JOURNAL OF BACTERIOLOGY, Sept. 1984, p. 940–945
0021-9193/84/090940-06$02.00/0

Vol. 159, No. 3

Dual System for Potassium Transport in *Saccharomyces cerevisiae*

ALONSO RODRÍGUEZ-NAVARRO* AND JOSÉ RAMOS

Departamento de Microbiología, Escuela Técnica Superior de Ingenieros Agrónomos, Córdoba, Spain

Received 27 December 1983/Accepted 26 May 1984

In a newly formulated growth medium lacking Na^+ and NH_4^+, *Saccharomyces cerevisiae* grew maximally at 5 μM K^+. Cells grown under these conditions transported K^+ with an apparent K_m of 24 μM, whereas cells grown in customary high-K^+ medium had a significantly higher K_m (2 mM K^+). The two types of transport also differed in carbonyl cyanide-*m*-chlorophenyl hydrazone sensitivity, response to ATP depletion, and temperature dependence. The results can be accounted for either by two transport systems or by one system operating in two different ways.

Like all other organisms, *Saccharomyces cerevisiae* accumulates K^+ from the external medium to fulfill cellular requirements. The transport of K^+ in yeasts has been the subject of very extensive studies (2) that have clarified many kinetic aspects of the process. All of the alkali cations compete for a single carrier which shows highest affinity for K^+ (K_m, 0.5 mM) (1). With respect to K^+ requirements (3), 0.5 mM supports a growth rate close to the maximum. At lower K^+ concentrations, both growth rate and K^+ content decrease, and at 0.2 mM K^+, growth is no longer detectable. In *Neurospora crassa*, K^+ requirements are quite similar to those of *S. cerevisiae* (20). By contrast, higher plants are capable of growing and accumulating K^+ at concentrations more than 1 order of magnitude less (14). With respect to the differences between yeasts and higher plants, two points are important: K^+ requirements in yeasts have been determined in the presence of high concentrations of NH_4^+ (3), and K^+ transport has been normally assayed in the absence of divalent cations (1, 4), which may be required (16). In the present paper we reexamine K^+ requirements and K^+ transport in yeast in a synthetic growth medium which lacks NH_4^+ and Na^+.

MATERIALS AND METHODS

Organisms. *S. cerevisiae* X2180.1B (α *SUC2 mal gal2 CUP1*) (Yeast Genetic Stock Center, University of California, Berkeley) was used throughout the work. To study the role of protein synthesis in adaptation to low K^+, we used the temperature-sensitive mutant 187 described by Hartwell and McLaughlin (8), kindly supplied by R. Sentandreu. A respiration-deficient strain derived from X2180.1B was obtained by treatment with ethidium bromide (21).

Growth media and culture conditions. Unless otherwise stated, we used a synthetic medium consisting of 8 mM phosphoric acid, 10 mM L-arginine, 2 mM $MgSO_4$, 0.2 mM $CaCl_2$, and 2% glucose, brought to pH 6.5 with arginine, plus vitamins and trace elements recommended previously (22). This medium contained 2 to 5 μM K^+ and 5 to 8 μM Na^+ (as measured by atomic absorption spectrophotometry). KCl was added to obtain the required amount of K^+. In some experiments (see Fig. 1), we used a similar medium in which NH_4^+ was substituted for arginine (3). Cells were grown in flasks (250 to 1,000 ml) in a shaker at 28°C.

Growth rates. Flasks were inoculated with about 10^3 cells ml^{-1}, and plate counts were performed before the cell density reached 10^5 cells ml^{-1}. Growth rates were calculated from the plots of cell counts versus time. When necessary, to check that the external conditions had not changed, the cells were removed by filtration, and the K^+ content and the pH of the medium were determined.

Uptake experiments. Two different types of cells were used in most of the experiments reported here: cells growing at 2 μM K^+ and cells growing at 2 mM K^+. The high K^+-grown cells were obtained by inoculating 10^5 cells ml^{-1} in 2 mM-K^+ medium and harvesting when the cell density was 0.1 to 0.2 mg (dry weight) ml^{-1}. The 2-μM-K^+ cells were obtained by inoculating 10^4 cells ml^{-1} in 20-μM-K^+ medium and allowing the culture to deplete the K^+ of the medium until it reached 2.5 to 2.0 μM K^+. The collected cells were always transferred to fresh medium or buffer to carry out the experiments.

