Biochimica et Biophysica Acta, 552 (1979) 322—330
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BBA 78326

CATION EXCHANGES OF YEAST IN THE ABSENCE OF MAGNESIUM

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(Received August 1st, 1978)

Key words: Cation exchange; Transport control; Antiport mechanism; Mg^{2+}; (Yeast)

Summary

Environmental Mg^{2+} was found to influence the K^+/Na^+ exchange rate of metabolizing yeast. Addition of EDTA increased the exchange rate and Mg^{2+} reversed the effect of EDTA.

Yeast starved in the absence of Mg^{2+} exchanged cellular K^+ or Na^+ for external H^+ when maintained at acidic pH. The exchange rate depended on cellular pH and showed the same kinetics for both K^+ and Na^+. At acidic pH, the presence of external cations neither inhibited H^+ absorption nor changed the cation/H^+ 1 : 1 stoichiometry. At neutral pH, external cations inhibited H^+ influx but did not change the cation efflux.

The K^+/Na^+ exchange is discussed as electrically coupled and the K^+/H^+ and Na^+/H^+ exchanges as electroneutral antiports.

Introduction

Yeast cells exchange K^+ for cellular H^+ [1,2], accumulating K^+ against steep gradients [3]. In media containing both Na^+ and K^+ the latter is preferentially accumulated [4], and if the yeast has been previously charged with Na^+, Na^+ is exchanged for K^+, even against chemical potentials [5]. In addition to these heterologous exchanges, the cell maintains a considerable K^+/K^+ exchange, and net gain or loss of K^+ can be explained as a result of the influx and efflux processes determined by the described exchanges [6].

The kinetics of these cation exchanges in yeast have been extensively studied [4,7,8], but there is still controversy about the mechanism. Conway et al. [5] and Ryan and Ryan [9] have proposed that two systems mediate the exchanges, while Rothstein [10] considers it unnecessary to postulate more than one cation transport system.

In the present paper we show that Mg^{2+} affects cation exchanges in yeast,

and that in the absence of Mg^{2+} a two transport system hypothesis gives a better explanation of the observed exchanges.

Materials and Methods

Washed commercial baker's yeast (Ebro Co. Azúcares y Alcoholes, S.A., Córdoba) was given the following sequential treatment, at 1/20 yeast concentration, with 10 mg/l tetracycline, and under agitation: (i) 3 h in 0.5% yeast extract/0.5% peptone/5% glucose/20 mM KH_2PO_4, pH adjusted to 4.5; (ii) 2 h in 50 mM potassium or sodium phosphate buffer, pH 7.5 unless otherwise stated/5% glucose; (iii) 18 h as in step ii but without glucose. The procedure was generally carried out in the absence of Mg^{2+} but in some experiments Mg^{2+} was included in steps ii and iii. In some experiments arsenate instead of phosphate was used in steps ii and iii. Cells from step i will be referred to as standard yeast, and after all the treatment, when Mg^{2+} was not added, will be referred to as Mg^{2+}-depleted starved yeast. Cells prepared in potassium buffer contained only K^+ and the cells prepared in sodium buffer almost only Na^+; these will be referred to as K^+-yeast and Na^+-yeast respectively. Strain X2180.1A from the Yeast Genetic Stock Center was used for comparative purposes in some experiments. All the experiments were carried out at 28° C. 2 mM $MgSO_4$ was used in all the experiments carried out in the presence of Mg^{2+}.

Cellular cations were extracted as previously described [11]. Cations in cell extracts and in supernatants were determined by atomic absorption spectrophotometry.

The absorption of H^+ was evaluated by maintaining the pH at a constant value by continuous addition of a HCl solution, with the aid of a Beckman Expandomatic SS-2 pH meter. Recordings of pH were obtained with a Radiometer PHM 62 pH meter and a REC 61 recorder.

Titration of internal H^+ was achieved by treating centrifuged cells with 500 mM NaCl, 9% *n*-butanol (by volume) at 40°C for 5 min. The cooled suspension was titrated with HCl or NaOH.

