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Membrane Transport Models with Fast and Slow Reactions: General Analytical Solution for a Single Relaxation

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Summary. Membrane transport models are usually expressed on the basis of chemical kinetics. The states of a transporter are related by rate constants, and the time-dependent changes of these states are given by linear differential equations of first order. To calculate the time-dependent transport equation, it is necessary to solve a system of differential equations which does not have a general analytical solution if there are more than five states. Since transport measurements in a complex system rarely provide all the time constants because some of them are too rapid, it is more appropriate to obtain approximate analytical solutions, assuming that there are fast and slow reaction steps. The states of the fast steps are related by equilibrium constants, thus permitting the elimination of their differential equations and leaving only those for the slow steps. With a system having only two slow steps, a single differential equation is obtained and the state equations have a single relaxation. Initial conditions for the slow reactions are determined after the perturbation which redistribute the states related by fast reactions. Current and zero-*trans* uptake equations are calculated. Curve fitting programs can be used to implement the general procedure and obtain the model parameters.

Key Words membrane transport models · relaxation models · transient analysis · cotransport

Introduction

Membrane transport models are frequently formulated according to the Eyring rate theory of chemical reactions. This approach has the advantage of providing a theoretical framework to calculate the flux equations on the basis of a transport model. It is assumed that there are structures in the membrane that can provide a favorable environment for a substrate binding and transport from one side of the membrane to the other. Given a sequence of reactions and their rates with the concentrations of the substrates, it is possible to calculate the transmembrane fluxes (for a review, *see* Läuger, 1987). The results of these calculations can be compared to experimental data, and it is possible to verify if the transport reaction sequence is appropriate. Usually, the calculations are performed assuming that the transport system is in the steady state, mainly because such calculations are more simple and provide analytical solutions (Turner, 1981; Sanders et al., 1984). Also experimental data are more easily obtained in these conditions. But such a treatment has a serious limitation, since it is not always possible to determine the individual rates of the reaction sequence only on the basis of a steady-state treatment. This approach will nevertheless provide evidence for a limited number of reaction sequences and take into account the influence of substrates and membrane potential. However, to compare a particular transport model to the measured fluxes or currents and to determine the individual rates, it would be necessary to calculate the time-dependent solution of the transport model and to compare this solution to the measured time dependence of the fluxes or currents. Such calculations and measurements have been performed on excitable membranes (Roy, 1975) and lipid bilayers containing ionophores (Läuger et al., 1981). With simple three- or four-state models, it was possible to obtain analytical solutions and to determine the rates of the reaction steps. In general, the time dependence of a transport system with n states will have n-1 time constants. To obtain them in relation to the reaction rates requires the solution of a polynomial of degree n-1. A major theoretical limitation arises when a system has five states or more: there is no general method to obtain an analytical solution for such a polynomial. Since transport models frequently involve many reaction steps, it appears rather difficult to consider all their relaxation processes. On the other hand, when a transport process contains many reaction steps, flux or current measurements would rarely provide all the relaxations, because some steps are too rapid to be observed. When relaxations are measured, there could

be one which is slower than the others, as observed for the Na-K ATPase current and fluorescence relaxations (Nakao & Gadsby, 1986; Borlinghaus, Apell & Läuger, 1987; Stürmer et al., 1989). The Na-K ATPase transport system contains a large number of steps, but only one time constant was measured. The relaxation of the Na-H exchanger was studied by Otsu et al. (1989) with radioactive Na uptake in vesicles, and a single time constant was calculated from the data. A single current relaxation of the Na-glucose transporter was also observed recently (Parent et al., 1990). Therefore, a complete theoretical treatment calculating all the time constants of a complex transport model would not always be necessary. Under these conditions, it would be more appropriate to provide an approximate calculation, taking into account that some reactions are more rapid than others and, thus, reach their equilibrium before other reactions start. Such a calculation would reduce the number of differential equations in the model and the number of time constants. If all the differential equations of a transport model could be reduced to only one, it would be possible to obtain an analytical solution for a single relaxation which could be compared to the measured relaxation. Standard curve fitting methods can be used to evaluate the unknown parameters. This approach has been recently introduced by Wierzbicki, Berteloot and Roy (1990) for the particular case of a simple carrier transport with four states. Assuming that there were two slow and two fast steps in the transport cycle, the model was transformed to give a single differential equation, which was solved to give a single relaxation. The uptake of a substrate was calculated for the zero-*trans* influx condition. It was shown that equations for the steady state, the time constant and the amplitude of relaxation could be obtained. These three functions depended differently on substrate concentrations and membrane potential. A comparison of the results calculated analytically with those obtained through a numerical integration has shown that both were identical after a period of time corresponding to the fast relaxations. Therefore, the analytical approximations were very accurate and could be used when a single slow relaxation is measured for some transport process to evaluate the unknown parameters. Although these calculations could not determine all the reaction rates, at least they could provide the rates of the slow steps.

