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KINETIC ANALYSIS OF A FAMILY OF COTRANSPORT MODELS

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The kinetic properties of a family of cotransport models are studied. The most general model allows the substrate and activator to bind in a random fashion to the transporter (iso random bi-bi mechanism). The other models require an ordered binding sequence (iso ordered bi-bi mechanism) and differ according to the order and symmetry of the binding events at the two membranes faces. In all cases it has been assumed that the translocation of the carrier is the rate-limiting step in the transport process. It is demonstrated that under zero trans, equilibrium exchange and infinite trans experimental conditions the usual kinetic parameters K_m and V can be expressed as simple functions of the activator concentration and a minimal set of model dependent constants with well defined kinetic interpretations. Kinetic criteria for distinguishing between the various models are established. The incorporation of the effects of membrane potential into the flux equations is also treated with the aid of certain simplifying assumptions. The usefullness of the concept of 'effective charge' for non mobile carrier mechanisms is emphasized.

Introduction

It is now well established that the active accumulation of many solutes by a variety of cell types is driven by secondary active cotransport processes [1,2]. The cotransported species, or 'activator', whose electrochemical gradient provides the necessary driving force for this accumulation is typically a monovalent cation and such as H^+ or Na^+. Given the ubiquity and importance of this type of transport system it is of considerable interest to attempt to determine the molecular mechanisms involved in the transport event. This problem can be approached in a variety of ways and our final understanding will undoubtedly be based on a synthesis of information obtained from many methodologies. In this paper the approach of transport kinetics is explored through a systematic theoretical treatment of a family of cotransport models. The usefulness of kinetic criteria for distinguishing between and characterizing certain reaction mechanisms is well-known from enzymology [3].

The kinetic properties of five closely related cotransport models of the carrier type are treated here. The most general model allows the substrate molecule and activator ion to bind in a random fashion to the transporter in analogy to an iso random bi-bi mechanism in enzyme kinetics [3]. The other four models, which may be regarded mathematically as special cases of the one just mentioned, require an ordered binding sequence analogous to an iso ordered bi-bi enzymatic mechanism. The models differ from one another according to the order and symmetry of the binding events at the two membrane faces. In all cases it has been assumed that the translocation of the carrier is the rate-limiting step in the transport process. In contrast to many previous treatments of cotransport models, in this paper (1) no assumptions of symmetry of binding constants or translocation rate constants are made, (2) a variety of possible

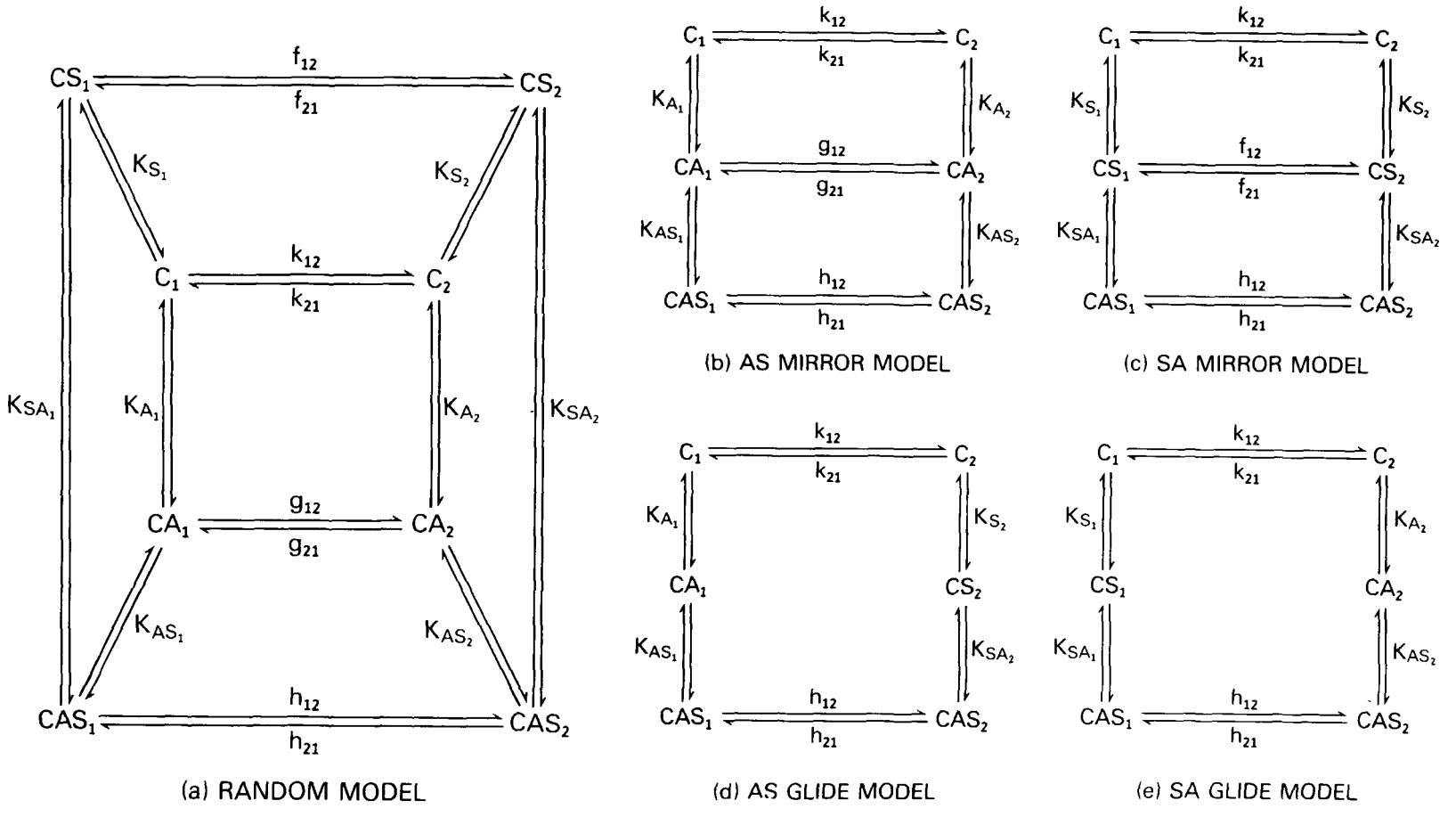

Fig. 1. Schematic representations of the five cotransport models analyzed in this paper (see text for details). The nomenclature is from Refs. 4 and 16.

experimental conditions (zero *trans*, equilibrium exchange and infinite *trans*) are considered and (3) the predictions of the various models are analyzed in the same theoretical framework and kinetic criteria are established for distinguishing between them.