The NaCl used in K^+ efflux experiments was Suprapur quality (Merck) with less than $5 \cdot 10^{-4}$% K^+.

Results

Cation exchanges in the absence of Mg^{2+}

When Mg^{2+} was not added to the external medium, standard yeast suspended in a high sodium buffer substituted Na^+ for cellular K^+, showing a high K^+/Na^+ exchange rate (50% substitution in about 10 min). Addition of EDTA increased the exchange, and addition of Mg^{2+} practically blocked the exchange when added in absence of EDTA or at a concentration that saturated the added EDTA. The effect caused by the lack of Mg^{2+} was observed before the cell had lost a significant amount of Mg^{2+}; in the presence of EDTA (2 mM), at pH 8.0, less than 5% of cellular Mg^{2+} was lost in 15 min.

The described K^+/Na^+ exchange was also observed with the strain X2180.1A, although it took place, at a significant rate, only in the presence of EDTA at pH 8.0 (Fig. 1).

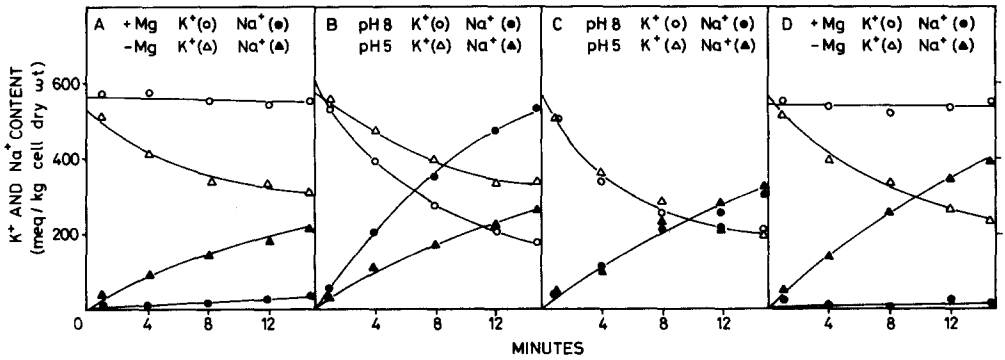


Fig. 1. Variation of the K^+ and Na^+ content of standard yeast suspended in sodium phosphate buffer: (A) at pH 7.0 with added Mg^{2+} and without added Mg^{2+}; (B) without Mg^{2+}, at pH 8.0 with 2 mM EDTA and at pH 5.0 with 20 mM EDTA; (C) without Mg^{2+}, cells treated with antimycin (18 μg/ml) and 2-deoxyglucose (5 mM), at pH 8.0 and pH 5.0; (D) strain X2180.1A, with Mg^{2+}, and without Mg^{2+} in the presence of 2 mM EDTA. Washed cells were suspended (1.5 mg dry wt./ml) at zero time in 50 mM sodium phosphate buffer plus NaCl to make the solution 200 mM in Na^+. In experiment C cells were preincubated for 10 min in 20 mM potassium phosphate buffer (pH 4.5) plus the drugs.

Conway and Moore [12] prepared Na^+-yeast by keeping the yeast fermenting for several hours in 0.2 M sodium citrate (Na^+ substituted for half of the K^+ content in 2 h). Kotyk and Kleinzeller [13] prepared Na^+-yeast by suspending the yeast in a solution of NaCl, but they obtained a lower degree of substitution than did Conway and Moore [12]. Our present results are in general agreement with those of the above-cited authors. The differences can be explained by differences between strains, yeast preparation methods and the availability of Mg^{2+} in the medium.

The K^+/Na^+ exchange was not energy dependent, since it was not inhibited by 2-deoxyglucose and antimycin A, although these drugs did inhibit the transport of Na^+ that was not exchanged for K^+. Thus, at pH 8.0, untreated cells took up more Na^+ than the K^+ lost, while in treated cells K^+ loss and Na^+ gain were equivalent (Fig. 1). This result is a consequence of the fact that antimycin and deoxyglucose stop energy metabolism and so prevent H^+ ejection [14]; an excess Na^+ gain would need an equivalent amount of H^+ loss.

