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Mutual interaction of ion uptake and membrane potential

G.W.F.H. Borst-Pauwels

Laboratory of Cell Biology, Catholic University, Faculty of Science, Nijmegen (Netherlands)

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The concentration dependence of cation uptake by the cell may be considerably complicated when this uptake is accompanied by a depolarization of the cell membrane. In case of carrier-mediated transport deviations from Michaelis-Menten kinetics may come to the fore comparable to those found in a dual mechanism of cation uptake or when substrate inhibition is involved. This remains true when only the maximum rate of uptake and not the K_m is dependent upon the membrane potential. We have proven this by means of computer simulation of cation transport mediated by a non-mobile carrier. Under restricted conditions still apparent Michaelis-Menten kinetics may be found despite the fact that the membrane potential varies with increasing substrate cation concentration. But even then there are still differences with 'normal' transport kinetics. A non-competitive inhibitor does not only affect the maximum rate of uptake but also the apparent K_m. Depolarization of the cells by a cation which passes the cell membrane by means of diffusion, affects the uptake of the substrate cation almost in the same way as a non-competitive inhibitor does and causes both a decrease in the maximum rate of uptake and an increase in K_m. In the case of competitive inhibition the apparent affinity of the inhibitor for the carrier depends upon the rate of transfer of this inhibitor through the cell membrane. The mutual influence of cation uptake and membrane potential is dealt with for uniport of either monovalent or divalent cations and for cotransport of monovalent cation with protons, as well. Possible effects of the surface potential are accounted for.

Introduction

If transport of an ion is driven by the membrane potential, one may expect that the membrane potential in turn is influenced by that transport. By this the concentration dependence of ion influx will become more complicated that when the membrane potential remains unaffected. Diffusion of a cation into the cell will show apparent saturation kinetics instead of a linear relation between uptake rate and cation concentration [1]. In a similar way ion transport mediated by a single-site carrier may show deviations from Michaelis-Menten kinetics when the cells are depolarized on increasing the substrate concentration [2]. For anion uptake in plant cells it has been shown that after accounting for changes in the membrane potential experimentally found on increasing the concentration of the anion with accompanying cation apparent dual mechanism kinetics may come to the fore [3]. For cation transport mediated by a single-site mobile carrier (or carrier of which the binding sites can switch between the two sides of the membrane) apparent two-site kinetics can be found when the membrane potential depends upon the concentration of the substrate cation [4]. These deviations from Michaelis-Menten kinetics were ascribed primarily to the fact that changes in the membrane potential influence the partition of the mobile carrier between the two sides of the cell membrane following which not only the maximum rate of transport is affected but also the apparent K_m. However, one may also expect deviations from Michaelis-Menten kinetics when only the maximum rate of uptake depends upon the membrane potential and the K_m is unaffected, which may be the case if an immobile carrier is involved. We now examine what type of deviations from Michaelis-Menten kinetics may be found in this case. Instead of making an assumption about the dependence of the membrane potential on the cation concentration as was done by Ref. 4 we have calculated the membrane potential by applying a modified Goldman equation [5]. This has enabled us not only to simulate the effect of the membrane potential on cation influx in a more general way but also to develop appropriate equations describing the expected relation between membrane potential and substrate

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Correspondence to: G.W.F.H. Borst-Pauwels, Laboratory of Cell Biology, R.C. University, Faculty of Science, Toernooiveld, 6525 ED Nijmegen, Netherlands.

cation concentration. Appropriate equations are derived for both monovalent and divalent cation uniport as well for monovalent cation-proton cotransport.

Theory

Derivation of the rate equation for monovalent cation uptake by means of a uniporter as function of the membrane potential

It is supposed that the ions are translocated by means of an immobile carrier with two fixed sites one at each side of the cell membrane. The ion species are designated by S with appropriate index and the corresponding concentrations by s with $s_{p,Z}$ for the concentration of S_p in compartment Z. $Z = \mathrm{I}$ refers to the medium and $Z = \mathrm{II}$ to the cell. Complexes between unloaded carrier C and S_p from the medium and from the cell are designated by $CS_{p,\mathrm{I}}$ and $CS_{p,\mathrm{II}}$, respectively, and that with S_p from the medium and S_q from the cell by $CS_{p,\mathrm{I}}S_{q,\mathrm{II}}$. The dissociation constant referring to S_p (K_p) is independent of the side of the cell membrane and is not affected by loading the opposite site of the carrier. At equilibrium $cs_{p,Z} = c \cdot s_{p,Z}/K_p$. We assume that transfer of substrate across the cell membrane can happen only from carrier cation complexes in which only one substrate is bound and not from $CS_{p,\mathrm{I}}S_{p,\mathrm{II}}$. The rate constants for transfer of cation S_p from $CS_{p,Z}$ across the cell membrane are indicated by $r_{p,Z}$, $Z = \mathrm{I}$ and II refer to transport from medium to cell and from cell to medium, respectively. The rate constants still depend upon the membrane potential. If a symmetrical Eyring barrier is involved [6], the relation between membrane potential and $r_{p,Z}$ is given by

$$r_{p,Z} = r_{0,p} y_Z \tag{1}$$

$$y_{\mathrm{I}} = \exp(-FE/2RT),\ y_{\mathrm{II}} = 1/y_{\mathrm{I}} \tag{2}$$

E is the membrane potential difference between compartment II and I. F, R and T have their usual meaning. $r_{0,p}$ the rate constant of transfer of S_p at zero membrane potential is assumed to be independent of the direction of translocation. The conservation equation is given by

$$C_{\mathrm{T}} = c + \sum(cs_{p,\mathrm{I}} + cs_{p,\mathrm{II}}) + \sum\sum cs_{p,\mathrm{I}}s_{q,\mathrm{II}} = c \cdot N \tag{3}$$

$$N = \left(1 + \sum s_{p,\mathrm{I}}/K_p\right)\left(1 + \sum s_{q,\mathrm{II}}/K_q\right) \tag{4}$$

