Ionic conductivity of electroporated lipid bilayer membranes

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Abstract

The ionic conductivity of lipid membrane pores has been theoretically analysed in terms of electrostatic interactions of the transported ions with the low-dielectric pore wall for a commonly encountered case of unequal concentrations of electrolyte on the two sides of curved lipid membranes. Theoretical analysis of the data on the conductivity of the electroporated membrane of lipid vesicles (Lecithin 20%) of radius \( a = 90 \) nm yields the molar energy of interaction of a small monovalent ion with a pore wall \( w_0 = 9 \pm 1 \) RT (or \( w_0 = 22 \pm \)kJ mol\(^{-1}\)), corresponding to a mean pore radius of \( r_p = 0.56 \pm 0.05 \) nm. The proposed theoretical approach provides a tool for the analysis and description of the nonlinear current–voltage dependencies in membrane pores and channels. © 2002 Published by Elsevier Science B.V.

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1. Introduction

The current–voltage characteristics of lipid membrane pores and protein channels frequently do not obey Ohm’s law. Rather, the electric current nonlinearly depends on the applied voltage [1–3]. The departure from the classical Ohm’s law in lipid membranes can be caused by the electrostatic interactions of the ions with the low-dielectric pore wall. Actually, if a point charge \( q \) is situated near the boundary of two dielectrics, say between water (dielectric constant \( \varepsilon_w = 80 \)) and the lipid phase of the membrane \( (\varepsilon_m = 2.5) \), \( q \) in water induces in the neighboring membrane \( (\varepsilon_m) \) an image charge \( q' \). Since \( \varepsilon_w > \varepsilon_m \), \( q' \) has the same sign as \( q \). Therefore, \( q \) experiences a repulsive force from \( q' \) tending to repel the ion from the lipid phase. Without aqueous pores or channels, the transfer of a monovalent ion through a lipid membrane encounters the Born free energy barrier of \( \Delta G_{\text{b}} = 62RT \), where \( R \) is the gas constant and \( T \) is the Kelvin temperature [4]. In pores and channels, the value of the barrier is lowered by a factor of 3–4 [5]. At constant radius of the pore, the height of the energy barrier can be reduced by an external electric field [1,2].

The conductivity of planar, UO\(_2\)\(^{2+}\)-ion modified asolectin bilayers has been studied previously at equal electrolyte contents on the two membrane sides [1,2]. In the present paper, we generalise the existing description to the common case of curved membranes of spherical vesicles and different salt contents on the two membrane sides.

2. Experimental

The approach for the determination of the surface fraction \( f_p \) of membrane electropores and the membrane conductivity \( \lambda_m \) from electro-optical and conductometrical measurements of salt-filled vesicles exposed to a rectangular electric field pulse has been described previously [6–8]; it is applied here to NaCl-filled lipid vesicles (Lecithin 20%) of radius \( a = 90 \) nm. The lipids of the total concentration \( [L] = 1 \) mM are suspended in 0.2 M NaCl solution and freeze–thawed five times in liquid nitrogen to obtain solute equilibration between trapped and bulk solution. In order to remove external NaCl, the vesicle suspension is dialyzed against degassed sucrose solution of the same osmolality (0.33 M sucrose). The final NaCl concentration is \( c_{\text{in}} = 0.2 \) mM in the bulk and \( c_{\text{in}} = 0.2 \) M in the vesicle interior, respectively. The lipid concentration of \( [L] = 1 \) mM and a vesicle radius of \( a = 90 \) nm correspond to the vesicle number density \( \rho_v = 2.4 \times 10^{15}/l \); the average distance between the vesicle surfaces is \( \ell = 0.566 \) \( \mu \)m, qualifying the suspension as diluted [8].

3. Results

The analysis of turbidity and conductometric relaxations in the salt-filled vesicles exposed to the rectangular field
pulse of the field strength 0 ≤ E MV m⁻¹ ≤ 7.5 and the pulse duration t_p=10 μs (data of T. Giese, not presented) shows that the surface fraction f_p of membrane electropores increases with E and levels off at E=2 MV m⁻¹ (Fig. 1a). The membrane conductivity λ_m nonlinearly increases with E without levelling off (Fig. 1b).

