Shift Reagents for ^{39}K Nmr

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Introduction

The two cations present at highest concentration inside living cells, Na^+ and K^+, are both observable by nmr spectroscopy through their isotopes ^{23}Na and ^{39}K [1]. Of these ^{23}Na has attracted by far the most attention since it is at least two orders of magnitude more receptive than ^{39}K. Despite this low sensitivity, nmr remains a potentially most attractive method for the study of K^+ especially in applications involving living cells, where it is the major intracellular cation, and in artificial vesicles acting as models for real cells, since it is a non-invasive, non-destructive technique. The major drawback to the use of ^{39}K nmr for these purposes is that the chemical shifts of aqueous alkali metal cations are virtually anion independent [1]. To overcome this drawback shift reagents have been developed that allow the observation of separate resonances from ions in contact with and out of contact with the shift reagent [2–4].

In an earlier communication we showed that Guptas' shift reagent (Dysprosium tripolyphosphate) [2] for Na^+, could also be applied to $^{39}K^+$ and we used this reagent to measure the concentration of K^+ inside human erythrocytes [5]. We now wish to report in more detail our preliminary observations on this shift reagent when applied to Potassium.

Aqueous shift reagents for Na^+ and K^+ consist of a paramagnetic lanthanide ion (Lan^{3+}) and a polyvalent ligand which complexes strongly with the Lan^{3+} and weakly with the Na^+ or K^+ ions. Springer *et al.* have shown that the essential feature of the 'strong' interaction is that the complex derived from the lanthanide and ligand should have large numbers of negatively charged oxygen atoms on its surface to which the alkali metal ions are attracted, thus bringing them close to the paramagnetic lanthanide ion [4]. Typical polyvalent ligands that have been used so far include diphosphate, tripolyphosphate, tetrapolyphosphate, nitrilotriacetate, and chelidamate [2–4]. Of these by far the largest ^{39}K shifts have been obtained with chelidamate. The chelidamate method, however, suffers from the disadvantage that large concentrations of lithium ions are needed to support the reagent meaning that very high ionic strengths are required to obtain appreciable ^{39}K shifts.

It seems probable from the description of the mode of operation of the shift reagents given above, that they operate largely *via* the so called pseudocontact (or through space dipolar) mechanism rather than by a contact (or through bond) mechanism. The theory of pseudocontact shifts shows that the magnitude and direction of the observed shift depends heavily on the distance apart of the two magnetic centres and the geometry of the instantaneous complex [6]. Recent work by Golding, however, shows that one cannot readily split such shifts into contact and pseudocontact terms [7]. Slight residual covalent bonding (*e.g.* 1–2% covalent interaction) or interaction distances <10 Å could lead to very different results from those predicted by the simple pseudocontact theory.

Moreover, one has no means of knowing *a priori* what the stoichiometries or geometries of the complexes are. There is thus no current method for predicting which shift reagents for sodium or potassium would be superior to others. Thus, the only resort available is to determine this experimentally.

Discussion

We have investigated the shifts of potassium ions in potassium tripolyphosphate on addition of the lanthanides, Dysprosium, Terbium, and Ytterbium. Pseudocontact theory suggests that the former two lanthanides should give substantial shifts to low frequency whilst Ytterbium should give much smaller shifts in the opposite direction [6, 7]. Our results are presented in Figs. 1–5.

With Dysprosium the shape of the titration curve is independent of the concentration of the potassium tripolyphosphate used: the maximum shift obtained is the same at all three concentrations, the curves

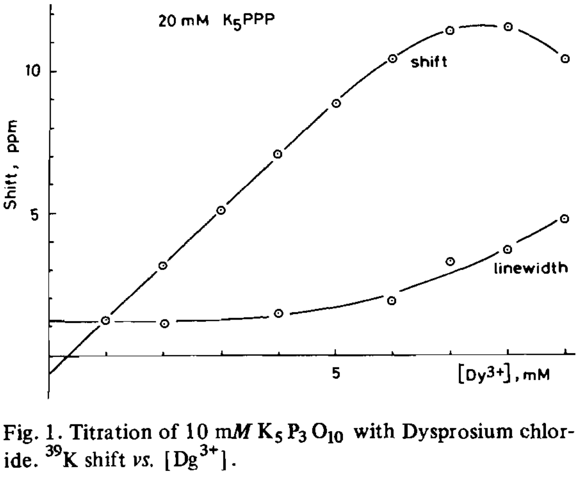

Fig. 1. Titration of 10 m*M* $K_5P_3O_{10}$ with Dysprosium chloride. ^{39}K shift *vs.* [Dg^{3+}].

