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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±1 RT (or w_0=22±kJ mol^{-1}), corresponding to a mean pore radius of $\bar{r}_p$=0.56±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.

Keywords: Lipid vesicles; Electroporation; Electrooptics; Conductometry; LambertW function; Nernst–Planck equation

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 ε_w=80) and the lipid phase of the membrane (ε_m=2.5), q in water induces in the neighboring membrane (ε_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 ΔG_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 λ_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_T]=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 osmolarity (0.33 M sucrose). The final NaCl concentration is c_{ex}=0.2 mM in the bulk and c_{in}=0.2 M in the vesicle interior, respectively. The lipid concentration of [L_T]=1 mM and a vesicle radius of a=90 nm correspond to the vesicle number density $\rho_v \approx 2.4 \times 10^{15}$/l; the average distance between the vesicle surfaces is $\ell \approx 0.566$ µm, 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

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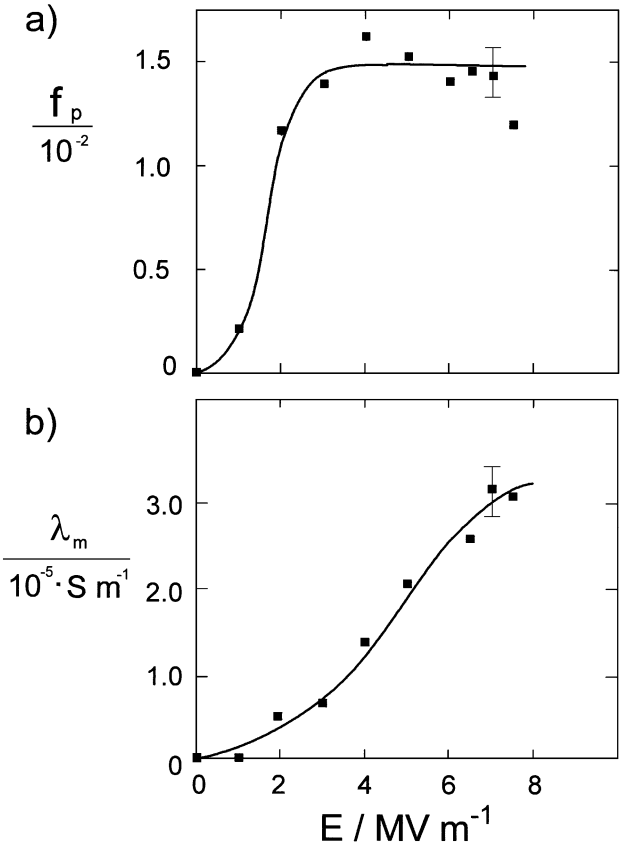

Fig. 1. (a) The surface fraction f_p of the membrane pores and (b) the membrane conductivity λ_m, respectively, as a function of the electric field strength E. Squares in (a) and (b) represent the experimental data, the solid lines refer to the theoretical predictions. Experimental conditions: one rectangular electric pulse of the field strength E and the pulse duration t_E=10 at T=293 K (20 °C); salt-filled unilamellar lipid vesicles (Lecithin 20%) of mean radius $a = 90$ (±10) nm, internal NaCl content $c_{in} = 0.2$ M, total lipid concentration $[L_t]$=1.0 mM, suspended in isotonic 0.33 M sucrose and 0.2 mM NaCl solution. Data of T. Griese, Bielefeld.

pulse of the field strength $0 \leq E$ MV m$^{-1} \leq 7.5$ and the pulse duration t_E=10 μs (data of T. Griese, 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:

$$J_i = -D_i\left(\frac{dC_i}{dx} + C_i\frac{z_i F}{RT}\frac{d\varphi}{dx}\right) \tag{1}$$

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, φ_{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 λ_p of the electrolyte in the pore is given by

$$\lambda_p = j/E_p \tag{2}$$

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

$$j = F\sum_i z_i J_i. \tag{3}$$

The strength of the external field across the pore is $E_p \approx -(\varphi_{ex}(d)-\varphi_{ex}(0))/d$=$-\Delta\varphi/d$ 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:

$$\lambda_p = \frac{FD}{E_p}\left(\frac{c_+(0)\exp(k_+\varphi(0)) - c_+(d)\exp(k_+\varphi(d))}{\int_0^d \exp(k_+\varphi(x))dx} - \frac{c_-(0)\exp(k_-\varphi(0)) - c_-(d)\exp(k_-\varphi(d))}{\int_0^d \exp(k_-\varphi(x))dx}\right) \tag{4}$$

where the approximation $D \approx D_+ \approx D_-$ has been introduced.

