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KINETICS OF ION TRANSLOCATION ACROSS CHARGED MEMBRANES MEDIATED BY A TWO-SITE TRANSPORT MECHANISM

EFFECTS OF POLYVALENT CATIONS UPON RUBIDIUM UPTAKE INTO YEAST CELLS

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SUMMARY

(1) The effect of surface charge upon the kinetics of monovalent cation translocation via a two-site mechanism is investigated theroretically.

(2) According to the model dealt with, typical relations are expected for the dependence of the kinetic parameters of the translocation process upon the concentration of a polyvalent cation, differing essentially from those derived for the case in which the membrane carries no excess charge.

(3) Even when a polyvalent cation does not compete with the substrate cation for binding to the translocation sites, apparently competitive inhibition may occur when the membrane is negatively charged.

(4) The model is tested experimentally by studying the effects of the polyvalent cations Mg^{2+}, Sr^{2+}, Ca^{2+}, Ba^{2+} and Al^{3+} upon Rb^+ uptake into yeast cells at pH 4.5. A good applicability is found.

(5) Equimolar concentrations of polyvalent cations reduce the rate of the Rb^+ uptake into yeast cells in the order $Mg^{2+} < Sr^{2+} < Ca^{2+} < Ba^{2+} \ll Al^{3+}$.

(6) The conclusion is reached that the reduction in the rate of Rb^+ uptake caused by the polyvalent cations applied results mainly from screening of the negative fixed charges on the membrane surface and binding to these negative sites rather than competition with Rb^+ for the transport sites.

(7) The results of our investigation indicate that the affinity of the alkaline-earth cations for the negative fixed charges on the surface of the yeast cell membrane increases in the order $Mg^{2+} < Sr^{2+} < Ca^{2+} < Ba^{2+}$.

(8) Probably mainly phosphoryl groups determine the net charge on the membrane of the yeast cell at a medium pH of 4.5.

INTRODUCTION

Theuvenet and Borst-Pauwels [1] showed that the kinetic parameters determining monovalent cation translocation across a biological membrane may be affected by variations in the surface potential of the membrane. The effect of variations in the surface potential upon the kinetic parameters may be investigated by studying the kinetics of the translocation process in the presence of increasing concentrations of a polyvalent cation.

In the present article it will be shown that essentially different relations between the kinetic parameters determining monovalent cation translocation mediated by a two-site mechanism and the concentration of a polyvalent cation may be expected for ion translocation across an uncharged membrane and for ion translocation across a membrane bearing negative fixed charges.

We have examined whether the relations derived for monovalent cation translocation across a negatively charged membrane can be applied to the description of the effects of polyvalent cations upon Rb^+ uptake into yeast cells, as this translocation process is described by a two-site transport model [2–5]. In addition we have tried to elucidate what type of charged groups on the surface of the yeast cell might be responsible for the surface charge density of the membrane.

THEORY

The rate equation for solute transport mediated by a two-site mechanism (see Borst-Pauwels [6]) has the mathematical form

$$v_i = \frac{A_i s_i + B_i s_i^2}{C_i + D_i s_i + s_i^2}, \tag{1}$$

where s_i represents the concentration in the medium of the substrate solute, denoted by s_i, and v_i is the rate of transport. The coefficients A_i–D_i are independent of s_i but depend on the concentration in the medium of other solutes that have an affinity for the transport sites.

We will now consider the case of monovalent cation transport across a biological membrane from a solution also containing other monovalent cations (s_j, s_k) and one polyvalent cation s_p of valency z_p. The rate equation will be derived for two limiting cases: (I) the membrane carries no excess charge; (II) the membrane carries an excess negative charge. The restriction will be made that the values of the rate constants for transfer of the substrate cation through the membrane are independent of variations in the surface potential.