As a basis for the present analysis it is demonstrated that the usual kinetic parameters, K_m and V, which characterize the substrate flux, can be expressed as simple functions of the activator concentration and a minimal set of model dependent constants with well defined kinetic interpretations. For a given set of experimental conditions these constants represent the maximum amount of information obtainable about the system from steady-state flux measurements.

The incorporation of the effects of membrane potential into the substrate flux equations is also treated with the aid of certain simplifying assumptions.

Theoretical derivations

Description of the models

The five models treated in this paper are shown schematically in Fig. 1. The two cotransported species are referred to as the substrate, S, and the activator, A. In the figure the 'free' carrier on side n of the membrane is represented by C_n, the carrier plus bound substrate by CS_n, the carrier plus bound activator by CA_n and the carrier plus bound activator and substrate by CAS_n. The external and internal faces of the membrane are labelled by $n = 1$ and $n = 2$, respectively. The rate constants for the translocation of the various free and loaded carrier species across the membrane are designated k_{12}, f_{12}, g_{12}, and h_{12} for inward diffusion and k_{21}, f_{21}, g_{21}, and h_{21} for outward diffusion. The dissociation constants K_{A_n}, K_{AS_n}, etc. which characterize the various binding events at the two membrane faces are discussed below.

In the Random model (Fig. 1a) the substrate and activator can bind to and dissociate from the carrier in either order whereas in the AS and SA models (Figs. 1b–1e) only one binding sequence is allowed at either membrane face. The models with 'mirror symmetry' have the same order of binding at both sides of the membrane while the models with 'glide symmetry' have the binding order reversed at opposite sides. Glide symmetry suggests a first in, first out mode of behavior which might, for example, be characteristic of certain types of pores [4]. Note that the AS Glide and SA Glide models are only distinguished from one another by their orientation in the membrane.

The kinetics of substrate flux for cotransport models related to those shown in Fig. 1 have been treated by a number of authors in the past [4–16], however, in many cases assumptions of symmetry of translocation rate constants [5–7,10,15], symmetry of binding events at the two membrane faces [8,10] or lack of mobility of partially loaded forms of the carrier [12,15] have limited the generality and usefulness of their results. In a few instances several of the schemes shown in Fig. 1 have been analyzed together [4,7,10,12,13] and kinetic criteria for distinguishing between them have been derived. But a general treatment of all the models under a variety of experimental conditions as presented here has been lacking. Moreover, no attempt seems to have been made to parametrize the kinetic equations in any useful or meaningful way.

As has been stressed previously [16] 'carrier-type' models such as those shown in Fig. 1 are considerably more general than the usual mobile carrier interpretation. These models assume only that the binding sites of the transporter are alternately exposed on one or the other side of the membrane and that these translocations are characterized by the rate constants k_{12}, k_{21}, g_{12}, etc. No further assumptions about the physical mechanism of the transport event are made.

Assumptions

The usual assumptions associated with the equilibrium carrier model [8–10] are made here, namely that:

1. The rate-limiting step in the transport process is the movement of the transport-binding site from one membrane surface to the other; thus the transporters are in equilibrium with the ligands S and A at the membrane faces.

2. The total number of transporters, free and loaded, is constant and equal to C_0.

3 There is no net movement of transporter binding sites from one face of the membrane to the other;

in other words, a steady state exists at the time of measurement

4. The transporter itself is not primary active.

In several cases [4,12–14] transport schemes similar to the various ordered models of Fig. 1 have been solved without making the assumption that the translocation step of the carrier is rate-limiting (assumption 1 above). The validity of this assumption for cotransport systems in general remains to be evaluated, however, it seems reasonable to assume that for a given transporter assumption 1 will hold at least for certain substrates and/or physical conditions. The effect of this assumption in simplifying the handling and predictions of kinetic models is well known [3].

Solution of the 'Random model'

The Random model of Fig. 1 can be solved in a straightforward manner by standard algebraic manipulation [16]. The points which are essential to the present work are given briefly below.

From assumption 1 above and Fig. 1a we have that on side n of the membrane

$$K_{S_n} = \frac{[C_n]\,[S_n]}{[CS_n]} \qquad K_{SA_n} = \frac{[CS_n]\,[A_n]}{[CAS_n]}$$

$$K_{A_n} = \frac{[C_n]\,[A_n]}{[CA_n]} \qquad K_{AS_n} = \frac{[CA_n]\,[S_n]}{[CAS_n]}$$

from which it follows directly that

$$K_{S_n} K_{SA_n} = K_{A_n} K_{AS_n} \tag{1}$$

Additionally from assumption 1 we have that the unidirectional flux of substrate from side 1 to side 2 of the membrane is given by.

$$J_S^{1\to 2} = f_{12}[CS_1] + h_{12}[CAS_1] \tag{2}$$

Using assumptions 2 and 3 we find that

$$[CS_1] = \frac{[S_1]}{K_{S_1}} Q_{12}, \qquad [CAS_1] = \frac{[S_1]\,[A_1]}{K_{A_1} K_{AS_1}} Q_{12} \tag{3a}$$

where

$$Q_{12} = \frac{C_0 F_{21}}{\alpha_1 F_{21} + \alpha_2 F_{12}} \tag{3b}$$

with F_{nm} and α_n given by

$$F_{nm} = k_{nm} + \frac{[A_n]}{K_{A_n}} g_{nm} + \frac{[S_n]}{K_{S_n}} f_{nm} + \frac{[S_n]\,[A_n]}{K_{A_n} K_{AS_n}} h_{nm} \tag{4a}$$

and

$$\alpha_n = 1 + \frac{[A_n]}{K_{A_n}} + \frac{[S_n]}{K_{S_n}} + \frac{[S_n]\,[A_n]}{K_{A_n} K_{AS_n}} \tag{4b}$$

The corresponding unidirectional flux from side 2 to side 1 of the membrane may be simply obtained by exchanging the roles of the subscripts 1 and 2 in Eqns. 2 and 3.