To determine $^{42}K^+$ or Rb^+ uptake, the cation was added to the suspension of cells at time zero. Then samples were taken and filtered, and the medium or the cells were analyzed. Uptake was determined from the increase of the cellular content. To analyze $^{42}K^+$ uptake, cell-free samples of the medium or samples of cells washed with nonradioactive medium were counted in a gamma counter. Samples were also analyzed for K^+ by atomic absorption spectrophotometry. To analyze Rb^+ uptake, the samples were filtered and washed with 20 mM $MgCl_2$ solution, and the cells were transferred to a new filter and washed again. The filters were acid extracted and analyzed by atomic absorption spectrophotometry (3). Transfer to a new filter was omitted when the Rb^+ concentration in medium was less than 100 μM.

To determine the initial rates of uptake, several samples were taken in short periods of time, and uptake was plotted versus time. In these plots, the first three or four datum points were very close to a straight line. For cells growing at 2 μM K^+, uptake was high, but the rate soon dropped, so all samples were taken within 5 min. For cells growing at 2 mM K^+, uptake was low, and the rate was constant for long periods of time. However, the sampling time was kept as short as possible. In $^{42}K^+$ experiments, the rate of K^+ influx was much higher than the rate required to compensate for the dilution of K^+ content owing to growth, and in all cases, the increase of cell volume during the experiments was insignificant. Thus, no correction for growth was necessary.

RESULTS

K^+ requirement for growth. Because both NH_4^+ (4) and Na^+ (1, 4) are known to compete with K^+ for transport, our first aim was to examine the K^+ requirement for the growth of yeast cells under NH_4^+- and Na^+-free conditions. We formulated a medium in which arginine served both as the nitrogen source and as the neutralizing cation for phosphate.

* Corresponding author.

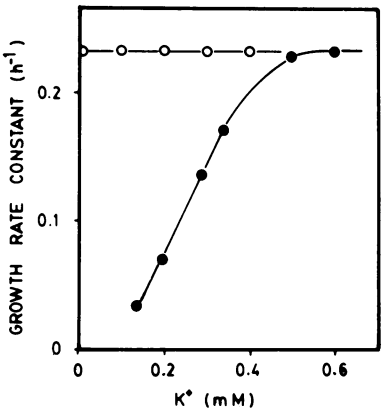

FIG. 1. K^+ dependence of the growth of *S. cerevisiae* in a NH_4^+ medium (●) and in arginine medium (○). Growth was followed by plate counting at low cell density to avoid any significant change of the medium during the assay. Growth rate constants (h^{-1}) were obtained from growth curves.

In this medium, the growth rate was maximum at 8 μM K^+, the lowest concentration that could be tested. In the presence of NH_4^+, at least 50 times more K^+ was required to obtain a similar growth rate (Fig. 1).

The increase in cell mass and decrease of external K^+ owing to depletion for growth (Fig. 2) showed that the cells took up K^+ at the rate required to increase the mass, and the cellular content remained constant until the external K^+ decreased below 5 μM. Then the rate of uptake decreased, but growth was not affected for an additional 45 min, resulting in a decrease of internal K^+ concentration. The growth rate decreased when the K^+ content of the cells reached about 80% of the normal amount (300 versus 380 nmol mg^{-1}).

From the results shown in Fig. 2, it was clear that yeast

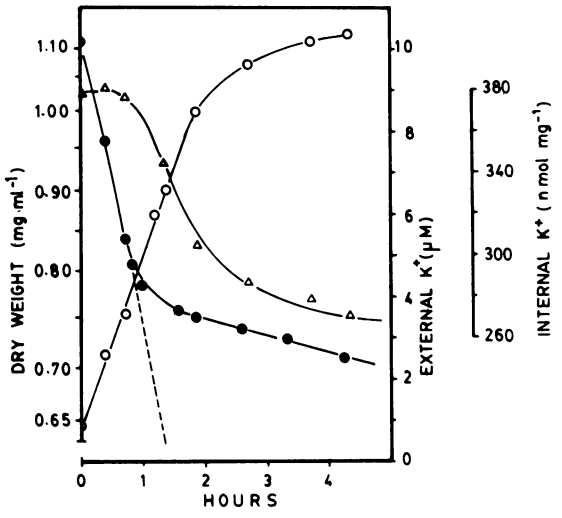

FIG. 2. Growth response of *S. cerevisiae* to the depletion of external K^+. Cells were grown in 20 μM K^+ medium and were harvested when the external K^+ was about 10 μM. Then the cells were concentrated and transferred to 10-μM-K^+ fresh medium. At times, samples were removed, and the external K^+ (●), dry weight of the culture (○), and K^+ content of the culture (△) were determined. The dotted line represents the expected decrease in external K^+ if the rate had not decreased.

