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A permeation theory for single-file ion channels: Corresponding occupancy states produce Michaelis–Menten behavior

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A kinetic theory is proposed for permeation in open ion channels. Within the model, the selectivity filter of the ion channel contains either n or $(n-1)$ single-file ions. Association and/or dissociation of the nth ion from the selectivity filter is the rate limiting step. Ion translocation occurs via concerted single-file motion of the $(n-1)$ ions and any water molecules contained within the selectivity filter. This concerted motion is assumed to be sufficiently rapid that translocation is not rate limiting. Ion permeation is thus sorption-limited, and the reduced permeation rate j^* is a universal function of the reduced concentration c^*, taking the form of a Langmuir adsorption isotherm. A corresponding occupancy-states explanation is thus provided for the Michaelis–Menten kinetics observed experimentally for many ion channels. Published experimental data for cation conduction in the open pores of the KcsA and gramicidin A channels are shown to exhibit the universal saturating behavior predicted by the theory. Additional validation of the theory by asymmetric-solution and tracer counterpermeation experiments is also discussed. © *2002 American Institute of Physics.* [DOI: 10.1063/1.1522709]

I. INTRODUCTION

The cytoplasmic membranes of biological cells provide a barrier between the interior of the cell and its surroundings, establishing the difference between ‘‘self’’ and ‘‘not-self’’ at a cellular level. Formed by a lipid bilayer, they are generally impermeable to water and most water-soluble ions. Ion channels are membrane-spanning proteins that catalyze transfer of ions into and out of the cell. They thus provide the basic conductive mechanism in biology, and are intimately involved in most forms of intercellular signaling. Ion channels possess the apparently paradoxical ability to conduct ions extremely rapidly while simultaneously maintaining high selectivity for a single ion species under physiological conditions.[1,2]

In their open state, ion channels form an aqueous pore capable of transporting ions and water molecules across the bilayer. The selectivity filter of the pore allows specific ions and water molecules to pass in a single-file arrangement.[1,2] Recently, a permeation theory was developed for permeation of tracer-tagged particles in single-file systems of finite extent.[3] This theory is based on a site-based model of adsorption and diffusion, and has been shown to be consistent with a matrix formulation of continuum diffusion theory for tracer-labeled particles. In long files, the self-diffusivity is inversely proportional to file-length and is proportional to intrafile diffusion limitations. However, for short files, correlations between the adsorbed particles make the self-diffusivity sensitive to sorption limitations. As the selectivity filters of ion channels are short (of order nanometers), sorption limitations might play a significant role in ion channel permeation.

Sorption limitations occur when transport of ions across the interface between the bulk aqueous phase and the partially dehydrated environment of the channel selectivity filter (or vice versa) is rate limiting. Continuum ion channel permeation theories have traditionally used partition coefficients (or similar concepts) to relate the concentrations at the boundaries of the two ‘‘phases.’’[1] Thus, they implicitly assume that sorption limitations are absent, and that electrodiffusion in the pore of the channel is the rate-controlling kinetic process.

Traditional site-based models of ion channels[1] do not make this assumption because adsorption and desorption events are explicitly included. However, like other single-file models,[3,4] they assume that particle transport is achieved via particle-vacancy exchanges. Thus, in single-file ion channels, either the ‘‘vacancy’’ is a water molecule (violating the single-file requirement), or a long-lived ion-sized void (which is expected to have a substantial formation energy in the aqueous channel). Another problem with traditional site-based models of ion channels is that they have too many adjustable parameters. As a result, the relationship between ion channel structure and model parameters is unclear.[1,5,6] In recent years, the relative merits of traditional continuum electrodiffusion and site-based models of ion channels have been debated at considerable length in the ion channel literature.[6,7] Subsequently, reviews of preferred modeling techniques for ion channels have omitted any detailed discussion of site-based models.[8,9]

The stated goal of ion channel permeation modeling is to develop a theoretical framework that relates structural information to the observed properties of the channel; provides an understanding of the underlying physical processes; and can

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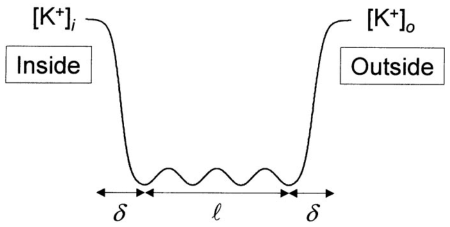

FIG. 1. A schematic representation of the host–guest potential for a pair of ions in the selectivity filter of the KcsA ion channel (with no voltage difference between the inside and outside ends), based on the (frozen) crystallographic distribution of K^+ ions (at 20 and 200 mM) reported by Morais-Cabral *et al.*[14]. In the proposed permeation theory, the model channel is conceptually divided into a central region ℓ and two end regions δ (where $2\delta+\ell=1$).