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

$$\lambda_p = \lambda^0\exp\left[(\alpha n|\Delta\varphi| - \varphi^0_{im})\frac{F}{RT}\right] \tag{5}$$

where n=h/d is the relative size of the pore entrance (Fig. 2b), λ^0=$F^2D(c(0)+c(d))/RT$ and α=$(1-RT/(F\varphi^0_{im}))$. It is noted that except for the term α, 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 λ_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 $G_m=\sum_i (G_p)_i$ 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 $S_m\lambda_m=\sum_i (S_p\lambda_p)_i+\lambda_L(S_m-\sum_i (S_p)_i)$. Usually, the conductivity λ_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 λ_p and a mean area S_p, the total surface fraction of pores is defined by $f_p=N_pS_p/S_m$. Insertions and rearrangements yield for this case:

$$\lambda_m=\lambda_p f_p \tag{6}$$

(for a single current event I_p, the conductance G_p is given by $G_p=I_p/U=\lambda_p S_p/d_p$). In the case of salt-filled vesicles, the inequalities $\lambda_{in}\gg\lambda_{ex}$ and $\lambda_m\ll 2\lambda_{ex}(d/a)$ hold, where λ_{in} and λ_{ex} are the conductivities of intravesicular and external media, respectively. Hence, $\Delta\varphi$ can be presented by $\Delta\varphi=\Delta\varphi_0 f(\lambda_m)$, where $f(\lambda_m)=1-\lambda_m a/(2d\lambda_{ex})$ is the conductivity function and $\Delta\varphi_0$ is the θ-average transmembrane field strength at $\lambda_m=0$, $\Delta\varphi_0=\surd 3aE/2$; θ is the angle between the membrane site and the direction of E [4]. Substitution of Eq. (5) and $\Delta\varphi$ into Eq. (6) yields the solution (by Maple 5):

$$\lambda_m=\beta\lambda_{ex}\mathrm{LambertW}\left(\frac{f_p\lambda^0}{\beta\lambda_{ex}}\exp\left[\frac{F(\sqrt{3}\alpha aEn/2-\varphi^0_{im})}{RT}\right]\right) \tag{7}$$

where $\beta=4d^2RT/(F\surd 3\alpha aEh)$ is a dimensionless factor and LambertW is a special function [10].

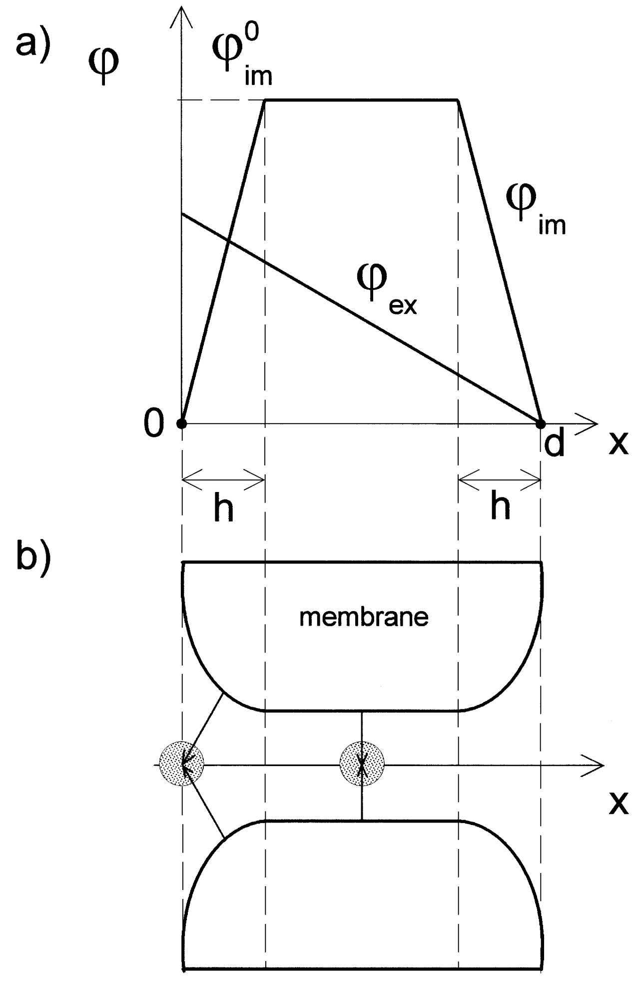

Fig. 2. (a) The electric potential φ_{ex} of an external transmembrane field and φ_{im} the image potential of an ion along the pore x-axis. (b) Forces on the moving ion (shown by arrows) from the induced image charge in the pore walls.