Transport models frequently involve a large number of reactions steps, and with larger models the extent of calculations becomes more important. This is particularly the case for active transport and cotransport models. Also, it is sometimes necessary to test different models or different positions of the slow steps within a model. Therefore, a rapid method to obtain the analytical solutions is necessary. The purpose of this paper is to provide a general calculation method for a single relaxation applicable to models of any size and for different external conditions. The method is similar to that introduced by Turner (1985) to calculate the steady-state solution of a rapid equilibrium model of any size. The single time constant, the relaxation amplitude and the steady-state solution are obtained. The method can be applied to calculate the flux, the zero-*trans* uptake and the current for different types of membrane transport models, whether active transport, cotransport, antiport or channels. Specific flux and current equations are derived only for transporters, although the method could also be applied to calculate the current in channels. The procedure can be introduced into a curve fitting program to provide a general approach to evaluate the parameters related to different models.

General Solution for Transport Models with a Single Relaxation

Assumptions

The method described to calculate approximate solutions giving a single relaxation in a complex transport system involves some specific assumptions.
i) The transport system can be described completely by a set of linear differential equations of first order.
ii) The total number of states in the system is constant. This is the conservation equation.
iii) The transport system has fast and slow reactions. For a closed sequence of reactions, there are two steps that are much slower than all the others.

The consequences of these assumptions are as follows:
i) The states of the fast reactions are related by equilibrium constants even though these reactions are time dependent and not strictly at equilibrium.
ii) After a perturbation, the states related by fast reactions redistribute themselves instantaneously to set the initial conditions for the slow reactions.
iii) The membrane flux is calculated from the slow steps with bound substrates.

Transport Model Reduction

A diagram for a typical transport model having one closed sequence of reactions is shown in the Figure. According to assumption (*iii*), there are only two slow reactions and all the others are fast. For exam-

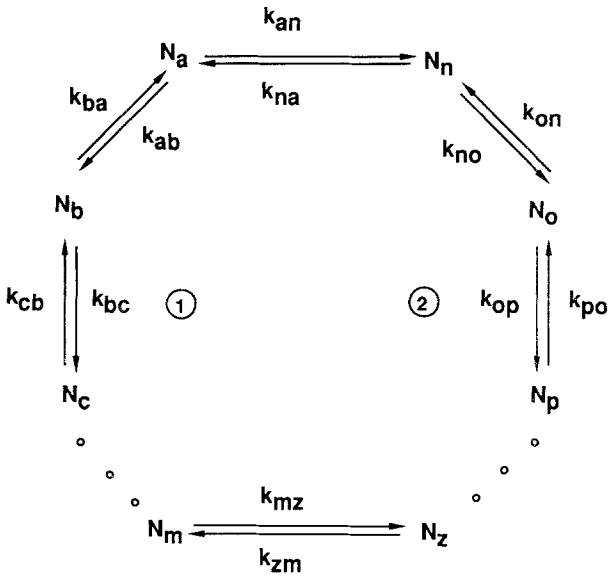

Fig. 1. General diagram for a membrane transport system with one closed cycle of reactions. On side (*1*), N_a, N_b . . . N_m and on side (*2*), N_n, N_o . . . N_z represent the states of the transporter on each side of the membrane. Substrate binding steps are not indicated explicitly; they are part of the rates expressed by k_{ab}, k_{ba} . . . and k_{no}, k_{on} . . . Each of the rates could also have a membrane potential dependence.

ple in the Figure all the side (*1*) reactions, N_a to N_m, and the side (*2*) reactions, N_n to N_z, are fast. These are expressed with equilibrium equations between adjacent states. Those of side (*1*) are as follows:

$$\begin{aligned} N_b &= (k_{ab}/k_{ba})N_a = K_bN_a \\ N_c &= (k_{bc}/k_{cb})N_b = K_cN_b \\ &\cdots\cdots\cdots\cdots \end{aligned} \tag{1}$$

and so on until the next slow step is reached. Similarly, on side (*2*)

$$\begin{aligned} N_o &= (k_{no}/k_{on})N_n = K_oN_n \\ N_p &= (k_{op}/k_{po})N_o = K_pN_o \\ &\cdots\cdots\cdots\cdots \end{aligned} \tag{2}$$

