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Effects of membrane potential and surface potential on the kinetics of solute transport

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A theoretical study has been made of the influence of the transmembrane potential difference and the surface potential of living cells on the kinetics of carried-mediated solute transport. It is assumed that the form of the free energy barrier within the membrane may be approximated by one dominant symmetrical peak, and that the electrical field is constant. Both single-ion transport kinetics and cotransport of an ion with a neutral solute are dealt with. Provided that the surface potential and the transmembrane potential are constant, the concentration dependence of the uptake rate is given by the Michaelis-Menten equation. The kinetic parameters, the maximal rate of uptake and the K_m, depend on both the surface potential and the membrane potential in a rather complex way. It is shown that the intuitive notion, that the maximal rate of cation uptake will increase when the cell membrane is hyperpolarized, is wrong in its generallity. Both an increase or a decrease may occur, depending on the characteristics of the transport system involved. If the magnitude of the membrane potential and the surface potential depends on the substrate concentration, marked deviations from Michaelis-Menten kinetics may come to the fore. This may result in either apparent positive or apparent negative homotrope cooperative effects. Enhancement of the uptake rate of the substrate ion may occur on adding another cation, despite the fact that the membrane will become depolarized. The same type of complex transport kinetics as found for Rb^+ and Na^+ uptake in yeast cells can be simulated by using a single-site transport model and including effects of the membrane potential.

Introduction

The effect of the membrane potential on the rate of ion translocation may be very great. This is especially true for mobile carriers and apparent mobile carrier systems. These are translocation systems in which the binding sites are not fixed at either the medium side or the cytoplasmic side of the cell membrane, but can 'move' from one side to the other. In such a case the distribution of the sites between the medium interface and the cytoplasmic interface is not constant but depends on e.g. the intracellular concentrations of solutes having affinity to the carrier and on the membrane potential. As a consequence, kinetic transport parameters such as the maximal rate V and the Michaelis constant K_m are not really constants, but depend on both the internal solute concentrations [1] and the membrane potential.

Several authors have dealt with the effects of the membrane potential on the kinetical parameters. For example, Stein [2] gives expressions for the simple carrier. Geck and Heinz [3] analyzed the effect of the membrane potential on the maxi-

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mal velocity and the Michaelis constant for the cotransport of a neutral solute with a cation, and studied in more detail the differences in the effects of the osmotic part and the electrical part of the driving force for some reduced models. Kotyk [4] also discusses qualitatively the behaviour of V and K_m upon changes in ion concentration and membrane potential for different types of cotransport carrier including the 'steady-state carrier', which for reasons of simplicity is not dealt with here. A fairly elaborated treatment is given by Sanders et al. [5] on basis of a generalized kinetic analysis.

In this paper, we shall discuss some important features of the way in which the electrical potentials interfere with the translocation rate, that have not been dealt with till now. These are the effect of the membrane potential on the relation between uptake rate and solute concentration, in case the substrate itself influences the membrane potential, and the effect of the surface potential on the kinetical parameters.

If the membrane potential changes on increasing the substrate concentration, V and K_m will also change, and we shall show that this may lead to deviations from Michaelis-Menten kinetics. In such a case apparent multi-site transport kinetics may come to the fore even though the substrate binds to only one site on the carrier. The situation becomes still more complicated when effects of the surface potential get involved. The negative surface potential of biological membranes may affect transport kinetics in two ways. In the first place the concentrations of ions near the membrane will differ from those in the bulk solution giving rise to an increase in K_m for anion uptake and a decrease in K_m for cation uptake [6]. Secondly, variations in the surface potential will introduce concurrent changes in the electrical field inside the membrane.

In the next sections equations will be derived which account for these effects. We shall consider translocation of a solute both via a single-site transporter and via a co-transport system in which the substrate is transferred together with an ion like Na^+ or H^+. In addition, some examples will be given of how the relation between the initial uptake rate and the substrate concentration may be influenced by the membrane potential for systems where this potential depends on the concentration of the substrates in the medium.

Theory

In deriving the transport equations we will use the general rate equations as given by one of us for mobile-carrier mediated transport [7]. Two cases will be dealt with, namely translocation of a substrate from the medium (side I) into the cytoplasm (side II) via a single-site carrier and cotransport of a substrate together with an ion via a two-site carrier. In the latter case one site binds only the substrate and the other site binds only the co-ion. In both cases the transport rate $v_{i,I}$ of a substrate i with concentrations $s_{i,I}$ is decribed by the simple Michaelis-Menten equation (Eqn. 1) as can be derived from Eqn. 26 in Ref. 7.

$$v_{i,I} = \frac{V s_{i,I}}{K_m + s_{i,I}} \tag{1}$$

where V and K_m are functions of the rate and binding constants.

The field E inside the membrane arises from the potential difference between the two membrane interfaces $\Delta\psi_m$, which in turn depends on the surface potentials $\psi_{0,I}$ and $\psi_{0,II}$ and on the potential difference $\Delta\psi_b$ between the two bulk phases (Fig. 1). We shall examine first how V and K_m depend on $\Delta\psi_m$ with constant surface potentials (in which case changes in $\Delta\psi_m$ equal those in $\Delta\psi_b$) and next how V and K_m behave as a function of the surface potentials at constant $\Delta\psi_b$, (thus when changes in $\Delta\psi_m$ are caused solely by variations of the surface potentials).

