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REVIEW

Membrane perturbation by an external electric field: a mechanism to permit molecular uptake

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Abstract Electropermeabilisation is a well established physical method, based on the application of electric pulses, which induces the transient permeabilisation of the cell membrane. External molecules, otherwise nonpermeant, can enter the cell. Electropermeabilisation is now in use for the delivery of a large variety of molecules, as drugs and nucleic acids. Therefore, the method has great potential in the fields of cancer treatment and gene therapy. However many open questions about the underlying physical mechanisms involved remain to be answered or fully elucidated. In particular, the induced changes by the effects of the applied field on the membrane structure are still far from being fully understood. The present review focuses on questions related to the current theories, i.e. the basic physical processes responsible for the electropermeabilisation of lipid membranes. It also addresses recent findings using molecular dynamics simulations as well as experimental studies of the effect of the field on membrane components.

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Abbreviations

PC	Phosphatidyl-Choline
PS	Phosphatidyl-Serine
PE	Phosphatidyl-Ethanolamine
SM	Sphingomyelin
DOPC	1,2-Dioleoyl-sn-Glycero-3-Phosphocholine
DOPS	1,2-Dioleoyl-sn-Glycero-3-Phosphoethanolamine
NBD	7-Nitrobenz-2-oxa-1,3-diazol-4-yl

Introduction

The cell membrane acts as a barrier that hinders the free entrance of most hydrophilic molecules into the cell. Their permeability can however be transiently increased by applying an electric field. This process, called electropermeabilisation and also named electroporation, has received increasing attention, particularly as an elegant and convenient means to gain access to the cytoplasm (Neumann 1989; Teissie et al. 2002; Weaver 1995). The phenomenon of electropermeabilisation has been observed for decades. Early reports were related with the death of cells caused by the irreversible permeabilisation of the cell membranes (Sale and Hamilton 1968). Studies in the 1970s revealed that short electric pulses in the μs and kV/cm range could induce transient vesicle as well as cell membrane permeabilisation leading to the transport of small molecules. Studies in the 1980s and 1990s showed that cells submitted

to longer electric pulses, i.e. in the ms range, can be transfected (Rols 2006; Teissie et al. 2005). Recent studies, coming from new technologies, concerns nanosecond pulsed electric fields, indicate that very short (10–300 ns) but high pulses (up to 300 kV/cm) induce effects that primarily affect intracellular structures and functions (Beebe et al. 2003). Therefore, the use of electric pulses to deliver therapeutic molecules has been rapidly developed over the last decade. This technology is relevant in a variety of researches and clinical settings including cancer therapy, modulation of pathogenic immune responses, delivery of therapeutic proteins and drugs (Gehl et al. 1999; Gilbert et al. 1997; Miklavcic et al. 2000; Rols 2006; Bigey et al. 2002). Electrochemotherapy, a new cancer treatment modality, has emerged (Belehradek et al. 1993; Mir et al. 1991a, b) and beside drugs, many other potentially therapeutic agents such as DNA can be electrotransfered (for review see Favard et al. 2007).

Whatever is the potentiality of this technique, there is a general agreement that very little is known about the underlying mechanisms brought into play in the cell and its membranes, following electropermeabilisation. Understanding these mechanisms is of vital importance, first to enhance the efficiency in in vitro use and secondly in order to optimise the safety of the method in its in vivo use. The major technical difficulties in the study of electropermeabilisation arise from the extreme complexity of the cell membrane and the lack of techniques available to study molecular phenomenon in living cells. The aim of this paper is to review current understanding of permeabilisation phenomena, i.e. the basic physical processes on lipid membranes. We will describe the classical electromagnetic theory behind the phenomenon and point out its limitations in its ability to give a full description of electroporation. The basic idea behind the semi-phenomenological theory for the formation of electropores is discussed, recent results coming from numerical simulations and effects of the external electric field at the molecular level are also reviewed.

The theory of electropermeabilisation

In developing a theory, it is helpful to start from a simplified model of cell membranes. In what follows, the term vesicle will be use for any kind of object constituted of an external medium and an internal medium separated by a lipid membrane (containing or not proteins) as depicted in Fig. 1.

Transmembrane electric potential modification

Most analytical theories of electropermeabilisation are based on a continuum point of view and do not take into account the precise details of the system at a molecular level. Here we will review the basic electrical theory of these systems and point out some of the underlying assumptions behind it.