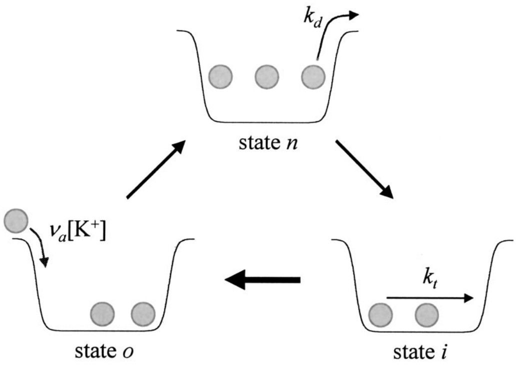

FIG. 2. Proposed *n*-occupancy permeation mechanism. Ions can only leave the selectivity filter when it contains *n* ions. The association step $(o\rightarrow n)$ is rate controlling at low concentrations and high voltages $(c^*\ll 1)$. The dissociation step $(n\rightarrow i)$ is rate controlling at high concentrations and low voltages $(c^*\gg 1)$. The translocation step $(i\rightarrow o)$ is assumed to be fast compared with the other steps. All steps are reversible.

make predictions that can be tested experimentally.[8] While this goal is hardly satisfied in most of the current model studies of ion channels,[8] it is the aim of the present article to present a first-order approximation that meets these goals while avoiding problems associated with traditional continuum and site-based models of ion channels.

In what follows, a kinetic theory is presented that is based on the common structural features of the KcsA and gramicidin A (gA) channels. The universal scaling predictions of the theory are then tested by comparison with KcsA experiments reported by Meuser *et al.*[10] and gA experiments reported by Busath *et al.*[11] The physical implications of the theory are then discussed and further experimental tests of the theoretical predictions are suggested.

II. PERMEATION THEORY

Recently, there has been much interest in the potassium-selective channel encoded by the kcsA gene from *Streptomyces lividans*. This channel is believed to be similar to other K^+ channels including voltage dependent K^+ channels in vertebrates and invertebrates; Ca^{2+}-activated K^+ channels in vertebrates; K^+ channels from plants and bacteria; and cyclic nucleotide-gated cation channels.[12] Permeation experiments, investigating the ion conduction and selectivity properties of KcsA, have confirmed it as a realistic model for ion permeation in eukaryotic K^+ channels.[13]

Figure 1 shows a schematic host–guest potential profile for a single-file channel based on x-ray analysis of the distribution of K^+ ions in the selectivity filter of KcsA.[14] It has been suggested that the selectivity filter of the KcsA channel (usually) contains two K^+ ions and two water molecules in a single-file arrangement. The two ions are able to move in a concerted manner between a K^+-water$-K^+-$water configuration and a water$-K^+-$water$-K^+$ configuration. Translocation between these two states is assumed to be rapid, but dissociation of an ion from the selectivity filter is thought to require that a third ion enter the selectivity filter.[14] This concerted motion hypothesis obviates the need for a long-lived high-energy vacancy within the file, and forms the basis for the $(n-1)$ ion translocation mechanism of the present theory.[15]

The fundamental structural approximation of the proposed theory is that the selectivity filter can be (conceptually) divided into a central region of width ℓ, and two end regions of width δ (Fig. 1). The selectivity filter always contains at least $(n-1)$ ions, and at most *n*, consistent with the concerted motion hypothesis for potassium in KcsA (Fig. 2).[16] An ion can only leave the selectivity filter when it contains *n* ions, and occurs with rate $k_d\theta_n$ (in the absence of a transmembrane voltage). k_d is a voltage-independent rate constant and θ_n is the probability of finding the model channel in the *n*-occupied state.

In its nonconductive state(s) the channel contains $(n-1)$ ions that can move between the *i*- and *o*-states at a rate characterized by k_t (Fig. 2). Ion translocation results from concerted single-file motion of the ions and any water molecules trapped between them, and thus does not require formation of high-energy ion-sized vacancies within the file. As no ions cross the high gradient δ-regions of the potential, translocation can be assumed to be relatively rapid, compared with transitions where an ion traverses a δ-region, i.e.; $k_t\gg k_d$ (see Appendix). Mutual electrostatic repulsion between the *n* ions is expected to raise the potential of the *n*-occupied state compared with the $(n-1)$-occupied states, thus making it conductive (as shown schematically in Fig. 2).

An ion can enter an $(n-1)$-occupied model channel if, and only if, the $(n-1)$ ions are located near the other end of the channel. Thus, the association step in Fig. 2 occurs with rate $\nu_a[S]_i\theta_o$. Entry of sorbate (or substrate) ions (S) into the selectivity filter from aqueous solution is assumed to be an essentially barrierless process that is independent of the applied voltage. ν_a is a concentration (and voltage) independent rate constant, and θ_o is the probability that the model channel is in an $(n-1)$-occupied state capable of accepting another ion from the interior solution, as shown in Fig. 2.