Introducing the conservation equation (assumption *ii*)

$$N_t = N_a + N_b + N_c + \ldots N_n + N_o + N_p + \ldots \tag{3}$$

and using Eqs. (1) and (2), it gives

$$N_t = A_aN_a + A_nN_n \tag{4}$$

where

$$A_a = 1 + K_b + K_bK_c + \ldots \tag{5}$$

$$A_n = 1 + K_o + K_oK_p + \ldots . \tag{6}$$

Many different choices of slow and fast steps could be made, but each choice implies the partition of the transport system in two sections, each one containing the fast reactions between the slow reactions. If the two slow reactions are not separated by a fast reaction, the value of A_a or A_n is equal to 1.

A consequence of the rapid equilibrium assumption for the fast reactions is that their derivatives are directly proportional to each other. Taking the time derivative of Eqs. (1) and (2) gives

$$\begin{aligned} dN_b/dt &= K_b\, dN_a/dt \\ dN_c/dt &= K_c\, dN_b/dt \\ &\cdots\cdots\cdots\cdots \\ dN_o/dt &= K_o\, dN_n/dt \\ dN_p/dt &= K_p\, dN_o/dt. \\ &\cdots\cdots\cdots\cdots \end{aligned} \tag{7}$$

The time derivative of the conservation equation, Eq. (3), is also calculated

$$\begin{aligned} &dN_a/dt + dN_b/dt + dN_c/dt + \ldots dN_n/dt \\ &\quad + dN_o/dt + dN_p/dt \ldots = 0. \end{aligned} \tag{8}$$

There are two groups of derivatives in Eq. (8). Using the set of Eq. (7) with Eqs. (5) and (6), they give

$$\begin{aligned} dN_a/dt + dN_b/dt + dN_c/dt + \ldots &= A_adN_a/dt \\ dN_n/dt + dN_o/dt + dN_p/dt + \ldots &= A_ndN_n/dt. \end{aligned} \tag{9}$$

These two derivatives are related to each other

$$A_adN_a/dt = -A_ndN_n/dt. \tag{10}$$

According to assumption (*i*) and the Figure, the time derivatives of each state are expressed as follows:

$$\begin{aligned} dN_a/dt &= k_{ba}N_b + k_{na}N_n - (k_{ab} + k_{an})N_a \\ dN_b/dt &= k_{cb}N_c + k_{ab}N_a - (k_{bc} + k_{ba})N_b \\ &\cdots\cdots\cdots\cdots \\ dN_n/dt &= k_{on}N_o + k_{an}N_a - (k_{no} + k_{na})N_n \\ dN_o/dt &= k_{po}N_p + k_{no}N_n - (k_{on} + k_{op})N_o. \\ &\cdots\cdots\cdots\cdots \end{aligned} \tag{11}$$

The left-hand side of Eq. (9) can be calculated by adding all the kinetic equations related by equilibrium conditions. Because of the symmetry of these equations, many terms will cancel each other when

they are added, leaving only the states next to a slow step.

$$A_a dN_a/dt = k_{zm}N_z + k_{na}N_n - k_{mz}N_m - k_{an}N_a. \tag{12}$$

Introducing the equilibrium equations, Eqs. (1) and (2), it gives

$$A_a dN_a/dt = -B_{an}N_a + B_{na}N_n \tag{13}$$

with

$$B_{an} = k_{an} + k_{mz}K_bK_c \ldots \tag{14}$$
$$B_{na} = k_{na} + k_{zm}K_oK_p \ldots . \tag{15}$$

With Eq. (4) it is possible to eliminate N_n from Eq. (13). It gives the single differential equation of this reduced system

$$dN_a/dt + N_a(B_{an}A_n + B_{na}A_a)/A_aA_n = N_tB_{na}/A_aA_n. \tag{16}$$

This equation can be easily integrated to give $N_a(t)$.

$$N_a(t) = N_a(s) - [N_a(s) - N_a(0)]\exp(-t/t_a) \tag{17}$$

where t_a is the time constant, $N_a(s)$ is the steady state and $N_a(0)$ is the initial state. An equation similar to Eq. (17) can be written for $N_n(t)$. The value of t_a is the same, but $N_n(s)$ and $N_n(0)$ are different.

