*Biochimica et Biophysica Acta*, 291 (1973) 274–279


BBA 76129

# ACTIVATION OF $Rb^+$ AND $Na^+$ UPTAKE INTO YEAST BY MONOVALENT CATIONS

G. W. F. H. BORST-PAUWELS, P. SCHNETKAMP AND P. VAN WELL
*Laboratory of Chemical Cytology, University of Nijmegen, Nijmegen (The Netherlands)*
(Received July 10th, 1972)

## SUMMARY

$^{86}Rb^+$ uptake by yeast was not only stimulated by $Rb^+$ or $K^+$ but also by $Na^+$. The uptake of $^{22}Na^+$ was enhanced by both $Rb^+$ and $K^+$, but not by $Na^+$, which was inhibitory at all concentrations applied. Inhibition of $^{22}Na^+$ uptake by inactive $Na^+$ occurred in two phases: one phase refers to inhibition at low $Na^+$ concentrations and the other to inhibition at high $Na^+$ concentrations. Our results can be qualitatively described by a two-site transport mechanism, having two cation binding sites, which must be occupied with monovalent cations before transport can occur.

## INTRODUCTION

Recently it has been shown that $Rb^+$ transport by yeast shows homotropic cooperativity; the dependence of the uptake rate upon the $Rb^+$ concentration appears to be sigmoidal[1]. The mechanism of $Rb^+$ transport could be described by a two-site transport mechanism, which functions only when both sites are occupied by $Rb^+$. We have now examined whether this is also true for $Na^+$ uptake. In addition we have investigated whether $Na^+$ can replace $Rb^+$ as the activating cation.

## METHODS

The yeast, *Saccharomyces cerevisiae*, Delft 2, was pre-aerated for 1 day at room temperature in distilled water. This yeast (22 g/l) was suspended in a solution containing 30 g/l glucose and 45 mM Tris–succinate buffer (pH 4.5) and was preincubated at 25 °C with $N_2$ bubbling through the suspension. After 60 min 27 ml suspension were rapidly added to 3 ml of a solution of $^{86}RbCl$ or $^{22}NaCl$ in Tris–succinate buffer provided with unlabelled RbCl, NaCl or KCl at different concentrations. Nitrogen was bubbled through the suspension continuously. The concentration of the radioactive cation did not exceed $10^{-5}$ M. Nine 1.8-ml samples of this mixture were taken at different times during a 5-min incubation period, the first sample being drawn after 15 s. The samples were added to 20 ml ice-cold 20 mM $MgCl_2$ placed 1 s earlier on a sintered glass funnel containing a No. 602 h Schleicher and Schüll filterdisk of 2.7 cm diameter. Upon mixing the fluid in the funnel, the solution was drawn off by suction. The yeast on the filter was washed successively by 1.5 ml of ice-cold water and 3 ml

of acetone. The radioactivity on the filter disks was determined with an end-window Geiger–Müller tube. Radioactive $Na^+$ or $Rb^+$ adsorbed to the cell wall were removed almost entirely by exchange with $Mg^{2+}$ during the filtration procedure, whereas no significant losses of intracellular $^{22}Na^+$ or $^{86}Rb^+$ occurred. The rate of uptake did not differ significantly when ice-cold distilled water was used instead of 20 mM $MgCl_2$, only the intercept of the ordinate of the uptake curves was much greater when using distilled water than on applying the $MgCl_2$ solution. Corrections for small losses of yeast during the filtration procedure were made by weighing the filterdisks before and after filtration, and referring the accumulated radioactivity to the dry weight of yeast present on the filter. Initial rates of uptake were obtained from the uptake curves which did not deviate significantly from a straight line, at least during the first 3 min of uptake, and in most cases not even during the full 5-min time interval.

The initial $Na^+$ and $K^+$ concentrations in the yeast preincubation medium were determined by centrifuging aliquots of the preincubated yeast suspension and using the supernatants obtained for flame-photometric analysis. The effect of addition of NaCl upon $K^+$ release from the yeast was studied by adding NaCl to the yeast suspension and taking 5-ml samples after 1.5 min incubation. The $K^+$ concentration was determined in the supernatant after centrifuging the yeast. Corrections for the contribution of $Na^+$ to the emission at 769 nm were made.