In the presence of a transmembrane voltage *V*, the ion permeation rates across the δ-regions of the model channel are given by

$$J=\nu_a[S]_i\theta_o-k_de^{-\phi}\theta_n \quad \text{and} \quad J=k_de^{\phi}\theta_n-\nu_a[S]_o\theta_i. \tag{1}$$

The transmembrane voltage increases the dissociation rate at the outside end of the model channel by a factor of e^{ϕ}

(where $\phi = ze_o \delta V/kT$), because the electric field does $ze_o\delta V$ work on the system as the ion dissociates from the channel. At the inner end of the selectivity filter, the dissociation rate is decreased by a factor $e^{-\phi}$, because the system must do electrical work when an ion dissociates from this end of the model channel. Hence, as long as the energy of the system increases, the dissociation step is assumed to be exponentially dependent on the magnitude of the energy change (at fixed temperature). In contrast, as long as the energy of the system decreases, the association step is assumed to be independent of the magnitude of the total energy change (at fixed temperature).[3,17,18] Thus, within the proposed theory, the association step is assumed to occur with rate $\nu_a[\mathrm{S}]_i\theta_o$, independent of the transmembrane voltage.[19]

As the selectivity filter must always contain either $(n-1)$ or n ions, the probability normalization criterion can be written as

$$\theta_i + \theta_o + \theta_n = 1. \tag{2}$$

A. Symmetric concentrations

When the model channel is exposed to symmetric bathing solutions ($[\mathrm{S}]_o = [\mathrm{S}]_i = [\mathrm{S}]$), Eqs. (1) and (2) can be solved simultaneously, at steady state, to find θ_n, which is given by a Langmuir adsorption isotherm,

$$\theta_n = \frac{c^*}{1+c^*}, \tag{3}$$

where the reduced concentration[20] is given by

$$c^* = [\mathrm{S}]/K_M. \tag{4}$$

The voltage-dependent Michaelis–Menten coefficient is given by

$$K_M = (e^{\phi} + e^{-\phi})k_d/\nu_a = K_d \cosh\phi, \tag{5}$$

where $K_d = 2k_d/\nu_a$ is a voltage-independent equilibrium dissociation constant. It is an important feature of the present model that no information about the rate of translocation (i.e., k_t) is necessary to derive Eq. (3) (see Appendix).

If the voltage-dependent translocation step in Fig. 2 is not rate limiting, then the $(n-1)$-occupied state probabilities are related by the Nernst equation,

$$\theta_o = \theta_i e^{\omega}, \tag{6}$$

where $\omega = ze_o \ell V/kT$, and

$$J = k_{\max}\theta_n, \tag{7}$$

where

$$k_{\max} = \frac{e^{\phi} - e^{-\phi}e^{-\omega}}{1+e^{-\omega}} k_d. \tag{8}$$

Hence, the ion permeation rate varies with concentration (activity) according to the Michaelis–Menten equation (7) at any fixed voltage. The experimentally measured permeation rate J can be divided by the maximum permeation rate $k_{\max}$, to provide a universal relationship,

$$j^* = \frac{c^*}{1+c^*}, \tag{9}$$

where the reduced permeation rate is given by

$$j^* = J/k_{\max} = \theta_n, \tag{10}$$

and c^* is given by Eq. (4).

Thus, within the model, j^* is a universal function of c^*, suggesting that ion channels might obey a "law of corresponding states,"[21] wherein the reduced permeation rate j^* is representative of the time-average occupancy state θ_n of the open channel.

B. Asymmetric concentrations

When the channel is exposed to asymmetric solutions of a single permeable species, the ion permeation rate J is once again given by Eqs. (7) and (9) and θ_n is given by Eq. (3). However, the differing concentrations break the symmetry required for j^* and c^* to be reduced by functions of voltage alone. In the asymmetric-solution case j^* is given by Eq. (10) but $k_{\max}$ is given by

$$k_{\max} = \frac{e^{\phi}[\mathrm{S}]_i - e^{-\phi}e^{-\omega}[\mathrm{S}]_o}{[\mathrm{S}]_i + e^{-\omega}[\mathrm{S}]_o} k_d, \tag{11}$$

and c^* becomes a voltage-weighted average (reduced) concentration,

$$c^* = \frac{[\mathrm{S}]_i + e^{-\omega}[\mathrm{S}]_o}{(1+e^{-\omega})K_M}. \tag{12}$$

For a specified concentration difference, the voltage V_{rev} at which the current is zero is given by

$$V_{\mathrm{rev}} = \frac{kT}{ze_o}\ln\frac{[\mathrm{S}]_o}{[\mathrm{S}]_i}, \tag{13}$$

the Nernst equation. Thus, the proposed theory is consistent with equilibrium thermodynamics.

