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Kinetical parameters of monovalent cation uptake in yeast calculated on accounting for the mutual interaction of cation uptake and membrane potential

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Kinetical parameters of monovalent cation uptake in yeast are calculated according to a model for mutual interaction of membrane potential and cation uptake. Apparent K_m values for monovalent cation uptake obtained from uptake studies are 3–5-times lower than the K_m values expected when the membrane potential remains constant at increasing cation concentrations instead of being reduced. The model accounts for various phenomena as (i) the increase in apparent K_m of Rb^+ uptake accompanying the decrease in maximum rate of uptake found on increasing the cellular K^+ content, (ii) the decrease in maximum uptake rate and increase in K_m in case of simultaneous transport of phosphate and Rb^+, (iii) the change in the maximum uptake rate without a change in K_m under conditions that the proton pump is effected and (iv) the absence of an increase in K^+ efflux at high depolarizing external cation concentrations.

Introduction

If carrier-mediated cation uptake is driven by the membrane potential one may expect that the cells are depolarized on increasing the cation concentration in the medium and by this the influx of the cations will be smaller than expected when the membrane potential is unaffected. The mutual interaction of ion uptake and membrane potential may complicate the kinetics of cation uptake [1]. For cation uptake via a single-site carrier driven by the membrane potential deviations from Michaelis-Menten kinetics are expected because of the dependence of the membrane potential upon the cation concentration. However, under appropriate conditions these deviations may be small and Michaelis-Menten kinetics may still prevail by approximation. The apparent maximum rate of uptake then depends upon the membrane potential expected at saturating cation concentrations and the K_m does not only depend upon the affinity of the cation for the carrier but also upon the extent of depolarization.

In yeast monovalent cation uptake is driven by the membrane potential [2], whereas in turn uptake of monovalent cations gives rise to a depolarization of the cell membrane [3,4]. The cation concentrations at which the influx of the fluorescent membrane potential probe DMP is reduced half-maximally appeared to be of the order of magnitude of the K_m values found for uptake of the cations applied. This indicates that the depolarization by the cations is due to cation influx via the monovalent cation carrier of the yeast.

We examined whether the model of mutual interaction of ion uptake and membrane potential could be applied to cation uptake in yeast. According to this model K_m values for cation uptake were calculated from previously obtained data on the dependence of the membrane potential upon the cation concentration [4]. In that study only cation concentrations were applied at which the uptake does not show significant deviations from Michaelis-Menten kinetics. Such deviations are expected at relatively low cation concentrations, at which besides the substrate binding site to which the above mentioned K_m refers a secondary high-affinity site, the socalled activation site, is still not saturated [5]. For Li^+ which has equal affinities to the two sites still Michaelis-Menten kinetics is found under conditions that the activation site is not saturated [6].

Probably monovalent cation uptake in yeast consists of cation-proton cotransport. The membrane potential

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Abbreviations: TPP, tetraphenylphosphonium; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DMP, 2-(dimethylaminostyryl)-1-ethylpyridinium.

of the yeast cells even at high pH is too small in order to account for the relatively large accumulation of K^+ found under equilibrium conditions, whereas cotransport of K^+ and proton accounts rather well for the large accumulation of K^+ over a wide range of pH values [7]. As a matter of fact in the related organism *Neurospora crassa*, a K^+-proton cotransport has been demonstrated [8].

According to Ref. 1 the concentration dependence of cotransport of a monovalent cation and a proton is given by

$$v_{j,I} = \frac{V_{m,j,I} y^2 s_{j,I}}{K_{m,j} + s_{j,I}} = \frac{V_m s_{j,I}}{K_m + s_{j,I}} = \frac{V_{m,j,I} y_\sim^2 s_{j,I}}{K_{m,j} y_\sim^2 / y_0^2 + s_{j,I}} \quad (1)$$

y is $\exp(-FE/2RT)$. F, R and T have their usual meaning, E is the difference in membrane potential between cytosol (II) and medium (I). y_0 and $y_\sim$ are the values of y at zero cation concentration and at saturating cation concentrations, respectively. $V_{m,j,I}$ is the maximum rate of uptake at $y_\sim = 1$, and $K_{m,j}$ is the half-maximum concentration for uptake of S_j expected when the depolarization caused by cation influx is virtually zero ($y_\sim = y_0$). Apparently K_m depends upon the extent of depolarization at saturating cation concentrations.