C_{T} is the sum of the concentrations of all carrier species. The second formulation in Eqn. 3 is obtained on eliminating the carrier-substrate complexes by applying the equilibrium conditions. The influx of S_p ($v_{p,\mathrm{I}}$) and the corresponding efflux ($v_{p,\mathrm{II}}$) are given by $r_{p,Z}cs_{p,Z}$. On applying the equilibrium conditions we get

$$v_{p,Z} = r_{p,Z}c \cdot s_{p,Z}/K_p = V_{0,p}s_{p,Z}y_Z/K_pN \tag{5}$$

The second formulation in Eqn. 5 is obtained on eliminating $r_{p,Z}$ and c by means of Eqn. 1 and Eqn. 3, respectively. $V_{0,p} = r_{0,p}C_T$. Rewriting Eqn. 5 as a function of the concentration ($s_{j,\mathrm{I}}$) of ion S_j, of which we wish to study the transport kinetics, gives

$$v_{p,Z} = \frac{V_{\mathrm{m},p,Z}s_{p,Z}y_Z}{K_{\mathrm{m},j} + s_{j,\mathrm{I}}} \tag{6}$$

For $V_{\mathrm{m},p,Z}$ and $K_{\mathrm{m},j}$, see Table I.

Dependence of the membrane potential upon the cation concentration in the medium

In Eqn. 6 y_Z still depends upon $s_{j,\mathrm{I}}$. Therefore for developing the rate equation of $v_{p,Z}$ as function of $s_{j,\mathrm{I}}$ we need to know the dependence of y_{I} on $s_{j,\mathrm{I}}$. The membrane potential resulting from all ion fluxes across the cell membrane can be obtained from the steady-state condition that no net charge flux across the cell membrane occurs. Besides cation fluxes by means of the carrier system, cation and anion fluxes via diffusion and a cation flux by means of an electrogenic pump are also considered. The rate equations for a monovalent cation S_c or anion S_a which pass the cell membrane by simple diffusion without mediation of a carrier are

$$v_{c,Z} = D_c s_{c,Z} y_Z \tag{7}$$

$$v_{a,Z} = D_a s_{a,Z}/y_Z \tag{8}$$

respectively. D with the appropriate index is a constant depending upon the ion involved.

The rate of extrusion of a cation S_e by means of an electrogenic pump or its reversal, $v_{e,\mathrm{II}}$ and $v_{e,\mathrm{I}}$, respectively, may be given by

$$v_{e,Z} = U_{e,Z}s_{e,Z}y_Z \tag{9}$$

The coefficient $U_{e,\mathrm{II}}$ still depends upon the metabolic state of the cells. It is to be expected that $U_{e,\mathrm{II}} \gg U_{e,\mathrm{I}}$.

The value of y_{I} is given implicitly by

$$\sum v_{p,\mathrm{I}} + \mathrm{Dep} \cdot y_{\mathrm{I}} = \sum v_{p,\mathrm{II}} + \mathrm{Hyp}/y_{\mathrm{I}} \tag{10}$$

The left hand side of Eqn. 10 contains the depolarizing fluxes, of which $\mathrm{Dep} \cdot y_{\mathrm{I}}$ represent the charge fluxes due to inward diffusion of cations, outward diffusion of anions and influx of cation by means of the reversal of the electrogenic cation pump. The right hand side of the equation contains the hyperpolarizing fluxes of which $\mathrm{Hyp}/y_{\mathrm{I}}$ refers to the charge fluxes via outward

TABLE I

Parameters for monovalent and divalent cation uptake

The parameters for divalent cation uptake also apply to monovalent cation-proton cotransport except those for $K_{m,j}$ and $V_{m,j,Z}$, which may still depend upon $s_{h,Z}$. The index g is 1 or 2 for monovalent cation and divalent cation uniport, respectively. $y_{\sim}$ is the value of y_I in Eqns. 12 and 19 at infinitely high values of $s_{j,I}$. See also the appendix for the effect of the surface potential upon $K_{m,j}$ and $V_{m,p,Z}$ and for an alternative transport model.

	Monovalent cation	Divalent cation	
$L_{m,j}$	$K_{m,j} + \mathrm{Vs}_{II}/\mathrm{Hyp}$	$K_{m,j} + \mathrm{Vs}_{II}/\mathrm{Hyp}\cdot y_I$	(T-1)
$M_{m,j}$	$\dfrac{K_{m,j}\mathrm{Dep} + \mathrm{Vs}_I}{\mathrm{Dep} + V_{m,j,I}}$	$\dfrac{K_{m,j,I}\mathrm{Dep}/y_I + \mathrm{Vs}_I}{\mathrm{Dep}/y_I + 2V_{m,j,I}}$	(T-2)
$Q_{m,j}$	$\mathrm{Hyp}/(\mathrm{Dep} + V_{m,j,I})$	$\mathrm{Hyp}/(\mathrm{Dep}/y_I + 2V_{m,j,I})$	(T-3)
$K_{m,j}$	$K_j(1 + \sum_{p \# j} s_{p,I}/K_p)$		(T-4)
$V_{m,p,Z}$	$K_j V_{0,p}/K_p(1 + \sum s_{q,II}/K_q)$		(T-5)
Hyp	$\sum D_c s_{c,II} + \sum D_a s_{a,I} + U_{e,II} s_{e,II}$		(T-6)
Dep	$\sum D_c s_{c,I} + \sum D_a s_{a,II} + U_{e,I} s_{e,I}$		(T-7)
Vs_I	$g \sum_{p \# j} V_{m,p,I} s_{p,I}$		(T-8)
Vs_{II}	$g \sum V_{m,p,II} s_{p,II}$		(T-9)
K_m	$K_{m,j}(y_{\sim}/y_0)^g$		(T-10)
V_m	$V_{m,j,I}(y_{\sim})^g$		(T-11)
V_m^{-2}	$\dfrac{\mathrm{Dep} + V_{m,j,I}}{\mathrm{Hyp}\cdot V_{m,j,I}^2}$		(T-12)
$V_m^{-3/2}$		$\dfrac{\mathrm{Dep}/y_{\sim} + 2V_{m,j,I}}{\mathrm{Hyp}\cdot V_{m,j,I}^{3/2}}$	(T-13)
$(K_m/V_m)^2$	$\dfrac{K_{m,j}^2(K_{m,j}\mathrm{Dep} + \mathrm{Vs}_I)}{V_{m,j,I}^2(K_{m,j}\mathrm{Hyp} + \mathrm{Vs}_{II})}$		(T-14)

