closer to the truth because $D$ is comparatively small.

In the simulations $w_f$ has been treated as empirical parameter.

It is expected to be independent of temperature and field strength as long as the latter is high enough to justify the Boyle and Gabriel model despite of a finite zero-field splitting. Due to the zero-field splitting the level spacings are of course not really equidistant, but this may be compensated in part by an inhomogeneous broadening of the levels. For the spectra of the trinuclear complex recorded under 6.21 and 2.47 T it was indeed possible to get satisfactory simulations for the whole temperature range from 4.2 to 50 K with one single value for $w_f$. The zero-field splitting, rhombicity, and hyperfine parameters were taken as determined from the low-temperature spectra except that, for the sake of simplicity, the Euler angles $\alpha$ and $\beta$ of the $efg$ tensor have been disregarded. The solid lines in Figure 8 show the simulations obtained for $S = 3/2$ and the parameters of Table IV with $w_f = 2.2 \times 10^8$ s$^{-1}$. The estimated uncertainty of this value is about 20%.

The relaxation rate in the tetrancular complex is obviously faster. The solid lines through the spectra in Figure 9 are simulations obtained for $S = 3/2$ and the parameters of Table IV with $w_f = 2.2 \times 10^8$ s$^{-1}$. Direct comparison with the trinuclear complex is possible, since the applied field of 6.21 T is so strong that the spin $1/2$ of the monomeric Cu(Mesalen) is coupled in a kind of Paschen-Back effect much stronger to the external field than to the spin $3/2$ of the trimener. The monomer may, however, mediate the cross-relaxation between two trimers, thus enhancing the relaxation rate by about 1 order of magnitude. This finding is not unexpected because it is well-known, e.g. from the work of Bhargava et al.,$^{24}$ that the presence of other paramagnetic ions shortens the spin-spin relaxation times substantially. Such an explanation is also consistent with the X-ray structure of the crystalline tetrancular entity described in the preceding section.

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Additional Material Available. Tables S1 and S2, listing interatomic distances and bond angles for the P$_4$As anions and anisotropic thermal parameters (2 pages); a listing of observed and calculated structure factors (19 pages). Ordering information is given on any current masthead page.


Multinuclear NMR Study of the Interaction of the Shift Reagent Lanthanide(III) Bis(triphosphate) with Alkali-Metal Ions in Aqueous Solution and in the Solid State

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Introduction

Several aqueous shift reagents (SRs) for metal cation NMR spectroscopy have been reported.$^{1-6}$ They have been shown to be useful in membrane transport biochemistry, for example in studies of alkali-metal ion transport across vesicles$^{7,8}$ and red blood cell membranes.$^{9-12}$ However, only two of them, Dy(TTHA)$^{3-}$ and Tm(DOTP)$^{3-}$, have found useful practical application for perfused heart studies$^{13,14}$ and in vivo rat brain $^{23}$Na NMR spectroscopy.$^{15,16}$ Despite considerable discussion, there seems still to be some controversy regarding the mode of interaction of one of the SRs most widely used for NMR studies in cell systems, namely Dy(PPP)$^{3-}$, with alkali-metal ions in aqueous solution.$^{17,18}$ Moreover, it has been shown recently that Dy(PPP)$^{3-}$ can change ion distribution and transport across red blood cell membranes as well as membrane potential.$^{19,20}$ These effects may be related to the mode of binding of alkali metal cations to Dy(PPP)$_3^-$. In this work we further analyze this problem by studying the $^6$Li NMR spin-lattice relaxation times of Dy(PPP)$_3^-$ and Tm(PPP)$_3^-$. In

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Abbreviations used in this paper are as follows: PPP = tripolyphosphate; SRs = shift reagents; TTHA$^{3-}$tetraethylenetetraminehexaacetate; DOTP$^{3-}$tetraazacyclododecane-$N,N',N'',N'''-$tetrakis(methanephosphonate); MAS, magic angle spinning; NMR, nuclear magnetic resonance; LnIRE, lanthanide-induced relaxation rate enhancement.

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Methods and Materials

DyCl₃, TmCl₃, D₂O, and sodium tripophosphate were obtained from Aldrich Chemical Co. ⁴LiCl (95.7% ⁶Li) was from Oak Ridge National Laboratories, TN. All chemicals were used as obtained, except sodium tripophosphate, which was purified by repetitive precipitation from 33% aqueous ethanol. The purity of the resulting product was established by ³¹P NMR spectroscopy.