Starvation of the yeast in phosphate buffer, in the absence of Mg^{2+}, drastically changed its cation exchange capacity. Thus, Mg^{2+}-depleted starved yeast showed a very low K^+/Na^+ exchange but showed a high H^+ influx when it was suspended in water and the pH was controlled by HCl (Fig. 2). The absence of Mg^{2+} during the starvation was essential to obtain a high H^+ influx, and starved yeast in the presence of Mg^{2+} neither exchanged K^+ for Na^+ nor absorbed H^+ when suspended in water (Fig. 2).

Depletion of cellular Mg^{2+} during starvation, and not the lack of external Mg^{2+}, proved to be the cause of the H^+ influx, since the addition of Mg^{2+} after starvation did not decrease H^+ absorption of Mg^{2+} depleted yeast. Also, addition of EDTA to the water did not increase the H^+ absorption of cells starved in the presence of Mg^{2+}. The H^+ influx took place in exchange for the cellular cation (K^+ in K^+-yeast and Na^+ in Na^+-yeast), and it was sensitive to high levels of KCl and NaCl in the water (Fig. 2). The efflux of the cation was able to

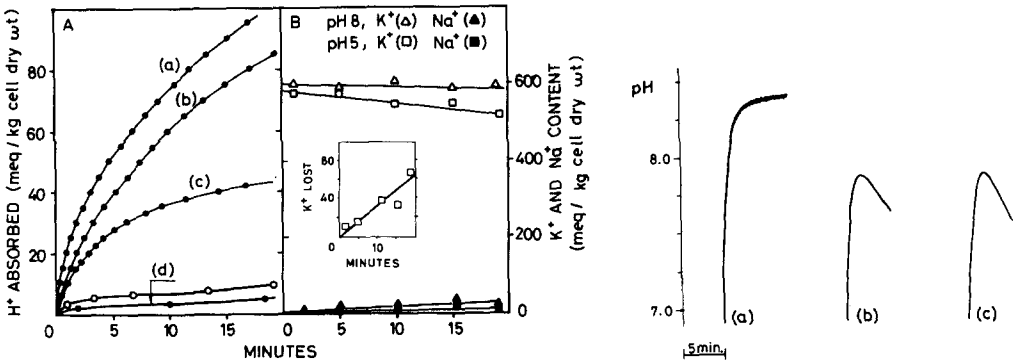


Fig. 2. Cation exchanges by yeast starved in potassium phosphate buffer. A. H^+ absorption in water at pH 5.0 by cells starved in the presence of Mg^{2+} (○), and H^+ absorption by Mg^{2+} depleted starved cells (●): (a) at pH 5.0 in water, (b) at pH 5.0 in 200 mM NaCl, (c) at pH 8.0 in water, (d) at pH 8.0 in 200 mM NaCl. B. Variation of the K^+ and Na^+ content of Mg^{2+} depleted starved yeast suspended in sodium phosphate buffer. In A, washed cells were suspended (1.5 mg dry wt./ml) as stated and the pH was controlled by addition of HCl. In B, conditions were as described in Fig. 1.

Fig. 3. pH changes in the suspension of Mg^{2+} depleted starved yeast (K^+-yeast) (a) in water, (b) in 200 mM NaCl, and (c) in 200 mM KCl. Washed cells were suspended and then the pH was recorded.

draw H^+ against its chemical potential, and when the medium pH was not controlled it reached values above 8.5. In the presence of high levels of KCl and NaCl, the external pH did not reach such a high value and showed a transient overshooting (Fig. 3).

The K^+/Na^+ exchange seemed to be independent of the K^+/H^+ exchange, since Mg^{2+} depleted starved yeast exchanged K^+ for H^+ but not K^+ for Na^+. A similar conclusion could be reached with standard yeast, since it did not show H^+ absorption when suspended in water at pH 5.0 although the cells, preincubated with deoxyglucose and antimycin (10 min), were suspended in water in the presence of EDTA (20 mM) and the drugs.