diffusion of cations, inward diffusion of anions and cation efflux by means of the electrogenic cation pump. For Dep and Hyp see also Table I. When there is no net charge transfer across the cell membrane by means of carrier-mediated ion translocation, then Dep and Hyp are interrelated according to

$$\mathrm{Hyp} = y_I^2 \cdot \mathrm{Dep} \tag{11}$$

y_I as function of $s_{j,I}$ can be obtained from Eqns. 6 and 10.

$$y_I^2 = \frac{(L_{m,j} + s_{j,I})Q_{m,j}}{M_{m,j} + s_{j,I}} \tag{12}$$

For the meaning of the parameters, see Table I. We will now introduce a function $q = 1 - (y_I/y_0)^2$, in which y_0 is the value of y_I at $s_{j,I} = 0$. This function depends upon $s_{j,I}$ according to a Michaelis-Menten equation.

$$q = \frac{q_{\max} s_{j,I}}{M_{m,j} + s_{j,I}} = q_{\max} - M_{m,j}(q/s_{j,I}) \tag{13}$$

$$q_{\max} = 1 - M_{m,j}/L_{m,j} \tag{14}$$

y_I may be obtained either by direct measurements of the membrane potential or from the equilibrium distribution of a lipophilic cation between cells and medium. It is also possible to obtain values of y_I/y_0 from the relative rates of uptake of a lipophilic cation into the cells provided that the relation between influx and membrane potential is known. $q_{\max}$ and $M_{m,j}$ can be obtained by plotting q against $q/s_{j,I}$. $L_{m,j}$ can then be obtained by means of Eqn. 14. $Q_{m,j}$ equals y_I^2 at saturating concentrations of $S_{j,I}$.

Rate equation for cation uptake taking into account the effect of the cation concentration upon the membrane potential

The rate equation for uptake of S_j as function of $s_{j,I}$, in which equation the dependence of the membrane potential upon $s_{j,I}$ is accounted for, is obtained by eliminating y_I from Eqn. 6 by means of Eqn. 12.

$$v_{j,I} = \frac{V_{m,j,I} s_{j,I}(L_{m,j} + s_{j,I})^{1/2} Q_{m,j}^{1/2}}{(K_{m,j} + s_{j,I})(M_{m,j} + s_{j,I})^{1/2}} \tag{15}$$

When $L_{m,j}/M_{m,j}$ approximates 1 the rate equation is expected to approximate a Michaelis-Menten equation.

$$v_{j,I} = V_m \cdot s_{j,I}/(K_m + s_{j,I}) = V_m - K_m(v_{j,I}/s_{j,I}) \tag{16}$$

From Eqns. 15 and 16 appropriate equations for V_m and K_m can be obtained by taking $s_{j,I}$ infinitely high and zero, respectively, see Table I.

Rate equation for divalent cation uptake

For carrier-mediated divalent cation transport similar rate equations apply as those derived for monovalent cation uptake. One has only to replace y_Z by y_Z^2 in Eqn. 6

$$v_{p,Z} = \frac{V_{m,p,Z} s_{p,Z} y_Z^2}{K_{m,j} + s_{j,I}} \tag{17}$$

At zero charge transfer we get

$$2\sum v_{p,I} + \mathrm{Dep}\cdot y_I = 2\sum v_{p,II} + \mathrm{Hyp}/y_I \tag{18}$$

For this it follows that

$$y_I^3 = \frac{L_{m,j} + s_{j,I}}{M_{m,j} + s_{j,I}} Q_{m,j} \tag{19}$$

For the meaning of the parameters see Table I. For divalent cation uptake q is defined as $1-(y_I/y_0)^3$. $L_{m,j}$, $M_{m,j}$ and $Q_{m,j}$ still depend upon y_I. Therefore Eqn. 19 is an implicit function of y_I and no straight line would be expected to come to the fore in a Hofstee plot of q.

On eliminating y_I from Eqn. 17 by means of Eqn. 19 we get for the influx of S_j

$$v_{j,I}=\frac{V_{m,j,I}s_{j,I}\left(L_{m,j}+s_{j,I}\right)^{2/3}Q_{m,j}^{2/3}}{\left(K_{m,j}+s_{j,I}\right)\left(M_{m,j}+s_{j,I}\right)^{2/3}} \tag{20}$$

If Eqn. 20 approximates a Michaelis-Menten relation, equations for K_m and V_m can be obtained in a similar way as for monovalent cation uniport, see Table I.

Monovalent cation-proton cotransport

Eqn. 17 may also apply to cotransport of a monovalent cation with a proton, provided that there is a strict coupling between transfer of proton and substrate cation. In this case $V_{m,p,Z}$ still depends on $s_{h,Z}$, the proton concentration in medium or cell and $V_{m,p,I}$ and $V_{m,p,II}$ are no longer equal to each other. This can be seen on applying the equilibrium condition $v_{p,I}=v_{p,II}$ to Eqn. 17 and taking into account that at equilibrium $s_{p,II}s_{h,II}/s_{p,I}s_{h,I}=y_I^4$. It then follows that

$$V_{m,p,II}/V_{m,p,I}=s_{h,II}/s_{h,I} \tag{21}$$

Eqns. 18–20 are expected also to apply to monovalent cation proton cotransport. $K_{m,j}$, $L_{m,j}$, $M_{m,j}$ and $Q_{m,j}$ may then still depend on $s_{h,Z}$.

