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MECHANOELECTRICITY OF GUEST-HOST MEMBRANE SYSTEMS: LIPID BILAYERS CONTAINING ION CHANNELS

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Abstract Mechano-electric responses of artificial guest-host membrane systems (liquid crystal lipid matrix containing ion channels) and native biomembranes from locust muscle to stretch and curvature deformation of the matrix were studied. Gating of ion channels by lateral tension and enhancement of the flexo-electric response during channel openings were observed.

Keywords: mechano-electricity, lipids, flexoelectricity, membranes, ion channels

INTRODUCTION

Mechanoelectricity is a fundamental property of membranes, relating to their mechanical and electrical degrees of freedom. It is closely related to mechanosensitivity (and mechanotransduction)[1], a basic property of living systems. An understanding of the mechanism(s) of mechanosensitivity would open the way for constructing artificial bioelectronic mechano-sensors.

The mechanical degrees of freedom which are of especial interest for mechanoelectric phenomena are membrane tension[2] and membrane curvature[4,8], although membrane curvature was not considered as being relevant to mechanosensitive gating of membrane ion channels[3]. Lateral tension may modify membrane permeability by influencing the open state probabilities of ion channels. In natural membranes two types of influence of tension (stretch) are well-documented and correlated stretch-dependent ion channels have been identified (i.e. stretch-activated channels[2] and stretch-inactivated channels[3]).

Membrane curvature is related to another basic mechanoelectric property of membranes, called curvature electricity or flexoelectricity[4]. Until now flexoelectricity was mainly studied in pure lipid bilayers[5,6] in the absence of membrane channels or in

native membranes under silent channel conditions[7], although the role of flexoelectricity in ion transport was suggested a long time ago[8,9].

MATERIALS AND METHODS

Application of finely controlled static and pulsed pressure differences across natural[2] and artificial[14] bilayer membranes became possible following the introduction of patch clamp methodology[10]. By either sucking or blowing a membrane patch fixed at the tip of a patch pipette, it is possible to vary both the curvature and the lateral tension of the patch; the lateral tension can be estimated from the applied pressure difference and patch curvature by using the Laplace law[2]. At a large pressure difference the tension effect prevails; it is also insensitive to the sign of curvature. Investigations of the electric effects related to curvature variations are best achieved using low oscillating pressure differences[7] so that induced tensions are below the threshold for channel gating.

The patch clamp technique was essentially the same as in our previous work[7], reported at the Schladming European LC Conference (1989). Pipettes were pulled from hard borosilicate glass (GC 150-19, Clark Electromedical Instruments) in two successive pulls. For model membranes a vertical Kopf puller was used. For natural membranes a horizontal puller was employed (Model M1, Industrial Science Assoc. Inc.).

Natural membrane patches were formed from the surface membranes of innervated metathoracic extensor tibiae muscle fibers of female adult locusts (Schistocerca gregaria) 7 - 10 days post-fledging[11]. The muscle was pretreated with collagenase (Sigma 1A, 1-2 mg ml^{-1}) for 1 h at room temperature (20 - 22 ^{o}C), before patching. Both the patch pipette and the muscle bath contained standard locust saline (180 mM NaCl, 10 mM KCl, 2 mM $CaCl_2$, 10 mM HEPES, pH 6.8). In some experiments locust saline of higher concentration of K^+ (100 mM KCL, the rest as above) in the pipette was used. Pipettes were fire-polished and coated with Sylgard. Tip diameters were ca. 1 μm. Membrane patches in either cell-attached, inside-out or outside-out configuration were studied. Occasionally, they contained one or two types of K^+ channels of ca 20 pS and 100 pS conductance.