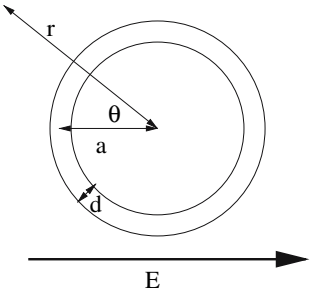

Fig. 1 Vesicle of radius a with membrane thickness d in an externally applied field $\mathbf{E}$ (direction of field indicated). r is the distance from the center of the vesicle

At the simplest level, the interaction of a vesicle with the applied electric field can be modelled by associating macroscopic electric properties with, in the simplest case, the three underlying materials of the system: the medium exterior to the vesicle, the vesicle membrane and the vesicle interior. Assuming that these components are homogeneous and have linear electromagnetic responses to applied fields the relevant electrical properties of a component are λ the electrical conductivity and ϵ the dielectric constant. In what follows we will denote the corresponding quantities by the subscripts e (exterior), m (membrane) and i (interior). The relevant Maxwell equation to compute the electrostatic potential for this system is (see for example Landau and Lifshitz 1975)

$$\nabla \cdot \left(\epsilon \frac{\partial}{\partial t} \nabla \phi + \lambda \nabla \phi \right) = 0 \tag{1}$$

and the boundary conditions are fixed away from the vesicle by the externally applied field. The local electric field $\mathbf{E}$ and current density $\mathbf{j}$ are then given by:

$$\mathbf{E} = -\nabla \phi \, ; \quad \mathbf{j} = \lambda \mathbf{E} \tag{2}$$

The second equation is simply Ohm's law and assumes a linear relationship between the induced current density and the local electric field. In most studies the local conductivity is taken to be a scalar but in principle for anisotropic systems such as a membrane it should be a tensor, there being a clear difference in the direction perpendicular to the membrane and that transverse in the membrane. The dielectric properties of the membrane are also anisotropic, we recall that the dielectric response of a material is due to the distribution and properties of its component dipoles and we thus expect different properties depending on whether we are in the region of the hydrophilic heads or the hydrophobic tail region within the bilayer. Another

important point is that the phenomenon of electropermeabilisation indicates a change in membrane structure at sufficiently high electric fields, this implies that the assumption of linear electrical response breaks down at this point and that nonlinear effects become important. However we can assume that up to this point the above set of equations gives a reasonable description of the electrical properties of the system.

The steady state equation in the presence of an applied field is the Laplace equation:

$$\nabla \cdot (\lambda \nabla \phi_s) = 0 \tag{3}$$

This equation is difficult to solve for an arbitrary geometry due to the spatial variation of λ but can be analysed in certain geometries and limits (Bernhardt and Pauly 1973; Cartree 1992; Neumann et al. 1989; Schwann 1957; Zimmermann 1974). For a planar membrane it is easily solved. If E is the applied field outside a flat membrane, the field within (perpendicular to its surface) the membrane is given by the continuity equation for the normal (in this case the only non-zero) component of the electric field

$$E_m = \frac{\lambda_e}{\lambda_m} E \tag{4}$$

This is a very simple result but has far reaching consequences. The external conductivity λ_e (which is for example of the order of 5 S/m for a weak electrolyte) is much greater than that of the membrane—typically the order of 10^{-6} S/m. This leads to a huge magnification effect with respect to the applied field. It is the creation of this large electric field within the membrane which is the basic reason for the electropermeabilisation phenomenon.

Another example which can be treated analytically is for a spherical vesicle placed in a uniform field [the case of an ellipsoidal vesicles can also be treated analytically (Bernhardt and Pauly 1973; Kotnik and Miklavcic 2000)]. The geometry is shown in Fig. 1, we denote by r the distance from the centre of the vesicle (the origin), by a the radius of the vesicle (distance from the centre of the vesicle to the middle of the membrane) and by d the thickness of the membrane. The angle θ at a given point is the angle between the position of that point from the origin and the direction of the applied field. The Laplace equation can be solved in this geometry and we find that the steady state jump in the electric potential, in the normal direction, across the membrane is given by

$$\Delta \phi_s(\theta) = \frac{3}{2} g a E \cos(\theta) \tag{5}$$

where g is a so called form factor dependent on a, d and the three electrical conductivities. In the limit where $d \ll a$, g is given by Neumann et al. (1989)

$$g = \frac{2\lambda_i \lambda_e \frac{d}{a}}{\lambda_m(\lambda_i + 2\lambda_e) + 2\frac{d}{a}(\lambda_i - \lambda_m)(\lambda_e - \lambda_m)} \tag{6}$$

In the limit where the membrane has an extremely low conductivity with respect to the other conductivities, the dielectric limit, we find that $g = 1$ and from (6) we see that the average electric field across the membrane is amplified, with respect to the applied field E by the factor a/d and its magnitude is maximal at the faces of the vesicle opposite the electrodes. Indeed it has been established for some time that it is in these electrode facing regions that the membrane becomes permeabilised. It is widely accepted that beyond a certain critical threshold $\Delta\phi_c$ permeabilisation occurs. This value of $\Delta\phi_c$ is found to be typically of the order of 100 mV (see later).