$$N_n(t) = N_n(s) - [N_n(s) - N_n(0)]\exp(-t/t_a). \tag{17a}$$

The time constant t_a and the steady state $N_a(s)$ and $N_n(s)$ are given by

$$t_a = A_aA_n/(B_{an}A_n + B_{na}A_a) \tag{18}$$
$$N_a(s) = N_tB_{na}/(B_{an}A_n + B_{na}A_a) \tag{19}$$
$$N_n(s) = N_tB_{an}/(B_{an}A_n + B_{na}A_a). \tag{19a}$$

From these equations, any other state function in the transport system can be obtained through the use of the equilibrium equations.

Initial Conditions

The initial state equation for $N_a(0)$ and $N_n(0)$ require additional calculations. It should be remembered that rapid transitions will occur immediately after the perturbation and produce new initial conditions that are different from those prevailing before the perturbation. The states related by equilibrium constants will redistribute themselves rapidly and provide the initial conditions of the slow system. Therefore, from the given initial conditions before the perturbation $N_a(*)$, $N_b(*)$ . . . and $N_n(*)$, $N_o(*)$ . . . a new set of initial conditions $N_a(0)$ and $N_n(0)$ are calculated. The sum of all the states related by equilibrium equations before and after the perturbation remain unchanged

$$N_a(*) + N_b(*) + \ldots = N_a(0) + N_b(0) + \ldots$$
$$N_n(*) + N_o(*) + \ldots = N_n(0) + N_o(0) + \ldots . \tag{20}$$

Introducing the equilibrium Eqs. (1) and (2), it follows that

$$N_a(0) = N_a(*)A_a(*)/A_a \tag{21}$$
$$N_n(0) = N_n(*)A_n(*)/A_n$$

with

$$A_a(*) = 1 + K_b(*) + K_b(*)K_c(*) + \ldots \tag{22}$$
$$A_n(*) = 1 + K_o(*) + K_o(*)K_p(*) + \ldots$$

where $K_b(*)$, $K_c(*)$ . . ., $K_o(*)$, $K_p(*)$ . . . represent the values of the equilibrium constants before the perturbation. They could be different from those after the perturbation if the equilibrium constants depend on substrate concentration or on membrane potential. Since the whole system is in equilibrium before applying the perturbation, the slow steps are also related by equilibrium equations initially

$$N_n(*) = K_n(*)N_a(*) \tag{23}$$

with

$$K_n(*) = k_{an}(*)/k_{na}(*). \tag{24}$$

Using Eq. (4)

$$N_t = A_a(*)N_a(*) + A_n(*)N_n(*). \tag{25}$$

It follows that

$$N_a(*) = N_t/[A_a(*) + K_n(*)A_n(*)] \tag{26}$$
$$N_n(*) = N_tK_n(*)/[A_a(*) + K_n(*)\,A_n(*)].$$

Using Eqs. (21) and (26) with Eqs. (5) and (6), $N_a(0)$ and $N_n(0)$ can be calculated as functions of equilibrium constants; the other initial states can also be obtained from Eqs. (1) and (2). Some initial states

could have a zero value depending on where substrate binding occurs (Wierzbicki et al., 1990).

Substrate Transport

We have developed a procedure to transform a complex transport system with many states into one with only four states. This is possible if there are only two slow steps and four slow rate constants in a closed system. There is only one time constant, and each state has its initial and steady-state amplitude. The transport system is completely characterized analytically and provides the basis to calculate substrate transport which can be compared to measured substrate transport. Since the transport system contains only two reaction steps that are not related by equilibrium constants, substrate transport can be calculated only from these two steps. It should be mentioned that relating the fast reaction states by equilibrium constants is an approximation and not a true equilibrium. Because the rates of the fast reactions are much larger than those of the slow reactions, the exchanges between the fast reaction states are nearly the same as those occurring in equilibrium conditions. For example, if substrate binding is fast compared to substrate translocation, the substrate spends most of its time binding and unbinding, and once in while it is translocated through a slow step. Therefore, there is a flux through a fast step as well as through a slow step, but the flux through a fast step cannot be calculated directly from that step because of the equilibrium approximation.

