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Regulation of ^{86}Rb influx during accumulation of Rb^+ or K^+ in yeast

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The influx rate of ^{86}Rb decreases during accumulation of K^+ or Rb^+ into metabolizing yeast cells under anaerobic conditions with glucose as substrate. Possible causes for the decrease in the influx rate are examined. It is ruled out that the decrease in the influx rate is caused by an increased turgor pressure of the cells or to an impairment of their energization. During the accumulation of K^+ or Rb^+, no decrease but an increase in the protonmotive force of the cells is found. The concomitant increase in cell pH accounts only in small part for the decrease in the influx rates. Acidification of the cells on adding butyrate to the suspension causes a transient increase in the influx rates, whereas the cellular monovalent cation content is still increased. Consequently, no direct relationship is found between the influx rate and the cellular content of K^+ and Rb^+.

Introduction

Most studies on ion transport in yeast are devoted to the question of how the ions are translocated through the yeast cell membrane and how the energy generated by metabolism is transduced to the transport mechanism [1,2]. The problem of how ion translocation stops after a certain time of incubation with the cations involved has gained less attention. In *Saccharomyces cerevisiae* with propanol-2 as substrate, Ryan and Ryan [3] found that the gradual decrease in net K^+ uptake rate was due to an increase in the efflux rate of K^+ rather than to a decrease in the influx of this cation. Apparently, a pump and leak system was involved in the regulation of the intracellular K^+ content. Rothstein and Bruce [4] using glucose as a substrate for *S. cerevisiae* found also a decrease in net K^+ uptake rate during accumulation of K^+ into the yeast. It was, however, less clear whether this decrease was due to an increase of the K^+ efflux or whether also decreases in the K^+ influx rate were involved. In plant roots, on the other hand, it has been unequivocally shown that the decrease in the net uptake rate of ^{86}Rb found on loading the plant roots with K^+ was mainly due to a decrease in the ^{86}Rb influx rate [5–9]. It has been supposed that the influx of monovalent cations into the roots was regulated by the cellular K^+ concentration via a change in the conformational state of the monovalent cation carrier. Hill plots of ^{86}Rb influx with cellular K^+ as the allosteric effector indicated that at least four and sometimes sixteen internally located allosteric binding sites were involved in the regulation of ^{86}Rb influx during K^+ accumulation [5,6].

We have now examined whether the influx rate of Rb^+ into the yeast *S. cerevisiae*, is also regulated by the intracellular monovalent cation content. The effect of loading the cells with nonradioactive Rb^+ or K^+ upon the influx rate of tracer amounts of ^{86}Rb was studied.

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One of the factors which may regulate the Rb^+ influx rate is the cell pH (pH_i) [3,10]. An increase in pH_i leads to a decrease in the monovalent cation influx rate. Since on loading the cells with K^+ or Rb^+ pH_i increases [11], an eventual decrease in the influx rate occurring on loading the cells with monovalent cations might be due to the concomitant increase in pH_i. In order to eliminate such an indirect effect, we added butyrate which passes readily the cell membrane in its undissociated form causing acidification of the cells on entry [12,13]. Another factor which may regulate the Rb^+ influx rate is the energy state of the cells. We therefore examined whether during Rb^+ or K^+ uptake changes occurred in the membrane potential, the protonmotive force and also in the rate of influx of another actively translocated cation namely Sr^{2+} [14].

Materials and Methods

Yeast cells (2%, w/v), *Saccharomyces cerevisiae*, strain Delft II, were exhausted of endogeneous substrate by aeration overnight at room temperature in distilled water. Then, the cells were washed twice with distilled water and transferred into 45 mM Tris buffer, adjusted with succinic acid to pH 4.5. Metabolism was started by adding 3%, w/v glucose under anaerobic conditions, i.e., by bubbling N_2 through the suspensions.

