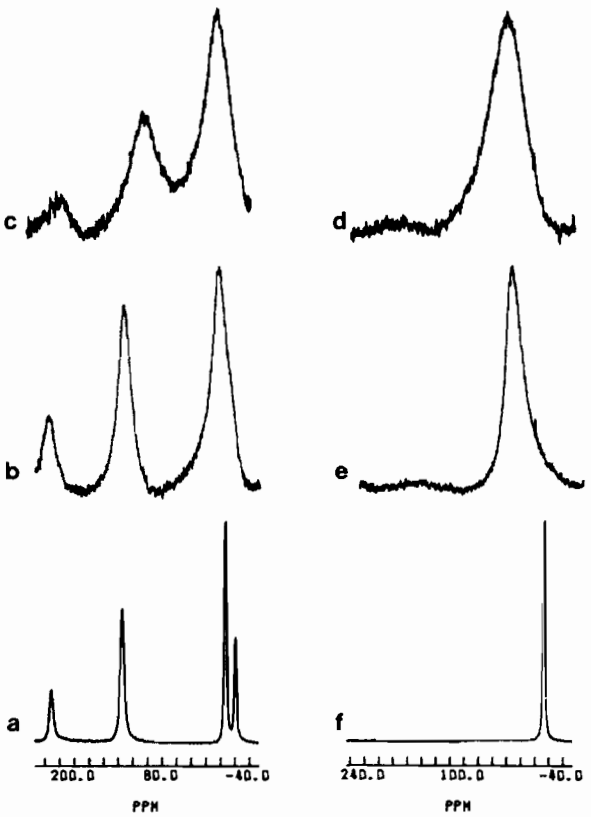


Fig. 1. 121 MHz ^{31}P NMR spectra of triphosphate in the presence of dysprosium(III) as a function of pH. The mole ratio of triphosphate to dysprosium is 4:1, dysprosium(III) 0.02 M: (a) pH 6.75; (b) pH 5.23; (c) pH 4.64; (d) pH 3.86; (e) pH 3.55; (f) pH 2.46.

Whilst illustrating the general features, spectra obtained at high magnetic field strengths suffer from the problem of signal loss at low pH due to excessive line broadening. At lower magnetic field strengths line broadening effects due to exchange between chemically shifted sites are less severe. In order to examine more closely the processes involved experiments were carried out in a field of 2T (^{31}P resonance frequency 40 MHz), under these conditions there was no loss of signal at low pH. When a 2:1 ratio of Dy^{3+}:PPP^{5-} was employed two lines were observed at any pH, indicating that at high pH (where the lines were narrow) the population of free ligand is very small, as pH is decreased the lines broaden and move towards the chemical shifts of the free ligand. The change in the chemical shift of the P(c) peak over the range pH 7.8 to pH 2.12, is 121 ppm, for P(t) it is 140 ppm. At pH 6.75 the differences in chemical shift between bound and free central phosphates was 252.8 ppm and for terminal phosphates it was 141.7 ppm. At pH 2.12 the P(t) peak has almost returned to the position of the free P(t) peak, but the P(c) peak has only shifted half way back. Protonation of triphosphate occurs first at P(t) [15], thus P(t) dissociates more readily from dysprosium, reducing its chemical shift as the pH decreases.

The central phosphate group takes up the final protons so its dissociation would only be expected to commence at lower pH. However some dissociation or change in structure is inferred from the decreasing shift of P(c) which reflects changes consequent upon partial protonation of the ligand. These protonation equilibria are on a time-scale fast compared to that of ligand exchange, if they were not then more than 2 peaks would be observed.

When both the chemical shift and populations of the exchanging species vary the conventional

approach to the calculation of exchange rates needs to be modified to take account of the changing shifts and populations of the species involved. Since the protonation equilibrium are complex and the shifts of the protonated complexes unknown, an attempt at fitting the exchange data would amount to little more than a parameterization process with little chemical significance. We have therefore made no such attempt, but have restricted ourselves to a qualitative description of the processes involved.

Although the dysprosium complex has been studied most thoroughly because it gives the largest ^{23}Na shifts, the other ten paramagnetic lanthanide ions studied also produced ^{31}P shifts, Table I. The magnitude and direction of the shifts are lanthanide dependent allowing the contributions to the observed shift to be separated and analysed.