Substrate transport functions can be calculated from the state functions related by slow steps. The initial and steady-state functions, $N(0)$ and $N(s)$, and the time constant, t_a, are normally dependent on substrate concentrations. When a specific model is considered and substrate binding occurs at a particular reaction step, the rate of this step is multiplied by the substrate concentration, S_1 or S_2, for side (*1*) or side (*2*) of the membrane. For example, if the binding of substrate S_1 occurs from N_a to N_b, k_{ab} becomes $k_{ab}S_1$ and K_b becomes K_bS_1. Similarly for the binding of S_2, k_{no} becomes $k_{no}S_2$ and K_o becomes K_oS_2. Introducing those changes into Eqs. (5), (6), (14) and (15), gives the concentration dependence of t_a and those of the initial and the steady-state functions. Calculating t_a from Eq. (18), gives

$$t_a = [1 + S_1(K_b + K_bK_c + \ldots)] \\ [1 + S_2(K_o + K_oK_p + \ldots)]/D \qquad (27)$$

With the denominator D given by

$$D = [1 + S_1(K_b + K_bK_c + \ldots)](k_{na} + S_2k_{zm}K_oK_p \ldots) \\ + [1 + S_2(K_o + K_oK_p + \ldots)](k_{an} + S_1k_{mz}K_bK_c \ldots). \qquad (27a)$$

The steady-state equations all have the same denominator, only the numerators being different. In this example, the slow steps with bound substrate are $N_m(s)$ and $N_z(s)$. Using Eqs. (19) and (19a) with Eq. (1), $N_m(s)$ and $N_z(s)$ are given by

$$N_m(s) = N_tS_1K_bK_c \ldots (k_{na} + S_2k_{zm}K_oK_p \ldots)/D \qquad (28)$$

$$N_z(s) = N_tS_2K_oK_p \ldots (k_{an} + S_1k_{mz}K_bK_c \ldots)/D \qquad (28a)$$

The initial states $N_m(0)$ and $N_z(0)$ are calculated from Eqs. (21), (22) and (26). If the substrate S is added at the beginning of the measurements, $N_a(*)$ and $N_n(*)$ are independent of S, because $K_a(*)$ and $K_o(*)$ are equal to zero. In this case $A_a(*) = A_n(*) = 1$ and $K_n(*) = K_n$

$$N_m(0) = \frac{N_t S_1K_bK_c \ldots}{[1 + K_n][1 + S_1(K_b + K_bK_c + \ldots)]} \qquad (29)$$

$$N_z(0) = \frac{N_t S_2K_oK_p \ldots}{[1 + K_n^{-1}][1 + S_2(K_o + K_oK_p + \ldots)].} \qquad (29a)$$

It is interesting to compare these functions regarding their concentration dependence. The steady-state functions $N_m(s)$ and the initial state functions $N_m(0)$ have a similar form, both saturating as S_1 is increased. But the half-saturation values are different for $N_m(s)$ and $N_m(0)$. The half-saturation value depends on the equilibrium constants for $N_m(0)$ while it depends also on the rate constants for $N_m(s)$. Also $N_m(s)$ is dependent on S_2 while $N_m(0)$ is not. The concentration dependence of $N_m(s)$ and t_a are also different. Both have the same denominator, but their numerators depend differently on S_1 and S_2. There are terms in the numerator of t_a that are independent of S_1 and S_2, meaning that the value of t_a becomes a constant as the substrate concentrations tend toward zero values. The half-saturation value for the numerator of t_a could be different from that for the denominator, depending on the values of the parameters. The concentration dependence of t_a could increase or decrease as S_1 or S_2 is increased and its half-saturation could be different from that of $N_m(s)$. For some particular combination of parameters, t_a could be independent of S_1 or S_2.

The rate and equilibrium constants could be voltage dependent. In this case the rates are multiplied by exponential functions of the membrane potential, according to the formalism of Läuger and Jauch (1986) and Apell (1989). If the membrane po-

tential is the applied perturbation, the functions of $A(*)$ and A will be different if one or more of the equilibrium constants are voltage dependent. If the perturbation affects only the slow rate constants, the initial state values before and after the perturbation are the same.