It will be shown that mutual interaction of monovalent cation uptake and membrane potential in yeast can account for various remarkable phenomena described in literature. One of these is that Rb^+ uptake is inhibited competitively by phosphate, though Rb^+ and phosphate uptake proceed via different carriers [9]. A second phenomenon concerns the concomitant increase in K_m and decrease in the maximum rate of Rb^+ uptake found on increasing the cellular K^+ content [10]. This has also been observed for Rb^+ uptake in plant roots [11,12]. A third phenomenon is that on increasing the external Rb^+ concentration efflux of Rb^+ from $^{86}Rb^+$-loaded cells does not increase significantly despite the expected depolarization of the cells [13]. At increasing K^+ concentrations in the medium even a decrease in K^+ efflux is found [14]. Finally, we will show that the finding that intracellular acidification of yeast cells causes an increase in the maximum rate of Rb^+ uptake and no change in K_m [15,16] is also compatible with our theory.

Results

The influx of the membrane potential probe DMP in yeast as function of the extracellular cation concentration of K^+, Rb^+, Cs^+, Na^+ and Li^+ [4] has been determined previously under almost the same conditions at which monovalent cation uptake has been studied by us [5]. Both studies were performed with 2% (w/v) yeast in 45 mM Tris succinate buffer in the presence of 3–5% (w/v) glucose under anaerobic conditions. In case of DMP uptake the pH was 7.0 and the time of preincubation was 30 min, whereas cation uptake was studied at pH 7.2 after 60 min preincubation. There are no indications that these minor differences affect the kinetics of cation uptake. The main difference between the two studies is that in the determination of DMP influx the ionic strength is kept constant by means of choline chloride, so that the sum of choline and added cation equals 30 mM, which is not done in the uptake studies.

The influx of DMP is assumed to be proportional to y/y_0. In the absence of added cation the membrane potential amounts to -111 mV at pH 7.0 [17], corre-

