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^7Li NMR Studies of Lithium Transport in Human Erythrocytes[1]

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Lithium transport in human erythrocytes was investigated by ^7Li NMR spectroscopy. The intra- and extracellular pools of Li^+ were distinguished by the addition to the red cell suspension of a cell-impermeable shift reagent, dysprosium(III) triphosphate. It was found that, for therapeutic levels of lithium used in the United States (where the typical plasma [Li^+] concentration range is 0.5-1.2 mM), a shift reagent concentration of 3 mM is sufficient to achieve clear chemical shift separation between the two $^7Li^+$ NMR resonances. Despite competition between Li^+ and other mono- and divalent cations for the shift reagent, the intra- and extracellular $^7Li^+$ NMR signals are clearly separated (approximately 3 ppm) even in the presence of physiologically relevant concentrations of Na^+, K^+, Mg^{2+}, and Ca^{2+}. Addition of an ionophore, monensin, to a K^+-only RBC suspension induces passive Li^+ transport, which can be monitored by following the relative intensities of the two $^7Li^+$ resonances. We conclude that the ^7Li NMR method is suitable for the noninvasive study of Li^+ transport in human erythrocytes and that it shows great promise as a tool for the investigation of the bioinorganic chemistry of lithium.

Introduction

Lithium is an effective drug in the treatment of manic depressive disorders and is currently being administered to approximately 1 in every 1000 individuals in the United States.[2] The mechanism(s) for the biological action of lithium is not understood, despite the important pharmacological properties of this drug. One possible mechanism of action involves the effect of lithium on the cell membrane transport properties. For our study, we chose red blood cells (RBCs) as the cell system. The importance of studying lithium transport in human erythrocytes is 2-fold. First, red blood cells provide a model system for nerve cells. Lithium transport in RBCs can be more easily characterized because of the ready availability of these cell types and their simplified morphology. Second, abnormalities in Li^+ transport across RBC membranes have been observed in families of hypertensive[3] and manic depressive[4] patients. For instance, it was observed that the rate of Li^+ efflux via the Na^+-Li^+ exchange pathway in RBCs of hypertensive patients (0.35 ± 0.02 mmol of Li^+/(L of RBCs × h)) was higher than that of control subjects (0.17 ± 0.02 mmol of Li^+/(L of RBCs × h)).[5] However, the reported transport rate values have varied drastically and have, in some cases, overlapped for both hypertensive patients and normal controls.[6] Similar controversy surrounds the reported transport rates of Li^+ in RBCs of manic depressive patients.[7,8] At this time, it is therefore uncertain whether abnormalities in lithium transport in RBCs are genetic markers of hypertension and manic depression.

Lithium transport in biological systems has been studied until recently by atomic absorption and flame photometry.[3-8] These techniques involve invasive procedures since physical separation of intra- and extracellular compartments is required prior to chemical analysis. In addition to being time-consuming, these separation methods occasionally lead to artifacts, which may be caused by non-specific-ion binding to membranes and additional ion transport during sample processing. The large variation in lithium transport data obtained in previous studies has been interpreted as being due to heterogeneity in etiology of these diseases, as it was found that varying prevalences of lithium transport abnormalities existed in patients from different countries and ethnic groups.[6-8] However, the invasive nature of the methods used previously may in part explain the observed discrepancies.

The recent discovery of highly negatively charged shift reagents, suitable for the study of Na^+ and K^+ fluxes in many cell types by metal NMR spectroscopy,[9-17] prompted us to investigate whether these noninvasive methods would be applicable to the study of lithium transport in RBCs. These new NMR shift reagents provide noninvasive probes of transport of alkali-metal cations in biological systems in that they are membrane impermeable and, thus, only affect the metal NMR resonance associated with the extracellular compartment. Moreover, phase microscopy[16] and ^{31}P NMR[15-17] studies indicate that these shift reagents have no effect on the shape and energy metabolism of RBCs.