Modifications of the rate equations derived

In the derivation of the rate equations developed by us we did not account for possible effects of the surface potentials at both sides of the cell membrane. When the surface potentials are non-zero, the concentrations of the cations near the membrane will differ from those in the bulk aqueous phases. Furthermore the electrical potential across the cell membrane will not longer be equal to the membrane potential between cytosol and medium. In the appendix we account for these effects.

In the transport model discussed by us the affinity of the substrate cation for the binding sites of the carrier was independent of the state of the carrier, allowing the formation of complexes of the form $CS_{p,I}S_{q,II}$. In the appendix we will show that similar equations are expected to apply if only carrier-cation complexes of the form $CS_{p,Z}$ can be formed. Only the equations for $K_{m,j}$ and $V_{m,p,Z}$ differ from those derived for the first transport model.

Results

The effect of substrate cation upon both the membrane potential and the rate of uptake is simulated according to the equations derived. The simulations are carried out on an Atari MegaST4. The simulation program has been developed by means of Maxon Pascal. The results are represented in the form of a so-called Hofstee plot [7], which consists of a plot of $v_{j,I}$ against $v_{j,I}/s_{j,I}$. When a Michaelis-Menten equation applies a straight line is found according to Eqn. 16.

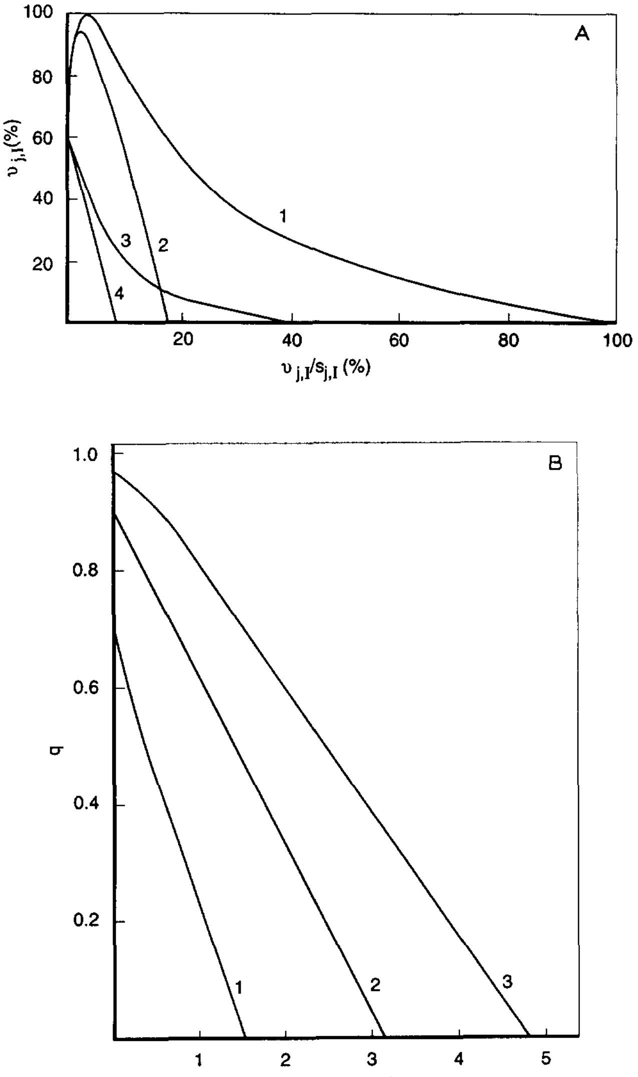

Fig. 1. (A) Hofstee plot of the dependence of the rate of uptake of a monovalent cation S_j by means of a uniporter upon its concentration in the medium at fixed values of $K_{m,j}$ and $Q_{m,j}$ and varying values of $L_{m,j}$ and $M_{m,j}$. See Table II for the values of the parameters referring to each curve and the values of the membrane potentials at both zero and infinitely high substrate concentrations. (B) Hofstee plot of $q=1-(y_I/y_0)^n$ at $L_{m,j}=3.5$ and $M_{m,j}=0.3$ for $n=1$, 2 or 3. The values of n are indicated in the figure.

Fig. 1A shows the simulation of monovalent cation uniport for both a high and a low value of $L_{m,j}$ at two different values of $M_{m,j}$. The values of the kinetic parameters and also of the membrane potentials at both zero substrate cation concentration and infinitely high substrate concentrations are given in Table II. At the lowest $M_{m,j}$ value a concave curve is found at both $L_{m,j}$ values. In addition at $L_{m,j} = 20$ apparent substrate inhibition is found at high substrate concentrations. The concave curves disappear on raising $M_{m,j}$ to 0.3. Then an almost linear relationship between $v_{j,I}$ and $v/s_{j,I}$ is found at $L_{m,j} = 3.5$, showing that under these conditions the uptake of S_j is described by a Michaelis-Menten equation by approximation. On the other hand the apparent substrate inhibition found at high $L_{m,j}$ persists on increasing $M_{m,j}$.

In Fig. 1B we show the dependence of q for monovalent cation uniport on $q/s_{j,I}$, for $q = 1 - (y_I/y_0)^n$, with $n = 1$, 2 or 3. At $n = 2$, q depends on $s_{j,I}$ according to a Michaelis-Menten equation, see Eqn. 13. We also include a plot of q at $n = 3$. In this case a slightly convex curve is found, whereas as will be seen in Fig. 4 for monovalent cation-proton cotransport a slightly concave curve is predicted. Recently we determined the effect of monovalent cations upon the uptake of the lipophilic fluorescent membrane potential probe 2-(dimethylaminostyryl)-1-ethylpyridinium (DMP) in yeast from which values of y_I were obtained as function of the cation concentration [8]. It appeared that in a Hofstee plot of q with $n = 1$ the data points could be fitted by a straight line. Fig. 1B shows that for monovalent cation uniport a concave curve is expected at $n = 1$. However, the expected deviations from linearity are relatively small and may be within the experimental error.