## RESULTS

We have determined the rate of radioactive $Rb^+$ uptake as a function of the concentration of added cations. The results are represented in plots of the relative rate of $^{86}Rb^+$ uptake expressed in percent of the control value without added cation against the logarithm of the concentration of added cation. Fig. 1 indicates that the the influx rate of $^{86}Rb^+$ is enhanced, not only by $Rb^+$ or $K^+$, but also by $Na^+$. The concentrations at which the ions stimulate $^{86}Rb^+$ transport to half of the maximal value estimated from Fig. 1 are tabulated in Table I. At much higher concentrations

TABLE I

ESTIMATED HALF-MAXIMAL CONCENTRATIONS OF MONOVALENT CATIONS NEEDED FOR STIMULATION AND INHIBITION OF $^{86}Rb^+$ AND $^{22}Na^+$ TRANSPORT, AND THE RELATIVE UPTAKE RATES OF THE ISOTOPE AT EITHER MAXIMAL ACTIVATION OR FIRST PHASE INHIBITION

$C_{0.5,1}$ is the half-maximal concentration for stimulation or first phase inhibition. $C_{0.5,2}$ is the concentration of added cation at which the rate of isotope uptake is decreased to 50% of the maximal value or to 50% of the rate obtained after first phase inhibition. $V/v_0$ is the ratio of uptake rates found at either maximal stimulation or first phase inhibition and the uptake rate found without added cation.

| *Transport process* | *$Na^+$ present (mM)* | *Added cation* | $C_{0.5,1}$ *(mM)* | $C_{0.5,2}$ *(mM)* | $V/v_0$ |
|---|---|---|---|---|---|
| $^{86}Rb^+$ uptake | 0.05 | $K^+$ | 0.018 | 0.52 | 1.80 |
| | | $Rb^+$ | 0.060 | 2.5 | 2.28 |
| | | $Na^+$ | 0.82 | ≈ 100 | 1.85 |
| $^{22}Na^+$ uptake | 0.05 | $K^+$ | ≤0.02 | 0.39 | ≥1.20 |
| | | $Rb^+$ | ≤0.06 | 2.0 | 1.26 |
| | | $Na^+$ | 0.35 | ≈ 90 | 0.10 |
| $^{22}Na^+$ uptake | 10 | $K^+$ | 0.11 | 2.4 | 2.26 |
| | | $Rb^+$ | 0.20 | 6.5 | 3.37 |

all three cations decrease the transport rate of $^{86}Rb^+$ into the cells. The concentrations, estimated from Fig. 1, at which the maximal rate of transport is decreased by 50% are also included in Table I. We have examined whether activation of $^{86}Rb^+$ transport by $Na^+$ might be only an indirect effect due to the increased amount of $K^+$ released from the cells in the presence of added $Na^+$ (*e.g.* the $K^+$ concentration in the medium increased from 0.028 to 0.072 mM in the presence of 50 mM NaCl within 1.5 min). The extent of activation, which would be expected on account of the increased $K^+$ concentration has been calculated without making corrections for competitive inhibition by the added $Na^+$. It is seen in Fig. 1 that the observed stimulation of $^{86}Rb^+$ uptake rate in the presence of increasing amounts of $Na^+$ is much greater than the maximal possible stimulation due to $K^+$ released from the yeast cells at concentrations of $Na^+$ lower than 10 mM. On the other hand, the observed stimulation at $Na^+$ concentrations above 10 mM appeared to be smaller than the calculated stimulation. This might be due to the fact that we did not account for competitive inhibition of $^{86}Rb^+$ transport by $Na^+$ in our calculation.