Materials and Methods

Lithium chloride, dysprosium chloride, sodium triphosphate, glucose, and deuterium oxide (99.8%) were supplied by Aldrich; HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] and monensin were supplied by Sigma; and cryptands 221 (4,7,13,16,21-pentaoxa-1,10-diazabicyclo[8.8.5]tricosane) and 211 (4,7,13,19-tetraoxa-1,10-diazabicyclo-[8.5.5]eicosane) were from Merck. All chemicals were used as received, except sodium triphosphate, which was recrystallized three times in 40% ethanol. Packed RBCs of healthy donors were supplied by the Chicago Chapter of the American Red Cross.

RBCs were washed three times by centrifugation at 2000 g for 10 min with isotonic 5 mM sodium phosphate–150 mM NaCl buffer, pH 7.4 at 4 °C, and were separated from the plasma and buffy coat by aspiration. Immediately before NMR measurements, the cells were washed once by centrifugation in the NMR buffer prior to the final resuspension at 13% hematocrit in the same NMR buffer. The NMR buffer contained 10 mM glucose, 5 mM $Na_7Dy(PPP_i)_2$·3NaCl, 50 mM HEPES at pH 7.5, and chloride salts of Li^+, Na^+, K^+, Ca^{2+}, and Mg^{2+}. The concentrations of chloride salts varied in the individual experiments and are indicated in the figure captions. The osmolarity of all the RBC suspensions was measured to be approximately 300 mosM with a Wescor vapor pressure osmometer. The shift reagent dysprosium(III) triphosphate, $Na_7Dy(PPP_i)_2$·3NaCl, was prepared from dysprosium chloride and sodium triphosphate according to published procedures.[14] The K^+ form of the shift reagent,[14] $K_7Dy(PPP_i)_2$·3KCl, was obtained from sodium triphosphate by passing it down a Chelex-100 column loaded with K^+.

^7Li, ^{23}Na, and ^{31}P NMR measurements were made at 104.8, 71.2, and

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(2) Jefferson, J. W.; Greist, J. H.; Baudhuin, M. *Lithium: Current Applications in Science, Medicine, and Technology*; Bach, R. O., Ed.; Wiley: New York, 1985; pp 345-352.
(3) Adragna, N. C.; Canessa, M. L.; Solomon, H.; Slater, E.; Tosteson, D. C. *Hypertension (Dallas)* **1982**, *4*, 795.
(4) Ehrlich, B. E.; Diamond, J. M. *J. Membr. Biol.* **1980**, *52*, 187.
(5) Woods, J. W.; Falk, R. J.; Pittman, A. W.; Klemmer, P. J.; Watson, B. S.; Namboodiri, K. *N. Engl. J. Med.* **1982**, *306*, 593.
(6) Wiley, J. S.; Clarke, D. A.; Bonacquisto, L. A.; Scarlett, J. D.; Harrap, S. B.; Doyle, A. E. *Hypertension (Dallas)* **1984**, *6*, 360.
(7) Pandey, G. N.; Dorus, E.; Davis, J. M.; Tosteson, D. C. *Arch. Gen. Psychiatry* **1979**, *36*, 902.
(8) Richelson, E.; Snyder, K. Carlson, J.; Johnson, M.; Turner, S.; Lumry, A.; Boerwinkle, E.; Sing, C. F. *Am. J. Psychiatry* **1986**, *143*, 457.
(9) Gupta, R. K.; Gupta, P. *J. Magn. Reson.* **1982**, *47*, 344.
(10) Pike, M. M.; Springer, C. S., Jr. *J. Magn. Reson.* **1982**, *46*, 348.
(11) Pike, M. M.; Fossel, E. T.; Smith, T. W.; Springer, C. S., Jr. *Am. J. Physiol.* **1984**, *246*, C528.
(12) Pike, M. M.; Yarmush, D. M.; Balschi, J. A.; Lenkiski, K. E.; Springer, C. S., Jr. *Inorg. Chem.* **1983**, *22*, 2388.
(13) Brophy, P. J.; Hayer, M. K.; Riddell, F. G. *Biochem. J.* **1983**, *210*, 961.
(14) Chu, S. C.; Pike, M. M.; Fossel, E. T.; Smith, T. W.; Balschi, J. A.; Springer, C. S., Jr. *J. Magn. Reson.* **1984**, *56*, 33.
(15) Ogino, T.; Shulman, G. I.; Avison, M. J.; Gullans, S. R.; Den Holander, J. A.; Shulman, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1099.
(16) Pettegrew, J. W.; Woesner, D. E.; Minshew, N. J.; Glonek, T. *J. Magn. Reson.* **1984**, *57*, 185.
(17) Boulanger, Y.; Vinay, P.; Desroches, M. *Biophys. J.* **1985**, *47*, 553.

