Volume 294, number 1,2, 35–37 FEBS 10433 December 1991

Turnover of the K^+ transport system in *Saccharomyces cerevisiae*

Begoña Benito, Enriqueta Riballo and Rosario Lagunas

Instituto de Investigaciones Biomédicas del CSIC, Arturo Duperier 4, 28029-Madrid, Spain

Received 30 September 1991

The stability of the K^+ transport system in *Saccharomyces cerevisiae* has been studied upon inhibition of protein synthesis with cycloheximide. Addition of the antibiotic gave rise to an inactivation of this transport. This activation followed first-order kinetics and was stimulated by the presence of a fermentable substrate. A half-life of about 4 h could be calculated in the presence of glucose. The results indicate that, similarly to sugar carriers, K^+ transport system is less stable than the bulk of proteins of this organism.

K^+ transport; Protein turnover; Plasma membrane ATPase; *Saccharomyces cerevisiae*

1. INTRODUCTION

Most enzymes in *Saccharomyces cerevisiae* are fairly stable in maintaining their catalytic activities for long periods under different metabolic conditions [1–4]. Sugar transport systems behave differently in this respect since a rapid inactivation is observed upon inhibition of protein synthesis [4]. This inactivation, that follows first-order kinetics indicating half-lives in the range of 1–5 h for these carriers [5–8], is an energy-dependent process stimulated by fermentable substrates. The characteristics of this inactivation suggest that it is due to proteolysis of these proteins [5–8]. To see whether a low stability is a peculiarity of the sugar transports or also affects other carriers of the yeast plasma membrane we have investigated the stability of the K^+ transport system.

K^+ transport has been extensively studied in *S. cerevisiae* [9–11]. This transport is dependent on plasma membrane potential [12] and exists in two interconvertible forms with different kinetic constants depending on the K^+ content of the cells [13,14]. To investigate the stability of this carrier we have followed its activity upon inhibition of protein synthesis by addition of cycloheximide.

2. MATERIALS AND METHODS

Cycloheximide, MES, diethylstilbestrol and ATP were from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade. Strain ATCC 42407 was grown aerobically with 2% glucose in minimal medium as previously described [15]. Strains RS-514 (MAT a, ade1-100, his4-519, leu 2-3,112, ura 3-52, PMA1), RS-515 (MAT a, ade1-100, his4-519, leu 2-3, 112, ura 3-52, pma1-204), RS-516 (MAT a, ade1-100, his4-519, leu 2-3,112, ura 3-52, pma1-205) constructed as described in [16] were grown with 2% glucose, 0.7% yeast nitrogen base without amino acids (Difco), 20 μg/ml adenine, and 20 μg/histidine buffered with 50 mM Mes-Tris, pH 6.5. Cell growth was monitored by optical absorbance measurement at 640 nm. Plasma membrane ATPase was measured in crude membrane preparations [17]. For this purpose cells were treated with glucose before homogenization as described in [18] and the enzyme activity was measured as described [17]. Protein content of the membrane preparations was determined by the method of Lowry et al. [19] after precipitation with 5% trichloroacetic acid. Activity of the K^+ transport system was measured by following Rb^+ uptake using atomic absorption spectrophotometry as described [13]. For this purpose cells were previously depleted of intracellular K^+ by incubation in the presence of NaN_3 as described in [14]. To recover the plasma membrane potential before Rb^+ uptake measurements, NaN_3-treated cells were washed with water and incubated for 5 min in the presence of glucose as described [14]. Rb^+ uptake was started by addition of 5 mM RbCl. At this concentration of Rb^+ the carrier is almost saturated and the velocity measured is very close to the V_{max} [14]. Protein content of the cells was determined as described in [20].

Correspondence address: R. Lagunas, Instituto de Investigaciones Biomédicas del CSIC, Arturo Duperier 4, 28029 Madrid, Spain.

