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Selective transfer of energy through an alamethicin-doped artificial lipid membrane studied at discrete molecular level

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Abstract

In this study we present novel evidence that strengthens the paradigm of selective transfer of energy mediated by a random gating of ion channels. Specifically, we investigated the spectral response of a noisy artificial biomembrane whose electrical properties were largely dictated by embedded alamethicin oligomers. In this respect, we first evaluated experimentally the linear transfer function of the system via the white-noise analysis method. We prove that such a system displays specific ranges of frequency over which input signals pass preferentially, depending on their spectral content and the holding potential across the artificial bilayer which contains alamethicin. By employing voltage-driven periodic stimulation of alamethicin oligomers, we demonstrate that overall response of the system obeys qualitatively the predictions inferred from the transfer function analysis of it. These results emphasize the exquisite ability of excitable membranes to behave as band-limited filters and allow for maximal transfer of energy from an external stimulus over well-defined frequency ranges.
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1. Introduction

The microscopic approach to describing the temporal evolution of chemical reactions leads to the observation of an inherent, natural-embedded feature known as stochastic behavior. Due to its ever-occurring collisions with the solvent molecules, time evolution of a protein dwelling in various states follows a stochastic path. The stochastic treatment of chemical reaction started with Kramer's work [1], and most models employed nowadays for such data interpretation rely extensively upon the differential form of the Chapman–Kolmogorov equation (the master equation) for a discrete set of states [2–4]. At the end, the solution of the master equation describes the gain–loss evolution for the probabilities of the separate states.

Numerous laboratory techniques based on single-channel recording on membrane proteins, confocal fluorescence microscopy, and single-photon detection have provided the possibility to study protein kinetics in aqueous solution with single proteins [5,6]. Regardless of the experimental method used to investigate dynamic features of collections of molecules, and in particular proteins, fluctuations among various states lead to what generally is known as 'noise'. In particular, electrical recordings on excitable cell ion channels show distinctly this type of 'molecular-noise', due to the fact that ion channels are continually opening and closing through thermal agitation [7,8]; hence, the instantaneous electrical current recorded will follow these fluctuations in the number of open and closed channels. Such quasi-random fluctuations of the membrane current give rise to subthershold fluctuations of the biomembrane potential, which may affect the dynamic behavior at the cellular level. Specifically, such fluctuations may influence the complex timing of action potentials, may lead to spontaneous action potential firing, and all computational processes which take place at the input of excitable cells are affected by the degree of linearity of the membrane which is more prone to exist in the subthreshold regime [9–11]. Recent studies have pinpointed another facet of the major role played by intrinsic voltage potentials on the biophysical processes that take place at the cellular level. That is, it has been shown that a weak input signal applied at the input of a specific nonlinear

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system, in particular an excitable biomembrane, can be amplified and optimized by the presence of electrical noise [12,13].

In this study we present novel evidence that strengthen the paradigm of selective transfer of energy mediated by the random gating of ion channels. The model ion channel used is alamethicin, which was made to fuse with artificial membranes. When added to one side of a membrane, alamethicin monomers get inserted into it and generate highly voltage-dependent pores [14]. The interplay of 'on'–'off' reactions among transmembrane alamethicin monomers generate aqueous pores with various sub-conductance states; the overall kinetics of such an oligomeric protein can be treated kinetically within the frame of Markov chains. Previous data have demonstrated that the voltage-dependence of the macroscopic conductance of alamethicin arises from voltage-dependent changes in the number of active channels in the membrane [15] and that the macroscopic steady-state conductance depends upon the concentration of alamethicin and of the salt in solution. Our main line of focus was to investigate the spectral response of a noisy artificial biomembrane whose electrical properties were largely dictated by embedded alamethicin oligomers. In this respect, we evaluated experimentally the linear transfer function of the system that was expected to show frequency and voltage-dependence, mainly due to the kinetic features of embedded alamethicin.

We prove that such an electrically noisy system as described above, displays specific ranges of frequency over which input signals pass preferentially, depending on their spectral content and the holding potential across the artificial membrane. Moreover, by employing voltage-driven periodic stimulation of alamethicin oligomers, we demonstrate that overall response of the system obeys qualitatively the predictions inferred from the transfer function analysis of it. We believe that such data derived from electrical measurements performed at the 'channel level' are useful to further extend the experimental approach of resonant transfer of energy mediated by model ion channels [16].