Case I

The rate equation can be derived directly from Eqn. *A*1 (see appendix), taking $s_{i,0} = s_i$, $s_{j,0} = s_j$, $s_{k,0} = s_k$ and $s_{p,0} = s_p$ in that equation, as, according to the Boltzmann distribution law (see Eqn. 6), the concentration of an ion near the membrane surface is equal to its concentration in the medium when the surface potential is zero. The relationships between the kinetic parameters (A_i–D_i) and the concentration of a polyvalent cation s_p are represented by:

$$A_i = \alpha_{i,0} + \alpha_{i,p} s_p \tag{2}$$

$$B_i = V_i \tag{3}$$

$$C_i = \gamma_{i,0} + \gamma_{i,p} s_p + \gamma_{i,pp} s_p^2 \tag{4}$$

$$D_i = \delta_{i,0} + \delta_{i,p} s_p \tag{5}$$

Note that the coefficients $\alpha_{i,p}$, $\gamma_{i,p}$, $\gamma_{i,pp}$ and $\delta_{i,p}$ are only non-zero when s_p has affinity for the transport sites. In such a case, competitive inhibition of monovalent cation transport will be observed. The maximal rate of transport V_i ($= B_i$) is independent of s_p.

Case II

In this case, the ion concentrations near the membrane surface are not equal to the corresponding ones in the medium. In order to obtain a mathematical expression for the rate of translocation as an explicit function of the ion concentrations in the medium alone, we have to know the relationship between the concentration of an ion near the surface of the membrane and its concentration in the medium. This relationship is given by the Boltzmann equation [7].

$$s_{n,0} = s_n \exp\left(-z_n q\psi_0/kT\right) = s_n y^{z_n} \tag{6}$$

where q is the absolute value of the charge on the electron and z_n is the valency of ion s_n, ψ_0 is the surface potential, T is the value of the temperature in degrees of Kelvin, k is the Boltzmann constant and y is defined by Eqn. 6. According to the Gouy-Chapman equation (see, for example, Haydon [8]) this factor y, which is related to ψ_0, is a function of the concentrations of both anions and cations present in the medium, including s_i:

$$\Phi = 2\pi\sigma^2/\varepsilon NkT = \sum_n s_n(y^{z_n}-1) \simeq \sum_m s_m y + s_p y^{z_p} \tag{7}$$

where the subscript m and p refer to monovalent and polyvalent cations, respectively. The approximated form applies when $y \gg 1$, because then the terms containing anion concentrations may be neglected. In this equation N is the Avogadro number, ε is the dielectric constant in the double layer near the membrane and Φ is related to σ, the surface charge density of the membrane. In the case that the concentrations of the monovalent cations are relatively low we may neglect the first term in the approximated form, as well. The surface potential may then be represented by the Gouy-Chapman expression for the polyvalent cation alone. By combining this reduced Gouy-Chapman equation with the Boltzmann equation (Eqn. 6) we obtain:

$$s_{p,0} = \Phi \tag{8}$$

If binding of the polyvalent cation to the fixed charges occurs, Φ becomes dependent of the type of the polyvalent cation, and Eqn. 8 turns into

$$s_{p,0} = \Phi_p \tag{9}$$

Φ_p ($< \Phi$) is lower at higher binding affinities of the polyvalent cation. As has been argued by McLaughlin et al. [9], for a limited range of concentration of s_p, that its concentration near the membrane surface and the surface charge density of the membrane are independent of the concentration of s_p in the medium.

On eliminating $s_{i,0}$, $s_{j,0}$, $s_{k,0}$ and $s_{p,0}$ in Eqn. $A1$ by means of Eqns. 6 and 9 and dividing both the nominator and the denominator of the rate equation by y^2 a similar relation to Eqn. 1 is obtained, with:

$$A_i = \alpha'_{i,0} + \alpha'_{i,p} s_p^{1/z_p} \tag{10}$$

$$B_i = V_i \tag{11}$$

$$C_i = \gamma'_{i,0} + \gamma'_{i,p} s_p^{1/z_p} + \gamma'_{i,pp} s_p^{2/z_p} \tag{12}$$

$$D_i = \delta'_{i,0} + \delta'_{i,p} s_p^{1/z_p} \tag{13}$$

These relationships differ essentially from those derived for the case in which the membrane carries no excess of charge (Eqns. 2–5). From Eqns. *A*1 and 10–13 it can be derived that the quotients $\gamma'_{i,p}/\delta'_{i,p}$ and $\gamma'_{i,pp}/\gamma'_{i,p}\,\delta'_{i,p}$ will be independent of the type of polyvalent cation of the same valency when the affinities of these cations to the transport sites are relatively low. In such a case coefficient $\gamma'_{i,pp}$ may be used as a measure for the affinity of a polyvalent cation to the fixed charges on the membrane. It should be noticed that, even when the polyvalent cation does not compete with the substrate cation for binding to the transport sites, apparently competitive inhibition may occur.