As shown in Fig. 1A for monovalent cation uptake Michaelis-Menten kinetics can be found by approximation at appropriate conditions. These conditions are characterized by the fact that $L_{m,j}$ does not differ much from both $K_{m,j}$ and $M_{m,j}$. We now examine the effects of both a competitive and a non-competitive inhibitor (S_i) on cation uptake. These effects are expected to differ from those for uncharged substrates, because besides effects on the maximum rate of uptake in case of a non-competitive inhibitor or on the K_m in case of a competitive inhibitor also indirect effects may come to the fore due to changes in the membrane potential. In addition we examine the effect of a depolarizing cation (S_c), which is able to pass the cell membrane by diffusion.

Fig. 2A shows that with a competitive inhibitor apparent competitive inhibition is still found. However, the K_m not only depends upon the concentration of the inhibitor (data not shown) but also upon the rate at which it may pass the cell membrane at zero membrane potential, which rate is proportional to $V_{0,i}$. With increasing $V_{0,i}$, K_m increases as well. Furthermore at low $V_{0,i}$ values concave deviations from linearity come to the fore in the Hofstee plot. At $V_{0,i} = 5$ the cells become hyperpolarized instead of depolarized on increasing the substrate cation concentration, see Table II.

In the presence of a non-competitive inhibitor, not only is the expected decrease in the maximum rate of uptake found, but also an increase in apparent K_m. Similar effects are found with a permeant cation,

TABLE II

Parameters referring to the simulations represented in Figs. 1 and 2

$K_{m,j}$, $L_{m,j}$, $M_{m,j}$, $s_{i,I}$ and $s_{c,I}$ are expressed in mM. E_0 and $E_{\sim}$, the values of the membrane potential E at $s_{j,I} = 0$ and $s_{j,I}$ at infinitely high concentrations, respectively, are expressed in mV.

Fig.	Curve	$K_{m,j}$	$L_{m,j}$	$M_{m,j}$	$Q_{m,j}$	Dep	$-E_0$	$-E_{\sim}$	
1	1	2	20	0.01	6	0	240	45.7	
	2	2	20	0.3	6	0	153	45.7	
	3	2	3.5	0.01	6	0	195	45.7	
	4	2	3.5	0.3	6	0	108	45.7	
2	1	1	1.99	0.195	81.3	0.0024	171	112	Control
									$V_{0,i}$
	a	2	2.99	0.390	81.3	0.0024	164	112	0
	b	2	2.99	1.20	81.3	0.0024	136	112	1
	c	2	2.99	4.41	81.3	0.0024	102	112	5
									$s_{i,I}$
	2	1	1.66	0.266	111	0.0024	167	120	0.5
	3	1	1.25	0.492	205	0.0024	159	136	3.0
									$s_{c,I}$
	2′	1	1.99	0.476	53	0.0090	138	101	2.2
	3′	1	1.99	0.901	10	0.090	79	59	29

though this cation does not show any affinity to the carrier. In the latter case at relatively high substrate cation concentrations more pronounced deviations from linearity are found in the Hofstee plot than with the non-competitive inhibitor.