Assumption 4 requires that the parameters of the model be related in such a way that when the electrochemical potential differences for both substrate and activator across the membrane are zero the net flux of these species is zero. This leads to the following thermodynamic constraints on the parameters of the Random model:

$$K_{S_2} f_{12} k_{21} e^{z_S u} = K_{S_1} f_{21} k_{12} \tag{5}$$

$$K_{A_2} g_{12} k_{21} e^{z_A u} = K_{A_1} g_{21} k_{12} \tag{6}$$

$$K_{SA_2} h_{12} f_{21} e^{z_A u} = K_{SA_1} h_{21} f_{12} \tag{7}$$

$$K_{AS_2} h_{12} g_{21} e^{z_S u} = K_{AS_1} h_{21} g_{12} \tag{8}$$

and

$$K_{S_2} K_{SA_2} k_{21} h_{12} e^{(z_S + z_A)u} = K_{A_1} K_{AS_1} k_{12} h_{21} \tag{9a}$$

or equivalently (cf., Eqn. 1)

$$K_{A_2} K_{AS_2} k_{21} h_{12} e^{(z_S + z_A)u} = K_{S_1} K_{SA_1} k_{12} h_{21} \tag{9b}$$

where

$$u = \frac{F\Delta\psi}{RT} \tag{10}$$

Here F, R and T have their usual thermodynamic definitions, z_S and z_A are the electrical charges on the

TABLE I

MATHEMATICAL CONDITIONS FOR DERIVING THE SOLUTIONS OF THE AS AND SA MODELS FROM THE SOLUTION OF THE RANDOM MODEL

AS Mirror Model
$f_{nm} \to 0$
$K_{S_n} \to \infty$ and $K_{SA_n} \to 0$ such that $K_{S_n}K_{SA_n} \to K_{A_n}K_{AS_n}$
Only Eqns. 6 and 8 apply to this model.
AS Glide Model
$f_{nm} \to 0, g_{nm} \to 0$
$K_{S_1} \to \infty$ and $K_{SA_1} \to 0$ such that $K_{S_1}K_{SA_1} \to K_{A_1}K_{AS_1}$
$K_{A_2} \to \infty$ and $K_{AS_2} \to 0$ such that $K_{A_2}K_{AS_2} \to K_{S_2}K_{SA_2}$
Only Eqn. 9a applies to this model.
SA Mirror Model
$g_{nm} \to 0$
$K_{A_n} \to \infty$ and $K_{AS_n} \to 0$ such that $K_{A_n}K_{AS_n} \to K_{S_n}K_{SA_n}$
Only Eqns. 5 and 7 apply to this model.
SA Glide Model
$g_{nm} \to 0, f_{nm} \to 0$
$K_{A_1} \to \infty$ and $K_{AS_1} \to 0$ such that $K_{A_1}K_{AS_1} \to K_{S_1}K_{SA_1}$
$K_{S_2} \to \infty$ and $K_{SA_2} \to 0$ such that $K_{S_2}K_{SA_2} \to K_{A_2}K_{AS_2}$
Only Eqn. 9b applies to this model.

substrate and activator, respectively, and $\Delta\psi = \psi_\lambda - \psi_1$ is the electrical potential difference (membrane potential) between side 2 and side 1 of the membrane. In fact, only three of the five constraints represented by Eqns. 5–9 are mathematically independent, since given any three the remaining two can be derived from them using Eqn. 1. The reason for including all five equations will be clear when the solutions to the AS and SA models are discussed below (cf Table I).

Solutions of the AS and SA models

The solutions of the AS and SA models may be taken directly from Eqns. 2–4 since these models may be regarded as limiting cases of the Random model. The mathematical conditions which reduce the solution of the Random model to those of the various simpler models are given in Table I.

The kinetics of substrate flux

The kinetics of substrate flux is studied here under the following substrate and activator conditions:

(i) zero trans conditions, i.e , $[S_2] = [A_2] = 0$.

(ii) equilibrium exchange conditions, i e., $[S_1] = [S_2]$, $[A_1] = [A_2]$, $\Delta\psi = 0$.

(iii) infinite trans conditions, i.e , $[S_2] \to \infty$, $[A_2] \to \infty$, or more specifically that $[S_2]$ and $[A_2]$ are sufficiently large that $F_{21} \to h_{21}[S_2][A_2]/K_{A_2}K_{AS_2}$ and $\alpha_2 \to [S_2][A_2]/K_{A_2}K_{AS_2}$ (cf Eqns. 4a and 4b).

