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ENERGY SOURCE FOR LITHIUM EFFLUX IN YEAST

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Summary

The efflux of Li^+ in yeast was found to depend on the protonmotive force. The ATP content of the cell regulated the efflux that was also sensitive to the decrease in the cell pH. We propose an electrogenic H^+/Li^+ antiport as the mechanism for the efflux of Li^+.

Introduction

Yeast cells exchange K^+ for cellular H^+ [1,2], accumulating K^+ against steep gradients [3]. The mechanism for this exchange has not been clearly established, but there is increasing evidence [4—7] for a chemiosmotic model similar to that accepted for bacteria [8], i.e., K^+ is drawn by an electrical potential created by an H^+-extruding ATPase. The model is indirectly supported by the work on H^+-dependent solute transport [9,10] and by the work on *Neurospora* [11,12] which is, like yeast, an ascomycete. The hypothesis of an electrical coupling between H^+ efflux and K^+ influx should be extended to the transport of other alkali cations, since all of these cations are accepted by the K^+ carrier [13—15]. However, if K^+ is present in culture medium, K^+ is accumulated and Na^+ and Li^+ are excluded from the yeast cell [16,17]. In the transport of Li^+ it has been shown that the cellular level is established by a steady state between influx and efflux. The efflux process takes place against the concentration gradient of Li^+ and follows first-order kinetics equal to those which take place when yeast cells charged with Li^+ are transferred to a medium without Li^+ [17]. In the present paper we show that the efflux of Li^+ depends on the pro-

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Tricine, *N*-tris(hydroxymethyl)methylglycine; Mes, 2-(*N*—morpholino)ethanesulfonic acid.

tonmotive force, and we propose an electrogenic H^+/Li^+ antiport as a model for the process.

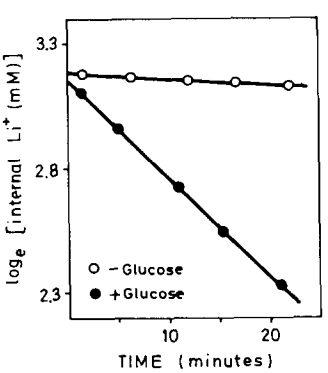
Material and Methods

The respiratory deficient strain of *Saccharomyces cerevisiae* 5252-32 D (his 4, ρ^-) was used in the present work. Growth of the yeast and analysis were performed as previously described [17], except that the medium was supplemented with 0.4 mg/l thiaminium dichloride, 0.4 mg/l pyridoxol hydrochloride and 20 mg/l L-histidine, and that addition of sorbitol was suppressed. In all cases cells were grown in the medium with 1 mM K^+ unless otherwise stated. The ATP content of the cells was determined by using the luciferine-luciferase method [10].

Results

The efflux of Li^+ from yeast to a Li^+-free medium (less than 10 μM) did not take place in the ρ^- strain when glucose was withdrawn from the medium (Fig. 1). By changing the glucose concentration, it was observed that the rate of Li^+ efflux varied concomitantly with the ATP content of the cell (Fig. 2). This relationship between Li^+ efflux and the ATP level was also observed when the ATP level was decreased by substituting arsenate for phosphate of the medium (data not shown).

The ATP dependence of Li^+ efflux resembled, in some ways, the ATP dependence of the H^+-pumping activity of the yeast cells [18]. To investigate whether an ATPase was involved in the Li^+ efflux, we studied the effect of ATPase inhibitors on this efflux. Diethylstilbestrol and *N,N'*-dicyclohexylcarbodiimide have been shown to inhibit the yeast plasma membrane ATPase strongly and also the H^+-pumping activity of yeast [18]. Fig. 3 shows that these compounds inhibited the efflux of Li^+ by about 65%, although they inhibited about 90% of



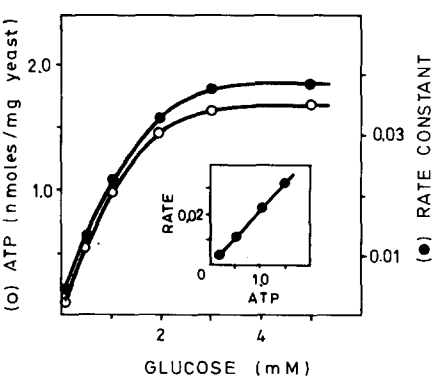


Fig. 1. Efflux of Li^+ from ρ^- yeast in the presence and absence of glucose. Cells were charged with Li^+ by incubation for 45 min with 100 mM Li^+, and then washed and transferred to a Li^+-free medium (pH 7.0) with and without glucose.