0020-1669/87/1326-4356$01.50/0

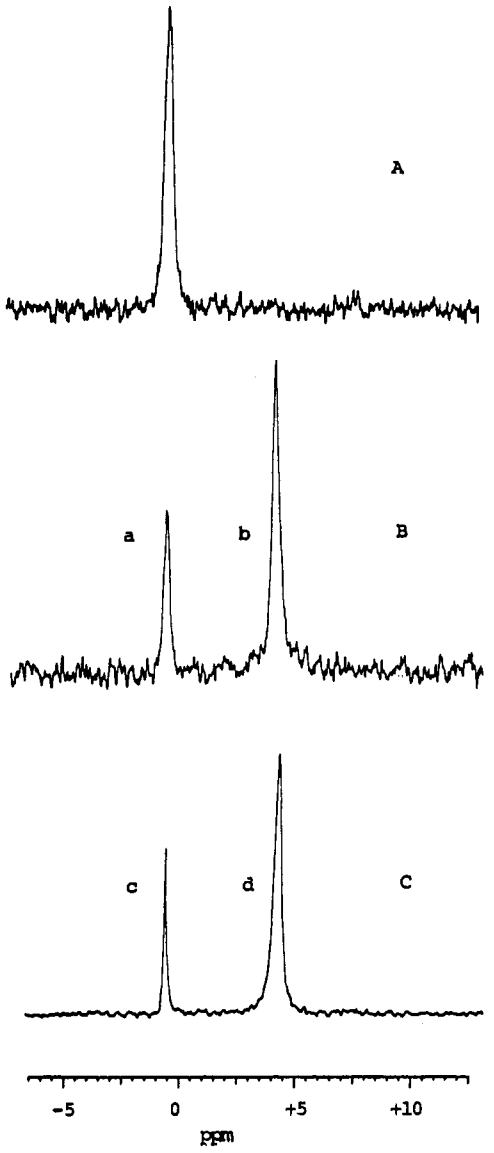

Figure 1. (A) ^{7}Li NMR spectrum (104.8 MHz, 37 °C) of gently packed RBCs in a medium containing 140 mM Na^+, 5 mM K^+, 3.5 mM Li^+, 10 mM glucose, and 50 mM HEPES at pH 7.5. Packed RBCs were incubated with 3.5 mM LiCl at 37 °C for 12 h prior to NMR measurements. 17% D_2O was present for field frequency lock. Hematocrit was 13%. (B) ^{7}Li NMR spectrum of the same RBC suspension as in part A with the exception that 5 mM $Na_7Dy(PPP_i)_2$·NaCl was present in the medium instead of 50 mM NaCl. (C) ^{7}Li NMR spectrum of a concentric sample (5-mm inner tube inside a 10-mm outer tube) containing 20 mM LiCl in distilled water in the inner tube while the outer tube contained the same suspension medium as in part B. All spectra were obtained in 1.1 min. Line broadening of 2.0 Hz was applied to accumulated free induction decays to improve the signal-to-noise ratio. a and c represent inner pools of Li^+ while b and d represent outer pools of Li^+.