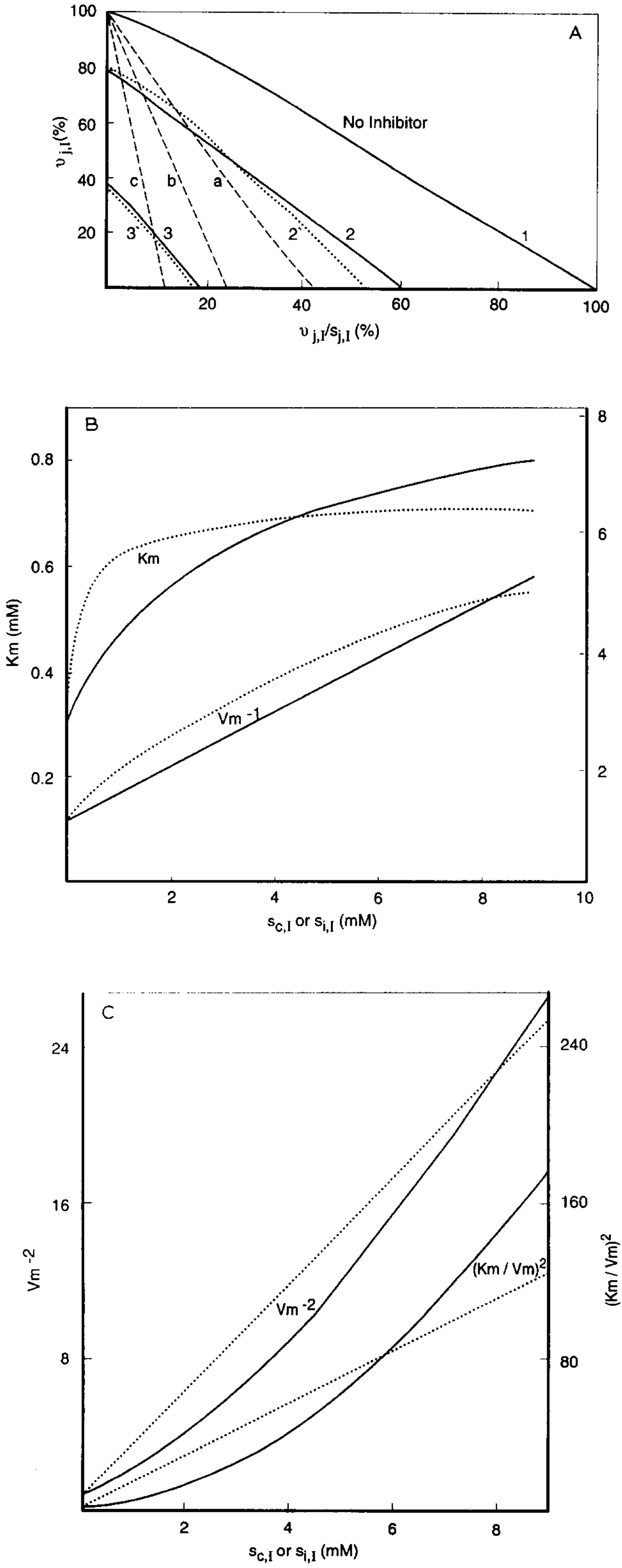

Fig. 2. Effects of a competing cation, a non-competitive inhibitor and a depolarizing cation diffusing through the cell membrane upon the kinetics of uptake of a monovalent cation S_j by means of a uniporter. Fig. 2A Hofstee plot of uptake of S_j. Full drawn line 1, control and line 2 and 3, effect of the non-competitive inhibitor (S_i) at 0.5 and 3 mM, respectively. Dotted lines 2′ and 3′, effect of the diffusing cation (S_c) at 2.2 and 29 mM, respectively. Dashed line a, b and c, effect of a competing cation (S_i) with $V_{0,i}$ is 0.2, 1 and 5, respectively. See further Table II. (B) Dependence of the apparent K_m and V_m^{-1} upon the concentrations of the non-competitive inhibitor or diffusing cation. (C) Plots of V_m^{-2} and $(K_m/V_m)^2$ against the concentrations of the diffusing cation and the non-competitive inhibitor according to Eqns. T-12 and T-14, respectively.

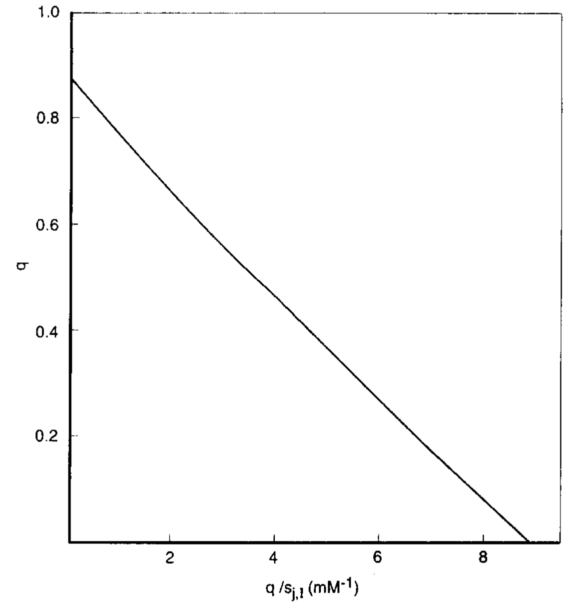

Fig. 3. Example of a Hofstee plot of $q=1-(y_I/y_0)^3$ for divalent cation uptake. $y_0=30$, $E_0=-173$ mV, $y_\sim=15$, $E_\sim=-138$ mV, $K_{m,j}=1$ mM, Dep = 5, Hyp = 4500, $Vs_I=0.1$ μM, $Vs_{II}=81$ mM, $V_{m,j,I}=1$.

Fig. 2B shows that the apparent K_m is not linearily related to the concentration of either the non-competitive inhibitor cation or the diffusing cation. On the other hand, V_m^{-1} is still proportional to the concentration of the non-competitive inhibitor, but not to the concentration of the diffusing cation.

According to Table I, on plotting V_m^{-2} or $(K_m/V_m)^2$ against the concentration of the depolarizing cation a straight line is expected, see Fig. 2C. However, for a non-competitive inhibitor a non-linear relationship is found in both cases.

For divalent cation uptake or monovalent cation-proton cotransport similar kinetics are expected as for monovalent cation uniport. Both apparent substrate inhibition and apparent dual mechanism may come to the fore (data not shown). Furthermore Michaelis-Menten kinetics may be found by approximation at appropriate conditions (data not shown). On the other hand no straight line is expected in a Hofstee plot of

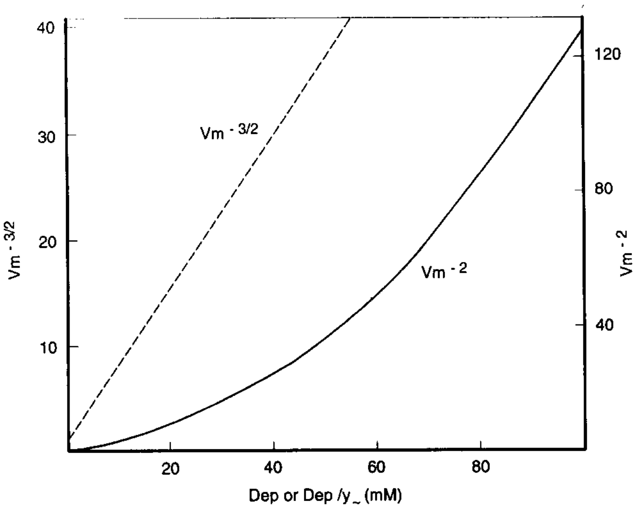

Fig. 4. Effect of the concentration of a depolarizing monovalent cation on the maximum rate of divalent cation uptake. Full drawn line plot of V_m^{-2} against Dep according to Eqn. T-12 and dashed line plot of $V_m^{-3/2}$ against Dep/$y_{\sim}$ according to Eqn. T-13.

$q = 1 - (y_I/y_0)^n$. According to Fig. 3 at $n = 3$ a concave curve deviating slightly from a straight line may be found in such a case. At $n = 1$ or 2 these deviations are still more pronounced (data not shown).

For monovalent cation uniport a linear relationship is expected between V_m^{-2} or $(K_m/V_m)^2$ and the concentration of a depolarizing cation, which is proportional to Dep. This does not apply any more for divalent cation uptake or monovalent cation-proton cotransport, as shown in Fig. 4. However, on plotting $V_m^{-3/2}$ against Dep/$y_{\sim}$ a straight line is expected according to Eqn. T-13.