METHODS

Yeast, *Saccharomyces cerevisiae* Delft 2, was starved under aeration for 12 h and washed three times with distilled water. After starvation the cells (2 % w/v) were preincubated in 45 mM Tris/succinate buffer (pH 4.5) provided with 3 % (w/w) glucose at 25 °C. N_2 was bubbled through the suspension. After 1 h a solution of ^{86}RbCl with or without appropriate concentrations of a polyvalent cation and Rb^+ (applied as chloride salts) was added to the yeast suspension. The uptake of Rb^+ was studied according to Borst-Pauwels et al. [5]. However, in the experiments described in the present article nine successive 1.8 ml samples of the yeast suspension were taken during a 1-min incubation period instead of a 5-min incubation period after the addition of the salts. In addition washing of the yeast was carried out with 50 mM instead of 20 mM ice-cold $MgCl_2$ solution. In a parallel experiment 1 min after the addition of the polyvalent cations without $^{86}Rb^+$, aliquots of the incubated yeast suspension were centrifuged and the concentrations of K^+ and Na^+ in the supernatant determined by flame photometry. Corrections for the contribution of the polyvalent cations to the emission at 769 nm, at which K^+ was determined, were made. The pH of the supernatant was also determined.

RESULTS

We have determined initial rates of Rb^+ uptake at various Rb^+ concentrations ranging from carrier-free Rb^+ (less than 10^{-3} mM) to 10 mM Rb^+, in the presence or absence of polyvalent cations at pH 4.5. The polyvalent cations were added together with the Rb^+ to the yeast suspension. In most cases a linear relationship between the amount of Rb^+ absorbed and the incubation time with radioactive Rb^+ was found. Only at concentrations of Rb^+ higher than 7 mM was a slightly bent curve observed. In these cases initial rates of uptake were obtained by drawing a tangent to the uptake curves at zero time. The concentration dependence of the uptake rates is represented graphically according to Hofstee [10]. These plots give a better distribution of the experimental data obtained at the various Rb^+ concentrations than the more generally applied Lineweaver-Burk plots [11]. In case the translocation process is described by Michaelis-Menten kinetics a straight line will be found in the Hofstee plot according to Eqn. 14.

$$v = \frac{Vs}{K_m + s} = V - K_m(v/s) \tag{14}$$

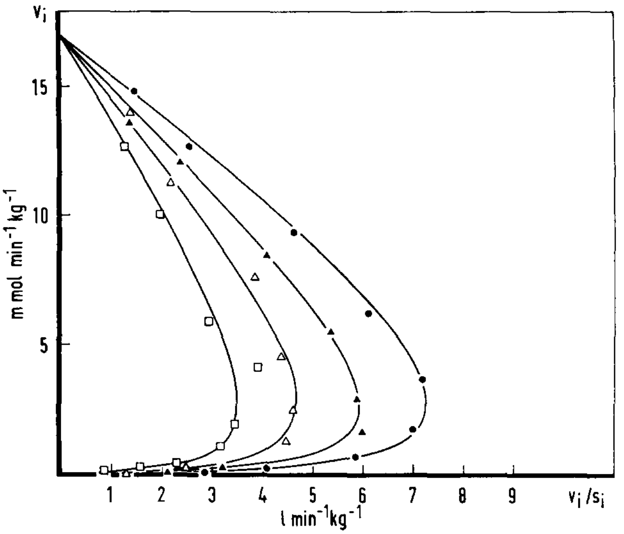

Fig. 1. Effect of Mg^{2+} upon the concentration dependence of the Rb^+ uptake rate at pH 4.5. The initial rate of Rb^+ uptake is plotted against the quotient of this rate and the Rb^+ concentration. The rate of uptake is expressed in mmol · min^{-1} · kg^{-1} dry weight of yeast. (●) Control; (▲) in the presence of 5 mM $MgCl_2$; (△) 25 mM $MgCl_2$; (□) 100 mM $MgCl_2$. Each point represents the mean value of triplicates.

