Voltage dependence of the carrier-mediated ion transport

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With regards to the common features of carrier-mediated transport, voltage dependence was studied, using an asymmetric, six-state model. Our study shows that for an ion exchanger, transporting one kind of ion via exchange with another kind, the ion flux as a function of the membrane potential shows a sigmoidal curve with a shallow slope, saturation behavior, and possibly a negative slope. These features are mainly due to the transport of ions with charges of the same sign in the opposite direction. Membrane potential depolarization can facilitate only one transport and hinder another. As a result, the ion flux cannot increase dramatically and has an upper limitation because the exchanging rate depends on competition of the two inversely voltage-dependent transport processes. In contrast, for unidirectional ion transporters, the ion flux will monotonically increase as a function of the membrane potential. Both the maximum ion flux and the voltage sensitivity are much higher than those of the ion exchanger.

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I. INTRODUCTION

In the living system, many proteins reside in cell membranes. They function as carriers to transport ions across the cell membrane. The underlying mechanisms involved in these transport systems are not diffusion, but the transporter's conformational change. Some of them consume ATP molecules; some do not. Some of them carry ions out of the cells, some of them bring ions into the cell, and some of them transport one kind of ion by exchanging another kind of ion. These carrier-mediated transporters in general are sensitive to the membrane potential, as they involve movement of ions. The voltage dependence of some of these transporters has been well studied, such as the Na/K pump molecules. Others are difficult to experimentally measure, such as those in the membrane of intracellular organelles. In this paper, we will discuss the general features of their voltage dependence.

The structure and function of these transporters may differ significantly from each other, but all share some common features. First, function of these transporters is generally envisioned as a loop [1–3]. There are one or two ion-translocation limbs in the loop depending on the transporter's functions. Because there is a charge associated with the transported ions, these ion translocations are inevitably sensitive to the membrane potential. The voltage dependence of each ion translocation depends on the transport direction with respect to the membrane potential. Second, for an ionic exchanger which transports two different ions in opposite directions, the two ion translocations may have opposite voltage dependence. Any potential change in the membrane, either depolarization or hyperpolarization, has reverse effects on the two opposite ion-translocation steps. The membrane potential change can only facilitate one transport, while hindering another. Finally, for those ion transporters whose whole functions are sensitive to the membrane potential, one of the voltage-dependent, ion-translocation steps must be either the rating-limit step or directly control the entrance level of the rate-limiting step. Due to these common features, ion transporters may have similar characteristics in their voltage dependence. In this paper, we will express the transport flux as a function of the membrane potential and plot the *I-V* curves in order to recover their common characteristics.

Consider an ion transporter which transports m number of ion A out of the cell by exchanging them with n number of B ions in each cycle. We can use an asymmetric six-state loop to describe the functions of this transporter without loss of generality. We attribute all of the voltage-dependent substeps in the two ion translocations into two voltage-dependent steps in the loop, respectively. Four voltage-independent steps represent other processes independent of the membrane potential, including binding and unbinding steps (Fig. 1). The binding and unbinding steps are only in a chemical reaction sense not including the related conformational change such as occlusion and deocclusion. The four-state model has been widely used to study ion transporters such as the Na/K pump molecules [3–5]. The difference between the six-state model and the four-state model is that the intermediate steps of ion binding and ion unbinding are separated. This arrangement will allow us to compare our theoretical results with currently available experimental results exploring the effects of ionic concentration on the pump's *I-V* curve. "Asymme-

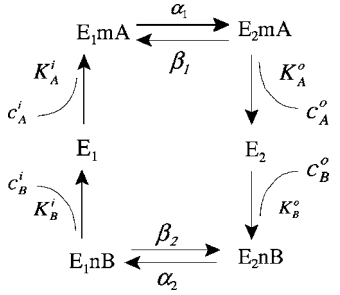

FIG. 1. A schematic drawing of a simple, asymmetric six-state model for a general carrier-mediated ion transporter.

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try" here means different ions having different binding affinities at the intracellular and extracellular sides of the membrane, respectively.