Experimentally, the vesicle or the cell is not permanently submitted to the external electric field. Therefore the variation of the transmembrane potential depends on the time t after which the pulse is applied. For a constant pulse the time dependent solution for the spherical vesicle gives the potential drop across the membrane to be

$$\Delta\phi(\theta, t) = \Delta\phi_s(\theta)\left(1 - \exp\left(-\frac{t}{\tau_m}\right)\right) \tag{7}$$

where $\Delta\phi_s$ is the steady state potential given by (5) and τ_m is the charging time of the membrane given by

$$\tau_m = aC_m\left(\frac{\lambda_i + 2\lambda_e}{2\lambda_i\lambda_e + \frac{a}{d}\lambda_m(\lambda_i + 2\lambda_e)}\right) \tag{8}$$

where C_m is the membrane capacitance per unit area. If the membrane is purely dielectric, $\lambda_m = 0$, the charging time is maximal and increases as the radius a is increased.

In order to achieve the steady state in potential drop, the external field should be applied for a time longer than τ_m. As an example, conventional electropermeabilisation protocols involve pulses of 0.1–1 kV/cm longer than 100 μs. Typically these pulses durations are much higher than the charging time constant of the outer membrane, i.e. $t_{\text{pulse}} \gg \tau_m$ (with $\tau_m \sim 1$ μs).

As stated above, when a critical value of $\Delta\phi$ is achieved across the membrane it has been observed that the lipid membrane becomes permeable (Hibino et al. 1993; Kotnik and Miklavcic 2000). The critical value has been experimentally determined to be of the order of 200–300 mV for cells (Gabriel and Teissie 1997; Teissie and Rols 1993). Clearly this critical value may be achieved before the system reaches its steady state. Thus intense but short pulses can induce the permeabilisation of the vesicle without reaching the steady state in the induced potential. For instance, recent technology authorise the use of nanosecond pulsed electric fields (nsPEFs), used in

so-called supra-electroporation, which consist in large external electric fields, i.e. 10–300 kV/cm applied in pulses shorter than 1 μs. As a confirmation of what is expected above when nsPEFs are applied to cell suspensions, they induce conduction currents in the cytoplasm exponentially decreasing with the charging time constant of the outer membrane. They also induce a corresponding displacement current through inner vesicular membrane structures (Gowrishankar et al. 2006; Vernier et al. 2004, 2006b) . For pulse durations shorter than or equal to the charging time of the outer membrane (i.e. $t_{\text{pulse}} \leq \tau_m$), a transient cytosolic field can develop membrane voltages across intracellular organelles and vesicles in excess of their critical transmembrane potential (Tekle et al. 2005; Beebe and Schoenbach 2005; Kolb et al. 2006).

The case where an electric field is applied across a suspension of cells or a tissue is more difficult to analyse, the electric field seen by a single cell is modified, with respect to the single cell case, by the presence of the others. This problem can be studied numerically and analytically using effective medium approximations (Susil et al. 1998; Pavlin et al. 2002; Yurong et al. 2005). The spirit of the effective medium approximation is that the presence of the other cells modifies the conductivity of the effective medium seen by a given cell. However for nearly dielectric membranes the potential difference generated is only weakly dependent on the conductivity of the external medium and thus the effect is not too drastic and the size the induced potential drop is not significantly changed.

In this section we have considered the effect on a vesicle of the distribution of the electric field. Of course the vesicle should be expected to react to the field at some point. Later we will discuss the theory of pore formation and it is interesting to consider how the field across a vesicle changes when pores are formed. Shown in Fig. 2 (bottom) is a spherical vesicle placed in a uniform field (from left to right) with a membrane conductivity one hundred time smaller than that of the external and internal media $\lambda_m = \lambda_e/100$. The colours on the figure (obtained by numerical solution of the Laplace equation) show the magnitude of the local electric field $|\mathbf{E}|$. We see clearly that the magnitude of the electric field is maximal at the two faces opposite the electrodes. In Fig. 2 (top) is the same vesicle but with two holes cut out at the poles opposite the electrodes. We see that the magnitude of the field within the pore regions collapses to the order of the applied field and that the maximal potential drop is across the membrane around the pore edges, however this potential drop is significantly lower than the initial drop at the poles for the membrane without holes. From these figures we see that the permeabilisation of one region of the membrane will prevent the permeabilisation of other regions by lowering the strength of the local electric field in these regions.