In the case of a two-site transport mechanism non linear curves will be obtained [6, 10].

Fig. 1 shows the effect of various concentrations of Mg^{2+} upon the kinetics of Rb^+ uptake at pH 4.5. It can be seen that the curves are convex, as found earlier [4]. These curves represent an apparent positive homotrope cooperative phenomenon [5]. On plotting the rate of uptake against the substrate cation concentration a sigmoidal curve will be found in such a case. Apparently, the Rb^+ uptake is inhibited competitively by Mg^{2+}. The maximal rate of the Rb^+ uptake (intercept with the ordinate in the Hofstee plot) and the shape of the isotherm are not affected when the concentration of Mg^{2+} is raised. Mg^{2+} decreases the rate of Rb^+ uptake at all Rb^+ concentrations applied, though to a lesser extent at higher concentrations. Similar results were obtained with Ba^{2+}, Sr^{2+} and Ca^{2+}. Equimolar concentrations of these cations inhibited the translocation of Rb^+ across the yeast cell membrane somewhat more than did Mg^{2+} ($Mg^{2+} < Sr^{2+} < Ca^{2+} < Ba^{2+}$).

According to McLaughlin et al. [9] the screening ability of a polyvalent cation increases greatly with the valency of the cation, giving rise to a greater decrease in the surface potential and thus to a greater reduction in the concentration of the substrate cation near the surface of the membrane. Therefore, a much stronger decrease in the Rb^+ uptake rate on addition of a trivalent cation may be expected than is found with equimolar concentrations of divalent cations, provided that the observed inhibitions are caused mainly by a screening phenomenon. Fig. 2 shows the effect of the trivalent cation Al^{3+} upon the kinetics of the Rb^+ uptake at pH 4.5. It can be seen that the effect of this trivalent cation is formally similar to that found with the divalent cations. However, the potency of this cation to inhibit the Rb^+ uptake is

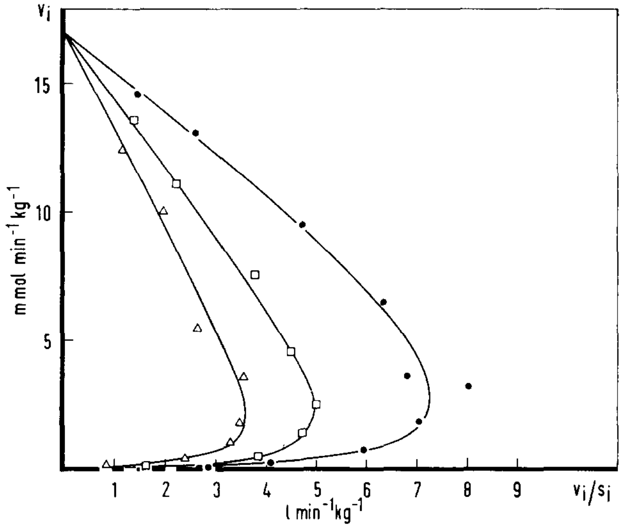

Fig. 2. Effect of Al^{3+} upon the concentration dependence of the Rb^+ uptake rate at pH 4.5. (●) Control; (☐) 0.1 mM $AlCl_3$; (△) 1 mM $AlCl_3$. See also legend to Fig. 1.

50–100 times greater than those of divalent cations. The possibility that this is due to an increase in the concentrations of K^+ and Na^+ in the medium or to a decrease in the pH of the medium has been ruled out. The Rb^+ uptake may also be inhibited seriously by monovalent cations [2, 3, 5].

As pointed out by Borst-Pauwels [6], the rate equation for Rb^+ uptake into yeast cells is of the form of Eqn. 1. With a curve-fitting program and the use of a digital computer we have computed the coefficients A_i, B_i, C_i and D_i of this rate equation, describing the isotherms of the Rb^+ uptake in the absence and presence of the various polyvalent cations. Coefficient B_i, representing the maximal rate of uptake, appeared to be independent of the polyvalent cation concentration, and

