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Effect of Agents Modifying the Membrane Dipole Potential on Properties of Syringomycin E Channels

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We evaluated the effect of agents modifying the membrane dipole potential: phloretin, 6-ketocholestanol and RH 421 on the properties of single channels formed by lipodepsipeptide syringomycin E (SRE) in planar lipid bilayers. SRE forms two conductive states in lipid bilayers: "small" and "large." Large SRE channels are clusters of several small ones, demonstrating synchronous openings and closures. The increase in the membrane dipole potential led to (i) an increase in SRE channel conductance, (ii) an increase in the channel's lifetime, and (iii) a decrease in a number of synchronously operating small channels in the clusters. Overall, the results support the model of the small SRE channel synchronization in the cluster as voltage-dependent orientation of the lipid dipoles associated with the channel pores.

Introduction

The membrane dipole potential originates from the dipole moments of the lipid molecules and from the adjacent water dipoles in the interfacial region.[1,2] The membrane interior is several hundred millivolts positive with respect to the external aqueous phases.[3,4] This value greatly exceeds the magnitude of typical transmembrane or surface potentials. The membrane dipole potential plays an important role in peptide−lipid interactions,[5−7] ion and glucose transport,[8−12] ATP-ase activity,[13,14] and other important biological processes.

Incorporating certain compounds possessing large dipole moments into the membrane can alter membrane dipole potential. For example, the sterols phloretin and phlorizin cause a decrease in the dipole potential of the lipid bilayer, whereas styryl dye RH-421 and 6-ketocholestanol increase the membrane dipole potential.[3,4,13,15] When phloretin inserts into the membrane interface, it is positioned in a way that a component of its molecular dipole is aligned against the intrinsic membrane dipoles. Upon membrane insertion of 6-ketocholestanol and RH 421, components of their molecular dipoles add to the intrinsic membrane dipoles. These agents have been widely used to study effects of the membrane dipole potential on ion channels and membrane transport.[16−21]

Here we show the effect of phloretin, RH 421, and 6-ketocholestanol on the activity of single channels produced by syringomycin E (SRE). Cyclic lipopeptide SRE is an important antifungal agent;[22−24] when incorporated into lipid bilayers, SRE forms two types of voltage-gated ion channels with small and large current amplitudes.[25,26] Large SRE channels are clusters of several small (elementary) ones, demonstrating synchronous opening and closure[26] and voltage-dependent channel synchronization in the cluster.[27,28] Ion channels formed by SRE are sensitive probes to the host membrane composition: both the single-channel conductance and the channel's gating depend on the surface charge.[20,29]

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We have studied the effect of phloretin, RH 421, and 6-ketocholestanol on the activity of the SRE channels. Assuming that the effects of phloretin, RH 421, and 6-ketocholestanol are related to changes in the membrane dipole potential, we report that the increase in the membrane dipole potential results in (i) an increase in the SRE elementary channel conductance, (ii) an increase in the channel's lifetime (both small and large), and (iii) a decrease in a number of synchronously operating elementary channels in the clusters. These results are in good agreement with the recently proposed model of the synchronization of SRE channel gating,[27,28] suggesting the involvement of the membrane dipoles in the clustering of elementary channels.

Experimental Section

All chemicals were of reagent grade. Synthetic 1,2-dioleoyl-*sn*-glycero-3-phosphoserine (PS), and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (PE) were obtained from Avanti Polar Lipids, Inc. (Pelham, AL). Water was distilled twice and deionized. All solutions were buffered with 5 mM MOPS, pH 6.0. SRE was purified as previously described.[30]

Bilayer lipid membranes were made according to the monolayer-opposition technique on a 50−100 μm diameter aperture in 10 μm thick Teflon film separating two (cis and trans) compartments of the Teflon chamber.[31] The aperture was pretreated with a solution of hexadecane in *n*-hexane (1:10, v/v) or squalene. The toxin was added to the aqueous phase of the cis-side compartment. Membranes were made from the mixture of PS and PE. The dipole potential of the membrane was reduced by the two-side addition of phloretin to the solution bathing the phospholipid membranes. The two-side addition of RH 421 to the membrane-bathing solution or 6-ketocholestanol to the membrane-forming solution were used to increase the dipole membrane potential of the phospholipid bilayers.

For phospholipid bilayers, the value of φ_d was an average taken from refs 9, 32, 33, 34, and 35. Modulation of φ_d by phloretin, RH421, and 6-ketocholestanol was performed according to refs 4, 20, and 36, respectively. According to ref 33, we considered φ_d to be the same for both 0.1 and 1.0 M NaCl bath solutions.