Effects of the membrane potential

A change in the membrane potential $\Delta\psi_m$ will cause a change in the electrical field E and thus in the initial uptake rate of the substrate. The way in which the field interferes with the translocation rate depends on the transport mechanism. It will be assumed that the electric field does not affect the binding of solutes to the transport sites, but only the translocation of the sites from one side of the membrane to the other. We shall deal with (apparent) mobile carriers or 'moving-site' systems. Thus not only the classical mobile carrier [1] which traverses the membrane like a ferry boat is an example of the systems that we shall consider, but also the membrane-spanning protein which

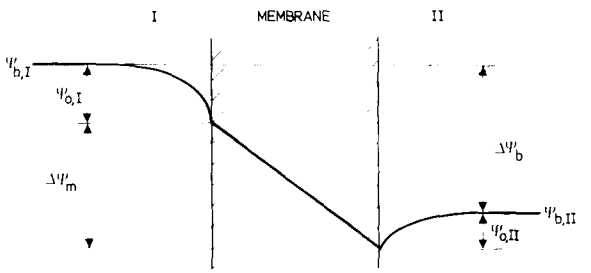

Fig. 1. Course of the electrical potential in the near membrane regions and inside the membrane. $\Delta\psi_m$ is the potential difference between the two membrane interfaces which causes the electrical field inside the membrane. $\psi_{0,I}$ and $\psi_{0,II}$ are the potential differences between the membrane surface and the bulk phase on side I and side II, respectively. $\Delta\psi_b$ is the potential difference between the two bulk phases.

can undergo one or more conformational changes, with the net effect that the orientation of the binding sites shifts from outside to inside or reversely.

In case of transport mediated by a mobile carrier, the field inside the membrane will not only affect the translocation rates of the electrically charged carrier forms, but will also indirectly affect those of the neutral forms, because the steady-state distribution of the carrier between the two membrane interfaces is influenced by E, too.

In systems, in which the transporting moiety does not actually move across the membrane, but in which the orientation of the binding sites shifts from one interface to the other by conformational changes of a membrane-spanning macromolecule, there exists an analogous situation. Proteins with their numerous charges have an electrical dipole moment μ, with a magnitude which depends on the conformational state of the macromolecule [8–10]. Since the free energy of a certain state depends on the magnitudes and directions of both μ and E, the electrical field will influence the transition rates and the steady-state distribution between the several conformational states. Thus translocation of a substrate and distribution of the transport sites between the membrane interfaces may be expected to be influenced by E in a way similar to the mobile carrier.

In both types of systems a free energy barrier must be passed via one or more steps in order to transpose a binding site from one interface to the other. If the form of the free energy barrier is approximated by one dominant symmetrical peak and if the electrical field is constant inside the membrane, the rate constant of the transposition from interface I (medium) to interface II (cytoplasm) of binding sites loaded with substrate i equals [11]:

$$r_{i,I} = r^0_{i,I} \exp(-n_i \Delta\psi_m/2) \tag{2}$$

and for the reverse direction:

$$r_{i,II} = r^0_{i,II} \exp(+n_i \Delta\psi_m/2) \tag{3}$$

where $r^0_{i,I}$ and $r^0_{i,II}$ are the rate constants in the absence of an electric field and $\Delta\psi_m$ is the potential difference between the two membrane interfaces. The corresponding expressions for the unloaded carrier are obtained by replacing in Eqns. 2 and 3 the subscript i by 0.

The meaning of n in Eqns. 2 and 3 depends on the transport mechanism. For the mobile carrier n equals the valency of the carrier-substrate complex. Thus if the valency of the unloaded carrier equals ν_0 and that of the substrate i equals ν_i then $n_i = \nu_0 + \nu_i$ for the loaded and $n_0 = \nu_0$ for the unloaded carrier. If transport is mediated by a membrane bound protein, n is a measure of the change in electrical dipole moment (parallel to the field E), associated with the conformational change. If the dipole moment of the protein with its site occupied by the substrate i equals $\mu_{i,I}$ when the substrate is bound at interface I, and $\mu_{i,II}$ if it is at interface II, the dependence of the rate constants on the membrane potential is given by [8–10]:

$$r_{i,I} = r^0_{i,I} \exp[-(\mu_{i,I} - \mu_{i,II})\Delta\psi_m/(dkT)] \tag{4}$$

and

$$r_{i,II} = r^0_{i,II} \exp[+(\mu_{i,I} - \mu_{i,II})\Delta\psi_m/(dkT)] \tag{5}$$

where $\mu_{i,I}$ and $\mu_{i,II}$ are the components of the dipole moments perpendicular to the membrane, d is the thickness of the membrane, k is the Boltzmann constant and T is the absolute temperature in K. The rate constants of the unloaded carrier are obtained by replacing in Eqns. 4 and 5 the subscript i by 0. Expressing the dipole moments in

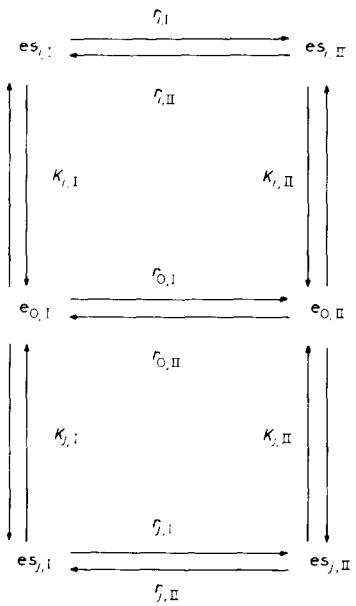

Fig. 2. Scheme of single-site carrier transport. I and II refer to the medium side and cytoplasmic side of the cell membrane, respectively. $s_{i,z}$ and $s_{j,z}$ (z = I or II) are solutes having affinity to the carrier site. $e_{0,z}$ is the empty carrier, and $(es_i)_z$ and $(es_j)_z$ are complexes formed by the carrier and $s_{i,z}$ and $s_{j,z}$, respectively. The dissociation constants of these complexes are $K_{i,z}$ and $K_{j,z}$. The rate constants for the movement of the carrier and the carrier-substrate complexes are $r_{0,I}$, $r_{i,I}$ and $r_{j,I}$ for the movement from side I to side II and $r_{0,II}$, $r_{i,II}$ and $r_{j,II}$ for the movement in the opposite direction.