109.3 MHz, respectively, on a JEOL FX-270 NMR spectrometer (Analytical Service Laboratory, Northwestern University) equipped with a multinuclear probe. Some of the multinuclear NMR studies were also done on a Varian VXR-300 NMR spectrometer (Chemistry Department, Loyola University of Chicago). The spectra were run on 10-mm NMR nonspinning tubes to avoid settling of RBCs. The 90° pulse was 18 μs. T_1 measurements of intra- and extracellular $^7Li^+$ NMR resonances in RBC suspensions were done by the inversion recovery method on a Varian VXR-400 NMR instrument (Analytical Service Laboratory, Northwestern University). The T_1 values for the intra- and extracellular pools of Li^+ were 4.9 and 0.1 s, respectively. Prior to the addition of shift reagent, the T_1 of extracellular Li^+ was 16.5 s. Since the relaxation times for the two $^7Li^+$ resonances were very different in magnitude, ^{7}Li NMR spectra were taken every $1.5T_1$ (repetition rate 7.5 s) with a flip angle of 45°. This spectrum acquisition procedure ensures that the relative intensities of the two measured NMR resonances reflect the relative amounts of intra- and extracellular $^7Li^+$ pools.

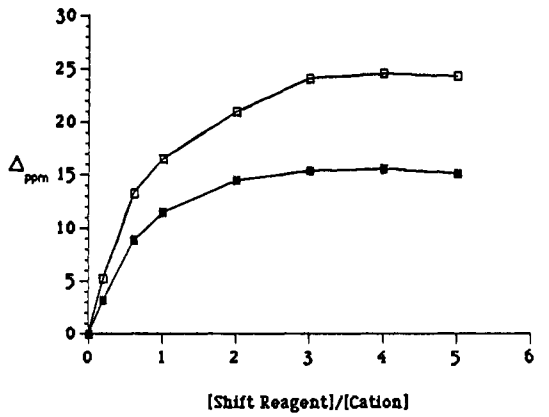

Figure 2. Plots of ^{7}Li (■) and ^{23}Na (□) chemical shift separation as a function of the molar ratio of shift reagent to lithium or sodium cation, respectively. The K^+ form of dysprosium triphosphate was used in these experiments. The Li^+ or Na^+ concentrations were kept at 5 mM while the shift reagent concentration was varied from 0 to 25 mM. Each sample was made in 50 mM HEPES buffer at pH 7.5 and contained 17% D_2O for field frequency lock. Data points shown represent the average of NMR measurements taken on two separately prepared samples.

Extracellular [Li^+] concentrations in RBC suspensions were determined from a calibration curve based on RBC suspensions including equal amounts of shift reagent and varying amounts of LiCl (1.0–5.0 mM). A plot of the ratio of the intensity of the extracellular RBC sample resonance to the intensity of the resonance of a reference sample (capillary tube containing 10 mM LiCl and 30 mM $DyCl_3$) versus millimoles of Li^+ was used as the calibration curve. Hematocrit concentrations were used to convert the resonance intensities into absolute intracellular and extracellular [Li^+] concentrations.

Atomic absorption studies were carried out on a Perkin-Elmer 5000 spectrophotometer with graphite furnace.

Results

Figure 1A indicates that the intra- and extracellular $^7Li^+$ resonances are not resolved in the ^{7}Li NMR spectrum of RBC suspensions to which no shift reagent was added. Similar observations were reported for ^{23}Na and ^{39}K NMR studies of RBCs.[9,13] These results follow from the almost total alkali metal NMR chemical shift independence of solvation and/or ligation.[18] Moreover, lithium is believed to be present mostly in the form of the free aquo ion in both the intracellular and the extracellular compartments of RBC suspensions. Separation of the two $^7Li^+$ NMR signals (Figure 1B) can be achieved by addition to the RBC suspension of the highly negatively charged shift reagent, dysprosium(III) triphosphate, $Dy(PPP_i)_2^{7-}$. A control experiment using an inner tube–outer tube combination (Figure 1C) gives the same chemical shift separation as for the RBC suspension shown in Figure 1B, indicating that (1) the shift reagent does not penetrate the cell membrane and (2) the upfield resonance is due to extracellular $^7Li^+$, which is in contact with the shift reagent, while the downfield resonance corresponds to intracellular $^7Li^+$.