3. RESULTS AND DISCUSSION

Addition of cycloheximide to glucose growing cells produced a decrease in the uptake of Rb^+ that followed first-order kinetics indicating a half-life for the K^+ transport system of about 3.5 h (Fig. 1A). These results are consistent with the low intracellular concentration of K^+ detected in yeast cells treated with this antibiotic [21]. When instead of glucose, ethanol was present as the energy source, the inactivation occurred at a lower rate and in this case a half-life of about 15 h could be calculated (Fig. 1B).

This decay of Rb^+ uptake could be due to the turnover of the carrier as well as to a decrease in the activity of the plasma membrane ATPase through its effect on plasma membrane potential and intracellular pH [12,22,23]. Actually a certain inactivation of this enzyme has been reported in conditions similar to the ones used in this work [24]. To check this possibility we investigated the behaviour of ATPase during our experiments.

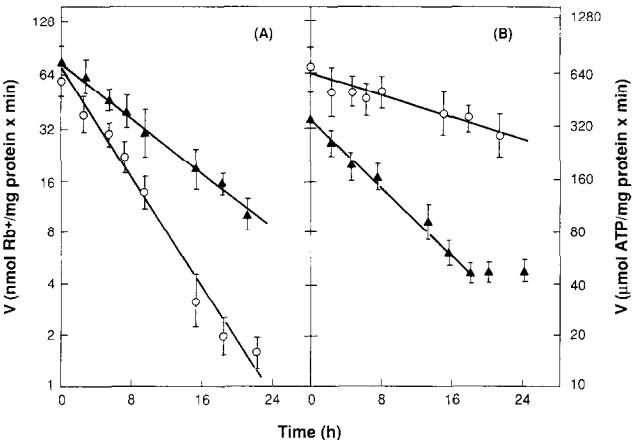

Fig. 1. Inactivation of the K^+ transport and plasma membrane ATPase upon addition of cycloheximide. Cells were harvested during exponential growth on glucose and transferred to 5 times the initial volume of fresh medium containing 10 μg/ml cycloheximide, 250 μg/ml tetracycline chlorhydrate and 2% glucose (A) or 2% ethanol (B). After incubation at 30°C for the indicated time, cells were harvested and assayed for K^+ transport (○) and ATPase (▲) activities. The results are means ± SD of 4 experiments.

We observed that addition of cycloheximide to glucose growing yeast gave rise to an inactivation of the ATPase that occurred at a lower rate than the one observed in the case of the K^+ transport system. To investigate the effect that these changes in ATPase could produce in the K^+ transport activity we used two mutants with a reduced expression of plasma membrane ATPase [16] and found that a decrease in the activity of this enzyme of as much as 60% was accompanied by a decrease in the activity of the carrier of only 25% (Table I). Similar results have been reported by Ramos et al. [12]. These results suggest that the slow decrease in ATPase activity observed upon addition of cycloheximide to glucose growing yeast ($T_{1/2}$ about 9 h) does not account for the rapid inactivation of the K^+ transport system ($T_{1/2}$ about 3.5 h) (Fig. 1A) and strongly indicate that this inactivation is mainly due to the unstability of the carrier itself. However, a much greater contribution of ATPase to the inactivation of the K^+ transport takes place when, instead of glucose, ethanol was present. This is suggested by the fact that, in this case, the inactivation of ATPase occurred at higher rate ($T_{1/2}$ about 7 h) than that of the carrier ($T_{1/2}$ about 15 h) (Fig. 1B).

In conclusion, the results shown in this work indicate that, similarly to sugar transport systems, K^+ transport system is less stable than the bulk of proteins of *S. cerevisiae* and that its instability is increased by the presence of fermentable substrates.

Rogers et al. [25] have observed that some proteins with short intracellular half-lives contain one or more regions rich in Pro (P), Glu (E), Ser (S), and Thr (T) that are flanked by positively charged amino acids. In addition, proteins containing Arg–Arg pairs are also rapidly degraded [25]. Taking these facts into account, it has been recently predicted that K^+ transport should be a target for rapid degradation because it contains three PEST sequences and six Arg–Arg pairs [26]. Our results confirm this prediction.

