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Contrast Reagents for the NMR Spectra of the Alkali Metals

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Abstract

Diamagnetic complexes of lanthanum and lutetium with linear tripolyphosphate anion are shown to be effective relaxation agents for NMR spectroscopy of all the alkali metals. The relaxation rates are independent of magnetic field strength, both isotopes of rubidium relax at almost the same rate, there is evidence of double Lorentzian lineshapes and possibly of dynamic frequency shifts all of which indicates that the relaxation mechanism is predominantly quadrupolar.

Introduction

The major cations in living cells are Na^+ and K^+. Both of these metals are observable by NMR and recently considerable interest has been displayed in using NMR techniques for studying these metals in biochemically or physiologically important systems [1].

Before such NMR measurements can be made it is essential that some means is found for distinguishing (contrasting) between metal ions in different compartments e.g. inside and outside the living cell. Reagents are required that enable one to contrast between intra- and extracellular ions. We call such reagents contrast reagents. Recent emphasis has been heavily biased towards the development of contrast reagents that rely on chemical shift differentiation (shift reagents) [2–6]. Gupta and Gupta showed that dysprosium tripolyphosphate was a very effective shift reagent for $^{23}Na^+$ [2] and we have demonstrated that this reagent can also be used for studies of $^{39}K^+$ [5, 6]. Other shift reagents that have been used include dysprosium and terbium in conjunction with EDTA, nitrilotriacetate, chelidamate etc.

An alternative form of contrast reagent as a means of distinguishing between intra and extracellular species by NMR would be one that established a relaxation time difference. For ions such as Rb^+ for which the Dy^{3+}/tripolyphosphate (PPP) shift reagent gives a maximum 9 ppm shift with an associated line-broadening of similar magnitude rendering this reagent unusable, relaxation may be the best means for distinguishing between the two sites.

In 1978, before the advent of shift reagents, Degani and Elgavish used a relaxation agent to distinguish between intra- and extravesicular sodium [7]. They used a paramagnetic gadolinium/EDTA reagent to relax the bulk Na^+ outside their vesicles. With relaxation agents an inversion recovery sequence containing appropriate time delays can allow the elimination of either the sharper or the broader signal. Alternatively, if the difference in relaxation is greater than a factor of say 50 and an appropriate spectral width is chosen, the broadened line effectively disappears into the baseline.

In our earlier observations on the use of paramagnetic lanthanide/tripolyphosphate complexes to shift ^{23}Na and ^{39}K resonances [5, 6, 8] we had assumed that the line broadenings observed as the shift difference was generated were paramagnetic in origin as had been suggested by Degani [7]. We now demonstrate, in this paper, that the line broadenings are almost certainly due to a quadrupolar interaction. We further demonstrate that they can be used to generate diamagnetic relaxation agents capable of being used in a biological context. These diamagnetic relaxation agents relax alkali metals more efficiently than the paramagnetic Dy^{3+} reagents. Further, since all the alkali metal isotopes are quadrupolar, relaxation can be studied in every element in this group of the periodic table.

The equation giving the quadrupolar relaxation rate is:

$$T_{1q}^{-1} = T_{2q}^{-1} = \frac{3\pi^2}{10} \frac{(2I+3)}{I^2(2I-1)} \chi^2 \left(1 + \frac{1}{3}\eta^2\right) \tau_c \quad (1)$$

where

$$\chi = \frac{e^2 q_{zz} Q}{h} \quad (2)$$

and q_{zz} is the largest component of E.F.G. This equation consists of several distinct terms and demonstrates how quadrupolar relaxation arises from interaction of the quadrupole moment with the electric field gradient. There are contributions from

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the spin quantum number (I), from the asymmetry of the electric field gradient (η) from the electric field gradient (q), from the quadrupole moment Q and from the correlation time for molecular tumbling τ_c.

It can be noted that the quadrupolar relaxation rate is independent of the magnetic field strength H_o. Also, for $I = 3/2$ nuclei the quadrupolar relaxation rate for the $-3/2 \rightarrow -1/2$ and $+1/2 \rightarrow +3/2$ transitions is, in general, different from the relaxation rate for the $-1/2 \rightarrow +1/2$ transition [1]. This gives rise to a double Lorentzian lineshape with the broad:narrow components in the integrated ratio of 60:40. There is also the possibility of observing a second order dynamic frequency shift difference between the broad and narrow components [9]. Thus, the observation of field independent relaxation, double Lorentzian lineshapes in the ratio 60:40 and possibly dynamic frequency shifts is good evidence for a quadrupolar relaxation mechanism.