2. Experimental

Current recordings through alamethicin oligomers were carried out by using folded bilayer membranes obtained as previously described [6]. A 25-μm thick Teflon septum was clamped between two Teflon chambers each of 1 ml volume. A bilayer was formed on an aperture of 100-μm diameter in the septum that had been pre-treated with 10% (v/v) hexadecane (Sigma-Aldrich) in highly purified *n*-pentane (Sigma-Aldrich). Both chambers contained 1 M NaCl, pH 7.5. Initially, the level of electrolyte was set just below the aperture and 1% (w/v) L-α-phosphatidylcholine (type IV S, >30% TLC, Sigma-Aldrich) in pentane (6 μL) was spread on the surface of each chamber. After the solvent has evaporated, the electrolyte level in the chambers was raised above the aperture. The formation of a bilayer was monitored by observing the increase in membrane capacitance to a value of approximately 150 pF. The bilayer chamber was properly grounded and enclosed into a home-made Faraday cage and all experiments were performed at a room temperature of ~25 °C.

Alamethicin monomers (Sigma-Aldrich) were added from a stock solution made in ethanol, in the *cis* chamber only, connected to the ground. In order to generate noisy voltage signals with an uniform spectral content that were used to evaluate the linear transfer function of the alamethicin-containing biomembrane, we employed an acquisition board (NI PCI 6014, National Instruments, Inc., USA) whose D/A output channel was fed with Gaussian distributed data generated numerically. The specific routine was written in the graphical program language LabView (National Instruments, Inc., USA), as previously described [17]. Currents from the bilayer chamber obtained in response to voltage white-noise stimuli were detected and amplified with an integrating headstage Axopatch 200 B amplifier (Axon Instruments, Foster City, USA), set on the voltage-clamp mode. Data acquisition of the amplified electrical signals was performed with a 16 bit resolution A/D card (NI PCI 6014, National Instruments, Inc., USA) at a sampling frequency of 5 kHz, and prior use such signals were filtered with a low-pass Bessel filter with the cutoff frequency set at 2 kHz.

The transfer function of the studied molecular system was estimated as the ratio of the power spectra of the resulting output electrical current to the input voltage white-noise stimulus. Thus, the above-mentioned quantity reflects the square modulus of the system's admittance. Fast Fourier analysis was performed on 2048 Hamming windowed data points, and at least 60 data segments were used to produce an averaged output. Experimental conditions were set such as to closely fulfill three main assumptions made with respect to this system identification method: the system under study was time-invariant, it obeyed the principle of causality and it behaved linearly. Specifically, before implementing the white-noise analysis protocol we waited a sufficient long time under stirring (~20 min) to make sure that alamethicin monomers reached their equilibrium in partitioning the biomembrane. In addition, the values of the voltage stimuli were kept small (under 10 mV), to maximize chances of not driving the system beyond its linearity region. In order to evaluate the spectral output of the alamethicin-containing biomembranes at a specific frequency, we used as a stimulus an analog periodic signal with a chosen frequency, generated at the analog output of the NI PCI 6014 card. Data was than fed into a PC-compatible computer for further numerical analysis including time-domain low-pass filtering, spectral analysis and graphing, done mainly with the help of the Origin 6.0 (OriginLab Corporation, USA) and Matlab software (The Mathworks, Inc., USA).

3. Results and discussion

In Fig. 1 we show original current traces which emphasize one of the major characteristics of alamethicin-induced transmembrane pores, namely a rather strong voltage-dependence of their kinetic activity. It is easily seen that at