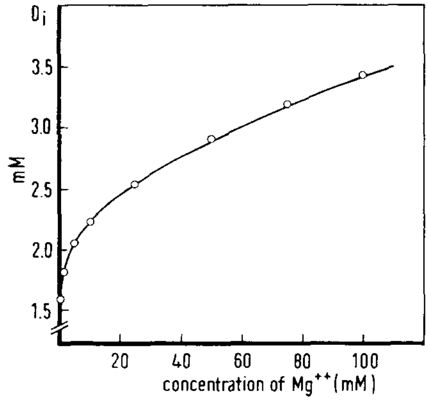

Fig. 3. Effect of Mg^{2+} upon the value of coefficient D_i of the rate equation (Eqn. 1) describing the Rb^+ uptake into yeast cells at pH 4.5. See also legend to Fig. 1.

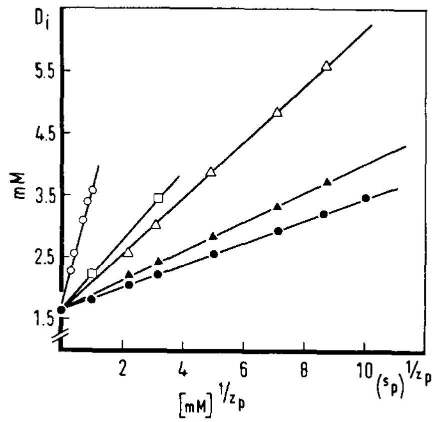

Fig. 4. The effects of Mg^{2+} (●), Sr^{2+} (▲), Ca^{2+} (△), Ba^{2+} (□) and Al^{3+} (○) upon the value of coefficient D_i of rate Eqn. 1. The value of D_i is plotted against the z_p-th root of the polyvalent cation concentration. See also legend to Fig. 1.

was on the average 17 mmol · min^{-1} · kg^{-1} on a dry weight basis. It appeared that the coefficient A_i is also virtually independent of the polyvalent cation concentration. The mean value of coefficient A_i is 0.54. On the other hand, the values of the coefficients C_i and D_i are increased when the polyvalent cation concentration is raised. When the yeast cell membrane is uncharged a linear relationship is expected between coefficient D_i and the concentration of a polyvalent cation. Fig. 3 clearly shows that, experimentally, a bent curve is obtained when the effect of the divalent cation Mg^{2+} is considered. This is also found with the other polyvalent cations applied. However, as shown in Fig. 4, linear relationships are found when coefficient D_i is plotted against the z_p-th root of the concentration of a polyvalent cation s_p of valency z_p. It is seen

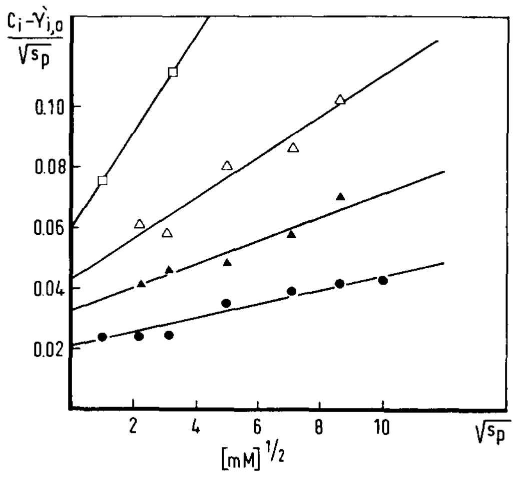

Fig. 5. The effects of Mg^{2+} (●), Sr^{2+} (▲), Ca^{2+} (△) and Ba^{2+} (□) upon the value of coefficient C_i of rate Eqn. 1. The experimental data are represented according to Eqn. 15. See also legend to Fig. 1.

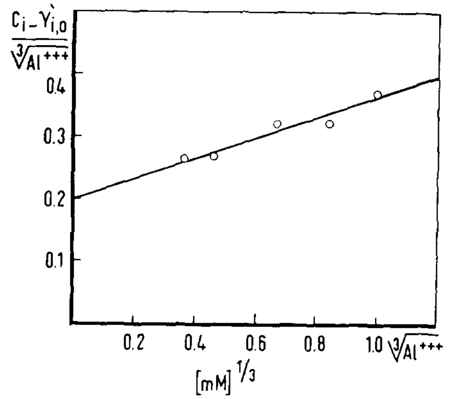

Fig. 6. Effect of Al^{3+} upon the value of coefficient C_i of rate Eqn. 1. See also legends to Figs. 1 and 5.

