

## ELECTRIC FIELD-INDUCED STRUCTURAL REARRANGEMENTS IN BIOMEMBRANES

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Structure and function of biological membranes are vitally dependent on the electric membrane polarization; prolonged depolarization of the plasma membrane causes cell death. The natural cross-membrane electric field affecting the protein/lipid dielectrics are on the order of  $100 \text{ kV cm}^{-1}$ . The inhomogeneous field originating from ionic groups and adsorbed small ions are of the same order of magnitude; these fields are restricted to the interfacial compartments adjacent to the membrane surface. Externally applied electromagnetic fields which are strong enough to compete with the intrinsic membrane fields, cause structural rearrangements in the proteins and in the lipid bilayer parts[1-6].

For example, electro-optic data of aqueous suspensions of purple membranes indicate that bacteriorhodopsin exhibits conformational flexibility in electric field pulses ( $1-30 \text{ kV cm}^{-1}$ ,  $1-100 \mu\text{s}$ ). The electric dichroism shows two kinetically different structural transitions within the protein molecule[1]. The electrically induced rearrangements comprise a rapid ( $\tau \approx 1 \mu\text{s}$ ), but concerted, change in the orientation of both retinal and tyrosine and/or tryptophan side chains. These angular changes of position are accompanied by changes in the local protein environment of the chromophores. A slower relaxation mode ( $\tau \approx 100 \mu\text{s}$ ) involves alterations in the microenvironment of aromatic amino acid residues and is accompanied by pK-changes of at least two types of proton binding sites, leading to a sequential uptake and release of protons.

Light scattering data are consistent with the maintenance of the random distribution of the membrane discs within the short duration of the applied electric fields.

The kinetics of the electro-optic signals and the steep dependence of the relaxation amplitudes on the electric field strength suggest a saturable induced-dipole mechanism and a rather large reaction dipole moment of:

$$\Delta m = 1.1 \times 10^{-25} \text{ C m} \quad (1)$$

( $= 3.3 \times 10^4$  Debye) per cooperative unit at  $E = 1.3 \times 10^5 \text{ V m}^{-1}$ [1]. The large reaction dipole moment:

$$\Delta M = 6.62 \times 10^{-2} \text{ C m mol}^{-1}, \quad (2)$$

is indicative of appreciable cooperativity in the probably unidirectional transversal displacement of ionic groups on the surfaces of, and within, the bacteriorhodopsin proteins of the membrane lattice.

The numerical value of  $\Delta M$  thus reflects ionic polarization involving ion pairs of the protein, in-

clusively the intrinsic Ca/Mg-ions and the ionic atmosphere of the entire membrane disc.

The data of detergent treated purple membranes suggests that detergent binding interferes with the protein/protein lattice interactions. At low concentrations of Triton X-100 the detergent appears to loosen the crystalline-like bacteriorhodopsin lattice structure. At higher detergent content, however, the protein-protein contacts seem to be strengthened, facilitating long-range interactions. Finally, at very high detergent concentration the protein lattice is dissolved.

Photobleaching disrupts the retinal Schiff's base bond. Thereby the internal fluidity of the protein increases as well as the internal flexibility of the neighbouring unbleached proteins. The bleaching experiments suggest that the protein polarization in external electric fields is independent of whether the retinal is covalently bound or not. Furthermore, the field-induced uptake and the release of protons are not affected by the state of the neighbouring proteins. The proton transfer is directly coupled to the existence of the intact Schiff's base.

Addressing the functional aspect of field-induced structural rearrangements in bacteriorhodopsin, there are some similarities between the photocycle and the electro-optic cycle of rotational chromophore displacements and conformational transitions in the protein part. In particular, there is a hyperpolarizing increase in the absolute value of the electric membrane

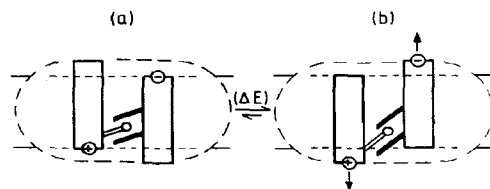


Fig. 1. Principle of the saturable induced-dipole mechanism causing positional changes of side chains in helical membrane proteins. In bacteriorhodopsin helical parts with different net charge may move transversal to the membrane plane in opposite directions when the electric membrane field is increased, (a)→(b). The geometrically limited increase in the distance of the charge centers is equivalent to a saturable induced dipole moment. The transversal displacement of at least one of the two helical parts can thereby cause a concerted rotational shift of the retinal ( $=0$ ) and of aromatic amino acid side chains which may sandwich the retinal chromophore.

potential difference during light induced proton pumping. This increase in the membrane field is concomitant with a decrease in the proton transport.

