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Charge Transfer Processes in Model and Biological Membranes: Defect and Mechano-Electric Aspects; Statics and Dynamics

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Mechano-electricity is a fundamental property of membranes, related to their mechanical and electrical degrees of freedom. Special importance in this respect is paid to the following pairs of variables: membrane tension and conducting defects' state; membrane curvature and polarization. Charge transfer processes are profoundly influenced by the interrelation between the tilted membrane characteristics.

The effect of membrane tension on the probability of opening of ion channels in locust muscle membrane has been studied using patch clamp techniques. The data emanating from these studies are consistent with a linear model of stress activation of these channels. Mechanosensitive defects (ion pores) in model dip-tip lecithin membrances containing peptide cyanobacterial toxins (microcystin and nodularin) have also been studied. In this case application of tension caused a progressive opening of defect states of higher conductance, according to a model for pore gating by lateral tension.

Membrane curvature is related to another fundamental mechano-electric property of membranes, *viz.* curvature electricity or flexoelectricity. By using patch clamp techniques combined with phase-sensitive amplification and by dynamic excitation of natural and artificial membranes by oscillating pressure it has been possible to study flexoelectric effects without and with ion pores or channels in the membranes. Striking enhancement of the flexoelectric res- ponse during pore/channel opening was observed. These data provide the first experimental evidence of our hypothesis that flexoelectricity is a driving force for ion transport through membrane channels.

Keywords: BLM; biomembranes; ion channels; pores; flexoelectricity; ion transport

INTRODUCTION

Mechano-electricity of a biological (or model) membrane implies a modification of its electric properties by mechanical forces (or vice versa). In other

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words mechano-electricity implies a relationship between mechanical and electrical degrees of freedom of a membrane system. Both passive (e.g. ion permeability) and active properties (membrane polarization, transmembrane electric potential) may be involved. In this way both electrostatics and electrodynamics of membranes are dependent on their mechanical state. The mechanical degrees of freedom that are of special importance for mechano-electricity are membrane tension and membrane curvature. Tension acts as a modifier of membrane permeability by means of changing the conducting state of membrane channels and pores. Curvature, on the other hand, induces electric polarization by virtue of a fundamental mechano-electric effect, viz. membrane flexoelectricity. Several other mechano-electric effects are still possible in membranes (i.e. piezoelectricity, ferroelectricity), though probably of a lesser importance.

MEMBRANE TENSION MODIFIES THE OPEN PROBABILITY OF ION CHANNELS

Mechanical regulation of the activity of an ion channel relies on the existence of slowly relaxing, mechanical degrees of freedom in such a protein molecule. It requires two conditions [6]: First, a force must be exerted on the membrane protein through an elastic element that is tensed by displacement. Second, a change in channel activity must entail a change in conformation that alters the tension in the elastic element. Stress-activated channels were discovered by Guharay and Sachs (1984) in chick skeletal muscle membrane [4]. The model of Howard *et al.* (1988) explains their behaviour by replacing the elastic element by the lipid bilayer surrounding the channel and the gating change by the difference in membrane area displaced by the open and closed channel conformation [6]. This area difference ΔA, envisaged to be of molecular dimension, would be multiplied by the membrane tension M to produce an energy difference between the open and closed states:

$$\Delta G = -M\,\Delta A \tag{1}$$

In this way the open probability p becomes tension-dependent:

$$p = 1/(1 + \exp(\Delta G/kT)) \tag{2}$$

where kT is Boltzmann constant times absolute temperature.

If ΔA is positive (open conformation larger than the closed one) the model predicts an increasing open probability with tension, i.e. a stress-activated channel (SAC). But if we assume an open conformation smaller in cross section than the closed one, i.e. ΔA negative, then the behaviour of the recently observed stress-inactivated channels (SIC) by Morris and Sigurdson [9] can be explained as well.

Recently, we have studied the tension dependence of the opening probability of a potassium-selective channel in the membrane of adult locust muscle by using patch clamp technique and by applying a static suction pressure P to the pipette interior ([20], and in preparation). The membrane tension M was varied in this way according to the law of Laplace:

$$P = M(1/R_1 + 1/R_2), \tag{3}$$

where R_1 and R_2 are two principal radii of membrane curvature. The channel having 35 pS conductance was found to be stress-activated one. The open probability was determined from the proportion of the area of the open state peak with respect to the total area of the amplitude histogram of channel currents. This was done at increasing values of the pressure difference, from 0 to -5 kPa. Applying the linear model of channel gating outlined above, channel gating area was evaluated as 2.4 nm [2]. Using the adhesion model of Opsahl and Webb [11] the initial tension of the patch originating from its adhesion interaction with glass micropipette wall could also be evaluated by a nonlinear fit of the *log* (probability) vs. pressure dependence and it was found to be about 0.5 mN/m.