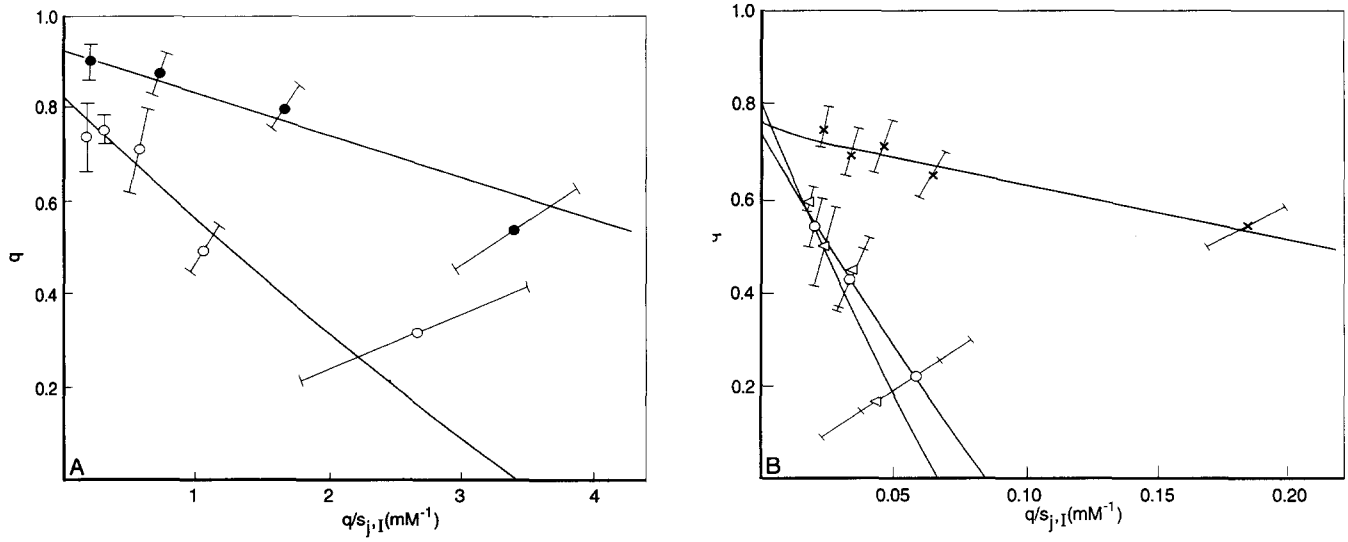

Fig. 1. Hofstee plot of q calculated from previously obtained experimental data [4] for various monovalent cations added to a suspension of metabolizing yeast cells at pH 7, under anaerobic conditions and at constant ionic strength. (A) Effects of K^+ (•) and Rb^+ (○). (B) Effects of Cs^+ (×), Na^+ (○) and Li^+ (△). The length of the bars denote the standard error of the mean.

TABLE I

Kinetical parameters for monovalent cation uptake in yeast

$K_{m,exp}$, K_m and $K_{m,j}$, are expressed in mM and V_m, $V_{m,exp}$, $V_{m,j,I}$ and $V_{m,j,II}$ in mmol/min per kg dry weight.

Ion	K^+	Rb^+	Cs^+	Na^+	Li^+
$y_\sim$	3.80	4.98	5.48	5.56	5.07
K_m	0.21	0.45	1.9	14.4	22
$K_{m,exp}$	0.15	0.43	3.0	29	33
$V_{m,j,I}$	1.24	0.46	0.31	0.29	0.43
$V_{m,j,II}$	0.300	0.111	0.075	0.070	0.104
V_m	17.8	11.4	9.3	9.1	11.1
$V_{m,exp}$	[19.0]	11.4	10.3	5.0	[10.2]
$K_{m,j}$	1.13	1.41	4.9	36.1	67
$K_{m,j}/K_m$	5.38	3.14	2.58	2.51	3.05

sponding with $y_0 = 8.82$. Knowing y_0 we are able to calculate y as function of the concentration of added cation $s_{j,I}$. Eqn. 2 gives the dependence of y upon $s_{j,I}$

$$y^3 = \frac{L_{m,j} + s_{j,I}}{M_{m,j} + s_{j,I}} Q_{m,j} \qquad (2)$$

The coefficients in this equation still depend upon y (see further Ref. 1). As shown by us a plot of q defined by

$$q = 1 - (y/y_0)^3 \qquad (3)$$

against $q/s_{j,I}$, is slightly concave in shape [1]. Fig. 1 shows the plots of the experimentally obtained data of q against $q/s_{j,I}$. The curves drawn are those fitting these data according to Eqn. 3. Table I gives the values of the kinetical parameters of cation uptake used in the calculation of the curves and also those being found experimentally ($K_{m,exp}$ and $V_{m,exp}$) [5,18,19]. For Li^+ and K^+ no uptake studies have been carried out under these conditions. For these cations $K_{m,exp}$ is calculated from competitive inhibition studies of Rb^+ uptake [18], whereas tentative values of $V_{m,exp}$ are obtained by multiplying $V_{m,exp}$ for Rb^+ found by us [18,19] with the quotient of the maximum rates of K^+ or Li^+ uptake and the maximum rate for Rb^+ uptake found in Ref. 20. $K_{m,exp}$ is corrected for differences in surface potential between the cation uptake experiments and the DMP uptake experiments because of the differences in ionic strength mentioned above according to Ref. 4. Since only relative values of V_m can be obtained from the dependence of y upon $s_{j,I}$, we took for the value of V_m for Rb^+ uptake $V_{m,exp}$ found for that cation and calculated the absolute values of V_m for the other cations relative to that of Rb^+. $V_{m,j,I}$ and $V_{m,j,II}$ are defined by Eqns. 1 and 5, respectively.