In this paper, we are studying the transporters' voltage dependence at steady state, therefore, we can simplify their kinetic differential equations to algebraic equations. The first equation describes the outwards flux, ϕ_1, for ion A as a function of forward and backward reaction rates, α_1 and β_1. The second equation describes the influx ϕ_2, for ion B as a function of reaction rates, α_2 and β_2. Since the transporter resides permanently within the membrane, the total flux must be zero, which is shown in the third equation. The fourth equation is the transporter conservation equation:

$$\phi_1 = c_{E_1 mA}\alpha_1 - c_{E_2 mA}\beta_1,$$

$$\phi_2 = c_{E_1 nB}\beta_2 - c_{E_2 nB}\alpha_2,$$

$$\phi_1 + \phi_2 = 0,$$

$$\sum_{i=6} c_i = c_{ET}.$$

The binding and unbinding processes with ions at the membrane interfaces are rapid when compared with the rates of the two ion translocations. For example, the time course for each individual step in the Na/K pump loop has been measured [17]. The results show that the two ion translocations have the slowest time courses in the loop, much slower than the binding and unbinding processes. Therefore, those membrane interface reactions can be considered to be at equilibrium represented by their dissociation constants [3–6]:

$$K^i_{mA}, K^i_{nB}, K^o_{mA}, K^o_{nB},$$

where the subscripts represent binding (unbinding) of m ions of type A ion or n ions of type B ion, and the superscripts represent the two sides of the cell membrane. Assuming that the dissociation constants for binding (unbinding) each ion are K_A and K_B, respectively, and that the binding (unbinding) process is a sequential procedure for individual ions, the corresponding dissociation constants for m ions and n ions can be expressed as follows, respectively [6]:

$$K^i_{mA} = (K^i_A)^m = \frac{c_{E_1}(c^i_A)^m}{c_{E_1 mA}},$$

$$K^i_{nB} = (K^i_B)^n = \frac{c_{E_1}(c^i_B)^n}{c_{E_1 nB}},$$

$$K^o_{mA} = (K^o_A)^m = \frac{c_{E_2}(c^o_A)^m}{c_{E_2 mA}},$$

$$K^o_{nB} = (K^o_B)^n = \frac{c_{E_2}(c^o_B)^n}{c_{E_2 nB}}.$$

Based on these equations, we can easily resolve the transport flux:

$$\phi_1 = -\phi_2 = \frac{c_{ET}(C_5\alpha_1\alpha_2 - C_6\beta_1\beta_2)}{C_1\alpha_1 + C_2\beta_1 + C_3\alpha_2 + C_4\beta_2}, \quad (1)$$

where

$$C_1 = \frac{(c^i_A)^m}{K^i_{mA}}\frac{K^i_{nB}}{(c^i_B)^n}\frac{(c^o_A)^m}{K^o_{mA}}\frac{K^o_{nB}}{(c^o_B)^n} + \frac{(c^i_A)^m}{K^i_{mA}}\frac{K^i_{nB}}{(c^i_B)^n}\frac{K^o_{nB}}{(c^o_B)^n} + \frac{(c^i_A)^m}{K^i_{mA}}\frac{K^i_{nB}}{(c^i_B)^n},$$

$$C_2 = \frac{(c^o_A)^m}{K^o_{mA}}\frac{K^o_{nB}}{(c^o_B)^n}\frac{(c^i_A)^m}{K^i_{mA}}\frac{K^i_{nB}}{(c^i_B)^n} + \frac{(c^o_A)^m}{K^o_{mA}}\frac{K^o_{nB}}{(c^o_B)^n}\frac{K^i_{nB}}{(c^i_B)^n} + \frac{(c^o_A)^m}{K^o_{mA}}\frac{K^o_{nB}}{(c^o_B)^n},$$

$$C_3 = \frac{(c^i_A)^m}{K^i_{mA}}\frac{K^i_{nB}}{(c^i_B)^n} + \frac{K^i_{nB}}{(c^i_B)^n} + 1,$$

$$C_4 = \frac{(c^o_A)^m}{K^o_{mA}}\frac{K^o_{nB}}{(c^o_B)^n} + \frac{K^o_{nB}}{(c^o_B)^n} + 1,$$