TABLE I

EXPLICIT EXPRESSIONS FOR THE DIMENSIONLESS PARAMETERS IN EQNS. 6, 7, 13 AND 14 FOR SINGLE-SITE AND COTRANSPORT MODELS

See also Figs. 2 and 3. It should be noted that the meaning of the r^0 values in this table depends on the equation in which they are inserted. For Eqns. 6 and 7 they represent rate constants in the absence of a membrane potential; for Eqns. 13 and 14 they represent rate constants at a vanishing surface potential. e_t, total concentration of carrier e.

Parameter	Single-site transport	Cotransport
V^*	$\frac{K_{iq,I}^{-1}V}{r_{i,I}^0 e_t}$	$\frac{\left(K_{i0,I}^{-1}+K_{iq,I}^{-1}s_{q,I}\right)V}{r_{iq,I}^0 K_{iq,I}^{-1}s_{q,I}e_t}$
K_m^*	$K_{i,I}^{-1}K_m$	$\frac{\left(K_{i0,I}^{-1}+K_{iq,I}^{-1}s_{q,I}\right)K_m}{\left(1+K_{0q,I}^{-1}s_{q,I}\right)}$
$r_{0,z}^*$	$\frac{r_{0,z}^0}{r_{i,I}^0}$	$\frac{\left(K_{i0,I}^{-1}+K_{iq,I}^{-1}s_{q,I}\right)r_{0,z}^0}{\left(1+K_{0q,z}^{-1}s_{q,z}\right)r_{iq,I}^0 K_{iq,I}^{-1}s_{q,I}}$
$r_{k,z}^*$	$\frac{r_{k,z}^0}{r_{i,I}^0}$	$\frac{\left(K_{i0,I}^{-1}+K_{iq,I}^{-1}s_{q,I}\right)r_{kq,z}^0 K_{kq,z}^{-1}s_{q,z}}{\left(K_{k0,z}^{-1}+K_{kq,z}^{-1}s_{q,z}\right)r_{iq,I}^0 K_{iq,I}^{-1}s_{q,I}}$
$s_{k,z}^*$	$K_{k,z}^{-1}s_{k,z}$	$\frac{\left(K_{k0,z}^{-1}+K_{kq,z}^{-1}s_{q,z}\right)s_{k,z}}{1+K_{0q,z}^{-1}s_{q,z}}$

Debye units (the dipole moment of a proton and an electron separated by a distance of 10 Å equals 48 Debye (D)) and d in Å, comparison of Eqns. 4 and 5 with Eqns. 2 and 3 yields $n_i = (\mu_{i,I} - \mu_{i,II})/(2.4\ d)$ when the transport site is occupied and $n_0 = (\mu_{0,I} - \mu_{0,II})/(2.4\ d)$ when it is empty.

The rate equations for the uptake of a substrate i via a single-site transporter, in the presence of a competitive solute j and of intracellular solutes having affinity to the carrier (Fig. 2), are obtained by inserting Eqns. 2 and 3 and the corresponding equations for $r_{0,I}$ and $r_{0,II}$ into the expressions which have been derived for carrier-mediated transport (Eqn. 26 in Ref. 7). This rate equation which accounts for the effect of the membrane potential on the transport still has the Michaelis-Menten form. The maximal uptake rate V and the Michaelis constant K_m are given in Eqns. 6 and 7. The kinetic parameters are expressed as dimensionless quantities denoted by an asterisk (*). The definitions of these quantities are given in Table I. $s_{k,II}$ represents the ion concentration inside the cell.

$$V^* = \frac{\left(r_{0,II}^* y^{-1/2n_0} + r_{k,II}^* s_{k,II}^* y^{-1/2n_k}\right) y^{1/2n_i}}{\left(r_{0,II}^* y^{-1/2n_0} + r_{k,II}^* s_{k,II}^* y^{-1/2n_k}\right) + \left(1 + s_{k,II}^*\right) y^{1/2n_i}} \quad (6)$$

and

$$K_m^* = \left[\left(r_{0,I}^* y^{1/2n_0} + r_{j,I}^* s_{j,I}^* y^{1/2n_j}\right)\left(1 + s_{k,II}^*\right) + \left(r_{0,II}^* y^{-1/2n_0} + r_{k,II}^* s_{k,II}^* y^{-1/2n_k}\right)\left(1 + s_{j,I}^*\right)\right] / \left[\left(r_{0,II}^* y^{-1/2n_0} + r_{k,II}^* s_{k,II}^* y^{-1/2n_k}\right) + \left(1 + s_{k,II}^*\right) y^{1/2n_i}\right] \quad (7)$$

with

$$y = \exp(-F\Delta\psi_m/RT) \quad (8)$$

Similar expressions are obtained for the 1-1

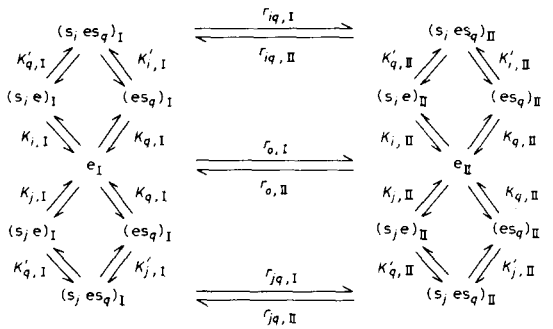

Fig. 3. Scheme for 1-1 cotransport. $s_{i,z}$ and $s_{j,z}$ are solutes competing for the same substrate site, $s_{q,z}$ is the co-solute having affinity to the other site. e_z is the empty carrier. $(s_i e)_z$ and $(s_j e)_z$ are the complexes formed between e_z and $s_{i,z}$, and between e_z and $s_{j,z}$, respectively. The dissociation constants of these complexes are $K_{i,z}$ and $K_{j,z}$. Likewise, $(es_q)_z$ is the complex formed between e_z and $s_{q,z}$. The complex $(s_i es_q)_z$ is formed when $s_{i,z}$ binds to $(es_q)_z$ (dissociation constant $K'_{i,z}$) or when $s_{q,z}$ binds to $(s_i e)_z$ (dissociation constant $K'_{q,z}$). The constant $K_{iq,z}$ is defined as:

$$K_{iq,z} = e_z s_{i,z} s_{q,z} / (s_i es_q)_z = K_{q,z} K'_{i,z} = K_{i,z} K'_{q,z}$$

