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INTERACTION OF MONOVALENT CATIONS WITH Rb^+ AND Na^+ UPTAKE IN YEAST

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Summary

The concentration dependence of both Rb^+ uptake and Na^+ uptake by yeast can be described by a quadratic rate equation. This equation is derived for translocation of cations via a two-site translocation system. In accordance with predictions made for such a two-site translocation system the shape of the uptake isotherm depends both upon the substrate cation species and upon the concentration of other added competing cations. On plotting the rate of Rb^+ uptake against the quotient of that rate and the Rb^+ concentration concave, convex and also linear curves are found depending upon the type and the concentration of added monovalent cations. The Na^+ uptake isotherm plotted in a similar way shows a shift from a concave curve to a straight line on adding increasing amounts of Rb^+ to the yeast suspension.

Decreasing the pH of the medium leads to a more pronounced convex

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LIST OF SYMBOLS

K_i,K_q, dissociation constants of s_i or s_q from the substrate site.
K'_i,K'_q, dissociation constants of s_i or s_q from the activation site.
k_i,k_q,k'_i,k'_q, apparent dissociation constants defined in Eqn. 7.
r_{qi},r_{iq},r_{ii}, rate constants of translocation of s_i,s_q and s_i respectively from the substrate site, if the activation site is occupied by s_q,s_i and s_i, respectively.
r'_{iq},r'_{qi},r'_{ii}, rate constants of translocation of s_i,s_q and s_i, respectively from the activation site with s_q,s_i and s_i, respectively on the substrate site.
s_ie,es_i,s_ies_i,s_qes_i,s_ies_q and s_ies_i, complexes of s_i and s_q with the translocator e. The left site is the activation site and the right site is the substrate site.
E_t, total translocator concentration.
ψ, surface potential.

uptake isotherm for Rb^+ uptake. This can be ascribed to an indirect effect of the surface potential upon Rb^+ uptake and to occupation of the binding sites by protons, which are replaced again by Rb^+ at increasing Rb^+ concentrations.

Replacement of one sodium ion from one of the two sites of the monovalent cation transport system by either K^+ or Rb^+ leads to an increase in the rate of Na^+ translocation across the yeast cell membrane.

Introduction

Uptake of monovalent cations in yeast proceeds via a multi-site translocation system. Armstrong and Rothstein [1,2] discovered that apart from the substrate site from which the substrate cation is translocated through the cell membrane, a modifier site is involved. This modifier site has no transport function. Occupation of this modifier site by cations other than the substrate ions leads to partial non-competitive inhibition of substrate cation uptake, which inhibition can be relieved again on replacing the inhibitory cation by substrate cation on the modifier site. We have shown that at low substrate concentrations also a third site is involved in monovalent cation uptake the so-called activation site [3]. Occupation of this site by K^+, Cs^+ or Na^+ leads to an increase in the rate of carrier-free radioactive Rb^+ uptake [4]. This was also found on adding non-radioactive Rb^+. On the other hand we found no indication for the involvement of the modifier site in Rb^+ translocation at low pH [3]. The latter is probably due to the fact that the concentrations of Rb^+ applied by us were relatively low and that occupation of the modifier site by Rb^+ was of minor importance under our experimental conditions. In fact we could describe the concentration dependence of Rb^+ uptake by a quadratic rate equation, which indicates that only two sites, the activation site and the substrate site, determined the rate of Rb^+ uptake at not too high Rb^+ concentrations. This opens the possibility to test predictions made theoretically for a two-site translocation process. It has been shown theoretically that the shape of the uptake isotherm, if plotted according to an Eadie or Hofstee plot [5] depends upon the properties of the substrate cation [6]. Both convex curves and concave curves may come to the fore. In addition we have shown that a shift from one type of curve to the other type of curve or to a straight line may occur on varying the concentration of an added competitive cation in the medium.

Methods

The yeast *Saccharomyces cerevisiae* Delft II was exhausted from internal substrate by aerating the cells in distilled water for one day. The cells were washed with 45 mM Tris-succinate buffer of either pH 4.5 or pH 7.2 and preincubated for one hour at 25°C in the buffer with 3% glucose (w/v) at a final concentration of 2.2% (w/v), while nitrogen was bubbling through the suspension. 18 ml of the yeast suspension was added after the preincubation to 2 ml of a solution of either $^{86}Rb^+$ diluted with appropriate concentrations of non-radioactive Rb^+ or $^{22}Na^+$ diluted with non-radioactive Na^+. If other

cations were added these cations were added together with the radioactive isotope. Nine successive samples of 1.8 ml were taken with intervals of approx. 5 s. The samples were filtrated and washed and the filters counted as described earlier [4]. Initial rates of uptake were taken from the slopes of the straight lines or from the tangents to the curves in the origins. Normally deviations from linearity were negligibly small.