When the transmembrane voltage is clamped at zero volts, the permeation rate is given by

$$J = \frac{[\mathrm{S}]_i - [\mathrm{S}]_o}{[\mathrm{S}]_i + [\mathrm{S}]_o} k_d \theta_n, \tag{14}$$

and c^* becomes the average (reduced) concentration,

$$c^* = \frac{[\mathrm{S}]_i + [\mathrm{S}]_o}{2K_M}. \tag{15}$$

Equation (14) is formally identical to single-component (co-permeation in *sorption-limited* Langmuirian membranes, where the single-component flux is given by[3,17]

$$J = \frac{[\mathrm{S}]_i - [\mathrm{S}]_o}{[\mathrm{S}]_i + [\mathrm{S}]_o} k_d \theta_T. \tag{16}$$

In the previous Langmuirian theory, the schematic host–guest potential profile has a similar form to that in Fig. 1,[17] but the occupancy (θ_T) relates to that of a single binding site, whereas in the present model the occupancy (θ_n) relates to the entire selectivity filter. The number of adsorbed particles in the (electrically neutral) Langmuirian single-file system ranges from 0 to L (where L is the number of sites), whereas, in the present system, the number of adsorbed ions can either be $(n-1)$ or n, reflecting the stronger (long-

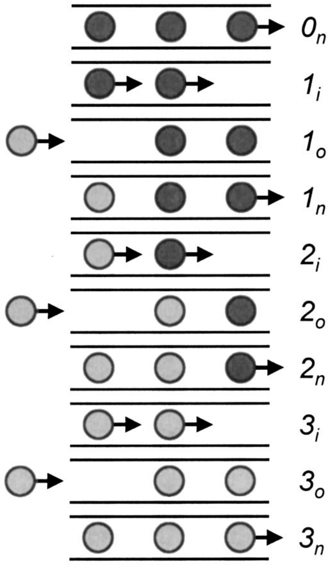

FIG. 3. Tracer counterpermeation through an $n=3$ single-file ion channel exposed to an inner solution of tracer-labeled A ions (light) and an outer solution of tracer-labeled B ions (dark). The illustrated sequence brings the channel from a BBB state (0_n) to an AAA state (3_n).

range) interactions of electrically charged systems. Thus, while the saturating behavior of both sorption-limited models is mathematically identical (with apparent Michaelis–Menten kinetics), the physical interpretations are different because the ion channel must always contain $(n-1)$ "helper ions," whereas the Langmuirian system has no minimum occupancy requirement.

C. Tracer counterpermeation

Tracer counterpermeation (TCP) occurs when the channel is exposed to antisymmetric solutions of tracer-labeled particles (pure A on the *i* side and pure B on the *o* side). With these boundary conditions (Fig. 3), the permeation rate of A-ions is given by the universal expressions (7), (9), and (10), where θ_n is the probability of finding the channel in any n-occupied state (i.e., neglecting particle labeling). j^* is now the reduced permeation rate of A-particles alone, $c^* = [\mathrm{A}]/K_M$ is the reduced concentration, and the permeation rate of tracer-labeled A-ions is given by

$$J_{\mathrm{A}} = \frac{e^{\phi} k_d \theta_n}{(1+e^{-\omega}) \sum_{m=0}^{n-1} \exp\left(\frac{-mze_oV}{kT} \right)}. \tag{17}$$

Because of the (anti)symmetry of the TCP boundary conditions, the occupancy of the 0_n state (θ_n^0) is related to the occupancy of the n_n state (θ_n^n) by

$$\theta_n^0(V) = \theta_n^n(-V), \tag{18}$$

and the ratio of the tracer-labeled permeation rates for an arbitrary electrical driving force is

$$\frac{J_{\mathrm{A}}}{-J_{\mathrm{B}}} = \exp\left(\frac{nze_oV}{kT} \right), \tag{19}$$

consistent with the result of Hodgkin and Keynes.[22]

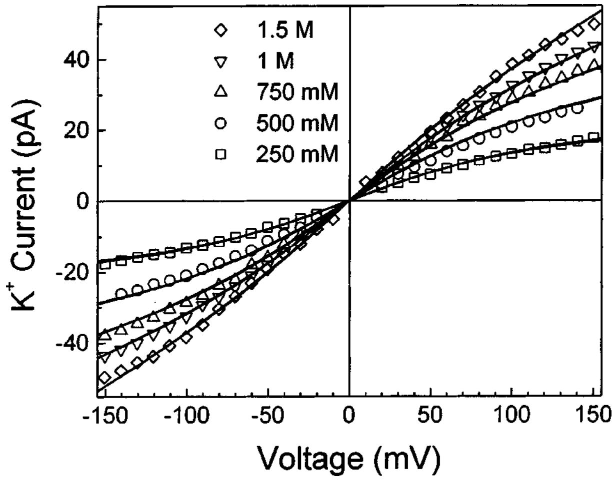

FIG. 4. Comparing theory (lines) with experiment (symbols) for K$^+$ ion permeation in KcsA under symmetric solution conditions. Fitted parameters, see Table I. The predictions of the 3- and 4-parameter models are virtually indistinguishable. Experimental data for $[\mathrm{K}^+]=250$, 500, 750, 1000, and 1500 mM are reproduced (with permission) from Meuser *et al.* (Ref. 10).

For zero applied voltage, equimolar counterpermeation results and the A-permeation rate reduces to

$$J_{\mathrm{A}} = \frac{k_d \theta_n}{2n}. \tag{20}$$

This result is consistent with the previous result for sorption-limited TCP via a compound diffusion (vacancy-mediated) mechanism (with θ_n replaced with θ_T).[3]

Thus, while the present model obviates the need for a vacancy translocation mechanism, it retains a similar formal structure at steady-state (long-times) if zero-voltage single-file permeation is sorption-limited, i.e., if the concerted translocation mechanism (cf. vacancy diffusion) is rapid compared with dissociation (desorption).