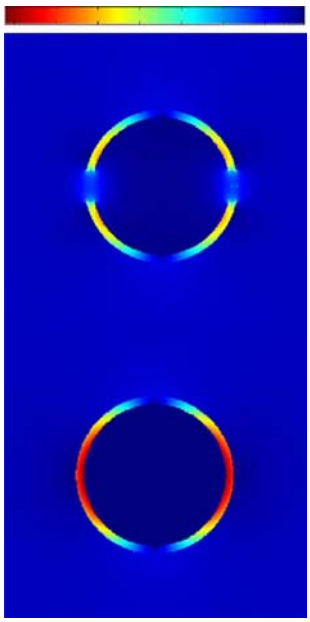

Fig. 2 Vesicles without (*bottom*) and with (*top*) pores, placed in a uniform electric field (applied horizontally), shown is the magnitude of the field $|\mathbf{E}|$ (*red high*, *blue low*)

Electromechanical effects on membranes—continuum description

Varying the different parameters of the applied electric field (value, duration, number and delay between pulses) can lead to different effects such as a slight increase in membrane conductivity, reversible electrical breakdown or irreversible breakdown leading to vesicle destruction. This latter phenomenon is often happening in systems subjected to extremely large applied electric field and was first observed in the early 1970s (Crowley 1973; Neumann and Rosenheck 1972). The electrically generated forces on the membrane can be calculated using the Maxwell stress tensor (see for instance Landau and Lifshitz 1975) and these forces will be balanced by the elastic and bending forces generated by the membrane. Deformations of the membrane are therefore determined by its mechanical properties. The elastic behaviour of a lipid membrane is generally characterised by a bending modulus and a stretching modulus. Typically bending a lipid membrane involves energy of the order of 1–10 k_BT while stretching requires much larger energy.

The first class of theories to explain electropermeabilisation were based on a continuum electrostatic and elastic description of the membrane (Crowley 1973). A perfectly flat dielectric membrane in a field is subject to an electrostatic compression. The field polarises the membrane and leads to a negative/positive polarisations charge on the surface of the membrane. The electrostatic compression

can be thought of as being generated by the attraction of these two surface charges across the membrane and this compression leads to an elastic response of the membrane. If the elasticity of the membrane (and its dielectric constant) are assumed to be independent on the thickness there is a critical field strength beyond which no equilibrium between the electrical and elastic forces is possible. This critical field strength only indicates that the model needs to be modified at high field strengths or becomes unphysical. It is however an indication that some new physics comes into play but does not tell us what it is. This model has a number of other drawbacks, the field necessary for the breakdown is too large with the experimentally observed permeabilisation threshold. Also a high degree of compression is predicted before the breakdown (40% compression), however this means that the membrane capacitance should vary up to the breakdown point but this is not observed.

For curved membranes the situation is however quite different, the stresses on spherical vesicles have been studied (Isambert 1998) and the resulting asymmetry leads to net lateral forces (which in the flat case above cancel exactly) and an increase in the surface tension Γ which is of the order

$$\Delta\Gamma \sim \frac{9\epsilon_m}{8d} E^2 a^2 \tag{9}$$

This increased surface tension is a result of the curved and closed geometry of the vesicle. In a planar membrane any local curvature, for example due to thermal fluctuations, induced asymmetry between the two sides and lead to net lateral stresses which can induced undulation instabilities beyond a critical field strength and these instabilities are again associated with an unspecified breakdown but not necessarily permeabilisation (Sens and Isambert 2002). We should however note that the lateral stress in this case leads to a negative contribution to the surface tension.

There seems to be a number of scenarios under which a membrane model can be destabilised by the application of an electric field. This destabilisation could be identified as the onset of electropermeabilisation. A common criticism made of this point of view is that this approach predicts an instability at a well defined field strength, however experimental evidence strongly suggests that there is a stochastic component in electropermeabilisation. Permeabilisation is seen to be achieved with a certain probability or after a certain time dependent on the applied field and other factors.

The creation of pores

When a membrane cannot be compressed or deformed it will need another way to minimise the electric stress. One solution is to become permeable, the defaults (or holes or pores) created in the membrane decrease the area of the membrane and strongly increase the membrane conductivity.

The theory of electroporation for cell membranes is similar to the theory of soap film rupture (Derjaguin and Gutop 1961). If one imagines a membrane with surface tension Γ_0 and then one cuts a circular hole in it of radius r, the energy change in the system due to the hole is

$$W(r) = -\Gamma_0 \pi r^2 + 2\pi\gamma_0 r \tag{10}$$

where γ_0 is a line tension associated with the pore, where the hydrophobic membrane interior is in contact with the water in the pore. Strictly speaking γ_0 should be r dependent as for large pores the hydrophilic lipid heads can line the pore interior and the energy cost is more associated with a bending one than a hydrophobic one. For pore radii smaller than $r_c = \gamma_0/\Gamma_0$ the effective force on the pore tries to close it while for $r > r_c$ the pore expands indefinitely—this scenario is associated with membrane rupture. In this simple model, pore formation in the membrane is clearly favoured by increasing the membrane surface tension Γ_0. In fact membrane pore formation has been observed in vesicles when the surface tension is increased by applying mechanical stress or even using intense light (Sandre et al. 1999). In these experiments the pore formation was seen to be temporary, pores open until leak-out of internal fluid relaxes the tension and the pores close. In order to slow down the time scale of this process to one over which it is observable the vesicles were immersed in a viscous environment. The line tension γ_0 in giant vesicles has been experimentally measured to be 10^{-11} N (Zhelev and Needham 1993). The energy function $W(r)$ can be interpreted as the energy of a particle at position r and in the limit where inertial and hydrodynamic effects can be neglected one can write a Smoluchowski equation for the number density of pores of radius r, denoted by $n(r)$ (Pastushenko et al. 1979; Powell and Weaver 1986)