Ag/AgCl electrodes with 2 M KCl/agarose bridges were used to apply transmembrane voltage (*V*) and to measure the single channel current (*I*). "Positive voltage" means the cis-side compartment is positive with respect to the trans side. All experiments were carried out at room temperature.

Transmembrane currents were recorded with a custom-made amplifier and digitized using a pClamp-compatible board. Data acquisition was performed with a 5 kHz sampling frequency and low-pass filtering at 200 Hz. Data were analyzed using pClamp 9.0 (Axon Instruments) and Origin 7.0 (OriginLab). Current transition and lifetime histograms were constructed for tested voltages (see also ref 27). The dwell time histograms of the elementary channels were fitted with an exponential density function.

Results and Discussion

Sample records in Figure 1 illustrate the effect of successively increasing the membrane dipole potential on the activity of the single SRE channels. Panels A, B, and C present the current fluctuations in the presence of 20 μM phloretin, no dipole modifiers, and 5 μM RH 421 in the membrane-bathing solution, respectively. The large current fluctuations (large SRE channels or clusters) correspond to the simultaneous opening or closure of several elementary SRE channels. These recordings show that the reduction of the membrane dipole potential (φ_d) to about 120 mV induced by addition of 20 μM phloretin leads to an increase in the number of elementary channels in the cluster and a decrease in both the elementary channel amplitude and channel lifetime. The increase of φ_d to ~300 mV by the addition of 5 μM RH 421 (or by the inclusion of 6-ketocholestanol into the membrane-forming solution, data not shown) results in opposite effects.

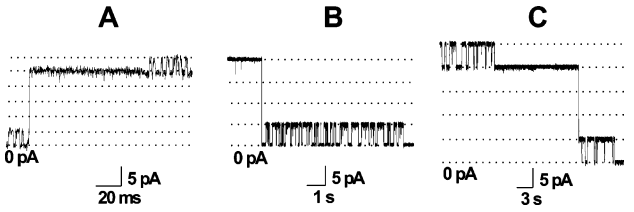

Figure 1. Current fluctuations induced by the elementary SRE channels and channel clusters in the bilayers of different dipole potentials (φ_d). The membranes were made from the equimolar mixture of PS and PE and bathed in 1.0 M NaCl, $V = 160$ mV. (A) $\varphi_d = (120 \pm 20)$ mV (20 μM phloretin); (B) $\varphi_d = (230 \pm 20)$ mV (no dipole modifier); (C) $\varphi_d = (300 \pm 40)$ mV (5 μM RH 421). The intervals between the dashed lines are equal to the amplitude of the elementary SRE channel.

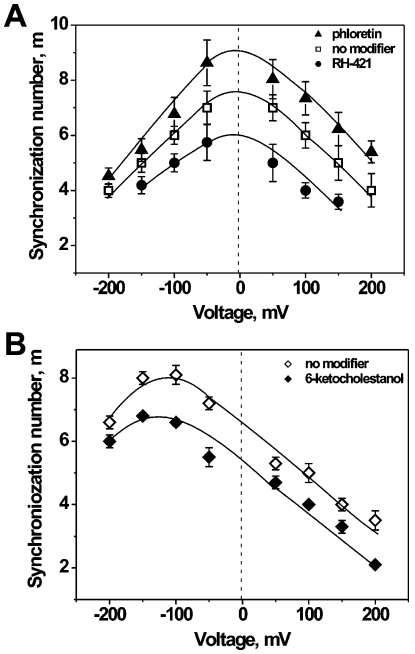

Figure 2. Voltage dependence of the mean number of the synchronized elementary channels in the cluster. (A) The membranes were made from the equimolar mixture of PS and PE and bathed in 1.0 M NaCl. (▲) $\varphi_d = (120 \pm 20)$ mV (with 20 μM phloretin); (□) $\varphi_d = (230 \pm 20)$ mV (no dipole modifier); (●) $\varphi_d = (300 \pm 40)$ mV (with 5 μM RH 421); (B) The membranes were made from the mixture of PS (33 mol %) and either PE or 6-ketocholestanol and bathed in 0.1 M NaCl: (◇) $\varphi_d = (230 \pm 20)$ mV (67 mol % PE, no dipole modifier); (♦) $\varphi_d = (430 \pm 20)$ mV (67 mol % 6-ketocholestanol).

