DIRECT HIGH-RESOLUTION NUCLEAR MAGNETIC RESONANCE STUDIES OF CATION TRANSPORT IN VIVO

Na^+ Transport in Yeast Cells

JAMES A. BALSCHI, VINCENT P. CIRILLO,* AND CHARLES S. SPRINGER, JR.
Departments of Chemistry and Biochemistry, State University of New York at Stony Brook, Stony Brook, New York 11794*

ABSTRACT A new nuclear magnetic resonance (NMR) method for monitoring transmembrane metal cation transport is reported. It is illustrated with a study of Na^+ efflux from Na^+-rich yeast cells. The technique involves the use of an anionic paramagnetic shift reagent, present only outside the cells, to induce a splitting of the sodium-23 NMR peak, in this case, into components representing intra- and extracellular Na^+. The time course of the efflux is in good agreement with the literature and can be well fitted with a double exponential decay expression. Splitting of the lithium-7 NMR signal from a suspension of Li^+-rich respiratory-deficient, petite yeasts is also reported.

The study of metal cation uptake by and efflux from isolated organelles and living cells is an extensive and important aspect of biophysical chemistry (1,2). The methods generally employed include conductance measurements, the use of radioactive tracers, the use of ion-selective electrodes, and the use of analytical techniques such as atomic absorption spectroscopy. In two separate communications, we have reported on a family of aqueous shift reagents for the direct high-resolution nuclear magnetic resonance (NMR) study of metal aquo ions (3) and their use in the study of transport across model membranes (4). Here, we report a preliminary study of transport in vivo.

Although yeast cells normally accumulate K^+ in preference to Na^+ in their intracellular aqueous spaces, they will take up Na^+ if incubated in a medium lacking K^+ and rich in Na^+. If subsequently resuspended in a Na^+-free environment, the intracellular Na^+ ions are transported out of the cells via either of two pathways (5,6).

Fig. 1 depicts the time-dependence of the sodium-23 NMR spectrum (132.3 MHz) of such a suspension of Na-rich yeast cells. Spectral details are given in the figure caption. A quantity of washed baker's yeast (*Saccharomyces cerevisiae*, Red Star brand; Universal Foods Corp., Milwaukee, WI) weighing 2.13 g (wet weight) was incubated with shaking in 110 ml of ~5% (wt/vol) glucose and ~0.2 M sodium citrate at room temperature for 4.5 h. A portion containing 410 mg (wet weight) of yeasts was removed, centrifuged at 10,000 rpm for 10 min (2.5°C), washed once in 25 ml cold water by recentrifuging at 10,000 rpm for 5 min (2.5°C), and finally suspended in 1.72 ml of 100% D_2O. The external aqueous phase was made 14.8 mM in $(HTEA)_3Dy(NTA)_2$ ($HTEA^+$ is $HN[CH_2CH_2OH]_3^+$ and NTA^{3-} is $N[CH_2CO_2]_3^{3-}$ [3]) and the spectrum seen in Fig. 1*a* was obtained 4 min after suspension in D_2O. Two relatively sharp peaks are clearly observed. The larger resonance is assigned to Na^+ ions inside the cells. If an analogous sample is prepared, except without the shift reagent, a single peak is observed at the same resonance frequency.

The smaller peak, 364 Hz upfield from the larger one, is assigned to Na^+ ions outside the cells where they are able to interact with $Dy(NTA)_2^{3-}$. This paramagnetic complex anion is known to induce an upfield shift in the $^{23}Na^+$ resonance of the sodium aquo ion (3). The outside Na^+ ions could be external ions that survived the washing, internal ions that have been transported out in the time elapsed after resuspension, or a combination of both. That the latter is the case, can be learned from an analysis of the spectra in Fig. 1*b–f*. Clearly inside Na^+ is transported out as time progresses. A plot of the fractional area under the inside peak (f_i = inner/total; determined by hand-resolution and planimetry) vs. time is shown in Fig. 2. The data are reasonably fitted by an expression with two exponential decay terms [$f_i = 0.34 \exp(-0.054t) + 0.46 \exp(-0.0011t)$, t in minutes] which extrapolates to 0.8 at zero time. This indicates that ~20% of the "NMR visible" Na^+ (see below) is present outside the cells immediately upon resuspension. The two first-order rate constants are

Address correspondence to Dr. Springer.

