THE MECHANISM OF SODIUM EFFLUX IN YEAST

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1. Introduction

Yeast cells accumulate K^+ against high transmembrane concentration gradients but accumulate little Na^+, except in very high [Na^+] media [1]. Na^+ enters into the cell through the K^+ carrier and possibly also through Na^+–substrate symporters, as described for phosphate [2]. If Na^+ can enter into the cell, some mechanisms must exist to exclude the cation and avoid its accumulation. In [3] a Na^+-pump was described that could pump out Na^+ against a transmembrane concentration gradient. Other authors have also studied the efflux movement of Na^+ in yeast [4–8] but the mechanism involved in the pumping-out process has not yet been established. The hypothesis of a redox pump [4] has not been confirmed, and even the independence of the Na^+-pump from the K^+ carrier remains in discussion [8].

We have described the accumulation of Li^+ in yeast [9] and proposed that the mechanism for the efflux of this cation is an electrogenic Li^+–H^+ antiporter [10]. Here, we report that the mechanism for Na^+ efflux is similar to that of Li^+ efflux.

2. Materials and methods

2.1. *Growth of yeast*

The respiratory deficient strain 5252-32D (*his4*, ρ^-) of *Saccharomyces cerevisiae* was used here. The growth medium was a slight modification of that in [11]. In brief, the basal medium was 25 mM $(NH_4)_2HPO_4$, 5 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.2 mM $CaCl_2$ (pH 7.0) plus histidine (24 μg/ml), glucose, vitamins and trace elements [11]. To this basal medium KCl was added as required.

2.2. *Na^+ content of the cells*

Cultures were collected in membrane filters, washed with 20 mM $MgCl_2$, transferred to a new filter and washed again. Filters with cells were extracted with 0.2 M HCl, 10 mM $MgCl_2$ for 24 h and Na^+ and K^+ were analysed by atomic absorption.

3. Results

When Na^+ was added to the medium in which yeast cells were growing, Na^+ was taken up by the cells and the internal [Na^+] increased to reach a steady value which was lower than that of the medium (control in fig.1A). A kinetic analysis of the accumulation [9]

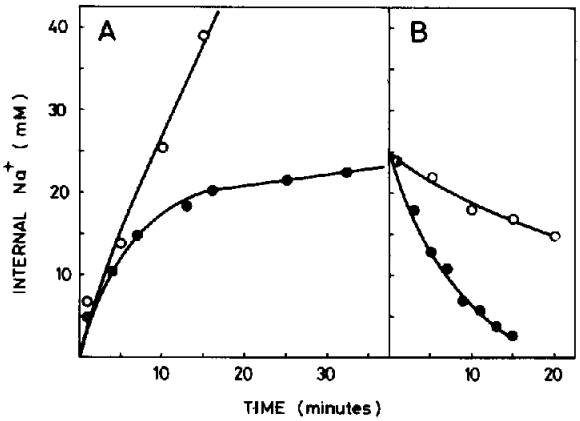

Fig.1. Effect of *p*CMBS on Na^+ fluxes. (A) To cultures in 1 mM K^+ (~0.2 mg dry wt/ml) NaCl was added to make the solution 50 mM Na^+ in the presence (o——o) and absence (•——•) of *p*CMBS (70 μM); at times samples were taken and the Na^+ content of cells were determined. In (B), cultures as in (A) were charged with Na^+ by making 50 mM Na^+ the culture medium and incubating for 45 min, then the cells were transferred to the same culture medium without Na^+, in presence (o——o) and absence (•——•) of *p*CMBS (70 μM); at times the Na^+ content of the cells were determined.

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showed that it was the result of a constant influx and an efflux with first-order kinetics. Efflux with first-order kinetics was observed up to the highest internal [Na^+] studied (60 mM). Analysis of the data in control experiments of fig.1 as in [9] gave kinetic constants of 0.12 min^{-1} and 0.14 min^{-1} in the accumulation and the zero–trans experiments, respectively. On the average, the kinetic constant of Na^+ efflux was 0.11 ± 0.02 min^{-1}. This efflux of Na^+ was inhibited by 75% by *p*-chloromercuribenzenosulphonate (*p*CMBS) without affecting the influx and, as a consequence, in presence of the drug, Na^+ accumulated nearly at a constant rate (fig.1).