$$\frac{\partial n}{\partial t} = \frac{\partial}{\partial r}\left[D\left(\frac{\partial n}{\partial r} + \frac{n}{k_B T}\frac{\partial W}{\partial r}\right)\right] \tag{11}$$

where D is the pore radius diffusion constant. This equation, although some what idealised, can be used to compute properties such as the number of pores $\int n(r)\,\mathrm{d}r$ and the area occupied by the pores $\int n(r) r^2\,\mathrm{d}r$. This is by no means a unique model for pore dynamics (although it is certainly the most popular) and versions dominated by inertial effects and hydrodynamics have also been studied (Wilhem et al. 1993; Karatekin et al. 2003).

The key point in electroporation theory is to quantify the dependence of the pore energy function $W(r)$ as a function

of r and also how to distinguish between small hydrophobic pores and larger hydrophilic pores. In most models the pores are taken to be nonconducting (Abidor et al. 1979; Barnett and Weaver 1991) and the energy change is taken into account by considering the change of the pores specific capacitance as water replaces lipid in a cylindrical region of radius r through the membrane thickness. This then gives a pore energy which depends on the potential drop across the membrane $\Delta\phi$ and effectively increases the surface tension term Γ to $\Gamma(\Delta\phi)$ which is given by

$$\Gamma(\Delta\phi) = \Gamma_0 + \frac{1}{2}\frac{\epsilon_m}{d}\left(\frac{\epsilon_e}{\epsilon_m} - 1\right)\Delta\phi^2 \tag{12}$$

where ϵ_e is the dielectric constant of the external medium, water or salt solution. The typical values of these dielectric constants are $\epsilon_e = 80\epsilon_0$ and $\epsilon_m = 2\epsilon_0$, where ϵ_0 is the vacuum permittivity. This increase in the effective surface tension then lowers the energy barrier for pore formation and also decreases the critical radius r_c beyond which the pore becomes unstable.

However the situation when the pores are considered to be conductive is quite different (Wilhelm 1993; Winterhalter and Helfrich 1987). Here the effect of the field decreases the surface tension (thus inhibits pore formation), however it decreases the line tension γ thus favouring the formation of pores for small radii. Explicitly it was shown

$$\Gamma(\Delta\phi) = \Gamma_0 - \frac{1}{2}\epsilon_m\frac{\Delta\phi^2}{d} \tag{13}$$

$$\gamma(\Delta\phi) = \gamma_0 - \frac{1}{2}\epsilon_e\frac{\Delta\phi^2}{\pi} \tag{14}$$

This means that conductive pores favour the early stage of pore formation but tend to stabilise larger pores and so the behaviour of conductive and non-conductive pores is quite different. It seems that a model reconciling these two limits is still to be proposed. Clearly we expect that the dielectric description is most relevant for small pores but beyond a certain radius conductive effects become dominant.

Equations of the form (10) describe hydrophilic pores where the lipid head groups are assumed to line the pore interior. These head groups will tend to repel each other due to steric and/or electrostatic interactions. These effects can be taken into account by including an interaction term of the pore with itself $W_{\text{int}}(r) = C/r^4$ where C is positive (Barnett and Weaver 1991). This repulsive interaction stabilises hydrophilic pores (gives a local minimum of W at $r \neq 0$). This modified potential cannot be valid at very small r as we know that the state with no pores exists. Thus there is a critical value R_* below which W must take another form. This is the radius below which there is no space to line the pores with the hydrophilic heads and thus the pore is hydrophobic. The simplest model for a hydrophobic pore is just to take $W(r) = \frac{1}{2}\kappa r^2$ for $r < r_*$. This form can be derived from microscopic modelling (Glaser et al. 1988) but in physics this simple harmonic oscillator form for the energy about a stable point is rather generic. The coefficients of the model can be taken to assure that W is continuous at r_* and that the minimum in the potential of W for $r > r_*$ occurs before r_* thus giving (meta-)stable hydrophilic pores.

The exposition above is applicable for single pores and does not take into account their interaction. Even without an applied field the pores should be coupled by the membrane tension, as a pore opens up it relaxes the tension of the others. This tension coupling can be taken into account in terms of A_p the total area occupied by the pores in a model proposed by Neu and Krassowska (2003). Apart from this the effective potential drop $\Delta\phi$ should also be modified by the presence of pores and the electrostatic energy for a hydrophilic toroidal pore is also different to that of a cylinder (Neu et al. 2003).

