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Measurement of intracellular potassium ion concentrations by n.m.r.

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^{39}K n.m.r. was used to detect and quantify K^+ within human erythrocytes. A shift reagent consisting of an anionic complex of dysprosium(III) with sodium tripolyphosphate permitted a distinction to be made between K^+ inside and outside erythrocytes. Intracellular K^+ concentrations determined by this method were similar to values obtained by flame photometry.

N.m.r. spectroscopy can be used to detect cations within intact cells (Civan & Shporer, 1978). The non-invasive nature of n.m.r. is particularly attractive when compared with the impalement of cells and tissues that is necessary in order to measure intracellular ion concentrations with cation-sensitive microelectrodes. N.m.r. also has the advantage over other methods such as atomic absorption or electron X-ray microanalysis in that it is non-destructive. Gupta & Gupta (1982) have developed a method for measuring intracellular $^{23}Na^+$ concentrations that exploits the ability of an anionic complex of dysprosium(III) with tripolyphosphate [$Dy(PPP_i)_2^{7-}$] to shift the extracellular Na^+ signal away from the resonance arising from intracellular Na^+. This method relies on the fact that the erythrocyte plasma membrane is impermeable to the shift reagent. The values that Gupta & Gupta (1982) obtained for the concentration of intracellular Na^+ within erythrocytes were within the expected range. Pike *et al.* (1982) have introduced a different anionic complex of dysprosium(III) for similar studies on the measurement of Na^+ concentrations within liposomes.

K^+ presents more severe problems to the n.m.r. spectroscopist than does Na^+ because ^{39}K is the least receptive of the alkali metals in the n.m.r. experiment. Fortunately, however, intracellular K^+ concentrations are typically an order of magnitude greater than those of Na^+, which allows their observation by n.m.r. In the present paper we show that the shift reagent developed by Gupta & Gupta (1982) can also be used to measure the concentration of K^+ within erythrocytes.

Materials and methods

Blood was drawn from a healthy donor (P. J. B.) into heparinized tubes. The erythrocytes were washed three times by centrifugation at 1000*g* for 10 min in 140 mM-NaCl/10 mM-glucose/5 mM-potassium phosphate buffer, pH 7.4, and the buffy coat was removed by aspiration. Immediately before the n.m.r. measurements were made erythrocytes were transferred to an n.m.r. tube (10 mm outside diameter) and washed twice in a resuspension medium containing (final concentrations) 10 mM-glucose, 5 mM-sodium phosphate, 6 mM-$DyCl_3$, 15 mM-sodium tripolyphosphate (pentasodium salt from Sigma Chemical Co.), 40 mM-NaCl and 20 mM-KCl in 25% (v/v) ^2H_2O adjusted to pH 7.4. The total Na^+ concentration was 120 mM. When the erythrocytes were suspended in media of higher KCl concentration (40 mM- or 60 mM-KCl), the amount of NaCl was decreased proportionately in order to maintain the same ionic strength. The erythrocytes were packed gently in the n.m.r. tube by centrifugation at 1000*g* for 10 min.

Spectra were obtained at 25°C with a Bruker WH 360 Fourier-transform spectrometer at 16.8 MHz. Preliminary experiments showed that the spin–lattice relaxation times for both shifted and unshifted resonances were approx. 5 ms. A 90° pulse was used with a repetition rate of 64 ms (more than 12 spin–lattice relaxation times). Free induction decays were collected into 256 data points with a sweep width of 2000 Hz in order to achieve a rapid pulse rate, but they were transformed in 4000 data points without any line broadening.

All Fourier transforms were performed under the same absolute intensity conditions. Therefore the integrated areas of the resonances arising from intra- and extra-cellular K^+ could be compared directly with a reference spectrum of shifted K^+ in resuspension medium alone (Fig. 2*a*). The spectra shown in Fig. 2 represent 20000 free induction decays collected in about 20 min.

By subtracting the spectrum derived from K^+ in

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the resuspension medium (Fig. 2*a*) from the spectrum obtained from the erythrocyte suspension (Fig. 2*b*), the fractional space occupied by the extracellular fluid and therefore that occupied by the erythrocytes could be determined. The end point of such a subtraction is shown in Fig. 2(*c*). The fractional space occupied by the cells in Fig. 2(*b*) was 0.70. The concentration of intracellular K^+ was measured by comparing the integrated intensity of the spectrum in Fig. 2(*c*) with the integrated intensity of the spectrum obtained from a known concentration of K^+ (20, 40 or 60mM) in resuspension medium (Fig. 2*a*). A correction was then made for the fractional space occupied by the erythrocytes.

Intracellular K^+ concentrations were measured with an EEL model 150 clinical flame photometer after haemolysis of the erythrocytes.

