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Effect of potassium on amino acid transport in yeast

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Abstract

Starved yeast cells accumulated potassium when the cation plus glucose was present in the incubation medium. Under these conditions, an increased amino acid transport capacity was developed within 30 to 60 min in comparison with cells incubated only with glucose. There seems to be a correlation between K^+ accumulation and an increase of the amino acid transport activity. Preincubation in the presence of potassium also produced an increased general protein synthesis. Transport systems are strongly inactivated by ammonium and this effect is partially reverted by potassium preincubation. Results also showed that potassium could 'protect' the amino acid transport systems from the inactivation produced by ammonium. It appears that potassium preincubation may have some effect on the rate of synthesis of the amino acid carriers, but effects of potassium appear to exist also on the degradation or inactivation of the carriers.

Keywords: Amino acid transport; Potassium; (Yeast)

1. Introduction

Starved yeast cells show very low rates of amino acid transport; to be expressed completely, they require preincubation with glucose [1,2]. Kotyk et al. [3] showed that the recovery of transport during preincubation was due to de novo synthesis of the specific transport systems. There seems to be also a more general increase of transport systems when yeast cells are preincubated with glucose or other substrates [1–5].

Potassium stimulates the transport of amino acids [6], phosphate [3] and sulfate [7]. Stimulation of glycolysis and respiration has been also observed when yeast is incubated with K^+ [8,9].

Besides protein synthesis, other factors modulate the activity of the membrane carriers of the yeast cell. Grenson et al. [10–13] studied the regulation of the adaptive transport systems by ammonium ions, detecting both inactivation–reactivation and repression of the general amino acid permease (GAP1). It seems that the transcription of the mRNA for the permease is diminished by ammonium.

The present work is an effort to characterize and to explain the potassium effect on amino acid uptake by *Saccharomyces cerevisiae*.

2. Materials and methods

2.1. Chemicals

L-[4,5-^{3}H]Leucine was obtained from ICN Radiochemicals (ICN Biomedicals), and L-[*carbamoyl*-^{14}C]citrulline from Amersham International (Amersham, UK). All reagents were of the highest purity available. Cycloheximide was obtained from Sigma (St. Louis, MO, USA), ammonium chloride was from Merck (Rahway, NJ, USA), and $DiSC_3(3)$ was obtained from Molecular Probes (Eugene, OR, USA).

2.2. Yeast

The experiments were performed with the facultative aerobic strains of *Saccharomyces cerevisiae* XT300.3A (a gift from Dr. A. Rodríguez-Navarro), Σ1278b and the general amino acid permease deficient mutant 2512c

Abbreviations: Mes, 2-(*N*-morpholino)ethanesulfonic acid; TEA, Triethanolamine; $DiSC_3(3)$, 3,3-dipropylthiacarbocyanine; CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

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(kindly donated by Dr. M. Grenson). The strains were grown aerobically at 30°C in Bacto Yeast Nitrogen Base Medium of Difco (Difco manual, 10th Edn.) using ammonium sulfate and glucose as nitrogen and carbon sources, respectively, in a gyratory shaker (at 250 rpm). Cells were harvested during the early stationary phase (13 to 15 h) and washed twice with double distilled water by centrifugation, and resuspended at 50% (w/v) in double distilled water.

2.3. Potassium accumulation

After preincubation of the cells, they were centrifuged and washed, and boiled in a water bath for 30 min. After centrifugation, the potassium concentration of an appropriate dilution of the supernatant was measured in a flame photometer.

2.4. Membrane potential

It was estimated as described [14], by following the changes of fluorescence at 540–590 nm of 125 nM $DiSC_3(3)$, added to a suspension of 10 mg of yeast cells in Mes-TEA 20 mM (pH 6.0); 0.25 μM $CaCl_2$; 50 mM glucose and 6 μM CCCP.

2.5. Proton pumping

It was followed by means of a glass electrode and a pH meter connected to a recorder. 15 mg wet wt./ml of cells were preincubated for 60 min with 20 mM Mes-TEA (pH 6.0) and 150 mM glucose with or without 10 mM potassium. After centrifuging and washing, 10 mg of cells were incubated in 4 mM Mes adjusted to pH 6.0 with TEA, and 50 mM glucose, to follow the change of the pH of the medium.