Fig. 2. Effect of glucose concentration on the ATP level and on the Li^+ efflux of ρ^- yeast cells. Conditions as in Fig. 1, except that the Li^+-free medium contained different amounts of glucose.

the H^+-pumping activity (data not shown). This suggests that the efflux of Li^+ may depend on the ATPase, but that the ATPase function can be substituted, at least partially, by other processes of the cell.

Studying the effect of uncouplers on Li^+ efflux to a Li^+-free medium, we found that the system was sensitive to the decrease in the cell pH (Fig. 4). 2,4-Dinitrophenol and NaN_3 at pH 4.5 inhibited Li^+ efflux, though they induced K^+ loss (dinitrophenol and NaN_3 did not affect the ATP content). The K^+ loss was supposed to be due to the uncoupling effect and shown to take place by a different porter than the Li^+ efflux. Other weak acids such as acetic, propionic and butyric acids also inhibited Li^+ efflux, as did dinitrophenol and NaN_3, but they did not induce K^+ loss. In ATP-depleted cells, dinitrophenol and NaN_3 also induced K^+ loss but not Li^+ loss. These data show that the efflux of Li^+ is independent of the efflux of K^+ induced by uncouplers, and also that the door opened for K^+ by dinitrophenol and NaN_3 is not available for Li^+. This corroborates the proposal of Peña [6] that the efflux of K^+ induced by dinitrophenol takes place through a component of the K^+ transport system of the cell. In fact, the K^+ carrier has a low affinity for Li^+ [13], and at the cellular levels of cations of our experiments (450 mM K^+, 25 mM Li^+), loss of Li^+ cannot be expected to take place through the K^+ carrier. With reference to the inhibition of Li^+ efflux by dinitrophenol, it is worth mentioning that effects of dinitrophenol on transport systems due to the decrease in the cell pH have been observed previously [19].

The uncoupler CCCP at pH 7.0 inhibited the efflux of Li^+ to a Li^+-free medium very slightly, but when added in uptake experiments on Li^+, we obtained very little information because CCCP affected the influx and the efflux simultaneously. As previously shown [17], influx of Li^+ takes place through the K^+ carrier and efflux takes place through the system we are studying. In order to clarify the effects of CCCP on the movements of Li^+, we designed some experi-

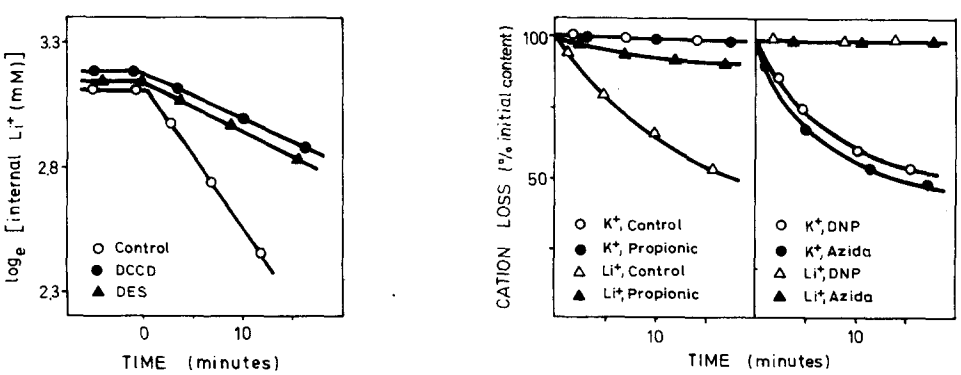


Fig. 3. Effect of dicyclohexylcarbodiimide (DCCD) and diethylstilbestrol (DES) on the efflux of Li^+. Cells charged with Li^+ as in Fig. 1 were transferred to Li^+-free medium (pH 7.0) with 200 mM K^+, without glucose and with the drug (150 μM dicyclohexylcarbodiimide, 50 μM diethylstilbestrol); after incubation (20 min for dicyclohexylcarbodiimide, 5 min for diethylstilbestrol) glucose (2%) was added (time zero). K^+ was included to avoid K^+/H^+ exchange in the presence of diethylstilbestrol.

Fig. 4. Efflux of Li^+ and K^+ in the presence of propionic acid (5 mM), dinitrophenol (DNP) (500 μM) and NaN_3 (Azida) (5 mM) at pH 4.5. Experiments were carried out as in Fig. 1 with glucose.

ments in which Li^+ moved only through the efflux system. In the presence of 200 mM external K^+, influx of Li^+, at an external concentration of 50 mM, was inhibited (Fig. 5) as a consequence of competition of both cations for the K^+ carrier [13,15]. Under these conditions (200 mM K^+, 50 mM Li^+), addition of CCCP induced a rapid increase in Li^+ content. As expected, this influx of Li^+ took place through the Li^+ efflux system because it was prevented by lack of glucose (ATP depletion) (Fig. 5). The fact that CCCP brought about 'downhill' inward movements through the Li^+ efflux system shows that this movement is electrogenic and requires depolarization of the membrane (the term electrogenic is employed here in the same sense as used in Ref. 20).

