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ELECTRICAL BREAKDOWN OF MEMBRANES

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A transient increase of membrane permeability and conductivity is observed when a cell membrane or an artificial lipid membrane is exposed to a transmembrane potential difference in the order of 1 Volt. The basic mechanism of this phenomenon, which is the same in cells as well as in BLM, involves the following steps: formation of hydrophobic pores, increase in radius and inversion of the pores to hydrophilic ones by molecular rearrangement of pore edge. The electric field enhances pore widening mainly by forces rectangular to the field lines.
The hydrophilic pores permit small molecules to cross the membrane. However, ions have to overcome a considerable energy barrier so that pore conductivity shows a strong and nonlinear dependence on voltage.
There exists neither a threshold voltage for pore formation nor any qualitative change of the electric response of the membrane during reversible electrical breakdown.

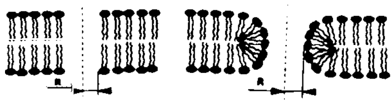
1. Introduction

Electrofusion and electroporation techniques have increasingly found new applications in biotechnology, medicine and other fields during the last few years /2/. For each new application parameters of the protocol have to be optimized /7/, or even new procedures have to be developed.
For that it is quite useful to know what primary processes go on in the membrane and to have a theory relating them to the phenomena observed. This topic is hardly found in reviews on cell electrofusion and electroporation; and according to our experience many of those who deal with biotechnological applications of these techniques still stick to rather inadequate models /9/ when they

try to imagine what goes on in their system.
Our poster (or this synopsis) is addressed to everyone who treats cells with electrical pulses. It introduces you to the primary processes which are relevant for the action of the electrical pulse and to the mechanism by which these processes lead to effects like electrofusion and electroporation. We are going to do this without presenting many theoretic formulae or much experimental evidence, which are presented elsewhere /3,5,6/.

2. Molecular Structure of the Pores

Hydrophobic pores are formed in the bilayer just by thermal fluctuations, so their life time is very short. However, they can reach a diameter in the order of 1 nm, as the calculation of their energy shows /6/.



a) hydrophobic b) hydrophilic
Fig. 1 Types of pores in the membrane

At this diameter and larger ones hydrophilic pores are energetically more favourable than hydrophobic ones. An inversion of the pore takes place, i.e. the lipids rearrange in a way that hydrophilic headgroups form the pore wall. SUGAR and NEUMANN /10/ proposed this reorientation not to be a single-step process but rather to involve the rearrangement of individual lipid blocks.

3. Forces acting on the Membrane

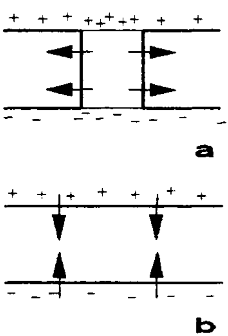


Fig. 2 Forces acting to form or widen pores

The basic mechanism by which the electrical field across the membrane enhances pore formation and enlargement are forces rectangular to the field lines (Fig. 2a) /1/. The water-filled pores represent an area of higher permittivity, and as long as the conductivity of the pore is negligible, the electrical field across the membrane produces a pressure inside the pore against the lower permittivity material of the membrane.
For some time the attraction of charges on both sides of the membrane (Fig. 2b) was believed to cause a membrane "breakdown" /4/, even in cell membranes /11/. But calculations

showed that this attraction force is not strong enough to explain pore formation adequately. However, pressure due to charge separation at the membrane may be involved in the creation of local fusion sites.

4. Kinetics of Pore Formation and Resealing

Hydrophobic pores only marginally contribute to the rise of membrane permeability and conductance under usual conditions, since their radius is too small. What we observe in electroporation studies is the development of number and size of hydrophilic pores.

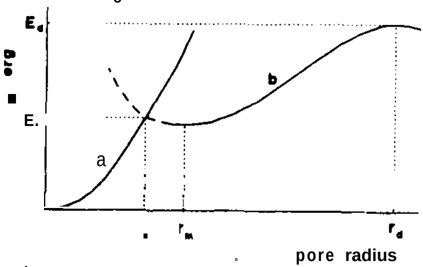


Fig. 3 Energy of both types of pores

Hydrophilic pores cannot exist at zero radius (Fig. 3). They develop from hydrophobic pores large enough, and thereby an energy barrier (E_*) has to be overcome. Considering the contribution of the force discussed above on free energy of the pore we get a decrease of this barrier with square of membrane voltage U_m /6/. Consequently, the rate of pore formation is proportional to $\exp(U^2)$. This was indeed found in the experiments, and for uranyl modified azolectin membranes this barrier is located at a pore radius of about 0.5 nm and amounts to about 40 - 50 kT in the absence of the field /6/.

The hydrophilic pore represents a metastable state. To disappear the pore has to overcome an energy barrier again. In pure lipid membranes the life time of the pores is in the order of milliseconds to seconds while in cell membranes interaction with proteins may stabilize the pores for much longer times.

5. Pore Conductance

Ions crossing a narrow pore in a membrane undergo a considerable interaction with the membrane surrounding the pore /8/. As a result pore conductivity is a function of voltage across the membrane, and the type of this function depends on pore shape and radius /3/.

It is quite important to take this interaction into account; otherwise one may underestimate the overall area of the pores by orders of magnitude.

The conductivity of the membrane at different voltages can be used to estimate simultaneously the size as well as the number of pores in the membrane /6/. In uranyl modified azolectin membranes we found pores of a radius between 0.6 and more than 1 nm.

6. Irreversible Breakdown

If pores with an overcritical radius (r^d, Fig. 3) develop in membranes under mechanical tension (e.g. in artificial BLM) the edge energy of the pore is no longer able to counterbalance pore enlargement; mechanical rupture is observed. Irreversible effects after pulsation of cells are usually due to secondary processes as osmotic swelling or leakage of metabolites.

7. Critical Voltage

If the membrane system under investigation isexposed to increasing voltages, then a point will be reached where the current through the membrane is so high that it produces a noticeable voltage drop in the solution surrounding the membrane. This reduces the voltage across the membrane to a value which depends on pulse length but is independent of the externally applied voltage /5/. A break in the electrical behaviour of the whole system is observed /11/. It was concluded that the properties of the membrane changed qualitatively, however, this was a clear misinterpretation.

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