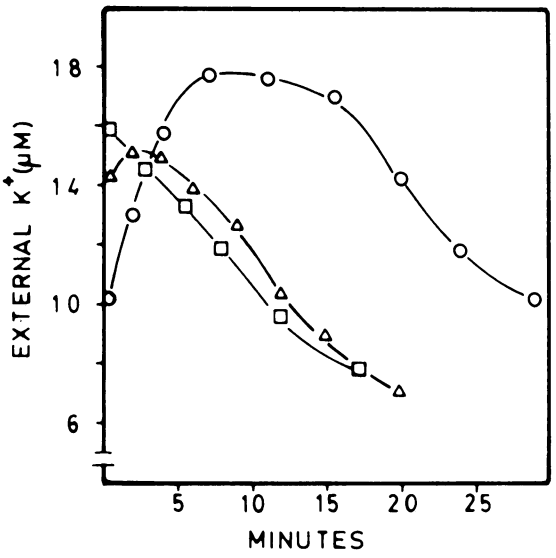

FIG. 3. Net uptake of K^+ in the micromolar range by cells grown at 2 mM K^+. Cells grown in 2-mM-K^+ medium were harvested when they reached a cell density of 0.15 mg ml^{-1} and were preincubated in fresh medium without K^+ at 0.05 mg ml^{-1} (in this medium, K^+ increased to less than 10 μM). At times, samples of cells were concentrated and transferred to fresh medium without added K^+, and the variation of the external K^+ was followed. Symbols: ○, 5 min preincubation, 0.7 mg ml^{-1}; △, 20 min preincubation, 0.52 mg ml^{-1}; □, 40 min preincubation, 0.64 mg ml^{-1}. Differences in external K^+ at time zero were not significant.

cells were able to take up K^+ efficiently at micromolar concentrations. However, we found that efficient uptake at micromolar K^+ took place only in cells that had been grown at micromolar K^+. When cells grown at millimolar K^+ were transferred to a medium without added K^+, they lost K^+ for some time before net uptake started (Fig. 3). After about 30 min, the cells took up K^+ at about 1.5 nmol mg^{-1} min^{-1}, a rate that not only could support maximum growth but also allowed the cells to recover the K^+ that had been lost.

Kinetics of K^+ and Rb^+ transport. The results above suggest that yeast cells may possess two modes of potassium transport: one with a relatively low affinity for K^+, present in cells grown at millimolar K^+ concentrations, and another with a considerably higher affinity, developed during growth at micromolar K^+. $^{42}K^+$ was used to determine the apparent K_m and V_{max} of K^+ transport in both types of cells (Fig. 4). For cells grown in the millimolar range, the results followed standard Michaelis-Menten kinetics, but for cells grown at micromolar K^+, there was a significant deviation from this behavior at the lowest concentrations tested. However, for comparison purposes a straight line was fitted to the latter set of data above 15 μM. It can be seen that, as predicted, cells grown at micromolar concentrations took up K^+ with a 100-fold greater affinity (K_m = 24 μM) than cells grown at millimolar concentrations (K_m = 2 mM). There was also a fivefold increase in the V_{max} of transport for cells grown at micromolar concentrations (34 versus 7 nmol mg^{-1} min^{-1}).

Similar information was gained from the measurement of Rb^+ transport by both types of cells (Fig. 5). In cells grown at micromolar K^+ concentrations, Rb^+ was taken up with an apparent K_m of 80 μM and a V_{max} of 30 nmol mg^{-1} min^{-1}, and uptake was competitively inhibited by K^+ with an apparent K_i of 12 μM (in good agreement with the K_m for K^+, above). The analysis of cells grown at millimolar K^+