$$C_5 = \frac{(c^i_A)^m}{K^i_{mA}}\frac{K^i_{nB}}{(c^i_B)^n},$$

$$C_6 = \frac{(c^o_A)^m}{K^o_{mA}}\frac{K^o_{nB}}{(c^o_B)^n}. \quad (2)$$

The quantities above, represented by C's, are functions of the ionic concentrations and dissociation constants. They are not functions of the reaction rate for either ion-translocation step. Therefore, they are insensitive to the membrane potential. Based on Boltzmann's distribution, each reaction rate, α_s or β_s, is proportional to an exponential of the ratio of an energy difference associated with the ion translocation event over the thermal energy KT. When a potential difference, V, is applied to the cell membrane, there will be two kinds of energies involved in the transporter: the intrinsic conformational energy of the transporter, which is independent of the membrane potential, and the electric energy supplied by the membrane potential, V.

Therefore, we can consider each reaction rate as a product of two parts. The first part reflects the intrinsic energy. Because of voltage independence, this part for all α_s and β_s can be attributed to the corresponding parameters, C's, respectively, in Eq. (1). For active transporters, such as the Na/K pumps, the energy provided by ATP hydrolysis belongs to this intrinsic energy. The energy value is constant and is independent of the membrane potential. The second part reflects the effects of the membrane potential, which can be expressed as follows [7,8]. Through these arrangements, both passive and active transport systems are covered in the model without loss of generality:

$$\alpha_1 = e^{A_1 V},$$

$$\beta_1 = e^{-B_1 V},$$

$$\alpha_2 = e^{-A_2 V},$$

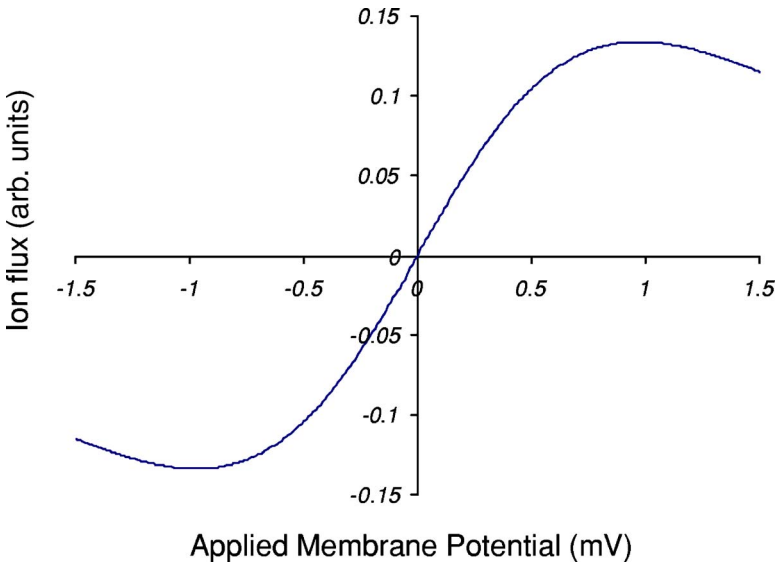

FIG. 2. (Color online) Trends of the ion flux versus membrane potential for ion exchangers. The ordinate is ion flux with an arbitrary unit and the abscissa is the applied membrane potential also with an arbitrary unit. The origin of the abscissa means at the membrane resting potential.

$$\beta_2 = e^{B_2 V}, \tag{3}$$

where the parameters represented with A's and B's are functions of the number of ions transported and the energy barriers involved in ion transport. It is necessary to point out that the ions A and B are moved in opposite directions, so that their corresponding reaction rates have opposite signs in the exponential.

By substituting Eq. (3) with Eq. (1), we get

$$\phi_1 = C_{ET} \frac{C_5 e^{(A_1 - A_2)V} - C_6 e^{-(B_1 - B_2)V}}{C_1 e^{A_1 V} + C_2 e^{-B_1 V} + C_3 e^{-A_2 V} + C_4 e^{B_2 V}}. \tag{4}$$

Equation (4) describes transport flux as a function of the membrane potential. Based on this equation, and by making some assumptions for each transport system, we can predict the voltage dependence of the transport flux.