2.6. Amino acid transport

Unless indicated differently, 15 mg wet wt./ml of yeast cells were placed in a buffer containing 20 mM Mes adjusted to pH 6.0 with TEA, and 150 mM glucose (control medium) and preincubated for 60 min at 30°C. KCl at variable concentrations and other additions are indicated in each case. After preincubation, cells were centrifuged and resuspended at the same concentration in the control medium without additions. After equilibrating the cell suspension for 2 min to 30°C in a water bath, the labeled amino acid to be transported was added (usually 200 μM L-[^{3}H]leucine or 80 μM ^{14}C-L-citrulline). After 2.0 min, aliquots (0.1 ml) were taken and filtered through a Millipore nitrocellulose filter (0.45 μm pore) under vacuum and washed with 10 ml of 5 mM cold amino acid. The filters were placed in a vial containing a toluene-based scintillation liquid. Vials were counted in a Packard Tri-Carb Scintillation Spectrometer.

2.7. Protein synthesis

It was followed by incorporation of L-[^{3}H]leucine (added at 200 μM during preincubation with or without potassium in the medium) into the trichloroacetic acid-insoluble material of the cells. Yeasts were incubated in the control medium plus the required additions at 30°C. At different times, samples were withdrawn and mixed with 1 ml of 10% trichloroacetic acid. The samples were placed 1 h at 4°C and then filtered and washed as above and used for scintillation counting. Where indicated protein synthesis was inhibited by adding 100 μM cycloheximide during or after preincubation.

2.8. Transport kinetic constants

They were calculated by the Inplot program (Graphpad Software, San Diego, CA, USA).

3. Results

When yeast cells were preincubated in the presence of K^+, L-leucine uptake was stimulated several fold over the control, preincubated only with glucose (Fig. 1). Some variability was observed from one experiment to another, and although the maximum effect was observed at 120 min, further experiments were performed preincubating the cells for 60 min, at which, the stimulation of leucine transport by 10 mM K^+ varied from 2.5- to 10-times. This increment was present at concentrations as low as 250 μM potassium (Fig. 2). The effect of K^+ was much higher than that of other monovalent cations (Table 1); the observed stimulation followed the sequence K^+, Rb^+, Na^+, Cs^+, Li^+. The effects of both K^+ and Rb^+ are similar to their relative rates of transport into yeast [15].

When yeast cells are incubated in the presence of potassium, there is an increase of the internal pH [9]. This

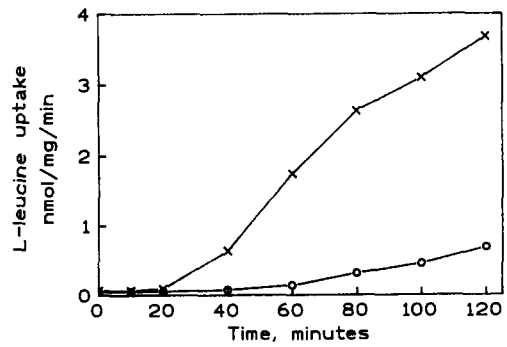

Fig. 1. Uptake of L-leucine by the yeast XT300.3A. Cells were preincubated at different times in the control medium (○) or in presence of potassium chloride 10 mM (×). The temperature was 30°C. After the preincubation they were centrifuged and resuspended in the control medium. The samples were preincubated for 2 min in the presence of 200 μM L-[^{3}H]leucine.