Random model

Using Eqns. 1–9 it may be shown in a straightforward (but somewhat tedious) way that for each of the experimental conditions given in the preceeding paragraph the unidirectional substrate flux from side 1 to side 2 of the membrane may be written in the form

$$J_S^{1\to 2} = \frac{V_S^{1\to 2}[S_1]}{K_S^{1\to 2} + [S_1]} \tag{11}$$

where $V_S^{1\to 2}$, the maximum velocity of transport, and $K_S^{1\to 2}$, the apparent Michaelis constant, can be written as simple functions of $[A_1]$ as follows:

$$V_S^{1\to 2} = \frac{V_{S_1}^0 K_{A_1}^\infty + V_{S_1}^\infty [A_1]}{K_{A_1}^\infty + [A_1]} \tag{12}$$

$$K_S^{1\to 2} = \frac{K_{S_1}^\infty}{K_{A_1}^\infty + [A_1]} \cdot (K_{A_1}^0 + [A_1]) \tag{13a}$$

$$= \frac{K_{S_1}^0 K_{A_1}^\infty}{K_{A_1}^\infty + [A_1]} \cdot \left(1 + \frac{[A_1]}{K_{A_1}^0}\right) \tag{13b}$$

The parameters in the above expressions have the following kinetic significance:

$V_{S_1}^0$ = maximum flux of S from side 1 to side 2 of the membrane when $[A_1] = 0$.

TABLE II

EXPRESSIONS FOR KINETIC PARAMETERS (see text)

Activator and substrate conditions	Zero *trans*	Equilibrium exchange	Infinite *trans*
$V^0_{S_1}$	$\frac{C_0 f_{12} k_{21}}{f_{12} + k_{21}}$	$\frac{C_0 f_{12} f_{21}}{f_{12} + f_{21}}$	$\frac{C_0 f_{12} h_{21}}{f_{12} + h_{21}}$
$V^\infty_{S_1}$	$\frac{C_0 h_{12} k_{21}}{h_{12} + k_{21}}$	$\frac{C_0 h_{12} h_{21}}{h_{12} + h_{21}}$	$\frac{C_0 h_{12} h_{21}}{h_{12} + h_{21}}$
$K^0_{A_1}$	$K_{A_1} \frac{k_{12} + k_{21}}{g_{12} + k_{21}}$	$\frac{k_{12} + k_{21}}{(k_{12}/K_{A_2}) + (k_{21}/K_{A_1})}$	$K_{A_1} \frac{k_{12} + h_{21}}{g_{12} + h_{21}}$
$K^\infty_{A_1}$	$K_{SA_1} \frac{f_{12} + k_{21}}{h_{12} + k_{21}}$	$\frac{h_{12} K_{SA_2} + h_{21} K_{SA_1}}{h_{12} + h_{21}}$	$K_{SA_1} \frac{f_{12} + h_{21}}{h_{12} + h_{21}}$
$K^0_{S_1}$	$K_{S_1} \frac{k_{12} + k_{21}}{f_{12} + k_{21}}$	$\frac{k_{12} + k_{21}}{(k_{12}/K_{S_2}) + (k_{21}/K_{S_1})}$	$K_{S_1} \frac{k_{12} + h_{21}}{f_{12} + h_{21}}$
$K^\infty_{S_1}$	$K_{AS_1} \frac{g_{12} + k_{21}}{h_{12} + k_{21}}$	$\frac{h_{12} K_{AS_2} + h_{21} K_{AS_1}}{h_{12} + h_{21}}$	$K_{AS_1} \frac{g_{12} + h_{21}}{h_{12} + h_{21}}$

$V^\infty_{S_1}$ = maximum flux of S from side 1 to side 2 of the membrane when $[A_1] \to \infty$.

$K^0_{S_1}$ = apparent Michaelis constant for the flux of S from side 1 to side 2 of the membrane when $[A_1] = 0$.

$K^\infty_{S_1}$ = apparent Michaelis constant for the flux of S from side 1 to side 2 of the membrane when $[A_1] \to \infty$.

$K^0_{A_1}$ = apparent Michaelis constant for the flux of A from side 1 to side 2 of the membrane when $[S_1] = 0$.

$K^\infty_{A_1}$ = apparent Michaelis constant for the flux of A from side 1 to side 2 of the membrane when $[S_1] \to \infty$.

Expressions for these quantities under zero *trans*, equilibrium exchange and infinite *trans* conditions are given in Table II. Note that in the models treated here half-saturation constants for transport by the carrier and binding to the carrier are identical (i.e. $K_d = K_m$). In analogy to Eqn. 1 it can be shown from Table I that in general

$$K^0_{A_1} K^\infty_{S_1} = K^\infty_{A_1} K^0_{S_1}$$

The expressions for the AS and SA models corresponding to Eqns. 12 and 13 are given below. For each model the quantities $V^0_{S_1}$, $V^\infty_{S_1}$, $K^0_{S_1}$, etc. are those defined in Table II with the additional constraints imposed by the conditions of Table I.

AS mirror model

In this model $V^0_{S_1} = K^\infty_{A_1} = 0$, and $K^0_{S_1} \to \infty$, thus

$$V_S^{1\to 2} = V^\infty_{S_1} \tag{14}$$

and

$$K_S^{1\to 2} = \frac{K^\infty_{S_1}}{[A_1]} (K^0_{A_1} + [A_1]) \tag{15}$$

AS glide model

Under zero *trans* and infinite *trans* conditions the solutions of the AS glide model are identical to those of the AS mirror model with the additional constraint that $g_{12} = 0$. Thus $V_S^{1\to 2}$ and $K_S^{1\to 2}$ have the same form as Eqns. 14 and 15 above. Under equilibrium exchange conditions, however, for the AS glide

model

$$V_S^{1\to 2} = \frac{V_{S_1}^{\infty}[A_1]}{K_{A_1}^{\infty} + [A_1]} \tag{16}$$

and $K_S^{1\to 2}$ has the same form as Eqn. 13.

