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The current-voltage relation of an aqueous pore in a lipid bilayer membrane

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In a recent paper, Glaser et al. ((1988) Biochim. Biophys. Acta 940, 275–287) use an inappropriate formula for the current-voltage relation of a pore to analyze the data from an electroporation experiment. They proceed to use their data analysis to support their contention that the voltage dependence of the membrane conductivity is caused entirely by the nonlinearity of the current-voltage relation of the pores, changes in pore size and number being unimportant. This contention is no longer justified. The formula that Glaser et al. use is valid for a one-dimensional problem, but they apply it to a pore that has a three-dimensional structure. Although the corresponding one-dimensional problem can be reduced to quadratures, only upper and lower bounds on the conductivity can be found for the three-dimensional problem without solving a partial differential equation.

Introduction

An important problem in the theory of electroporation is the calculation of the electric current I through an aqueous pore in a lipid bilayer membrane as a function of the voltage V across the pore. In a simple continuum model of this system, the membrane is a uniform insulator with dielectric constant $\epsilon_m \approx 2$ containing aqueous pores with dielectric constant $\epsilon_w \approx 80$. The membrane is embedded in an electrolytic solution. Fig. 1 shows a cross-section of the membrane through the center of a pore and the cylindrical coordinate used in this paper. Table I lists typical values for the parameters of the system. The mathematical formulation of this three-dimensional problem consists of a set of coupled partial differential equations that cannot be solved analytically. In contrast, if the potential as a function of position can be found, the Nernst-Planck equation of the corresponding one-dimensional problem can be reduced to quadratures and, if the energy of an ion in the pore has a sufficiently simple form, the integral can be evaluated analytically. The simplicity of the solution of the one-dimensional problem makes it desirable to use it to estimate the current flow in the three-dimensional case.

In a recent paper Glaser et al. [1] use the formula

$$G = \pi R_0^2 \sigma \left[\frac{\exp(U) - 1}{U \int_0^h \exp\left(U \frac{h-z}{h} + w(z) \right) \mathrm{d}z} \right] \tag{1}$$

(their Equation 9) for the conductance G of a planar membrane containing a single pore and surrounded by identical electrolytes consisting of aqueous solutions of two species of singly charged ions. In Eqn. 1, σ is the conductivity of the bulk solution, h is the thickness of the membrane, $w(z)$ is the energy in units of kT of an ion inside the channel a distance z from the edge of the membrane in the absence of an applied field, U is the potential difference across the membrane in units of kT/e, k is Boltzmann's constant, T is the temperature of the solution, and R_0 is the radius of the pore entrance (see Fig. 1). (I have changed the notation slightly and corrected a misprint in the original. The

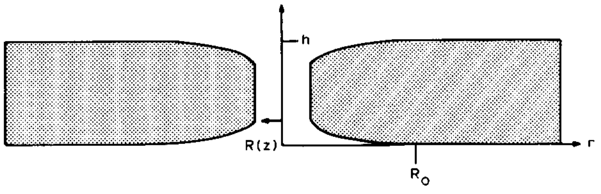

Fig. 1. An aqueous pore in a lipid bilayer membrane. The pore is axi-symmetric about the z-axis, the membrane extends from $z = 0$ to $z = h$, and $R(z)$ is the radius of the pore as a function of z.

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TABLE I

Typical values of parameters

ϵ_m	dielectric constant of membrane	2
ϵ_w	dielectric constant of water	80
n_α	ion density (0.1 M solution)	0.006 nm^{-3}
λ_D	Debye length	1 nm
g	plasma parameter	18
h	membrane thickness	5 nm
R	pore radius	0.5 nm
L	electrode spacing	1 cm
A	membrane area	1 cm^2

original from of the formula applies to a membrane with N identical non-interacting pores, and so contains a factor of N missing from my Eqn 1.) Glaser et al. quote Eqn. 1 without derivation. Neumke and Läuger [2] derive an equivalent formula for the related one-dimensional problem of conduction by poorly conducting membrane with no pores.

In this paper, I show that Eqn. 1 is not valid for the three-dimensional case, and I derive an upper bound and a lower bound on the conductance of an axially symmetrical pore in a lipid bilayer membrane.

Formulation of the problem

Consider the continuum model of a pore in a membrane shown in Fig. 1. In an experiment to measure the V-I characteristic of the membrane, a voltage is applied between two electrodes, one on each side of the membrane. The electrode spacing L is much larger than the membrane thickness $h (L \gg h)$. On a macroscopic scale the system consists of two good condutors (the electrolytes) with a current dependent voltage drop at their junction. On a microscopic scale, the source of the strong electric field at the junction is a layer of space charge in the electrolyte near the membrane surface, and the electric current flows only through the pores in the membrane.