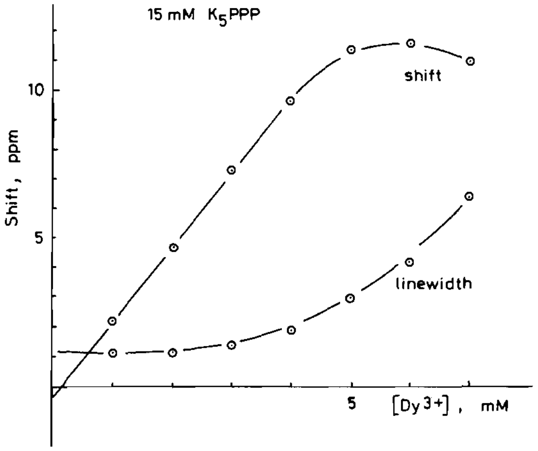

Fig. 2. Titration of 15 m*M* $K_5P_3O_{10}$ with Dysprosium chloride. ^{39}K shift *vs.* $[Dg^{3+}]$.

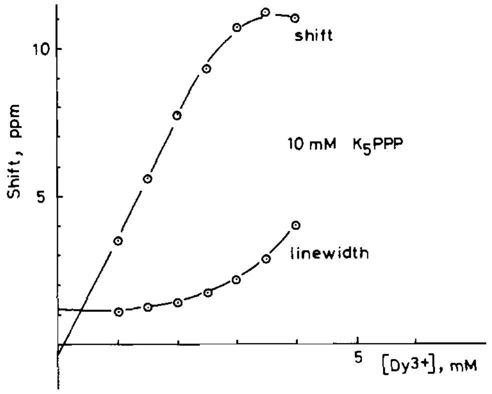

Fig. 3. Titration of 20 m*M* $K_5P_3O_{10}$ with Dysprosium chloride. ^{39}K shift *vs.* $[Dg^{3+}]$.

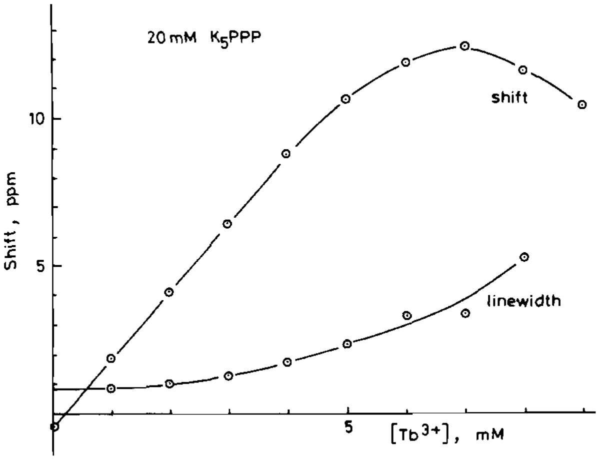

Fig. 4. Titration of 20 m*M* $K_5P_3O_{10}$ with Terbium nitrate. ^{39}K shift *vs.* $[Tb^{3+}]$.

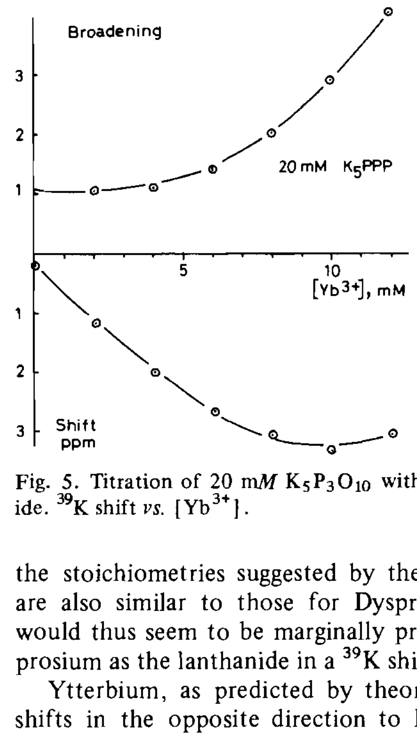

Fig. 5. Titration of 20 m*M* $K_5P_3O_{10}$ with Ytterbium chloride. ^{39}K shift *vs.* $[Yb^{3+}]$.