K^+/H^+ and Na^+/H^+ exchanges in Mg^{2+} depleted starved yeast

The time course of H^+ absorption by cells starved in potassium or sodium phosphate buffer and maintained at a constant pH showed a rapid initial rate that slowed down progressively with time, with no quantitative differences between K^+- and Na^+-yeast. The extension of the H^+ absorption and the initial rate were dependent on the pH of the buffer used for the preparation of the yeast, both increasing with pH (Fig. 4). The rate of exchange was stimulated at acidic pH values, but no great differences were found in the 3.0—4.0 pH range. High levels of KCl and NaCl, which inhibited H^+ influx at pH 5.0 and 8.0, did not produce significant effects at more acidic pH values, i.e., the inhibition was undetectable at pH 3.5 (Fig. 4 and compare with Fig. 2). The influx pattern was the same when arsenate was used for the starvation instead of phosphate buffer, showing that phosphate energy was not involved in the exchange.

The H^+ added to control the pH of the yeast suspension was readily absorbed by the yeast. The loss of anions with p*K* values higher than the pH of the suspension, which could have neutralized the H^+ added, was very low, as can be

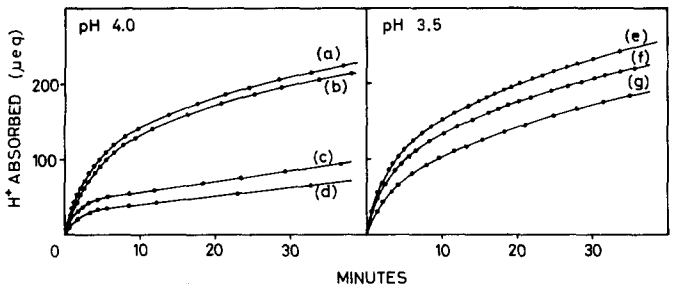
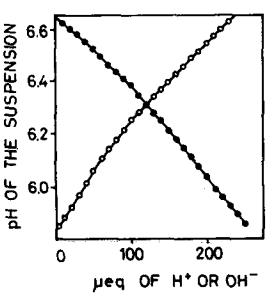


Fig. 4. Time course of H^+ absorption by Mg^{2+} depleted starved yeast at pH 4.0 and at pH 3.5; (a) in water, K^+-yeast starved at pH 7.5; (b) in water, Na^+-yeast starved at pH 7.5; (c) in water, K^+-yeast starved at pH 6.5; (d) in water, K^+-yeast starved at pH 5.5; (e) in water, K^+-yeast starved at pH 7.5; (f) in 100 mM KCl, Na^+-yeast starved at pH 7.5; (g) in 500 mM NaCl, K^+-yeast starved at pH 7.5. Washed cells (1.5 g) were suspended in 150 ml water and the pH was controlled by addition of HCl.

deduced from the following observations (Fig. 5): (i) cells that had been kept for 40 min at pH 4.0 and then treated with butanol, gave lower pH values than those directly treated; (ii) the amount of base needed to bring the pH of cells treated after 40 min at pH 4.0 to that of cells treated at time zero was only slightly lower than the amount of acid needed to perform the opposite operation; and (iii) the amount of acid needed to maintain the pH during the 40 min incubation was between the two amounts cited in ii.

In agreement with previous reports [10], the H^+ inflow described was due to the exchange of H^+ for K^+ or Na^+ with an approximately 1 : 1 stoichiometry (Fig. 6), independent of the pH of the suspension in the 3.0 to 5.0 pH range. At pH values of 4.0—5.0, low levels of Na^+ or K^+ (10 mM Na^+ in K^+-yeast and 10 mM K^+ in Na^+-yeast) did not affect the stoichiometry, and at pH values lower than 4.0 the stoichiometry was not significantly affected even with high levels of K^+ or Na^+ (100 mM) (Fig. 6). However, high levels of the cations at more neutral pH values did affect the stoichiometry. Thus, at pH 6.6, 500 mM