SA mirror model

In this model $K_{A_1}^0 \to \infty$ and $K_{S_1}^{\infty} = 0$, thus

$$V_S^{1\to 2} = \frac{V_{S_1}^0 K_{A_1}^{\infty} + V_{S_1}^{\infty}[A_1]}{K_{A_1}^{\infty} + [A_1]} \tag{17}$$

and

$$K_S^{1\to 2} = \frac{K_{S_1}^0 K_{A_1}^{\infty}}{K_{A_1}^{\infty} + [A_1]} \tag{18}$$

SA glide model

Under zero *trans* and infinite *trans* conditions the solutions of the SA glide model are identical to those of the SA mirror model with the additional constraint that $f_{12} = f_{21} = 0$, and accordingly that $V_{S_1}^0 = 0$. Thus $V_S^{1\to 2}$ and $K_S^{1\to 2}$ have the form of Eqns. 16 and 18 above. Under equilibrium exchange conditions $V_S^{1\to 2}$ and $K_S^{1\to 2}$ have the form of Eqns. 16 and 13, respectively *.

Special cases

Some special cases of the models analysed here have been treated in the literature. Several of these are discussed briefly below.

Several authors have assumed [12,15] or speculated [5–7,10] that the translocation rate constants f_{nm} and g_{nm} of the partially loaded forms of the carrier are zero or at least small enough to be neglected. At present there seems to be no conclusive experimental evidence on this point. Moreover given that the unloaded and fully loaded forms of the carrier are mobile, there seems to be no a priori reason that the partially loaded forms are not mobile as well. Accordingly, in order to retain generality, f_{nm} and g_{nm} have not been set to zero here. A glance at the above derivations shows that assuming $f_{nm} = g_{nm} = 0$ results in some simplification of the expressions given in Table II The most notable is that $V_{S_1}^0$ vanishes, thus simplifying the form of $V_S^{1\to 2}$ in the Random and SA models (Eqns. 12 and 17).

Heinz et al. [9] and Geck and Heinz [11] have considered two special cases of the Random model. These are the so-called 'velocity-type' and 'affinity-type' models. In velocity-type models it is assumed that the activator increases the mobility of the carrier without changing its affinity for the substrate (i.e. $h_{nm} > k_{nm}$ but $K_{AS_n} = K_{S_n}$) while in affinity-type models the activator increases affinity rather than mobility (i.e. $k_{nm} = g_{nm} = f_{nm} = h_{nm}$ but $K_{AS_n} < K_{S_n}$). Examination of Table II shows that the form of Eqns. 12–18 are not changed by the velocity-type model assumptions. On the other hand for affinity-type models one finds that $V_{S_1}^0 = V_{S_1}^{\infty}$ and thus that the expressions for $V_S^{1\to 2}$ in the Random and SA mirror models (Eqns. 12 and 17) reduce to

$$V_S^{1\to 2} = V_{S_1}^{\infty}$$

Note, however, that this simplification of $V_S^{1\to 2}$ only occurs if $f_{nm} = h_{nm}$. If it is assumed that $k_{nm} = h_{nm} \neq f_{nm}$ (e.g. if $f_{nm} = 0$) then $V_S^{1\to 2}$ in the Random and SA mirror models retains the form of Eqn. 13 (or Eqn. 16 if $f_{nm} = 0$) and no simplification of steady-state kinetics is obtained from the affinity-type model assumptions.

Although the velocity- and affinity-type assumptions may be applicable to some experimental systems it seems more likely that the usual situation in Nature is a combination of these effects. Thus these and other special cases of the model parameters will not be treated further here but should be kept in mind by the interested reader.

Properties and tests of the models

Dependence of kinetic parameters on activator concentration

The expressions for $K_S^{1\to 2}$ and $V_S^{1\to 2}$ as functions of $[A_1]$ (Eqns. 12–18) are quite different for the various models considered here. Thus if $K_S^{1\to 2}$ and

* The inhibition of the equilibrium exchange flux of one co-transported species by high concentrations of the other as described for glide models by Hopfer and Groseclose [4] is not seen in the models treated here. This can be shown to be due to assumption 1 of the present analysis which requires the rate-limiting step in the transport process is the translocation event.

$V_S^{1\to2}$ are determined experimentally over a broad range of activator concentrations (and ideally under more than one of zero *trans*, equilibrium exchange and infinite *trans* conditions) these predictions may be used as quantitative tests of the models. Such an analysis would lead not only to the identification of the appropriate theoretical model (if any) but also to the evaluation of the various kinetic constants $V_{A_1}^{\infty}$, $K_{S_1}^{0}$, etc.

In this regard the usefulness of the ratio $V_S^{1\to2}/K_S^{1\to2}$ should also be stressed. Like $V_S^{1\to2}$ and $K_S^{1\to2}$ this quantity can also be determined from and Eadie-Hofstee or Lineweaver-Burke-type analysis (e.g. it is the intercept on the $J_S^{1\to2}/[S_1]$ axis of an Eadie-Hofstee plot); however, provided sufficiently low substrate concentrations are used, it can also be measured directly since when $[S_1] \ll K_S^{1\to2}$, Eqn 11 reduces to

$$J_S^{1\to2} = \frac{V_S^{1\to2}}{K_S^{1\to2}}[S_1]$$

The predicted dependence of $V_S^{1\to2}/K_S^{1\to2}$ on activator concentration is particularly simple in the case of the SA models where it is a linear function of $[A_1]$ (except for the SA glide model under equilibrium exchange conditions).

Several other simple functional relationships are also predicted in various models. For example, in the AS mirror model $V_S^{1\to2}$ is a constant and $K_S^{1\to2}$ is a linear function of $1/[A_1]$ In the SA mirror model $1/K_S^{1\to2}$ is a linear function of $[A_1]$. Similar relations hold for the corresponding Glide models under zero *trans* and infinite *trans* conditions Also, under equilibrium exchange conditions both Glide models predict that $1/V_S^{1\to2}$ is a linear function of $1/[A_1]$.