$K_{jq,z}$ is defined in an analogous way. The r values with appropriate indexes are the rate constants for the movements of the carrier complexes across the membrane.

cotransport system. In this case the translocator has two binding sites: one site for the substrate i or a competitive solute j and one for the co-ion q (Fig. 3). As the substrate i binds to one site only, the expression for $v_{i,I}$ still has the Michaelis-Menten form. If translocation of the carrier occurs only when both sites are occupied or both are unoccupied, the dependence of V and K_m on the membrane potential has the same form as in the single-site case. The difference with the single-site translocator is, that the dimensionless rate constants and concentrations in Eqns. 6 and 7 become dependend on the co-solute concentration s_q. The meaning of the dimensionless quantities for the cotransport case are also given in Table I. In addition to V and K_m we have also calculated the behaviour of a third parameter $F = V/K_m$. This parameter is useful in experimental analysis, since it equals the ratio $v_{i,I}/s_{i,I}$ for very low concentrations $s_{i,I}$ (for example carrier-free concentrations in case of the uptake of labelled compounds). In Fig. 4 the dependence of V^*, K_m^* and F^* on $\Delta\psi_m$ has been plotted for a number of reduced cases:

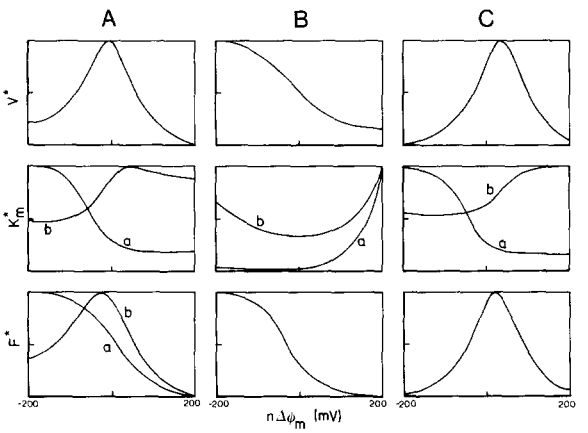

Fig. 4. The dimensionless parameters V^*, K_m^* and $F^* = V^*/K_m^*$ as a function of the membrane potential $\Delta\psi_m$ according to Eqns. 6–8. Vertical scaling is arbitrary. The curves have been calculated for the next values of the model parameters: $r^0_{0,I} = r^0_{0,II}$; $r^0_{p,I} K_{p,I} = r^0_{p,II} K_{p,II}$ ($p = i$ or j); the internal cation, having affinity to the carrier is $s^*_{i,II} = 1$ and further:

Model	Parameter	Curve	$s^*_{j,I}$	$s^*_{j,II}$	$r^*_{0,I}$	$r^*_{j,I}$
A	V^*		–	10	1	1
	K_m^*	a	1	10	1	10
($n_i \neq 0$,	K_m^*	b	1	1	1	1
$n_0 = 0$)	F^*	a	0.1	10	1	1
	F^*	b	1	10	1	1
B	V^*		–	10	10	0.1
	K_m^*	a	1	10	1	0.1
($n_i = 0$,	K_m^*	b	10	10	0.1	0.1
$n_0 \neq 0$)	F^*		10	10	0.1	0.1
C	V^*		–	10	1	0.1
	K_m^*	a	10	10	10	0
($n_i = -n_0$	K_m^*	b	1	1	0.1	1
$\neq 0$)	F^*		1	1	0.1	1

(A) $n_i \neq 0$ and $n_0 = 0$ (effect of $\Delta\psi_m$ only on loaded carrier, no effect on unloaded carrier).

(B) $n_i = 0$ and $n_0 \neq 0$ (effect of $\Delta\psi_m$ on unloaded carrier, no effect on loaded carrier).

(C) $n_i = -n_0 \neq 0$ ($\Delta\psi_m$ has opposite effects on loaded and unloaded carrier).

It appears that V^*, K_m^* and F^* may be increasing, decreasing or have a minimum or a maximum as a function of the membrane potential. Although the curves have been calculated for some specific values of the rate constants, dissociation constants and solute concentrations it can be shown that the curves in Fig. 4 represent all possi-

ble forms (as far as it concerns increase, decrease and occurrence of maxima or minima) that may occur for each reduced case [12].

Effects of the surface potential

As was mentioned in the introduction, the surface potential has two kinds of effects: one on the concentration of ions near the membrane, and one on the magnitude of the potential difference between the two membrane interfaces. Since we are interested in the translocation of solutes from the medium into the cytoplasm we consider the effect of the surface potential on the medium side ($\psi_{0,I}$) only. Due to the presence of a negative surface potential, the concentration $s^0_{i,I}$ of an ion i near membrane interface I will differ from the concentrations in the bulk solutions $s_{i,I}$. Hence a change in $\psi_{0,I}$ will be accompanied by a change in the concentration $s_{i,I}$ near the transport sites. Neglecting differences in activity coefficients of the ions in the bulk phase with those in the near membrane region, we have according the Boltzman equation:

$$s^0_{i,I} = s_{i,I} \exp(-\nu_i F\psi_{0,I}/RT) \tag{9}$$

As the uptake rate is determined in the first instance by $s^0_{i,I}$, all ion concentrations occurring in the rate equations must be replaced by Eqn. 9 in order to express $v_{i,I}$ as a function of the bulk concentrations $s_{i,I}$.