Kinetical coefficients were obtained by applying a suitable curve-fitting program to the series of data of uptake rates and of the concentrations of added cations according to Eqn. 2. The computations were carried out on a IBM 360/50 computer.

Na and K contents of the medium were determined by means of flame photometry in the supernatant of the yeast suspension obtained after centrifugation just before adding the cations to the yeast suspension.

Results

The Rb^+ and Na^+ uptake isotherms were plotted according to an Eadie or Hofstee plot [5]. On plotting the rate of uptake against the quotient of the rate of uptake and the substrate cation concentration a straight line will be found if the uptake isotherm is described by a Michaelis-Menten equation:

$$v = \frac{Vs_i}{K_m + s_i} = V - K_m v/s_i \tag{1}$$

where s_i is the substrate cation concentration. If a two-site translocation system is involved a quadratic rate equation will be found according to Eqn. 2.

$$v = \frac{As_i + Bs_i^2}{C + Ds_i + s_i^2} = \frac{(A_0 + A_q s_q)s_i + Bs_i^2}{C_0 + C_q s_q + C_{qq} s_q^2 + (D_0 + D_q s_q)s_i + s_i^2} \tag{2}$$

where s_q is the concentration of an added cation. For the deriviation of Eqn. 2, see Ref. 6.

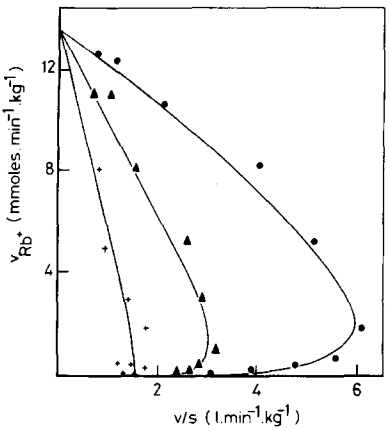
In the case of a two-site translocation system generally non-linear curves will

TABLE I

KINETICAL COEFFICIENTS FOR Rb^+ AND Na^+ UPTAKE BOTH AT pH 4.5 AND AT pH 7.2 ACCORDING TO A TWO-SITE TRANSLOCATION MODEL

A_0 was expressed in mmol · min^{-1} · kg^{-1} · mM^{-1} (per dry weight). A_q in mmol · min^{-1} · kg^{-1} · mM^{-2}, B in mmol · min^{-1} . kg^{-1}, C_0 in mM^2, C_q and D_0 in mM, C_{qq} and D_q are dimensionless. pH means the pH at which the ion uptake was studied, s_i refers to the cation of which the uptake was studied and s_q is the added cation.

pH	s_i	s_q	A_0	A_q	B	C_0	C_q	C_{qq}	D_0	D_q
4.5	Rb^+	Li^+	0.64	0.015	13.7	0.235	0.0049	0.0000238	1.55	0.0207
7.2	Rb^+	Li^+	0.21	0.014	13.0	0.0068	0.00075	0.0000207	0.38	0.0210
4.5	Rb^+	K^+	0.50	16	17.0	0.21	2.95	5.52	2.03	5.57
4.5	Rb^+	Cs^+	0.60	0.34	17.5	0.21	0.116	0.0095	1.76	0.75
4.5	Rb^+	Na^+	0.38	1.56	12.3	0.18	0.33	0.0037	1.52	0.25
7.2	Rb^+	Na^+	0.10	1.26	9.9	0.0050	0.050	0.0033	0.27	0.22
4.5	Na^+	Rb^+	42	903	4.25	70	755	755	154	176
7.2	Na^+	Rb^+	3.0	484	4.20	2.34	86	406	18	107



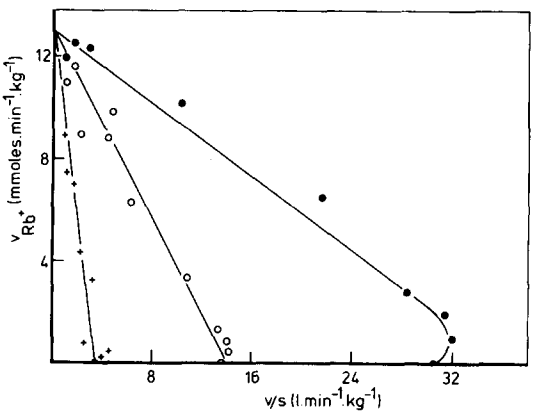


Fig. 1. Rb^+ uptake isotherm at pH 4.5 plotted according to an Eadie or Hofstee plot. Effect of Li^+ upon Rb^+ uptake. ●, ▲, +; 0, 100, 300 mM Li^+, respectively. The curves drawn were computed on applying a curve fitting program according to Eqn. 2. Each experiment was carried out in triplicate.