Uptakes of ^{86}Rb, ^{89}Sr, tetra[^{14}C]phenylphosphonium and [^{14}C]butyrate were determined according to Refs. 10, 14, 15 and 16, respectively. Initial rates of uptake were obtained from the tangents to the uptake curves at zero time. pH was determined according to Ref. 17. For the determination of cell K^+, 2 ml yeast samples were mixed rapidly with 2 ml ice-cold 50 mM $MgCl_2$ solution, immediately filtrated and washed with 2 ml ice-cold distilled water. The filters were extracted with 2 ml 35% nitric acid for 1 h and neutralized with 2 ml diluted ammonia. The K^+ content of the extracts was determined by means of flame spectrophotometry after appropriate dilution. Where necessary, corrections were made for the contribution of Rb^+ to the light emission. All experiments were carried out at least in duplicate.

Cell sizes were determined by means of a Coulter counter model ZF equiped with a size-distribution analyzer model P641.

^{86}Rb, ^{89}Sr, [^{14}C]butyrate and tetra[^{14}C]phenylphosphonium were purchased from Amersham International, U.K.

Results

Fig. 1 shows the results of a typical experiment. Uptake of 10 mM Rb^+ was followed during 75 min by determining the radioactivity of the cells at appropriate times. The net influx rate decreased with time. In order to define whether this decrease was due to a gradual increase in the efflux rate as the cellular Rb^+ concentration increased or that in fact only the influx rate was decreased, we determined in a parallel experiment the uptake of ^{86}Rb added at appropriate times after the addition of nonradioactive 10 mM Rb^+. The initial influx rate was calculated from the tangent to these uptake curves at zero time. The influx rate decreased greatly during Rb^+ accumulation. The efflux rate calculated from the difference between influx and net flux amounted to 3.6% (with a standard error

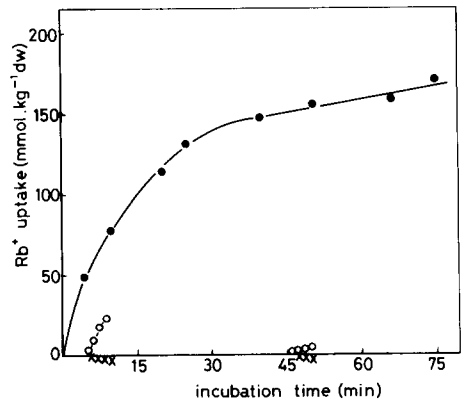

Fig. 1. Time-course of uptake of 10 mM Rb^+ into metabolizing yeast cells and Rb^+ influx and efflux determined by addition of ^{86}Rb at appropriate times. The yeast was preincubated for 60 min with 3%, w/v glucose anaerobically. Then, 10 mM RbCl was added. Net Rb^+ uptake was determined by adding ^{86}Rb together with 10 mM nonradioactive Rb^+ (●). In two parallel experiments in which also 10 mM nonradioactive Rb^+ was added after the 60 min preincubation period, ^{86}Rb was added at 5 and 45 min, respectively, and uptake of ^{86}Rb was determined. From these data influx of Rb^+ was calculated (○). Efflux of Rb^+ (×) was calculated by subtracting the corresponding net flux at the appropriate time from the influx. At 45 min, the mean influx rate and efflux rate found in four different experiments amounted to 1.36 ± 0.30 and 0.43 ± 0.16, $mmol \cdot kg^{-1}$ dry wt., respectively. Results are quoted $\pm$ S.E.

of the mean of 1.5%) of the initial influx rate found when ^{86}Rb was added together with 10 mM nonradioactive Rb^+. Therefore, the efflux did not contribute much to the net fluxes showing that the main cause for the decrease in net flux was the decrease in the influx rate.

Fig. 2 shows the results obtained from several experiments in which we compared the decrease in the ^{86}Rb influx rate with changes in cellular K^+ and Rb^+ content and with the pH_i value. In addition, the effect of addition of butyrate upon these parameters was determined. Butyrate penetrated the yeast cells as the undissociated acid giving rise to an acidification of the cells. Because pH_i was much higher than pH_o, much butyrate