The effects of varying concentrations of RbCl, KCl or inactive NaCl on the rate of $^{22}Na^+$ uptake are shown in Fig. 2. The medium of the control contains 0.05 mM

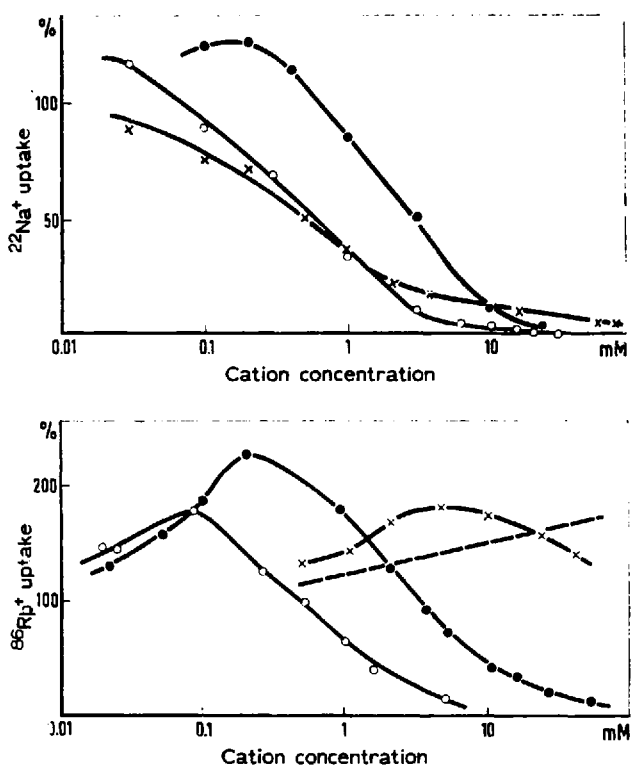


Fig. 1. Effect of monovalent cations upon the rate of uptake of $^{86}Rb^+$ by yeast. The concentrations of $K^+$ and $Na^+$ in the medium without added cations were 0.03 and 0.05 mM, respectively. The uptake rate is expressed in percent of the control rate. The latter rate, calculated from the uptake curves of $^{86}Rb^+$ into the cells, corresponds with a rate of disappearance of radioactivity from the medium into the cells of 2.5% per min at a yeast concentration of 20 g wet wt per l. The broken line represents calculated stimulation of $^{86}Rb^+$ uptake as a function of $Na^+$ concentration due to the added $K^+$, except that released from the cells within 1.5 min. No correction was made for competitive inhibition by either $K^+$ or $Na^+$. ○, effect of $K^+$; ●, effect of $Rb^+$; ×, effect of $Na^+$.

Fig. 2. Effect of monovalent cations upon the rate of uptake of $^{22}Na^+$ by yeast. The control uptake rate amounts to 0.55% decrease of the radioactivity of the medium per min at a yeast concentration of 20 g wet wt per l. ○, effect of $K^+$; ●, effect of $Rb^+$; ×, effect of $Na^+$.

$Na^+$ which must be due to leakage of $Na^+$ from the cells. Apparently, the addition of extra $Na^+$ does not increase the rate of $^{22}Na^+$ transport into the cells. On the contrary, even low concentrations of $Na^+$ lead to a large decrease in the $^{22}Na^+$ uptake rate. The inhibition of $^{22}Na^+$ uptake occurs in two phases: $Na^+$ concentrations up to about 10 mM inhibit half-maximally at 0.35 mM and give a maximum 90% inhibition, whereas $Na^+$ concentrations above 10 mM inhibit the residual 10% activity half maximally at about 90 mM.

$Rb^+$ and $K^+$ appear to enhance $^{22}Na^+$ uptake, though to a much smaller extent than is found for $^{86}Rb^+$ transport. Only upper limits for the concentrations needed for half-maximal stimulation of the rate of $^{22}Na^+$ uptake could be established from the curves in Fig. 2, see Table I. The estimated concentrations needed for half-maximal inhibition of $K^+$- or $Rb^+$-activated $^{22}Na^+$ transport are of the order of magnitude of those found for inhibition of $^{86}Rb^+$ transport. At a concentration of 10 mM $Na^+$, a much greater stimulation of $^{22}Na^+$ uptake is found than in the absence of added $Na^+$, as shown in Fig. 3. The rate of disappearance of radioactive $Na^+$ from the medium, which decreased from 0.55%·min$^{-1}$ to 0.06%·min$^{-1}$ by addition of 10 mM $Na^+$ to the yeast suspension, increased again to 0.20%·min$^{-1}$ in the presence of 1 mM $Rb^+$. The concentrations of $Rb^+$ and $K^+$ needed for half-maximal stimulation of $^{22}Na^+$ uptake were several times higher than those found at 0.05 mM $Na^+$. This applies also to the half-maximal concentrations for inhibition of radioactive $Na^+$ uptake, see Table I.