Fig. 2. Effect of Li^+ upon Rb^+ uptake isotherm at pH 7.2. ●, ○, +; 0, 30, 150 mM Li^+, respectively. See also subscript to Fig. 1.

be found in the Hofstee plot. The Rb^+ uptake isotherm at pH 4.5 showed a convex shape, see Fig. 1. This convex shape became less pronounced if Li^+ was added to the medium. This was due to the fact that Li^+ inhibited the uptake of Rb^+ more at medium Rb^+ concentrations than at low or at high Rb^+ concentrations. The kinetical coefficients calculated by means of a suitable curve fitting program according to Eqn. 2 are tabulated in Table I. We have also computed the values of apparent dissociation constants of Rb^+ for the two sites, k_i and k_i' for the substrate site and the activation site, respectively, see Appendix for the relation of the apparent dissociation constants and the kinetical coefficients of Rb^+ uptake according to Eqn. 2. Also apparent dissociation constants for

TABLE II

APPARENT DISSOCATION CONSTANTS FOR THE TWO BINDING SITES OF THE MONOVALENT CATION TRANSLOCATION SYSTEM

The apparent dissocation constants were expressed in mM. These values were calculated from the kinetical coefficients given in Table I. For the way of calculation, see the appendix. The values for Cs^+ obtained via Cs^+ uptake were from Ref. 7. k' and k referred to the activation site and the substrate site, respectively.

Ion uptake	Cation	pH 4.5		pH 7.2	
		k'	k	k'	k
Rb^+ uptake	Li^+	139	71	18	18
	Na^+	0.54	89	0.10	15
	K^+	0.085	0.45		
	Rb^+	0.13	1.63	0.020	0.31
	Cs^+	10	2.2		
Na^+ uptake	Na^+	0.45	154	0.13	18
	Rb^+	0.10	0.90	0.032	0.18
Cs^+ uptake	Cs^+	10	2.2	2.0	0.3

binding of Li^+ to the two sites were computed, see Table II. Apparently the affinities of Li^+ for the two sites did not differ much, and were probably not significantly different, whereas the apparent affinity of Rb^+ for the activation site was much greater than the apparent affinity of Rb^+ for the substrate site.

At pH 7.2, the convex shape of the Rb^+ uptake isotherm was less pronounced than at pH 4.5, see Fig. 2. In the presence of added Li^+ a shift to a straight line occurred. The values of the kinetical coefficients calculated and those of the apparent dissociation constants of both Rb^+ and Li^+ for the two sites are given in Table I and II, respectively. The apparent dissociation constants of both Li^+ and Rb^+ were reduced considerably on increasing the pH from 4.5 to 7.2.

Addition of K^+ up to 1 mM led to an increase in the rate of Rb^+ uptake at low Rb^+ concentrations being maximal at carrier-free $^{86}Rb^+$, as seen in Fig. 3. At the higher K^+ concentrations applied inhibition of Rb^+ uptake was found also at low Rb^+ concentrations. The uptake isotherm shifted from a convex curve to a straight line on increasing the K^+ concentration in the medium. As shown in Table I the kinetical coefficients of $s_q = [K^+]$ were much higher than those found for Li^+. The apparent dissociation constants of K^+ for the two sites were much lower than those for Li^+ and were also lower than those for Rb^+, which means that the affinity of K^+ for the two sites was greater than the affinity of Rb^+ for these sites.

The rate of Rb^+ uptake at pH 4.5 was increased by Cs^+ added to the medium at low Rb^+ concentrations, see Fig. 4. The percentual increase in the rate of uptake was maximal for carrier-free $^{86}Rb^+$. The affinity of Cs^+ for the activation