4. Theory

The ion flux \( J_i = (dn_i/dt) / S_p \) across a pore, where \( n_i \) is the amount of ions of the type i, \( t \) is the time, and \( S_p \) is the surface cross-section of the pore, is classically described by the Nernst–Planck equation:

\[
J_i = -D_i \left( \frac{dC_i}{dx} + C_i \frac{z_i F}{RT} \frac{d\varphi}{dx} \right)
\]  

where \( C_i(x) \) is the ion concentration profile in the pore along the x-axis (membrane normal), \( D_i \) is the average diffusion coefficient of the ion in the pore range, \( z_i \) is the charge number (with sign), \( F \) is the Faraday constant, \( R \) is the gas constant, and \( \varphi(x) \) is the electric potential in the pore: \( \varphi(x) = \varphi_{ex}(x) + \varphi_{im}(x) \). Here, \( \varphi_{im} \) is the electric image potential induced by the moving ion and \( \varphi_{ex} = - \int E_p(x) dx \) is the potential of the transmembrane field \( E_p(x) \). We assume that the concentrations \( c_i(0) \) and \( c_i(d) \) of the ions at the two pore ends at \( x=0 \) and \( x=d \) are equal to the bulk ion concentrations \( c_i(0) \) and \( c_i(d) \), respectively. Hence, \( c_i(0) = c_i(0) \) and \( c_i(d) = c_i(d) \). The conductivity \( \lambda_p \) of the electrolyte in the pore is given by

\[
\lambda_p = j / E_p
\]

where the total current density \( j \) refers to the flux contributions of all ion types:

\[
j = F \sum_i z_i I_i.
\]

The strength of the external field across the pore is \( E_p = (\varphi_{ex}(d) - \varphi_{ex}(0))/d = -\Delta \varphi / d \) and \( d \) is the membrane thickness. For the simple case of a monovalent 1:1 electrolyte where \( |z_i| = 1 \), the insertion of Eq. (1) in Eqs. (3) and (2) and integration yields:

\[
\lambda_p = \frac{F D}{E_p} \frac{c_i(0) \exp(k_i \varphi(0)) - c_i(d) \exp(k_i \varphi(d))}{\int_0^d \exp(k_i \varphi(x)) dx} - \frac{c_i(0) \exp(-k_i \varphi(0)) - c_i(d) \exp(-k_i \varphi(d))}{\int_0^d \exp(-k_i \varphi(x)) dx}
\]

where the approximation \( D = D_i = D \) has been introduced.

Different from the traditional integration of the Nernst–Planck equation using \( \varphi_{im}(x) = \text{constant} \) [9], we consider a specific trapezium shape of the image potential: \( \varphi_{im}(x) \) at \( x < 0 \) and \( x > 0 \), \( \varphi_{im}(x) = \varphi_{im}^0 (x/h) \text{sign}(z) \) at \( 0 \leq x \leq h \), \( \varphi_{im}(x) = \varphi_{im}^0 (d-x/h) \text{sign}(z) \) at \( d-h \leq x \leq d \), where \( h \) is the thickness of the pore entrance (Fig. 2a) and \( \text{sign}(z) \) is the sign of the ion. Integration of Eq. (4) for the case \( |\Delta \varphi| \gg 25 \text{ mV} \) yields:

\[
\lambda_p = \lambda_0 \exp \left[ \left( z n h |\Delta \varphi| - \varphi_{im}^0 \right) \frac{F}{RT} \right]
\]

where \( n = h/d \) is the relative size of the pore entrance (Fig. 2b), \( \lambda_0 = F D (c_i(0) + c_i(d))/RT \) and \( z = (1 - RT/F \varphi_{im}^0) \). It is noted that except for the term \( z \), Eq. (5) is similar to the analogous approximation of Glaser et al. [2] applied to one pore, but Eq. (5) is closer to the exact Eq. (4) than the approximation of Glaser et al. (Fig. 3).

For practical purposes, it is recalled that the membrane conductance \( G_m = \lambda_m S_m / d \), where \( S_m \) is the total membrane surface area and \( \lambda_m \) is the membrane conductivity, refers to
the measured current \( I \) and the voltage \( U \) as \( G_m = I/U \). If the membrane pores are specified by the additive parallel conductances, \( G_p = \lambda_p S_p/d_p \), where \( S_p \) and \( d_p \) are the surface area and the length of one pore, respectively, the relation \( G_m = \sum_i (G_p) \) holds. Here, \( i \) is the summation index: \( i = 1, 2, \ldots, N_p \), where \( N_p \) is the total number of pores. If \( d = d_p \) holds, we obtain \( S_m \lambda_m = \sum_i (S_p \lambda_p) + \lambda_l (S_m - \sum_i S_p) \). Usually, the conductivity \( \lambda_l \) of the closed lipid membrane is very small (\( \lambda_l \approx 5 \times 10^{-13} \) S m\(^{-1}\)). When the pores are represented by a mean conductance \( \lambda_p \) and a mean area \( S_p \), the total surface fraction of pores is defined by \( f_p = N_p S_p/S_m \). Insertions and rearrangements yield for this case:

\[
\lambda_m = \lambda_p f_p
\]