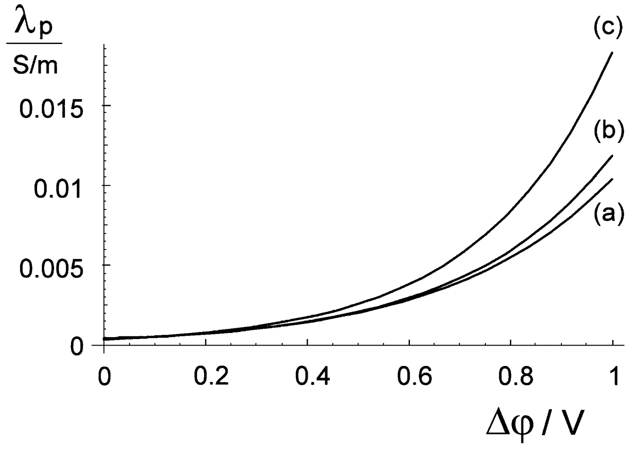

Fig. 3. Conductivity λ_p of a single pore as a function of the potential difference $\Delta\varphi$ across the membrane calculated with: (a) exact (Eq. (4)), (b) approximate (Eq. (7)), and (c) approximate (Eq. (11)) from Glaser et al. [2] applied to one pore. Calculations were performed for monovalent 1:1 electrolyte, $C(0)=C(d)=0.2$ M, the membrane thickness $d=5$ nm, the size of the pore entrance $h=0.5$ nm (see Fig. 2), and the image potential $\varphi^0_{im}=0.23$ V, corresponding to the energy barrier $w_0=9RT$.

5. Data analysis

Analysis of the λ_m data (Fig. 1b) with Eq. (7) yields the relative size $n=0.12\pm0.01$. With $d=5$ nm, we obtain $h=0.6$ nm and the value of the image potential of a monovalent ion is $\varphi^0_{im}=0.23\pm0.003$ V, corresponding to the molar energy $w_0=F\varphi^0_{im}=9\pm1$ RT (or $w_0=22\pm2$ kJ mol^{-1}). According to Parsegian [5], the value $w_0=9RT$ refers to themean pore radius $\bar{r}_p=5RT/w_0=0.56\pm0.5$ nm. Total electrical energy w_{el} of an ion in a pore can be characterized by the energy difference $w_{el}=F(\alpha naEf(\lambda_m)\surd 3/2-\varphi^0_{im})$. With increasing external field strength $0\leq E/\mathrm{MV\ m^{-1}}\leq 7.5$, w_{el} becomes less negative: $-9\leq w_{el}/RT\leq -7$. For f_p and λ_m in the limits $0\leq f_p\leq 1.5\times10^{-2}$ and $5.0\times10^{-13}\leq\lambda_m/\mathrm{S\ m^{-1}}\leq 3.2\times10^{-5}$ (Fig. 1), respectively, insertion of the values $h=0.6$ nm, $\bar{r}_p=0.56$ nm, and $\varphi^0_{im}=0.23$ V into Eq. (5) yields the conductivity and conductance of a single pore, $1.8\times10^{-4}\leq\lambda_p/\mathrm{S\ m^{-1}}\leq 6.2\times10^{-13}$ and $0.036\leq G_p/\mathrm{pS}\leq 1.2$, respectively.

Table 1
Key electric parameters of electroporated lipid membranes at two limiting field strengths E

E/ (MV m^{-1})	w_{el}/ (RT)	f_p/ (10^{-2})	λ_m/ (μS m^{-1})	λ_p/ (mS m^{-1})	G_p/ (pS)
0	-9 ± 1	0	5×10^{-7}	0.18	0.036
7.5	-7 ± 1	1.5 ± 0.1	32 ± 6	6.2 ± 0.5	1.2 ± 0.1

$w_{el}=F(\alpha naEf(\lambda_m)\sqrt{3/2}-\varphi^0_{im})$ is the electric energy of a monovalent ion in a pore, f_p is the surface fraction of membrane pores, λ_m is the total conductivity of the membrane, λ_p is the conductivity of a single pore, and G_p is the conductance of a single pore. Here, the mean pore radius is $\bar{r}_p=0.56\pm0.5$ 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 $n=h/d=0.12\pm0.01$ 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 $w_0=9\pm1$ 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: $0.036\leq G_p/\text{pS}\leq1.2$ at $0\leq E/\text{MV m}^{-1}\leq7.5$. 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 $\Delta W=W_p-W_{lin}=8.5$ kT exceeds the manifold kT. Here, W_p is the electric polarization energy of a single pore in the pole caps of the vesicles, $W_p=9\pi a^2\varepsilon_0(\varepsilon_w-\varepsilon_m)\bar{r}_p{}^2E^2/(8d)=17$ kT, W_{lin} is the edge energy of the hydrophilic pore, $W_{edge}=2\pi\lambda\bar{r}_p=8.5$ kT, $\bar{r}_p=0.56$ nm, $a=90$ nm, and $\varepsilon_0=8.854\times10^{-12}$ C^2 J^{-1} m^{-1} [2]. Additionally, after switching off the electric field, the characteristic time of pore resealing 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 trapezium 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_p of pores from the membrane conductivity λ_m in lipid vesicles.

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