Addition of CCCP in efflux experiments inhibited partially the initial rate of Li^+ efflux, but inhibited only very slightly the total loss in 20 min (Fig. 6) (200 mM K^+ stimulated Li^+ efflux, compare Fig. 4 and Fig. 6; see Ref. 17). Taking into account that yeast cells have the capacity to compensate for positive charges as they move inward (i.e., K^+ transport and H^+ symport processes), the hypothesis of an electrogenic transport for the efflux of Li^+ can be completed with the idea that in this efflux a positive charge moves inward (or a negative charge outward). In the presence of CCCP, the depolarized membrane would allow both downhill influx and downhill efflux through the efflux system, but in the absence of uncoupler the influx would be impossible since it would involve a positive charge moving outward that could not be compensated. The existence of a membrane potential, negative inside, would further prevent the influx but would help the efflux. The inhibition of the initial rate of Li^+ efflux by CCCP (Fig. 6) is in accordance with the proposed model because it would be a consequence of the depolarization of the membrane.

To investigate whether H^+ movement takes part in the efflux of Li^+, it was necessary to depolarize the membrane without involving H^+ permeability and, therefore, we tried to induce free movements of K^+ through the membrane.

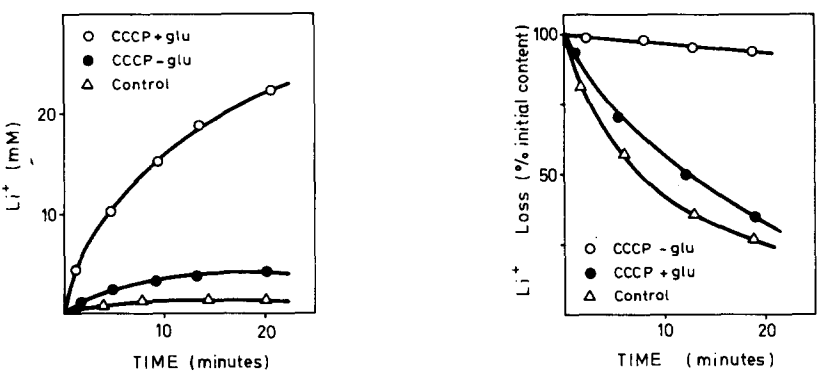


Fig. 5. Entry of Li^+ in ρ^- yeast induced by CCCP (150 μM) in the presence of 200 mM K^+. Cells were suspended in culture medium (pH 7.0) containing 200 mM K^+ and 50 mM Li^+, and the Li^+ content was analyzed as various times. In the control, the medium contained glucose (glu). In the presence of CCCP, experiments were carried out with and without glucose.

Fig. 6. Effect of CCCP (150 μM) on the efflux of Li^+ in the presence of 200 mM K^+. Cells charged with Li^+ as in Fig. 1 were suspended in culture medium (pH 7.0) with 200 mM K^+. In the control, the medium contained glucose (glu). In the presence of CCCP, experiments were carried out with and without glucose.

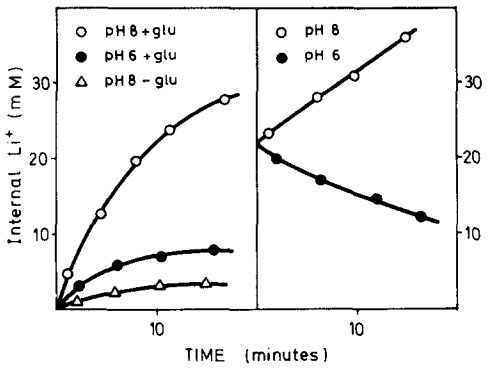


Fig. 7. pH dependence of Li^+ movement in the presence of EDTA and 200 mM K^+. (A) Cells were suspended in Tricine (5 mM)/Mes (5 mM) buffer, pH 6.0 and 8.0, with 200 mM K^+, 50 mM Li^+ and 2 mM EDTA, with or without glucose (glu). (B) Cells were charged with Li^+ as in Fig. 1 and then suspended as in A with glucose and 20 mM Li^+.