III. COMPARISON WITH EXPERIMENT

Two widely-studied channels of known structure are the KcsA K channel (discussed above) and gramicidin A.[8,9] Gramicidin A (gA) is an antibiotic, channel-forming polypeptide, which has long been considered a useful model for more complicated biological ion channels.[1,23] The schematic potential profiles in Figs. 1 and 2 are also consistent with the known structure of gA.[1] The universal j^*-c^* predictions of the theory will now be tested by comparison with published experiments for these two ion channels.

A. K$^+$ in KcsA

Figure 4 shows symmetric-solution experimental $I-V$ (current–voltage) permeation data for K$^+$ ions in open KcsA channels as reported by Meuser *et al.*[10] The lines represent a least-squares fit of the model to the experimental data with the parameters shown in Table I.

At $\theta_n=0.5$ and $V=0$ (i.e., $[\mathrm{K}^+]=K_M=K_d=630\,\mathrm{mM}$ and $(c^*, j^*)=(1, 0.5)$), the outward permeation rates associated with the 3-parameter model are $\nu_a[\mathrm{K}^+]\theta_o = 1.0\times10^8\ \mathrm{s}^{-1}$; $(k_t\theta_i \gg 1.0\times10^8\ \mathrm{s}^{-1})$; and $k_d\theta_n = 1.0\times10^8\ \mathrm{s}^{-1}$. Whereas, in the 4-parameter model, the outward

TABLE I. KcsA K^+ permeation parameters.

δ	$k_d(\mathrm{s}^{-1})$	$\nu_a(\mathrm{s}^{-1}\,\mathrm{M}^{-1})$	$k_t(\mathrm{s}^{-1})$
0.18	1.9×10^8	6.0×10^8	$\cdots$
0.16	2.6×10^8	5.9×10^8	1.9×10^9

permeation rates are: $\nu_a[\mathrm{K}^+]\theta_o = 1.3\times10^8\,\mathrm{s}^{-1}$; $k_t\theta_i = 5\times10^8\,\mathrm{s}^{-1}$; and $k_d\theta_n = 1.3\times10^8\,\mathrm{s}^{-1}$. Hence, the assumption that k_t is not a rate-limiting parameter is supported by the kinetic model at equilibrium. As shown in Fig. 4, the fitted curves are essentially unchanged when k_t is added to the model, indicating that k_t is not rate determining, and may be omitted from the model. This provides support both for the assumption that any potential barriers between the *i* and *o* configurations are low,[14] and for the schematic potential representation shown in Fig. 1.

The experimental data in Fig. 4 are reduced and replotted in Fig. 5 to show the universal j^*-c^* behavior predicted by the theory. The data for a single $I-V$ curve in Fig. 4 are spread out by the voltage dependencies of $k_{\max}$ and K_M. The points with the lowest voltages in Fig. 4 correspond to the highest reduced permeation rates and concentrations in Fig. 5, because $k_{\max}$ and K_M have their smallest values at low voltages. Within the present theoretical framework, the reduced flux j^* in Fig. 5 corresponds to the time-average occupancy state θ_n of the open ion channel [Eq. (10)].

B. Na^+ in gA

Figure 6 shows the symmetric-solution experimental gA data reported by Busath *et al.*[11] together with the least-squares fit to the theory. In these experiments the permeant ion is Na^+, and the gA channel spans a diphytanoylphosphatidylcholine (DPhPC) bilayer. The universal j^*-c^* behavior of the proposed theory appears to be a reasonable first-order approximation over the entire range of concentrations examined experimentally for the Na^+/DPhPC/gA system (with reduced concentrations ranging from about $c^*=0.4$ to about $c^*=20$). The fitted parameters are $\delta=0.22$, $k_d=3.8\times10^6\,\mathrm{s}^{-1}$, $\nu_a=8.3\times10^7\,\mathrm{s}^{-1}\,\mathrm{M}^{-1}$ so that $K_d=91\,\mathrm{mM}$.