Figure 2 shows the voltage dependence of the mean number of synchronously operating elementary channels in the cluster (*m*) at 1.0 M NaCl (Figure 2A) and 0.1 M NaCl (Figure 2B) solutions bathing membranes of different dipole potentials. At high NaCl concentration (1.0 M) (Figure 2A), the $m-V$ curves are symmetrical with respect to *V*, but their position along the

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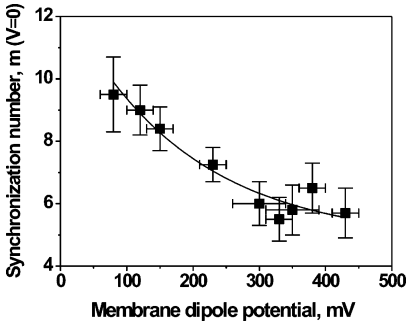

Figure 3. The mean number of the synchronized elementary channels in the cluster at $V \rightarrow 0$ ($m_{V=0}$) as a function of the membrane dipole potential. The membranes were bathed in 1.0 M NaCl.

ordinate is determined by the φ_d value. The application of V of either sign or an increase in φ_d induces desynchronization of elementary channels. As a result, SRE clusters with fewer (less than 9) synchronously opened channels appear. These findings are consistent with the idea of lipid dipoles impacting SRE cluster opening/closure.[27,28] From Figure 2B we can also see that, at low NaCl concentration (0.1 M), the maximum of the $m-V$ curves appear to be shifted to lower voltages (by ~100 mV) in membranes without dipole modifiers and in membranes containing 6-ketocholestanol.

We addressed the issue of SRE channel synchronization in recent publications.[27,28] We attributed the asymmetry of the $m-V$ curves at low NaCl concentration to the influence of asymmetrically distributed positive charges of SRE molecules along the channel pore. At 0.1 M NaCl, the charge−dipole interactions and the dipole−dipole interactions between cluster-forming molecules are presumably involved in the synchronization of the elementary channels. At 1.0 M NaCl (symmetrical $m-V$ curves), the SRE charges are screened, and only the dipole−dipole interactions regulate the synchronization of the elementary channel. In refs 27 and 28, we assumed that the transmembrane voltage affects the orientation of the channel-associated lipid dipoles, which, in turn, regulates synchronization of the elementary channels in the cluster. The orientation of the lipid dipoles under the applied voltage depends on the dipole moments of the lipid molecules and on the intensity of the transmembrane field. Assuming the cluster synchronization is regulated by dipole−dipole interactions between closely arranged elementary channels, we expect that the decrease in φ_d promotes the elementary channel synchronization in the cluster. We estimated the number of synchronously operating elementary channels at $V \rightarrow 0$ ($m_{V=0}$) in all studied ranges of φ_d. Figure 3 shows the dependency of $m_{V=0}$ as a function of the membrane dipole potential at 1.0 M NaCl. From Figure 3 it occurs that a 5-fold increase in φ_d results in a 2-fold decrease in $m_{V=0}$. First-order exponential fitting of this data allows us to suggest that ~13 elementary channels is the maximum number of synchronized units in one SRE channel cluster.

As mentioned above, SRE elementary channel current amplitude depends on φ_d (Figure 1). We measured the conductance of SRE channels (g) as a function of V in 1.0 M (Figure 4A) and 0.1 M NaCl (Figure 4B) at different φ_d values. According to Figure 4, the increase in φ_d produces an increase in the elementary channel conductance at all tested V values. Figure 4 shows that, at a given NaCl concentration, the shape of the conductance−voltage curves is not influenced by the dipole potential. At the same time, the changes in the channel conductance are not significant upon large changes in the dipole potential. Therefore, we suggest that the SRE pore geometry is not influenced by φ_d.

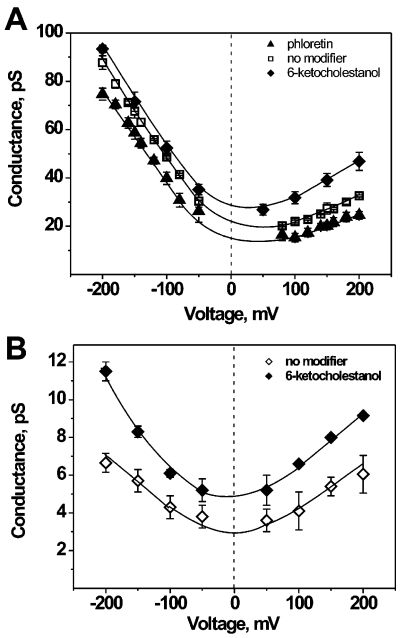

Figure 4. Conductance-voltage curves of the elementary SRE channels in the membrane of different dipole potentials. (A) Membranes were made from the equimolar mixture of PS and PE or PS and 6-ketocholestanol, bathed in 1.0 M NaCl: (▲), $\varphi_d = (120 \pm 20)$ mV (with 20 μM phloretin); (□), $\varphi_d = (230 \pm 20)$ mV (no dipole modifier); (♦), $\varphi_d = (380 \pm 20)$ mV (50 mol % PS and 50 mol % 6-ketocholestanol). (B) Membranes were made from the mixture of PS (33 M%) and either PE or 6-ketocholestanol. The membrane bathing solution was 0.1 M NaCl. (◇), $\varphi_d = (230 \pm 20)$ mV (67 mol % PE, no dipole modifier); (♦), $\varphi_d = (430 \pm 20)$ mV (67 mol % 6-ketocholestanol).