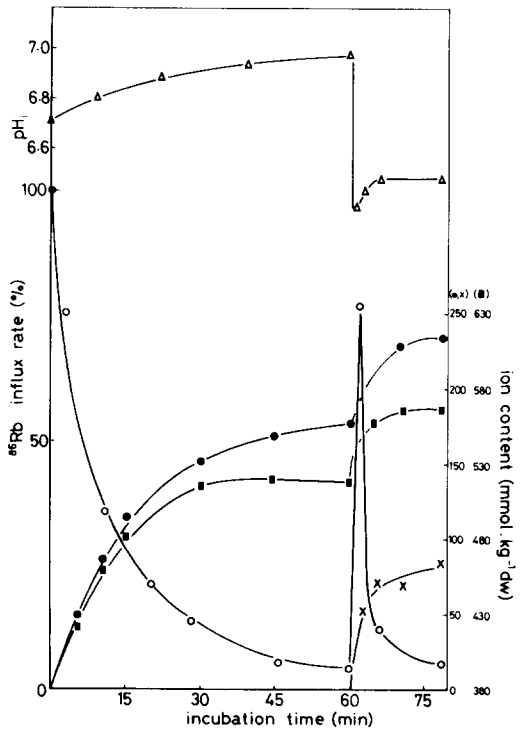

Fig. 2. Effect of loading the cells with 10 mM RbCl upon the influx rate of ^{86}Rb, the cellular Rb^+ content, the sum of cellular Rb^+ and K^+ contents and pH_i. The cells were treated as described in Fig. 1. ○, ^{86}Rb influx rate expressed in percentage of the influx rate found when ^{86}Rb is added together with nonradioactive Rb^+; ●, cellular Rb^+ content; ■, sum of the cellular Rb^+ and K^+ contents; △, pH_i and ×, cellular butyrate content. At 60 min after the addition of 10 mM RbCl, 4 mM butyrate was added. The results presented are those of one of five similar experiments.

was accumulated. Rb^+ added at an external concentration of 10 mM accumulated into metabolizing cells to an amount exceeding 150 mmol · kg^{-1} dry wt. The cellular K^+ content only decreased from 380 to 345 mmol · kg^{-1} dry wt. Therefore, the sum of the Rb^+ and K^+ contents increased considerably. Concomitantly with the accumulation of Rb^+, pH_i increased from 6.7 to 7.0. Addition of butyrate to a final concentration of 4 mM led to an additional increase in the cellular Rb^+ content. After addition of butyrate, pH_i first decreased rapidly, thereupon it slightly increased. The ultimate steady-state pH_i, however, was much lower than the pH_i observed immediately before adding the butyrate. The decrease in the net uptake rate of Rb^+, which developed during Rb^+ uptake was mainly due to a decrease in the influx rate as determined by adding carrier-free ^{86}Rb at appropriate times during accumulation of nonradioactive Rb^+. The influx rate was decreased 95% after 1 h accumulation of 10 mM Rb^+. A transient increase in the ^{86}Rb influx rate up to 75% of the initial influx rate found on adding ^{86}Rb together with 10 mM Rb^+ occurred after addition of 4 mM butyrate. 20 min later, the influx rate was again equal to the influx rate found just before addition of butyrate. The increase in total monovalent cation content found after the addition of butyrate was much lower than the amount of butyrate absorbed. The values amounted to 47 and 83 mmol · kg^{-1} dry wt., respectively.

Similar results were found for ^{86}Rb influx during accumulation of 10 mM K^+ into the cells (see Fig. 3). Addition of 4 mM butyrate led to a transient increase in the rate of influx of ^{86}Rb. In addition, the cellular K^+ content increased. An initial decrease in pH_i was followed by a slight increase. The increase in K^+ content found after addition of butyrate amounted to 51 mmol · kg^{-1} dry wt., whereas the butyrate content was 83 mmol · kg^{-1} dry wt.

During accumulation of K^+ alone and of K^+ together with butyrate, the volume of the cells increased slightly, namely, 2.0% and 2.5%, respectively. Taken into account an estimated volume of nonosmotic material of the cells, the cell water increased 3% and 4%, respectively. Fig. 4 shows a plot of the influx rate of ^{86}Rb against the mean concentration of K^+ inside the cell water. These