In the presence of 0.2 mM phosphate and at pH 7.2 the maximum rate of Rb^+ uptake decreases by 10%, whereas the apparent K_m increases by 75% [9]. Uptake of orthophosphate in yeast consists of a cotransport of monovalent phosphate and two or three protons [21,22]. The influx at 0.2 mM phosphate amounts to about 6 mmol/min per kg dry weight causing partial depolarization of the cells which depends upon the number of protons cotransported, see Table II. We simulated Rb^+ uptake in the presence of 0.2 mM phosphate, see Fig. 2 for the corresponding Hofstee [23] plots of Rb^+ uptake. Cotransport of phosphate with both two and three protons is considered. If the concentration dependence of the influx is described by a Michaelis-Menten equation, then a straight line is found according to

$$v_{Rb,I} = V_m - K_m(v_{Rb,I}/s_{Rb,I}) \qquad (4)$$

Actually slightly concave curves are found. However, in the range of cation concentrations at which the activation site is virtually saturated, these deviations are quite small and in the presence of phosphate these

TABLE II

Calculated effect of 0.2 mM phosphate uptake upon the membrane potential of yeast at pH 7 in the absence of added cation (E_0) and in the presence of saturating concentrations of Rb^+ ($E_\sim$)

$E_\sim - E_0$ is the depolarization caused by saturating concentrations of Rb^+. Equil. means equilibration of K^+ between cells and medium. $s_{K,I}$ is the concentration of K^+ in the medium. n is the number of protons cotransported with phosphate.

Phosphate	n	Equil.	E_0 (mV)	$E_\sim$ (mV)	$E_\sim - E_0$ (mV)	$K_{m,Rb}$ (mM)	$s_{K,I}$ (μM)
−		−,+	−111	−82	29	1.41	10
+	2	−	−104	−80	24	1.41	10
+	3	−	−98	−79	19	1.41	10
+	2	+	−103	−80	23	1.44	19
+	3	+	−95	−78	17	1.49	34

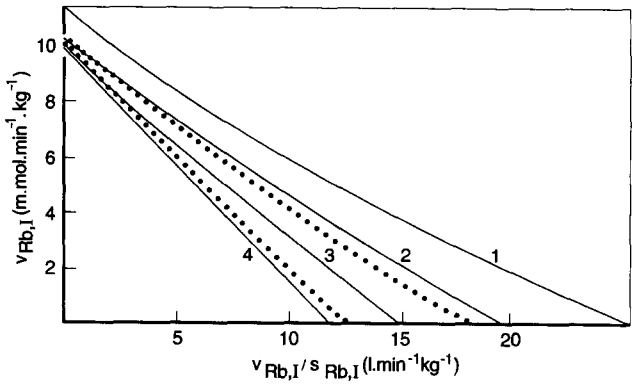

Fig. 2. Simulation of the effect of 0.2 mM phosphate uptake upon Rb^+ uptake at pH 7 by metabolizing cells under anaerobic conditions [9]. Curve 1 control without added phosphate, curve 2 and 3 expected curves for cotransport of phosphate with two or three protons, respectively, and curve 4 curves expected on the ground of the experimentally found Rb^+ uptake at full activation of the transport mechanism. Dotted lines represent the uptake curves expected if K^+ is allowed to equilibrate between cells and medium, see also Table II.

deviations almost disappear. Also the Rb^+ uptake curve, calculated from the experimentally found increase in K_m and decrease in V_m [9] expected under conditions that the activation site of the carrier is saturated with Rb^+ and 30 mM choline chloride is present, is given. The slopes of the uptake curves, which are a measure for the K_m of uptake, increase in the presence of phosphate. This increase is higher for cotransport of phosphate with three protons than with two protons, but is still smaller than the increase found experimentally. On accounting for redistribution of K^+ between cells and medium due to depolarization of the cells by phosphate, the discrepancies between the uptake curves calculated and that found experimentally become still smaller. The maximum rates of Rb^+ uptake are decreased by phosphate to about the same extent as has been found experimentally. Table II shows that not only the membrane potential at zero Rb^+ concentrations is reduced by phosphate influx but also the depolarization at saturating concentrations of Rb^+. Due to the depolarization of the cells caused by phosphate influx, the external K^+ concentration will increase causing a further reduction of the membrane potential and an increase in $K_{m,Rb}$. Both factors contribute to the increased slopes of the curves expected on accounting for redistribution of K^+ in Fig. 2.