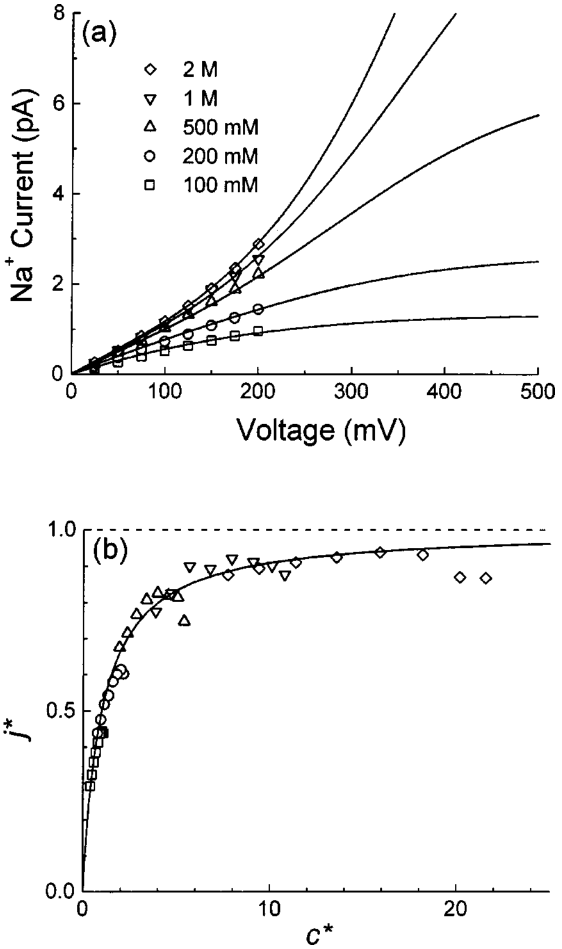

FIG. 6. Comparing theoretical predictions with experiment for permeation of Na^+-ions in gramicidin A. Symbols, experimental data reproduced (with permission) from Busath *et al.* (Ref. 11) for $[\mathrm{Na}^+]=100$, 200, 500, 1000, and 2000 mM. Lines, fitted theory. (a) Current–voltage $(I-V)$ curves. The currents increase monotonically with concentration as expected from the proposed model. (b) Corresponding occupancy-states (j^*-c^*) plot.

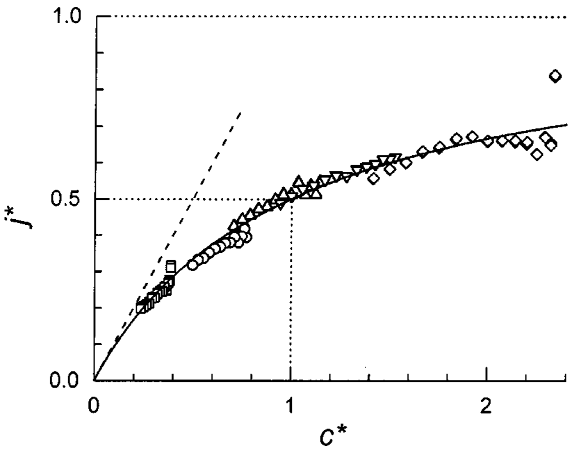

FIG. 5. Reduced permeation-concentration (j^*-c^*) relationship for the KcsA K^+ ion channel. Solid line, theory. Symbols, as in Fig. 4 [experimental data from Meuser *et al.* (Ref. 10)]. Within the proposed theory, $j^*=\theta_n$, the probability of finding the channel in the *n*-occupied state.

IV. DISCUSSION

A permeation theory has been proposed that is based on the common structural features of the gramicidin A channel and the KcsA selectivity filter (Figs. 1 and 2). Within the model, the channel provides an extended, uniform, ion-attracting region capable of holding either $(n-1)$ or n single-file ions (and water molecules). In general, the predictions of the theory depend on the rate controlling step(s), which in turn depend on the boundary conditions.

A. Association

Within the proposed model, association is a voltage-independent process. At low concentrations and high voltages (where $c^*\ll1$) the association step becomes rate limiting. According to Eq. (1), association occurs with rate $\nu_a[\mathrm{S}]_i\theta_o$, where θ_o is the probability that the channel is in a state capable of accepting another S-ion from the interior solution. At high voltages, $\theta_o\rightarrow1$, and the association rate approaches its maximum possible value of $\nu_a[\mathrm{S}]_i$. For sufficiently large voltages, the inward dissociation rate tends to zero $(k_d e^{-\phi}\theta_n \to 0)$, and the (net) permeation rate approaches a value of $\nu_a[\mathrm{S}]_i$. Thus, at high voltages (and $c^* \ll 1$) the experimentally measured current is expected to be independent of voltage, and proportional to concentration (for any of the concentration boundary conditions considered herein).

The voltage scale in Fig. 6(a) has been expanded beyond the experimental range of Busath *et al.*[11] to show extrapolation of the present model to very high voltages. Unlike the previous site-based model,[24] the present theory successfully predicts the voltage independence that is observed experimentally at high voltages and low concentrations.[25] Previous site-based models of gA[24] and KcsA,[10] include a potential barrier for the association step that produces an exponential voltage dependence at high voltages, which is qualitatively inconsistent with experiment.