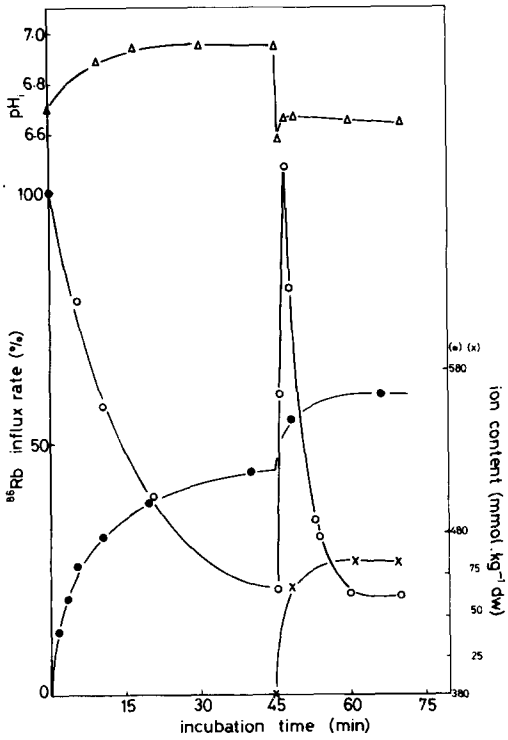

Fig. 3. Effect of loading the cells with 10 mM KCl upon the influx rate of ^{86}Rb, the cellular K^+ content and pH_i. ●, Cellular K^+ content. 4 mM butyrate was added 45 min after the addition of 10 mM KCl. See also legend to Fig. 2.

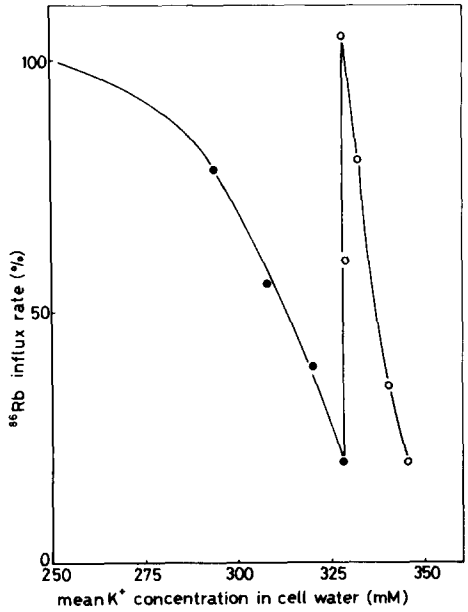

Fig. 4. Plot of the ^{86}Rb influx rate against the mean cellular K^+ concentration for yeast cells found during accumulation of 10 mM K^+, before and after addition of 4 mM butyrate. ●, ○, Data points found before and after addition of 4 mM butyrate, respectively. Data from Fig. 3. The K^+ concentrations refer to the cell water. Appropriate corrections for the increase in cell water volume during K^+ and butyrate accumulation have been applied.

concentrations were calculated from the K^+ contents of the cells given in Fig. 3 and the corresponding cell water volumes. It is clearly shown, that no single relationship exists between the influx rate of ^{86}Rb and the mean cellular K^+ concentration. After the addition of butyrate, the influx rate was increased greatly without a concomitant decrease in the mean cellular K^+ concentration, which would be expected when, as assumed for plant roots, the rate of ^{86}Rb influx is determined by the intracellular K^+ concentration [5–9].

Theuvenet et al. [10] showed that the maximal rate of Rb^+ influx into yeast decreased with increasing pH_i, whereas the affinity constants of Rb^+ for the carrier were not changed. A single relationship between the maximal rate of Rb^+ uptake and pH_i was found on varying pH_i in different ways. If the decrease in the influx rate of ^{86}Rb found on loading the cells with 10 mM Rb^+

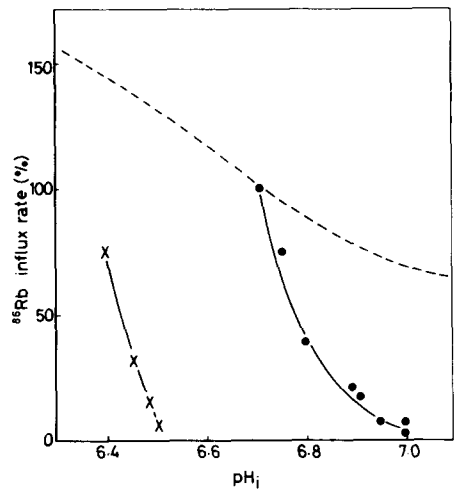

Fig. 5. Plot of the ^{86}Rb influx rate against pH_i for cells loaded with 10 mM Rb^+. The dotted line represents the dependence of the maximal rate of Rb^+ uptake found previously by us [10]. ● and ×, Data of Fig. 2 representing a plot of the influx rate of ^{86}Rb against pH_i during accumulation of 10 mM Rb^+ before and after addition of 4 mM butyrate, respectively.

