AN EFFLUX MECHANISM DETERMINES THE LOW NET ENTRY OF LITHIUM IN YEASTS

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1. Introduction

Lithium is a toxic cation for yeasts and many strains keep a lower concentration in the cytoplasm than that present in the medium [1]. A transmembrane electrical potential, negative inside, is in increasing evidence in yeasts [2–5], similar to that found in *Neurospora* [6] and also in bacteria, where it draws K^+ into the cell [7]. This potential would draw any cation into the cytoplasm, provided the appropriate channel exists and it is known that Li^+ enters into the yeast cell through the K^+ carrier [8] which seems to be dissociated from the energy coupling [4]. So the maintenance of a lower Li^+ concentration in the cytoplasm than in the medium would require a system to exclude this cation. In this paper we present kinetic evidence for such a system.

2. Materials and methods

2.1. *Growth of the yeast*

Saccharomyces cerevisiae strain X2180. 1A was maintained and grown as described previously [1]. The medium KNa (x,y) (x mM K^+ and y mM Na^+) at pH 6.5, unless otherwise stated, was used through the work.

2.2. *Li^+ content of the cells*

Analysis of Li^+ content of the cells were done as described previously [1] except that sorbitol was included in the culture medium, at a concentration approximately isotonic to the concentration of Li^+ to be assayed thereafter. When cell concentration was approx. 0.1 mg dry wt/ml (log phase, pH unchanged) cells were centrifuged and resuspended in fresh medium with Li^+ and without sorbitol. For efflux experiments, cells were kept for 90 min with Li^+, centrifuged and resuspended in fresh medium with sorbitol and without Li^+, after drying the tube walls with filter paper.

3. Results

Figure 1 shows the time course of Li^+ uptake by the yeast. Cell Li^+ concentration reached steady values after about 30 min for an external Li^+ concentration range of 50–200 mM. The final values were higher, the higher the external concentration, but always inferior to it. Analysis of the Li^+ uptake curve revealed a straight-line for the plot of log (maximal cell Li^+ minus cell Li^+ at time t) versus time.

Figure 2 shows that the 'downhill' efflux of Li^+ from the precharged cells to a Li^+ free medium (contamination <0.5 mM) followed a first-order kinetic. This efflux was practically unaffected by HCl-adjusted pH changes in the range 3.5–8.0.

If we suppose that the 'uphill' Li^+ efflux follows

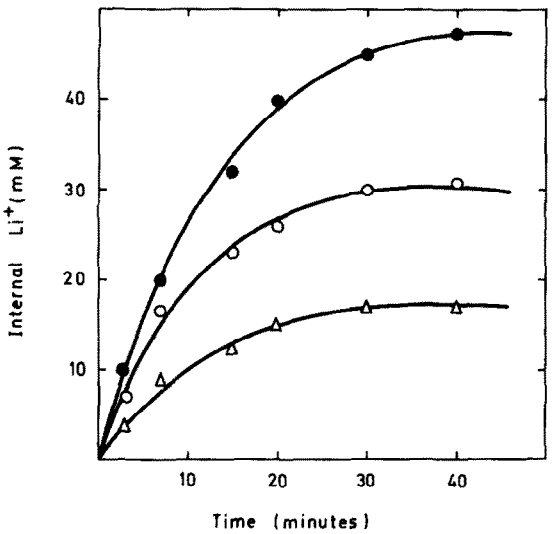

Fig.1. Time course of the accumulation of Li^+ by the yeast. Cells grown in KNa (10.0) were suspended, at $t = 0$, in fresh medium plus different amounts of Li^+: (△——△) 50 mM, (○——○) 100 mM, (●——●) 200 mM.