Despite some impressive success in describing experimental results the above theories still need to be tested at a molecular level, for instance by molecular dynamics simulations. While they have more microscopic details than continuum models they still rely on certain continuum concepts such as line and surface tensions.

Molecular dynamics simulation

Advantages and limitations

Biophysical and biological methods used to study the electropermeabilisation process present kinetics and sensitivities which, respectively, permit only millisecond and micrometre scale resolution of post-pulse membrane modifications. Moreover, these methods are rather indirect and only permit the visualisation of the consequences of permeabilisation such as the entrance or efflux of molecules. Therefore, they can not be used to visualise molecular phenomena with nanosecond resolution. Recently (for instance see the review by Tieleman 2006) these small distance and short time aspects has however been studied using molecular dynamics simulations. Nevertheless, molecular simulations present some limitations. Indeed, the complexity (one or two lipids components) and size of the simulations systems are still rather limited and certain quantitative aspects found in these simulations, for instance the critical permeabilisation field threshold, do not agree with experimental data. However these simulations give vital insight into the permeabilisation process and we may

have some confidence in the qualitative results of such simulations. In addition simulations can also be used to test the validity of theoretical models of pore formation by evaluating the energy change associated with a pore (Leontiadou et al. 2004; Wohlert et al. 2006).

Pore formation under applied tension

In a typical atomistic molecular dynamics simulation the system consists of 500 lipids in the bilayer and there are 20 or so water molecules in the system for each lipid. Before examining the effect of an electric field on these bilayers it is interesting to examine the effect of membrane surface tension on pore formation. In these systems a negative surface tension is applied, this can be achieved by choosing the area occupied per lipid be less than the equilibrium one (Leontiadou et al. 2004; Wohlert et al. 2006). For instance imposing an area per lipid of 0.60 nm^2 rather than the simulation equilibrium one of 0.62 nm^2 imposes a surface tension of about −10 mN/m (Wohlert et al. 2006). It is found that beyond a critical lateral pressure (–50 to –10 bar) pores form in the simulations and are stable over the life time of the simulations. The pores that are formed in these simulations are hydrophilic : when a hole is formed through the hydrophobic chains of the lipids, the lipid head groups reorientate in order to face the water entering the membrane. In Leontiadou et al. (2004) the line tension γ of (10) is estimated to be 3×10^{-11} N, and is the same order of magnitude as the experimental estimate mentioned in Sect. ''Creation of pores''.

Electropermeabilisation

Simulations involving electric fields across a membrane is a heavy computational task in molecular dynamics simulations as the field must be calculated from the charge distribution and acts not only on charged components of the system but also on the dipole moments of the system, and in particular the solvent water. The force acting on a dipole of moment **d** is given by

$$\mathbf{f} = \mathbf{d} \cdot \nabla \mathbf{E} \tag{15}$$

and is zero in a constant field. However where the field varies strongly the dipole is subjected to strong forces. In the simulations of Tieleman (2004), on phospholipid bilayers, it was shown that the initial step in the electroporation process is quite different to that of electroporation under an applied lateral tension. The field applied were of the order of 0.5 V/nm and the poration proceeds by penetration of water molecules into the lipid bilayer due to the strong electrical forces acting on their dipole moments. As the water molecules penetrate the bilayer the local variations of the electric field in these defects lead to further forces on the dipoles, thus accelerating the process of pore formation. In this initial step of pore formation the pores are hydrophobic but eventually are lined with the phospholipid head groups and thus become hydrophilic. The presence or absence of salt is found to have little effect on the initial process of pore formation. Indeed simulations on an octane layer in water, with no charged head groups or salt, shows exactly the same behaviour. Similar results were found in the simulations of Tarek (2005), where wire-like chains of water were seen to penetrate the bilayer. The pores are then again stabilised by a lining of lipid head groups.

Vernier et al. (2004) showed that when an electric field of 0.45 V/nm is applied on DOPC-DOPS bilayer, the poration is observed on time scales shorter than 5 ns and PS translocation occurred only along pore walls (see Fig. 3). PS lipids do not facilitate water pore formation, consistent with the idea that interactions in the hydrophobic interior of the membrane govern the relative ease of spread of water column into the bilayer (Tieleman 2004). When the field polarity is changed, PS translocation is reversed. This result suggested that pulse-driven PS externalisation appears to be an electrophoretic transport of PS head groups through hydrophilic pores (Vernier et al. 2006a).