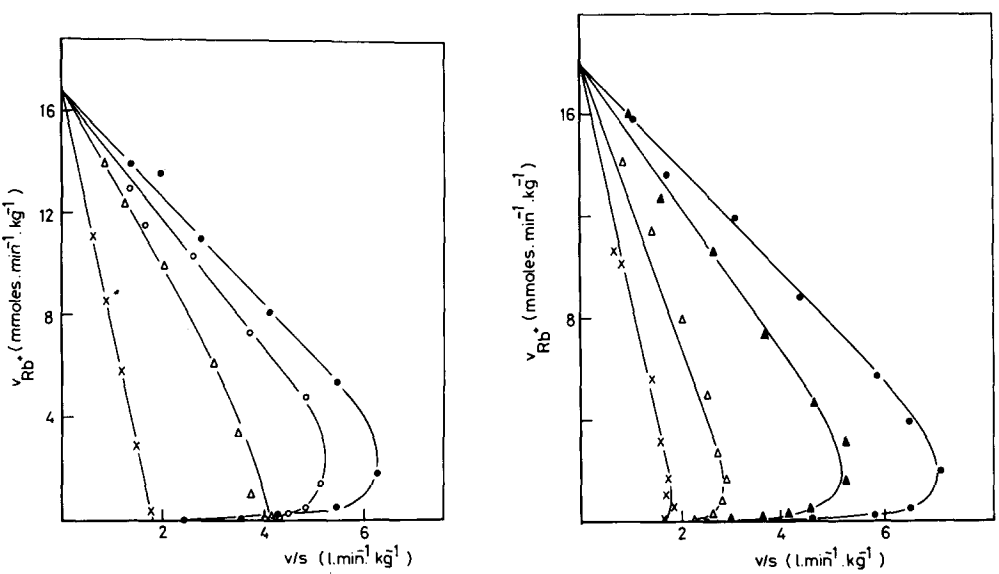


Fig. 3. Effect of K^+ upon Rb^+ uptake isotherm at pH 4.5. ●, ○, △, X; 0, 0.1, 0.3, 1.0 mM K^+, respectively. See also subscript to Fig. 1.

Fig. 4. Effect of Cs^+ upon Rb^+ uptake isotherm at pH 4.5. ●, ▲, △, X; 0, 1, 5, 10 mM Cs^+, respectively. See also subscript to Fig. 1.

site appeared to be lower than the affinity for the substrate site. The values of the apparent dissociation constants for the two sites were equal to those found for Cs^+ uptake at pH 4.5, recently [7]. In that study, however, we assigned the low dissociation constant to the activation site and the high dissociation constant to the substrate site. Our study of the interaction of Cs^+ with Rb^+ uptake showed that this was wrong.

Na^+ added to the yeast suspension appeared to have a dramatical effect upon the shape of the uptake isotherm of Rb^+ at pH 4.5, see Fig. 5. On increasing the Na^+ concentration a shift was found from a convex curve to a concave curve. Uptake of Rb^+ was enhanced by Na^+ at low Rb^+ concentrations. The percentual stimulation was again maximal for carrier-free $^{86}Rb^+$. The values of the apparent dissociation constants of Na^+ for the two sites differed considerably. The dissociation constant of Na^+ for the substrate site was of the order of magnitude of that of Li^+, whereas the affinity of Na^+ for the activation site was far greater.

At pH 7.2 also a shift from a convex curve to a concave curve was found on adding Na^+ to the yeast suspension, see Fig. 6. The affinity of Na^+ to the two sites increased considerably on increasing the pH from 4.5 to 7.2.

The concentration dependence of Na^+ uptake was quite different from that found for Rb^+ uptake. A concave curve was found at pH 4.5, see Fig. 7. In the presence of Rb^+, the rate of Na^+ uptake was increased except at the lower Na^+ concentrations. Then a decrease in the rate of Na^+ uptake was found at relatively high Rb^+ concentrations. At 2.5 mM, the uptake isotherm was straight. Stimulation by Rb^+ was maximal at moderate Na^+ concentrations of about 1—10 mM. The dissociation constants of Na^+ and Rb^+ for the two sites were of the order of magnitude of the dissociation constants computed from the kinetical coefficients obtained from Rb^+ uptake experiments.

Na^+ uptake at pH 7.2 gave also rise to a concave uptake isotherm, see Fig. 8. Rb^+ enhanced the uptake of Na^+. Stimulation of Na^+ uptake was maximal at