The effect of increasing the molar ratio of shift reagent to lithium on the $^7Li^+$ chemical shift separation was investigated (Figure 2). For comparison, the effect of increasing the molar ratio of shift reagent to sodium on the $^{23}Na^+$ chemical shift is also included in Figure 2. In order to compare the $^7Li^+$ and $^{23}Na^+$ chemical shifts afforded by dysprosium(III) triphosphate, the K^+ form of this shift reagent was used in the measurements plotted in Figure 2. For [shift reagent]/[cation] ratios greater than or equal to 3, both the $^7Li^+$ and $^{23}Na^+$ chemical shift separations remain approximately constant. Although the affinity of Li^+ for the shift reagent is less than that of Na^+, it is still possible to obtain a clear separation of the two $^7Li^+$ pools with the help of $Dy(PPP_i)_2^{7-}$. The chemical shift separations between the two $^7Li^+$ NMR resonances of Li^+-loaded RBC suspensions containing 1, 3, 5, 10, 15, and 20 mM shift reagent (in the Na^+ form) and 1

(18) Detellier, C. *NMR of Newly Accessible Nuclei*; Laszlo, P., Ed.; Academic: New York, 1983; Vol. 2, pp 105–151.

Table I. Competition between Li^+ and Other Metal Cations for the Shift Reagent, $Dy(PPP_i)_2^{7-}$

ion concn, mM[a]					^7Li chem shift sepn, ppm[b]
Na^+	K^+	Mg^{2+}	Ca^{2+}	Li^+	
50				1	11.16[c]
140				1	5.89
50	5			1	10.84
50		1		1	9.66
50			2	1	6.11
140	5			1	5.16
140	5	1		1	4.48
140	5	1	2	1	2.93

[a] Sample medium contained, in addition to the ions indicated above, 10 mM glucose, 5 mM $Na_7Dy(PPP_i)_2 \cdot 3NaCl$, 17% D_2O, and 50 mM HEPES at pH 7.5 and 37 °C. [b] The ^7Li chemical shift separation was measured for concentric samples (5-mm inner tube containing 5 mM LiCl in distilled water inside a 10-mm outer tube containing the sample medium described in footnote *a*). [c] The [shift reagent]/[Li^+] ratio in this experiment is 5, and the chemical shift separation is 11.16 ppm. For the same ratio in Figure 2, a chemical shift separation of 15 ppm is observed. The higher [Li^+] concentration used in the experiment represented in Figure 2 forces a larger proportion of Li^+ to interact with the shift reagent. Similar observations were made for Na^+ (see Figure 2, ref 10).

mM Li^+ in the extracellular compartment were found to be 0.2, 1.7, 3.7, 7.8, 10.7, and 10.8 ppm, respectively. Thus, for the therapeutic levels of Li^+ used in the United States, i.e., where the plasma [Li^+] concentration varies between 0.5 and 1.2 mM, a minimal concentration of 3 mM dysprosium(III) triphosphate ensures that the two $^7Li^+$ NMR resonances are completely resolved. The largest possible $^7Li^+$ chemical shift separation between the two Li^+ pools in RBCs of manic depressive patients is attainable with a 15 mM concentration of shift reagent, although a significant amount of broadening of the extracellular $^7Li^+$ resonance is observed under these conditions.

Competition for dysprosium(III) triphosphate between Li^+ and Na^+, K^+, Mg^{2+}, and/or Ca^{2+}, at their physiologically relevant concentrations, was studied. The results are presented in Table I. It was found that divalent cations compete more effectively with Li^+ than monovalent cations. Moreover, experiments carried out in the presence of two or more competing cations show that the decrease observed in the ^7Li chemical shift separation is not additive. We conclude from this study that the two $^7Li^+$ resonances in RBCs are separated by approximately 3 ppm even in the presence of physiologically relevant concentrations of Na^+, K^+, Mg^{2+}, and Ca^{2+}.