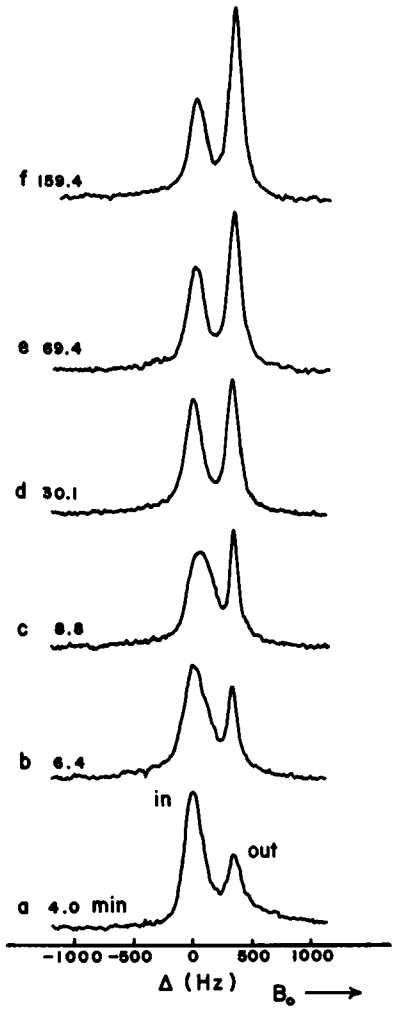

FIGURE 1 The time dependence of the ^{23}Na NMR spectrum (132.3 MHz, 11.74 T [tesla]) of a D_2O suspension (24% [wt/vol]) of Na^+-rich yeast cells. The spectra are labeled with the time elapsed since resuspension. Each spectrum is the result of the accumulation of 128 free-induction decays in 52 s. The time labels correspond to the midpoints of the accumulation intervals. The suspension was occasionally stirred, to prevent settling, by a gentle pipetting. The temperature was 297°K.

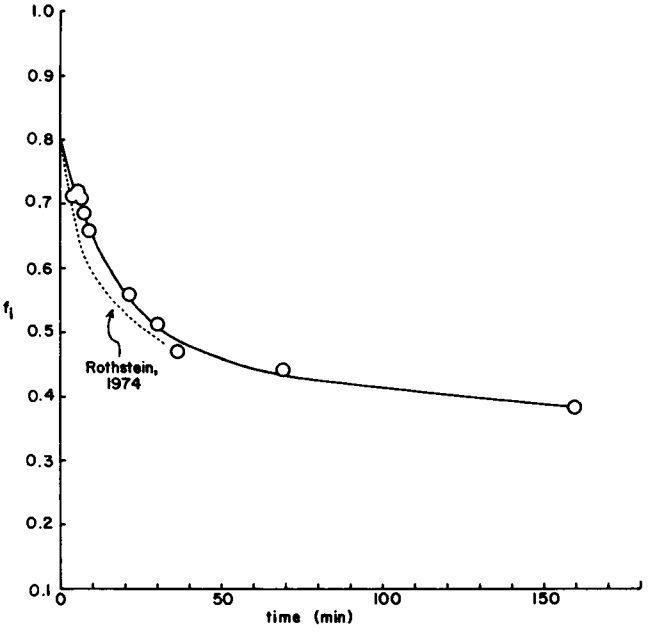

FIGURE 2 The fractional area of the inside resonance (f_i) of Fig. 1 as a function of time. The solid curve is a computer fitting. The results of Rothstein (dashed curve) are taken from reference 6 (Fig. 2). The initial value of f_i was adjusted to coincide with our initial value.