are initially linear with slopes varying inversely with the tripolyphosphate concentration, and the relative concentrations needed to obtain the maximum shift are identical. In all three cases titration was stopped when the curve had gone through a maximum and the first signs of a precipitate appeared in the tube. In all three cases line broadening is slight at first but becomes more important at mol ratios $Dy^{3+}/PPP^{5-} > 0.3$. Such behaviour is indicative of complex equilibria involving at least two different stoichiometries for the interaction between Dy^{3+} and PPP^{5-}. In the initial stages, with a low molar Dy^{3+}/PPP^{5-} ratio, only one species appears to be involved, and since the maximum in the graph appears at just below a 1:2 ratio we suggest that this complex is $Dy^{3+}(PPP^{5-})_2$ as was suggested for ^{23}Na by Gupta and Gupta [2].

With Terbium a very similar result was obtained. The maximum shift observed was, however, slightly greater (12.4 ppm *vs.* *ca.* 11.5 ppm) and the line broadenings were slightly smaller. The shape of the curve is similar to that obtained for Dysprosium and the stoichiometries suggested by the titration curve are also similar to those for Dysprosium. Terbium would thus seem to be marginally preferable to Dysprosium as the lanthanide in a ^{39}K shift reagent.

Ytterbium, as predicted by theory, gave inferior shifts in the opposite direction to Dysprosium and Terbium. It is extremely interesting to compare the shifts obtained with Ytterbium to the line broadenings. At the maximum shift (*ca.* 3.5 ppm) the line broadening is comparable (*ca.* 3 ppm) to the shift difference. In general lanthanides such as Praseodimium, Europium and Ytterbium are used in shift reagents for ^1H and ^{13}C applications in preference to Dysprosium and Terbium because the latter lanthanides produce much larger line broadenings which outweigh their advantages in terms of larger shifts [6]. With ^{23}Na and ^{39}K the reverse is true, and the large shifts obtained with Dysprosium and Terbium are not at the expense of unacceptable line broadenings.

Experimental

Spectra were obtained at 25 °C on a Bruker WH 360 Fourier Transform Spectrometer at 16.8 MHz operated by the SERC in Edinburgh University. We thank the SERC for an allocation of time on this instrument. Typically, free induction decays were collected into 512 data points with a sweep width of 2000 Hz and transformed in 8 K data points.

The potassium tripolyphosphate (K_5PPP) was prepared from Na_5PPP by passing the latter down an ion exchange column loaded with the K^+ form of Dowex 50W-X8 (200–400 BSS mesh size). The eluent was evaporated to dryness on a rotary evaporator and further dried under vacuum at 50 °C overnight. The K_5PPP contained less than 1% Na_5PPP (^{23}Na nmr) and *ca.* 20% diphosphate (^{31}P nmr).

Dysprosium and Ytterbium trichlorides, supplied by Alfa Inorganics, were used after drying under vacuum overnight at 50 °C. Terbium was used as its nitrate pentahydrate, supplied by Aldrich, because of the limited solubility of the chloride. It was transferred to a closed weighing bottle from the container in which it was received under an atmosphere of dry nitrogen.

The K_5PPP was added to the outer compartment of a coaxial nmr tube (5 mm internal and 10 mm external diameter) and to the inner tube was added a solution of 100 m*M* KCl in ^2H_2O. The spectrometer was field-frequency locked on the ^2H resonance of the ^2H_2O in the inner tube. The isotropic hyperfine shift was measured as the difference of the observed resonance position from the resonance of the cations in the centre tube in the absence of shift reagent. Shifts to higher frequency are defined as positive.

No attempt was made to control the pH during titrations. In all cases the initial pH was *ca.* 9.0 and decreased linearly throughout the titration to a final value of *ca.* 7.7.

In separate experiments the influence of bulk susceptibility effects was shown to be small (<10% of the observed shift and in the opposite direction in the case of Dy^{3+}).

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