(for a single current event \( f_p \), the conductance \( G_p \) is given by \( G_p = f_p U = \lambda_p S_p/d_p \)). In the case of salt-filled vesicles, the inequalities \( \lambda_m \gg \lambda_{\text{ex}} \) and \( \lambda_m \ll 2\lambda_{\text{ex}} (d/a) \) hold, where \( \lambda_m \)

\[\varepsilon\phi \] and \( \lambda_{\text{ex}} \) are the conductivities of intravesicular and external media, respectively. Hence, \( \Delta \phi \) can be presented by \( \Delta \phi = \Delta \phi_0 f(\lambda_m) \), where \( f(\lambda_m) = 1 - R_m a/(2d \lambda_{\text{ex}}) \) is the conductivity function and \( \Delta \phi_0 \) is the \( \theta \)-average transmembrane field strength at \( \lambda_m = 0 \), \( \Delta \phi_0 = \sqrt{3} a E/2 \); \( \theta \) is the angle between the membrane site and the direction of \( E \) [4]. Substitution of Eq. (5) and \( \Delta \phi \) into Eq. (6) yields the solution (by Maple 5):

\[
\lambda_m = \beta \lambda_{\text{ex}} \text{LambertW} \left[ \frac{f_p \lambda^0_{\text{ex}}}{\beta \lambda_{\text{ex}}} \exp \left[ \frac{F(\sqrt{3}a E / 2 - \phi^0_{\text{im}})}{RT} \right] \right]
\]

where \( \beta = 4a^2 RT/(F \sqrt{3} \pi a E h) \) is a dimensionless factor and LambertW is a special function [10].

5. Data analysis

Analysis of the \( \lambda_m \) data (Fig. 1b) with Eq. (7) yields the relative size \( n = 0.12 \pm 0.01 \). With \( d = 5 \) nm, we obtain \( h = 0.6 \) nm and the value of the image potential of a monovalent ion is \( \phi^0_{\text{im}} = 0.23 \pm 0.003 \) V, corresponding to the molar energy \( w_0 = F \phi^0_{\text{im}} = 9 \pm 1 \) RT (or \( w_0 = 22 \pm 2 \) kJ mol\(^{-1}\)). According to Parsegian [5], the value \( w_0 = 9 \) RT refers to the mean pore radius \( r_p = 5 \) RT/\( w_0 = 0.56 \pm 0.5 \) nm. Total electrical energy \( w_{\text{el}} \) of an ion in a pore can be characterized by the energy difference \( w_{\text{el}} = F(\alpha \sigma a E(f(\lambda_m)) / 3 \phi^0_{\text{im}}) \). With increasing external field strength \( 0 \leq E \) m V m\(^{-1}\) \( \leq 7.5 \), \( w_{\text{el}} \) becomes less negative: \(-0.9 \leq w_{\text{el}} / RT \leq -7 \). For \( f_p \) and \( \lambda_m \) in the limits \( 0 \leq f_p \leq 1.5 \times 10^{-2} \) and \( 5.0 \times 10^{-13} \leq \lambda_m \) S m\(^{-1}\) \( \leq 3.2 \times 10^{-5} \) (Fig. 1b), respectively, insertion of the values \( h = 0.6 \) nm, \( r_p = 0.56 \) nm, and \( \phi^0_{\text{im}} = 0.23 \) V into Eq. (5) yields the conductivity and conductance of a single pore, \( 1.8 \times 10^{-4} \leq \lambda_p S m^{-1} \leq 6.2 \times 10^{-13} \) and \( 0.036 \leq G_p / pS \leq 1.2 \) respectively.
Table 1