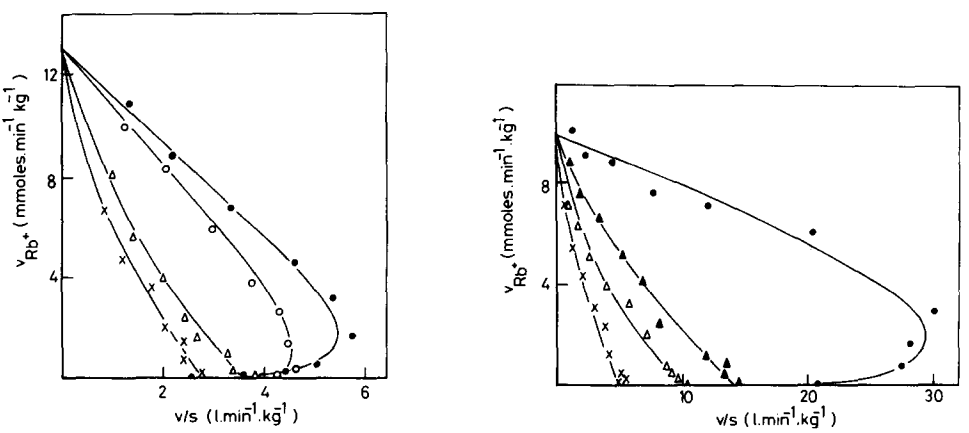
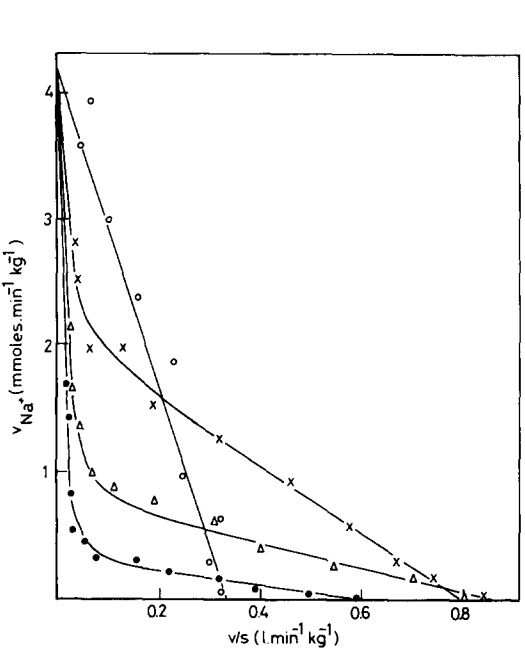


Fig. 5. Effect of Na^+ upon Rb^+ uptake isotherm at pH 4.5. ●, ○, △, X; 0, 3, 30, 60 mM Na^+, respectively. See also subscript to Fig. 1.

Fig. 6. Effect of Na^+ upon Rb^+ uptake isotherm at pH 7.2. ●, ▲, △, X: 0, 10, 20, 40 mM Na^+, respectively. See also subscript to Fig. 1.



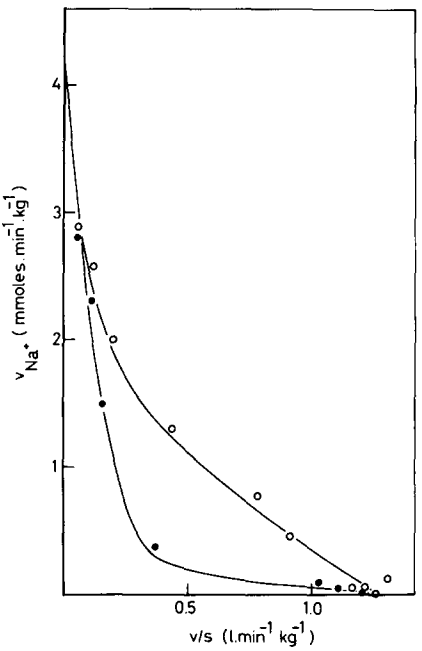


Fig. 7. Effect of Rb^+ upon Na^+ uptake isotherm at pH 4.5. •, △, X, ○; 0, 0.1, 0.5, 2.5 mM Rb^+, respectively. See also subscript to Fig. 1.

Fig. 8. Effect of Rb^+ upon Na^+ uptake isotherm at pH 7.2. • and ○; 0 and 0.3 mM Rb^+, respectively. See also subscript to Fig. 1.

moderate Na^+ concentrations just as was found at pH 4.5. The apparent dissociation constants for both Rb^+ and Na^+ at pH 7.2 were much smaller than those obtained at pH 4.5.

Discussion

The concentration dependence of the rates of uptake for both Rb^+ and Na^+ is described well by a quadratic rate equation. Apparently the kinetics of uptake of these monovalent cations can be described by a two-site transport mechanism, though probably three sites are involved in monovalent cation translocation in yeast [1—4]. In fact we have found recently on applying a very large range of concentrations that Cs^+ uptake is described by a cubic rate equation, which is typical for a three-site translocation mechanism [7]. However, under the experimental conditions applied by us, apparently only the activation site and the substrate site are involved, whereas interaction of the cations with the modifier site does not occur at all or does not have a detectable effect upon the kinetics of cation uptake. This allows us to describe the uptake of both Rb^+ and Na^+ by the rate equation (Eqn. 2). It has been shown theoretically [6], that in case of a two-site translocation system the shape of the uptake isotherm depends upon the relative values of the kinetical coefficients, see also Table III. The kinetic coefficients A, C and D depend upon the concentrations of other added monovalent cations s_q. The shape of the uptake