Acidifying metabolizing yeast cells under anaerobic conditions at pH 4.5 by addition of varying amounts of butyric gives rise to an increase in V_m for Rb^+ uptake [15]. Similar results were obtained in Ref. 16 at pH 5.0. Remarkably this stimulation is not accompanied by a change in K_m. Possibly the increase in V_m is due to stimulation of the proton pump accompanying the acidification of the cells [24]. We now examined whether our model may account for an increase in V_m without accompanying change in K_m on varying the contribution of the proton pump to the charge fluxes across the cell membrane. Fig. 3 shows that according to the model for mutual interaction of membrane potential and cation uptake hyperpolarization or depolarization of the cells due to an increase or decrease in the proton pump activity is expected to give rise to large changes in V_m for Rb^+ uptake, whereas the K_m only slightly varies. Accordingly, the theoretically expected depolarization at saturating Rb^+ concentrations is almost constant.

In yeast Rb^+ influx in cells metabolizing in the presence of 3% (w/v) glucose under anaerobic conditions at pH 4.5 decreases on increasing the cellular K^+ or Rb^+ content [25]. This decrease is not due to a depolarization of the cell membrane. Neither the uptake of TPP nor that of Sr^{2+} are significantly affected by loading the cells with K^+. The decrease in Rb^+ influx results from both a decrease in V_m and an increase in apparent K_m as is found by Ref. 10 under slightly different conditions. The latter may be due to a

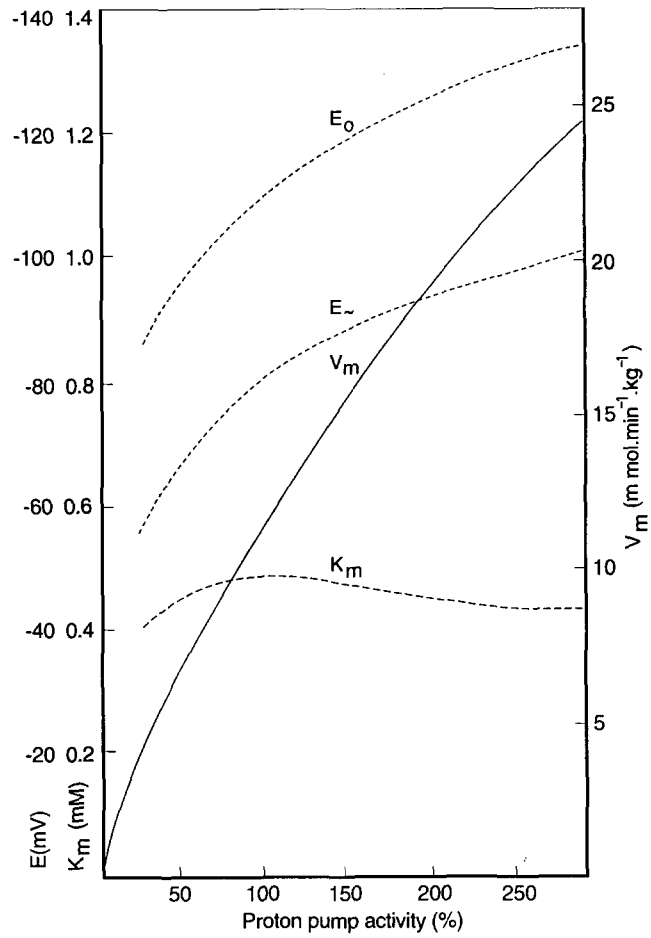

Fig. 3. Calculation of the expected effect of changes in the proton pump activity on both K_m and V_m for Rb^+ uptake at pH 7 and upon the membrane potential in the absence of added cation (E_0) and at saturating concentrations of Rb^+ ($E_\sim$). 100% proton pump activity refers to the condition that the cells are preincubated for 30 min in the presence of 5% glucose at pH 7.0 under anaerobic conditions.

decrease in affinity of Rb^+ for the carrier. This, however, is not necessarily true. According to Eqn. 1 the apparent K_m depends upon $y_\sim/y_0$, that means upon the extent of depolarization of the cell membrane. When the rate of transfer of a cation through the cell membrane decreases the depolarization decreases, too, and by this K_m will increase. We simulated the effect of a change in the transfer rate of the monovalent cations which pass the cell membrane by means of the carrier upon the kinetics of Rb^+ uptake at pH 7. Fig. 4 shows that the depolarization of the cell membrane due to influx of Rb^+ increases with increasing values of the transfer rate. Not only V_m varies appreciably on varying the cation transfer rate, but also K_m. The changes in K_m are less pronounced at high values of the transfer rate than those in V_m.