Table I

Activity of the plasma membrane ATPase and the K^+ transport system in mutants with reduced expression of ATPase

Strain	ATPase activity	K^+ transport activity
RS-514 (PMA1)	100	100
RS-515 (pma1-204)	42	75
RS-516 (pma1-205)	37	72

Values are relative to those of the wild-type strain. The absolute values of the activities of the wild-type were 0.45 μmol $ATP \cdot min^{-1} \cdot mg^{-1}$ protein for ATPase and 61 nmol $Rb^+ \cdot min^{-1} \cdot mg^{-1}$ protein for K^+ transport.

Acknowledgements: We are very grateful to Dr. A. Rodriguez-Navarro for help in the Rb^+ uptake measurements and for the stimulating discussions, to Dr. R. Serrano for the gift of the ATPase mutants and to Dr. C. Gancedo for critical reading of this manuscript. This work was supported by Dirección General de Investigación Científica y Técnica (PB87-0294), by Commision of the European Communities (BAP 0389E), and by Fondo de Investigaciones Sanitarias (88/1448).

REFERENCES

[1] Halvorson, H. (1958) Biochim. Biophys. Acta 27, 255–266.
[2] Halvorson, H. (1958) Biochim. Biophys. Acta 27, 267–276.
[3] López, S. and Gancedo, J.M. (1979) Biochem. J. 178, 769–776.
[4] Lagunas, R., Dominguez, C., Busturia, A. and Sáez, M.J. (1982) J. Bacteriol. 152, 19–25.
[5] Alonso, A. and Kotyk, A. (1978) Folia Microbiol. 23, 118–125.
[6] Busturia, A. and Lagunas, R. (1985) Biochim. Biophys. Acta 820, 324–326.
[7] Busturia, A. and Lagunas, R. (1986) J. Gen. Microbiol. 132, 379–385.
[8] DeJuan, C. and Lagunas, R. (1986) FEBS Lett. 207, 256–260.
[9] Amstrong, W.M.D. and Rothstein, A. (1967) J. Gen. Microbiol. 50, 967–988.
[10] Derks, W.J.G. and Borst-Pauwels, G.W.F.H. (1980) Biochim. Biophys. Acta 596, 381–392.
[11] Borst-Pauwels, G.W.F.H. (1981) Biochim. Biophys. Acta 650, 88–127.
[12] Ramos, J., Haro, R. and Rodriguez-Navarro, A. (1990) Biochim. Biophys. Acta 1029, 211–217.
[13] Rodriguez-Navarro, A. and Ramos, J. (1984) J. Bacteriol. 159, 940–945.
[14] Ramos, J. and Rodriguez-Navarro, A. (1986) Eur. J. Biochem. 154, 307–311.
[15] Lagunas, R. (1976) Biochim. Biophys. Acta 440, 661–674.
[16] Vallejo, C.G. and Serrano, R. (1989) Yeast 5, 307–319.
[17] Serrano, R. (1988) Methods Enzymol. 157, 533–544.
[18] Serrano, R. (1983) FEBS Lett. 156, 11–14.
[19] Lowry, O.H., Rosebrough, N.S., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–271.
[20] Jayamaran, J., Cotman, C., Mahler, H.M. and Sharp, C.V. (1966) Arch. Biochem. Biophys. 116, 224–252.

[21] Conor, R., Fuhrmann, G.F. and Rothstein, A. (1970) Biochim. Biophys. Acta 203, 583–585.
[22] Ulaszewski, S., van Herck, J.C., Dufur, J.P., Kulpa, J., Nieuwenhuis, B. and Goffeau, A. (1987) J. Biol. Chem. 262, 223–228.
[23] Goffeau, A. (1988) J. Med. Biol. Res. 21, 1233–1240.
[24] Benito, B., Moreno, E. and Lagunas, R. (1991) Biochim. Biophys. Acta 1063, 265–268.
[25] Rogers, S., Wells, R. and Rechsteiner, M. (1986) Science 234, 364–368.
[26] Anderson, J.A., Best, L.A. and Gaber, R.F. (1991) Gene 99, 30–46.