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 NH₄⁺, *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⁺ media 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 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 NH₄⁺ (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 NH₄⁺ 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 MgSO₄, 0.2 mM CaCl₂, 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 NH₄⁺ 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 10⁶ cells ml⁻¹, and plate counts were performed before the cell density reached 10⁷ 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 10⁵ 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 10⁷ 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 ⁴²K⁺ 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 ⁴²K⁺ 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 μM MgCl₂ 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 ⁴²K⁺ 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 NH₄⁺ (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 NH₄⁺- and Na⁺-free conditions. We formulated a medium in which arginine served both as the nitrogen source and as the neutralizing cation for phosphate.
In this medium, the growth rate was maximum at 8 μM K+, the lowest concentration that could be tested. In the presence of NH₄⁺, 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 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⁺. The 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 (Kₘ = 24 μM) than cells grown at millimolar concentrations (Kₘ = 2 mM). There was also a fivefold increase in the Vₘₐₓ 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 Kₘ of 80 μM and a Vₘₐₓ of 30 nmol mg⁻¹ min⁻¹, and uptake was competitively inhibited by K⁺ with an apparent Kᵢ of 12 μM (in good agreement with the Kᵢ for K⁺, above). The analysis of cells grown at millimolar K⁺.
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; \(V_{\text{max}}\) = 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.

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

<table>
<thead>
<tr>
<th>Type of cells</th>
<th>Treatment</th>
<th>(K_m) for Rb(^+) (mM)</th>
<th>(V_{\text{max}}) (nmol mg(^{-1}) min(^{-1}))</th>
<th>(K_i) for K(^+) (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (\mu)M K(^+)</td>
<td>Control</td>
<td>0.15</td>
<td>21</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Propionic acid</td>
<td>0.3</td>
<td>50</td>
<td>0.035</td>
</tr>
<tr>
<td>2 mM K(^+)</td>
<td>Control</td>
<td>6</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Propionic acid</td>
<td>18</td>
<td>9</td>
<td>4</td>
</tr>
</tbody>
</table>

* 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.

\(^{a}\) The 5-\(\mu\)M-K\(^+\) cells were obtained essentially as the 2-\(\mu\)M-K\(^+\) cells, except that they were used when they reached 5 \(\mu\)M 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: Δ, 100 μM Rb⁺; O, 10 mM Rb⁺; (B) Δ, 10 mM Rb⁺; O, 100 mM Rb⁺. Buffers (all 5 mM): 2,3-dimethylglutaric acid for pH 3.5 and 2,3-dimethylglutaric acid for pH 7.0 and 7.5; N-(2-hydroxyethyl)piperazine-N'-(2-hydroxyethyl)ethanesulfonic acid for pH 5.5 and 5.7; N-(2-hydroxyethyl)piperazine-N'-(2-hydroxyethyl)ethanesulfonic acid for pH 8.0. The acids were brought to pH with NaOH and contained 0.1 mM MgSO₄ and 2% glucose. The base was brought to pH with HCl and contained 1 mM CaCl₂, 0.1 mM MgSO₄, and 2% glucose.

was lowered still further. At pH 4.3 in the 2-μM-K⁺ cells, the apparent Kₘ rose fivefold to 44 μM, and the Vₘₐₓ decreased by 30% to 20 nmol mg⁻¹ min⁻¹; in 2-mM-K⁺ cells, the apparent Kₘ increased about twofold to 10.5 mM, but the Vₘₐₓ 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 Vₘₐₓ and the apparent Kₘ 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-chlorophenylhydrazone (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 mM 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: O, control; Δ, 60 μM CCCP; □, 150 μM CCCP.

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

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⁺ (O), 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-\(\mu\)M-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-\(\mu\)M-K\(^+\) cells were assayed at 10 mM Rb\(^+\) (again, a near-saturating concentration) and at 100 \(\mu\)M Rb\(^+\) (a concentration near the \(K_m\) for those cells). The plots for 2-\(\mu\)M-K\(^+\) cells exhibited a breakpoint at 27\(^\circ\)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 \(\mu\)M 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\(^\circ\)C) and nonpermissive (36\(^\circ\)C) temperatures in medium without added K\(^+\) and then was assayed for the uptake of 100 \(\mu\)M Rb\(^+\). At the nonpermissive temperature, inhibition of protein synthesis was verified by measuring the incorporation of \(L\)-[guanido-\(\text{\textsuperscript{14}}\)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 Michaelis 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 \(K_m\)s of 2 mM K\(^+\) and 6 mM Rb\(^+\). By contrast, when the K\(^+\) concentration during growth was reduced to 2 \(\mu\)M, the kinetics of transport became complex, and the apparent \(K_m\)'s decreased to 20 \(\mu\)M K\(^+\) and 80 \(\mu\)M 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\(^\circ\)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-\(\mu\)M- 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 trans-inhibition 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 *Chlorella 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 and reviewers.

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

**LITERATURE CITED**