Flux and Uptake

Transport across membranes is frequently measured with radioactive substrates. In this case, the radioactive tracer S_1 is introduced on side (*1*) of the membrane and its content or uptake S_2 is measured on side (*2*) of the membrane. From the transport model, it is necessary to calculate the time-dependent release of substrate S_2, assuming a constant concentration of S_1 introduced initially on side (*1*). The uptake of a substrate S_2 inside vesicles in zero-*trans* conditions has been treated in detail for a four-state model by Wierzbicki et al. (1990). The same method applies to calculate the uptake for our generalized model which has been reduced to a four-state model. If the substrate releasing step is slow, it is used to calculate the uptake. If this step is fast, a slow step preceding substrate release determines the rate of uptake; it could be a translocating or a binding step. The uptake of a substrate S_2 on side (*2*) of the membrane is related to the flux J_{12} from side (*1*) to side (*2*) as follows:

$$dS_2/dt = J_{12}(t). \tag{30}$$

The flux $J_{12}(t)$ is determined by one of the four state functions $N(t)$ related by slow steps, and it has the following form:

$$J_{12}(t) = J_{12}(s) - [J_{12}(s) - J_{12}(0)]\exp(-t/t_a). \tag{31}$$

The functions for $J_{12}(s)$ and $J_{12}(0)$ are obtained from one of the four states related by slow reactions. In the example developed above, these functions are

$$\begin{aligned} J_{12}(s) &= k_{mz}N_m(s) \\ J_{12}(0) &= k_{mz}N_m(0). \end{aligned} \tag{32}$$

If Eq. (31) is independent of S_2 and if the initial value of $S_2 = 0$, the integration of Eq. (30) gives the following uptake function:

$$S_2(t) = J_{12}(s)t - [J_{12}(s) - J_{12}(0)]t_a[\exp(-t/t_a) - 1]. \tag{33}$$

This function is compared to experimental data and values of $J_{12}(s)$, $J_{12}(0)$ and t_a are obtained for each uptake curve. Graphs of $J_{12}(s)$, $J_{12}(0)$ and t_a as functions of substrate concentration are obtained, and they can be compared to those calculated from the model.

Current

Substrate transport across membranes can also be studied from current measurements, if the substrates carry an electric charge. Current relaxations produced by a rapid change of membrane potential or substrate concentration can be measured for time constants in the millisecond range. The calculation of the time-dependent current across a membrane will be performed for a transporter model, either active transport, cotransport or antiport. The theory of electrical relaxation in membranes has been reviewed by Läuger et al. (1981) and has been applied to many different experimental measurements of ion transport in lipid bilayers. This theory has been recently used to calculate the relaxation of ionic currents produced by the Na-K ATPase (Apell, 1989). The basic hypothesis of this calculation is that the time-dependent current $I(t)$ measured in the external circuit is an *average current* resulting from the summation of individual charge translocations in the transporter molecules associated with transitions between states of the reaction cycle. This current $I(t)$, is given by the sum of the currents through each of the charged carrying steps in the transport system. It is also assumed in this calculation that the voltage step induces rapidly a constant electric field across the membrane. This is possible if the capacitive effect is rapid and if the charge density inside the membrane is much less than that on the surface. A detailed discussion of these assumptions is given in Läuger et al. (1981). In addition, it is assumed that membrane transport is much slower than diffusion through unstirred layers.

This average current is given by

$$I(t) = F[aJ_a(t) + bJ_b(t) + \ldots mJ_m(t) + nJ_n(t) + oJ_o(t) + \ldots]. \tag{34}$$

The coefficients a, b, m, n, and o are called the "dielectric coefficients" of the transitions. In the case of a homogeneous dielectric environment, they can be interpreted as the translocation of charges over a fractional membrane distance. Their values would be given by $a = z_a l_a/d$, $b = z_b l_b/d$, $m = z_m l_m/d$, $n = z_n l_n/d$ and $o = z_o l_o/d$, representing the displacement of a charge of valency z_a, z_b . . . over a distance l_a, l_b . . . perpendicular to the membrane surface; d is the membrane thickness. In general, these coefficients depend on the dielectric properties of the transport protein.

$J_a(t)$, $J_b(t)$ . . . represent the net average flux across a particular step. Since there is only one relaxation in the transport system, each of these net fluxes is given by the following equations:

$$J_a(t) = J_a(s) - [J_a(s) - J_a(0)]\exp(-t/t_a) \qquad (35)$$
$$J_b(t) = J_b(s) - [J_b(s) - J_b(0)]\exp(-t/t_a)$$

and so on for the other net fluxes.
Introducing Eq. (35) into Eq. (34), it gives

$$I(t) = I(s) - [I(s) - I(0)]\exp(-t/t_a) \qquad (36)$$

where $I(s)$ is the steady-state current and $I(0)$ is the initial current. Since in the steady state all the fluxes are equal

$$J(s) = J_a(s) = J_b(s) = \ldots$$
$$I(s) = F\,J(s)[a + b + \ldots m + n + o + \ldots] = zF\,J(s) \qquad (37)$$

where $z = z_a 1_a/d + z_b 1_b/d + \ldots$ and zF is the total charge transported across the whole membrane.