ION PORES PRODUCED IN LIPID BILAYERS BY CYANOBACTERIAL TOXINS, ARE MECHANOSENSITIVE

Application of lateral tension to lipid bilayers leads to the appearance of defects with the structure of semitoroidal pores [7]. The edges of these pores are formed by a strongly curved lipid monolayer connecting the outer and inner face of the bilayer. Litster's approach [7] based upon the concept of a line energy per unit length of the pore edge, called "edge energy" γ has further been developed by us in order to include the dependence of the edge energy on the pore radius R [17, 19]. The concept of generalized molecular asymmetry [2,15] was used to show how wedge-shaped lipids (lysophospholipids, free fatty acids, glycolipids, gangliosides, etc.) decrease the edge

energy and promote pore opening [17]. They do so by accumulating in the strongly curved part of the lipid monolayer lining the pore. If a stable pore of radius r is then formed in an unstressed bilayer, its increment Δr caused by application of lateral tension M is calculated [10]:

$$\Delta r = -Mr^2/(2\gamma - 3Mr). \tag{4}$$

When M is low, Δr increases linearly with M; when M approaches a critical value of $2\gamma/3r$, there is an irreversible growth of the pore leading to disruption of the bilayer.

If the molecular structure of the pore is taken into account considering the pore as a semi-toroidal multimer of asymmetrically shaped wedge-like molecules, than the pore growth would be inherently step-wise, each step being produced by recruitment of another monomeric unit of the pore-forming molecules. In this way step-wise fluctuations of the ion curents through the pore would be observed. Such an approach was applied to the barrel-stave model of alamethicin pores [11], and the gating area increment by a single alamethicin molecule was determined to be 1.2 nm [2] using an expression for the pore energy under lateral tension of the type of Eq. (1).

We have investigated diphytanoyl lecithin bilayers containing oligopeptide toxins (microcystin, nodularin) from blue-green algae [19,23]. These weakly biphilic (as revealed by surface tension measurements) and wedge-like toxin molecules may aggregate to form semi-toroidal, hydrophilic pores. The dip tip technique of Coronado and Latorre [1] was used and various protocols of toxin emplacement in the lipid bilayer were attempted. The pores formed by the toxins exhibited many conductance states (from 5 pS to over 1000 pS). Tension-induced switchings from low to high conductance states were observed when both blowing and suction pressures were applied to the bilayer. Nodularin pores were described by a combination of the semi-toroidal and barrel-stave models. From the experimentally found relationship between occurrence probability and pore conductance (i.e. pore radius) the edge energy g of a nodularin pore was determined [23] as $1.4 \cdot 10^{-12}$ J/m, considerably lower than pure egg lecithin value [5] of $2.0 \cdot 10^{-11}$ J/m. These studies demonstrate that unlike membrane channels bilayer tension can modulate not only pore open probability, but also pore open conductance, a result in line with the theoretical model outlined above (Eq. 3). The results may have also importance for native membranes, as it was demonstrated that nodularin can form stable long-lived open pores in locust muscle membrane patches [23].

FLEXOELECTRICITY: A DIRECT MECHANISM FOR TRANSFORMATION OF MECHANICAL INTO ELECTRICAL ENERGY IN BIOMEMBRANES

Flexoelectricity is an effect related to the liquid crystal structure of the membrane. It stands for the curvature-induced membrane polarization [13,21,22]:

$$P_s = f(1/R_1 + 1/R_2), \tag{5}$$

where P_s is the membrane polarization per unit area in C/m and f is the flexoelectric coefficient in Coulombs. Note the formal analogy between the constitutive equation of flexoelectricity (5) and the Laplace law (3).

Flexoelectricity is manifested in liquid crystals and in liquid crystalline membrane structures where curvature causes an orientational splay deformation of the membrane constituents (*cf.* [8]) and modulates the surface density of charges and dipoles on the membrane surface. The theory of membrane flexoelectricity was extensively developed (see [14, 22, 26]). Typical order of magnitude of f is from 1.10^{-20} to 1.10^{-18} C, depending on the kind of electric multipoles giving the leading contribution: dipoles or charges [24, 26].

As with piezoelectricity in solid crystals, flexoelectricity can also be manifested by a direct flexoeffect (i.e. curvature-induced polarization, Eq. (5)) and by a converse flexoelectric effect (i.e. electric field-induced membrane curvature):

$$(1/R_1 + 1/R_2) = fE/(K + Ma^2/8), \tag{6}$$

where E is the transmembrane electric field, K is the curvature elasticity modulus of the membrane and a is the membrane radius. Eq. (6) predicts a substantial curvature for the usual values of the membrane potential, but it can only be observed if membrane tension M is identically zero. First observation of the converse flexoelectric effect in BLM, where the damping effect of M is substantial, was done very recently [25] by using stroboscopic interferometry.