Discussion

The kinetics of cation influx by means of carrier-mediated transport may be complicated when the uptake of the cation influences the membrane potential between cell and medium. Either deviations from Michaelis-Menten kinetics may come to the fore or, when the Michaelis-Menten equation still applies, anomalous inhibition kinetics may be expected. We still discuss these now in more detail.

Deviations from Michaelis-Menten kinetics for monovalent cation uptake

Eqn. 15 gives the relation between the uptake rate of a monovalent cation and its concentration in the medium. This equation may approximate a Michaelis-Menten type relation when $L_{m,j}$ and $M_{m,j}$ do not differ much. On the other hand when $L_{m,j}/M_{m,j} \gg 1$ deviations from Michaelis-Menten kinetics may come to the fore resulting in a concave Hofstee plot, as is also found in case of a dual transport mechanism [7]. As shown by Gerson and Poole [3] also for anion uptake apparent dual mechanism may occur when the cells are depolarized with increasing salt concentration. Besides depolarization of the cell membrane by the substrate cation, changes in the surface potential or the involvement of a two-site transport mechanism may also give rise to apparent dual mechanism [9]. A correct interpretation of the kinetic data obtained in studies of ion uptake in biological cells will therefore be favoured if additional data are available concerning the dependence of both the surface potential and the membrane potential upon the substrate ion concentration. When $L_{m,j}$ is not only higher than $M_{m,j}$ but also much higher than $K_{m,j}$ apparent substrate inhibition may be found. In such a case the depolarization of the cell membrane still increases on increasing the substrate cation concentration far above $K_{m,j}$, when the externally located sites of the carrier will be already saturated for the greater part.

In this publication we applied a relatively simple model for cation transport according to which only the maximum rate of uptake depends upon the membrane potential, whereas the K_m is independent of the membrane potential. For mobile carrier mediated transport the K_m also depends upon the membrane potential [4,10]. Then on accounting for changes in the membrane potential expected on increasing the substrate cation concentration convex deviations may come to the fore in the Hofstee plot [4], corresponding to a sigmoidal relation between the rate of uptake and the substrate concentration, which are not expected to occur according to the transport model discussed in this paper. These deviations should be ascribed to the fact that the partition of the sites of the mobile carrier between the two sides of the cell membrane depends on the membrane potential.

Apparent Michaelis-Menten kinetics for monovalent cation uptake

Under limited conditions Michaelis-Menten kinetics may still be found approximately, namely when the quotients $L_{m,j}/M_{m,j}$ and $L_{m,j}/K_{m,j}$ do not deviate much from unity. Even under these conditions there are still important differences from 'normal' transport kinetics.

The effect of a competitive inhibitor depends upon the rate at which the competitive inhibitor is translocated through the cell membrane, an effect not being expected when the membrane potential remains constant. When $V_{m,i,Z} \gg V_{m,j,Z}$ it is even possible that the cells become hyperpolarized on increasing the substrate cation concentration.

The effects of a non-competitive inhibitor or a permeant cation on the kinetics of cation uptake are qualitatively the same. Both decrease the maximum rate of uptake and increase the apparent K_m. However, the two types of inhibition can be distinguished by plotting V_m^{-2} or $(K_m/V_m)^2$ against the concentration of the inhibitory cation, see Fig. 2C. The increase in K_m must be ascribed to a reduction in the depolarization of the cells by the substrate cation in the presence of the inhibitory cation.

Monovalent cation uptake in yeast is driven by the membrane potential [11], and gives rise to depolarization of the cell membrane [8,12]. In accordance with the theory developed by us both the protonophore 2,4-dinitrophenol and proton-phosphate cotransport, which also is expected to depolarize the yeast cell membrane [13], increase the K_m for Rb^+ uptake with a concomitant decrease in the maximum rate of uptake [14,15].

Divalent cation uptake or monovalent cation-proton cotransport

For divalent cation uptake and this also applies for cation-proton cotransport a more complicated relation is expected to come to the fore for the dependence of the membrane on the cation concentration as is illustrated in the corresponding plots of $q = 1 - (y_I/y_0)^n$ in Figs. 1B and 3. At $n = 2$ and 3 a straight line and a convex curve, respectively, are expected for monovalent cation uniport, whereas for divalent cation uptake and monovalent cation-proton cotransport in both cases a concave curve is found.

Further for divalent cation uptake and monovalent cation-proton cotransport a depolarizing cation may lead to a decrease in V_m and concomitant increase in K_m. However, the relations between V_m^{-2} or $(K_m/V_m)^2$ and the concentration of the depolarizing cation are quite different from those expected for monovalent cation uniport, see Table I and Figs. 2C and 4. Possibly these differences may be used in order to distinguish between monovalent cation uniport and cation-proton cotransport.

Effect of the surface potential

In the appendix we show that accounting for effects of the surface potential at both sides of the cell membrane leads to some modifications of the equations derived. The main difference is that when the surface potentials are non-zero, apparent dissociation constants are changed. Furthermore the value of $V_{m,j,Z}$ is changed with varying surface potentials. The latter effect was not accounted for in our previous studies of the effect of the surface potential on monovalent cation uptake in yeast being reviewed in Ref. 9. Interpretation of the effects of the surface potential on monovalent cation uptake may be still more complicated, when changes in the values in Dep and Hyp are also involved in varying the surface potential.

Comparison between two models for ion transport

In the appendix we compared the rate equations for ion transport via the fixed site carrier model described under theory in which the two opposite sites of the carrier can be occupied simultaneously by substrate ion with those expected when only one of the two sites can be occupied at the same time. The main difference between the two models is that in the first case $K_{m,j}$ only depends upon the concentration of competing cations and $V_{m,j,Z}$ depends upon the concentration of cations having affinity to the carrier inside the cell, whereas in the second case $K_{m,j}$ also depends on the concentration of ions inside the cell and $V_{m,j,Z}$ is just independent of the intracellular cation concentration.