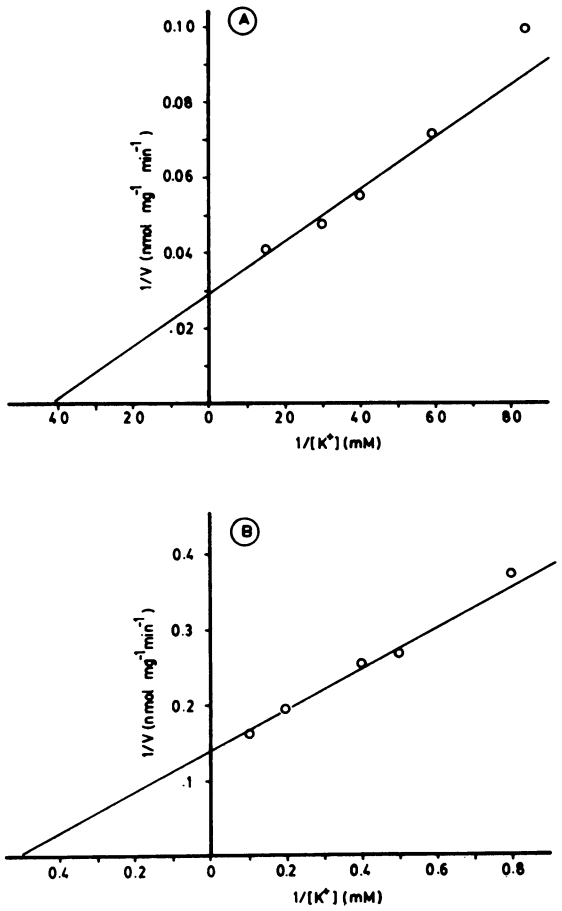

FIG. 4. Double reciprocal plots of $^{42}K^+$ transport in cells growing at 2 μM K^+ (A) and 2 mM K^+ (B). (A) Cells were grown in 20 μM K^+ medium and collected at 2 μM K^+. The cells were transferred to fresh medium at 0.1 to 0.3 mg (dry weight) ml^{-1} (lower densities were used at higher concentrations of K^+ and were preincubated until the external K^+ dropped to about 2 μM. Then, $^{42}K^+$ was added. Samples were filtered, and the medium was analyzed for $^{42}K^+$ and K^+. (B) Cells were grown in 2 mM K^+, harvested at a cell density of 0.2 mg ml^{-1}, and transferred to 2-mM-K^+ fresh medium at about the same cell density. Then, $^{42}K^+$ was added, samples were taken at the indicated times, and the cells were analyzed for $^{42}K^+$. In the assay carried out at 1.25 mM K^+, a concentrated sample of cells in 2 mM K^+ was added to the medium with $^{42}K^+$

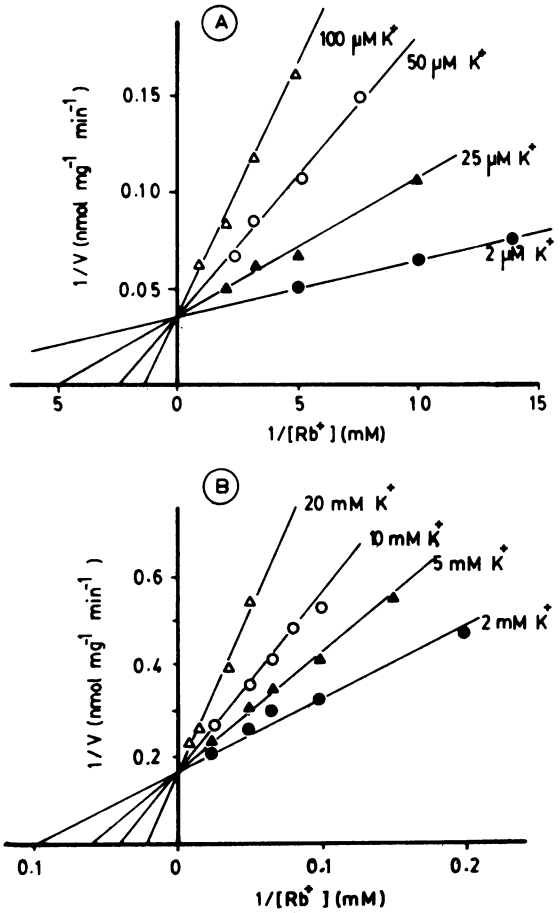

FIG. 5. Double reciprocal plots of Rb^+ transport, at several K^+ concentrations, in cells growing at 2 μM K^+ (A) and 2 mM K^+ (B). (A) Cells were prepared as described in the legend to Fig. 4A, except that the cell density was 0.1 mg ml^{-1}, and Rb^+ or Rb^+ plus K^+ was then added. At 2 μM K^+, the line was fitted making use of points off the scale of the figure. (B) Cells were prepared as described in the legend to Fig. 4B.

concentrations was complicated by the fact that, in the absence of added K^+ during the transport assay, the rate of Rb^+ uptake increased with time (see Fig. 7B, control). The initial rate at 10 mM Rb^+ was 1 nmol mg^{-1} min^{-1}, but the rate accelerated to 5 to 8 nmol mg^{-1} min^{-1} after 10 min and was then maintained for some time. In the presence of millimolar K^+, the uptake of Rb^+ was constant for more than 20 min. Therefore, we routinely assayed Rb^+ transport in the presence of K^+. Under these conditions, transport exhibited Michaelis-Menten kinetics (apparent K_m = 6 mM; V_{max} = 6 nmol mg^{-1} min^{-1}), with competitive inhibition by K^+ (apparent K_i = 3 mM).