Tetramethylammonium tripophosphate was prepared from the sodium form by ion-exchange chromatography and recrystallized as described earlier. The residual sodium content in crystallized tetramethylammonium tripophosphate was analyzed by atomic absorption and ⁷³Na NMR spectroscopy and was found to be 2%. ³¹P NMR analysis of the above solution did not show any (phosphorous containing) impurities.

The shift reagents Dy(PPP)₂⁺ and Tm(PPP)₂⁺ were prepared as reported earlier. The ratio of Dy⁺⁺ or Tm⁺⁺ to PPP⁻ was maintained before and after NMR measurements and was observed to be the same. pH values reported are not corrected for deuterium isotope effects. All the NMR samples had 15% (v/v) D₂O.

The Na₃La(PPP)₂ salts were prepared by mixing a solution of 1 mmol LaCl₃·6H₂O in 5 mL of water with a solution of 2 mmol of Na₃(PPP) in 5 mL of water. The solution was filtered and then poured into 100 mL of ethanol. The precipitate obtained was filtered off, washed with cold aqueous ethanol, and dried in the air.

Results and Discussion

⁶Li Spin–Lattice Relaxation Studies in Solution.

For most alkali-metal ions the quadrupolar mechanism dominates the relaxation. As a result the relaxation rates are usually so high that paramagnetic ions are hard to measure accurately. The ⁴Li nucleus, however, has a very small quadrupole moment, and consequently its relaxation is extremely slow. Therefore, ⁶Li is a very suitable probe for studying the location of counterions of negatively charged Ln(III) complexes via the lanthanide-induced relaxation rate enhancements (Ln1RE).

Figure 1 shows the variation of the measured ⁶Li spin–lattice relaxation rate of Na⁺–free 5 mM solutions of Dy(PPP)₂⁺ and Tm(PPP)₂⁺ SRs at 25 °C and pH 7.5 when the concentration of LiCl present varied from 1.0 to 100 mM (the value of ρ [Li⁺] / [SR] changed from 0.2 to 20). The exchange rate between free and the various forms of SR-bound ⁶Li was fast on the ⁶Li NMR time scale; only a single ⁶Li resonance was observed, the chemical shift of which was dependent on pᵢ. The values of 1/T₁ were increased by about 30% when pᵢ increased from 2.0 to 0.2. After a maximum at pᵢ = 7, R₁ decreases steadily, as a result of exchange of the fast relaxing bound ⁶Li⁺ nuclei with the slow relaxing ⁶Li⁺ nuclei in the bulk. The difference in magnitude of the relaxation rates for the Dy⁺⁺ and the Tm⁺⁺ complexes can be explained by the differences in electron spin relaxation times and effective magnetic moments of these lanthanides (see below).

Assuming that isotropic relaxations occurs, that the contact contributions to the Ln1RE is negligible, the value of R₁ for ⁶Li⁺ bound to a Ln(PPP)₂⁻ complex can be related to the distance between the Ln(III) ion and the ⁶Li ion by using the reduced Solomon–Bloembergen equation:

\[ R₁ = \frac{g²(μ₀/4π²)(μ²-γ²Tₑ/Tₚ)}{1/Tₚ} \]  

(1)

Figure 1. Spin–lattice relaxation rates of ⁶Li in the presence of the SRs Ln(PPP)₂⁻ (A, Ln = Dy; B, Ln = Tm), at 25 °C and pH 7.5. The SRs were used in the tetramethylammonium form. The Ln(PPP)₂⁻ concentration was held constant at 5 mM, whereas the Li⁺ concentration was varied between 1 and 100 mM by addition of LiCl.
Figure 2. Simulated curves of Li spin–lattice relaxation rates in the presence of a Ln(PPP)$_2^-$ complex. (A) shows the model in which only one Li$^+$ binds strongly. Each triphosphate ligand is assumed to bind both Ln$^{3+}$ and Li$^+$ ions. Parameters used are as follows: concentration SR = 5 mM, stability constant adduct $K_n = 10000$ M$^{-n}$, $R_1$ bound = 17.4 s$^{-1}$, $R_1$ free = 0.05 s$^{-1}$. (B) shows the model in which seven Li$^+$ ions are bound in the second coordination sphere of Ln$^{3+}$ in the Ln(PPP)$_2^-$ complex. Parameters used are as follows: concentration Ln(PPP)$_2^-$ = 5 mM; stepwise stability constants $K_1 = 4000$, $K_2 = 2000$, $K_3 = 1800$, $K_4 = 1600$, $K_5 = 1400$, $K_6 = 1200$, $K_7 = 1000$ M$^{-7}$. The corresponding Li$^+$ complexes are assumed to have the following $R_1$ values: 2.8, 3.0, 3.2, 3.6, 3.7, and 3.8 s$^{-1}$. For free Li$^+$ an $R_1$ value of 0.05 was assumed.