Model lecithin membranes were formed at the tips of nonpolished patch pipettes from monolayers of diphytanoyl lecithin (DPhL from Avanti Polar Lipids) using the method of Coronado and Latorre[12]. Lipid monolayers were spread on the air/buffer interface from chloroform or n-pentane solutions. Pipettes with larger tip openings (up to 5 μm) were used in this case. The tip diameter was estimated from the pipette resistance when the pipette was filled with 150 mM KCl, 10 mM BES, pH 7.0 and dipped in a bath containing solution of the same composition. Due to its branched alkyl chains, the lipid (DPhL) is in a liquid crystal state at room temperature. Ion pores in DPhL bilayers were induced by a blue-green algae toxin[13], microcystin-LR (MCYST) isolated from Microcystis aeruginosa. It was added in the form of a methanol solution either to the pipette sline or to the bath saline, to give a final concentration of 10 - 20 ng ml^{-1}. According to our studies MCYST is capable of inducing ion-selective pores in DPhL bilayers with a broad range of pore conductances[14]. Static pressure differences (up to 50 torr) and slowly varying pressure ramps were mechanically achieved using a reversible peristaltic pump (Camlab) and were monitored by a pressure meter (a bridge based on a Motorola MPX100AP silicon piezoresistive pressure sensor). Oscillating pressures (in the range of 20 Hz, 10 torr (pp)) were generated by a pressure system driven by a loudspeaker. The effects of static or variable tension on channel activity in a patch were recorded using a List EPC7 patch-clamp amplifier with current and voltage output directed either to a modified Sony PCM linked to a standard videorecorder or to a four-channel RACAL tape recorder. This arrangement permitted simultaneous recordings of pressure signals. Stimulating voltages of various waveforms and slow voltage ramps were generated by a VCF sweep generator. Changes in membrane patch capacitance were used to estimate patch deformation under pressure. Capacitance changes were recorded by the method of Neher and Marty[15] by linking a SR530 dual lock-in amplifier to the List amplifier, and by using a 5 KHz 5 mV(pp) carrier sine wave from the internal oscillator of SR530 which was applied to the stimulating input of the List EPC7. Data analysis was undertaken on a Masscomp MC5500 computer with FORTRAN 77 software. Recordings of membrane currents were filtered at 1-3 KHz on playback, AD converted, sampled at 10 KHz and

stored on a 450 Mbyte hard disc. Current amplitude distributions were obtained and fitted as previously described[16]. Analysis of channel gating in response to voltage ramps presented at different static pressures was performed by the method of current-voltage surfaces[17].

Flexoelectric measurements were obtained using another combination of List EPC7 amplifier and SR530 dual lock-in amplifier[7]. It was possible, in this manner, to measure either the first or the second harmonic component of the membrane current with respect to the reference curvature oscillation frequency (e.g. 20 Hz), in a regime when at the same time the rms amplitude and the phase of the flexoelectric signal could be displayed.

RESULTS AND DISCUSSION

The number of giga-seal patches obtained from locust muscle membranes was about 50. In about 30 % of them the 20 pS K^+ channel[11] was observed and every time this channel was present, it was found stretch-sensitive.

In Fig. 1 a. K^+ channels in a cell-attached patch of locust muscle membrane are illustrated. The patch was subjected to -50 torr pressure (pipette negative). Double openings can also be seen. When the pressure differential was removed the K^+ channels closed immediately, only to open again when the pressure difference was restored (not shown). Fig. 1 b. demonstrates the frequency histogram of the channel currents from the record shown on Fig. 1 a. Two open state peaks with fitted maxima of 3.6 pA and 6.9 pA were obtained. Since the second peak is approximately a double of the first one, a conclusion about the presence of more than one channel in this patch can be drawn.

Fig. 2 demonstrates the response of a K^+ channel in a muscle membrane patch to a pressure ramp (from 0 torr to -20 torr to 20 torr). Activation of this channel closely follows the pressure changes induced by the ramp,the activation thresholds being - 4 torr and +5 torr respectively. This patch clearly responded to changes in lateral tension rather than to changes in its area: the capacitance (i.e. area) changes (middle trace) were delayed with respect to the peaks and zeroes of pressure by about 5 sec.