Addition of a lithium ionophore,[19] monensin, to a RBC suspension in a K^+-only medium (Figure 3) induces passive Li^+ transport. It was found that the gain in peak area of the extracellular $^7Li^+$ resonance was exactly matched by the loss in peak area of the intracellular $^7Li^+$ resonance. Addition of different concentrations of monensin, 0.06, 0.09, and 0.23 mM, to a Li^+-loaded RBC suspension led to apparent k values of 0.013, 0.017, and 0.49 min^{-1}, respectively. The apparent rate constants were determined from a linear regression analysis of a plot of ln $([Li^+]_t/Li^+]_0)$ versus time, where $[Li^+]_0$ and $[Li^+]_t$ represent the initial Li^+ concentration and that after time t. The ratio k/[monensin] was approximately constant and equal to 200 ± 10 M^{-1} min^{-1}. Thus, the pseudo-first-order rate constants for Li^+ transport across RBC membranes are dependent on monensin concentration, indicating that the $^7Li^+$ NMR resonance intensity changes observed in Figure 3 are indeed due to Li^+ transport.

^{31}P NMR studies of inorganic phosphate present in the RBC suspension medium indicate that no pH change occurs as a result of monensin-induced Li^+ transport. However, the chemical shift separation afforded by the shift reagent dysprosium(III) triphosphate is strongly pH-dependent, particularly around pH 7.5.[14] Since no changes in chemical shift separation between the two $^7Li^+$ NMR resonances or the position of the inorganic phosphate