Here $\mu_0/4\pi$ is the magnetic permeability under vacuum, $\mu$ is the effective magnetic moment, $\gamma$ is the magnetogyric ratio, $B$ is the Bohr magneton, and $T_1$ is the electron spin relaxation time. This latter parameter has been found to be rather independent of the ligation of the Ln(III) cation. If the adduct would have the previously suggested binuclear structure, in which each triphosphate is bound to both Ln$^{3+}$ and M$^+$, the distance Ln$^{3+}$-Li$^+$ would be about 4 A. Then for Ln = Tm, an $R_1$ value of 11.3 s$^{-1}$ can be calculated with eq 1 and a $T_1\text{eq}$ value of $4.6 \times 10^{-13}$ s. Analogously it can be calculated that in the corresponding Dy(PPP)$_2^-$ system, the $R_1$ value of Li$^+$ is 17.3 s$^{-1}$. These calculated relaxation rates are considerably larger than the experimental ones. The curves of $R_1$ as a function of $\rho$ were simulated with the use of the above-mentioned bound values of $R_1$, a value of 0.05 s$^{-1}$ for free Li$^+$, and eq 2, where $R_1 = R_1\text{bound} + R_1\text{free}$.

$R_1\text{free}$ are the mole fractions of bound and free Li$^+$, respectively, which were calculated by using a stability constant of the complex of 10000 M$^{-1}$. The calculated curves have shapes that differ dramatically from the experimental ones (see e.g. Figure 2A). The simulated curves show a steeper decrease of $R_1$ at $\rho > 1$, whereas the experimental ones have a maximum at $\rho$ about 7. On the basis of these shapes and the lower $R_1$ values, the binuclear structure of the Ln(PPP)$_2^-$-Li$^+$ adduct can be rejected. The experimental data strongly suggest that Ln(PPP)$_2^-$ complex binds rather strongly up to seven Li$^+$ ions in the second coordination sphere of the Ln$^{3+}$ ion, which is in agreement with a previous proposal. The increase of $R_1$ between $\rho = 0.2$ and 7 indicates that successive binding of the seven Li$^+$ ions to Ln(PPP)$_2^-$ causes the SR-Li$^+$ structure to become more compact, probably as a result of the electrostatic repulsion between the ligands upon charge neutralization by the Li$^+$ ions. Simulation of the dependence of $R_1$ of $\rho$ for the Dy(PPP)$_2^-$-Li$^+$ adduct with a procedure similar to that described above, by using bound values for $R_1$ of 2.8-3.8 for $n = 1-7$ and a somewhat arbitrary chosen set of stepwise stability constants for the respective adducts ($K_n = 4000-1000$ M$^{-n}$), gives a curve that is in good agreement with the experimental one (see Figure 2B). A fitting of the experimental data with stability constants and relaxation rates was not attempted, since the model with seven bound alkali-metal ions requires 15 variables (7 stepwise stability constants and 8 $R_1$ values). The good agreement between the experimental and simulated curve indicates, however, that the estimates of the bound $R_1$ values were correct. Then with eq 1, it can be calculated that for the Dy(PPP)$_2^-$-Li$^+$ system the Dy$^{3+}$-Li$^+$ distances vary between 5.4 and 5.1 Å, going from $n = 1$ to 7. Analogously it can be calculated that in the Tm(PPP)$_2^-$ system the Tm$^{3+}$-Li$^+$ distance is between 5.9 and 5.7 Å. These distances show that the seven monovalent cations are coordinated to the outer oxygens of the triphosphate ligands.