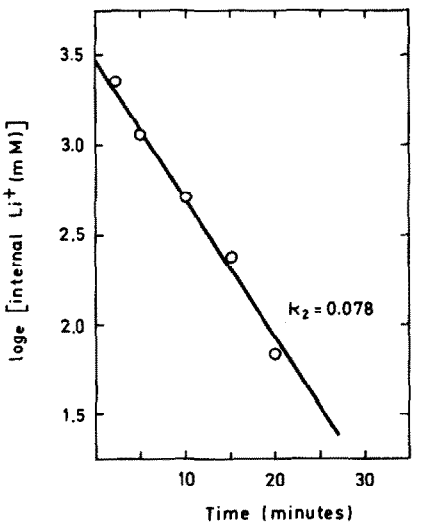

Fig.2. Efflux of Li^+ from the yeast. Cells precharged of Li^+ in KNa (10.0) plus 100 mM Li^+ were resuspended in Li^+-free medium at $t = 0$.

the same kinetic as the 'downhill' efflux and that the influx is constant for a given external Li^+ concentration, the Li^+ concentration of the cell, at time t in an uptake experiment, can be calculated as the difference between the Li^+ input and the Li^+ output. Provided that the taken time does not allow significant growth (doubling-time in KNa (10.0) was 4.5 h) calculations are as follows:

$$\text{Influx} = Ek_1 = K_E \quad (E, \text{ external } Li^+ \text{ concentration})$$

$$\text{Efflux} = Ik_2 \qquad (I, \text{ internal } Li^+ \text{ concentration})$$

$$dI = (K_E - Ik_2)\,dt \text{ (for one liter cell water)}$$

$$t = -\frac{1}{k_2} \log_e \frac{K_E - I_t k_2}{K_E}$$

in the steady state $K_E = I_\infty k_2$

$$t = -\frac{1}{k_2} \log_e \left(1 - \frac{I_t}{I_\infty}\right)$$

The above hypothesis explains the observed uptake kinetic (fig.3) and, in addition, the k_2 obtained from the uptake and from the 'downhill' efflux are reasonably coincident (fig.2 and fig.3).

External K^+ affected both the influx and the efflux

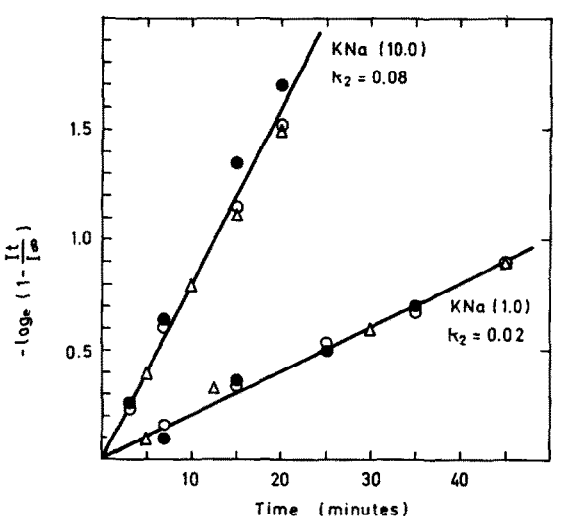

Fig.3. Plot of $-\log_e (1 - I_t/I_\infty)$ versus time in KNa (10.0) and KNa (1.0). Conditions as in fig.1. I_∞ was taken as the internal lithium concentration at $t = 90$ min. (△——△) 50 mM, (○——○) 100 mM and (●——●) 200 mM of external Li^+.

Table 1
Li^+ concentration in the yeast cells at equilibrium with the external Li^+ (I_∞) and the influx of Li^+, in media with 10 mM and 1 mM of external K^+

Medium	External Li^+ (mM)					
	50		100		200	
	I_∞	K_{50}	I_∞	K_{100}	I_∞	K_{200}
KNa (10.0)	17	1.4	30	2.4	45	3.6
KNa (1.0)	50	1.0	90	1.8	140	2.9

I_∞, mmol/liter cell-water. K_E, mmol/min/liter cell-water, was calculated from I_∞ and the k_2 of fig.3.

of Li^+, which were higher at the higher concentration tested. The efflux at 10 mM K^+ was increased more than the influx, with reference to 1 mM K^+, resulting in a lower internal Li^+ concentration at 10 mM than at 1 mM of external K^+ (fig.3 and table 1). An effect of K^+ from the inside can be ruled out as the K^+ cell content at the two tested external concentrations were similar.