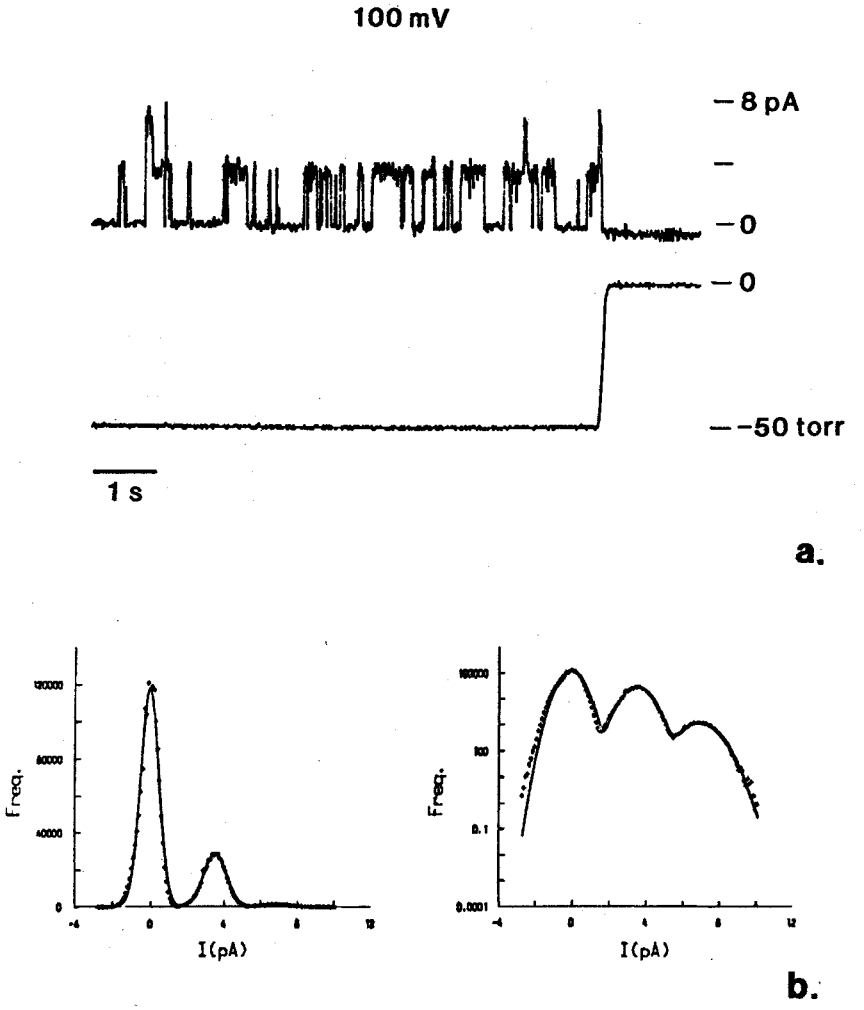

FIGURE 1. Stretch-activated K^+ channels in locust muscle membrane: a. Cell attached patch. Pipette filled with locust saline containing 100 mM KCl. Seal resistance, 7 Gohm. Pipette potential, 100 mV; corresp. to a membrane potential of -140 mV. Pressure jump from -50 torr to zero.
b. Frequency histogram of channel current amplitudes from Fig. 1 a. in lin-lin (left) and log-lin (right) form. Data fit by Gaussians: closed state 0 pA; single open state 3.6 pA (corresponding conductance 25.7 pS at membrane potential -140 mV); double open state 6.9 pA.