Valinomycin and gramicidins did not induce Li^+ influx and we carried out the experiments in the absence of Mg^{2+}. It has been shown previously that in the absence of Mg^{2+}, cells equilibrate alkali cations on both sides of the membrane, supposedly through a K^+ uniporter [21]. It can be presumed, therefore, that in the presence of EDTA and 200 mM K^+ (theoretical $\Delta\psi$ about 20 mV) the efflux of Li^+ would respond to the external pH if H^+ were transported with Li^+. Fig. 7 shows that this was the case. Under conditions in which no physiological anions were present in the medium and therefore inward movement of anions could be discarded, the rate of Li^+ entry was dependent on the external pH. Furthermore, when the cells were suspended in a medium with a Li^+ concentration similar to that of the cell, 'uphill' influx or efflux took place in response to the external pH. Again, these movements of Li^+ did not take place in the absence of glucose, showing that they occurred through the efflux system.

Discussion

The role of ΔpH in the movements of Li^+ in presence of EDTA and 200 mM K^+ shows that H^+ moves in the opposite direction to Li^+. Thus, an alkaline pH in the medium drove uphill influx, and an acidic pH in the medium drove uphill efflux (Fig. 7). Taking into account that the movement of Li^+ should be electrogenic (see above), the most obvious hypothesis that could give a satisfactory explanation to the above observations is an electrogenic H^+/Li^+ antiport. In this hypothesis, the partial inhibition of Li^+ efflux by diethylstilbestrol and dicyclohexylcarbodiimide can be explained by the necessity for eliminating an excess of positive charge during the electrogenic H^+/Li^+ antiport. This could take place through several transport systems, but the extrusion of H^+ by an ATPase may be the natural mechanism for this function [18]. Therefore, the inhibition of the H^+-extruding ATPase can explain the effect of diethylstilbestrol and dicyclohexylcarbodiimide on the Li^+ efflux.

In yeast, Li^+ is taken up by the same porter as K^+ [13], which is exchanged

for H^+ [1,2], suggesting that Li^+ is also exchanged by H^+. If we consider that influx and efflux of Li^+ occur simultaneously [17], in the hypothesis of an H^+/Li^+ antiport for the efflux, the reason why the transport of Li^+ does not result in uncoupling could arise from the fact that Li^+ efflux is low compared to the fluxes of other ions, as demonstrated in bacteria [22]. At 100 mM internal Li^+ (an extremely high internal Li^+ concentration at which Li^+ is toxic, see Ref. 23), the rate of efflux of Li^+ would be lower than 5 nmol/min per mg, whereas an activity of 30—60 nmol/min per mg has been measured for the H^+ pump [18]. A point to be considered in relation to uncoupling is the situation in starved cells. If no regulation exists, ATP-depleted cells suspended in water should lose Li^+ in exchange for H^+ and the excess charges would be neutralized by the loss of K^+ (coupling of the electrogenic H^+/Li^+ antiport to a K^+ uniport). This efflux of K^+ in response to a positive charge accumulation is well documented (for a review see Ref. 9). These losses have not been observed, and the ATP dependence of Li^+ efflux could explain this fact. The role of ATP in the efflux of Li^+ (Fig. 2) is more easily understood as a regulator of the process than as a fuel. In fact, there is no point in considering ATP as a fuel for the transport of Li^+ through the Li^+ efflux system since ATP is necessary, both for inward or outward movements, though ATP is always inside. The regulation by ATP makes it clear that in starvation, the activity of the efflux system is controlled and, consequently, an unnecessary loss of cations is prevented.

The existence of a transmembrane electrical potential, negative inside, in yeast and its role in transport are in increasing evidence [4—7,9,10]. The present results support this hypothesis. If an electrical potential exists, an electrogenic antiport for the efflux of Li^+ fulfils the theoretical requisites that its function requires. Driven by the protonmotive force, the porter could pump Li^+ out of the cell in a diversity of environmental conditions. At acidic pH values, in which $\Delta\psi$ could be low, as it is in other eukaryotic micro-organisms [24—26], the driving force would be ΔpH, and at alkaline pH values, in which ΔpH is pressing in the opposite direction, the driving force would be $\Delta\psi$. The sensitivity of the system to the decrease in the cell pH points to a simultaneous control of the cell pH and cation content. Under conditions of low cellular pH the system would not exchange Li^+ for H^+ and the cell would retain more Li^+ and less H^+. It is worth mentioning that competition between H^+ and Li^+ for the efflux system could give an explanation for this fact.

The mechanism for the efflux of Li^+ proposed in this paper (Fig. 8) is not limited to this cation but seems to be a general mechanism for the efflux of other alkali cations including K^+, though the latter cation shows a lower efflux rate than those of Li^+ and Na^+ (unpublished results).

The cooperation of cation-proton antiport systems in the control of cell

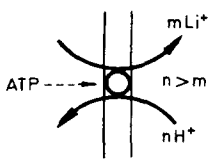


Fig. 8. Model of the Li^+ efflux system. The dashed line indicates interaction with the system.

cation content, and probably cell pH, suggests the generality of these mechanisms, since they have been previously described in bacteria [27].

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