$9.0(\pm 2.0) \times 10^{-4}\ s^{-1}$ and $0.18(\pm 0.11) \times 10^{-4}\ s^{-1}$. We can calculate approximate permeability coefficients, P, by assuming the cells are spherical and employing the relationship $P \simeq$ (first-order rate constant) (cytoplasmic volume) (cell surface area)$^{-1}$ (7). Taking the cytoplasmic volume to be 0.47×10^{-10} cm^3 (8) and the average cell radius to be 2.5×10^{-4} cm (9), we calculate the two values of P to be 5.4×10^{-8} cm/s and 1.1×10^{-9} cm/s.

The initial rate of exit observed here is similar to that reported by Rothstein (6) whose results (obtained by quenching, extraction, and subsequent flame photometry) are reproduced in Fig. 2. Any discrepancies could certainly be caused by yeast strain differences, differences in history, and/or differences in conditions such as the significantly greater cell density (by an order of magnitude) and/or the uncontrolled pH of the external medium in our experiment.

In the absence of other external cations, Na^+ efflux is rate-limited by the efflux of organic anions and is accompanied by proton influx (6). In other experiments, we have added exogenous K^+ ions during the course of Na^+ efflux and have observed the expected stimulation (6). The reason why the efflux observed here is biphasic is not known. One possibility is that the high cell density causes the solution to be quickly depleted of dioxygen. Interestingly, the efflux of Li^+ (and, presumably, Na^+) from yeasts requires energy even when it is down a concentration gradient (5). Thus the faster process could represent the aerobic metabolism of endogenous glucose reserves using available dioxygen. The slower rate might represent a process limited by the proton efflux leakiness. The Na^+ efflux pathway may involve an electrogenic Na^+/H^+ antiporter (5).

Although the $^{23}Na^+$ resonances observed here are broader than is the case either in the presence of model membranes (4), or in the absence of any membrane (3), they are still sharp and certainly within the "fast motional narrowing" condition (10). The sodium-23 nucleus is quadrupolar ($I = 3/2$) and as such is susceptible to a potent relaxation mechanism not suffered by nonquadrupolar nuclei. Whenever $^{23}Na^+$ is bound tightly, or in a motionally hindered situation, its NMR line can become extremely broad; so much so, in certain circumstances, that it becomes "NMR invisible" under high-resolution conditions (10–12). Obviously, most of the $^{23}Na^+$ we are observing, both inside and outside the cells, is present as the unbound aquo ion (13). In some experiments, we have noted indications of a slight increase (<12%) in the total

area of the two peaks as transport progressed. Such a result could be explained as a decrease in a small amount of tightly bound, and thus "invisible", Na^+ as the distribution of Na^+ between inside and outside is changed. This could be related to the transient line broadening and possible development of upfield shoulders seen on the inside resonance in Fig. 1*b*, *c*, and *d*, although this phenomenon is not yet proven to be nonartifactual. Besides possible anionic macromolecular binding sites within the cells, yeasts have an anionic cell wall outside the cell membrane. This could bind some Na^+ (6) and sequester it from the anionic shift reagent although this would be expected to affect the position and/or intensity of the outside line. Protoplasts can be prepared from intact yeast cells by removal of their cell walls (14) and might prove the subject of interesting experiments.