The second effect of the surface potential is that a change in $\psi_{0,I}$ will also introduce a change in the potential difference $\Delta\psi_b$ between the two membrane interfaces (see Fig. 1):

$$\Delta\psi_m = \Delta\psi_b + \psi_{0,II} - \psi_{0,I} \tag{10}$$

When the potential difference between the two bulk phases ($\Delta\psi_b$) and $\psi_{0,II}$ do not change on varying $\psi_{0,I}$, the dependence of the rate constants on the surface potential becomes:

$$r_{i,I} = r^0_{i,I} \exp(+n_i\psi_{0,I}/2RT) \tag{11}$$

and

$$r_{i,II} = r^0_{i,II} \exp(-n_i\psi_{0,I}/2RT) \tag{12}$$

where $r^0_{i,I}$ and $r^0_{i,II}$ are the rate constants in the absence of a surface potential. The corresponding equations for the unloaded carrier are obtained by replacing the subscript i by 0.

The meaning of n_i depends again on the translocation mechanism. For a mobile carrier n_i equals the valency of the carrier-substrate complex and for a membrane-spanning protein n_i is a measure of the change in dipole moment. But note that in Eqn. 9 the near membrane concentration always depends on the valency of the ion. Inserting Eqn. 11 and 12 and the corresponding equations for $r_{0,I}$ and $r_{0,II}$ into the rate equations one obtains expressions for the kinetic parameters as a function of the surface potential. For transport of an ion with valency ν via a single-site mobile carrier with valency ν_0 (thus taking $n_i = \nu_0 + \nu$ and $n_0 = \nu_0$) we obtain in dimensionless form:

$$V^* = \frac{\left(r^*_{0,II} + r^*_{k,II}s^*_{k,II}y_0^{1/2\nu}\right)}{\left(r^*_{0,II} + r^*_{k,II}s^*_{k,II}y_0^{1/2\nu}\right)y_0^{1/2(\nu+\nu_0)} + (1 + s^*_{k,II})\,y_0^{-1/2\nu_0}} \tag{13}$$

and

$$K^*_m = \left[\left(r^*_{0,II} + r^*_{k,II}s^*_{k,II}y_0^{1/2\nu}\right)\left(1 + s^*_{j,I}y_0^{\nu}\right)y_0^{\nu_0} + \left(r^*_{0,I} + r^*_{j,I}s^*_{j,I}y_0^{1/2\nu}\right)(1 + s^*_{k,II})\right] \Big/ \left[\left(r^*_{0,II} + r^*_{k,II}s^*_{k,II}y_0^{1/2\nu}\right)y_0^{\nu_0} + (1 + s^*_{k,II})\,y_0^{-1/2\nu}\right] \tag{14}$$

where

$$y_0 = \exp(-F\psi_{0,I}/RT) \tag{15}$$

V^*, K^*_m, the concentration s^* and the rate constants r^* still have the same meaning as given in Table I. It should be noted, however, that the r^0 values now apply to the case where $\psi_{0,I}$ and not $\Delta\psi_b$ vanishes. Fig. 5 shows how V, K_m and F behave as a function of $\psi_{0,I}$ in two cases:

(A) The empty carrier bears no net charge ($\nu_0 = 0$, $\nu \neq 0$).

(B) The carrier substrate complex bears no net charge ($\nu_0 = \nu \neq 0$).

Again V and K_m may be increasing, decreasing or have an extremum.

The Eqns. 13 and 14 do in general not apply directly to the cotransport system, since the normalized quantities V^*, K^*_m, r^* and s^* still depend

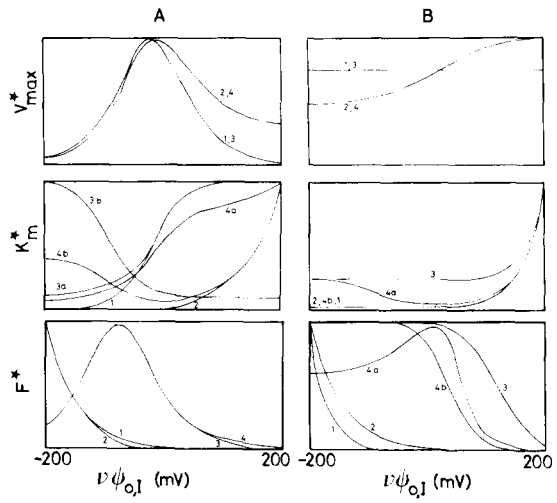

Fig. 5. The dimensionless parameters V^*, K^*_m and F^* plotted vs. $\psi_{0,I}$ according to Eqns. 13–15, for the uptake of $s_{i,I}$ in the presence of a competitive solute $s_{j,I}$. Vertical scaling is arbitrary. For each of the reduced models A and B, there are four subcases (1) $r^*_{0,I} = 0$, $s^*_{j,I} = 0$; (2) $r^*_{0,I} \neq 0$, $s^*_{j,I} = 0$; (3) $r^*_{0,I} = 0$, $s^*_{j,I} \neq 0$; (4) $r^*_{0,I} \neq 0$, $s^*_{j,I} \neq 0$. The values of the model parameters are: for all (sub)cases $\Delta\psi_b = -50$ mV, $\psi_{0,II} = 0$ mV, $r^0_{0,I} = r^0_{0,II}$, $r^0_{p,I}K_{p,I} = r^0_{p,II}K_{p,II}$ ($p = i$ or j), $s^*_{i,II} = 1$. The values of the parameters $r^*_{0,I}$, $r^*_{j,I}$, $s^*_{j,I}$ and $s^*_{j,II}$ are for each subcase, respectively:

Model	Curve	$r^*_{0,I}$	$r^*_{j,I}$	$s^*_{j,I}$	$s^*_{j,II}$
A	1	0	10	0	1
	2	1	10	0	1
	3	0	10	10	1
	3a	0	10	10	1
	3b	0	0.1	10	10
	4	0.1	10	1	1
	4a	0.1	10	1	1
	4b	0.1	10	1	10
B	1	0	10	0	0.1
	2	1	10	0	0.1
	3	0	10	10	0.1
	4	1	10	1	0.1
	4a	1	10	1	0.1
	4b (K^*_m)	1	10	10	0.1
	4b (F^*)	1	10	1	1

on the near-membrane concentration of the co-ion q, which in turn depends on $\psi_{0,I}$. Eqns. 13 and 14 and Fig. 4 can only be applied directly to the case of the uptake of a co-ion i, which is cotransported along with a neutral solute q. However, the V and K_m of the uptake kinetics of a substrate which is transported in the presence of a co-ion will in general be quite different from that shown in Fig. 4, depending on the valencies of the substrate and the co-ions, and on their relative affinities to the transport sites [12].

The v-s relation

Although V and K_m may depend on the membrane potential and the surface potential the relation between the uptake rate and the solute concentration remains of the simple Michaelis-Menten type as long as $\Delta\psi_m$ and $\psi_{0,I}$ remain constant. However, it is well known that many substances cause a concentration-dependent change in the membrane potential or the surface potential or both. Consequently, V and K_m may change on increasing the substrate concentrations; this in turn will result in deviations from the Michaelis-Menten relation. In addition, a competitive solute may have – apart from its inhibitory action due to competition for the same carrier site – and extra stimulatory or inhibitory effect due to changes in the electric potentials. Therefore, the effects of a competitive solute may be quite different from what would be expected on basis of the classical kinetical schemes as will be shown below. The same type of effects may of course be expected for the co-ions in a cotransport system. Since V and K_m as a function of $\Delta\psi_m$ may be increasing, decreasing or have an extremum, virtually any kind of deviation from Michaelis-Menten kinetics may come to the fore.

By way of illustration, we consider the uptake of a monovalent cation i in the presence of a competitive monovalent cation j via a neutral mobile carrier. It is assumed, that the membrane potential depends on the concentrations $s_{i,I}$ and $s_{j,I}$ according to:

$$\Delta\psi_m = \Delta\psi_{m,0} - b \cdot \log(1 + a_i s^*_{i,I} + a_j s^*_{j,I}) \qquad (16)$$

For simplicity we shall not include additional effects of the surface potentials, and put $\psi_{0,I}$ and $\psi_{0,II}$ equal to zero. In Fig. 6 the results of some simulations are shown in the form of plots of $v_{i,I}$ vs. $v_{i,I}/s_{i,I}$ (Hofstee plots [13]). If V and K_m do not change as a function of the membrane potential the Hofstee plot yields a straight line. However, it is seen, that both apparently positive (the convex

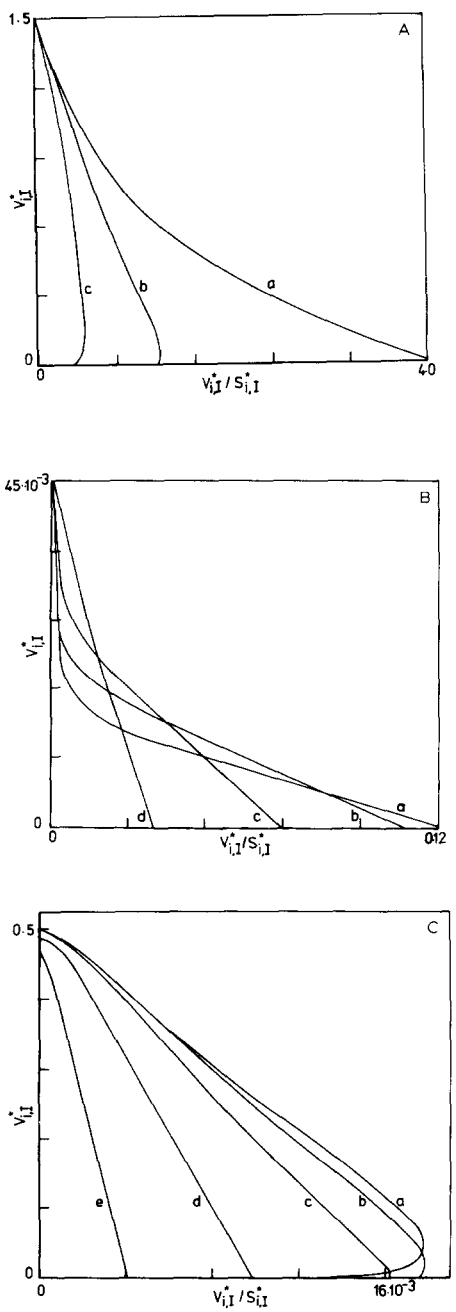

Fig. 6. Hofstee plots calculated for the uptake of a monovalent cation $s_{i,I}$ via a neutral single-site carrier at different concentrations of a competitive ion $s_{j,I}$ In Plots A and B a second competitive cation l is present at a fixed concentration $s_{l,I}$. The membrane potential depends on $s_{i,I}$ and $s_{j,I}$ according to Eqn. 16. The plots have been computed for the next parameter values: $\nu_0 = 0$, $\nu_i = \nu_j = \nu_l = 1$, $\Delta\psi_{m,0} = -150$ mV, the only internal cation having affinity to the carrier is $s_{j,II} = 10$, $r^0_{0,I} = r^0_{0,II}$, $r^0_{p,I}K_{p,I} = r^0_{p,II}K_{p,II}$ ($p = i, j, l$) and further:

curves) and negative (the concave curves) homotrope cooperative effects may come to the fore. In Fig. 6A, a convex plot changes into a concave one on increasing the concentration of the competitive ion j. In Fig. 6B the apparent double concave Hofstee plot changes into a straight one on increasing $s_{j,I}$. Moreover, the uptake of the substrate i is stimulated at the higher concentrations by the competitive ion j whereas it is inhibited at the lower concentrations. In Fig. 6C the competitive ion has a stimulating effect at low concentrations of the substrate and cause a disappearance of the convex deviations.