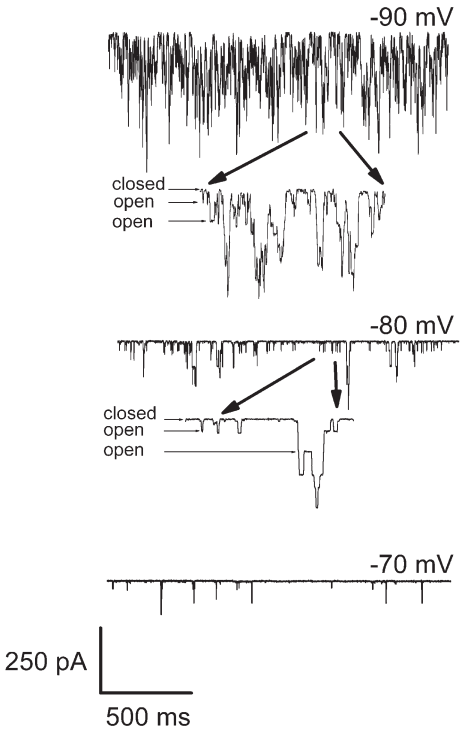

Fig. 1. Original traces which show changes in the electrical conductance of an artificial lipid membrane imposed by alamethicin added on one side of the membrane. It is easily seen that larger negative potentials augment the probability of alamethicin oligomers formation within the membrane, due to a favorable interaction between the alamethicin monomers and the external electric field imposed. The insets of traces recorded at −90 and −80 mV show a detailed view of some sub-conductance states of the alamethicin oligomers, where 'closed' and 'open' states are represented.

more hyperpolarized potentials, more alamethicin oligomers cross the lipid membrane and aggregate reversibly to generate conductance-fluctuating pores. Although the kinetics of insertion of alamethicin monomers is highly voltage-dependent, the 'on–off' interactions among transmembrane alamethicin oligomers and neighboring monomers are rather voltage-insensitive (data not shown). Since the upper limit of the voltage-driven translocation rate of alamethicin monomers is bounded by the hopping rate over the hydrophobic core of the membrane, we argued that depending on the frequency of the applied voltage signal, alamethicin 'push-in' across the membrane may not keep up the pace with the applied signal.

Therefore, we anticipated that the lipid membrane-alamethicin system would respond differently with respect to the current elicited in response to an input stimulus, depending on the ability of alamethicin monomers to be driven across the membrane by a time-varying voltage signal.

To investigate this, we first carried out a white-noise system-identification protocol to determine the electrical transfer function of the lipid membrane-alamethicin aggregate. In Fig. 2 we plotted the square modulus of the system's admittance, as derived at different holding potentials (i.e., −90, −80, −70 and 0 mV). Interestingly, one may notice that as soon as the holding potential of the lipid membrane becomes more negative, the shape of system's admittance changes pointing to particular

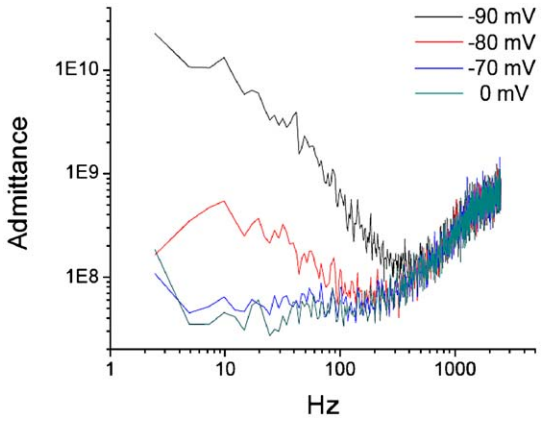

Fig. 2. The square modulus of the admittance of an artificial lipid membrane doped with alamethicin oligomers, measured at different holding potentials (0, −70, −80 and −90 mV). As described in the text, these traces were derived from the ratio of power spectra of the output currents (pA) resulting from application of white-noise voltage (V) stimuli across the membrane.

frequency ranges over which the total current assume rather minimal values. At a zero value of the holding potential, the system's admittance resembles that of a capacitor connected in parallel with a resistor. This makes sense, since under such circumstances alamethicin monomers will not have enough energy to surpass the dielectric barrier imposed by the hydrophobic core of the lipid membrane. Consequently, the electrical features of the system will be mostly dictated by its capacitance and residual electrical resistance. When the holding potential changes and helps to push alamethicin monomers across the lipid membrane, a major passive pathway for current flow will be constituted by oligomeric structures of alamethicin within the membrane. This can be mostly seen at low frequencies of a voltage stimulus applied across the lipid membrane. Concomitant with a rise in the frequency of the