The efflux of Li^+ in yeast may be mediated by an electrogenic antiporter with H^+ [10]. To investigate the dependence of the efflux of Na^+ on ΔpH and $\Delta\Psi$, we studied the movements of Na^+ through the efflux system at pH 8.0 when the membrane had been depolarized with high K^+ in the absence of Mg^{2+}, to make the membrane permeable to K^+ [12]. In [10] cells were treated with ethylene diamine tetraacetate (EDTA) but in presence of EDTA, *p*CMBS did not inhibit efflux. Therefore, here we used 50 mM citrate to complex Mg^{2+}. In the presence of 200 mM K^+, at pH 8.0 with Mg^{2+} and at pH 6.0 without Mg^{2+}, addition of *p*CMBS increased the rate of Na^+ accumulation, as a result of Na^+ efflux inhibition (fig.2). However, at pH 8.0, 200 mM K^+ and without Mg^{2+}, addition of *p*CMBS did not affect the initial rate of Na^+ accumulation. Furthermore, the accumulation of Na^+ in the latter conditions (fig.2C) lasted ~25 min; thereafter the cells lost Na^+. Addition of *p*CMBS at 25 min suppressed the loss of Na^+ (not shown). This change in the direction of the net transport can be explained by the change of the transmembrane potentials that drive the efflux process. In fact, during the first 25 min, cells increased their K^+ content from ~300–>400 mM and Na^+ from 0–90 mM, thus increasing $\Delta\Psi$ (negative inside). During this time the cellular pH should have increased as a result of K^+ and Na^+ accumulation which necessarily should have taken place in exchange for H^+. The increase of these parameters should stimulate the activity of a Na^+–H^+ electrogenic antiporter. The higher Na^+ accumulation that took place during the first 25 min at pH 8.0 without Mg^{2+} (fig.2) could be due equally to an influx through the efflux system or to a lower discrimination of the influx system between K^+ and Na^+, in these conditions. The lack of effect of *p*CMBS suggests that the efflux system did not mediate influx, contrarily to that observed with Li^+ [10]. However, it can not be ruled out that the efflux system mediated influx but that *p*CMBS does not affect the efflux system when operating in reverse.

Efflux of Li^+ in yeast does not take place in ATP-depleted cells [10], but Na^+ loss has not been described in such circumstances [13]. In our respiratory-deficient yeast, the loss of Na^+ in the absence of glucose (ATP depletion) was pH-dependent. At pH 3.5 there was no loss but a higher pH the cells lost Na^+ and at pH 7.0 loss of Na^+ was similar in presence or absence of glucose. The efflux of Na^+ in the absence of glucose was slightly inhibited by *p*CMBS, by 30–40% depending on experiments.

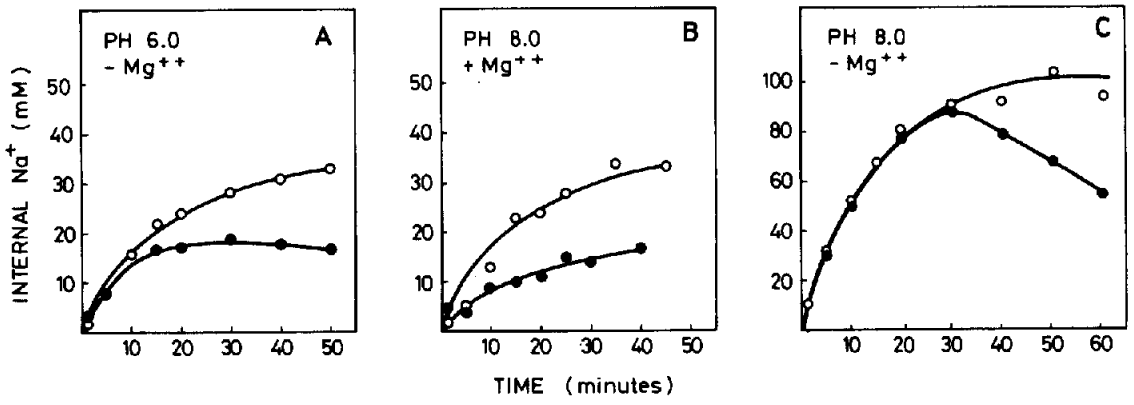

Fig.2. Effect of external pH and depolarization on Na^+ entry. Cultures as in fig.1 were washed and transferred to tricine (5 mM)/Mes (5 mM) buffer with 200 mM K^+, 50 mM Na^+, 2% glucose and, alternatively, 2 mM $MgSO_4$ or 50 mM citrate; the pH was adjusted to 6.0 or 8.0 with HCl or KOH, as required. Experiments were conducted in presence (o——o) or absence (•——•) of *p*CMBS (70 μM).