that the slope of the straight lines (denoted coefficient $\delta'_{i,p}$) depends upon the kind of polyvalent cation, increasing in the order $Mg^{2+} < Sr^{2+} < Ca^{2+} < Ba^{2+} \ll Al^{3+}$. As shown previously these "z_p-th root relations" are expected only when the yeast cell membrane is negatively charged and the surface potential is relatively high. Under these conditions the relationship between coefficient C_i and the concentration of a polyvalent cation s_p of valency z_p may be represented by Eqn. 12. This equation can be transformed into

$$\frac{C_i - \gamma'_{i,0}}{s_p^{1/z_p}} = \gamma'_{i,p} + \gamma'_{i,pp}\, s_p^{1/z_p} \tag{15}$$

On plotting the left-hand term of Eqn. 15 against s_p^{1/z_p} a straight line may be expected in such a case. The intercept of this line with the ordinate equals the value of coefficient $\gamma'_{i,p}$, whereas the slope of this line equals the value of coefficient $\gamma'_{i,pp}$. As a matter of fact, the experimental data lie approximately on straight lines (see Figs. 5 and 6). The values of the coefficients $\gamma'_{i,p}$, $\gamma'_{i,pp}$ and $\delta'_{i,p}$ for the various polyvalent cations applied, evaluated from the data in Figs. 4, 5 and 6, are given in Table I. The values of these coefficients increase in the order $Mg^{2+} < Sr^{2+} < Ca^{2+} < Ba^{2+} \ll Al^{3+}$.

TABLE I

VALUES OF THE COEFFICIENTS $\gamma'_{i,p}$, $\gamma'_{i,pp}$ AND $\delta'_{i,p}$ FOR THE VARIOUS POLYVALENT CATIONS APPLIED

The values of the coefficients are calculated from the data in Figs. 4–6.

Polyvalent cation	$\gamma'_{i,p}$	$\gamma'_{i,pp}$	$\delta'_{i,p}$
Mg^{2+}	0.021	0.0023	0.19
Sr^{2+}	0.033	0.0039	0.24
Ca^{2+}	0.043	0.0067	0.46
Ba^{2+}	0.060	0.0160	0.58
Al^{3+}	0.20	0.169	2.0

DISCUSSION

The relations for the kinetic parameters determining Rb^+ uptake into yeast cells with the concentration of a polyvalent cation found agree well with those theoretically derived for ion translocation mediated by a two-site mechanism across a negatively charged membrane and the case in which the translocational steps through the membrane are not affected by variations in the surface potential. The coefficients A_i and B_i are independent of the concentration of a polyvalent cation applied, whereas D_i is linearly related to the z_p-th root of that concentration and C_i is a quadratic relation of that root. In the context of our theoretical considerations these findings indicate that, under our experimental conditions, the yeast cell membrane is negatively charged and the surface potential is relatively high, at least in the micro-environment of the sites of the translocation mechanism.

According to the model, linear relations may be expected between the coefficients $\gamma'_{i,p}$ and $\delta'_{i,p}$, and also between $\gamma'_{i,pp}$ and the product of $\gamma'_{i,p}$ and $\delta'_{i,p}$, when the affinity of the polyvalent cations to the translocation sites is relatively low. As a matter of fact, to a good approximation, linear relationships are found (see Figs. 7 and 8), indicating that the affinity of the polyvalent cations applied to the translocation sites is relatively low. The conclusion is reached that the reduction in the rate of Rb^+ uptake by the polyvalent cations is due mainly to screening of the negative fixed charges on the surface of the yeast cell membrane and to binding of the polyvalent cations to the negative sites, rather than to competition with Rb^+ for the translocation sites. For a series of polyvalent cations of the same valency coefficient $\gamma'_{i,pp}$ is proportional to the affinity of a polyvalent cation for the negative sites.