A. Case 1: Ion exchanger

In order to represent the movement of two kinds of ions in opposite directions, both of the ion-translocation steps have to exist in the loop. The transport flux is expressed as Eq. (4). The denominator is a weighted summation, where the parameters in the exponential are A_1, B_1, A_2, and B_2, respectively. In contrast, the numerator is a weighted subtraction, and the parameters in the exponential are also subtractions, $(A_1 - A_2)$ and $-(B_1 - B_2)$, respectively.

Even without any detailed information, we can discuss the trends of the transport flux as a function of the membrane potential or the I-V curve of the transporter. Consider a simple situation. Assume that in each ion-translocation step the forward and backward reaction rates have the same value, $A_1 = B_1$, $A_2 = B_2$, respectively. This assumption is, in general, correct if the ion moving across the cell membrane is only under a membrane potential difference. That is because an electric field always applies energies of the same value but reverse signs to the opposite ion movements. If the transporter's conformational change is also involved, please see the application section.

We can also consider a simple situation in which all of the coefficients, C's, are the same in the numerator and denominator, $C_5 = C_6 = C_4 = C_3 = C_2 = C_1 = C$. Later, we will show that both our theoretical study and other experimental results show that changing the value of these parameters will affect only the detail of the I-V curve and not its trends. As a first step to study the trends of voltage dependence, this assumption is reasonable. When we study the detailed I-V curve for a specific kind of transporter, this assumption will be eliminated. With these assumptions, we have

$$\begin{aligned}\phi_1 &= c_{ET} C \frac{e^{(A_1 - A_2)V} - e^{-(A_1 - A_2)V}}{e^{A_1 V} + e^{-A_1 V} + e^{-A_2 V} + e^{A_2 V}} \\ &= \frac{\dfrac{e^{(A_1 - A_2)V} - e^{-(A_1 - A_2)V}}{2}}{\dfrac{e^{A_1 V} + e^{-A_1 V}}{2} + \dfrac{e^{-A_2 V} + e^{A_2 V}}{2}} = \frac{\sinh (A_1 - A_2)V}{\cosh A_1 V + \cosh A_2 V}.\end{aligned} \tag{5}$$

The numerator is a $\sinh x$ function, while the denominator is a summation of two $\cosh x$ functions. The $\cosh x$ function has an upside-down bell shape, having a minimum value when the variable $x = 0$. The value of $\cosh x$ monotonically increases when x moves away from $x = 0$. In the numerator, $\sinh x$ monotonically increases from the third quadrant to the first quadrant as a function of x. The ratio of $\sinh x$ over $\cosh x$ is a sigmoidal curve. The value increases when x increases and reaches saturation, and conversely decreases when x decreases, reaching a negative saturation.

Let us assume that $A_1 = B_1 = 2$, $A_2 = B_2 = 1$, and $C_5 = C_6 = C_4 = C_3 = C_2 = C_1 = C = 1$. We can then plot the transport flux, Eq. (5), as shown in Fig. 2.

The curve has a sigmoidal shape. The characteristics of this I-V curve can be described as follows: First, the slope of the curve is very shallow, which indicates a low sensitivity of the transport flux to the membrane potential. Second, when the membrane potential is largely depolarized, or the membrane potential, V, is significantly increased, the I-V curve

becomes saturated, showing a plateau. Finally, when the membrane potential is further depolarized, the curve starts to decrease so that the slope becomes negative.

All these characteristics mainly result from competition between the two opposite voltage-dependent transitions, which is reflected by A_1-A_2 and B_1-B_2 in the numerator. Let us assume that the first ion-translocation step is a rate-limiting step having the slowest reaction rate, and that a membrane potential depolarization accelerates this step. Then, the membrane depolarization will decelerate the second ion translocation step, making this step slower because of movement of ions in the opposite direction. Due to the first translocation being the rate-limiting step, the whole transport flux increases. However, when the membrane potential is depolarized to a specific value, the time needed for the two ion-translocations becomes comparable. The acceleration in the first step will be compensated by the deceleration in the second step. Therefore, the membrane potential depolarization can no longer increase the whole transport flux, resulting in the fact that the *I-V* curve shows a saturation behavior. If the membrane potential is continuously depolarized, the second ion-translocation step becomes the rate-limiting step. As a result, the whole transport flux decreases, showing a negative slope of the *I-V* curve.