In general Glide and Mirror type models can be distinguished by comparing the activator dependence of influx ($1 \to 2$) and efflux ($2 \to 1$) kinetics under zero *trans* (or infinite *trans*) conditions or by comparing the activator dependence of equilibrium exchange kinetics to zero *trans* (or infinite *trans*) results

Dependence of flux on activator concentration

In addition to studying the dependence of $K_S^{1\to2}$ and $V_S^{1\to2}$ on activator concentration it is also interesting to look directly at the dependence of $J_S^{1\to2}$ on $[A_1]$. From Eqns. 11–13 we have that for the Random model under zero *trans*, equilibrium exchange or infinite *trans* conditions

$$J_S^{1\to2} = \frac{(V_{S_1}^{0}K_{A_1}^{\infty} + V_{S_1}^{\infty}[A_1])[S_1]}{K_{A_1}^{\infty}(K_{S_1}^{0} + [S_1]) + [A_1](K_{S_1}^{\infty} + [S_1])} \qquad (19)$$

In general a plot of $J_S^{1\to2}/[A_1]$ vs. $J_S^{1\to2}$ is curvilinear with the form shown in Fig. 2. For large $[A_1]$ this curve approaches a straight line with intercept $V_{S_1}^{\infty}[S_1]/(K_{S_1}^{\infty} + [S_1])$ on the $J_S^{1\to2}$ axis and slope $-(K_{S_1}^{\infty} + [S_1])/K_{A_1}^{\infty}(K_{S_1}^{0} + [S_1])$. This asymptotic behavior can be seen directly from Eqn. 19 since for $[A_1]$ large ($\gg V_{S_1}^{0}K_{A_1}^{\infty}/V_{S_1}^{\infty}$) this equation is of Michaelis-Menten form. Furthermore, when $V_{S_1}^{0} = 0$ a plot of $J_S^{1\to2}/[A_1]$ vs $J_S^{1\to2}$ is linear with the above

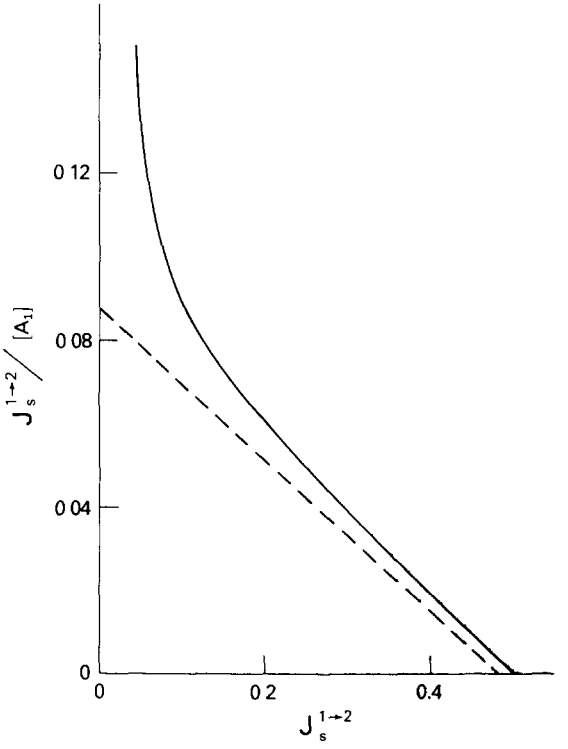

Fig 2. Dependence of flux on activator concentration. The solid line represents a plot of $J_S^{1\to2}/[A_1]$ vs. $J_S^{1\to2}$ for the Random model (see text for details). The following parameterization was used; $V_{S_1}^{0} = 0.2\ V_{S_1}^{\infty}$, $[S_1] = K_{S_1}^{\infty} = 0.1\ K_{S_1}^{0}$. $J_S^{1\to2}$ is plotted in units of $V_{S_1}^{\infty}$; $[A_1]$ is plotted in units of $K_{A_1}^{\infty}$. The broken line is a plot of $J_S^{1\to2}/[A_1]$ vs. $J_S^{1\to2}$ for the same parameterization. Note that the slope of the solid line approaches that of the broken one as $J_S^{1\to2}$ increases (i.e. as $[A_1] \to \infty$).

intercept and slope. Thus one can think of the deviation from linearity at low $[A_1]$ in Fig. 2 as being due to substrate flux moving via the carrier without co-transported activator.

The above method of plotting flux data has several interesting properties. For example, under zero *trans*, equilibrium exchange or infinite *trans* conditions

(1) The plot is always linear for the AS mirror, AS glide and SA glide models. It is curvilinear for the Random and SA mirror models if and only if $V^0_{S_1} \neq 0$, i.e. if and only if the partially loaded carrier, CS_n, is mobile.

(2) The intercept on the $J_S^{1\to2}$ axis is independent of substrate concentration for the SA mirror model (and for the SA glide model except under equilibrium exchange conditions).

(3) The intercept on the $J_S^{1\to2}/[A_1]$ axis is a linear function of substrate concentration for the AS mirror model (and for the AS glide model except under equilibrium exchange conditions).

A simple test for random vs. ordered binding schemes may be carried out using zero *trans* (or infinite *trans*) flux data taken over a suitable range of substrate and activator concentrations as follows. If the $J_S^{1\to2}$ intercept on an Eadie-Hofstee plot (i.e. $V_S^{1\to2}$) is found to depend on $[A_1]$ then both AS models are ruled out (cf. Eqn. 14); while if the $J_S^{1\to2}$ intercept on a $J_S^{1\to2}/[A_1]$ vs. $J_S^{1\to2}$ plot is found to depend on $[S_1]$, both SA models are ruled out. If neither intercept is found to be constant only the Random model is consistent with the data.