Since monovalent cation uptake gives rise to a depolarization of the yeast cells, one may expect an increase in the efflux of the main cellular cation, K^+, at increasing substrate cation concentrations. However, K^+ ef-

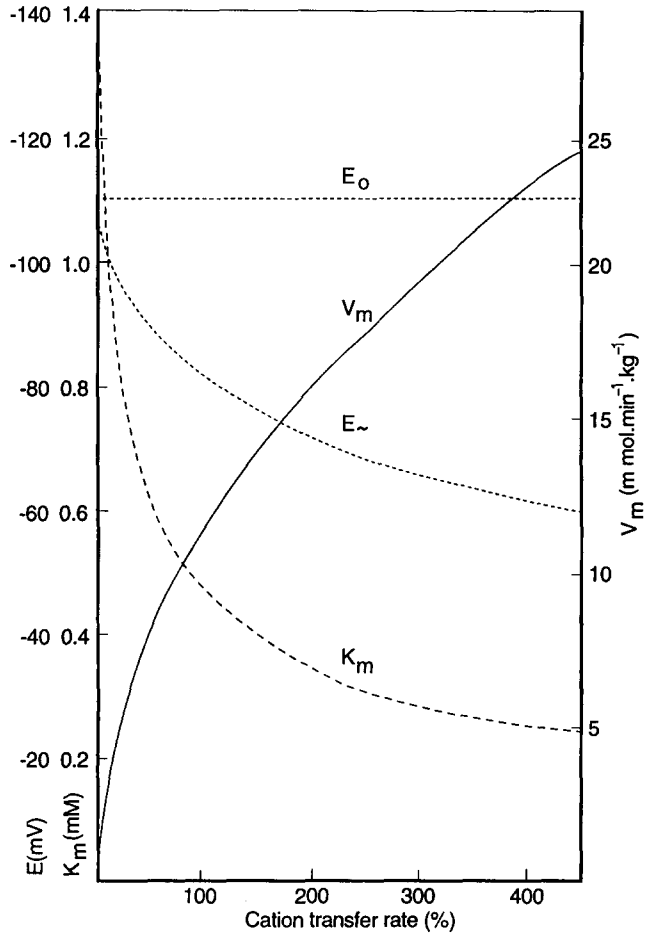

Fig. 4. Simulation of the effect of a change in the rate of cation transfer of across the yeast cell membrane upon both K_m and V_m for Rb^+ uptake at pH 7 and upon the membrane potential in the absence of added cation (E_0) and at saturating concentrations of Rb^+ ($E_\sim$).

flux is is not only proportional to the driving force (y^{-2}) but also depends upon the extent of saturation of the carrier according to

$$V_{K,II} = \frac{V_{m,K,II} s_{K,II} y^{-2}}{K_{m,Rb} + s_{Rb,I}} \quad (5)$$

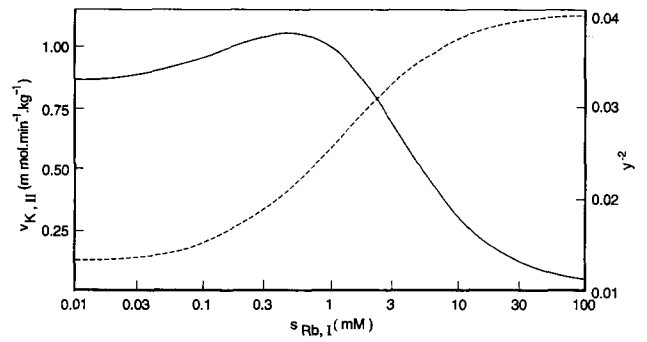

Fig. 5. Calculated efflux of K^+ from yeast as function of the external Rb^+ concentration at pH 7. Full drawn line, K^+ efflux; dotted line, y^{-2}.

Fig. 5 shows that K^+ efflux increases slightly with increasing Rb^+ concentrations in the medium up to 0.5 mM. At concentrations above 1 mM, however, the efflux decreases steeply, despite the fact that y^{-2} still increases.