It is necessary, however, to point out that although we only considered the simplest situation, the results of a sigmoid shaped *I-V* curve have general consequences. In fact, changing the values of parameters of *C*'s and the values of A_1, B_1, A_2, and B_2 will only change the details or the parameters of the sigmoid curve, for example shifting the curve or changing the slope. The curve will remain sigmoidal in shape.

B. Case 2: Unidirectional ion transport

For these transporters, ions are transported across the cell membrane in one direction. The ions may be transported by exchanging nonionic molecules, such as glucose or some form of nutrient. Therefore, there is only one voltage-dependent step in the loop, $A_2=B_2=0$. For a simple case $C_3=C_4=0$, we have

$$\phi_1 = c_{ET} C \frac{e^{A_1 V} - e^{-A_1 V}}{e^{A_1 V} + e^{-A_1 V}} = \frac{\sinh A_1 V}{\cosh A_1 V}. \tag{6}$$

Here we assume that the energy barriers for the forward and backward reactions of the ion transition steps are the same, $A_1=B_1=1$. The flux ϕ_1 in Eq. (6) can be plotted as in Fig. 3.

The flux is monotonically increased as the membrane potential increases. At a membrane potential close to zero, the *I-V* curve has the steepest slope. When the membrane potential increases, the curve gradually becomes shallower. Indeed, when the membrane potential is large enough, the slope can become very small, but can never reach a plateau. There exists no negative slope in the *I-V* curve. By comparing Figs. 3 and 2, we realize that the maximal slope of the curve in Fig. 3 is about 0.8, which is much larger than that shown in Fig. 2, only 0.2. Therefore, the voltage sensitivity for the unidirectional ion transporter is much higher than that for the ion exchanger. In other words, the same membrane potential depolarization will generate a much larger ion flux for a unidirectional ion transporter than that for the ion exchanger. In addition, the possible maximal current of the unidirectional ion transporter is about 1 arbitrary unit, which is much larger than that of the ion exchanger, which is only 0.14 arbitrary units.

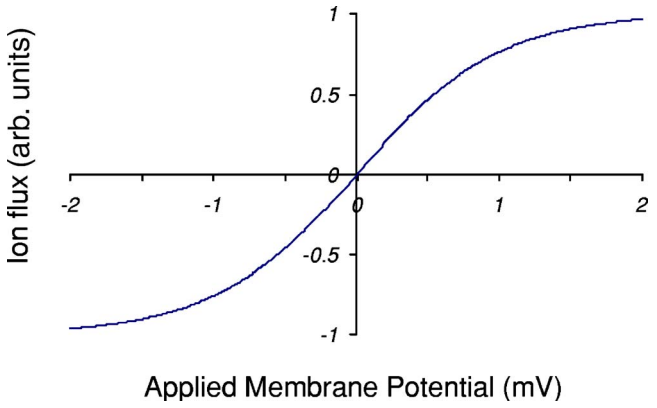

FIG. 3. (Color online) Trends of the ion flux as a function of membrane potential for unidirectional ion transporters. The abscissa and ordinate are the applied membrane potential and ion flux, respectively, with arbitrary units. The origin of the abscissa means at the membrane resting potential.

Again, we have discussed only the simplest situation, but the results do not lose generality. Changing the parameter *C*'s and A_1 and B_1 will only modify the details of the sigmoid curve but will not change the sigmoid shape.

To summarize, the characteristics of the voltage dependence of an ion exchanger and of unidirectional ion transporter can be concluded as follows:

(1) The voltage dependence, or the slope of the *I-V* curve, of the unidirectional ion transporter is much higher than that of the ion exchanger.

(2) The possible field-induced transport flux, or the current, of the unidirectional ion transporter can be much larger than that of the ion exchanger.

(3) The transport flux, or the current, of a unidirectional ion transporter monotonically increases as the membrane potential increases. The slope gradually becomes small, but can never reach zero or negative. Rather, saturation behavior, or a plateau, and possible negative slope are all characteristics of the voltage dependence of the ion exchanger.