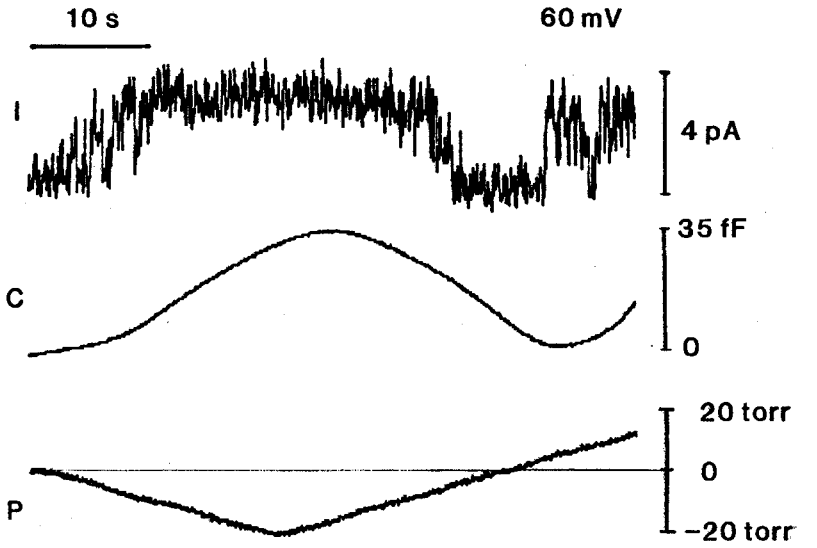

FIGURE 2. Stretch activation of K^+ channels and capacitance changes induced by a pressure ramp in locust muscle membrane: Cell-attached patch. Standard locust saline. Seal resistance, 2 Gohm. Pipette potential, 60 mV. P is the record of the pressure ramp from 0 to -20 to 0 to 20 torr. C is the change in patch capacitance. I is the channel current trace filtered at 0.5 KHz in an attempt to partially suppress the 5 KHz, 5 mV(pp) carrier sine wave. Experimentally determined sensitivity to capacitance change by decompensation of C fast of List amplifier was 2.7 mV/fF ; cf. theoretically calculated 2.777 mV/fF.

The peak amplitude (35 fF) of the membrane capacitance change was very similar to the initial value of the patch capacitance. Therefore, it seems reasonable to conclude that the patch had a hemispherical configuration at the time of the peak capacitance change, i.e. its radius of curvature (R) was half that of the pipette tip diameter (0.85 μm). With a pressure difference (P) of 20 torr (= 2666 Pa) the Laplace law yields a tension $T = P.R/2 = 2.3$ mN m^{-1}

Artificial ion pores induced by MCYST in DPhL bilayers were also found to be stress sensitive. Progressive openings of more than one conductance state under tension were conveniently studied in voltage ramp experiments. Current-voltage surfaces of a MCYST-containing membrane at 0 torr and four suction pressures of -11, -20, -30 and -50 torr respectively are shown in Fig. 3. There was a threshold for pore opening; e.g. at the maximum negative voltage studied (- 200 mV) it was -11 torr.

Voltage and lateral tension act in concert: higher conductance states being more probable at low membrane voltages when lateral tension is high, and vice-versa. In the patch of Fig. 3, with -50 torr 3 conductance states could be identified at negative voltages and 2 at positive voltages. We do not know if these conductance states represent openings of different pores or if they are sub-conductance states of a single pore, but they are not multiples of a single conductance state, thus favouring an interpretation in terms of a progressive opening of a single pore. Interestingly, there was a tendency for tension-induced higher conductance states to switch back to lower ones at high transmembrane potentials; the solid contours in Fig. 3 surrounding the second largest number of opening events (100 in this case) become discontinuous (island-like) and eventually terminate above + - 100 mV. This phenomenon, which was seen in many other patches, was especially pronounced at higher ionic strength (0.5 - 1 M KCl)[14]. It can be understood in terms of the counteracting dielectric ponderomotive force opening the pore (this will tend to saturate as the pore diameter becomes large, i.e at high open pore conductance, due to the shorting out of the field in the pore centre) and the overall decrease of the bilayer tension in electric field predicted by the Lippman equation[18] (this has no tendency of saturation). An attractive feature of these findings is that both mechanical and electrical components of lateral membrane tension could be involved and, correspondingly utilized, in switching of pores between closed and open states.