The next step was to find whether the two kinetically distinct modes of K^+ and Rb^+ transport differed in other respects. Experiments were carried out to determine the dependence of transport on extracellular pH, intracellular pH, metabolic energy, and temperature.

Dependence of Rb^+ transport on extracellular pH. Figure 6 shows the variation of Rb^+ transport with the pH of the medium in cells grown at micromolar and at millimolar K^+. In both cases, transport remained relatively constant as the external pH was decreased from 8.0 to 5.5 but fell as the pH

TABLE 1. Apparent kinetic parameters for Rb^+ transport at pH 5.6 in the presence and absence of 5 mM propionic acid[a]

Type of cells	Treatment	K_m for Rb^+ (mM)	V_{max} (nmol mg^{-1} min^{-1})	K_i for K^+ (mM)
5 μM K^+[b]	Control	0.15	21	0.015
	Propionic acid	0.3	50	0.035
2 mM K^+	Control	6	6	2
	Propionic acid	18	9	4

[a] Obtained essentially as in Fig. 5, except that assays were carried out in 5 mM MES [2-(*N*-morpholino)ethanesulfonic acid], pH 5.6, with $Ca(OH)_2$ containing 2 mM $CaCl_2$, 0.1 mM $MgSO_4$, and 2% glucose or in 5 mM MES–5 mM propionic acid, pH 5.6, with $Ca(OH)_2$ containing 0.1 mM $MgSO_4$ and 2% glucose. The contaminant K^+ in these buffers was very low, and no preincubation in buffer before Rb^+ addition was carried out.

[b] The 5-μM-K^+ cells were obtained essentially as the 2-μM-K^+ cells were, except that they were used when they reached 5 μM K^+.

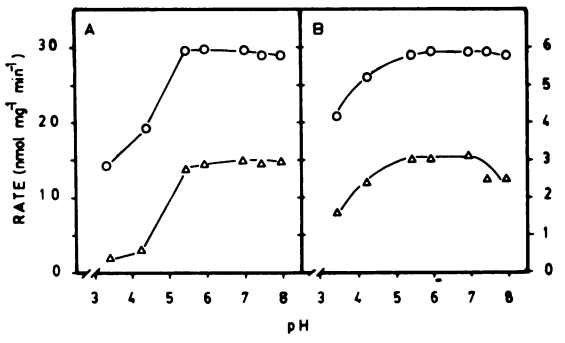

FIG. 6. pH dependence of Rb^+ transport in cells growing at 2 μM K^+ (A) and 2 mM K^+ (B). Cells were prepared as described in the legend to Fig. 4 and were transferred to buffers with 2 μM K^+ (A) and 2 mM K^+ (B). (A) Symbols: △, 100 μM Rb^+; ○, 10 mM Rb^+; (B) △, 10 mM Rb^+; ○, 100 mM Rb^+. Buffers (all 5 mM): 2,3-dimethylglutaric acid for pH 3.5 and 4.3; 2-(*N*-morpholino)ethanesulfonic acid for pH 5.5 and 6.0; *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid for pH 7.0 and 7.5; *N*-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine for pH 8.0. The acids were brought to pH with $Ca(OH)_2$ and contained 0.1 mM $MgSO_4$ and 2% glucose. The base was brought to pH with HCl and contained 1 mM $CaCl_2$, 0.1 mM $MgSO_4$, and 2% glucose.

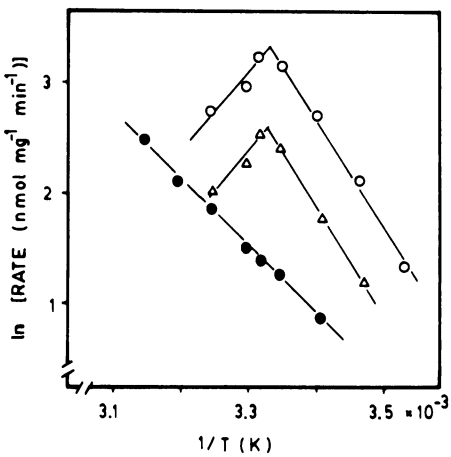

FIG. 8. Arrhenius plots of Rb^+ transport rates by cells growing at 2 μM K^+ (○, △) and 2 mM K^+ (●). Assays were carried out as described in the legend to Fig. 5. Symbols: ○, 10 mM Rb^+, △, 100 μM Rb^+; 40 mM Rb^+.

was lowered still further. At pH 4.3 in the 2-μM-K^+ cells, the apparent K_m rose fivefold to 44 μM, and the V_{max} decreased by 30% to 20 nmol mg^{-1} min^{-1}; in 2-mM-K^+ cells, the apparent K_m increased about twofold to 10.5 mM, but the V_{max} was not affected.