4. Discussion

In [6] Na^+ efflux in yeast followed roughly a sigmoid curve with reference to $[Na^+]$, thus contrasting with the first-order kinetics reported here. In addition to this difference, the efflux rates of Na^+ that we have found are higher than those reported previously: 0.6 mmol . min^{-1} . kg^{-1} at 60 mmol . kg^{-1} internal Na^+ [3]; 0.9 mmol . min^{-1} . kg^{-1} at 65 mmol . kg^{-1} internal Na^+ [7]; 2.9–3.3 mmol . min^{-1} . kg^{-1} at 80–60 mmol . kg^{-1} in [8]. In [6], the maximum increase in rate in relation to an increase in $[Na^+]$ (the slope of the tangent at the inflexion point) was 0.046 min^{-1} which is also lower than our constant rate of 0.11 min^{-1}. Even if all of these fluxes corresponded to the activity of the same porter, differences between strains and preparation of the yeast may account for the discrepances in efflux rates mentioned above. However, it is necessary to consider that Na^+-yeasts [14] were used in the works mentioned. Na^+-Yeasts were prepared by fermenting in Na^+-citrate which implies some Mg^{2+} drain. Since Mg^{2+} depletion changes the cation exchanges of yeast [12] caution should be taken when comparing our results with those obtained with Na^+-yeast. In [15], for the efflux of Na^+, a first-order kinetics (probably at very low $[Na^+]$) with a constant rate of 0.075 min^{-1} was found. This efflux is intermediate between that obtained with Na^+-yeast and that with growing yeast (present results) and was obtained in cell yeasts that had been aerated in water for 24 h. With reference to [6], the sigmoid curve followed for the rate of Na^+ efflux in Na^+-yeast might be the result of changes in the cellular pH. When a Na^+-yeast is suspended in presence of K^+ or Rb^+, the K^+ or Rb^+ taken up exceeds the Na^+ loss, and H^+ loss compensates the difference [4,8]. Therefore, in an experiment of Na^+ efflux the cell pH would increase during the experiment. Since the rate of Na^+ efflux from Na^+-yeast is pH-dependent and is maximal at pH 6.5 [7], a kinetics that were first-order at constant pH would not be first-order kinetics if the cellular pH increased. If pH 6.5 were within the range of pH variation, the plot of the rate *vs* concentration would be sigmoidal.

In Na^+-yeast, there is no agreement about the independence of the Na^+-efflux system from the influx system [3,4,7,8]. The efflux system described here seems to be independent from the influx system. The different sensitivity to *p*CMBS of the two pathways, influx and efflux, and the manner in which ΔpH and $\Delta\Psi$ participate in the efflux strongly support their independence. In fact, an electrogenic antiporter in which positive charge moves from outside to inside when the outward flux of Na^+ takes place can not be considered to correspond to an accumulation mechanism.

We had reported on the efflux of Li^+ in yeast [9,10]; an obvious question is the relationship between Li^+ and Na^+ porters. Because of the low affinity of the porters/porter for their substrate, competition experiments are not possible, and only some speculations can be done. Some similarities between Li^+ efflux and Na^+ efflux (rates, mechanism, sensitivity to *p*CMBS) suggest that both systems can be the same, but the different sensitivity to ATP depletion (Li^+ efflux is sensitive [10] but not Na^+ efflux) may suggest the opposite. However, in ATP-depleted cells, the loss of Na^+ was pH-dependent and was less sensitive to *p*CMBS than in the presence of glucose. This points to the idea that Na^+ efflux can be more complicated than Li^+ efflux. In presence of glucose both cations might use the same porter but, in the absence of glucose, Na^+ could use more carriers, except at a pH <3.5. In connection with this fact it is interesting to consider that the efflux of K^+ in the presence and absence of glucose is insensitive to *p*CMBS (unpublished).

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References

[1] Tolberg, A. B. and Pace, N. (1960) Proc. Soc. Exp. Biol. 103, 488–490.
[2] Roomans, G. M., Blasco, F. and Borst-Pauwels, G. W. F. H. (1977) Biochim. Biophys. Acta 467, 65–71.
[3] Conway, E. J., Ryan, H. and Carton, E. (1954) Biochem. J. 58, 158–167.
[4] Foulkes, E. C. (1956) J. Gen. Physiol. 39, 687–704.
[5] Kotyk, A. and Kleinzeller, A. (1958) J. Gen. Microbiol. 20, 197–212.
[6] Dee, E. and Conway, E. J. (1968) Biochem. J. 107, 265–271.
[7] Ryan, J. P. and Ryan, H. (1972) Biochem. J. 128, 139–146.

[8] Rothstein, A. (1974) J. Gen. Physiol. 64, 608–621.
[9] Rodríguez-Navarro, A. and Asensio, J. (1977) FEBS Lett. 75, 169–172.
[10] Rodríguez-Navarro, A., Sancho, E. D. and Pérez-Lloveres, C. (1981) Biochim. Biophys. Acta 640, 352–358.
[11] Asensio, J., Ruiz Argüeso, T. and Rodríguez-Navarro, A. (1976) Antonie van Leeuwenhoek 42, 1–8.
[12] Rodríguez-Navarro, A. and Sancho, E. D. (1979) Biochim. Biophys. Acta 552, 332–330.
[13] Eddy, A. A. (1978) Curr. Top. Membr. Transp. 10, 279–360.
[14] Conway, E. J. and Moore, P. T. (1954) Biochem. J. 57, 523–528.
[15] Borst-Pauwels, G. W. F. H., Walters, G. H. L. and Henricks, J. J. C. (1971) Biochim. Biophys. Acta 225, 269–276.