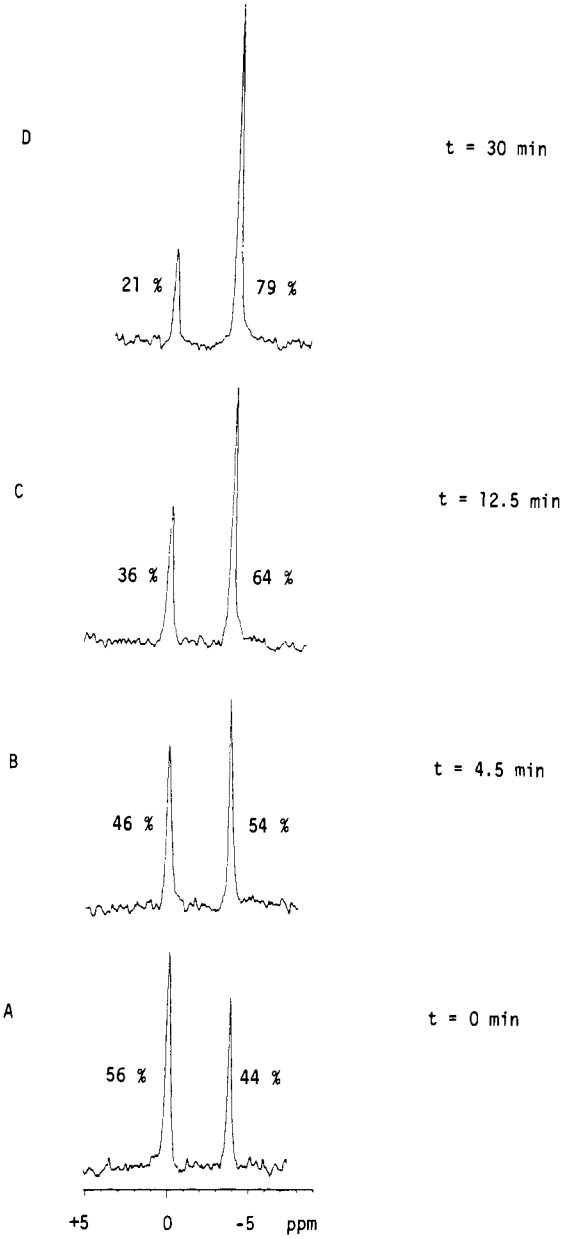

Figure 3. Time dependence of the ^7Li NMR (104.8 MHz, 37 °C) spectrum of a 17% D_2O suspension of RBCs that had been incubated overnight with 150 mM LiCl and 5 mM HEPES, pH 7.5. The suspension medium contained 1 mM LiCl, 149 mM KCl (including shift reagent contribution), 10 mM glucose, 5 mM $K_7Dy(PPP_i)_2 \cdot 3KCl$, and 5 mM phosphate at pH 7.5. The hematocrit was 13%. Spectra A–D are labeled with the time elapsed after the RBC suspension was made 0.06 mM in monensin (ethanol solution). The time labels represent the midpoints of the accumulation periods. The normalized percentage areas of the inner and outer pools of Li^+ are indicated on the plot. At the end of 75 min, all of the intracellular Li^+ had been transported out to the extracellular medium.

^{31}P NMR resonance were observed, we conclude that Li^+–H^+ exchange is not taking place. Monensin is known to have a higher affinity for Na^+ than for Li^+,[19] and thus a Na^+–Li^+ exchange mechanism could in principle be occurring. However, a Na^+–Li^+ exchange mechanism is not probable in this case since the cells are suspended in a K^+-only medium and virtually no intracellular Na^+ is present in RBCs (as measured by ^{23}Na NMR spectroscopy) as a result of extensive Li^+ loading of red cells. Experiments similar to that described in Figure 3 were tried where K^+ in the suspension medium was partially or completely replaced by Na^+, and it was found that no Li^+ transport occurred. Absence of monesin-induced Li^+ transport in RBCs suspended in a Na^+ medium is probably a result of strong Na^+ binding to monensin.

(19) Riddell, R. G.; Hayer, M. K. *Biochem. Biophys. Acta* **1985**, *817*, 313.

Thus, we postulate that a Li^+–K^+ exchange mechanism is taking place in Figure 3. Direct evidence for the proposed mechanism is difficult to obtain by metal NMR spectroscopy because of the inherently low sensitivity of the ^{39}K nucleus.

C221 and C211 were briefly tested as potential lithium ionophores[20] by ^7Li NMR spectroscopy. In a K^+-only suspension medium, addition of C221 led to slow Li^+ transport. Equilibrium of the two Li^+ pools took more than 1 day in the case of C221 as opposed to 1 h for monensin. It was also found that addition of C211 to a RBC suspension induced slow lithium transport as well as binding of lithium to C211 (data not shown). With both cryptands, however, the unfacilitated Li^+ transport (obtained without addition of cryptand) amounted to approximately 50% of that induced by the ionophores. Atomic absorption studies carried out on similar Li^+-loaded RBC suspensions that had been incubated with either cryptand, but in the absence of shift reagent, confirmed that C221 and C211 can induce slow Li^+ transport in a K^+-only RBC suspension.

Discussion

We show in this paper that the shift reagent, dysprosium(III) triphosphate, that was first introduced by Gupta and Gupta[9] to achieve separation of $^{23}Na^+$ pools in RBCs also provides good resolution of the two $^7Li^+$ pools for Li^+-loaded RBCs (Figure 1B).[1,21] A pulse gradient NMR method had previously been used to study Li^+ transport in RBCs.[22] Springer and his co-workers[23] reported previously the transport of Li^+ in yeast cells by ^7Li NMR spectroscopy. It is interesting to note that these investigators used a 19 mM concentration of another shift reagent dysprosium(III) tris(dipicolinate), and obtained a chemical shift separation of less than 2 ppm with the intra- and extracellular components only partially resolved as opposed to that of 5 ppm observed by us with a smaller concentration (5 mM) of dysprosium(III) triphosphate. Clearly, dysprosium(III) triphosphate is a better $^7Li^+$ shift reagent than dysprosium(III) tris(dipicolinate). We are presently investigating other dysprosium and thulium complexes for their potential as $^7Li^+$ NMR shift reagents and analyzing their toxicity properties.