Effect of lowering intracellular pH. In yeast cells, it has been found that intracellular pH has a significant effect on alkali cation transport (17). To test this effect in cells grown at micromolar and millimolar K^+ concentrations, we treated the cells with propionic acid at pH 5.6 to lower the intracellular pH (17). In both types of cells, the V_{max} and the apparent K_m of Rb^+ transport increased after such treatment (Table 1).

Effect of CCCP and ATP depletion. The 2-μM-K^+ cells and 2 mM K^+ cells differed substantially in the sensitivity of transport to carbonyl cyanide-*m*-chlorophenyl hydrazone (CCCP) (Fig. 7). When cells of a respiration-deficient strain (obtained from strain X2180.1B) were grown at 2 μM K^+, CCCP inhibited Rb^+ transport up to 90% as the concentration was raised to 150 μM. With 2-mM-K^+ cells, on the other hand, CCCP did not affect the initial rate of Rb^+ uptake (no K^+ added) but did abolish the time-dependent increase in rate that took place in the controls. This effect did not increase from 40 to 150 μM CCCP.

A similar difference was seen when respiration-deficient cells, grown at 2 μM K^+ or 2 mM K^+, were depleted of ATP by withdrawing glucose from the medium (19). Transport of Rb^+ was greatly inhibited in 2-μM-K^+ cells despite the chemical gradient of the cation. In 2-mM-K^+ cells, however, the effect of ATP depletion was similar to that of CCCP. The

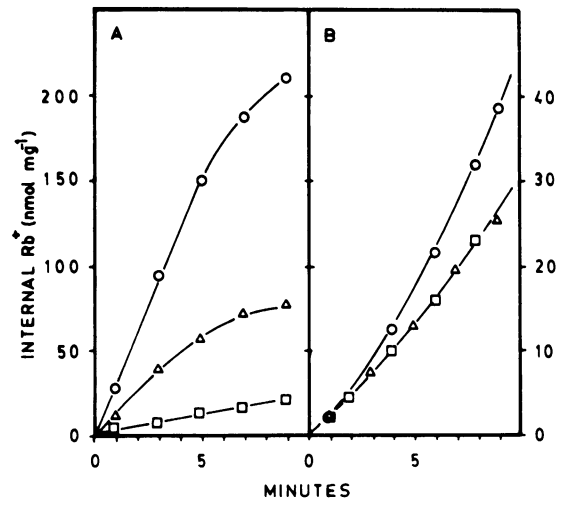

FIG. 7. Effect of CCCP on Rb^+ uptake by cells growing at 2 μM K^+ (A) and 2 m M K^+ (B). The cells were prepared as described in the legend to Fig. 5, and uptake was carried out at 20 mM Rb^+ without K^+ added. Symbols: ○, control; △, 60 μM CCCP; □, 150 μM CCCP.

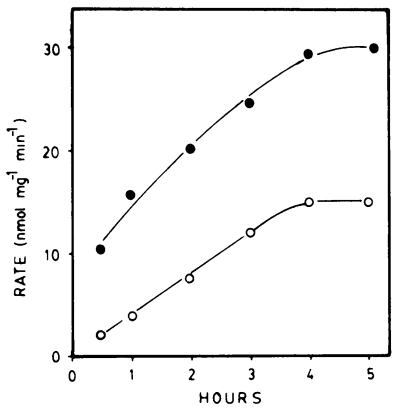

FIG. 9. Rates of Rb^+ transport by cells grown at 2 mM K^+ and preincubated in low-K^+ medium. Cells were prepared as described in the legend to Fig. 3, except that preincubation was carried out at a higher cell density (0.2 mg ml^{-1}). At the indicated times, samples of cells were taken, washed, and transferred to fresh medium (about 5 μM K^+) with 100 μM Rb^+ (○), 10 mM Rb^+ (●), and Rb^+ transport was assayed.

initial rate of Rb^+ uptake was inhibited less than 50% in comparison with the rate observed in control cells, but the uptake rate did not increase with time (about 1 nmol mg^{-1} min^{-1} during more than 20 min; data not shown).