Gene electrotransfer

DNA is an anionic and hydrophilic molecule which under normal circumstances can not cross the plasma membrane. However, electropermeabilisation allows an increased membrane permeability with respect to DNA. The molecular basis behind this process of gene electrotransfer is highly debated. Indeed, there are two hypotheses: the first suggests that DNA is transported through large stable pores that form without significant interaction with DNA molecules and then reseal. Krassowska's theoretical model and Tieleman's molecular simulation back up this hypothesis

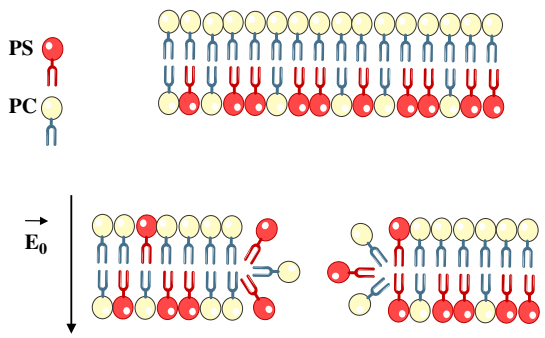

Fig. 3 Phosphatidylserine translocation: pulse-driven PS externalisation appears to be an electrophoretic transport of PS headgroups through hydrophilic pores

(Krassowska and Filev 2007; Smith et al. 2004; Tieleman 2004). The second hypothesis, which is more widely supported, is as follows. Experimental investigations (Golzio et al. 2002; Sukharev et al. 1992) and molecular simulations suggest that DNA molecules migrate into cells by direct interaction with lipids and by forming intermediates that involve lipid/DNA complexes. In this hypothesis, electric field application induces many pores of a diameter which is too small to allow DNA transport (steric, entropic and image charge forces prevent the passage). However, when the electric field is switched off, structural modifications can assure DNA translocation into the cytoplasm. Indeed, plasma membrane is a dynamic and functional assembly which consists of several molecules and presents heterogeneity of lateral and transversal distribution of these molecules. Consequently, one can suggest that such induced-electric field perturbations on membrane components distribution could promote DNA translocation.

Electric field effect on membrane components

We have previously seen how analytical theories and molecular simulation can predict the existence of pores in idealised or simplified vesicles. Nevertheless, in order to fully understand the effect of electric field on membranes, it is necessary to analyse its effects on the membrane components.

Transbilayer mobility

Phospholipid distribution in two leafs of plasma membrane is asymmetric. Indeed, PE and PS phospholipids are presents in the inner leaf whereas PC phospholipids set in the outer leaf of plasma membrane. Transmembrane asymmetry is supported by proteins like flippases and floppases (Janmey and Kinnunen 2006). Some investigations showed that this transmembrane asymmetry is lost when electric field is applied in cells (Dressler et al. 1983; Haest et al. 1997). Haest et al. (1997) described that the passive transbilayer mobility of fluorescently labeled (using NBD) lipids in erythrocytes increase with the strength, the duration and the number of field pulses (Fig. 3). Moreover, this study revealed that the enhanced mobilities induced by electropermeabilisation differ for the NBD-lipids used (the following mobility ordering is observed: SM $\ll$ PS $<$ PC $<$ PE). These enhanced transbilayer mobilities of the phospholipid probes do not return to their normal values even during the prolonged resealing of field-pulse-treated cells (24–27 h at 37°C, Haest et al. 1997). Moreover, ATP-dependent components of the flip-flop of lipid probes are suppressed in electroporated and resealed cells. This inhibition is partly due to loss of cellular Mg^{2+} during the electropermeabilisation. Indeed, the electropermeabilisation induces the entry and the exit of, respectively, water and ATP. Translocated lipids contribute to the stabilisation of water channels (Kotulska et al. 2007; Tarek 2005). Moreover, Devaux showed that membrane lipids translocation can induce microvesicles formation (100–200 nm) (Devaux 2000). Consequently, one can suppose that electro-translocated lipids play also a role in endocytosis mediated by electric field. Such electroendocytosis process has been observed on cells several hours after the electropermeabilisation (Mahrour et al. 2005; Antov et al. 2005). The key point in these investigations is that if the electropermeabilisation phenomenon is very fast (induced at the nanoseconds to milliseconds time range of order) its consequences can persist for a long time (from minutes to hours).