The model for pore gating by tension proposed by us[14] involves a direct coupling of the pore area change to the tension. Very similar in sense and suitable for explanation of locust channels' gating is the linear model of Howard et al.[21] which removes the need of

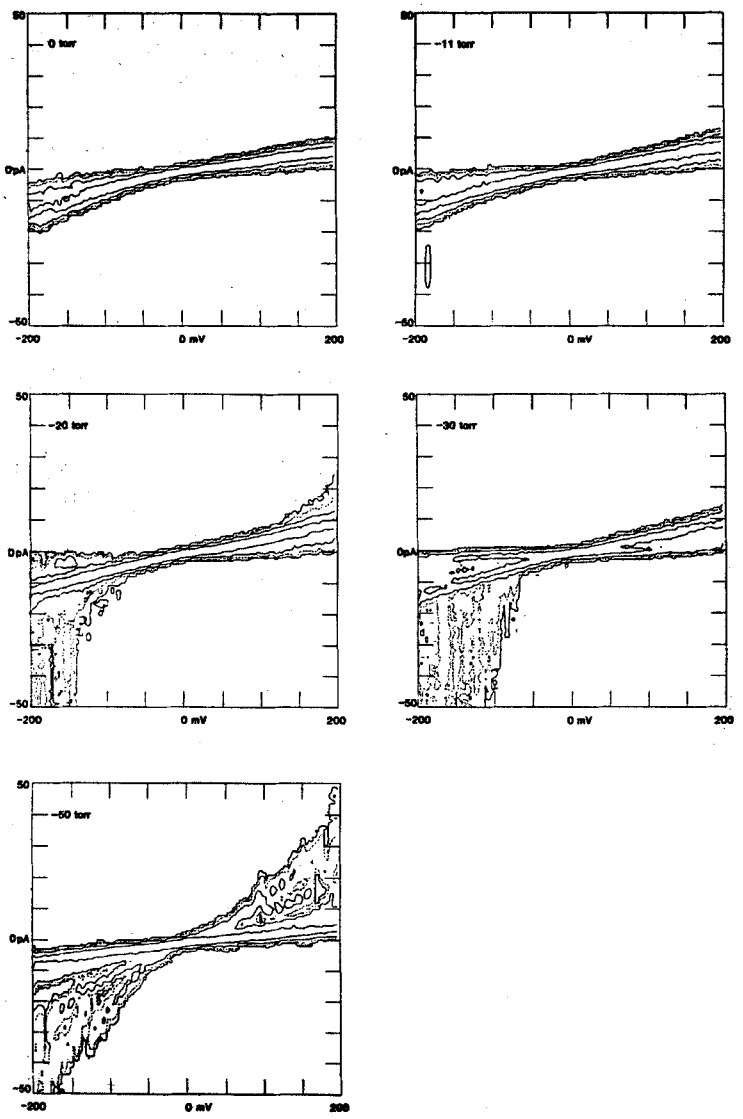

FIGURE 3. Progressive opening of higher conductance states of a MCYST pore in DPhL bilayer under suction. Voltage ramp -200 to 200 mV, duration 10 s, data averaged over 15 successive ramps. Current-voltage surfaces of a MCYST-containing bilayer at 0 , -11, -20, -30 and -50 torr, as indicated. Seal resistance 40 Gohm. 10 ng/ml MCYST in the pipette saline(containing 100 mM KCl). At -50 torr 3 conductance states at negative potentials (88 pS, 147 pS and 220 pS) and 2 conductance states at positive potentials (71 pS and 119 pS) can be identified.