Direct flexoelectric effect can be studied experimentally by dynamic excitation of the membrane with oscillating pressure that makes the membrane curvature oscillate [16]. With a high imput impedance electrometric amplifier the transmembrane potential oscillations due to the flexoelectric polarization (following the Helmholtz equation) can be measured [3]. With a low imput

impedance amplifier the displacement current, proportional to the time derivative of the surface polarization can be measured accordingly [16].

To apply these ideas to the investigation of flexoelectricity in living membranes, it was necessary to develop a technique for excitation of curvature oscillations in small membrane patches, isolated or cell-attached. Patch clamp technique combined with phase-sensitive amplification of the membrane currents was found particularly suitable for this purpose [18, 21, 22]. Oscillation pressures in the range of 10 to 1000 Hz, generated by a loudspeaker-driven bellows, or by a piezoelectric sounder, were applied to the pipeline of the patch pipette holder. Apparantus and analysis of pressure dissipation and generated curvatures are given in [22].

Using a combination of patch clamp amplifier and lock-in amplifier interfaced at the frequency of curvature oscillations of the membrane patch flexoelectric currents from model diphytanoyl lecithin (DPhL) membranes and membrane patches of adult locust muscle membrane (inside-out, outside-out and cell-attached) were observed for the first time [18, 21]. With membranes containing no channels or pores the amplitude of the flexoelectric current is a measure of membrane flexoelectric coefficient. To calculate it, the amplitude of the patch curvature should also be evaluated: this was done by the capacitance microphone effect [12], expressed in the generation of 2nd harmonic capacitive current through a membrane, clamped to a non-zero holding potential (flexoelectric response is 1st harmonic with respect to the oscillating frequency). Flexoelectric coefficients [21] of synthetic lecithin bilayers (DPhL) were found to be $1.8 \cdot 10^{-20}$ C and those of locust membrane patches with no channels of the order of $1.0 \cdot 10^{-21}$ C.

In lipid bilayers containing pores and membrane patches containing electric gated channels a striking enhancement of the flexoelectric current was observed during the channel/pore opening. Partially, this effect could be accounted for by considering the in-phase component of the membrane current depending on the membrane conductance and flexoelectric voltage. By comparing the amplitude histograms of the total channel currents and of their 1st harmonic components a correspondence between 1st harmonic increments and conductance increments at channel/pore opening was found in some cases both with model and biomembranes. From this correspondence it was calculated that during a half period of the curvature oscillation (10 Hz) the flexoelectric effect was able to transport about 5300 elementary charges (ions) through the open channel/pore. The direction of transport depends on the sign of flexocoefficient: at a positive flexocoefficient the depolarizing electric field moves the positive ions towards the center of membrane curvature [21].

However, in some other cases the enhancement of the 1st harmonic during channel/pore opening was huge (up to 3 orders of magnitude). Complete reversibility after closing was observed as well. For instance, in the case of electrically gated channels of locust muscle membrane with a cell-attached patch oscillating at 20 Hz under pressure amplitude of 10 mm Hg(pp), a r.m.s. flexoresponse of 5 fA was measured at 0 mV pipette potential (when channels were silent). By increasing pipette potential step-wise to + 50 mV, a step-wise increase of the rms flexocurrent was observed, reaching a maximum value of about 250 fA (50 times amplification). By returning pipette potential to zero the original value was restored.

With negative pipette potentials some weak amplification (a few times) was observed only at −80 mV, when rare channel openings started to show up. Even more dramatic amplification was observed in pore-containing DPhL bilayers during the stochastic pore openings under voltage clamp ramp. The explanation of these striking findings is still an open question. A modulation of open channel conductivity by oscillating lateral tension could, in principle, produce a 1st harmonic component of the current, providing the patch has a substantial initial curvature. However, at least with ion channels in locust membranes such modulation should be excluded on the basis of the static pressure experiments (see above) which indicated a change of the open probability only, but not of the open conductivity.

CONCLUSION

The mechano-electric effects described above are of utmost relevance to the mechano-sensitivity of cell membrances at the first place [22]. Some of them, like flexoelectricity, are of fundamental importance for the energy transformation in membrane systems. In a flexoelectric membrane the mechanical and electrical degree of freedom can not be considered separately any more. They are interrelated through the direct and converse flexoelectric effect. The electromechanical coupling coefficient for membrane flexoelectricity could easily approach the value of 1, while for membrane piezoelecticity it is much lower, 10^{-4} (see [22]). Transport processes in cellular or sub-cellular membrane systems with variable curvature (erythrocytes, leukocytes, mitochondria, chloroplasts, retinal discs, brush borders, stereocilia, etc.) should be reconsidered now in closer relation to the possible role of flexoelectricity. Variable, dynamic membrane curvature could be generated by reversible contraction/expansion of the muscle-like cytoskeletal submembrane structures at the expense of ATP hydrolysis. Long ago a hypothesis that

flexoelectricity could be a driving force for ion transport in membranes [13], in particular through membrane channels [27], was advanced by us. The experimental findings outlined above provide the first evidence in confirmation of this hypothesis.

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