Lateral diffusion

Several investigations have suggested that when low electric fields (LEFs) are applied on cells, electrophoretic segregation of charged lipids and proteins occurs in the plane of the cell membrane (McLaughlin and Poo 1981). Indeed, the tangential electric field (E_θ) in the cell membrane is a driving force which induces electromigration towards the anode or cathode sides of the cell, either by electrophoretic mobility of charged components or by electrophoresis (Fig. 4). For weakly conducting membranes this tangential components is given by

$$E_\theta = \frac{3}{2} E \sin(\theta). \tag{16}$$

An asymmetric distribution of acetylcholine receptors in embryonic muscle cells or Concanavalin A receptors in

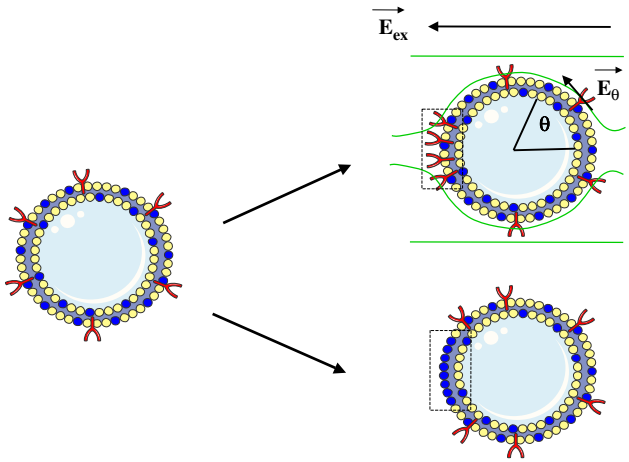

Fig. 4 Proteins and lipids electromigration: the external E_{ex} and tangential electric field, E_θ, is a driving force which induce electrophoretic mobility of proteins and/or lipids toward cathodic sides of the cell. *Green lines* represent electric field lines

Xenopus myoblasts, is induced when a DC electric field of 10 V/cm is applied for 45 min up to 3 h (Lin-Liu et al. 1984; McLaughlin and Poo 1981). McLaughlin and Poo 1981 have described that the the additional exposure of Xenopus myoblasts to the same strength of field but with opposite polarity for 10 min completely reverses the asymmetric accumulation of Con A receptors towards other side of the cell. Moreover, the lateral electromigration of epidermal growth factor receptors on corneal epithelial cells (Zhao et al. 1999) and $F_{c\epsilon}$ receptors on rat basophilic leukaemia cells toward the cathodic sides of the cell occurs when a DC electric field of 1.5 V/cm is applied for 12 h and when a 10 V/cm field is applied for 30 min to 1 h. The common point made in these investigations is that protein electromigration occurs only when electric fields of these orders are applied over a relatively long period of time. Consequently, one can speculate that low electric field application during short time scale causes limited electrophoretic motility which induces limited and reversible protein aggregation, as opposed to what happens for long exposure times. These results may have direct consequences on subsequent transport process across the membrane. Antov et al. (2005) have suggested that the electrophoretic segregation of charged components (e.g., glycolipids and glycoproteins) in the outer leaflet on the cell membrane is responsible for both enhanced adsorption and stimulated uptake via changes in the membrane elastic properties that enhance budding and fission processes. This phenomenon is called *electroendocytosis*.

Protein conformations

Several investigations have suggested that membrane proteins (i.e. voltage-sensors in skeletal muscle fibers) may undergo some structural and functional damage during the electric injury (Teissie and Tsong 1980; Chen and Lee 1994). Membrane proteins consist of amino acids which carry electrical charges (Tsuji and Neumann 1983) and each peptide unit is an electric dipole with a moment of about 3.5 Debyes. The major functional structure of the membrane protein is α-helical. In this structure, many peptide dipoles are aligned to form larger dipoles of the order of 120 Debyes across the plasma membrane (Hol 1985). In response to physiological membrane potential changes, these electric dipoles are perturbed in voltage-dependent membrane proteins (i.e. channel proteins, voltage sensors). An external electric field will strongly affect the electric dipoles in membrane proteins and this may cause conformational and functional damage (Chen 2005). Indeed, a 4 ms transmembrane potential pulse of –600 mV results in a reduction of both Na^+ and K^+ channel conductivities, and a reduction of ionic selectivity of the K^+ channels against Na^+ ions. This phenomenon induces a depolarisation of the membrane resting potential in frog skeletal muscle membrane. The kinetics of the spontaneous reversal of the electroconformational damage of channel proteins was found to be dependent on the magnitude of imposed membrane potential pulse (Chen and Lee 1994).

Conclusion

The current state of our theoretical understanding of membrane permeabilisation due to electromagnetic fields, and the associated transport of molecules, is some what incomplete and limited. Certain effects of the electric field parameters on membrane permeabilisation, and the associated transport of molecules, are well established but a great deal of what happens at the molecular level remains speculative. Molecular dynamics simulations are now giving interesting new insight into the process. However, a cell membrane is highly complex and can not be considered as the simple assembly of one or two classes of lipids. Electroinduced destabilisation of the membrane includes both lateral and transverse redistribution of lipids and proteins, leading to mechanical and electrical modifications which are not yet fully understood. One may suggest that such modifications can be involved in the subsequent transport of molecules interacting with them such as the DNA molecules. Experimental verification of the basic mechanisms leading to the electropermeabilisation and other changes in the membrane remain a priority given the importance of these phenomena for processes in cell biology and in medical applications.

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