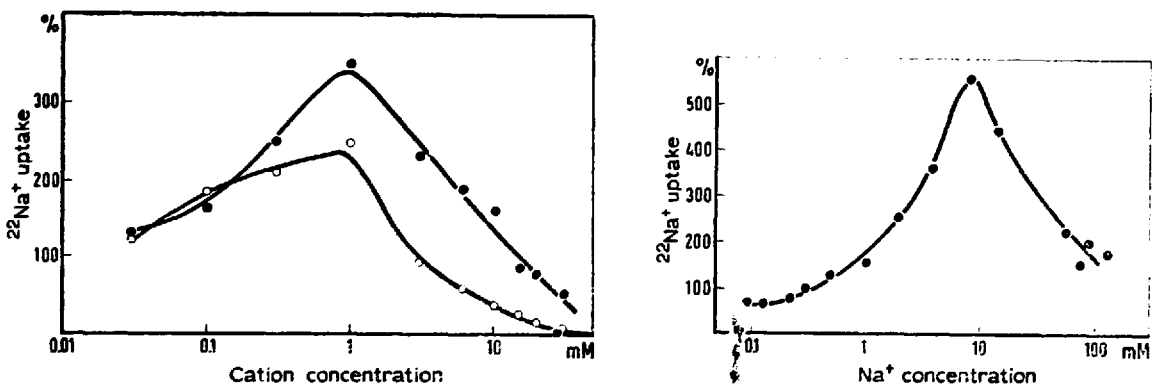


Fig. 3. Effect of monovalent cations upon the rate of uptake of $^{22}Na^+$ by yeast in the presence of 10 mM NaCl. The control uptake rate amounts to 0.06% decrease of the radioactivity of the medium per min at a yeast concentration of 20 g wet wt per l. ○, effect of $K^+$; ●, effect of $Rb^+$.

Fig. 4. Effect of 2.5 mM RbCl on $^{22}Na^+$ uptake rate at varying $Na^+$ concentrations, expressed in percent of the uptake rate in the absence of added RbCl.

Fig. 4 shows that at a given $Rb^+$ concentration the stimulation of $^{22}Na^+$ uptake increases with higher $Na^+$ concentrations in the medium up to 10 mM, and decreases again when the $Na^+$ concentration exceeds this value. The $Na^+$ concentration at which the relative increase of $^{22}Na^+$ uptake is maximal appeared to depend upon the $Rb^+$ concentration. At 0.3 mM $Rb^+$ this concentration is 2 mM.

## DISCUSSION

The model for $Rb^+$ transport in yeast that we recently proposed[1] implies a two-site transport mechanism; one site having a transport function and a second

site being the activation site. We showed that transport of $Rb^+$ occurs only when both sites are filled with $Rb^+$ or one site with $K^+$ and the other one with $Rb^+$. It was shown that the affinity of $Rb^+$ and of $K^+$ for the activation sites is much greater than the affinity of these ions for the substrate site. Accordingly, stimulation of $^{86}Rb^+$ uptake occurs at much lower concentrations of inactive $Rb^+$ and $K^+$ than those at which inhibition due to replacement of $^{86}Rb^+$ from the substrate sites occurs.

Apparently, $Na^+$ is also able to activate the $Rb^+$ transport mechanism. The affinity of $Na^+$ for the activation sites is much lower than the affinity of $Rb^+$ or $K^+$ for these sites, as judged from the concentrations needed for 50% stimulation of $Rb^+$ uptake. Also, much higher concentrations of $Na^+$ are needed in order to obtain 50% inhibition of $Rb^+$ uptake. The latter value is somewhat higher than the value of 30 mM reported for the $K_m$ of $Na^+$ uptake at pH 4.5 (ref. 2).