TABLE III

DEPENDENCE OF THE SHAPE OF AN EADIE OR HOFSTEE PLOT FOR MONOVALENT CATION UPTAKE UPON THE RELATIVE VALUES OF THE KINETICAL COEFFICIENTS OF THE SUBSTRATE CATION s_i ACCORDING TO EQN. 2

Relative values	Shape of the curve
$A/B + BC/A < D$	convex
$A/B + BC/A = D$	straight
$A/B + BC/A > D$	concave

isotherm will depend upon the concentration of added cation and also upon the features of the other cation. This is typical for a two-site translocation process. In this respect a two-site translocation process distinguishes itself from other translocation processes, which also give rise to a quadratic rate equation. In the case of a dual mechanism also a quadratic rate equation is found. However, the uptake isotherm has always a concave shape, which cannot be altered by adding competing cations [5,6]. This is also true for cation uptake via a single-site translocation process across a negatively charged cell membrane, which under certain conditions may give rise to deviations from Michaelis-Menten kinetics [8].

The uptake isotherm for Rb^+ uptake at high pH in the presence of added Na^+, see Fig. 8, has a similar shape as the uptake isotherm observed by Armstrong and Rothstein [2] for K^+ uptake under comparable conditions. These authors, however, attributed the deviations from Michaelis-Menten kinetics to an interaction of Na^+ with the so-called modifier site, by which a partial non-competitive inhibition of K^+ uptake was caused. It was assumed that K^+ at relatively high concentrations could replace the Na^+ from the modifier site by which the non-competitive inhibition was relived again. Our results show, however, that the interaction of Na^+ with monovalent cation uptake at high pH can be described by a two-site translocation system consisting of the activation site and the substrate site, and that the modifier site is probably not involved in this interaction.

At pH 4.5 the convex deviation from linearity in the Hofstee plot is more pronounced than at pH 7.2. This can be explained as follows. The surface potential of the yeast cell is decreased considerably on decreasing the pH of the medium [9]. As follows from Eqn. 7 (see Appendix) the apparent affinity constants of Rb^+ and of other cations present in the medium will increase. Consequently the number of unoccupied sites of the translocator will increase too. It has been shown earlier, that Rb^+ is not or only very slowly translocated, if one of the two sites of the translocation system is unoccupied [10,11]. On increasing the Rb^+ concentration in the medium, the number of unoccupied sites will decrease and therefore the rate of Rb^+ translocation will increase. A second factor which is probably involved, is that on decreasing the pH protonation of the transport sites will increase. If replacement of protons on the translocation sites by Rb^+ leads to an increase in the rate of Rb^+ uptake, this will also contribute to the occurrence of a convex curve, see also Eqn. 9 in the Appendix.

An increase in the rate of Rb^+ uptake at pH 4.5 at low Rb^+ concentrations by added monovalent cations K^+, Cs^+ and Na^+ was found by us earlier [4]. This increase was attributed to the replacement of protons from the activation site by the cations added and it was assumed that the rate of Rb^+ translocations is larger if one site of the transport system is occupied by one of these cations than if one site is occupied by a proton. According to Eqn. 11 the chance whether enhancement of Rb^+ uptake occurs depends upon the values of the affinity constants of the added cation. In addition, part of the enhancement of Rb^+ uptake observed at low pH may be due to occupation of sites of the transport system which are still unoccupied, a situation which may be favoured by a decrease in the negative surface potential as outlined above.

Stimulation of Na^+ uptake at pH 4.5 by K^+ or Rb^+ has been found by us earlier [4]. Apparently, stimulation of Na^+ uptake is also found at high pH. Therefore this enhancement cannot be attributed to a replacement of protons from one of the binding sites by K^+ or Rb^+. A more likely explanation is that replacement of Na^+ from one of the two sites of the translocation system by either K^+ or Rb^+ leads to an increase in the rate of Na^+ translocation through the yeast cell membrane. An indication that this may be true is that the mean value of A_q/BD_q observed at pH 4.5 and at pH 7.2 is greater than one for $s_q = [Rb^+]$, see also Appendix, Eqn. 12. In addition occupation of unoccupied sites of the transport system by Rb^+ may also contribute to the enhancement of Na^+ uptake. Since Na^+ has a very low affinity to the substrate site, this effect will persist still at relatively high Na^+ concentrations, in contradiction with the situation in Rb^+ transport.