Temperature dependence of Rb^+ transport. The 2-μM-K^+ cells and 2-mM-K^+ cells also differed in the temperature dependence of Rb^+ transport (Fig. 8). The 2-mM-K^+ cells were assayed for uptake at 40 mM Rb^+ (a near-saturating concentration); the 2-μM-K^+ cells were assayed at 10 mM Rb^+ (again, a near-saturating concentration) and at 100 μM Rb^+ (a concentration near the K_m for those cells). The plots for 2-μM-K^+ cells exhibited a breakpoint at 27°C, but the plot for 2-mM-K^+ cells was linear.

Appearance of high-affinity K^+ transport. To investigate the time course with which high-affinity K^+ transport appeared during incubation in low-K^+ medium, cells were grown at 2 mM K^+, transferred to fresh medium without added K^+, and assayed for Rb^+ uptake at intervals. A significant increase in uptake of 100 μM Rb^+ was seen in less than 1 h, and by 4 h, the uptake rate was maximal (Fig. 9). Protein synthesis was not required for the appearance of the high-affinity transport system. A temperature-sensitive protein synthesis mutant was incubated at 1 h at the permissive (23°C) and nonpermissive (36°C) temperatures in medium without added K^+ and then was assayed for the uptake of 100 μM Rb^+. At the nonpermissive temperature, inhibition of protein synthesis was verified by measuring the incorporation of L-[*guanido*-^{14}C]arginine into protein. High-affinity Rb^+ transport developed with a normal time course, even in the absence of protein synthesis (data not shown).

DISCUSSION

Previous studies on K^+ transport by *S. cerevisiae* have been performed with fresh bakers' yeast previously aerated for several hours in deionized water. Under such conditions, yeast cells display a single K^+ transport system with simple Michelis kinetics and a K_m of approximately 0.5 mM (1). In the present study, we show that the kinetics of transport can vary significantly depending upon the growth and assay conditions. Our experiments have been carried out with cells grown in a medium in which NH_4^+ and Na^+ were replaced by arginine. When a relatively high K^+ concentration (2 mM) was present during growth, the kinetics of transport were similar to those described previously: a single system following Michaelis kinetics, with K_ms of 2 mM K^+ and 6 mM Rb^+. By contrast, when the K^+ concentration during growth was reduced to 2 μM, the kinetics of transport became complex, and the apparent K_ms decreased to 20 μM K^+ and 80 μM Rb^+

In addition to the different affinities for substrate, the two modes of K^+ transport were also different in temperature dependence, sensitivity to CCCP, and response to ATP depletion. Of these differences, the break in the Arrhenius plot was the most significant. Breaks in Arrhenius plots at the lipid-phase transition temperature are commonly found with transport systems, but they usually involve a change in the increase in transport rate rather than a decrease above the breakpoint. One example of deactivation above 30°C has been described for D-xylose transport in *Rhodotorula glutinis* and has been interpreted in terms of structural changes above the breakpoint (9). If one invokes a similar explanation for the break observed in micromolar K^+ transport in *S. cerevisiae*, the results shown in Fig. 6 suggest structural or mechanistic differences in Rb^+ transport between 2-μM- and 2-mM-K^+ cells.

Further work will be required to establish the relationship of the two modes of K^+ transport to one another. From kinetic experiments, it does not appear that the low-affinity mode persists during K^+ limitation, with the high-affinity mode simply added on. Rather, the data suggest that the former is somehow converted into or replaced by the latter. A well-known mechanism of regulation of transport is transinhibition by intracellular substrate. In the case of ions, regulation by transinhibition operates in the transport of K^+ in plants (7, 10), the transport of sulfate in *Penicillium notatum* (5), and the transport of Cl^- in *Chara corallina* (18). In the present case, it is difficult to explain all of the changes in the kinetics of K^+ and Rb^+ transport in terms of internal K^+. Alternatively, there is good precedent in other organisms for the existence of more than one transport system to cover a wide range of concentrations of an ion. The transport of phosphate by yeast cells (2) and *Neurospora* spp. (12, 13), the transport of K^+ by *Escherichia coli* (6) and *Anabaena variabilis* (15), and the transport of Rb^+ in *Chorella pyrenoidosa* (11) are all mediated by two transport systems with different affinities for substrate.

ACKNOWLEDGMENTS

We are most grateful to C. W. Slayman and C. L. Slayman, Yale University, in whose laboratories A.R.-N. performed the experiments shown in Fig. 4. We thank C. W. Slayman for critical reading of the manuscript. We also acknowledge the many valuable suggestions of the editor-in-chief.

This work was supported by grant 4696-79 from Comisión Asesora para la Investigación Científica y Técnica, Spain.