II. APPLICATION

We will now use Na/K pump molecules as an example to discuss their voltage dependence. To do so, detailed information of each reaction rate, α_1, β_1, α_2, and β_2, is needed. Let us consider three procedures involved in transport of either Na or K ions across the cell membrane: binding access channels or "ion wells," proteins' conformational changes, and releasing access channels or "ion wells" [9–13]. We assume apportionment factors *a*, *r*, and *b*, which represent partial membrane potential change, *aV*, *rV*, and *bV* affecting the three steps, respectively. In terms of proteins' conformation

change, we can further define an apportionment factor, h. Membrane potential hrV affects the reaction rates from state E_1 to E_2. The rest of the portion, $(1\text{-}h)rV$, influences the reaction rates from state E_2 to E_1. If the pump molecule's conformational change is independent of membrane potential, $r=0$, or has the same apportionment factor, $h=0.5$, the exponential parameter in α_1 will be the same as that in β_1, except for having a negative value. This is again a simple situation, like the one we have discussed in case 1, $A_1=B_1$, $A_2=B_2$.

Considering that the stoichiometric ratio of the Na/K pump molecules is 3:2 [14–17] and that the thermal molar energy is equivalent to 26 mV at temperature or 30 °C, we have

$$\alpha_1 = D_1\exp\{[3a + (3+z)hr + 3b]V/26\},$$

$$\beta_1 = D_2\exp\{[-3a - (3+z)(1-h)r - 3b]V/26\},$$

$$\alpha_2 = D_3\exp\{[-2a - (2+z)(1-h)r - 2b]V/26\},$$

$$\beta_2 = D_4\exp\{[2a + (2+z)hr + 2b]V/26\},$$

where z stands for the intrinsically charged particles moved by the pump molecules during the conformation changes. Let $a=b=\frac{1}{5}$, $r=\frac{3}{5}$, and, $z=-2$ [10]. Substituting these reaction rates into Eqs. (3) and (4), we have

$$\phi = C_{ET}\frac{C_5\exp[(0.4+0.6h)V/26] - C_6\exp[(-1+0.6h)V/26]}{C_1\exp[(1.2+0.6h)V/26] + C_2\exp[(-1.8+0.6h)V/26] + C_3\exp[-0.8V/26] + C_4\exp[0.8V/26]}. \quad (7)$$

The ionic flux can be plotted as a function of the membrane potential, V, as shown in Fig. 4.

When the membrane potential is depolarized, the pump flux increases and finally reaches saturation at a membrane potential around zero. When the membrane potential is hyperpolarized, the pump flux decreases and reaches zero. This predicted sigmoidal curve is very similar to the experimental results from the Na/K pump molecules [18–21]. Figure 5 shows the measured I-V curve of the Na/K pump in skeletal muscle fibers [21]. On changing the values of the parameters represented by C's, the slope of the I-V curve and the regions of saturation will change, but the curve retains a sigmoidal shape. As expressed in Eq. (2), the C's are functions of ionic concentration gradients and dissociation constants. Nakao and Gadsby have found that varying the concentration of extracellular K or intracellular Na merely leads to an up- or down-scaling of the I-V curve without appreciably changing the shape of the sigmoidal curve [22].

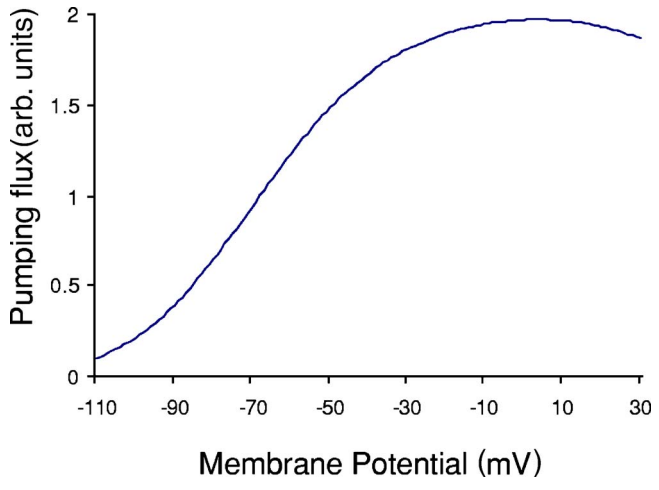

FIG. 4. (Color online) A plot of the predicted ion flux of the Na/K pump versus the membrane potential. The apportionment factor, h, is 0.8, where a membrane holding potential of −90 mV has been considered.