It has been shown in the appendix that it is not excluded that two cations like Rb^+ and Na^+ are translocated at the same rate of translocator of which the activation site is occupied by Na^+ and the substrate site by Rb^+, or just reversely. In this respect the activation site differs from the modified site. It has been shown by Armstrong and Rothstein [2], that the modifier site is not involved at all in the translocation of cations through the yeast cell membrane. The possibility of a cotransport of two cations by the activation site and the substrate site may have important implications for the energetisation of monovalent cation transport in yeast. Apart from energetisation via the membrane potential [12,13] also energetisation via the electrochemical gradient of the other cation which is translocated together with the substrate cation may contribute to the accumulation of the substrate cation. If protons are translocated together with the substrate cation energetisation by the proton motive force [14] may be involved.

Appendix

A general rate equation for cation transport via a two-site translocation system is given in Eqn. 20 of Ref. 6. It is assumed that both sites of the translocation system are independent. That means that occupation of one site by a cation does not affect the affinity of the cations to the other site. The translocator e binds the substrate cation s_i or a competing cation s_q either at the activation site arbitrarily designated as left site, and which is characterized by a high affinity for Rb^+ or at the substrate site (right site), which has a low

affinity to Rb^+ [3]. The kinetic coefficients referring to the activation site are marked with a prime, those referring to the substrate site are not marked. The following combinations of s_i with translocator e can come to the fore. The substrate cation s_i is translocated with rate constant r with appropriate index through the cell membrane after binding to the translocator. The dissociation constants of the translocator cation complexes are designated by K with appropriate index.

$s_i + e = s_i e$,	$K'_i = s_i \cdot e / s_i e$,	r'_i for s_i
$s_i + e = e s_i$,	$K_i = s_i \cdot e / e s_i$,	r_i for s_i
$s_i + e s_i = s_i e s_i$,	$K'_i = s_i \cdot e s_i / s_i e s_i$,	$r'_{ii} + r_{ii}$ for s_i
$s_i + s_i e = s_i e s_i$,	$K_i = s_i \cdot s_i e / s_i e s_i$,	$r'_{ii} + r_{ii}$ for s_i
$s_i + e s_q = s_i e s_q$,	$K'_i = s_i \cdot e s_q / s_i e s_q$,	r'_{iq} for s_i, r_{qi} for s_q
$s_i + s_q e = s_q e s_i$,	$K_i = s_i \cdot s_q e / s_q e s_i$,	r_{qi} for s_i, r'_{qi} for s_q

Dissociation constants for cation s_q are defined K'_q and K_q in a similar way. The rate equation (Eqn. 20 of Ref. 6) can be written as a function of both the substrate cation concentration s_i and the concentration of an added cation s_q, see Eqn. 2 under results. The kinetical coefficients of Eqn. 2 are functions of the rate constants and of the dissociation constants. The coefficients of the numerator also depend upon the total translocator concentration e_t.

$$A_0 = \sum_{\substack{j \neq i \\ j \neq q}} A_j s_j = \sum_{\substack{j \neq i \\ j \neq q}} (r'_{ij} k_i k_j^{-1} + r_{ji} k'_i k'^{-1}_j) s_j e_t \tag{3}$$

$$A_q = (r'_{iq} k_i k_q^{-1} + r_{qi} k'_i k'^{-1}_q)\, e_t ; B = (r_{ii} + r'_{ii})\, e_t \tag{4}$$

$$C_0 = k_i k'_i ; C_q = k_i k'_i (k_q^{-1} + k'^{-1}_q) ; C_{qq} = k_i k'_i k_q^{-1} k'^{-1}_q \tag{5}$$

$$D_0 = k_i + k'_i ; D_q = k_i k_q^{-1} + k'_i k'^{-1}_q \tag{6}$$

The apparent dissociation constants, k,k' with proper index depend upon the concentrations of other competing cations still present in the medium, like the small amounts of K^+, which are released by the yeast cell, some Na^+ and protons. Competitive inhibition by the Tris cation is relatively small as has been shown by Dr. A. Theuvenet in our laboratory. In addition the apparent dissociation constants depend upon the surface potential and decrease if the surface potential becomes more negative [8].

$$k_i = K_i \left(y^{-1} + \sum_{\substack{j \neq i \\ j \neq q}} \frac{s_j}{K_j} \right) \quad k'_i = K'_i \left(y^{-1} + \sum_{\substack{j \neq i \\ j \neq q}} \frac{s_j}{K'_j} \right) \tag{7}$$

$y = \exp(-F\psi/RT)$, F, R and T have their usual meaning, ψ is the surface potential. Similar relations apply for k_q and k'_q. They are found on replacing index i by q in Eqn. 7. If the concentrations of cations added are relatively high, y will decrease due to screening of the negative groups on the cell membrane [15] and the apparent dissociation constants will increase. The large apparent dissociation constants found for Li^+ for both sites and the large dissociation constant for Na^+ for the substrate site may reflect partly the decrease in y expected at the high concentrations of added cation.