The function for $J(s)$ can be obtained from the net flux through any one of the slow reactions, because in the steady stae the net flux through any step is the same.

$$J(s) = k_{mz}N_m(s) - k_{zm}N_z(s). \qquad (38)$$

The initial membrane current $I(0)$ is given by the sum of the initial current through each of the charge carrying steps. Since each of these currents can be different, they have to be calculated separately.

$$I(0) = F[aJ_a(0) + bJ_b(0) + \ldots mJ_m(0) + nJ_n(0) + oJ_o(0) + \ldots] \qquad (39)$$

where $J_a(0)$, $J_b(0)$ . . . , $J_m(0)$, $J_n(0)$, $J_o(0)$ . . . are the initial net fluxes through each step. The initial net flux through the slow steps are given by

$$J_a(0) = k_{na}N_n(0) - k_{an}N_a(0)$$
$$J_m(0) = k_{mz}N_m(0) - k_{zm}N_z(0). \qquad (40)$$

From Eq. (12), the difference between these fluxes is obtained

$$J_a(0) - J_m(0) = A_a dN_a/dt = -A_n dN_n/dt. \qquad (41)$$

As stated above, there is also a net flux through each of the fast steps. They are represented by J_b between N_a and N_b, J_c between N_b and N_c, J_n between N_n and N_o, J_o between N_o and N_p and so on. The net flux through each of the fast steps is calculated from the time derivative of each of these states.

$$dN_a/dt = J_a(0) - J_b(0)$$
$$dN_b/dt = K_a dN_a/dt = J_b(0) - J_c(0)$$
$$\ldots\ldots \quad \ldots\ldots$$
$$dN_n/dt = -J_a(0) + J_n(0)$$
$$dN_o/dt = K_o dN_n/dt = -J_n(0) + J_o(0). \qquad (42)$$
$$\ldots\ldots \quad \ldots\ldots$$

From Eqs. (41) and (42), each of the initial net flux equation for the fast reactions can be calculated. For example, $J_b(0)$ is given by

$$J_b(0) = [J_a(0)\,(A_a - 1) + J_m(0)]/A_a. \qquad (43)$$

From these equations it is possible to determine the total initial membrane current $I(0)$. This current is more complicated to calculate than the steady-state current $I(s)$, because the initial currents at each reaction step can be different after the perturbation is applied. Binding and unbinding of charges from external media and their transport through the system produce these initial currents. In some particular cases, the form of $I(0)$ could be simplified, depending on which reaction step is carrying a charge.

Curve Fitting

Membrane transport models usually contain many unknown parameters. It is therefore necessary to compare the measured flux or current with that obtained from the model and to evaluate the unknown parameters which are usually the rate and equilibrium constants and their voltage dependence. When an analytical equation has been obtained to calculate the flux or the current, it is much easier to use standard curve fitting methods to evaluate the unknown parameters. The first step of this evaluation is to compare the flux, the uptake or the current equation, given respectively by Eqs. (31), (33) or (36), with the measured data to determine the initial and steady-state values and the time constant. Normally these measurements are performed for different substrate concentrations and/or different voltages. From these results, graphs of the initial and steady-state values and the time constant are obtained as functions of substrate concentration or voltage. These graphs can be compared with those calculated from a transport model. For example, in uptake relaxation measurements obtained at different substrate concentrations S_1, graphs of $J_{12}(s)$, $J_{12}(0)$ and t_a would be obtained as functions of S_1. Then using Eqs. (27)–(29), it is possible to determine if the data can

be reproduced with these equations and to evaluate the unknown parameters. It should be realized that not all the parameters can be evaluated by curve fitting. When products or sums of equilibrium constants always appear together, as factors of a substrate concentration, they have to be replaced by a single parameter. In the case of Eqs. (27)–(29), the group of equilibrium constants $(K_b + K_bK_c + \ldots)$ multiplying S_1 and $(K_o + K_oK_p + \ldots)$ multiplying S_2 cannot be evaluated individually. A single parameter must be used to replace each parenthesis. Also the products $k_{mz}K_bK_c \ldots$ and $k_{zm}K_oK_p \ldots$ must be replaced each one by a single parameter. But the rates of the other slow step, k_{an} and k_{na}, can be evaluated. Even with these limitations, the measurement of a single relaxation provides much more information on the rates of the transport process than steady-state measurements.