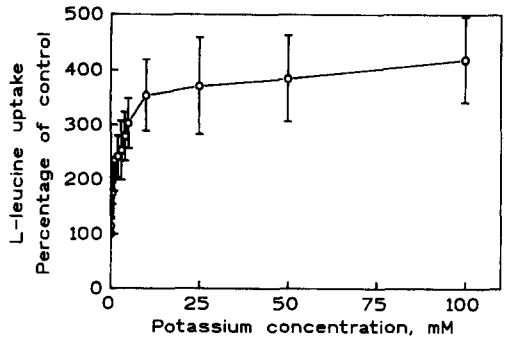

Fig. 2. Effects of potassium on the rate of transport of L-leucine. Yeast (XT300.3A) was preincubated for 60 min at 30°C in the control medium (glucose 150 mM, Mes-TEA 20 mM, pH 6) and in the presence of increasing concentrations of potassium chloride. Then the cells were centrifuged and resuspended in the same medium to which 200 μM L-[^{3}H]leucine was added. The samples were incubated for 2 min, and then the cells were filtered and washed as described in Section 2. The results are shown as percent of the control.

same internal alkalinization can be observed if the cells are preincubated at high pH values [9] even in the absence of potassium. In order to test if an internal alkalinization was responsible for the stimulation of the uptake of leucine, yeast cells were preincubated for 60 min at different pH values in the absence or presence of potassium, and then the rate of uptake of the amino acid was measured at pH 6. In the pH range tested (not shown), leucine uptake seemed to be independent of the preincubation pH under both conditions (with or without K^+), and the preincubation at high pH did not increase the rate of leucine transport.

When yeast cells were preincubated with potassium ions, an important accumulation of the cation was observed (Table 2). It is also interesting that when these cells were placed in a potassium-free medium, a larger efflux of potassium was observed than with cells preincubated in the absence of potassium (not shown). The membrane potential difference, estimated with $DiSC_3(3)$ [14] did not show significant differences between the cells that had been preincubated with glucose or glucose plus potassium. The proton pumping activity was also followed by the changes of the medium pH, and no differences were observed also between the cells preincubated under these two conditions (not shown).

Table 1
Stimulation of L-leucine transport by preincubating yeast cells for 60 min in the presence of 10 mM monovalent cations. Correlation between cation transport affinities and its effects on leucine transport

Cation	Cation transport relative affinity [15]	Amino acid transport (nmol min^{-1} mg^{-1})
Li^+	2	0.67
Na^+	3	1.49
K^+	100	4.80
Rb^+	50	1.80
Cs^+	8	1.15

Experimental conditions were as for Fig. 2. Transport in the absence of cations was 1.0 nmol mg^{-1} min^{-1}.

Table 2
Intracellular potassium after preincubating different strains of yeast with glucose or glucose plus potassium

Preincubation	XT300.3A	Σ1278b	2512c
Not preincubated	187 ± 1.1	192 ± 0	191 ± 4
Glucose	212 ± 28	190 ± 18	193 ± 40
Potassium 10 mM	336 ± 44	297 ± 18	323 ± 38

The cells (7.5 mg/ml) were preincubated for 60 min in the control medium or in presence of KCl 10 mM. Then the samples were centrifuged and the pellet was resuspended in bidistilled water and the tubes were immersed in boiling water for 30 min. The cells were centrifuged and potassium was determined in the supernatant in a Zeiss flame photometer. Results are indicated as the intracellular concentration (mM), calculated on the basis of a water content equal to 50% of the wet weight of the cells. The standard deviations are from 6 separate experiments. The wild-type strain Σ1278b was included to compare with the 2512c mutant strain.

Kotyk et al. [3] reported that when yeast cells are preincubated for long periods in the presence of glucose, a parallel increment between the transport activity and the synthesis of the proteins mediating transport is observed. In *S. cerevisiae* preincubated in the presence of glucose alone, or with 10 mM K^+, the same correlation between transport and general protein synthesis was observed (Fig. 3); a higher incorporation of L-[^{3}H]leucine into trichloroacetic insoluble material was observed when the cells were preincubated in the presence of potassium than in the cells preincubated with glucose alone. If these experiments were carried out in the presence of cycloheximide, the incorporation of labeled amino acid was prevented (not shown).