The values of k_i and of k'_i are calculated from the values of C_0 and D_0.

The values of k_q and k'_q are calculated by means of Eqn. 8:

$$k_q k'_q = C_0 C_{qq}^{-1}; k_q + k'_q = C_q C_{qq}^{-1} \tag{8}$$

In order to see which of the two values obtained by means of Eqn. 8 should be assigned to k_q and which one to k'_q, the value of D_q calculated according to Eqn. 6 is compared with the value of D_q found actually.

An increase of v/s_i on increasing the concentration of s_i as is found for Rb^+ uptake at low Rb^+ concentrations, that means an increase in the rate of uptake of $^{86}Rb^+$ by added non-radioactive Rb^+, will occur if $BC_0/A_0D_0 > 1$. This can be seen by differentiating v/s_i to s_i near $s_i = 0$.

$$\frac{BC_0}{A_0D_0} = \frac{(r'_{ii} + r_{ii})k_i k'_i}{[\sum (r'_{ij}k_i k_j^{-1} + r_{ji}k'_i k_j'^{-1})s_j](k_i + k'_i)} \tag{9}$$

Taking into account that $k_i k'_i/(k_i + k'_i) \approx k'_i$ for Rb^+ uptake, it is seen that the chance that v/s_i increases with increasing concentration of s_i is greater if k'_i is large than if k'_i is small and if A_0 is small. The coefficient A_0 depends upon the concentration of cations present in the medium and upon the rate constants of translocation of substrate s_i from translocator of which one site is occupied by s_j thus from either $s_j es_i$ or $s_i es_j$.

Enhancement of uptake of a substrate cation s_i on adding a competitive inhibitor to the yeast suspension will occur if

$$A_q/(A_0 + Bs_i) > (C_q + D_q s_i)/(C_0 + D_0 s_i + s_i^2) \tag{10}$$

If s_i approximates to zero, that means if one is studying the effect of an added competitive inhibitor upon the rate of uptake of carrier-free radioactive substrate, enhancement of uptake of s_i will occur if $A_q C_0/A_0 C_q > 1$.

$$\frac{A_q C_0}{A_0 C_q} = \frac{(r'_{iq}k_i k_q^{-1} + r_{qi}k'_i k'^{-1}_q)k_q k'_q}{[\sum (r'_{ij}k_i k_j^{-1} + r_{ji}k'_i k_j'^{-1})s_j](k_q + k'_q)} \tag{11}$$

If $k'_q << k_q$ as is the case for $s_q = Na^+$ or K^+, see Table II, $k_q k'_q/(k_q + k'_q) \approx k'_q$.

Enhancement of uptake of carrier-free radioactive substrate will be favoured if the apparent dissociation constant of the added cation s_q for the activation site is relatively large, and if the rate constants of translocation of substrate cation s_i from translocator of which one site is occupied by s_q and the other one by s_i ($s_q es_i$ or $s_i es_q$) are relatively large.

Enhancement of uptake of a substrate cation s_i by added cation s_q at relatively high concentrations of s_i is expected to occur if $A_q > BD_q$, as can be seen by taking s_i in Eqn. 10 infinitely high.

$$\frac{A_q}{BD_q} = \frac{r'_{iq}k_i k_q^{-1} + r_{qi}k'_i k'^{-1}_q}{(r_{ii} + r'_{ii})(k_i k_q^{-1} + k'_i k'^{-1}_q)} \tag{12}$$

This condition will be fulfilled if both $r'_{iq} > r'_{ii}$ and $r_{qi} > r_{ii}$.

If $r'_{qi} = r_{qi}$ and $r_{iq} = r'_{iq}$, that means if the rate of translocation of a cation s_i from $s_q es_i$ or from $s_i es_q$ equals the rate of translocation of cation s_q from these two complexes, a stochiometric contransport of ions s_i and s_q will occur. It can be easily seen that in such a case the value of A_q/D_q will be the same for

the effect of for example Na^+ upon Rb^+ uptake and Rb^+ upon Na^+ uptake. The values of A_q/D_q are 6.2 and 5.1 at pH 4.5 for the effect of Na^+ upon Rb^+ uptake and that of Rb^+ upon Na^+ uptake, respectively, and 5.7 and 4.5 for the corresponding values at pH 7.2. Probably these figures do not differ significantly, which means that Rb^+ and Na^+ might be translocated with the same rate from translocator of which one site is occupied by Rb^+ and the other one by Na^+.

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