A useful variation on the $J_S^{1\to2}/[A_1]$ vs. $J_S^{1\to2}$ plot is also worth discussing. Consider the quantity $\hat{J}_S^{1\to2}$ defined by

$$\hat{J}_S^{1\to2} = J_S^{1\to2} - J_S^{1\to2}\,([A_1] = 0) \tag{20}$$

where $J_S^{1\to2}$ and $J_S^{1\to2}$ $([A_1)] = 0)$ are substrate fluxes measured under the same experimental conditions in the presence and absence of activator, respectively. It can be easily shown that a plot of $\hat{J}_S^{1\to2}/[A_1]$ vs. $\hat{J}_S^{1\to2}$ is linear for all models and experimental conditions treated here. The slope of this line is identical to that of the $J_S^{1\to2}/[A_1]$ vs. $J_S^{1\to2}$ plot and the intercept on the $\hat{J}_S^{1\to2}$ axis is

$$\frac{V^\infty_{S_1}[S_1]}{K^\infty_{S_1} + [S_1]} - \frac{V^0_{S_1}[S_1]}{K^0_{S_1} + [S_1]}$$

A $\hat{J}_S^{1\to2}/[A_1]$ vs. $\hat{J}_S^{1\to2}$ plot has been shown in Fig. 2 (see figure caption).

Dependence of activator flux and kinetics on substrate concentration

In the above treatment only substrate fluxes and kinetics have been considered, however, if the activator flux through the transporter can also be reliably measured, analyses and tests complimentary to all those discussed above can be carried out simply by reversing the roles of substrate and activator.

Dependence of substrate flux on membrane potential

Since many cotransport events are electrogenic, considerable experimental and some theoretical emphasis has been placed on the dependence of coupled fluxes on membrane potential ($\Delta\psi$). But since so little is known at present about the interaction of the transporter with the transmembrane electric field or about the way the electric field itself varies across the membrane, a number of assumptions must be made [16] in order to analyze the predicted dependence of flux on $\Delta\psi$. It is the purpose of this section to illustrate how the models discussed in this paper can be extended to include the effects of membrane potentials and, provided certain assumptions hold, solved to yield tractable testable predictions. It will quickly become clear, however, that more work is required to determine the validity of these assumptions before membrane potential effects can be used to test transport models in a quantitative way. A related analysis from a different approach has been carried out by Geck and Heinz [11,17].

First, let us restrict ourselves to a simplified set of experimental conditions. Assuming that $[A_1]$ and $[S_1]$ are sufficiently small that $[A_1] \ll K^0_{A_1}$ and $[S_1] \ll K_S^{1\to2}$, then $\hat{J}_S^{1\to2}$ (cf. Eqn. 20) has the form

$$\hat{J}_S^{1\to2} = \frac{V^\infty_{S_1}[S_1]\,[A_1]}{K^\infty_{S_1}K^0_{A_1}} \tag{21}$$

for all models and experimental conditions treated here (cf. Eqns. 11–18). Note that under these conditions $\hat{J}_S^{1\to2}$ is linear in both $[A_1]$ and $[S_1]$. For zero *trans* experimental conditions Eqn. 21 can be rewritten as

$$\hat{J}_S^{1\to2} = \frac{C_0 k_{21} h_{12}[S_1]\,[A_1]}{K_{AS_1}K_{A_1}(k_{12} + k_{21})} \tag{22}$$

We now follow the treatment of membrane potential for carrier type models given previously by Turner and Silverman [16]. In principle both the binding constants and the rate constants in Eqn. 22 could be functions of $\Delta\psi$, however, for carrier type models it seems reasonable to assume that the most significant effect of $\Delta\psi$ will be on the translocation rate constants since these parameters characterize processes involving the net transfer of electrical charges across the membrane. Thus assuming that the binding constants of the models are not affected by electric fields and that the rate constants are simple exponential functions of $\Delta\psi$ it can be shown (cf. Ref. 16) that

$$k_{12} = k_{12}^0 e^{-\beta\eta u} \qquad k_{21} = k_{21}^0 e^{\beta(1-\eta)u} \tag{23}$$

$$h_{12} = h_{12}^0 e^{-(\beta+z_S+z_A)\eta u}$$

$$h_{21} = h_{21}^0 e^{(\beta+z_S+z_A)(1-\eta)u}$$

Here u is defined in Eqn. 10, k_{12}^0, k_{21}^0, h_{12}^0 and h_{21}^0 are the values of k_{12}, k_{21}, h_{12} and h_{21} when $\Delta\psi = 0$, β is the 'effective charge' of the transporter (see below) and η characterizes the point in the electric field where the transition from C_1 to C_2 occurs. The parameters β and η are discussed briefly below and in more detail in Ref. 16.

In a mobile carrier model β can be seen to represent the electrical charge of the free carrier [16]. As mentioned earlier, however, the kinetic models shown in Fig. 1 are not limited to the mobile carrier interpretation. Thus the translocation rate constants k_{nm}, h_{nm}, etc. simply characterize some unspecified conformational change in the transporter which results in a reorientation of binding sites, and β, in turn, characterizes the electrical dependence of these events. β is referred to as the 'effective charge' of the transporter since it is the charge that would be associated with an 'electrically equivalent' mobile carrier. Note that for non mobile carrier mechanisms there is no reason to expect β to assume only integer values.

The parameter η is restricted to the range $1 \geqslant \eta \geqslant 0$ and $-\eta\Delta\psi$ is the electrical potential difference between side 1 of the membrane and the point at which the transition state of the carrier from form C_1 to C_2 occurs (e.g. if the transition state occurs at the mid-point of the transmembrane potential drop then $\eta = 0.5$). It has been assumed in deriving Eqns. 23 that η is identical for the loaded and unloaded forms of the carrier [16].

Substituting Eqns. 23 into Eqn. 22 yields

$$\hat{J}_S^{1\to 2} = \frac{C_0 k_{21}^0 h_{12}^0 [S_1]\,[A_1]}{K_{AS_1} K_{A_1}} \cdot \frac{e^{-(\beta+z_S+z_A)\eta u}}{(k_{21}^0 + k_{12}^0 e^{-\beta u})}$$

With the further assumption that $k_{12}^0 = k_{21}^0$ one can write

$$\hat{J}_S^{1\to 2} = \mathring{J}_S^{1\to 2} \frac{2e^{-(\beta+z_S+z_A)\eta u}}{1 + e^{-\beta u}} \tag{24}$$

where

$$\mathring{J}_S^{1\to 2} = \frac{C_0 [S_1]\,[A_1] h_{12}^0}{2K_{AS_1} K_{A_1}}$$

Here $\mathring{J}_S^{1\to 2}$ is the flux of S when $\Delta\psi = 0$. It is clear from Eqn. 24 that even with the above simplifying assumptions the dependance of $\hat{J}_S^{1\to 2}$ on membrane potential is rather complex and in general not easy to test experimentally. Also the magnitude of the effect of the membrane potential on flux will be a function of the parameters β and η. Several simple cases are discussed below.