Added after preincubation in the presence of glucose or glucose plus KCl, cycloheximide stopped the general protein synthesis and the increase of the uptake of leucine. However, in yeast preincubated with potassium, although the increase in the rate of transport was stopped by cycloheximide, a much slower decay of the transport was observed, compared with the yeast preincubated with glu-

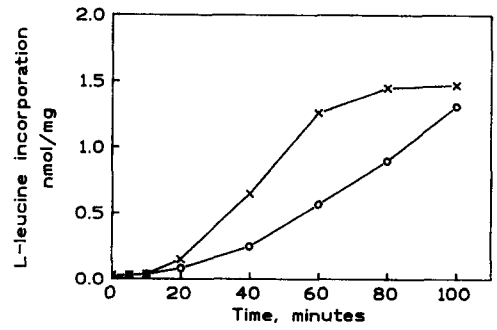

Fig. 3. Rate of incorporation of L-[^{3}H]leucine into trichloroacetic acid-insoluble material by yeast cells preincubated in the presence of glucose (○), or glucose plus 10 mM KCl (×). Experimental conditions were similar to Fig. 1, and the incorporation of labeled leucine was measured as described in Section 2.

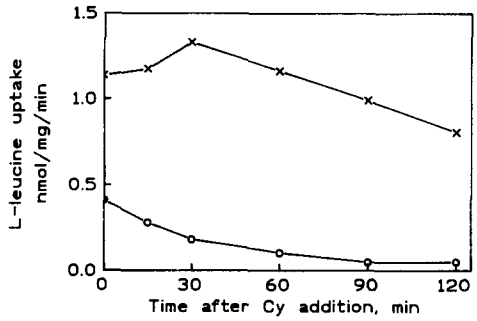

Fig. 4. Decay of transport activity after cycloheximide (Cy) addition. After the wild-type strain XT300.3A cells were preincubated 60 minutes at 30°C with glucose (○) or glucose plus 10 mM of potassium chloride (×), 100 μM cycloheximide was added and at several times samples were centrifuged and resuspended in the control medium. The samples were preincubated for 2 min in the presence of 200 μM L-[^{3}H]leucine and its transport was measured.

cose only (Fig. 4). While those cells preincubated with glucose lost close to 90% of the transport activity 120 min after the addition of cycloheximide, those preincubated with glucose plus potassium maintained around 80% of the transport activity after the same time of addition of the inhibitor.

There is a mutant (gap$^-$, 2512c), described by Grenson et al. [10], lacking the general amino acid permease, which offers the possibility of defining the involvement of this permease in the activation of amino acid transport by potassium. As expected, no citrulline transport activity was observed in the mutant strain gap$^-$ (not shown), independently of the preincubation conditions. On the other hand, when the mutant strain was compared to its corresponding parental wild type strain (Σ1278b), no potassium stimulation of leucine uptake was observed in the mutant cells, compared to the wild-type yeast (Fig. 5), in which the increase in the transport by potassium preincubation was similar to that observed in the wild-type strain XT300.3A used in the other experiments. Fig. 6 also shows that following the time-course, neither leucine uptake rate nor

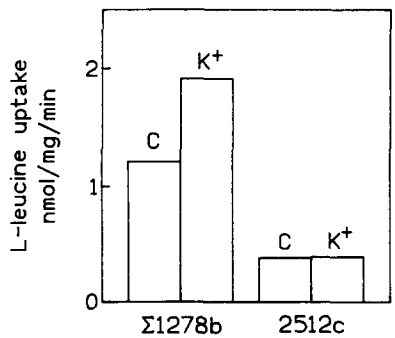

Fig. 5. Effect of potassium preincubation on L-leucine transport in the wild-type yeast Σ1278b and in the mutant yeast 2512c lacking GAP1. After preincubation for 60 min with glucose or with glucose plus potassium, centrifugation and resuspension in control medium, the cells were incubated for 2 min in the presence of 200 μM of L-[^{3}H]leucine, to measure its transport as described in Section 2.