<table>
<thead>
<tr>
<th>$E'$</th>
<th>$w_0/\varphi$</th>
<th>$f_0$</th>
<th>$\lambda_m/\mu$</th>
<th>$\lambda_p/m$</th>
<th>$G_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(MV m$^{-1}$)</td>
<td>(RT)</td>
<td>(10$^{-3}$)</td>
<td>(μS m$^{-1}$)</td>
<td>(mS m$^{-1}$)</td>
<td>(pS)</td>
</tr>
<tr>
<td>0</td>
<td>$-9 \pm 1$</td>
<td>0</td>
<td>$5 \times 10^{-7}$</td>
<td>0.18</td>
<td>0.036</td>
</tr>
<tr>
<td>7.5</td>
<td>$-7 \pm 1$</td>
<td>1.5±0.1</td>
<td>32±6</td>
<td>6.2±0.5</td>
<td>1.2±0.1</td>
</tr>
</tbody>
</table>

$w_0 = F(\nu a E' / (3/2 - \varphi_m))$ is the electric energy of a monovalent ion in a pore, $f_0$ is the surface fraction of membrane pores, $\lambda_m$ is the total conductivity of the membrane, $\lambda_p$ is the conductivity of a single pore, and $G_p$ is the conductance of a single pore. Here, the mean pore radius is $\bar{r}_p = 0.56 \pm 0.5$ nm.

The key electric parameters of the porated lipid membrane are summarized in Table 1.

6. Discussion

The small length, $h = 0.6$ nm, of the curved pore entrance suggests that the pore shape is close to cylindrical (Fig. 2b). The relative size of the pore entrance $n = h/d = 0.12 \pm 0.01$ is similar to the value $n = 0.15$ calculated for the electropores in planar, UO$_2^{2+}$-ion modified asolectin bilayers [2]. The value of the interaction energy $w_0 = 9 \pm 1$ RT for ions in the membrane pores of lipid vesicles compares well with $w_0 = 7.7RT$ in electropores of asolectin planar membranes [2]. The conductance $G_p$ of a single pore is rather small: $0.036 \leq G_p/pS \leq 1.2$ at $0 \leq E/MV m^{-1} \leq 7.5$. For a comparison, the $G_p$ of membrane protein channels is a factor 10–100 larger than the $G_p$ of the membrane electropores [3]. For larger pores, spontaneous rapid flickering of pores is improbable, at least at $E = 7.5$ MV m$^{-1}$. Note that the minimum energy difference $\Delta W = W_p - W_{\text{lin}} = 8.5$ kT exceeds the manifold kT. Here, $W_p$ is the electric polarization energy of a single pore in the pole caps of the vesicles, $W_p = 9a^2 \varepsilon_c (\varepsilon_m - \varepsilon_m) \bar{r}_p^2 E^2 / (8d) = 17$ kT, $W_{\text{lin}}$ is the edge energy of the hydrophilic pore, $W_{\text{edge}} = 2\pi a \bar{r}_p = 8.5$ kT, $\bar{r}_p = 0.56$ nm, $a = 90$ nm, and $\varepsilon_0 = 8.854 \times 10^{-12}$ C$^2$ J$^{-1}$ m$^{-1}$ [2]. Additionally, after switching off the electric field, the characteristic time of pore rescaling is larger than 7 μs [11]. Therefore, within the 10 μs of the electric pulse duration, the net pore closing can be safely neglected. The small value of $G_p$ for the lipid pores can be rationalized by the small value of $\bar{r}_p = 0.56$ nm, leading to the large electrostatic barrier $w_0 = 5RT/\bar{r}_p = 9RT$ for ions [5].

Note that already, the simple trapezoid shape of the image potential is sufficient to describe the field dependence of the membrane conductivity. Alternatively to the electrostatic energy of image charge, the barrier $w_0$ can be attributed to the energy of eventual partial dehydration of the ion entering the pore. In any case, the effect of the energy barriers of different nature and shape on the conductivity of pores and channels can be calculated with Eq. (4) numerically.

7. Conclusion

The dependence of the membrane conductivity on the field strength can be consistently described with an integrated Nernst–Planck equation combined with a trapezium-shaped ionic energy barrier by a LambertW function. The analytical expressions account for asymmetrical concentrations of electrolyte on the two membrane sides and the partial discharging of the membrane due to a finite pore conductivity ($f(\lambda_m) < 1$). The theoretical approach and the experimentally accessible parameters can be used for an exact determination of the fraction $f_0$ of pores from the membrane conductivity $\lambda_m$ in lipid vesicles.

References