B. Dissociation

The voltage dependence of the model is determined by the electrical dissociation distance δ. At a fixed value of $[\mathrm{K}^+]/K_d$, the shape of the $I-V$ curve is determined by δ. For smaller values of δ, the $I-V$ curves will level off more rapidly. For larger values of δ, the $I-V$ curves will level off less rapidly, particularly at high concentrations. At sufficiently high concentrations $(c^* \gg 1)$, $\theta_n \to 1$ and the rate of dissociation $k_d e^{\phi}\theta_n$ will become rate-limiting and the current approaches an exponential dependence on voltage (proportional to $k_d e^{\phi}$). The model thus successfully predicts [Fig. 6(a)] the experimentally-observed change to supralinear $I-V$ behavior at high $(\approx 2\,\mathrm{M})$ concentrations in gA.[25] This situation occurs as long as the association rate is not rate limiting. For intermediate voltage-scaled concentrations, the $I-V$ curves appear nearly Ohmic for low voltages (e.g., $[\mathrm{Na}^+] = 200\,\mathrm{mM}$; $V < 200\,\mathrm{mV}$, Fig. 6).

C. Translocation

Within the present theoretical framework, the Michaelis–Menten kinetics of ion channels are caused by permeation being sorption limited. Translocation of ions (and water molecules) in $(n-1)$-occupied channels is sufficiently rapid that it is not rate-limiting. This concerted single-file motion is conceptually distinct from the translocation mechanism of site-based models, because formation of long-lived voids in the aqueous pore of the channel is not required.[26] Despite this conceptual difference, the steady-state properties of the present model are similar to the sorption-limited extreme of the previous single-file theory[3] [at long times, see Eqs. (16) and (20) above]. Hence, the short-time "diffusivity" of ions in the $(n-1)$-occupied state of the channel (proportional to k_t) is not representative of the steady-state permeation rate.

D. Ion occupancy

For symmetric (and TCP) solutions, the scaling quantities ($k_{\max}$ and K_M) depend only on the identity of the system and the transmembrane voltage, but are independent of concentration. Thus, the present model provides a corresponding occupancy-states explanation for the Michaelis–Menten dependence of ion channel conductance (at fixed voltage) that has been observed experimentally for many multi-ion channels.[1] At fixed voltage, the conductance is proportional to the permeation rate, which in turn is proportional to θ_n—the probability of finding the model channel in the n-occupied state (given by a Langmuir adsorption isotherm). The open model channel contains either $(n-1)$ or n ions and thus differs from the (minimum free energy) ensemble-average number of ions by less than one ion.

The net permeation rate j^* of a single component is not sensitive to the preferred number of single-file ions n in the channel (for $n>1$) or the length of the channel (as would be expected for diffusion-limited permeation). The model is sorption-limited, and hence the permeation rate is independent of file length. Thus, if the present theory is applicable, experimental determination of the net permeation rate cannot be used to determine the value of n. Comparison of tracer counterpermeation (TCP) experiments with Eq. (17) should provide a stringent additional test of the model assumptions and predictions, and, if the present theory is applicable, should provide a macroscopic method for determining n. Comparison with Eq. (17) is preferred over Eq. (19) (the tracer-labeled flux ratio), because the form of Eq. (19) does not depend on the permeation mechanism, but only the number of ions in the channel.[22]

E. Previous ion channel models

Previous continuum models and simulations have focused on properties measured in the interior ℓ-region of the channel (such as D_{pore}).[8,9] In contrast, the present model is sorption-limited, and transport through the δ-regions is rate controlling. The mobility of ions in the pore of the channel k_t (cf. D_o in the previous model[3,17]) is *not* related to the experimentally observed permeation rate, and ion conduction is *not* controlled by the electrodiffusion equations that produce the voltage-independent conductance of Ohm's law. A diffusion formalism of the present theory utilizes a compound self-diffusivity[3] D_S, related to the sorption-limited transitions between the m_n and $(m+1)_n$ states of the channel (e.g., $1_n \to 2_n$ in Fig. 3), which occur on a timescale comparable the sorption time ($\sim 10\,\mathrm{ns}$ for K^+ in KcsA).

In light of the present theory, the failure of previous site-based models to provide meaningful physical models of the permeation process can be understood as a result of their including unnecessary adjustable parameters that did not relate to the rate-limiting steps of the permeation process. As illustrated by the 4-parameter example (discussed above), addition of just a single unnecessary parameter can significantly affect the physically meaningful parameters because of the exponential dependence on voltage (viz, δ and k_d in Table I). As the steady-state solution of the traditional 7-parameter model [that was originally fitted to the data of Figs. 3 and 6(a)[10,24]] includes the present (3-parameter) model (with $n=2$) as a special case, the 7-parameter model will always fit any finite set of experimental data more precisely. However, extrapolation of the present model at low concentrations to high voltages [see Fig. 6(a)] is qualitatively consistent with experiment,[25] whereas similar extrapolation of the 7-parameter model is not.[24,25]

F. Model validation and development

The present theory makes predictions that can be directly tested by permeation experiments. The theoretical prediction of universal j^*-c^* behavior has been confirmed experimentally for voltage-driven permeation between symmetric solutions of a single permeable species for permeation of K^+ ions in KcsA and Na^+ ions in gA. The predictions of the theory for asymmetric solutions and TCP boundary conditions rely on no new parameters except for the value of n, and thus suggest additional experimental tests of the model. If a third impermeable (and nonblocking) cation is added to the solutions, then mixed-mode permeation experiments[27] could also be conducted between solutions of the same ionic strength to test the theoretical predictions under these more general boundary conditions. The present theory has been developed for two-component permeation wherein the permeation properties of the tracer-tagged ions are *identical*. Generalization of the model to ion channels that conduct chemically distinct ions is in progress.