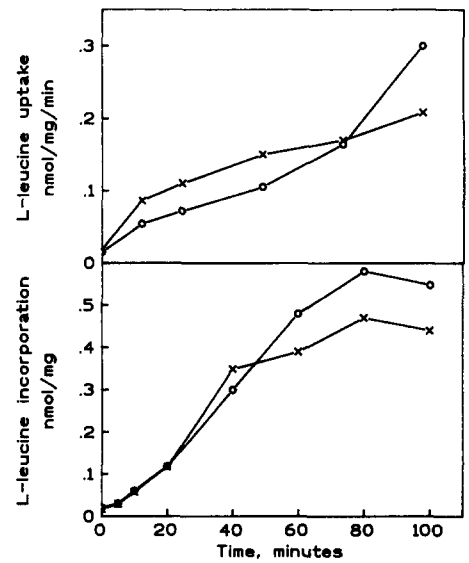

Fig. 6. Effects of preincubation with glucose (○) or glucose plus potassium (×) of the gap$^-$ mutant 2512c, on the amino acid transport activity (upper panel) and amino acid incorporation into trichloroacetic acid-insoluble material (lower panel). The experiment was carried out as described for Figs. 1 and 3.

protein synthesis were increased in the 2512c mutant strain 2512c, by preincubation with 10 mM potassium.

L-citrulline is transported in yeast through the general amino acid permease [10]. The use of this amino acid then, offers another possibility to study the effects of potassium preincubation on this transport system in a more specific way. To this purpose, we compared the kinetics of citrulline transport in cells preincubated with glucose, as compared to those preincubated with potassium. Fig. 7 shows that when yeast cells of the strain XT300.3A were preincubated with potassium, a clear one-component kinetics was observed, with K_m values of 125 and 103 μM, and V_{max} of 3.57 and 3.47 nmol mg^{-1} min^{-1}, obtained in two separate experiments. However, in the cells preincubated with glucose alone, only a very small uptake of the amino acid was observed, which had no relation to its added concentration (Fig. 7).

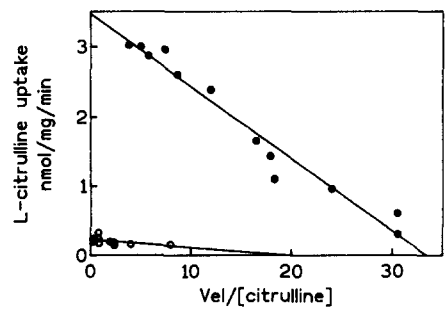

Fig. 7. Kinetics of L-citrulline transport in yeast cells preincubated with glucose (○) or with glucose plus 10 mM KCl (●) for 60 min. Experimental conditions were as for Fig. 1, but varying concentrations of citrulline were used.

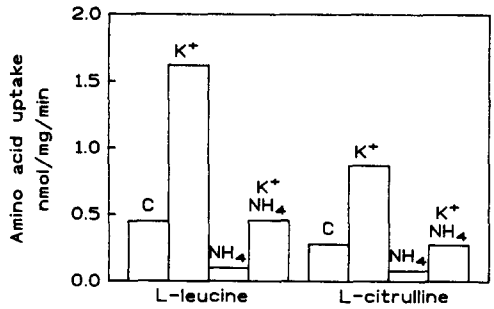

Fig. 8. Effects of preincubation of yeast cells (XT300.3A) with ammonium and potassium, on leucine and citrulline transport activity. Control (C); KCl 10 mM (K^+); NH_4Cl 10 mM (NH_4); equimolar concentrations (10 mM) of KCl and NH_4Cl (K^+–NH_4). 200 μM of L-[^{3}H]leucine and 80 μM of L- [^{14}C]citrulline were used. Experimental conditions were as for Fig. 5.

As reported before by Grenson [12], the addition of ammonium greatly decreased the transport of leucine, tested in our wild-type XT300.3A strain preincubated with glucose; Fig. 8 (bar #3) shows the results. However, the preincubation in the presence of equimolar (10 mM) concentrations of potassium and ammonium partially prevented the inactivation produced by ammonium (Fig. 8, bar #4). Besides, similar results were obtained for the transport of leucine or citrulline.