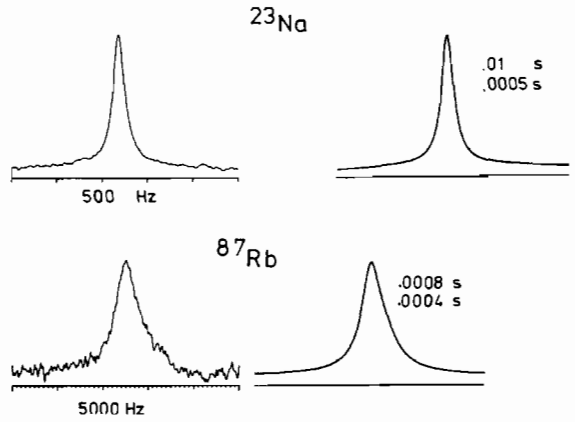

Fig. 1. ^{23}Na and ^{87}Rb spectra observed and calculated to fit a double Lorentzian lineshape using the two relaxation times indicated.

Results and Discussion

In checking the effect of lanthanide (Lan) tripolyphosphate (PPP) complexes on ^{23}Na we originally observed that diamagnetic lanthanum (La^{3+}) gave no chemical shift difference, confirming the paramagnetic nature of the shift reagents, but that they gave some line broadening [8]. We have now demonstrated that the tripolyphosphate complexes of the diamagnetic lanthanides La^{3+} and Lu^{3+} relax all the alkali metal nuclei. In the case of ^{23}Na, ^{39}K, ^{85}Rb and ^{87}Rb these complexes generate substantial relaxation time differences and are, therefore, of potential use as contrast reagents in compartmentalised systems. We further show that intracellular $^{87}Rb^+$ can be observed in the presence of strongly relaxed extracellular Rb^+.

We have previously found that the tetramethylammonium (Me_4N^+) ion interacts less strongly with PPP than do the alkali metal ions (M^+). We therefore chose a standard system with 20 mM M^+, 20 mM Cl^-, 40 mM (Me_4N^+) and 8 mM PPP in order to emphasise the M^+/PPP interaction. 2 ml quantities of these solutions were titrated with microlitre amounts of 100 mM $LanCl_3$ and linewidths at halfheight were recorded.

We examined all the magnetically active alkali metal isotopes except ^{6}Li, ^{40}K in the presence of La^{3+} on the high field WH360 spectrometer in Edinburgh. Measurements on ^{7}Li, ^{23}Na and ^{87}Rb with La^{3+} and Lu^{3+} were performed on our lower field WP80 spectrometer in Stirling. The results are presented in Table I.

The observations of linewidth *versus* [Lan^{3+}] for ^{7}Li, ^{23}Na and ^{87}Rb are essentially independent of field as would be expected for quadrupolar relaxation. In many cases the lineshapes are clearly double Lorentzians (Fig. 1). The isotopes ^{85}Rb and ^{87}Rb possess different quadrupole moments and different spin quantum numbers. Since the chemistry of these isotopes is indistinguishable in the complexes with Lan/PPP only terms in I and Q should determine the relative relaxation rates. Evaluation of these terms in eqn. (1) shows that they cancel each other and the quadrupolar relaxation rates for these nuclei should be virtually identical. This is in accord with our observations. In the case of ^{87}Rb there is some indication that small second order dynamic frequency shifts may be present but the data are not good enough to allow quantitative confirmation. In all cases the linewidths of the M^+/PPP solutions in the absence of Lan^{3+} are approximately three times broader than that of the 100 mM M^+Cl^- reference solutions. These observations are all consistent with quadrupolar relaxation being the dominant mechanism for the line broadening.

The linewidths observed for La^{3+} and Lu^{3+} are similar but not identical. The lutetium reagent relaxes Li^+ somewhat more efficiently but relaxes Na^+ and Rb^+ less efficiently. Lu^{3+} is a smaller ion than La^{3+} meaning that the geometry of the complex and therefore the associated electric field gradient will differ when Lu^{3+} replaces La^{3+}.