TABLE I

EFFECT OF LOADING THE CELLS WITH 10 mM Rb^+ OR K^+ FOR 45 MIN UPON THE RATES OF ^{86}Rb, ^{89}Sr AND TETRA[^{14}C]PHENYLPHOSPHONIUM INFLUX

Rb^+- and K^+-loaded cells were obtained by incubating 2%, w/v yeast for 60 min in the presence of 3%, w/v glucose, and then an additional period of 45 min in the presence of both glucose and 10 mM RbCl or 10 mM KCl, respectively. In one series (+B) 4 mM Tris/butyrate was added 30 min after the addition of the RbCl or KCl, whereas in the other series (−B) no butyrate was added. After the 45 min incubation with RbCl or KCl, trace amounts of either ^{86}Rb, ^{89}Sr or tetra[^{14}C]phenylphosphonium (TPP) were added and the uptake of these tracers was determined during 1, 5 and 10 min, respectively. Initial rates of influx were calculated from the tangents to the uptake curves. The initial rates of influx were expressed as percentage of the influx rates found on adding the radioactive tracers together with RbCl or KCl instead of 45 min later. The experiments were carried out in triplicate. Results are expressed as % ± S.E.

	Rb^+-loaded cells		K^+-loaded cells	
	−B	+B	−B	+B
$^{86}Rb^+$ influx	4 ± 0.3	2.8 ± 0.3	17 ± 4	6.6 ± 1.5
$^{89}Sr^{2+}$ influx	107 ± 8	110 ± 10	123 ± 10	119 ± 13
[^{14}C]TPP influx	138 ± 33	170 ± 41	91 ± 22	154 ± 24

is due to the concomitant increase in pH_i, then the data points for the relative ^{86}Rb influx rate would lie on a single curve as was found earlier by us for the dependence of the maximal rate upon pH_i. Fig. 5 shows that this was not true. In addition, the data points for the dependence of the influx rate upon pH_i before and after the addition of butyrate did not lie on the same curve. Similar results were found for the dependence of the ^{86}Rb influx rate upon pH_i for cells loaded with K^+ (data not shown).

We have also considered the possibility that the decrease in the rate of ^{86}Rb influx found on loading the cells with K^+ or Rb^+ is due to an impairment of the energization of Rb^+ transport. There are, however, no indications that the yeast cells were depolarized during Rb^+ or K^+ uptake. The rate of influx of the lipophilic cation tetraphenylphosphonium did not decrease under conditions that caused a considerable decrease in the ^{86}Rb influx rate both in the absence and in the presence of butyrate. Also, the rate of influx of $^{89}Sr^{2+}$ was not decreased under these conditions (see Table I).

Discussion

Rothstein and Bruce [4] have shown that the influx rate of K^+ for 10 mM K^+ at pH 4.0 in *S. cerevisiae* remains constant for at least 5 min. The efflux of K^+ is negligible during this period when compared to the efflux observed in the absence of K^+. At intermediate K^+ concentrations, the initial efflux is also very low, but increases with a lag time of approx. 30 min to 0.68 $mmol \cdot kg^{-1} \cdot min^{-1}$, giving rise to a decrease in net efflux rate. A similar rate of efflux has been found by us for K^+ efflux after addition of 10 mM Rb^+ (data not shown). Ryan and Ryan [3] who examined the K^+ uptake in *S. cerevisiae* with propanol-2 as substrate also found a decrease in the net K^+ uptake rate during accumulation of 10 mM K^+. This decrease, however, could be accounted for by an increase in leakage of K^+ from the cells, whereas the influx rate hardly decreased. The differences between their results and ours can be attributed to the fact that the rate of K^+ influx under the conditions applied by Ryan and Ryan is approx. 10-times lower than the rate of K^+ influx in our cells (data not shown), whereas the K^+ efflux rates observed by Ryan and Ryan are of the same order of magnitude as those found by us and also by Rothstein and Bruce [4]. This will lead rapidly to a balance between K^+ influx and efflux, whereas under the conditions applied by us the initial influx rate being determined with ^{86}Rb exceeds the efflux rate greatly.