The temporal coincidence and the opposite sequence of field-induced pH-changes compared with the light-induced pH-changes suggest a direct functional role of the electric field changes. The increase in the electric membrane field resulting from proton pumping may exert a negative feed-back (reducing proton transport) *via* an electric field effect directly on the structure of bacteriorhodopsin, switching off pump activity.

A further functional aspect of the bacteriorhodopsin data may be of general fundamental importance. Compared with the rapid induction of the electric field-mediated structural changes ( $\mu\text{s}$  range), annealing of the changes after the electric impulse is very slow (ms). The field-induced conformational transitions therefore exhibit memory properties.

It may be recalled that electric field-induced, long-lived structural changes have been suggested as a mechanism for the initial, physical step of biological memory recording.

Besides the field-induced conformational changes in membrane proteins the phenomena of electroporation and electrofusion are impressive examples for

electrically induced structural rearrangements most probably in the lipid part of biomembranes. Both electroporative gene transfer and electrofusion are of great importance in cell biology and biotechnology.

The mechanisms, *ie* the electric field-induced structural rearrangements leading to the formation of the porous membrane patches, which furthermore are fusogenic, are not known. In any case the electric field effect is indirect. The externally applied electric field is at first amplified interfacial membrane polarization before the membrane structure responds to the field forces, exceeding a threshold value  $E_m$ .

The natural membrane potential difference is  $\Delta\varphi_m \approx -100$  mV; *ie* the natural average field across the membrane of thickness  $d = 10$  nm is  $E_{m,n} \approx \Delta\varphi_m/d = 100$  kV cm $^{-1}$ . The stationary value of the actual transmembrane voltage  $V_m$ , relative to the direction of the (constant) external field vector  $E$ , results from contributions from the (diffusion) potential  $\Delta\varphi_m$ , from asymmetric surface charges and the interfacial polarization. For a spherical cell of radius  $a$ ,  $V_m$  is given by[6]:

$$V_m \approx \Delta_m \frac{|\cos\delta|}{\cos\delta} - \frac{3}{2} f(\lambda) E a |\cos\delta|, \quad (3)$$

Table 1. Fundamental processes of the electroporation hysteresis of membranes

Physical-chemical processes	Electric terms
<b>I. REVERSIBLE PRIMARY PROCESSES</b>	
1. <i>Primary electric events</i>	
1.1 Electric dipole induction and dipole orientation	Dielectric polarization
1.2 Redistribution of mobile ions at phase boundaries (membrane-solution), including (a) ionic atmosphere shifts, (b) local activity changes of effectors, <i>eg</i> H $^{+}$ - (pH-changes) or Ca $^{2+}$ -ions	Ionic-dielectric interfacial polarization (Maxwell-Wagner, $\beta$ -dispersion)
2. <i>Structural rearrangements</i>	
2.1 Conformational changes in protein and lipid molecules	Electro-restructuring
2.2 Phase transitions in lipid domains, resulting in pores, cracks ( <i>via</i> pore coalescence) and percolation	Electroporation Electroporeses, electrocrack Electropercolation
2.3 Annealing and resealing processes	
<b>II. IRREVERSIBLE SECONDARY PROCESSES</b>	
1. <i>Transient material exchange</i>	
1.1 Release of internal compounds, <i>eg</i> hemolysis	Electropermeabilization
1.2 Uptake of external material, <i>eg</i> drugs, antibodies	Electrorelease Electroincorporation, Electrosequencing
1.3 Transfer of genetic material, <i>eg</i> DNA, mRNA, viroids, ... with stable cell transformation	Electrotransfection Electrotransformation, Electroporative gene transfer
2. <i>Membrane reorganizations</i>	
2.1 Cell fusion (if membrane contact)	Electrofusion
2.2 Vesicle formation (budding)	Electrovesiculation Electrobudding
2.3 Electromechanical rupture	Dielectric breakdown
3. <i>Tertiary effects</i>	
3.1 Temperature increase due to dissipative processes	Joule heating, dielectric losses
3.2 Metal ion release from metal electrodes	Electroinjection
3.3 Electrode surface H and O <i>in situ nascendi</i>	Electrolysis