$Na^+$ uptake probably also proceeds *via* a two-site transport mechanism. $Rb^+$ and also $K^+$ are able to stimulate the $Na^+$ transport, pointing to the existence of at least one additional site of the $Na^+$ transport mechanism. The concentrations of $K^+$ and $Rb^+$ needed for half-maximal stimulation of $Na^+$ uptake are of the same order of magnitude as the concentrations needed for half-maximal stimulation of $Rb^+$ transport, which might indicate that the same or similar sites are involved in the activation of both processes. The first phase inhibition of $^{22}Na^+$ uptake by added $Na^+$ occurs in the same range of concentrations at which stimulation of $Rb^+$ uptake occurs. This suggests that the decrease in the rate of $^{22}Na^+$ uptake by low concentrations of $Na^+$ is due to occupation of the activation sites by $Na^+$ leading to a 90% reduction of the $^{22}Na^+$ transport rate. This would mean that when $Na^+$ is on the activation sites the $^{22}Na^+$ uptake rate is much lower than when the activation sites are partly occupied by $K^+$, which is the situation when no extra ions are added, as was found for $Rb^+$ uptake[1]. In agreement with this view, activation of $^{22}Na^+$ transport by both $Rb^+$ and $K^+$ under conditions of maximal first phase inhibition (10 mM $Na^+$) is much greater than at 0.05 mM $Na^+$. Stimulation of $Na^+$ transport at high $Na^+$ concentrations by 2.5 mM $Rb^+$ is much lower than at 10 mM $Na^+$, see Fig. 4. Competition of $Na^+$ with $Rb^+$ or $K^+$ for occupation of the activation sites is thus very effective in that case.

The half-maximal concentrations of second phase inhibition of $^{22}Na^+$ transport by $Na^+$ and the half-maximal concentrations for inhibition of $^{22}Na^+$ uptake by both $Rb^+$ and $K^+$ are of the order of magnitude of the half-value concentrations needed for inhibition of $^{86}Rb^+$ transport, supporting the view that the mechanism of $Na^+$ transport is identical to the mechanism of $Rb^+$ transport.

The model for ion uptake by yeast described is in some way similar to that developed by Armstrong and Rothstein[3], namely, as far as the assumption that at least two sites are involved in cation uptake is concerned. However, they did not find any indication for enhancement of cation uptake, only inhibition was found. In addition, the $K$ values reported by these authors, which are a measure of the affinity for the modifier site in their model, are higher than the values found by us. This might indicate that, instead of two sites, three sites are involved in monovalent cation transport by yeast: modifier and substrate sites with a relatively low affinity for the cations and activation sites with a high affinity for the cations. The efflux of $Na^+$ from yeast cells can also be described by a three-site model[4]. For activation of cation transport in *Neurospora crassa* we refer to refs 5 and 6.

We are now developing a mathematical model which describes ion uptake *via*

a two-site transport mechanism with the aim determining of whether cation uptake by yeast can be described by a two-site transport model or whether a more complicated mechanism underlies the rate data. (G. W. F. H. Borst-Pauwels, unpublished.)

ACKNOWLEDGEMENT

The yeast was kindly supplied by the Gist- en Spiritusfabriek at Delft. The authors are very grateful to Prof. Dr Ch. M. A. Kuyper from this laboratory and to Prof. Dr S. L. Bonting from the laboratory of Biochemistry for their valuable remarks.

REFERENCES

1 G. W. F. H. Borst-Pauwels, G. H. J. Wolters and J. J. G. Henricks, *Biochim. Biophys. Acta*, 225 (1971) 269.
2 W. McD. Armstrong and A. Rothstein, *J. Gen. Physiol.*, 48 (1964) 61.
3 W. McD. Armstrong and A. Rothstein, *J. Gen. Physiol.*, 50 (1967) 967.
4 E. Dee and E. J. Conway, *Biochem. J.*, 107 (1968) 265.
5 C. W. Slayman and C. L. Slayman, *J. Gen. Physiol.*, 55 (1970) 758
6 C. W. Slayman, *Biochim. Biophys. Acta*, 211 (1970) 502.