Discussion

The mechanism of monovalent cation uptake in yeast is certainly more complicated than that underlying the transport model applied in this paper. At low substrate concentrations deviations from Michaelis-Menten kinetics are found [5], which may be accounted for by a two-site transport model. However, as already stated in the introduction, at not too low substrate concentrations deviations from Michaelis-Menten kinetics are small and the uptake can still be described by a single-site uptake model. Theoretically even then deviations from Michaelis-Menten kinetics may come to the fore, because of the dependence of the membrane potential upon the substrate cation concentration [1]. As shown in Fig. 2, for Rb^+ uptake these deviations are small. At concentrations at which the activation site is saturated the deviations are at maximum 2%, which will be hardly detectable experimentally. This is also true for Cs^+ and Na^+ uptake. Only for K^+ uptake the deviations are more pronounced (data not shown).

The dependence of the relative depolarization of the cells, for which y/y_0 is a measure, upon the monovalent cation concentration is as expected. The experimental data of q (Eqn. 3) as function of $q/s_{j,I}$ can be fitted well to the theoretically expected curves indicating that our model for the mutual interaction between cation uptake and membrane potential [1] is applicable to monovalent cation uptake in yeast. This is further supported by the fact that apparent K_m values obtained from the simulations do not differ much from those being found experimentally ($K_{m,exp}$). They appear to be 3–5-times lower than the 'real' K_m for cation transport ($K_{m,j}$) and do not only depend upon the affinity of the cations for the carrier but also upon the maximum extent of depolarization for which the quotient $y_\sim/y_0$ is a measure. By this for example the relative affinities of Rb^+ and K^+ for the carrier are much smaller, than one would conclude from the values of $K_{m,exp}$.

According to the model for mutual interaction between cation uptake and membrane potential an increase in K_m for Rb^+ uptake is expected when the cells are depolarized by influx of phosphate with two or three protons. The difference between the calculated effect of phosphate uptake upon Rb^+ uptake and that observed experimentally is smaller when three protons are cotransported than when two protons are cotransported, which may be an argument for the notion that

phosphate uptake is accompanied by influx of three protons [21] instead of two protons [22]. The discrepancy between the theoretically expected effect of phosphate upon Rb^+ uptake and the experimentally found effect diminishes on accounting for redistribution of K^+ between cells and medium. Not only cotransport of phosphate and protons but also the protonophore 2,4-dinitrophenol gives rise to a decrease in V_m and an increase in K_m [26], which may be ascribed to depolarization of the cells, as well. Possibly the increase in K_m of Rb^+ uptake found in the presence of various organic cations [27] may also be ascribed to depolarization of the cells by these cations.

Stimulating the proton pump or blocking this pump is expected to give rise to an increase or decrease in V_m for Rb^+ uptake, whereas K_m remains almost constant as shown in Fig. 3. The effect of blocking the proton pump differs essentially from the effect of a depolarizing ion upon Rb^+ uptake, because in the latter case K_m increases. The at low pH experimentally found stimulatory effect of butyric acid upon Rb^+ uptake by metabolizing cells via an increase in V_m, without concomitant change in K_m may be ascribed to an increase in the proton pump activity due to acidification of the cells [15,16]. However, whether actually the cells are hyperpolarized under these conditions should still be confirmed. Remarkably the protonophore CCCP which is expected to have a similar effect as phosphate or 2,4-dinitrophenol only decreases V_m, whereas K_m is unaffected [16]. This may indicate that the effect of CCCP relies on an inhibition of the proton pump rather than on an increased proton influx.

On increasing the cellular K^+ content V_m for Rb^+ uptake decreases and K_m increases [10]. As shown by us the increase in K_m is not necessarily due to a decrease in affinity of Rb^+ to the so-called substrate site but may be due to the decrease in the extent of depolarization of the yeast cell membrane expected when the rate of transfer of the cation through the cell membrane decreases. This may also apply to Rb^+ uptake in plant roots, for which concomitant decreases in V_m and increases in K_m are found on increasing the K^+ content of the roots, as well [11,12]. For these plant roots the percentual changes in K_m are of the order of magnitude as those being calculated by us for Rb^+ uptake in yeast.