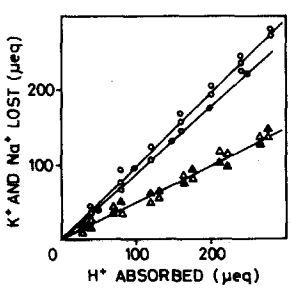


Fig. 5. Titration of Mg^{2+} depleted starved yeast (1.5 g dry wt.) after butanol treatment. •, titrated at time zero; ○, titrated after 40 min in water at pH 4.0. Conditions were as described in Fig. 4. After 40 min in water the yeast had absorbed 250 μequiv. of H^+.

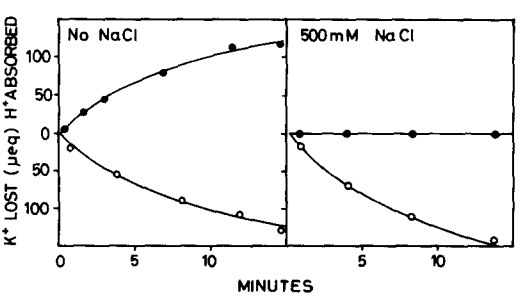
Fig. 6. Stoichiometry of the K^+-Na^+/H^+ exchange. K^+ and Na^+ lost were plotted versus H^+ absorbed. Cells were maintained as stated in Fig. 4, and the K^+ and Na^+ lost were measured in the liquid after removing the cells by filtration. ○, K^+-yeast in water at pH 4.0; •, K^+-yeast in 100 mM NaCl at pH 3.5; △, K^+ lost, and ▲ Na^+ lost, by a (K^+ + Na^+)-yeast (50% of each cation) in water at pH 4.0.

NaCl completely inhibited net H^+ movements, but the cell continued losing K^+ (K^+-yeast) at a rate close to that of the K^+/H^+ exchange observed in the absence of NaCl and at the same pH (Fig. 7). The loss of K^+ in these conditions took place in exchange for Na^+, but the exact stoichiometry was difficult to establish because the net accumulation of Na^+ was low and, under our experimental conditions, the presence of high amounts of Na^+ in the envelopes masked the Na^+ content of the cell.

Cells prepared with K^+ and Na^+ in the starvation buffer acquired the same cation content ratio as the buffer. These cells, when kept at pH 4.0 in water, did not show any difference from cells containing only K^+ or only Na^+ and lost the cations without discrimination between them. In all cases, the loss of K^+ plus Na^+ was equivalent to the H^+ absorbed (Fig. 6). It must be indicated, however, that during the time spent in adjusting the suspension pH to 4.0, the loss of Na^+ was greater than the loss of K^+, indicating that at alkaline pH values Na^+ efflux was greater than K^+ efflux.

The decreasing rate of the exchange of K^+ (or Na^+) for H^+ with time (Fig. 4) was not due to the dissipation of the transmembrane gradients of K^+ (or Na^+) and H^+ during the experiment, since the transfer of cells that had been kept at pH 4.0 for some time to cation-free water at pH 3.0 did not increase the rate. From kinetic considerations, the decrease in K^+ content of the cell would have been the factor determining the decreasing rate of K^+/H^+ exchange. However, it seems not to be the case, since the rate decreased more rapidly than expected on the basis of a first order kinetics for the K^+ loss (Fig. 8), and the rate proved to be also dependent on cellular pH. The different exchange rates observed between cells starved at different pH values (Fig. 4) could be related to the pH value of the butanol treated yeast (Table I). More alkaline cells always showed a higher H^+ absorption rate than more acidic ones, and after 40 min at pH 4.0 all the preparations showed similar cellular pH and H^+ absorption rates. However, analysis of H^+ absorption as a function of cellular K^+ and H^+ concentrations did not follow a simple kinetic model.