4. Discussion

The data obtained from this study suggest that the stimulation on the amino acid transport activity by K^+ may be related to the transport of the cation, because of the following facts: (a) the potassium effect was observed starting at low concentrations of the cation, similar to those required for its transport. (b) There is a similar selectivity of the transport system for monovalent cations [15] and their effect on the transport of leucine. (c) Potassium was accumulated when the cells were preincubated in its presence, suggesting a relation between its accumulation and the amino acid transport increase. Intracellular K^+, when the cation was present at 10 mM in the medium during preincubation, was increased around 60%. Another possibility to explain the results is the internal alkalinization resulting from the transport of potassium into the cells [9], which may result from the increased H^+ pumping observed in the presence of the monovalent cation(s), which has been observed also by Kotyk et al. [16]. However, our experiments carried out (not shown) in which this internal alkalinization was provoked by preincubating the cells at high pH values showed that it is not responsible for the increase of amino acid transport; the cells preincubated at high pH in the presence of glucose did not show an increased transport, and this transport was still stimulated by K^+ to a similar extent to that observed in cells preincubated at lower pH values.

The increase of the amino acid transport is blocked by cycloheximide, probably because, as shown before for glucose incubation [3], it requires the synthesis of the carriers. Besides, the time-course of the transport capacity was similar to that of protein synthesis, as shown by following the incorporation of labelled leucine into trichloroacetic acid insoluble material. Cycloheximide also inhibited the further increase of leucine transport activity after preincubation with potassium. This indicates that the potassium effect during preincubation may require an increase of the carrier molecules, although a role of the monovalent cation on the activation of the carriers cannot be ruled out. Since it was also possible to demonstrate a major general increase in protein synthesis when the cells were preincubated in the presence of glucose plus potassium, as compared to glucose alone, it is possible that the potassium accumulation during preincubation increased the rate of synthesis of the carrier.

The increased activity of the carrier could be observed when following the uptake of L-citrulline, which is transported through the general amino acid permease (GAP1, Ref. [10]) and it was not found in the mutant cells which lack this system. Besides, it is striking that citrulline transport, occurring only through this permease seemed not to be active at all, unless potassium was present during preincubation of the cells. This may further indicate that the accumulation of K^+ during preincubation could stimulate the synthesis of this carrier.

Similar to what Sychrová et al. described [17,18] in *Schizosaccharomyces pombe*, the transport of L-leucine in *Saccharomyces cerevisiae* does not appear to be constitutive; a preincubation with glucose was required to observe leucine transport (Fig. 1). Besides, in the presence of K^+, the cation is rapidly accumulated (data not shown) by the cells, and it seems to further stimulate the synthesis of the amino acid carrier.

As reported by Grenson et al., the transport systems were found to be inactivated to very low levels by ammonium ions [10]. This effect was partially reverted by potassium preincubation (Fig. 8); the lower inactivation of these systems by ammonium might be related to the increased rate of the general protein synthesis obtained when yeast was preincubated in the presence of potassium. Horák et al. [19] also described that the inactivation of L-lysine transport by ammonium preincubation could be overcome by the de novo synthesis of the carrier protein in *Schizosaccharomyces pombe*. It seems that the presence of potassium in the medium could 'protect' the amino acid transport systems from the inactivating effect of ammonium, suggesting opposite effects by the two cations.

The repression of GAP1 permease synthesis due to the presence of ammonium ions, seems to involve the formation and/or stability of the GAP1 messenger RNA [20]. In the light of these results, potassium might be acting at the transcription level competing against ammonium. The data on the disappearance of the amino acid transport capacity

of the cells after the addition of cycloheximide also indicate that K^+ has an effect resulting in a higher stability of the carrier than in cells preincubated with glucose alone. This may be due to effects of the cation on the inactivation or degradation of the permease, indicating that something else besides protein synthesis is responsible for the difference of transport activity.

In summary, it appears that K^+, accumulated by the cells during preincubation, could have some effect on the rate of synthesis of the amino acid carriers, and this effect is more evident on the general amino acid permease. However, the effects of K^+ appear to be also on the degradation or inactivation of the carriers.

Acknowledgements

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