Depolarization of the cells is expected to give rise to a stimulation of K^+ efflux from the cells as is found for example on adding the uncoupler 2,4-dinitrophenol to yeast cells [26]. However, depolarization of yeast by Rb^+ does not give rise to an increase in Rb^+ efflux from Rb^+ loaded cells [133, whereas K^+ efflux even decreases with increasing K^+ concentration in the medium [14]. This remarkable phenomenon should be ascribed to the fact that the efflux of cellular cations does not only depend upon the extent of depolarization, but also upon the extent of saturation of the carrier at the medium side according to Eqn. 5, which is counteracting the stimulation of efflux by depolarization.

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References

1 Borst-Pauwels, G.W.F.H. (1993) Biochim. Biophys. Acta 1145, 15–24.
2 Calahorra, M., Ramírez, J., Clemente, M. and Peña, A. (1987) Biochim. Biophys. Acta 899, 229–238.
3 Peña, A., Uribe, S., Pardo, J.P and Borbolla, M. (1984) Arch. Biochem. Biophys. 231, 217–225.
4 Borst-Pauwels, G.W.F.H., Van de Mortel, J.B.J. and Theuvenet, A.P.R. (1992) Biochim. Biophys. Acta 1107, 207–212.
5 Borst-Pauwels, G.W.F.H. (1981) Biochim. Biophys. Acta 650, 88–127.
6 Rodríguez-Navarro, A. and Asensio, J. (1977) FEBS Lett. 75, 169–172.
7 Boxman, A.W., Dobbelmann, J. and Borst-Pauwels, G.W.F.H. (1984) Biochim. Biophys. Acta 772, 51–57.
8 Rodríguez-Navarro, A., Blatt, M.R., Slayman, C.L. (1986) J. Gen. Physiol. 87, 649–674.
9 Roomans, G.M. and Borst-Pauwels, G.W.F.H. (1977) Biochim. Biophys. Acta 470, 84–91.
10 Ramos, J. and Rodríguez-Navarro, A. (1986) Eur. J. Biochem. 154, 307–311.
11 Glass, A.D.M. (1976) Plant Physiol. 58 33–37.
12 Siddiqi, M.Y. and Glass, A.D.M. (1983) Can. J. Bot. 61 671–678.
13 Azoulay, L. and Borst-Pauwels, G.W.F.H. (1977) Acta Bot. Neerl. 23, 505–512.
14 Rothstein, A. and Bruce, M.R.A. (1958) J. Cell. Comp. Physiol. 51, 145–159.
15 Theuvenet, A.P.R., Roomans, G.M. and Borst-Pauwels, G.W.F.H. (1977) Biochim. Biophys. Acta 469, 272–280.
16 Ramos, J. Haro, R. and Rodríguez-Navarro, A. (1990) Biochim. Biophys. Acta 1029, 211–217.
17 Van de Mortel, J.B.J., Mulders, D., Korthout, H., Theuvenet, A.P.R. and Borst-Pauwels, G.W.F.H. (1988) Biochim. Biophys. Acta 936, 421–428.
18 Derks, W.G. and Borst-Pauwels, G.W.F.H. (1980) Biochim. Biophys. Acta 596, 381–392.
19 Derks, W.G. and Borst-Pauwels, G.W.F.H. (1979) Physiol. Plant. 46, 241–246.
20 Armstrong, W. McD. and Rothstein, A. (1964) J. Gen. Physiol. 48, 61–71.
21 Cockburn, M., Earnshaw, P. and Eddy, A.A. (1975) Biochem. J. 146, 705–712.
22 Roomans, G.M. and Borst-Pauwels, G.W.F.H. (1979) Biochem. J. 178, 521–527.
23 Hofstee, B.H.J. (1952) Science 116, 329–331.
24 Serrano, R. (1984) Curr. Top. Cell. Regul. 23, 87–126.
25 Boxman, A.W., Theuvenet, A.P.R., Peters, P.H.J., Dobbelmann, J. and Borst-Pauwels, G.W.F.H. (1985) Biochim. Biophys. Acta 814, 50–56.
26 Borst-Pauwels, G.W.F.H., Wolters, G.H.J. and Henricks, J.J.G. (1971) Biochim. Biophys. Acta 225, 269–276.
27 Peña, A. Mora, M.A. and Carrasco, N. (1979) J. Membr. Biol. 47, 261–284