The limiting shift (δ_l) is that observed when all of the coordinating groups are bound to the lanthanide ion. Thus for ^{31}P shifts it is the value obtained when the pH is above 7, since under these conditions there is no protonation and all the coordinating groups may be considered to be bound. The limiting shift is a combination of contact, dipolar and complex formation shifts [16] expressed by eqn. (1).

$$\delta_l = F\langle S_z\rangle + GC^D + K_N \quad (1)$$

K_N is the complex formation shift which excludes any paramagnetic effects. It is the limiting shift observed on complexation to a diamagnetic lanthanide ion resulting from electronic and structural distortions in the ligand upon complexation. $F\langle S_z\rangle$ is the contact shift term arising from through bond effects and orbital overlap, it is composed of the lanthanide specific terms, $\langle S_z\rangle$, and the ligand specific hyperfine interaction constant, F. The $\langle S_z\rangle$ terms have been calculated by Golding and Halton [17] incorporating bonding effects and spin–orbit coupling, the values used here are taken from column 5 of Table I of their paper [17].

The dipolar shift, GC^D, has a lanthanide dependent parameter C^D for which the most appropriate values are those calculated by Golding and Pyykkö [18], and listed as set 4 in Table II of their paper. G is the ligand dependent term which is given by eqn. (2) for an axially symmetric complex. G is related to the distance of the ligand atom from the lanthanide, r, and the angle, θ, which it makes with the principal axis of the complex, the crystal field parameters, $A_2\langle r^2\rangle$, are assumed to be constant [16].

$$G = \beta^2(3\cos^2\theta - 1)2A_2\langle r^2\rangle/60(kT)^2r^3 \quad (2)$$

Both sets of parameters [17, 18] follow the currently preferred positive high frequency convention defined by Bleaney [19], to which we also adhere. Care is required in the choice of the source of C^D values since another paper by Bleaney [20] uses the opposite sign convention.

K_N was determined from the shifts observed with La^{3+} complexes, Table I, and several of the methods introduced by Reilley *et al.* [16] were evaluated for separating the contact and dipolar shifts. Methods A1 and A2 involve plots of eqns. (3) and (4) respectively

Method A1

$$(\delta_l - K_n)/C^D = F(\langle S_z\rangle/C^D) + G \quad (3)$$

Method A2

$$(\delta_l - K_n)/\langle S_z\rangle = G(C^D/\langle S_z\rangle) + F \quad (4)$$

Method A2, which is preferred when the dipolar shifts are large, gave the most satisfactory plots, Fig. 2, although the scatter is much greater for the central phosphorus than for the terminal phosphorus. The results obtained by method A2 are in agreement with those obtained through a full multiple regression analysis of the data which yielded for P(t) $G = 2.3$, $F = 2.2$ and for P(c) $G = 2.7$, $F = -1.3$ when the data for samarium are excluded.

The contact and dipolar shifts for phosphorus in $[Ln(PPP)_2]^{7-}$ have been determined previously [9], however, the authors used the C^D values of the wrong sign resulting in incorrect F and G values. When their data are recalculated using the sign convention employed here their results are similar to our own. These authors [9] also omitted the terbium induced shifts from their calculations, our data, Fig. 2, show that the points for terbium lie where expected. The omission of the data for samarium is justified on the grounds that there are low lying excited states contributing to $\langle S_z\rangle$ [17], and its position on the plots of Fig. 2 weigh it heavily. Inclusion of the data for samarium increases the values of G, but as there is no

TABLE I. $[Ln(PPP)_2]^{7-}$ Induced ^{31}P and ^{23}Na Shifts at pH 8

Ln^{3+}	$\delta_{P(t)}$ (ppm)	$\delta_{P(c)}$ (ppm)	$\delta_{Na(l)}$ (ppm)
La	−6	−17	20
Ce	13.4	0.9	
Pr	26.1	−7.7	
Nd	16.5	−16.8	
Sm	−2.4	−11.4	
Eu	−54	−19.5	
Tb	115	364	−309
Dy	131	225	−477
Ho	31.3	99	−226
Er	−140	−134	151
Tm	−199	−231	229
Yb	−62	−57	

[a]Limiting shifts obtained from eqn. (5), where no value appears the shifts were too small for the precise determination of $\delta_{Na(l)}$.