For the alkaline-earth cations this affinity increases in the order $Mg^{2+} < Sr^{2+} < Ca^{2+} < Ba^{2+}$ at pH 4.5. This is partially in agreement with the results of the study of Rothstein and Hayes [12] on the cation-binding properties of the yeast cell surface at pH 5. They also found that the affinity of Ba^{2+} is the greatest. However, little difference between Mg^{2+}, Sr^{2+} and Ca^{2+} was observed. This discrepancy with our results might be due to the difference in the pH of the medium. It is well

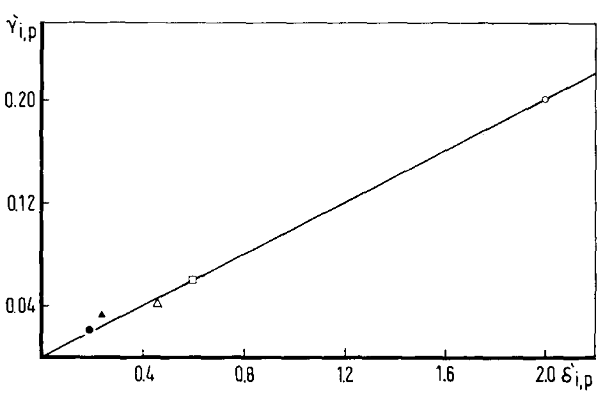

Fig. 7. A plot of coefficient $\gamma'_{i,p}$ against coefficient $\delta'_{i,p}$ for the various polyvalent cations. The values of the coefficients are calculated from the data in Figs. 4–6. (●) Mg^{2+}, (▲) Sr^{2+}, (△) Ca^{2+}, (□) Ba^{2+} and (○) Al^{3+}.

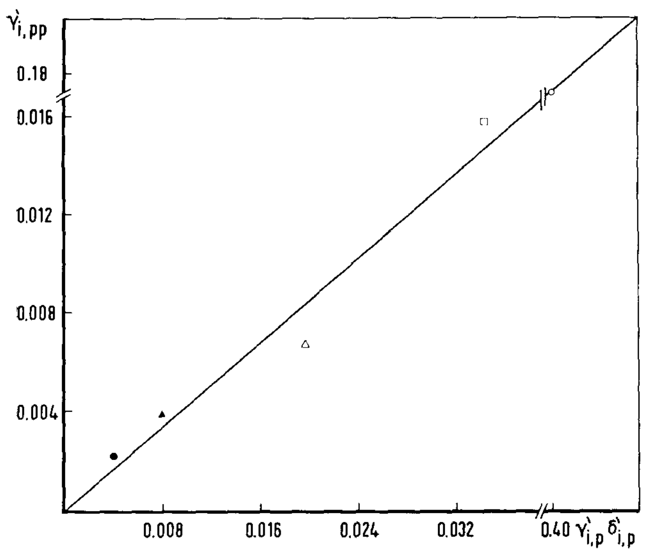

Fig. 8. A plot of coefficient $\gamma'_{i,pp}$ against the product of the coefficient $\gamma'_{i,p}$ and $\delta'_{i,p}$ for the various polyvalent cations. See also legend to Fig. 7.

established both theoretically and experimentally in artificial systems (see the detailed review by Diamond and Wright [13]) that, when alkaline-earth cations genuinely bind to a negatively charged surface, the selectivity pattern shifts with variation of the pH, giving rise to a nonselective situation at a certain pH value (see, for example. D'Arrigo [14, 15]). We are now examining the effect of the pH upon the selectivity pattern for the potency of the alkaline-earth cations in reducing the rate of the Rb^+ uptake into yeast cells. The selectivity sequence we have found at pH 4.5 corresponds to sequence II described by Sherry [16]. A similar sequence is found experimentally for binding of alkaline-earth cations to the phosphate colloid nucleate [17]. It could be speculated, therefore, that phosphoryl groups determine the net charge on the membrane surface of the yeast cell at pH 4.5. This is supported by recent findings that the isoelectric point of plasma membrane vesicles isolated from the yeast is below pH 3 [18].

Our present study shows that one should be very careful in interpreting effects of polyvalent cations upon monovalent cation uptake in terms of competitive inhibition. Analogous effects may be obtained when the surface potential is affected. We are aware that changes in membrane permeability introduced by polyvalent cations (see, for example, Viets [19]) may also occur. However, in such a case changes in the maximal rate of uptake should be expected [20]. As may be judged from the independence of coefficient B_i from the concentrations of the polyvalent cations applied, there are no indications that these polyvalent cations affect the K^+ (Rb^+) permeability of the yeast cell membrane.