III. DISCUSSION

In this paper we present our results of the study of voltage dependence of the carrier-mediated ion transporter at steady state. We started from a general six-state model without focusing on any specific proteins. We found that for an ion exchanger that transports two kinds of ions in opposite directions, the transport flux as a function of the membrane potential shows a sigmoid shaped I-V curve with saturation behavior and a possibly negative slope at large membrane potential depolarization. For the unidirectional ion transporter, the transport flux is monotonically increased as the

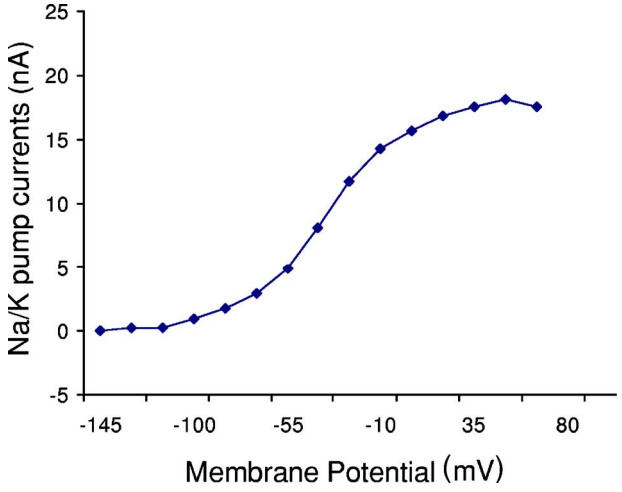

FIG. 5. (Color online) I-V curve of the Na/K pumps obtained from skeletal muscle fibers.

membrane potential is depolarized. When the membrane potential is largely depolarized, the slope of the *I-V* curve can become very small but it will never show a negative slope.

While applying our results to the Na/K pumps, the predicted *I-V* curve is consistent with both the experimentally measured *I-V* curve and the results previously obtained by Lauger and Apell in the study of Na/K pumps [9].

A. Ion transporters versus ion channels

The underlying mechanisms involved in carrier-mediated transport are different from those of the ion channels. Diffusion helps the movement of ions through the ion channel. In contrast, the ion transporter-assisted movement of ions across cell membrane is not by diffusion but is mediated by the transporter's conformational changes. Because it involves different mechanisms, the ion channel and transporter have different voltage dependence and, hence, different *I-V* curves.

For ion channels, when the membrane potential is largely depolarized or when the channels are fully opened, the *I-V* curve generally shows a straight line, indicating a constant channel conductance. The channel currents do not show saturation behavior. This has been approved both by the macroscopic measurements showing a straight line *I-V* curve when the membrane potential is far beyond the channel's open threshold and microscopic measurement using single channel recording techniques, showing the all-or-none feature of channel currents. An ion transporter does not have this feature.

B. Four-state model versus six-state model

A similar model has been widely used to study the Na/K pump molecules. For example, many papers have been published by using a four-state model to study the functions of the Na/K pump molecules [4,5]. In this study, we purposely used the six-state model, where the intermediate steps of ion binding and unbinding are explicitly included in order to study the effects of changing the ionic concentration and the dissociation constant on the trends of voltage dependence. Our results predicted that changing these values will only modify the details of the *I-V* curve but not the sigmoidal shape.

C. Passive versus active transporter

The purpose of this study is to investigate the general trends of the voltage dependence of the ion transporter. The results suit both the passive and the active transporters. Though there is no explicit step regarding energy source in the six-state model, the energy provided either by hydrolysis ATP or other chemical potential has been considered. Because these energy sources are constants for individual transport systems and insensitive to the membrane potential, they have been included in the first part of the reaction rates, α and β, and attributed to the corresponding parameters C's, in Eq. (4). In other words, utilizing energy in ion transportation does not affect the transporter's voltage dependence as long as the energy process is not sensitive to the membrane potential.