It should be noted that the previously reported Dy(III)-induced Li shifts of the Li$^+$-Dy(PPP)$_2^-$ adduct as a function of $\rho$ also showed a break at $\rho = 7.2$. The induced shifts, however, decreased steeply between $\rho = 0.2$ and 7. If it is assumed that the induced Li shifts are of a pseudococontact origin and that internal reorientations in the complexes result in effective axial symmetry, the induced shifts are determined by

$$\Delta = k(3 \cos^2 \theta - 1)/r^3 \quad (3)$$

where $\Delta$ is the induced shift and $\theta$ is the angle between the vector $r$ and the effective magnetic axis. The variation of the chemical shifts as a function of $\rho$ indicates that at low $\rho$ values binding sites with a high $\Delta$ value are occupied by Li$^+$ preferentially. As outlined previously, the signs of the pseudocontact portion of the Ln(III)-induced $^{31}$P and $^{13}$O shifts indicate that the triphosphate ligands are in the equatorial region ($53 < \theta < 125^\circ$), whereas the water ligand is in the axial region (0 $< \theta < 5^\circ$ or $155 < \theta < 180^\circ$) (see Figure 3). Similarly, the sign of the induced shift for the counterion nucleus indicates that the counterion also is located in the axial region. The coordination geometry of nine-coordinated Ln$^{3+}$ complexes can often best be described by a tricapped trigonal prism. If it is assumed that this geometry also applies here, and if the binding fashion of the triphosphate ligands established previously is taken into account, a model can be constructed in which the water molecule and oxygens of the central phosphates of the triphosphate ligands occupy the capping positions and oxygens of the terminal phosphates the remaining positions (see Figure 3). Then the effective magnetic axis is most likely in the direction of the Ln$^{3+}$-water bond, and the $^{13}$P nuclei are indeed in the equatorial region. If one considers the axial region opposite to the water ligand is relatively small, which may explain some preference of the counterions for this region.

Further addition of Li$^+$ leads to exchange of the Li$^+$ ions on this preferred site with those at other locations. Since each site has a different $\theta$ value, this will result in a decrease of the Li$^+$ shifts due to averaging of the 3 cos$^2 \theta - 1$ term in eq 3. In this way only one of the bound Li$^+$ ions contributes mostly toward the observed Li$^+$ shifts. The Li$^+$ relaxation measurements, however, unambiguously show that about seven monovalent counterions can be bound in the second coordination sphere of Ln(III) in the Ln(PPP)$_2^-$ complexes.

Solid-State $^{23}$Na NMR Studies of Na$_7$La(PPP)$_2$.

In the solid-state MAS $^{23}$Na NMR spectrum of Na$_7$La(PPP)$_2$ only a single broad line ($\Delta v/2 = 1.3$ kHz) was observed. The NMR spectra...
Interaction of Ln(PPP)_2^- with Alkali-Metal Ions

Figure 3. Schematic representation of the structure of the Ln(PPP)_2^-alkali-metal ion adduct. A tricapped trigonal-prismatic coordination of the Ln^{3+} ion is assumed. ● = P, and ○ = O.

of quadrupolar spin nuclei such as ^23Na (I = 3/2) contain information on both the chemical shift and the quadrupole interaction, which depends on the local symmetry around the ^23Na nucleus, giving in principle direct information on the nature of the Na^+ sites. The two-dimensional solid-state nutation NMR spectrum allows one to separate the quadrupolar interaction from the chemical shift interaction. These spectra have been calculated theoretically for various half-integer quadrupolar spins, including I = 3/2. Figure 4 shows the two-dimensional MAS ^23Na nutation spectrum obtained for a polycrystalline sample of Na_4La(PPP)_2. No separation is observed in the F_1 direction; only a single peak at 2ω_QC is present. A comparison with simulated spectra shows that the absence of peaks at ω_QC and between ω_QC and 2ω_QC indicates that all ^23Na nuclei have a nuclear quadrupolar coupling constant (QCC) of at least 1.4 MHz. This implies that in the solid state the seven ^23Na counterions are all in environments with high electric field gradients. These data, therefore, suggest that all Na^+ ions are coordinated to phosphates; for Na^+ ions with an octahedral coordination with water a lower QCC should be expected. It has, for instance, been reported that in a single crystal of Na_4P_2O_7·10H_2O the Na^+ ions that are fully coordinated by water have a QCC of 0.467 MHz, whereas those coordinated to both waters and pyrophosphate have a QCC of 1.907 MHz.

The proposed coordination of seven Na^+ ions to the outer oxygens of the triphosphate ligand is supported by the crystal structure of Na_4(PPP)_2·14H_2O, in which four of the five different Na^+ ions are coordinated to triphosphate oxygens. In conclusion, in both liquid and solid phases up to seven alkali-metal cations bind to the second coordination sphere of Ln(PPP)_2^- complexes via the outer oxygens of the triphosphate ligands (Figure 3) rather than having one preferential binding site in the proximity of the lanthanide ion. However, in the second coordination sphere some preference of the counterions for the axial region opposite the water ligand may exist.

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References