In plant roots, the rate of ^{86}Rb influx also decreases during K^+ accumulation [5–9]. The gradual decrease in the influx rate of ^{86}Rb in plant roots is attributed to a feedback regulation by cellular K^+ of the roots. It is assumed that the transport mechanism responsible for K^+ uptake

bears a set of K^+-binding sites located at the cellular side of the plasma membrane [5,6]. Occupation of these sites by cellular K^+ should lead to a change in the conformational state of the transport system by which the carrier is inactivated. This theory is supported by the finding that the inhibition of K^+ uptake (I) relates to the cellular K^+ concentration (S) according to an equation of the form of:

$$I = \frac{I_m S^n}{K + S^n} \quad (1)$$

in which equation I_m represents maximal inhibition, n is the number of sites binding intracellular K^+, and K is the dissociation constant of K^+ with the multi-site translocator. With the data points found before addition of butyrate, a good fit is obtained according to Eqn. 1 with $n = 19$ (data not shown). However, on adding butyrate, the cellular K^+ content, the mean concentration of K^+ in the cell water and the rate of ^{86}Rb influx are increased. This is not compatible with the quantitative predictions of Eqn. 1. Our results indicate that the influx rate of ^{86}Rb is not regulated by the K^+ content of the cells. It might, however, be possible that uptake of butyrate in the cells induces a redistribution of K^+ between the cytosol and, e.g., the vacuole by which the cytosolic K^+ concentration is transiently decreased and that still a direct relation exists between the ^{86}Rb influx rate and the cytosolic K^+ concentration. Therefore, an allosteric regulation of the monovalent cation transport system by the cations translocated into the cytoplasm cannot be completely ruled out at this stage.

In yeast not only K^+ influx but also influx of amino acids is regulated by the cellular substrate content [18].

That the decrease in ^{86}Rb influx rate found on increasing the cellular Rb^+ or K^+ content is due to an impairment of the energization of monovalent cation transport is not very probable. There are no indications that metabolism is stopped. On the contrary, glycolysis is increased 10% (data not shown) and the membrane potential becomes more negative. This apparent hyperpolarization could possibly be accounted for by the increase in glycolysis. Also, the protonmotive force, which may be the driving force of K^+ uptake [19] is increased, since not only the membrane potential becomes more negative but also the pH gradient across the yeast cell membrane is increased.

We have also excluded that the decrease in the ^{86}Rb influx rate found on loading the cells with 10 mM Rb^+ is due to the increased pressure on the cell membrane. Decreasing this pressure by addition of 800 mM sorbitol does not lead to an increase in ^{86}Rb influx in Rb^+-loaded cells (data not shown).

Ryan and Ryan [3] have shown that the rate of ^{42}K exchange with ^{39}K depends upon pH_i. This pH_i dependence is almost identical to the pH_i dependence of the maximal rate of Rb^+ uptake observed by Theuvenet et al. [10] and indicated in Fig. 5. Apparently, the decrease in the ^{86}Rb influx rate observed during accumulation of K^+ or Rb^+ into the yeast cells cannot be ascribed to the alkalinization of the cells. The decrease in the influx rate found is much greater than would be expected from the observed increase in pH_i. In addition, the ^{86}Rb influx rate in cells acidified with butyrate is, after the transient increase in ^{86}Rb entry, very low despite the fact that the cells still have a low pH_i.

In conclusion, it is difficult at this stage to say which mechanism is involved in the regulation of ^{86}Rb influx during Rb^+ or K^+ accumulation into metabolizing yeast cells. The physiological role of the regulation of the monovalent cation influx rate may be, that the cells conserve energy which would be otherwise used for the accumulation of the cations. Such a feedback mechanism would be more beneficial for the cell than a regulation of its monovalent cation via a pump and leak system.

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