Discussion

There exists no simple relation between the membrane potential and the initial uptake rate in 'mobile-site' transport systems. In fact, an increase in the electrical part of the driving force may cause an increase or a decrease in the uptake rate. This is due to a redistribution of the binding sites in the membrane under the influence of the electrical field, which may result in a more or less depletion of the sites at one membrane interface. Thus the intuitive notion that for example a hyperpolarization should increase and a depolarization should decrease the initial rate of cation uptake is in its generality not correct. Although V and K_m may depend in a complex way on the membrane potential, the relation between the uptake rate and the substrate concentration remains hyperbolic as long as $\psi_{0,I}$ and $\Delta\psi_m$ do not change on varying the

Fig.	b (mV)	a_i	a_j	$r^*_{0,I}$	$r^*_{j,I}$	$r^*_{l,I}$	$s^*_{l,I}$	$s^*_{j,I}$	
6A	40	0.1	0.1	0	10	0	0	0.01	(a)
								0.04	(b)
								0.16	(c)
6B	50	1	0.1	0.1	0.1	0.1	1	0.01	(a)
								0.63	(b)
								2.5	(c)
								10.0	(d)
6C	50	0.1	1	1	1	10	1	0.1	(a)
								1.6	(b)
								6.3	(c)
								25	(d)
								100	(e)

substrate concentration. However if the magnitude of one of these potentials changes with the substrate concentration, V and K_m are no constants any more, but become dependent on $s_{i,1}$ too, due to their dependencies on $\Delta\psi_m$ or $\psi_{0,1}$. As a consequence, the v-s relation will deviate from the Michaelis-Menten scheme and may show for example apparent homotrope cooperative effects. In an analogous way the dependence of the v-s relation on competitive solutes or co-solutes may be more complex than what would be expected on basis of the simple single-site or cotransport model. For example, a competitive solute may have, apart from its inhibitory effect, an additional inhibitory or stimulating effect on the rate of substrate uptake.

In our approach of the transport kinetics, we have made the simplifying assumption that the translocation step is rate limiting, so that the binding of the ligands at the membrane interfaces may be treated as equilibrium reactions. This assertion may be too restrictive for a number of transport systems as discussed by Sanders et al. [5]. These authors and also Kotyk [4] use the more general 'steady-state carrier'. Sanders et al. find that with this type of model very diverse kinetics may come to the fore. They argue that their model obviates the need for refinements, such as making additional assumptions about the surface potential. Although this may be true from a purely descriptive point of view, cell membranes do bear negative charges, which do have a great influence on the transport kinetics; thus effects of the surface potential should be taken into account in many cases. According to our analysis, very complex kinetics may also occur in a simple 'equilibrium-binding model', due to effects of the ligands on the membrane potential and the surface potential.

We have applied our analysis to the kinetically simple 'mobile-site' transport system in which the binding sites are distributed between the cytoplasmic side and the medium side of the cell membrane, and in which the distribution is governed by the solute concentrations on both sides and by the membrane potential. This is in contrast to for example 'fixed-site' transport systems like the tetramer model of Lieb and Stein [14] where the number of transport sites available at the medium side of the membrane remains constant, independent of the internal solute concentrations [15]. As was argued under Theory the 'mobile-site' mechanism comprises not only the mobile carrier, but also those systems where translocation occur via a membrane spanning protein. As long as in the latter system the total number of binding sites is constant and as long as the binding sites of each transporting moiety are exposed to either of the two membrane interfaces, they are kinetically equivalent to the mobile carrier system [16]. In addition, in both cases the effect of the membrane potential on the kinetics may be described by multiplying the translocation rate constants with a Boltzmann factor (see Eqns. 2–5).

The extent to which the initial uptake rate varies with $\Delta\psi_m$ and the degree of deviation from the Michaelis-Menten kinetics depend greatly on the value of n in Eqns. 2 and 3. The Hofstee plots in Fig. 6 have been calculated for the uptake of monovalent cations via a neutral mobile carrier, thus for $n = 1$. The question remains of course how representative this value is for transport via membrane bound proteins. If one assumes that the membranes is 50 Å thick, than a change of 120 D in the dipole moment corresponds to a unity value for n. As the dipole moments of proteins range from several hundreds to several thousands Debye units a change in dipole moment of several hundreds D seems not unreasonable. Tsuji and Neumann [17] have shown that an electrical field pulse of $15 \cdot 10^5$ V/m causes a conformational change in bacteriorhodopsine molecules, associated with a change in electrical dipole moment of the order of magnitude of 30000 D. They ascribe the conformational change and the induction of the dipole moment to a slight transversal displacement of helical parts of the molecule caused by the electric field. In addition, by comparing his model for the gating currents in axon membranes, Schwarz [8] calculates a change in dipole moment of 400 D for a membrane bound protein. Hence the effects shown in Fig. 6 may be expected for transport via membrane bound proteins as well.