The agreement of our experimental results with the theory presented in the present report is perhaps surprising, in view of the many assumptions inherent in the derivation of the Gouy-Chapman equation and the rate equation. However, as discussed by several investigators (e.g. Haydon [21], Davies and Rideal [22] and

Barlow [23]), the simple double-layer theory should be applicable to most surfaces, As a matter of fact McLaughlin et al. [9] found a good applicability of the "double-layer theory" to phospholipid bilayers and they suggested that this theory might be applied to biological membranes as well.

APPENDIX

As pointed out by Theuvenet and Borst-Pauwels [1], the rate equation for an enzymic process may be used to describe an ion translocation process across a biological membrane, provided that the concentrations in the rate equation are replaced by those near the surface of the membrane. These concentrations may differ from those in the medium when the membrane bears a charge. The rate equation for ion translocation mediated by a two-site mechanism can be derived from Eqn. 12 in the article of Borst-Pauwels [6] on replacing the notations for the ion concentrations in the medium by those for the ion concentrations near the membrane surface. For the case of monovalent cation translocation across a biological membrane from a solution also containing other monovalent cations (s_j,s_k) and one polyvalent cation (s_p) summation over j in Eqn. 12 in the article of Borst-Pauwels [6] has to include p. Then this rate equation becomes

$$\begin{aligned} v_i &= \frac{A_{i,0}\, s_{i,0} + B_{i,0}\, s_{i,0}^2}{C_{i,0} + D_{i,0}\, s_{i,0} + s_{i,0}^2} \\ &= \biggl[V_i \biggl\{ K'_{ii}\, b_i + K_{ii}\, b'_i + \sum_{j \neq i,p} s_{j,0} \left(\frac{K'_{ii}\, b_{ji}}{K'_{ji}} + \frac{K_{ii}\, b'_{ij}}{K_{ij}} \right) \\ &\qquad + s_{p,0} \left(\frac{K'_{ii}\, b_{pi}}{K'_{pi}} + \frac{K_{ii}\, b'_{ip}}{K_{ip}} \right) \biggr\} \biggr] s_{i,0} + V_i\, s_{i,0}^2 \Big/ \\ &\qquad \biggl\{ \biggl[K_{ii}\, K'_i \biggl\{ 1 + \sum_{j \neq i,p} s_{j,0} (1/K_j + 1/K'_j) + \sum_{j \neq i,p} \sum_{k \neq i,p} \frac{s_{j,0}\, s_{k,0}}{K_{kj}\, K'_k} \\ &\qquad + s_{p,0} \left(1/K_p + 1/K'_p + \sum_{j \neq i,p} \frac{s_{j,0}}{K_{pj}\, K'_p} + \sum_{k \neq i,p} \frac{s_{k,0}}{K_{kp}\, K'_k} \right) + \frac{s_{p,0}^2}{K_{pp}\, K'_p} \biggr\} \biggr] \\ &\quad + \biggl[K_{ii} + K'_{ii} + \sum_{j \neq i,p} s_{j,0} \left(\frac{K_{ii}}{K_{ij}} + \frac{K'_{ii}}{K'_{ji}} \right) + s_{p,0} \left(\frac{K_{ii}}{K_{ip}} + \frac{K'_{ii}}{K'_{pi}} \right) \biggr] s_{i,0} + s_{i,0}^2 \biggr\} \qquad (A1) \end{aligned}$$

In this equation, the symbols K with appropriate subscripts are the equilibrium constants for the complexes of the transport mechanism with the various cations present near the surface of the membrane. The prime denotes that equilibrium applies to binding of a cation to the "left" side of the mechanism; when no prime is present then the equilibrium applies to binding of a cation to the "right" side of the mechanism. By this the two sites of the mechanism are formally distinguished from each other. The symbols b with appropriate subscripts are the rates of translocation relative to the rate of translocation via the complex in which both sites of the mechanism are occupied by s_i. The rate of translocation at infinitely high concentrations of s_i is represented by V_i. The additional subscript 0 assigned to the notations for the ion concentrations refers to the ion concentrations near the membrane surface.

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