The general analytical method we have developed to calculate the time constant and the initial and steady-state transport functions are very useful to test different models on a set of experimental results. Equations (18)–(19a) and (21) provide the basic expressions to calculate explicit equations for these transport functions. It is possible to introduce these equations into a subroutine of a curve fitting program, followed by equations to calculate the flux or the current. Once this procedure is established, it remains the same for the different models. The difference between the models appears in the calculation of A_a, A_n, $A_a(*)$, $A_n(*)$, B_{an} and B_{na}, with their substrate concentration and voltage dependence. These functions can be introduced at the beginning of the subroutine, and they can be changed from one model to another. Depending on where the slow steps, substrate binding and voltage dependence are introduced in the system, these expressions could be different from one model to another. The advantage of this method is that different models can be studied with minor changes in the program. It is possible to compare the parameters obtained by curve fitting for different position of the slow steps in the cycle and also for different number of reaction steps. If the transport model contains more than one closed cycle of reactions, each cycle should be treated separately.

Discussion

The theoretical framework proposed in this paper gives the possibility to calculate analytically a single relaxation from an arbitrarily complex transport model. The basic assumption is that there are two reaction steps in a closed system that are slower than all the others. The fast reactions reach their equilibrium state immediately after the application of a perturbation which is either a voltage or a substrate concentration change. The states of these fast reactions are related to each other by their equilibrium constants and are redistributed among themselves after the perturbation. This procedure provides the initial conditions for the slow reactions. The whole system is therefore described by four slow rate constants and many equilibrium constants. By selecting two likely slow steps, analytical expressions are obtained for the steady state, the initial amplitude and the time constant of the membrane flux. The rationale behind this calculation is that in many experimental flux or current measurements it is not possible to determine more than one relaxation. By comparing those equations with available data, it is possible to determine the rate and equilibrium constants using standard curve fitting programs. The advantage of such calculations is to provide a more detailed description of a transport system as compared with the steady-state treatment. It is possible to use numerical integration to obtain the time-dependent solutions of a transport system and to compare the calculations with the data. When the rate constants are not known, this method requires much trial and error calculations to obtain a reasonable fit with the data. When experimental results show a single time constant, our analytical treatment could be used to compare the flux or current measurements to those calculated from a model and to determine the rates of the slow steps, using curve fitting programs. Afterwards, numerical integration could be use to determine the accuracy of the analytical calculation.

An important aspect of our method is the selection of the slow steps in the transport model. From the type of relaxation observed, whether the flux or current increases or decreases toward the steady state, it is possible to reduce the number of possible configurations of slow and fast steps to be studied. In a complex transport system, it would not be useful to try all the possible configurations of slow and fast steps; for example, a six-state model has 15 possible configurations. It is possible that in certain cases the substrate concentration dependence of the equations is different depending on the position of the slow steps, but many different choices could lead to similar calculated results. It should be emphasized that relaxation measurements are a refinement in the study of a membrane transport system which has to be characterized first in its steady state to determine its general properties. Once the configuration of the transport system is more or less determined and some indication of the rates of the various steps is

available, relaxation measurements should be performed to determine more precisely those rates, using our analytical treatment.

The relaxation of current calculation was made to be applied to transporters rather than channels. It is possible to perform a calculation to describe a single relaxation of the macroscopic current in a population of channels using the same approach. The kinetic system can be used to describe the average number of open and closed states and to calculate the time dependence of the average number of open channels, $N_{open}(t)$. The current relaxation is given by this function multiplied by the single-channel current. Such a calculation will be useful if the measured current shows a single relaxation and if there is evidence that there are more than three channel states. For example, in the Figure, the state N_n would be an open state and the other closed states. Agonist activation of the channels could be fast reactions, while the voltage-dependent activation could be slow or vice versa. This approach was used by Gunning and Ciani (1983) to calculate a single voltage-dependent relaxation of the average current in a three-state system with one open and two closed states.

It is possible that relaxation measurements give more than one time constant. If two of them are measured, our approach can still be used but instead of two slow steps, three will be required. In that case the transport system can be reduced to two differential equations to be solved instead of one. It is possible to solve analytically such a system, although the solutions are more complex expressions of the rate and equilibrium constants. It seems therefore that the approach proposed in this paper to obtain approximate analytical solutions to describe relaxations in membrane transport has many possibilities of applications, and the generalized method to calculate them should make its use more accessible.

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