LITERATURE CITED

1. **Armstrong, W. M., and A. Rothstein.** 1964. Discrimination between alkali metal cation by yeast. I. Effect of pH on uptake. J. Gen. Physiol. **48**:61–71.
2. **Borst-Pauwels, G. W. F. H.** 1981. Ion transport in yeast. Biochim. Biophys. Acta **650**:88–127.
3. **Camacho, M., J. Ramos, and A. Rodríguez-Navarro.** 1981. Potassium requirements of *Saccharomyces cerevisiae*. Curr. Microbiol. **6**:295–299.
4. **Conway, E. J., and F. Duggan.** 1958. A cation carrier in the yeast cell wall. Biochem. J. **69**:265–274.
5. **Cuppoletti, J., and I. H. Segel.** 1974. Transinhibition kinetics of the sulfate transport system of *Penicillium notatum*: analysis based on an iso uni uni velocity equation. J. Membr. Biol. **17**:239–252.
6. **Epstein, W., and B. S. Kim.** 1971. Potassium transport loci in *Escherichia coli* K-12. J. Bacteriol. **108**:639–644.
7. **Glass, A. D. M.** 1976. Regulation of potassium absorption in barley roots. An allosteric model. Plant Physiol. **58**:33–37.
8. **Hartwell, L. H., and C. S. McLaughlin.** 1968. Temperature-sensitive mutants of yeast exhibiting a rapid inhibition of protein synthesis. J. Bacteriol. **96**:1664–1671.
9. **Heller, K. B., and M. Höfer.** 1975. Temperature dependence of the energy-linked monosaccharide transport across the cell membrane of *Rhodotorula gracilis*. J. Membr. Biol. **21**:261–271.
10. **Jensen, P., and S. Petterson.** 1978. Allosteric regulation of potassium uptake in plant roots. Physiol. Plant. **42**:207–213.
11. **Kannan, S.** 1971. Plasmalemma: the seat of dual mechanism of ion absorption in *Chlorella pyrenoidosa*. Science **173**:927–929.
12. **Lowendorf, H. S., G. F. Bazinet, and C. W. Slayman.** 1975. Phosphate transport in *Neurospora*. Derepresion of a high-affinity transport system during phosphorus starvation. Biochim. Biophys. Acta **389**:541–549.
13. **Lowendorf, H. S., C. L. Slayman, and C. W. Slayman.** 1974. Phosphate transport in *Neurospora*. Kinetic characterization of a constitutive, low-affinity transport system. Biochim. Biophys. Acta **373**:369–382.
14. **Pitman, M. G., and Lütge.** 1983. The ionic environment and plant ionic relations, p. 5–34. *In* O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegel (ed.), Encyclopedia of plant

physiology, new series, vol. 12C. Springer-Verlag, KG, Berlin.
15. **Reed, R. H., P. Rowell, and W. D. P. Stewart.** 1981. Uptake of potassium and rubidium by the cyanobacterium *Anabaena variabilis*. FEMS Microbiol. Lett. **11**:233–236.
16. **Rodríguez-Navarro, A., and E. D. Sancho.** 1979. Cation exchanges of yeast in the absence of magnesium. Biochim. Biophys. Acta **552**:322–330.
17. **Ryan, J. P., and H. Ryan.** 1972. The role of intracellular pH in the regulation of cation exchanges in yeast. Biochem. J. **128**:139–146.
18. **Sanders, D., and U.-P. Hansen.** 1981. Mechanism of Cl^- transport at the plasma membrane of *Chara corallina* II. Transinhibition and the determination of H^+/Cl^- binding order from a reaction kinetic model. J. Membr. Biol. **58**:139–153.
19. **Serrano, R.** 1980. Effect of ATPase inhibitors on the proton pump of respiratory-deficient yeast. Eur. J. Biochem. **105**:419–424.
20. **Slayman, C. W., and E. L. Tatum.** 1964. Potassium transport in *Neurospora*. I. Intracellular sodium and potassium concentrations, and cation requirements for growth. Biochim. Biophys. Acta **88**:578–592.
21. **Slonimski, P. P., G. Perrodin, and J. H. Croft.** 1968. Ethidium bromide-induced mutation of yeast mitochondria: complete transformation of cells into respiratory deficient nonchromosomal "petites". Biochem. Biophys. Res. Commun. **30**:232–239.
22. **van der Walt, J. P.** 1970. Chapter II, criteria and methods used in classification, p. 34–113. *In* J. Lodder (ed.), The yeasts, a taxonomic study, 2nd ed. North-Holland Publishing Co., Amsterdam.