The electric current I through a pore is the sum of the electric currents I_α carried by each type of ion in the solution

$$I = \sum_\alpha I_\alpha \tag{2}$$

where the subscript α labels the ion species. Let $\mathbf{j}_\alpha$ be the particle current density of species α. The particle current C_α and the electric current I_α are related $\mathbf{j}_\alpha$ by

$$C_\alpha = \int\int \mathbf{j}_\alpha \cdot \mathbf{da} \tag{3}$$

where the integral is over any open surface that caps the pore, and

$$I_\alpha = Z_\alpha e C_\alpha \tag{4}$$

where e is the proton charge and $Z_\alpha e$ is the charge of an ion of type α. To calculate I we must know $\mathbf{j}_\alpha$, which is related to n_α, the number density of ions of type α, by the Nerst-Planck equation

$$\mathbf{j}_\alpha = -D_\alpha\left(\nabla n_\alpha + \frac{Z_\alpha e n_\alpha}{kT}\nabla\Phi_\alpha\right) \tag{5}$$

where D_α is the diffusion constant of ion species α and Φ_α is the effective potential for an ion of type α. If chemical reactions can be neglected, $\mathbf{j}_\alpha$ obeys the continuity equation

$$\nabla \cdot \mathbf{j}_\alpha + \frac{\partial n_\alpha}{\partial t} = 0 \tag{6}$$

Since the product of the volume of a pore and the ion density is small compared to one ($\pi R^2 h n_\alpha < 1$), the probability of finding an ion in the pore is small and we must interpret I_α, C_α, $\mathbf{j}_\alpha$, n_α, and Φ_α as ensemble averages. In this interpretation, $n_\alpha d^3x$ is not the number of particles in the volume d^3x, but is rather the probability of finding a particle in d^3x.

If there are l different ion species, the continuity equation (Eqn. 6) with the particle current densities $\mathbf{j}_\alpha$ defined by Eqn. 5 constitute a set of l equations in $2l$ unknowns. To close the set, additional equations are required to define the Φ_α's. $\Phi_\alpha(\mathbf{x})$ is the ensemble average of the electric potential at $\mathbf{x}$ when an ion of type α is at $\mathbf{x}$. It includes contributions from all of the other ions in the system and from image charges on the boundary surfaces.

Φ_α can be written as

$$\Phi_\alpha = \Phi_{ext} + \Phi_{\alpha,B} \tag{7}$$

where Φ_{ext}, is the same for all types of ions, and $Z_\alpha e \Phi_{\alpha,B}$ can be different for different types of ions. Setting up the equations for Φ_α is difficult due to spatial correlations among the particles. The sources of the potential $\Phi_{\alpha,B}$ are the induced polarization charge on the membrane and the space charge due to spatial correlations between the ions. This term, also called the Born energy, can only be computed if correlations are neglected. The sources of the potential Φ_{ext} are the charges on the electrodes and the space charge in the electrolyte near the membrane. A measure of the strength of the correlations is the plasma parameter g, defined by

$$g = \frac{1}{\lambda_D^3 n_\alpha} \tag{8}$$

where λ_D is the Debye length. Spatial correlations are weak if the plasma parameter is small. For a 0.1 M solution of ions, $g \approx 18$ (see Table I), and correlations are important. This means that the simple Debye shielding theory is not valid. The proper treatment of correlations remains an unsolved problem.

Inside the pore itself conditions are different. Due to the small size of the pore and the electrostatic repulsion between the membrane and an ion, the probability of there being more than one ion inside a pore at any given time is small. We are therefore justified in neglecting correlations in the calculation of $\Phi_{\alpha,\mathrm{B}}$, and Φ_{ext} is the Laplacian field (no space charge) due to sources outside the pore and polarization charge on the membrane surfaces. For the case where space charge can be neglected, Levitt [3] and Jordan [4] have computed $\Phi_{\alpha,\mathrm{B}}$ and Φ_{ext} on the axis of an axially symmetric pore. (The source charge in their calculations of Φ_{ext} is a uniform sheet of charge outside the membrane).

Derivation of the conductance for the one-dimensional case

If Φ_α is known, the following shortcut can be used to find the V-I relation.