Figure 4 shows that the influx of Li^+, calculated when it is at equilibrium with the efflux, is a saturating process that is enhanced by external K^+.

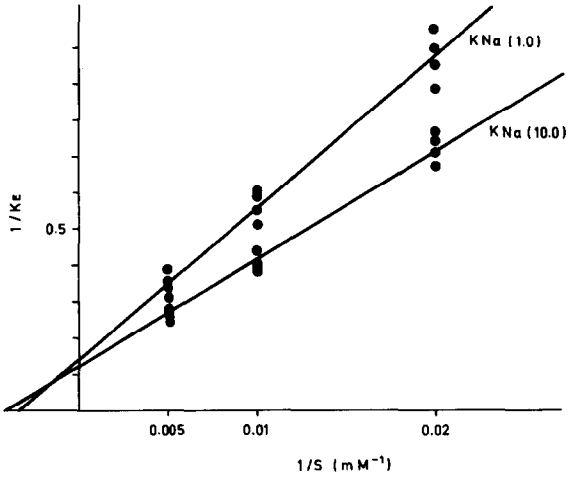

Fig.4. Reciprocal plots of the influx of Li^+. K_E was calculated from I_∞ and k_2, as in table 1.

4. Discussion

An electrical potential, negative inside, would draw all cations into the cell. At thermodynamical equilibrium, the ratios between cation concentrations inside the cell would be the same as those outside. However, experimental data show that Li^+ and K^+ are in opposite gradients in many yeast strains [1], in spite of the fact that both cations enter into the cell through the same carrier [8]. It must be considered that the binding effects of the cell macromolecules cannot account for these differences (reviewed in ref. [9]).

The present results show that the Li^+ content of the cell is kept in kinetical equilibrium between the influx and the efflux of the cation in two well mixed compartments. A similar mechanism has been proposed for K^+ in *Escherichia coli* [10] and could, in fact, be operating for other alkali metal cations. The sodium pump in yeasts [11,12] and the Na^+/H^+ antiporter in bacteria [13,14], would be the efflux mechanism for this cation. By the effect of the outward pumping, thermodynamical equilibrium would not be reached, and the cell cation contents would be determined by the inward–outward pumping balance.

The efflux of Li^+, as it takes place in strain X2180. 1A, has to be an active transport, whether it is electrogenic or mediated by a Li^+/H^+ antiporter. In the latter case, neither the difference between the activity coefficients, inside and outside, nor the osmotic pressure could justify the observed gradient, taking into account that we have found the efflux insensitive to acidic pH values.

Our data on the influx are in agreement with those in the literature [8,15,16] obtained by different experimental approaches. Differences in the K_m can be explained by the different capacities for Li^+ accumulation shown by different yeast strains and by the sensitivity of the process to culture conditions [1]. The slight differences in the Li^+ content in the present work versus our previous one with the same strain [1] were probably due to a higher aeration of the cultures in this work. The enhancement of Li^+ influx by K^+ is in accordance with the fact that K^+ stimulates Na^+ and Rb^+ uptake [17].

A large content of lithium in a lithium yeast has been associated with low rates of fermentation, oxygen consumption and growth [18]. In our experimental

conditions significant effects of lithium on the metabolism of the yeast were not observed. Only 200 mM Li^+ in KNa (1.0) decreased growth slightly, but it did not affect oxygen consumption and CO_2 production in agreement with a previous publication [1]. In fact the internal Li^+/K^+ ratio in the lithium yeast [18] was much higher than the maximal ratio found in the present work, 5 versus 0.5.

The efflux kinetic is compatible with a saturating process with a carrier of low affinity for Li^+, as it has been shown for the influx.

The existence of a non-available cell-water or an error in its measurement would not significantly modify most of our results as it would affect both I_t and I_∞.

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