To have optimum effect as a contrast reagent a relaxation agent should be capable of causing the broadened signal from one compartment to disappear into the background noise. Given equal signals from metal ions in two compartments one would expect a tenfold increase in linewidth in one compartment to make them distinguishable from one another but not for one resonance to disappear entirely. The normalised integral results presented for ^{39}K and ^{87}Rb at high lanthanide concentrations show clearly that up to 90% of the integrated intensity is lost and the remaining 10% of the signal is broadened tenfold.

TABLE I. Linewidths

[Lan^{3+}] (mM)	Linewidth (Hz) (139.9 MHz, 100 mM LiCl, linewidth 0.2 Hz)	Linewidth (Hz) (31.14 MHz, 100 mM LiCl, linewidth 1.5 Hz)	
	La^{3+}	La^{3+}	Lu^{3+}
^{7}Li			
0.0	1.9	1.7	2.2
0.5	1.9		
1.0	1.7	1.4	3.4
2.0		2.5	3.9
3.0	3.2	3.1	4.1
4.0		3.4	4.6
5.0	3.9	3.2	4.9
6.0		3.5	4.8
7.0	4.2	3.4	6.1
8.0		3.5	5.5
9.0	2.3	3.4	5.3
10.0		3.6	5.4
11.0	2.0	3.5	

	Linewidth (Hz) (95.2 MHz, 100 mM NaCl, linewidth 9 Hz)	Linewidth (Hz) (21.19 MHz, 100 mM NaCl, linewidth 8.5 Hz)	
	La^{3+}	La^{3+}	Lu^{3+}
^{23}Na			
0.0	19	19	16
1.0	21	20	28
2.0	23	21	31
2.5		25	
3.0	39	43	38
3.5		61	
4.0	41	86	46
4.5		110	
5.0		118	53
5.5		160	
6.0			62

	Linewidth (Hz) (16.8 MHz, 100 mM KCl, linewidth 6 Hz)	Relative integral (%)
	La^{3+}	
^{39}K		
0.0	17.5	100
0.5	19.5	110
1.0	21.4	95
2.0	26.3	89
3.0	53.7	
4.0	102	25
5.0	220	9

	Linewidth (Hz) (34.8 MHz, 100 mM RbCl, linewidth 132 Hz)
	La^{3+}
^{85}Rb	
0.0	367
1.0	482
2.0	600
3.0	804
4.0	1300

(continued)

TABLE 1. *(continued)*

[Lan^{3+}]	Linewidth (Hz) (117.8 MHz, 100 mM RbCl, linewidth 132 Hz)	Relative integral (%)	Linewidth (Hz) (26.22 MHz, 100 mM RbCl, linewidth 134 Hz)	
	La^{3+}		La^{3+}	Lu^{3+}
^{87}Rb				
0.0	376	100	363	356
0.5	450	110	424	493
1.0	466		459	507
1.5	593		590	671
2.0	640		609	695
2.5	640		763	669
3.0	783		784	661
3.5				709
4.0	976			772
5.0	1230			1229
6.0	>1300	12		
7.0	>1500	9		

	Linewidth (Hz) (47.2 MHz, 100 mM CsCl, linewidth 0.2 Hz)
	La^{3+}
^{133}Cs	
0.0	0.5
0.5	0.7
1.0	0.8
2.0	0.8
4.0	4.4
5.0	6.5
6.0	5.5

The relative signal heights in the two compartments will now be *ca.* 100:1 making the broadened signal effectively disappear into the noise. This forms the basis of a very effective contrast reagent.

It has been presumed that the shift reagents containing paramagnetic Lan^{3+} ions operate by a 2:1 PPP:Lan^{3+} complex carrying 7 negative charges. Such a complex would also be expected to exist in our experiments at low [Lan^{3+}]. Complexes of different stoichiometry would become more important at higher [Lan^{3+}]. The M^+ ions are then attracted to these paramagnetic complexes and shifted by a pseudocontact interaction. A similar chemical explanation can account for the quadrupolar line broadening. Interaction of M^+ with the diamagnetic Lan/PPP complex should bring the M^+ into a region of high electric field gradient causing quadrupolar relaxation. Rapid exchange of M^+ on the complex with M^+ in the bulk solution would then lead to relaxation of the Na^+ in solution broadening its resonance.