First, rewrite the Nerst-Planck equation (Eqn. 5) as

$$\mathbf{j}_\alpha = -D_\alpha \exp\left(-\frac{Z_\alpha e\Phi_\alpha}{kT}\right)\nabla\left[n_\alpha \exp\left(\frac{Z_\alpha e\Phi_\alpha}{kT}\right)\right] \tag{9}$$

Multiply both sides of Eqn. 9 by $\exp((Z_\alpha e\Phi_\alpha)/kT)/D_\alpha$ and integrate along a path from a point A on one side of the membrane through the pore to a point B on the other side of the membrane. The right member of the resulting equation is the line integral of a gradient and is equal to the change in value of the argument of the gradient operator. We then have

$$\int_A^B \frac{\exp\left(\frac{Z_\alpha e\Phi_\alpha}{kT}\right)}{D_\alpha}\mathbf{j}_\alpha\cdot\mathrm{d}s = n_\alpha(\mathrm{A})\exp\left(\frac{Z_\alpha e\Phi_\alpha(A)}{kT}\right) - n_\alpha(\mathrm{B})\exp\left(\frac{Z_\alpha e\Phi_\alpha(\mathrm{B})}{kT}\right) \tag{10}$$

Eqn. 10 is not useful without further simplifying assumptions. Consider the case of quasi-steady flow in one dimension. For quasi-steady flow, the continuity equation (Eqn. 7) becomes

$$\nabla\cdot\mathbf{j}_\alpha = 0 \tag{11}$$

For one-dimensional flow along the z-axis, Eqn. 11 has the solution

$$\mathbf{j}_\alpha = j_\alpha\mathbf{e}_z;\ j_\alpha = \text{constant} \tag{12}$$

Take j_α outside of the integral in Eqn 10, solve for j_α, and multiply the numerator and denominator of the left member of the result by $\exp(-Z_\alpha e\Phi_\alpha(h)/kT)$ to get

$$j_\alpha = \frac{\left[n_\alpha(0)\exp\left(\frac{Z_\alpha e(\Phi_\alpha(0)-\Phi_\alpha(h))}{kT}\right) - n_\alpha(h)\right]}{\int_o^h \frac{\exp\left(\frac{Z_\alpha e(\Phi_\alpha(z)-\Phi_\alpha(h))}{kT}\right)}{D_\alpha}\,\mathrm{d}z} \tag{13}$$

where point A is $z=0$ and point B is $z=h$. Note that Eqn. 13 is unchanged by the operation $\Phi(z)\rightarrow\Phi(z)+a$, where a is an arbitrary constant.

To write an expression for I, use the definitions of I, C_α, and I_α (Eqns. 2–4). In one dimension, these equations can be combined and written as

$$I = \pi R_0^2 e\sum_\alpha Z_\alpha j_\alpha \tag{14}$$

where R_0 is the radius of the pore, which is independent of z in a one-dimensional problem. Combine Eqns. 13 and 14 to obtain

$$I = \pi R_0^2 e\sum_\alpha \frac{Z_\alpha\left[n_\alpha(0)\exp\left(\frac{Z_\alpha e(\Phi_\alpha(0)-\Phi_\alpha(h))}{kT}\right) - n_\alpha(h)\right]}{\int_0^h \frac{\exp\left(\frac{Z_\alpha e(\Phi_\alpha(z)-\Phi_\alpha(h))}{kT}\right)}{D_\alpha}\,\mathrm{d}z} \tag{15}$$

Eqn. 15 is the general form of the solution for steady-state transport of charged particles in one dimension.

To derive Glaser's formula (Eqn. 1), we must make further restrictive assumptions:

(1) There are only two different ion species.

(2) Both ion species are singly charged ($Z_\alpha = +1$ or -1). Since the bulk solution is electrically neutral, this implies that $n_+(0) = n_-(0) = n(0)$ and $n_+(h) = n_-(h) = n(h)$.

(3) The diffusion constant D_a is not a function of position.

(4) The ion concentrations are the same on both sides of pore, $(n(0) = n(h))$.

(5) The potential $\Phi_{\alpha,\mathrm{B}}$ can be written in the form $\Phi_{\alpha,\mathrm{B}} = Z_\alpha e f(z)$.

(6) The function $f(z)$ has even symmetry about the membrane midplane $(f(z) = f(h-z))$.

(7) The electric field $E_z = -\mathrm{d}\Phi_{\mathrm{ext}}/\mathrm{d}z$ has even symmetry about the membrane midplane $(E_z(z) = E_z(h-z))$, which implies that $\Phi_{\mathrm{ext}}(z) - \Phi_{\mathrm{ext}}(h) = -(\Phi_{\mathrm{ext}}(h-z) - \Phi_{\mathrm{ext}}(0))$.