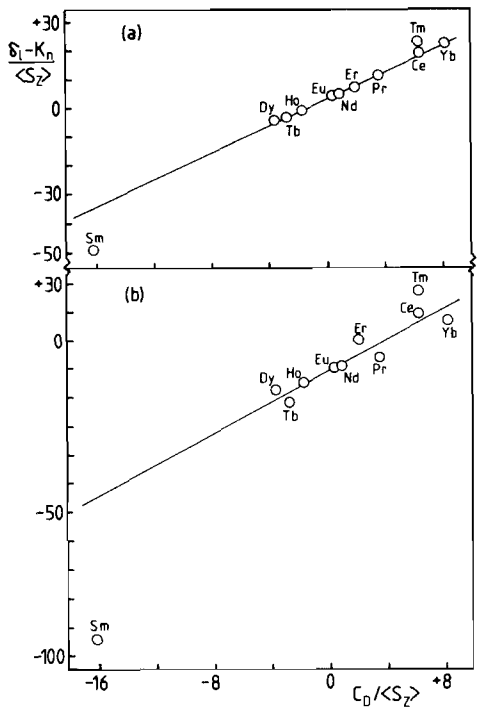


Fig. 2. Plot of eqn. (4) for the separation of the contact and dipolar shifts produced by lanthanide(III) ions on the ^{31}P NMR spectra of (a) the terminal phosphates and (b) the central phosphate of triphosphate.

sign change it does not influence the later discussion on the structure of the complex.

^{23}Na NMR Spectra

Over the pH range 3–11 the 79 MHz ^{23}Na spectra show only a single peak in the presence of $[Dy(PPP)_2]^{7-}$, the peak exhibits a pH dependent shift from an external reference. This is consistent with the NMR fast exchange limit in which the exchange processes are occurring at a rate which is rapid with respect to the chemical shift difference, expressed in frequency units, between the free and bound sodium ions. Under these conditions the observed shifts are a population weighted average of those for free sodium ions and those bound to the dysprosium complex. It is impracticable to add enough of the shift reagent to determine the limiting shift directly, instead of a 1:1 complex is assumed and $1/\delta_{obs}$ plotted against $[Na^+]$ in accord with eqn. (5),

$$1/\delta_{obs} = (1/\delta_1 K_{Na}[Cx]) + ([Na^+]/\delta_1[Cx]) \qquad (5)$$

where K_{Na}, the ratio of the slope to the intercept, is the association constant for the binding of Na^+ to $[Ln(PPP)_2]^{7-}$ which is present at a constant total concentration [Cx]. It should be noted that a 2:1 mole ratio of triphosphate to lanthanide was found to give the maximum shift, the 2.5:1 ratio reported [10] may have arisen from impurities in commercial

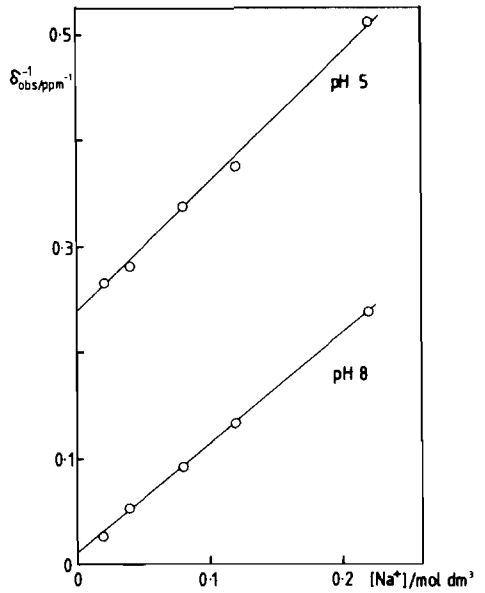


Fig. 3. Dependence of the reciprocal of the ^{23}Na NMR chemical shift on sodium ion concentration in the presence of 0.002 M $[Dy(PPP)_2]^{5-}$ at pH 5.0 and pH 8.0, 25 °C.

sodium tripolyphosphate. Plots of eqn. (5) at pH 5.0 and 8.0 for the dysprosium shift reagent are shown in Fig. 3. The linearity of the plots bears out the assumed 1:1 stoichiometry, the similar slopes at the two pHs indicate similar limiting shifts, but the differing intercepts show that the association constant is lower at pH 5. The limiting shifts obtained with several lanthanide bistriphosphate complexes are collected in Table 1, the association constants at pH 8 ranged from 120 to 360 dm^3 mol^{-1}, except for the erbium complex which consistently, and so far inexplicably, gave a value of 30 dm^3 mol^{-1}. These constants are significantly less than that reported for Ca^{2+} (1.2×10^4 dm^3 mol^{-1}) [21] which is in accord with its higher charge, and the electrostatic nature of the interaction between the anionic lanthanide complex and the alkali or alkaline earth cation.