Experimentally, deviations from Michaelis-Menten kinetics have been found in a diversity of systems like fungi [18], higher plant cells [19], and animal cells [20]. Generally the uptake is then supposed to be mediated by a mechanism with two binding sites, or by two single-site transport sys-

tems operating simultaneously in parallel [21]. However, as we have shown here, one single-site system may account for these phenomena as well. For example the complex kinetics of monovalent cation uptake in yeast have been ascribed to the binding of the ions to two or more sites of a system in which the total number of sites at the medium side of the membrane remains constant ('multi-site immobile carrier' mechanism) [22]. In such a system, a depolarization or a hyperpolarization would only affect the maximal uptake rate but not the apparent affinity constants. However, if the yeast cell membrane is depolarized by the addition of the proton ionophore 2,4-dinitrophenol or by adding phosphate, which is transported with two or more protons [23,24], not only the maximal uptake rate of Rb^+ is changed but also the apparent affinity constants [25]. Hence it is very well possible that Rb^+ is taken up via a mobile site transporter. In addition, the membrane potential of the yeast cell depends on the concentration of cations in the medium added in the same concentration range as applied in the transport studies [26,27]. Thus the apparently positive and negative homotrope cooperativity that have been found for the Na^+ and Rb^+ uptake, respectively, might be caused by effects via the membrane potential. In fact, a similar type of kinetics as found experimentally [28] can be simulated with our present single-site model (Figs. 6B and 6C).

In cotransport systems, the transport of one solute (amino acid, sugar, inorganic anion or cation) is assumed to be driven by the electrochemical gradient of another solute (mainly protons in plant cells and sodium ions in animal cells [29–31]. Although the membrane potential thus plays a fundamental role in these systems, its possible effects have not been taken into account in many studies of cotransport kinetics. Solute translocation via cotransport is accompanied by a concentration dependent depolarization [32–34]. Thus it is very well possible, that for example deviations from Michaelis-Menten kinetics which have been found in a number of cases may be brought about by effects via the membrane potential.

References

1 Wilbrandt, W. and Rosenberg, T. (1961) Pharmacol. Rev. 13, 109–183
2 Stein, W.D. (1977) Biochim. Biophys. Acta 467, 376–385
3 Geck, P. and Heinz, E. (1976) Biochim. Biophys. Acta 443, 49–63
4 Kotyk, A. (1983) J. Bioenerg. Biomembranes 15, 307–319
5 Sanders, D., Hansen, U.-P., Gradman, D. and Slayman, C.L. (1984) J. Membrane Biol. 77, 123–152
6 Theuvenet, A.P.R. and Borst-Pauwels, G.W.F.H. (1976) J. Theor. Biol. 57, 313–329
7 Borst-Pauwels, G.W.F. (1974) J. Theor. Biol. (1974) 48, 183–195
8 Schwarz, G. (1978) J. Membrane Biol. 43, 127–148
9 Neumann, E. (1983) Stud. Biophys. 94, 107–114
10 Stevens, C.F. (1978) Biophys. J. 22, 295–36
11 Eyring, H. and Eyring, E.M. (1963) Modern Chemical Kinetics, Reinhold, New York
12 Barts, P.W.J.A. (1981) Thesis, Nijmegen
13 Hofstee, B.H.J. (1952) Science 116, 239–231
14 Lieb, W.R. and Stein, W.D. (1970) Biophys. J. 10, 585–609
15 Kotyk, A. and Janacek, K. (1975) Cell Membrane Transport. Principles and Techniques, Plenum Press, New York
16 Heinz, E. (1978) Mechanics and Bioenergetics of Biological Transport, Springer, Berlin
17 Tsuji, K. and Neumann, E. (1983) Biophys. Chem. 17, 153–163
18 Slayman, C.W. and Slayman, C.L. (1970) J. Gen. Physiol. 55, 758–786
19 Mettler, I.J. and Leonard, R.T. (1979) Plant Physiol. 63, 191–194
20 Panet, R. and Atlan, H. (1980) J. Membrane Biol. 52, 273–280
21 Borst-Pauwels, G.W.F.H. (1976) J. Theor. Biol. 56, 191–204
22 Theuvenet, A.P.R., Roomans, G.M. and Borst-Pauwels, G.W.F.H. (1977) Biochim. Biophys. Acta 469, 272–280
23 Roomans, G.M. and Borst-Pauwels, G.W.F.H. (1977) Biochim. Biophys. Acta 470, 84–91
24 Cockburn, M., Earnshaw, P. and Eddy, A.A. (1975) Biochem. J. 146, 705–712
25 Borst-Pauwels, G.W.F.H., Wolters, G.H.J. and Henricks, J.J.G. (1971) Biochim. Biophys. Acta 225, 269–276
26 Barts, P.W.J.A., Hoeberichts, J.A., Klaasen, A. and Borst-Pauwels, G.W.F.H. (1980) Biochim. Biophys. Acta 597, 125–136
27 Boxman, A.W., Barts, P.W.J.A. and Borst-Pauwels, G.W.F.H. (1982) Biochim. Biophys. Acta 686, 13–18
28 Derks, W.J.G. and Borst-Pauwels, G.W.F.H. (1980) Biochim. Biophys. Acta 596, 381–392
29 Eddy, A.A. (1978) in Current Topics in Membranes and Transport. (Bronner, F. and Kleinzeller, A., eds.), pp. 279–360, Academic Press, New York
30 Heinz, E. (1972) Na-linked Transport of Organic Solutes, Springer, Betlin
31 West, I.C. (1980) Biochim. Biophys. Acta 604, 91–126
32 Felle, H., Luehring, H. and Bentrup, F.-W. (1979) Z. Naturforsch. 34C, 1222–1226
33 Felle, H. and Bentrup, F.-W. (1980) Planta 147, 471–476
34 Slayman, C.L. and Slayman, C.W. (1974) Proc. Natl. Acad. Sci. USA 71, 1935–1939