Although dysprosium(III) triphosphate is clearly a better shift reagent for $^{23}Na^+$ NMR than for $^7Li^+$ NMR studies, a 3 mM concentration of this shift reagent is however sufficient to give complete separation of the two Li^+ pools (Figure 2). This $^7Li^+$ chemical shift separation in RBC suspensions is maintained even in the presence of physiologically relevant concentrations of other metal cations such as Na^+, K^+, Mg^{2+}, and Ca^{2+} (Table I). The observation that the decrease in the ^7Li chemical shift separation is not additive is in agreement with the postulated solution structure for this shift reagent,[24] where seven alkali-metal countercations present in the second coordination sphere neutralize the −7 charge on dysprosium(III) triphosphate. Other metal ions, in particular divalent metal ions, compete with Li^+ for the limited number of sites available in the second coordination sphere of the shift reagent, thus neutralizing the high negative charge on the dysprosium complex.

Figure 3 indicates that addition of monensin to a RBC suspension induces passive Li^+ transport. Monensin is specific for Na^+.[19] Enhanced affinity of monensin for Li^+ was achieved by having only Li^+ and K^+ ions present in the suspension medium. These experimental conditions are nonphysiological since Na^+ (as opposed to K^+) is the major extracellular countercation. Similar experiments carried out in a Na^+ medium failed because monensin preferentially binds Na^+ without transporting it. Clearly, chemical modification of monensin, C221, and C211 is necessary in order to obtain ionophores with sufficiently high specificity for Li^+ over Na^+ that will transport Li^+ even in a Na^+ medium.[21a] The good match obtained between the gain in peak area of the extracellular Li^+ resonance and the loss in peak area of the intracellular Li^+ (Figure 3) upon addition of monensin to the RBC suspension cannot be interpreted as indicating 100% visibility for intracellular $^7Li^+$ in the NMR experiment since a change in the hematocrit value was observed during passive Li^+ transport (data not shown). A more detailed determination of cell volume is therefore necessary in order to settle the visibility question. It is interesting to note that intracellular $^{23}Na^+$ in RBCs was found to be only 80% NMR visible when the cell volume was monitored by the isotope dilution method.[17] ^7Li, like ^{23}Na, is a quadrupolar nucleus, and strongly immobilized $^7Li^+$ may be invisible in the NMR experiment as a result of extensive quadrupolar relaxation.[18] Whatever the amount of immobilized intracellular $^7Li^+$ may be, it is clear from Figure 3 that the metal NMR method described in this paper is uniquely suited for monitoring transport of free intracellular lithium in RBCs.

The NMR method described in this paper may turn out to be an excellent probe for the study of the bioinorganic chemistry of lithium. Moreover, the noninvasive nature of this method could most likely make it the preferred tool (as opposed to atomic absorption and flame photometry) for the characterization of abnormalities in lithium transport in RBCs of manic depressive and hypertenisve patients. Testing of its applicability to the latter problems is now under way in our laboratory.

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Registry No. Li, 7439-93-2; ^7Li, 13982-05-3.

(20) Shamsipur, M.; Popov, A. I. *J. Phys. Chem.* **1986**, *90*, 5997.
(21) After submission of this paper for publication, two related reports appeared in the literature describing the application of the shift reagent $Dy(PPP_i)_2^{7-}$ to the study of Li^+ transport in vesicles and RBCs, respectively: (a) Shinar, H.; Navon, G. *J. Am. Chem. Soc.* **1986**, *108*, 5005. (b) Pettegrew, J. W.; Post, J. F. M.; Panchalingam, K.; Withers, G.; Woessner, D. E. *J. Magn. Reson.* **1987**, *71*, 504.
(22) Andrasko, J. *J. Magn. Reson.* **1976**, *21*, 479.
(23) Balschi, J. A.; Cirillo, V. P.; Springer, C. S., Jr. *Biophys. J.* **1982**, *38*, 323.
(24) Nieuwenhuizen, M. S.; Peters, J. A.; Sinnema, A.; Kieboom, A. P. G.; Van Bekkum, H. *J. Am. Chem. Soc.* **1985**, *107*, 12.