The contact and dipolar shifts for sodium were separated using both methods A1 and A2 to give $F = 1.4$ and $G = -4.3$. The non-zero value of F confirms the suspicion [10] that there is a contact contribution to the shift, implying a degree of electron delocalisation over both metal nucleii. The contact and complex formation shifts are of similar magnitude, but opposite sign, so they effectively cancel for most lanthanide ions, eqn. (1). This validates the estimation of G by using the data from a single paramagnetic lanthanide ion [8].

The pH dependence of the ^{23}Na chemical shift is illustrated in Fig. 4, the data are fitted approximately by a sigmoidal curve which has been drawn by assuming that the protonation of a group with a pK_a of 6.3 destroys the ability of the complex to

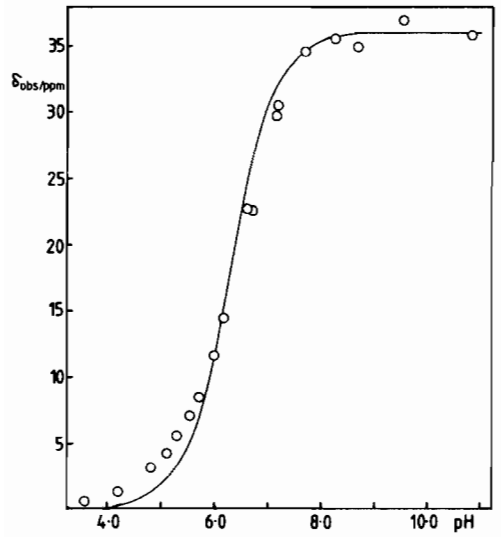


Fig. 4. pH dependence of the ^{23}Na chemical shift, 0.02 M Na^+, 0.002 M $[Dy(PPP)_2]^{5-}$, 25 °C. The solid line shows the pH dependence calculated for a pK_a of 6.3.

shift the ^{23}Na resonance. It is known that some phosphate groups titrate in this pH range [22]. The data presented here show a similar trend to that presented by Chu *et al.* [8], but their shifts are smaller due to their higher sodium ion concentration, and their curve is displaced to lower pH, which may be due to higher ionic strength. A fuller understanding of the pH dependence is obtained from the data in Fig. 3 which show that the association constant dropped to 5 dm^3 mol^{-1} at pH 5, but that the limiting shift was little changed. This suggests that the structure of the dysprosium complex responsible for the shift in the ^{23}Na resonance remains unchanged and that the reduced affinity for Na^+ is a consequence of the decreased proportion of this unprotonated complex present at the lower pH.

At room temperature and pH 6–9 the shift reagent gives a constant ^{23}Na shift over a period of at least 12 h. At higher temperatures the shift decreases, above 70 °C there is an irreversible decrease with time. The reversible effect is partially due to a decrease in K_{Na}, but there is an inherent inverse temperature dependence of the dipolar shift, eqn. (2). At high pH there are also time dependent irreversible decreases in the chemical shift. The irreversible changes can be attributed to hydrolysis of the complex.

Structure of the Na^+ $[Dy(PPP)_2]^{7-}$ Complex

Although the dysprosium ion is at least hexacoordinated by the tripolyphosphate ions, its coordination number is likely to be 8 or 9 [23], and at least some of the extra ligands will be water. For several of the lanthanide ions the number of coordinated water molecules can be derived from luminescence lifetime measurements [24, 25]. A major non-radiative pathway for excited lanthanide ions in aqueous solution is energy transfer to the O–H vibrational manifold. In D_2O the lower vibrational energy reduces the transfer efficiency and increases the radiative lifetime. The lifetime of Dy^{3+} is too short for our equipment so the adjacent lanthanide, terbium was used. The relationship between lifetime, t, and the number of coordinated water molecules, q, has been established for terbium [25], eqn. (6).

$$q = 4.2((1/t_{H_2O}) - (1/t_{D_2O})) \quad (6)$$

Use of this equation indicates that there are 2 ($q = 1.9$) water molecules coordinated to the $[Tb(PPP)_2]^{7-}$ complex at pH 7. The luminescence lifetime decreases with pH, Fig. 5, until on the plateau at pH 2–4 the number of coordinated water molecules has increased to 4. There is a similarity between the pH dependencies of the ^{23}Na shift, Fig. 4, and the luminescence lifetime, Fig. 5, which suggests that destruction of the sodium binding site on the complex is accompanied by increased hydration, although the lifetime data cannot be fitted by the titration of a single phosphate.