Under such a mechanism the addition of further amounts of Lan^{3+} to an M^+/PPP preparation in our experiments should lead to increasing amounts of Lan/PPP complexes of higher molecular weight, and hence to an increase in the linewidth of the bulk M^+. The observation of double Lorentzian lineshapes and possibly of dynamic frequency shifts requires a further condition, that of a slowly tumbling complex with which the M^+ ions are exchanging. The only reasonable explanation for this is the formation of very large LanPPP complexes in which several Lan^{3+} ions bind to a larger number of PPP^{5-} ions to give a large, slowly tumbling and very complex ion.

Finally, to demonstrate the utility of these reagents in biological systems, we have incubated purified human erythrocytes in a rubidium-containing medium to encourage the exchange of intracellular K^+ for extracellular Rb^+. The medium also contained extracellular relaxation agent. During incubation the intracellular $^{87}Rb^+$ signal was seen to grow from zero intensity and the variation of signal intensity with time over a 24 h period is shown in Fig. 2. After 24 h there was no $^{87}Rb^+$ observable in either the rubidium-containing supernatant from the cell-containing medium or in the rubidium-containing incubation medium that had not been exposed to the cells (Fig. 3). Similarly in a system of vesicles it has proved

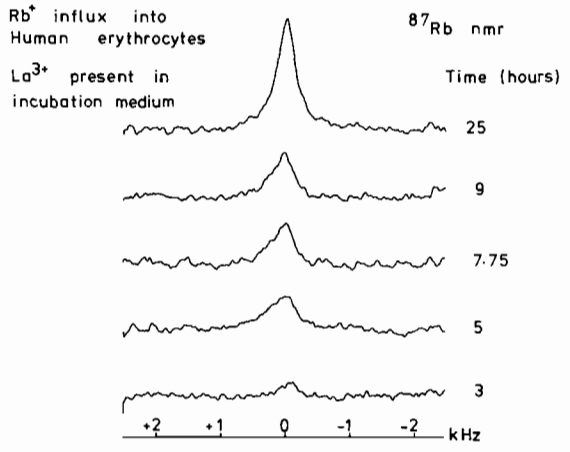

Fig. 2. The time course of Rb^+ influx into human erythrocytes followed by ^{87}Rb NMR; the suspension medium contains relaxation agent and only the intracellular ^{87}Rb is visible.

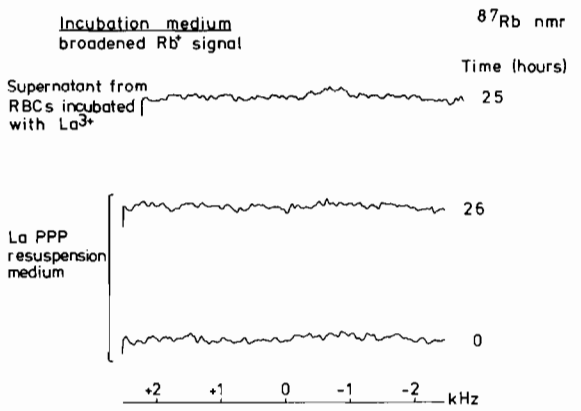

Fig. 3. The absence of detectable $^{87}Rb^+$ signals in the initial and final resuspension medium.

possible to relax the extravesicular $^{87}Rb^+$ signal into the baseline and observe dynamic NMR effects brought on by the addition of nigericin due to rapid ionophore mediated exchange between intra- and extravesicular Rb [10].

Experimental

Spectra were run on a Bruker WH360 FT NMR spectrometer in Edinburgh and a Bruker WP80 spectrometer in Stirling. Solutions were made by mixing standard solutions of tetramethylammonium tripolyphosphate and the alkali metal chloride to give 2 cm^3 of a solution that was 20 mM in M^+ and Cl^-, 40 mM in Me_4N^+ and 8 mM in tripolyphosphate. Samples were field/frequency locked on the ^2H resonance of ^2H_2O in the inner compartment of a coaxial tube. Lanthanide additions were carried out by injecting microlitre amounts of a 100 mM solution of the lanthanide as its chloride.

Tetramethylammonium tripolyphosphate was prepared by neutralising a known quantity of tetramethylammonium hydroxide with tripolyphosphoric acid prepared from sodium tripolyphosphate on a cation exchange column in its H^+ form.

Spectra were calculated, where appropriate, on a BBC model B microcomputer in BBC BASIC and plotted on a PLOTMATE plotter.

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