Substitute restrictions (1) – (5) into Eqn. 15 and expand the sum to get

$$I = \pi R^2 ne\left\{\frac{D_+[\exp(U)-1]}{\int_0^h \exp\left(\frac{e(\Phi_{\mathrm{ext}}(z)-\Phi_{\mathrm{ext}}(h))}{kT} + \frac{e^2}{kT}(f(z)-f(h))\right)\mathrm{d}z} - \frac{D_-[\exp(-U)-1]}{\int_0^h \exp\left(-\frac{e(\Phi_{\mathrm{ext}}(z)-\Phi_{\mathrm{ext}}(h))}{kT} + \frac{e^2}{kT}(f(z)-f(h))\right)\mathrm{d}z}\right\} \tag{16}$$

where

$$U = \frac{e(\Phi_{\mathrm{ext}}(0)-\Phi_{\mathrm{ext}}(h))}{kT} \tag{17}$$

Consider the second term in brackets in Eqn. 16. Change variables to $y = h - z$ and the integral becomes

$$\int_0^h \exp\left(\frac{e(\Phi_{\text{ext}}(h-y) - \Phi_{\text{ext}}(h))}{kT} + \frac{e^2}{kT}(f(h-y) - f(h))\right) \mathrm{d}y$$

Use assumptions (5) and (6) and multiply the numerator and denominator of this expression by $\exp(U)$ to get

$$-\frac{D_-\left[1 - \exp\left(\frac{eE_0h}{kT}\right)\right]}{\int_0^h \exp\left(\frac{e(\Phi_{\text{ext}}(y) - \Phi_{\text{ext}}(h)}{kT} + \frac{e^2}{kT}(f(y) - f(h))\right) \mathrm{d}y}$$

The coefficient of D_- in this term is clearly the same as the coefficient of D_+ in the first term in the brackets in Eqn. 16 and can be factored out.

Define $w(z)$ by

$$w(z) = \frac{e^2}{kT}(f(z) - f(h)) \tag{18}$$

and note that σ, the conductivity of the bulk solution, is

$$\sigma = \frac{ne^2(D_+ + D_-)}{kT} \tag{19}$$

Conductance is the ratio of current to voltage. Divide both sides of Eqn. 16 by $U\,kT$ and use the result, Eqns. 17–19, and the definition of conductance to obtain

$$G = \pi R_0^2 \sigma \left[\frac{\exp(U) - 1}{U \int_0^h \exp\left(\frac{e(\Phi_{\text{ext}}(z) - \Phi_{\text{ext}}(h))}{kT} + w(z)\right) \mathrm{d}z}\right] \tag{20}$$

Eqn. 20 reduces to Glaser's formula [1] for the special case E_z = constant.

Derivation of conductance for the three-dimensional case

Unfortunately, after Eqn. 11 the above derivation is valid only for a one-dimensional system. In three dimensions, the best we can do without solving the partial differential equations is obtain an upper bound and a lower bound for G.

Consider a pore that has axial symmetry about the z-axis. The line integral in Eqn. 10 involves the current $\mathbf{j}_\alpha$. Steady flow in three dimensions is divergence free (Eqn. 11), but divergence free flow does does *not* mean that $\mathbf{j}_\alpha$ is a constant (Eqn. 12 is not valid). To proceed, notice that, since Φ_α considered as a function of r has a minimum on the z-axis, j_α considered as a function of r has a maximum there:

$$j_\alpha(r, z) \le j_\alpha(0, z) \tag{21}$$

Similarly, j_α has a minimum at the pore wall, $r = R(z)$:

$$j_\alpha(r, z) \ge j_\alpha(R(z), z) \tag{22}$$

To obtain an upper bound for G, note that $\mathbf{j}_\alpha = j_\alpha \mathbf{e}_z$ on the axis of the pore, integrate Inequality 21 over the area of the pore, and use the definition of C_α (Eqn. 3) to obtain

$$C_\alpha \le \pi R^2(z)\, j_\alpha(r = 0, z) \tag{23}$$

Inequality 23 is valid for all values of z. Solve Inequality 23 for j_α $(r = 0, z)$ and use the result in Eqn. 10, evaluating the line integral along the z-axis, to obtain

$$C_\alpha \le \frac{\left[n_\alpha(0)\exp\left(\frac{Z_\alpha e(\Phi_\alpha(0) - \Phi_\alpha(h))}{kT}\right) - n_\alpha(h)\right]}{\int_0^h \frac{\exp\left(\frac{Z_\alpha e(\Phi_\alpha(z) - \Phi_\alpha(h))}{kT}\right)}{\pi R^2(z) D_\alpha} \mathrm{d}z} \tag{24}$$