where  $E$  is the amount of  $\mathbf{E}$  and the conductivity factor  $f(\lambda) \leq 1$  may be approximated by  $f(\lambda) = 1$  for a non-conducting membrane. Equation (3) correctly covers the signs and the angular position dependence of  $V_m$  relative to  $\mathbf{E}$ . At the pole caps in the  $\mathbf{E}$ -direction,  $|\cos \delta| = 1$ , yielding the maximum values of  $V_m$ .

An average value for the stationary transmembrane electric field strength  $E_m$  relative to the external field vector may be estimated from:  $E_m \approx -V_m/d$ .

It has been outlined previously[6] that membrane electroporation represents a cycle of structural rearrangements, where the intermediate states of the unidirectional annealing process are probably different from those of the unidirectional electroporation process in the presence of the external field.

The physical conception that comprises both (reversible) metastable states and (irreversible) unidirectional transitions in a cyclic manner is called hysteresis. Therefore the electroporation/resealing cycle may be analyzed and understood in terms of a structural relaxation hysteresis.

The actual processes which are involved and/or accompany the electroporation hysteresis are summarized in Table 1. The compilation in this table aims at a conceptual clarification as a necessary basis for studies of the mechanisms of electric field-induced structural reorganizations in biomembranes.

*Acknowledgement*—Support by DFG (grant Ne 227/4) is gratefully acknowledged.

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## EFFECTS OF ION PAIR CORRELATIONS IN ELECTRIC DOUBLE LAYERS

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The problem of diffuse ion layers near charged surfaces immersed in electrolyte solutions can be analysed with a number of increasingly elaborate physical models. Since the statistical mechanics equations of these models cannot be solved exactly, they are treated at different levels of analytical approximation or numerical simulation. In the simplest model, ions are replaced by point charges and the solvent is a dielectric continuum. The appropriate mean field theory dates back to the work of Gouy and Chapman. With a more elaborate "primitive" model, ions are represented as charged hard spheres with a given radius.

We are now able to evaluate the behaviour of the primitive model of the double layer with great accuracy. At the level, even such a simple model presents many surprises. The collective behaviour of ions in the electrolyte, most easily described *via* ion-ion pair correlation functions, modifies mean field results and leads to interesting effects. While the most dramatic changes from the expected behaviour are found in the evaluated surface-surface interaction, other quantities like the ion density or the electrostatic potential are also affected. Experimental data obtained in direct measurements with the surface forces apparatus in Canberra support our conclusions.

The theoretical advances have been made *via* two major routes. In the first case one considers two surfaces at a separation relatively large compared to the Debye screening length. By approximating the

long range behaviour of the direct pair correlation function with its asymptotic limit,  $c(\mathbf{r}, \mathbf{r}') \sim -U(\mathbf{r}, \mathbf{r}')/k_B T$ , one can derive different limiting laws for the surface-surface interaction. This work has re-emphasised the intrinsic link between the Van der Waals forces and the double layer interaction. The temperature-dependent zero-frequency term in the dispersion force calculations is screened by the fluctuation of the ionic cloud. The effect has been described in a classical book on the dispersion forces[1] and some of the formulas derived earlier for the simpler cases work remarkably well. New results have extended this work to a more difficult case of charged[2, 3] and dipolar[2, 4] surfaces.

The second theoretical method involves solving the standard integral equations of liquid-state physics for the inhomogeneous ionic fluid between two surfaces. In planar geometry, we consider such fluid as consisting of many layers of uniform density, which are then equivalent to a multi-species two-dimensional fluid. With this method, we have achieved[5] the long-standing goal of treating the inhomogeneous ionic fluid near a charged surface as accurately as if it were a bulk fluid.

The results of our calculations of the ion correlation effects depends on the strength of the coupling in the system. For low surface charges (when area per unit charge is above about  $5 \text{ nm}^2$ ) and low monovalent electrolyte concentrations (up to about 0.1 M) the