However, there is an implicit assumption in our derivation. We assume the energy process cannot be the rate-limiting steps. This assumption is satisfied for most situations. For example, in the Na/K pump, ATP hydrolysis is much quicker than the ion-translocation steps [17]. In order to study the voltage dependence, we only have to focus on two kinds of steps—those which are sensitive to the membrane potential and those which have the slowest time courses. Therefore, the energy source is generally not specified in the cycle. This has been widely used in many studies [3–5]. However, in order to obtain details of the *I-V* curve for a specific transporter such as the location of the plateau and the value of the slope, the energy source must be included.

D. Saturation behavior and negative slope mainly due to competition of two opposing ion transports

One of the distinguishing characteristics of the ion exchangers' voltage dependence is their saturation behavior and possible negative slope when the membrane potential is largely depolarized. What is the fundamental mechanism behind this characteristic?

One possible explanation is due to the transporters' molecular basis. Like ion channels, due to the fact that the size of the channels' narrowest pore is determined by the molecular structure, ion permeation rate through channels is limited due to this molecular basis.

For an ion exchanger, the binding site and binding affinity are determined by the molecular structure. However, this molecular basis cannot be used to explain the saturation of the ion exchanger. First, the results both predicted in this paper and experimentally proven are that the saturation behavior occurs only when the membrane potential is largely depolarized and not when the ionic concentrations are increased. Second, it is well known that the stoichiomietric numbers of the Na/K pumps remain constant throughout a wide range of membrane potential. In other words, neither membrane potential change nor ionic concentration change can affect the pump molecules binding with three Na and two K ions. Therefore, the saturation behavior and the negative slope cannot be attributed to the saturation of binding ions and binding sites in the transporter.

Indeed, the reaction rates α and β depend on the molecular structure. Therefore, the saturation behavior of the ion exchanger might be due to the limited values of the reaction rates. For example, for a unidirectional ion transporter in which there is no electrical competition, the slope of the *I-V* curve will become smaller and smaller when the membrane potential is depolarized, as shown in Fig. 3.

However, by comparison of the two *I-V* curves, saturation of the ion exchanger (Fig. 2) occurs much sooner than that of the unidirectional ion transporter (Fig. 3) in response to the membrane potential's depolarization. For the ion exchanger, the transport flux is saturated to 0.14 arbitrary units at a membrane potential of 0.8 arbitrary units, where the unidirectional ion transport flux keeps increasing until reaching 1 arbitrary unit at an infinite potential. Clearly, the saturation

of the ion exchanger's flux is not the same as that of the unidirectional ion transporter. This early-coming saturation of the ion exchanger cannot be explained by the limited value of the reaction rates.

Due to the fact that any membrane potential change, either depolarization or hyperpolarization, can only facilitate one transport but hinder another, the competition of the two ion transports inevitably influences the whole pump rate. De Weer [23] and Stein [24] estimated that 80% of energy from ATP hydrolysis in physiological conditions is required for Na/K pump to transport Na and K ions against their electrochemical potential. Clearly, any membrane potential change which alters the energy barrier to be overcome by the two ion transports will directly affect the pump rate. In addition to this, the two ion transports have the slowest time courses in the pump loop [17]. Experiments also showed that there is no significant difference between the two transports even though the Na transport is the rate-limiting step [17]. When a membrane potential depolarization accelerates the Na transport and decelerates the K transport, soon the time courses for the two transports becomes comparable. As a result, further membrane potential depolarization can no longer increase the ion flux. Instead, it will decrease the pump current. Therefore, this electrical competition is the primary reason generating this current limitation or the sigmoidal shaped *I-V* curve for the ion exchanger.

E. Significance

This study, on the basis of a general six-state model, predicts the voltage dependence of the carrier-mediated ion transporter without focusing on any specific proteins. This study shows general trends of the transport flux as a function of membrane potential for both an ion exchanger and a unidirectional ion transporter. Except for the Na/K pump, many transporters, such as those found within the membranes of intracellular organelles, are difficult to be experimentally characterized. This study provides some insight into the mechanism involved in their voltage dependence.

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