To illustrate the expected generalization of this technique to studies using magnetic nuclei of any metal cationic species, we have conducted a few experiments with the lithium-7 nucleus. In our hands, commercial baker's yeast of the type used in Fig. 1 could not be made to take up Li^+. Yeast strains do show wide variation in the ability to accumulate Li^+ (15). Fig. 3 depicts a low-field $^7Li^+$ NMR spectrum (38.9 MHz) of a suspension of respiratory-deficient, petite yeasts (16) (*Saccharomyces cerevisiae*, Y185ρ^-) which were induced to take up Li^+ in a manner similar to the treatment of the cells of Fig. 1. Details are given in the figure caption. The spectrum, obtained ~18 h after resuspension, clearly shows two peaks. Since the external aqueous phase is 19 mM in $(HTEA)_3Dy(DPA)_3$ (DPA^{2-}, dipicolinate), the upfield line is assigned to those Li^+ ions outside the cells. The downfield resonance is attributed to Li^+ inside the cells. The relative intensities of

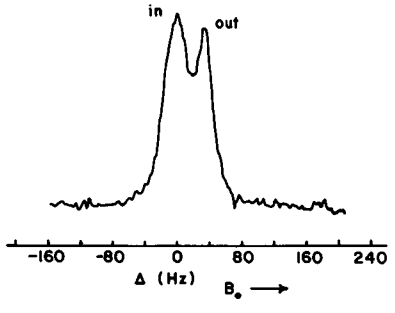

FIGURE 3 The ^7Li NMR spectrum (38.9 MHz, 2.35 T) of a D_2O suspension (40% [wt/vol]) of Li^+-rich ρ^- yeast cells prepared as follows. Yeast cells, strain Y185ρ^-, were suspended to a cell density of 2% (wt/vol) in a 0.2 M Na_3citrate, 5% (wt/vol) glucose solution for 45 min with shaking at 30°C. They were then centrifuged at 3,000 rpm for 5 min and resuspended in a 0.2 M LiCl, 5% glucose solution for 2 h. They were again centrifuged at 3,000 rpm and resuspended in fresh Li^+ medium. 2 h later the yeast were centrifuged at 10,000 rpm for 10 min (T = 2°). After the supernatant was removed, the yeast were resuspended in ice-cold water and centrifuged. The cells were finally suspended in 1.1 ml 100% D_2O and the external solution was made 19 mM in $(HTEA)_3Dy(DPA)_3$. The spectrum shown is the result of the accumulation of 720 free-induction-decays in 6.1 min. It was obtained ~18 h after the cells were suspended in D_2O.

the two peaks have changed little, if any, since resuspension. Transport is much slower in this case because the respiratory-deficient yeasts cannot avail themselves of the dioxygen present and also must rely on exogenous glucose for Li^+ efflux (5). Glucose was not present in the resuspension medium. We have noted in other analogous experiments on Na^+-rich ρ^- yeasts, however, that Na^+ efflux occurs quite readily (unpublished results).

Thus, it seems clear that, at high fields, this technique holds promise for the study of passive and active transport of magnetic isotopes of any of the physiological metal aquo cations ($^{23}Na^+$, $^{39}K^+$, $^{25}Mg^{2+}$, or $^{43}Ca^{2+}$) in living systems. Erythrocytes and erythrocyte "ghosts" (17) would seem to be very attractive next candidates.[1] Earlier $^{23}Na^+$ NMR studies have indicated that most of the Na^+ present inside erythrocyte cells is "NMR visible" (13). Of particular interest might be the investigation of Li^+ transport in erythrocytes, a process monitored in the study of manic-depressive illness (18). An alternative pulse gradient ^7Li NMR method has been reported (19). Even more intriguing is the possibility of monitoring transport into and out of whole organs by perfusing them in a bath containing the shift reagent species. A report of gated images of isolated perfused working rat hearts using ^{23}Na NMR has recently appeared (20).

Some advantages of this NMR approach which may prove of benefit include the following: (*a*) the use of stable isotopes, (*b*) the simultaneous determination of inside and outside concentrations, (*c*) the continuous, nondestructive monitoring of the approach to transport equilibrium (or steady state), (*d*) the study of transport kinetics at equilibrium (or steady state) by the use of magnetization transfer or exchange broadening (4), (*e*) the possibility of stable isotope labeling, and (*f*) the possibility of observing clear spectral distinctions of transport mechanism (4, 7).

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