The presence of Mg^{2+} during starvation, which determined a low H^+ absorption by these cells, also determined a lower cellular pH than that of cells



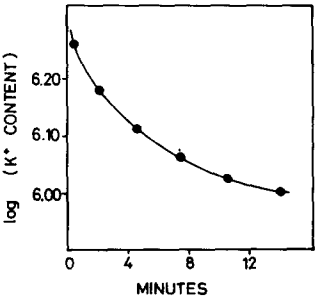


Fig. 7. K^+ loss and H^+ absorption by Mg^{2+} depleted starved yeast at pH 6.6 in water and in 500 mM NaCl. Conditions were as described in Fig. 4 and Fig. 6.

Fig. 8. Plot of the logarithm of the K^+ content versus time of Mg^{2+} depleted starved yeast when suspended in water at pH 4.0. The K^+ content at times was deduced from the initial K^+ content and the H^+ absorbed. Conditions were as described in Fig. 4.

TABLE I

K^+ CONTENT, CELLULAR pH AND H^+ INFLUX RATES OF K^+-YEAST STARVED IN THE PRESENCE OF Mg^{2+} AND IN ITS ABSENCE, WHEN IT WAS MAINTAINED IN WATER AT pH 4.0

Conditions as were described in Fig. 4.

Starvation	Zero time			40 min		
	K^+ content (mequivalent per kg cell dry wt.)	pH of cells by butanol treatment	H^+ influx (mequivalent per kg per min)	K^+ content (mequivalent per kg cell dry wt.)	pH of cells by butanol treatment	H^+ influx (mequivalent per kg per min)
pH 7.5, $+Mg^{2+}$	580 ± 40	6.10 ± 0.05	3.6 ± 0.8	—	—	—
pH 7.5, $-Mg^{2+}$	590 ± 50	6.60 ± 0.05	35.3 ± 3.0	430 ± 40	5.75 ± 0.05	1.35 ± 0.09
pH 6.5, $-Mg^{2+}$	510 ± 40	6.00 ± 0.05	15.5 ± 1.6	440 ± 30	5.65 ± 0.05	0.74 ± 0.08
pH 5.5, $-Mg^{2+}$	450 ± 50	5.90 ± 0.05	9.0 ± 0.9	400 ± 40	5.50 ± 0.05	0.62 ± 0.08

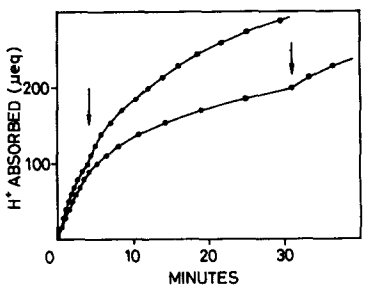


Fig. 9. Effect of 2,4-dinitrophenol on the H^+ absorption by Mg^{2+} depleted starved yeast. The arrows indicate the times at which the drug (1 mM) was added. Conditions were as described in Fig. 4.

starved without Mg^{2+}. However, by comparing cellular pH values and rates of H^+ absorption of yeast starved in Mg^{2+} and those starved at different pH values in the absence of Mg^{2+}, it seems clear that the lower cellular pH is not the only factor determining the low H^+ absorption (Table I).

Addition of the uncouplers 2,4-dinitrophenol or carbonylcyanide *m*-chlorophenylhydrazone to Mg^{2+} depleted starved yeast increased the H^+ absorption in exchange for K^+ or Na^+. This increase in the rate was higher just after addition (Fig. 9).

Discussion

The controlling effect of Mg^{2+} on the permeability of mitochondrial [15] and erythrocyte [16,17] membranes is well documented. Present results point to an essential role of Mg^{2+} in maintaining the impermeability of yeast to alkali cations. It is unlikely that cations other than Mg^{2+}, which could have been added as impurities in the Mg^{2+} salt, could be responsible for the cell impermeability because when EDTA and an excess of Mg^{2+} were added together (2 mM Mg^{2+}, 1 mM EDTA) cells were impermeable. In fact, the Mg^{2+}-EDTA complex is much weaker than are such complexes of other cations such as Fe^{3+}, Zn^{2+}, Mn^{2+}, Co^{2+} or Ca^{2+} [18] and small amounts of any of these cations should be

complexed even in the presence of Mg^{2+}. The mechanism by which Mg^{2+} maintains the high impermeability is out of the scope of the present work but it must be pointed out that, in standard yeast, only the lack of external Mg^{2+}, and not depletion of cellular Mg^{2+}, was the cause of the high level of K^+/Na^+ exchange.