In principle, all of the microscopic assumptions (and predictions) of the present model can be tested using atomistic simulations of the open state(s) of the channel, given sufficient computer resources and realistic interatomic potential functions. As the theory relates to long-time (ensemble steady-state) sorption-limited behavior, dynamic simulations (e.g., MD) of long duration compared with the average dissociation time $(1/k_d)$ are required to directly test the kinetic predictions of the model. Alternatively, steady-state sampling simulations (e.g., MC) might be utilized to investigate the steady-state (or equilibrium) corresponding occupancy-states predictions of the model.

As a consequence of the sorption-limited property of the model, simulations will require the use of interatomic potential functions capable of realistically modeling the passage of ions (and water) from the three-dimensional bulk aqueous phase into the partially dehydrated one-dimensional environment of the selectivity filter (and vice versa).

V. CONCLUSION

A novel permeation theory has been presented for single-file ion channels containing a maximum of n ions. While the theory is formally similar to the sorption-limited extreme of previous theories for uncharged systems, it encompasses a conceptually distinct, concerted-motion mechanism for ion translocation within the file. This concerted motion mechanism does not require the formation of long-lived high-energy voids within the file or a violation of the single-file constraint. In addition, the open channel always contains $(n-1)$ "helper" ions that maintain the open channel in a form capable of accepting and transferring an ion of the same species. The helper ions are strongly bound to the channel (explaining the high selectivity of KcsA for K^+ ions over Na^+ ions, for example), while electrostatic repulsion between the n ions decreases the differential adsorption energy for the nth ion (explaining the high permeation rates for K^+ ions in KcsA—of order 10^8 ions per second). These "autoselective" properties of biological ion channels containing helper ions will be investigated further in future work.

Unlike previous continuum theories, it is assumed that intrapore diffusion may not be the rate-limiting process, but rather, that interfacial transport between the bulk aqueous phase and the partially dehydrated interior of the channel (and vice versa) is rate limiting. Unlike previous single-file ion channel models, the association step is voltage-independent, and as a result, the theory successfully predicts voltage-independent ion transfer at low concentrations and high voltages in qualitative agreement with experiment. Within the theory, the occupancy of the channel θ_n relates to the selectivity filter in its entirety (rather than to discrete ion binding sites) and the number of ions in the channel differs from the (minimum free energy) ensemble average value (of the open channel) by less than one ion, resulting in a principle of corresponding occupancy states that produces universal (Michaelis–Menten) permeation-concentration (j^*-c^*) behavior.

The universal behavior of the theoretical model has been shown to be a reasonable first-order approximation for two ion channels of known structure using previously published experimental data for symmetric-solution single-component permeation in KcsA and gA channels. Theoretical predictions for asymmetric concentrations and tracer counterpermeation boundary conditions have been presented, suggesting further experimental tests for the theory and a macroscopic technique for determining the number of ions in the conductive state(s) of the channel.

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APPENDIX: GENERAL DERIVATION

Generally, for an ion channel exposed to symmetric bathing solutions $([S]_o=[S]_i=[S])$, permeation by the three-step mechanism of Fig. 2 (with a translocation rate of $k_t e^{\omega/2}\theta_i$) is given by

$$J=k_d\theta_n\frac{e^{\phi}-e^{-\phi}e^{-\omega}}{(1+e^{-\omega})+e^{-\omega/2}(e^{\phi}+e^{-\phi})\left[\frac{k_d}{k_t}\frac{\theta_n}{1-\theta_n}\right]}, \quad \text{(A1)}$$

where θ_n, c^* and K_M are given by Eqs. (3), (4), and (5), respectively. Equation (A1) can be simplified if

$$\frac{e^{-\omega/2}(e^{\phi}+e^{-\phi})}{(1+e^{-\omega})}\left[\frac{k_d}{k_t}\frac{\theta_n}{1-\theta_n}\right]\ll 1, \quad \text{(A2)}$$

and Eq. (A1) becomes

$$J = k_d \frac{e^{\phi} - e^{-\phi} e^{-\omega}}{1 + e^{-\omega}} \theta_n, \text{ or } \quad j^* = \frac{c^*}{1 + c^*} \tag{A3}$$

as before. Thus, if the present model is applicable, Eq. (A2) can be justified because it is required for the model to reproduce the Michaelis–Menten permeation kinetics (A3) that are observed experimentally. The simplifying assumption (A2) is applicable if the translocation rate constant k_t is large compared with the dissociation rate constant k_d (or more generally, $k_t e^{\omega/2} \theta_i \gg k_d e^{\phi} \theta_n$), where $\theta_i = (1 - \theta_n)/2$ at zero applied voltage. Thus, within the present model, the sorption-limited assumption, that translocation is rapid compared with dissociation, can be justified, in general, if the channel exhibits universal Michaelis–Menten kinetics.

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