Use the same arguments and make the same assumptions that led from Eqn. 15 to Eqn. 20 to obtain

$$G \le \pi\sigma \left[\frac{\exp(U) - 1}{U \int_0^h \frac{\exp\left(\frac{e(\Phi_{\text{ext}}(0, z) - \Phi_{\text{ext}}(0, h))}{kT} + w(z)\right) \mathrm{d}z}{R^2(z)}}\right] \tag{25}$$

To obtain a lower bound for G, evaluate the integral in the definition of C_α (Eqn. 3) over a surface that intersects the pore boundary at the point (R, z) and has the property that the normal $\mathbf{da}$ is everywhere parallel to $\mathbf{j}_\alpha$. Use Inequality 22 to obtain

$$C_\alpha \ge \pi R^2(z)\, j_\alpha(R, z) \tag{26}$$

Arguments analogous to those used to derive Inequality 25 lead to

$$G \ge \pi\sigma \left[\frac{\exp(U) - 1}{U \int_0^h \frac{\exp\left(\frac{e(\Phi_{\text{ext}}(R, z) - \Phi_{\text{ext}}(R, h))}{kT} + w(z)\right) \mathrm{d}z}{R^2(z)}}\right] \tag{27}$$

Inequalities 25 and 27 are derived with the tacit assumption that the ions are point particles. The finite size of the ions can be taken into account by substituting $(R(z) - r_\alpha)$ for $R(z)$, where R_α is the ion radius. If $R(z) < r_\alpha$ anywhere in the pore, $G = 0$.

Discussion

Inequalities 25 and 27 describe the conductance of a single pore, a quantity that is not directly measurable. In a typical electroporation experiment, a membrane is placed between two parallel electrodes, voltage is applied between the electrodes, and the current to the electrodes is measured as a function of time. Many small pores are required to account for the observed current. If the pores are sufficiently far apart, the electric field in the vicinity of a pore will be unaffected by the presence of the other pores, and the total current through the membrane will be simply the sum of the currents flowing through each pore.

The voltage U in Inequalities 25 and 27 is the voltage drop between $z = 0$ and $z = h$; in general, this is not equal to the voltage applied between the electrodes. In addition to the voltage drop through the pore, the applied voltage includes the half-cell potentials at each electrode, the ohmic drop in the bulk solution far from the membrane, and the voltage drop in the region of converging or diverging current flow in the bulk solution near the ends of the pore. The two half-cell potentials sum to zero if the two electrodes are made of the same material and the solutions on the two sides of the membrane are identical. The ohmic drop in the bulk solution is easy to measure or calculate. The voltage drop near the pore entrance is difficult to determine, as it involves the electric field in the region near the membrane for which no adequate theory exists. Modeling this region as a simple Ohmic conductor leads to the 'spreading resistance' computed by Newman [5].

Implicit in the use of Eqn. 19 for σ in the derivation of Inequalities 25 and 27 are two assumptions:

(1) the number density $n(0)$ of the ions at the entrance to the pore is the same as the number density of the ions far from the membrane, and

(2) The diffusion constants D_+ and D_- are the same in the pore and in the bulk solution.

Neither of these assumptions is true, and the error in each tends to increase the conductance estimate.

Glaser et al. analyze their data to calculate, among other things, the number and average radius of the pores. To do this, they assume that the pores do not interact and that the membrane can be adequately characterized by an average pore conductance, given by Eqn. 1 evaluated at the average pore size. The conductance of a membrane containing N pores is then N times Eqn. 1. Since the fractional change of the pore entrance radius R_0 is small even for large changes of the pore minimum minimum radius, they claim that the only dependence of G on pore size is through the function $w(z)$. They then analyze their data to find the values of several model parameters. Careful study of Inequalities 25 and 27 shows that G depends strongly on the shape of the pore both explicitly and implicitly through the dependence of Φ_{ext} and w on the pore shape. In particular, the approximation that $\mathrm{d}\Phi_{\text{ext}}/\mathrm{d}z$ is constant in the mouth of the pore is not valid. Glaser et al. conclude by using the results of their analysis to argue that the observed dependence of the membrane condutance on the membrane voltage is caused entirely by the nonlinearity of the V-I characteristic for each pore. This conclusion should be reexamined using a formula appropriate for a three-dimensional pore.

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