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Appendix

In the derivation of the rate equations we did not account for eventual effects of the surface potentials at both sides of the cell membrane ($E_{s,Z}$). If these potentials are non-zero, for monovalent and divalent cations the concentrations near the membrane are x_Z^g-times higher than the corresponding bulk concentrations with $g = 1$ and 2, respectively, and for monovalent anions they are x_Z-times smaller.

$$x_Z = \exp(-FE_{s,Z}/RT) \tag{A-1}$$

As shown previously [16] for carrier mediated cation transport we can account for effects of the surface potentials by replacing K_p in the rate equations by modified apparent dissociation constants.

$$K_{p,Z} = K_p/x_Z^g \tag{A-2}$$

It should be stressed that whereas K_p is independent of the side of the cell membrane, $K_{p,\mathrm{I}}$ will differ from $K_{p,\mathrm{II}}$, unless $E_{s,\mathrm{I}} = E_{s,\mathrm{II}}$.

A second modification in the derivation concerns the membrane potential. The driving force for carrier-mediated ion transport or ion diffusion is the membrane potential across the cell membrane E_m. Only when the surface potentials are zero, $E_m = E$, which we applied tacitly in the derivation of the rate equations. However, when the surface potentials are non-zero E should be replaced by E_m, which is related to E by

$$E_m = E - E_{s,\mathrm{I}} + E_{s,\mathrm{II}} \tag{A-3}$$

Also y_I in Eqn. 1 should be replaced by $y_m = \exp(-FE_m/2RT)$ and y_{II} by $1/y_m$. From Eqns. A-1 and A-3 it follows that y_m is related to y_I by

$$y_m = (x_{II}/x_I)^{1/2} y_I = \alpha y_I \tag{A-4}$$

On accounting for the effects of the surface potentials in Table I Eqns. T-4 and T-5 become

$$K_{m,j} = K_{j,I}\left(1 + \sum_{p \neq j} s_{p,I}/K_{p,I}\right) \tag{A-5}$$

$$V_{m,p,Z} = K_{j,Z} V_{0,p} \alpha^g / K_{p,Z}\left(1 + \sum s_{q,II}/K_{q,II}\right) \tag{A-6}$$

In order to account for the differences in ion concentrations between near membrane region and bulk aqueous phase concentrations and the difference between y_I and y_m, D_c and $U_{e,Z}$ in Eqns. T-6 and T-7 should be multiplied by $x_I \cdot \alpha$ and D_a should be divided by $x_I \cdot \alpha$.

The transport model dealt with until now consists of a carrier with two opposite independent sites, which can bind both cations from the medium and from the cells at the same time. In a similar way one may derive the rate equations for the case that the carrier shows only affinity to the cations when both sites are unoccupied, which means that only carrier cation complexes of the form of $CS_{p,Z}$ may appear and that $CS_{p,I}S_{q,II}$ cannot exist. Then $K_{m,j}$ and $V_{m,j}$ are given by

$$K_{m,j} = K_{j,I}\left(1 + \sum_{p \neq j} s_{p,I}/K_{p,I} + \sum s_{p,II}/K_{p,II}\right) \tag{A-7}$$

$$V_{m,p,Z} = K_{j,Z} V_{0,p} \alpha^g / K_{p,Z} \tag{A-8}$$

List of symbols

Z,	index for the medium side of the cell membrane (Z = I) or the cell side (Z = II);
p,q,j,	indices indicating the substrate species for carrier-mediated transport, respectively;
h,	symbol for proton;
c,a,	indices indicating the cation and anion species for diffusion across the cell membrane, respectively;
e,	index for a cation translocated via an electrogenic pump;
g,	1 for monovalent cation uniport and 2 for divalent cation uptake or monovalent cation-proton cotransport;
S_p,	substrate species;
C,	unloaded carrier;
$CS_{p,Z}$,	carrier loaded with S_p at side Z of the cell membrane;
$CS_{p,I}S_{q,II}$	carrier loaded with S_p at the medium side and with S_q at the cell side of the cell membrane;
C_T,	total concentration of carrier;
K_p,	dissociation constant of $CS_{p,Z}$ at zero surface potential;
$K_{p,Z}$,	apparent dissociation constant at non-zero surface potential;
$r_{p,Z}$,	rate constant of transfer of substrate across the cell membrane;
$r_{0,p}$,	rate constant at zero membrane potential;
D_c, D_a,	diffusion constants for S_c and S_a, respectively;
$U_{e,Z}$,	coefficient in the rate equation of the electrogenic cation pump;
$V_{0,p}$,	expression for $r_{0,p}C_T$;
E,	membrane potential between cell and medium;
E_m,	membrane potential between near membrane region at cell side and medium side;
E_0,	E at $s_{j,I} = 0$;
$E_\sim$,	E at saturating concentrations of $s_{j,I}$;
$E_{s,I}$,	surface potential between membrane and medium;
$E_{s,II}$,	surface potential between membrane and cytosol;
y_I,	$\exp(-FE/2RT)$;
y_{II},	$1/y_I$;
y_m,	$\exp(-FE_m/2RT)$;
y_0,	y_I at $s_{j,I} = 0$;
$y_\sim$,	y_I at saturating concentrations of $s_{j,I}$;
x_Z,	$\exp(-FE_{s,Z}/RT)$;
α,	$(x_{II}/x_I)^{1/2}$;
q,	$1-(y_I/y_0)^n$, n may be 1, 2 or 3;
q_{max},	maximum value of q;
K_m,	apparent K_m for cation uptake;
V_m,	corresponding maximum rate of uptake;
$K_{m,j}$,	K_m for uptake of S_j expected when the membrane potential is not changed on increasing $s_{j,I}$;
$L_{m,j}$, $M_{m,j}$ and $Q_{m,j}$,	factors determining y_I;
Dep,	depolarizing factors via diffusion or reversal of the cation pump;
Hyp,	hyperpolarizing factors via diffusion or cation pump;
Vs_I,	factor depending upon carrier-mediated inward charge flux besides that by means of S_j;
Vs_{II},	factor depending upon carrier-mediated outward charge flux.

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