Case 1. $\beta = 0$, i.e. the parameters k_{12} and k_{21} are independent of $\Delta\psi$ (or in terms of a mobile carrier, the unloaded carrier is electrically neutral). With this assumption

$$\hat{J}_S^{1\to 2} = \mathring{J}_S^{1\to 2} e^{-(z_S+z_A)\eta u}$$

In this case flux varies exponentially with $\Delta\psi$ but the parameter η plays an important role in the magnitude of this effect Note in particular that when $\eta = 0$, $\hat{J}_S^{1\to 2}$ is independent of $\Delta\psi$.

Case 2. $\beta = -(z_S + z_A)$ i.e. the parameters h_{12} and h_{21} are independent of $\Delta\psi$ (or in terms of a mobile carrier, the fully loaded carrier is electrically neutral). With this assumption

$$\hat{J}_S^{1\to 2} = \frac{2\mathring{J}_S^{1\to 2}}{1 + e^{(z_S+z_A)u}}$$

In this case flux is independent of η. Note also that the maximum value of $\hat{J}_S^{1\to 2}$ is $2\mathring{J}_S^{1\to 2}$ (obtained

when $(z_S + z_A)u \ll 0$). This is in marked contrast to the previous case where flux could be increased without limit by a favorably directed membrane potential.

Case 3. $\eta = 1/2$, i.e. the transition $C_1 \rightleftharpoons C_2$ occurs at the mid point of the transmembrane electrical potential difference. With this assumption

$$\hat{J}_S^{1\to 2} = 2\mathring{J}_S^{1\to 2}\frac{e^{-(z_S+z_A)u/2}}{(e^{\beta u/2} + e^{-\beta u/2})}$$

(Note that in this case $\hat{J}_S^{1\to 2}$ does not depend on the sign of β). The effect of changing β when $\eta = 1/2$ is illustrated in Fig. 3. It is clear that $\beta = 0$ is the most favorable arrangement for coupling the electrical potential difference to the substrate flux. Nevertheless, for typical physiological values of $\Delta\psi$ ($\simeq -50$ mV) we have $u = F\Delta\psi/RT \simeq -2$ and it can be seen from Fig. 3 that for β values substantially different from zero this coupling can also be quite effective.

Case 4. $z_S + z_A = 0$ i.e., a complete cycle of the transporter results in no net transfer of electrical charge across the membrane With this assumption

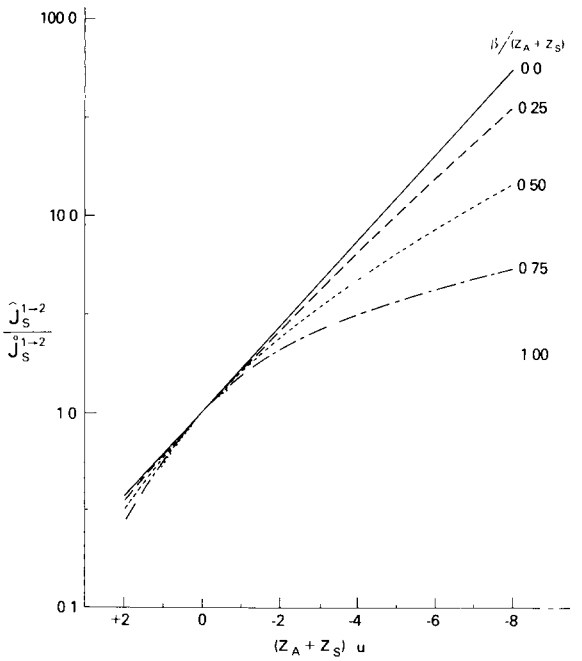

Fig. 3. The dependence of $J_S^{1\to 2}$ on membrane potential for various values of β, the effective charge of the transporter (see text for details).

$$\hat{J}_S^{1\to 2} = \mathring{J}_S^{1\to 2}\frac{2e^{-\beta\eta u}}{1 + e^{-\beta u}}$$

This example has been included to demonstrate the interesting fact that even when the net transport process is electroneutral a membrane potential can still result in stimulation or reduction of flux provided $\beta \neq 0$

Concluding remarks

In this paper the kinetic properties of a family of cotransport models of the carrier type have been studied in some detail. A number of tests of the models have also been discussed. Using these the interested investigator should be able to test the appliability of a given model to his/her own experimental system In contrast to many earlier treatments no assumptions regarding the symmetry of the transporter or lack of mobility of partially loaded forms of the carrier have been made and a variety of experimental conditions have been analyzed In addition, it has been shown that the flux equations can be written in terms of a set of model dependent constants with well defined kinetic interpretations. (cf. Eqns 11–18 and Table II). It has also been demonstrated that the effects of transmembrane electrical potentials can be incorporated into the flux equations in a straightforward way, however, owing to the number of as yet untested assumptions which must be made in order to do so, considerable caution is required in the interpretation of experimental results

The analysis of carrier models given in this paper is complimentary to the work of Heinz et al [9] and Geck and Heinz [11] who concentrate on the systematics of energetic coupling of substrate flux to the activator electrochemical gradient using several variants of the 'random model' treated here. As an extension of the work of these authors it is emphasized in the present paper and in Ref. 16 that the usual interpretation of the parameter β as the charge on the free carrier can be generalized to apply to non-mobile-type mechanisms using the concept of 'effective charge'.

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