The K^+/Na^+ exchange of standard yeast in the absence of Mg^{2+} could be explained by the independent, electrically coupled exit of K^+ and entrance of Na^+. In fact, yeast cells appear to be permeable to K^+. K^+ influx seems to be dissociated from H^+ ejection in K^+ transport [19] and the efflux of K^+ is the source of energy that draws H^+-cotransported substrates in ATP depleted yeast [20–23]. It is conceivable that if K^+ moves out of the cell, Na^+ could move into the cell, although both movements may have different kinetics. The lack of Mg^{2+} may have increased the natural fluxes. An electrical coupling for the K^+/Na^+ exchange is also compatible with the insensitivity of the process to deoxyglucose and antimycin, but these results do not rule out the presence of a K^+/Na^+ electroneutral antiporter.

With reference to the mechanism of the K^+/H^+ and Na^+/H^+ exchanges observed in Mg^{2+} depleted starved yeast there are two possible hypotheses: (i) an electrical coupling, K^+ (or Na^+) moving out by the same mechanism as that described above and a leak of H^+ induced by the starvation; and (ii) an electroneutral cation/H^+ antiport. The results reported here strongly favour the antiport hypothesis. The results presented in Fig. 6 show that NaCl did not modify the stoichiometry of the K^+/H^+ exchange in spite of Na^+ having a favourable gradient and being able to move through the membrane, as can be deduced from the Na^+/H^+ exchange of Na^+-yeast. These data agree with the concept of an antiporter, in which the influx of Na^+ (H^+/Na^+ antiport) and the efflux of K^+ (K^+/H^+ antiport) will depend on the cations and H^+ concentrations in the corresponding side of the membrane. Since the external H^+ concentration was 100 times higher than the internal, and the concentrations of external Na^+ and internal K^+ were very similar, the influx of Na^+ can be predicted to be insignificant in comparison with K^+ efflux. The results in Fig. 7 can also be explained by the electroneutral antiport: high levels of Na^+ outside would not stimulate the K^+ efflux observed at neutral pH, because the rate of the efflux would be determined by the K^+ and H^+ concentrations. In the case of no net H^+ movement (Fig. 7) the exchange of K^+ for Na^+ would be the result of the coupling of both antiports (K^+/H^+ and H^+/Na^+); although some insignificant exchange by electrical coupling cannot be rejected.

It is difficult to estimate the significance of the K^+/H^+ and Na^+/H^+ exchanges in relation to the overall transport of cations in the growing yeast. However, it seems more probable that they correspond to natural exchanges increased by the lack of Mg^{2+}, rather than ionophore-mediated exchanges created de novo. Indeed, the efflux system of cations in yeast has some similarities with the cation/H^+ exchanges described here. Thus, Ryan and Ryan [9] reported that the Na^+ efflux is inhibited by the decrease of the cellular pH, as we have found in the Na^+/H^+ exchange, and Rothstein [10] reported that the Na^+ efflux system has a low discrimination between K^+ and Na^+, as we have found in the K^+/H^+ and Na^+/H^+ exchanges (Fig. 6). Quantitative differences in the discrimination between K^+ and Na^+ reported by Rothstein [10] and those reported here

could be explained by differences in the experimental conditions.

The idea of an antiporter for the efflux of cations is not a novel one, since antiport mechanisms for this function has been postulated in *Streptococcus faecalis* [24], *Escherichia coli* [25] and *Halobacterium halobium* [26].

Acknowledgments

We thank Mrs. M.C. Ruiz for her assistance in many of the experiments. This work was financially supported by Fondo Nacional para el Desarrollo de la Investigación Científica.

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