Results and discussion

We determined the ratio of Dy^{3+} to tripolyphosphate that resulted in the maximum chemical shift of the K^+ resonance. Resuspension medium that contained 15mM-tripolyphosphate but no Dy^{3+} was added to the inner and the outer tubes of a coaxial n.m.r. tube (5mm inside and 10mm outside diameter). Addition of $DyCl_3$ to the outer tube caused the K^+ signal to shift to a maximum at about 6.5mM-$DyCl_3$. As shown in Fig. 1, this optimal concentration of Dy^{3+} was independent of the K^+ concentration, although the chemical shifts were greater with 20mM-KCl than with 40mM- or 60mM-KCl. Gupta & Gupta (1982) used a 2:1 molar ratio of tripolyphosphate to Dy^{3+} to shift $^{23}Na^+$. Our data show that a molar ratio of 2.5:1 induces a greater shift with $^{39}K^+$. In other experi-

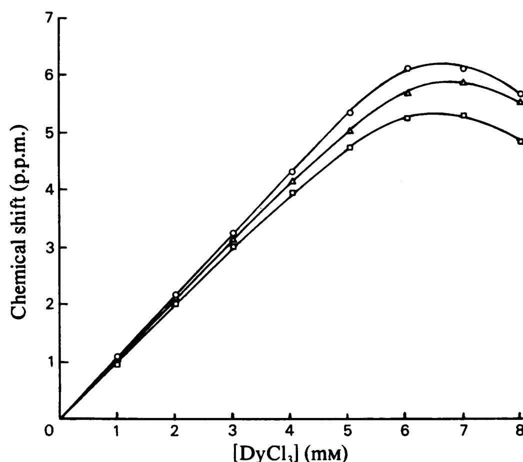

Fig. 1. *Effect of increasing concentrations of Dy^{3+} on the chemical shift of K^+ at three different concentrations of K^+*

Suspension medium containing 15mM-sodium tripolyphosphate was added to the inner and the outer tubes of a coaxial n.m.r. tube, and increasing amounts of $DyCl_3$ were added to the outer tube. The chemical shift between the signals from K^+ in the inner and the outer tubes was measured. Symbols: ○, 20mM-K^+; △, 40mM-K^+; □, 60mM-K^+.

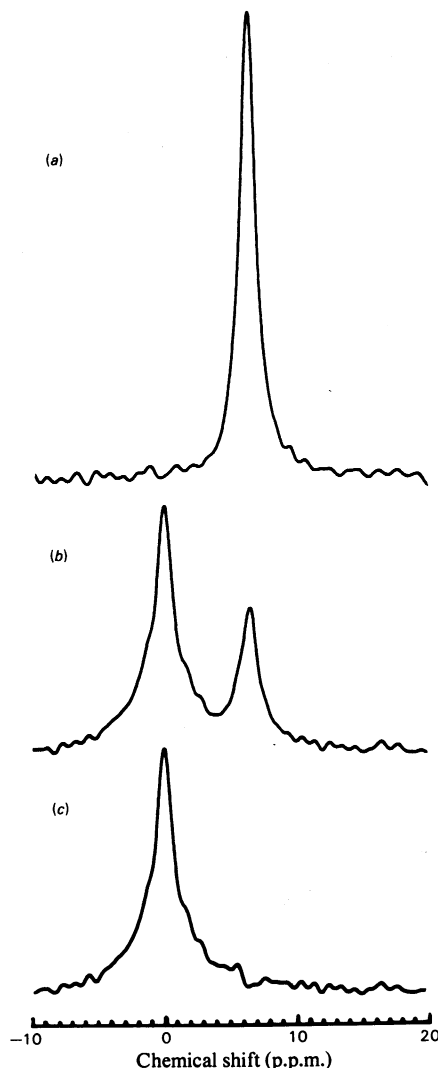

Fig. 2. *^{39}K n.m.r. spectra of (a) resuspension medium containing 60mM-K^+, 6mM-Dy^{3+} and 15mM-tripolyphosphate, (b) human erythrocytes in the same medium, and (c) difference spectrum after the subtraction of 0.3 of the integrated intensity of spectrum (a) from spectrum (b)*

For each spectrum 20000 free induction decays were collected in approx. 20min.

ments we have found that this ratio is also optimal for $^{23}Na^+$. The shift reagent presumably works by binding Dy^{3+} to the trypolyphosphate, which brings them into close proximity to each other, thereby inducing a contact shift. Literature values for the stability constants suggest that K^+ binds somewhat less firmly to tripolyphosphase than does Na^+. Therefore we expected K^+ to be less shifted than Na^+, and this is indeed the case.

Fig. 2(*b*) shows the resonance arising from unshifted intracellular K^+ and extracellular K^+ that has been chemically shifted by the $DyCl_3$ reagent. The spectrum of 60mM-K^+ in resuspension medium in the presence of shift reagent is shown in Fig. 2(*a*). Fig. 2(*c*) represents the difference spectrum when spectrum (*a*) is subtracted from spectrum (*b*). Calculation of the fractional space that was intracellular followed by comparison of the integrated area of the intracellular K^+ peak (Fig. 2*c*) with that of the reference spectrum (Fig. 2*a*) gave a value for the intracellular K^+ concentration of 81mM. This value agrees well with similar concentrations of 81mM and 85mM that were calculated when the erythrocytes were resuspended in media containing 20mM-KCl and 40mM-KCl respectively. We estimate the error in the spectral subtraction to be about $\pm 10\%$. These values also agree well with the intracellular K^+ concentration of 88mM that was measured by classical flame photometry.

In summary, measurement of intracellular K^+ concentrations by ^{39}K n.m.r. is reliable and has the clear advantages of being non-invasive and non-destructive. This technique should be applicable to a wide variety of living cells and tissues.

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