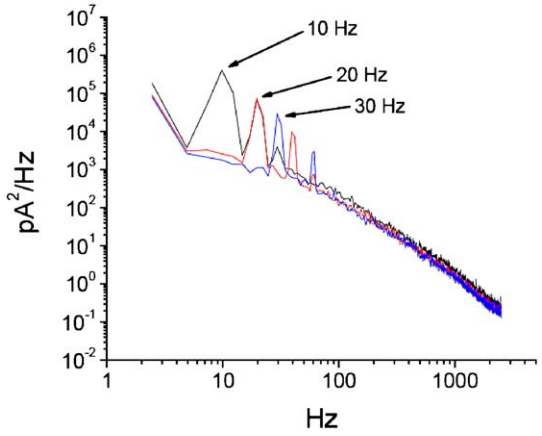

Fig. 3. Power-spectra of electrical currents mediated by an artificial lipid membrane doped with alamethicin oligomers when the stimulus consisted of periodic waveforms (10, 20 and 30 Hz) having similar 'peak-to-peak' amplitudes and a dc bias of −80 mV. The arrow-indicated peaks correspond to main spectral components which result under the above-mentioned conditions, whereas the remaining peaks are upper (2nd and 3rd) spectral harmonics.

voltage stimulus applied, lesser and lesser alamethicin monomers will be able to follow changes of the overall potential across the membrane, so that the likelihood of further oligomer formation will tend to decrease. With an increase in the frequency of the voltage stimulus (>330 Hz at −90 mV holding potential), the total current across the membrane will be mostly represented by its capacitive component. Accordingly, this can be seen in Fig. 2 by an increase of the system's admittance. Apparently, the threshold frequency value of the voltage stimulus at which the system's admittance assumes a local minimum depends upon the holding potential imposed across the membrane (~130 Hz at −70 mV and ~330 Hz at −90 mV); that is, higher holding potentials seem to shift this particular frequency to ascending values. At the present moment we are still investigating this phenomenon for a reasonable explanation.

To further test the predictions derived from the analysis of the transfer function, we excited our system with three different periodic voltage waveforms of 10, 20 and 30 Hz, respectively. According to Fig. 2, one would expect that in the range of low frequencies, ascending frequency stimuli would elicit descending responses. As it can bee seen from Fig. 3, the system's response was maximal when the voltage stimulus with the lowest frequency was employed (3.8×10^5 pA^2/Hz at 10 Hz, as compared to 0.6×10^5 pA^2/Hz at 20 Hz and 0.2×10^5 pA^2/Hz at 30 Hz). Note that power spectra derived above for the main harmonic component do include contributions from the Lorenzian noise of alamethicin oligomers which reflect their association–dissociation kinetics within the membrane. As in any other case of electrophysiology-related investigation, the sampling frequency of the A/D board used to acquire electrical traces and the cutoff frequency of the low-pass filter employed may play a distinctive role on the outcome of the analysis [8,18]. Under the worst conditions, under-sampling and under-filtering of electrophysiological traces will definitely obscure molecular events that make up the kinetics of studied proteins, leading to erroneous read-outs of the inferred data. Bearing this in mind and having documented the issue, we chose such conditions as to be able to distinctly resolve time-domain transitions of alamethicin oligomers among various conductive substates. Therefore, we ensured a proper evaluation of the admittance of the lipid membrane doped with alamethicin oligomers. In the presented case, higher sampling and filtering frequencies would not improve such estimations, leading only to a supplementary thermal noise increase. Among others, the physiological state of a cell regulates biochemical changes of its plasma membrane, so that cells may continuously adapt their ability to interact with exogenous stimuli by changes imposed on the overall kinetics of ion channels. In this respect, we plan further experiments in which the elasticity of a lipid membrane will be changed in a controllable manner via addition of cholesterol. Therefore, the elastic stress imposed on the membrane will expectedly decrease the likelihood of alamethicin monomers to hop across the membrane. Consequently, one may envision that the frequency range over which the membrane responds maximally to time-variable stimuli will be shifted to lower values as compared to the case of a cholesterol-free planar lipid membrane. In addition, since properties of artificial lipid membranes depends on the solvent used we plan to extend further investigations by employing membranes composed of various solvents [19].

4. Conclusion

In this work we prove that a system consisting of alamethicin oligomers inserted into an artificial lipid membrane displays specific ranges of frequency over which input signals pass preferentially, depending on their spectral content and the holding potential across the artificial bilayer. By employing a voltage-driven periodic stimulation protocol of alamethicin oligomers, we demonstrate that overall response of the system obeys qualitatively the predictions inferred from the transfer function analysis of it. These results come to further strengthen the exquisite ability of excitable membranes to behave as band-limited filters and allow for maximal transfer of energy from external stimuli over well-defined frequency ranges.

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