Although there is a general trend to lower coordination number along the lanthanide series [23] it is assumed that there is no abrupt decrease on going from the terbium to the dysprosium triphosphate complex. Thus at pH 7 the shift reagent can be represented as $[Dy(PPP)_2(H_2O)_2]^{7-}$ with a coordination number of at least 8. Following Bryden *et al.* [26] the dipolar shift parameter G can be used to establish the positions of the phosphate groups and sodium ion around the central lanthanide ion, negative values of G occur for atoms in the axial region, within 54.7° of the principal axis of the complex. The positive G values for the phosphorus atoms indicate that they all occupy the equatorial region.

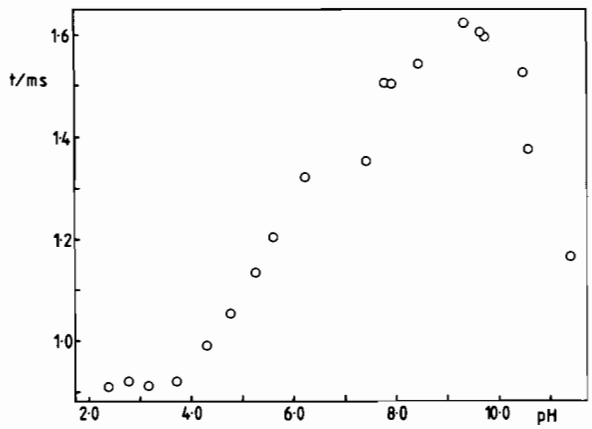
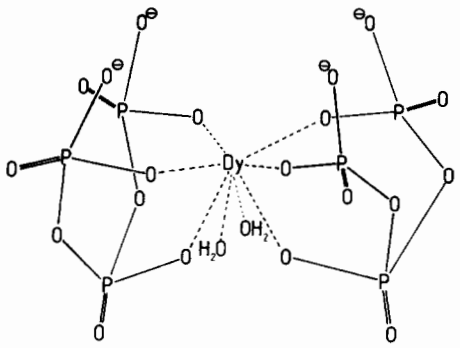


Fig. 5. pH dependence of the terbium luminescence lifetime, triphosphate:terbium(III), 2:1, terbium(III) 0.02 M in water.



Structure 1

Structure **1** places the two tripolyphosphate ions so that the four terminal phosphorus atoms are in a plane. The near fourfold symmetry of these atoms, contrasted to the twofold axis relating the central phosphate atoms, could account for the more successful separation of the contact and dipolar shifts for the terminal phosphorus atoms, as the method employed assumed axial symmetry. Analysis of the ^{17}O data published for solutions of lanthanides in D_2O [9] gives a positive value of G, thus also putting the coordinated water molecules in the equatorial region, as there are two of them it seems reasonable to place them between the central phosphates. The four terminal phosphates provide a negatively charged region above them which would serve as a binding site for the sodium ion in the axial region, which is required by the negative G value found for sodium.

There is evidence from the relatively small change in chemical shift of the central phosphorus atoms with pH, and from potentiometric titrations [15, 27], that the central phosphate group bonds more strongly to metal ions than the terminal groups. Protonation occurs first at the terminal phosphates [15, 27] which when dissociated will allow coordination of further water molecules, as indicated by the luminescence measurements, thus destroying the postulated Na^+ binding site and reducing the ^{23}Na chemical shift.

A binuclear structure for the complex has been suggested [8], with the coordinating sodium ion displacing a terminal phosphate, but this is not supported by the ^{31}P NMR data. A more recent suggestion [9] incorporates NMR evidence that one water is coordinated and that two of the terminal phosphate groups are bidentate, as in the 1:1 ATP:Dy^{3+} complex [28]. Such bidentate terminal phosphate groups have also been suggested for the triphosphate cobalt(II) complex [29], and may be sterically favoured in such 1:1 complexes. The structure proposed here, which incorporates the two water molecules required by the luminescence results, makes the dysprosium eight coordinate, if two of the terminal phosphates were bidentate the very high coordination number of ten would be required. In structure **1** the monodentate terminal phosphates provide a highly charged binding site for the sodium ion on the principal axis which can account for the high binding constant and large shift which are observed.

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