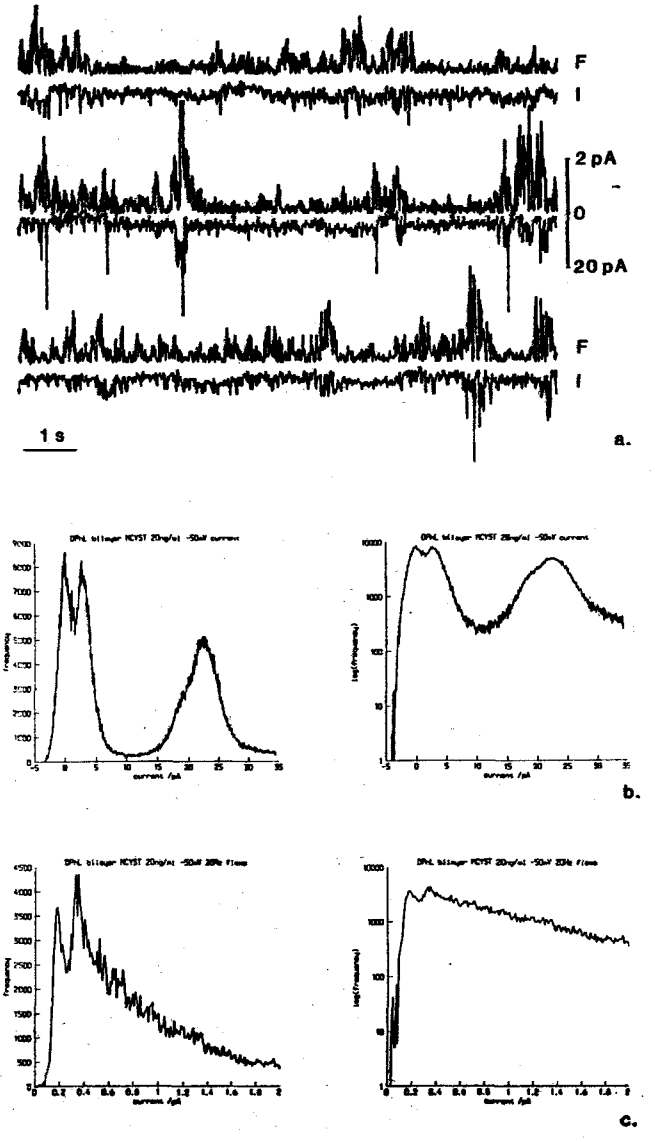

FIGURE 4. Correlation between the amplitudes of the pore currents and rms flexoelectric response. DPhL bilayer, MCYST (20 ng/ml) added to the bath saline (containing 150 mM KCl). 20 Hz, 10 torr(pp) oscillating pressure. Seal resistance 5 Gohm. a. Successive 3KHz filtered current traces at -50 mV (I, downward) and rms flexoelectric amplitude (F, upward). b. Current histogram showing a closed and two open states (60 pS and 440 pS, this one having two substates) of the MCYST pore. Current data were 3 KHz filtered, sampled at 10 KHz and numerically filtered. Histogram bin width 0.05 pA. c. Flexoelectric histogram showing two peaks at 0.2 pA and 0.4 pA and possibly a third one at 0.6 pA. Flexoelectric data were sampled at 2 KHz and digitally filtered. Histogram bin width 0.01 pA.

channel attachement to cytoskeleton for collection of stress[2] and, perhaps, the involvement of an intracellular messenger[3].

The threshold-like tension response of channels leads one to believe that with low amplitude oscillating pressures the net effect of curvature on membrane polarization could be studied, together with the possible correlation of the resulted flexoelectric current to the stochastic opening and closings of channels. Fig. 4 demonstrates a correspondence between the distribution of current pulses across an oscillating MCYST-containing DPhL membrane due to opening and closing of pores and the rms amplitude of the flexoelectric response of the membrane at the oscillation frequency (20 Hz). Fig. 4 a. shows simultaneous records of the total membrane current (I) and its rms flexoelectric component (F). Fig. 4b. is the pore current amplitude histogram and Fig. 4 c. is the flexoelectric component rms amplitude histogram. The histogram in Fig. 4 b. shows one closed and three open states for the MCYST pore; there are clearly two main components to the histogram in Fig. 4 c. (corresponding to the closed and the first open state of 4 b.), although a third component may also be present.

Similar data about enhancement of the flexoelectric current amplitude were obtained from locust muscle membrane patches (cell-attached, inside-out and outside-out) containing K^+ channels (data not shown). These findings provide supporting evidence for the hypothesis that flexoelectricity is a driving force for ion transport through membrane channels[8,9,19,20]. Enhancement of the flexoelectric response during pore (channel) opening could also be utilized in some biomolecular devices of flexoelectric type (oscillating pressure sensors).

The results reported here suggest the possible use of a liquid crystal lipid matrix containing ion channels in molecular switches and mechanical sensors, i.e. a nowel application of the old guest-host liquid crystal concept in molecular electronics and ionics.

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