Table 1. Experimental and Calculated Ratios of the SRE Channel Conductance in the Membranes of Different Dipole Potentials

NaCl concentration	ratio of SRE channel conductance	calculated ratio[a]	calculated ratio[b]	experimental ratio
1.0 M	$g^*_{\varphi_d=120mV}/g^*_{\varphi_d=230mV}$	0.013	0.57	0.71
	$g^*_{\varphi_d=230mV}/g^*_{\varphi_d=300mV}$	0.063	0.68	0.77
	$g^*_{\varphi_d=230mV}/g^*_{\varphi_d=380mV}$	0.0011	0.43	0.72
0.1 M	$g^*_{\varphi_d=230mV}/g^*_{\varphi_d=430mV}$	0.00036	0.31	0.60

[a] $g^*_{\varphi d}{}^1/g^*_{\varphi d}{}^2 = \exp[-ze(\varphi_d{}^1 - \varphi_d{}^2)/kT]$; $\varphi_d{}^1 < \varphi_d{}^2$ [b] $g^*_{\varphi d}{}^1/g^*_{\varphi d}{}^2 = \exp[-0.15ze(\varphi_d{}^1 - \varphi_d{}^2)/kT]$; $\varphi_d{}^1 < \varphi_d{}^2$ *conductance at $V \rightarrow 0$ (Figure 4)

According to ref 3, the dependence of the ion membrane conductance (G) on the ion mobility (λ), the ion concentration in the aqueous phase (C), and the membrane dipole potential (φ_d) is expressed by the equation

$$G \sim \lambda C \exp(-ze\varphi_d/kT) \quad (1)$$

where ze is the ion charge, k is the Boltzmann constant, and T is the absolute temperature. Equation 1 predicts a decrease in anionic and an increase in cationic membrane conductance with the reduction of φ_d. For the SRE channels that are preferentially anion-conductive,[25,37] eq 1 qualitatively explains the results presented in Figure 4. The single-channel conductance of the cationic channels gramicidin A and alamethicin increases with the decrease of φ_d.[17−19,21,38] However, the calculated changes in the SRE channel conductance as a function of φ_d using eq 1,

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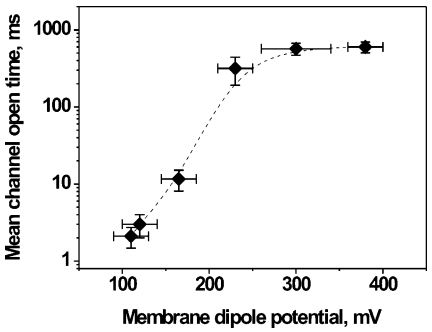

Figure 5. The mean open time of the elementary SRE channel at $V = -200$ mV as a function of the membrane dipole potential. The membranes were bathed in 1.0 M NaCl.

should be more significant than the experimental ones (see Table 1, columns 3 and 5). According to ref 39, the discrepancy between the experimental and calculated values of the channel conductance is attributed to the shielding of the dipole potential in the interior of the channel pore. The shielding of φ_d in the channel lumen originates from the difference between dielectric constants in the channel interior and in the membrane hydrocarbon core; the shielding depends on the channel geometry. Assuming the SRE channel radius is about 1 nm[26] and supposing the binding sites for the conducting ions (anions) are located in the center of the pore, we predict that φ_d in the pore is 85% lower than φ_d in the bilayer.[13,39] The experimental and calculated ratios of the SRE channel conductance in the membranes of different φ_d values are summarized in Table 1 (columns 4 and 5, respectively). These calculated values are close to experimental ones.

In contrast to the insignificant changes in channel conductance, the lifetime of the elementary SRE channels changed dramatically as a function of φ_d. The increase in φ_d from ~80 to ~380 mV produces a 3000-fold increase in channel lifetime ($V = -200$ mV, 1.0 M NaCl) (Figure 5). The membrane dipole potential has a similar effect on the open time of the SRE clusters (data not shown). The mechanism of a regulation of channel lifetime by φ_d may be rationalized using the model proposed for gramicidin channels.[38] The processes of gramicidin channel dimer formation and dissociation involve the movement of the indole dipoles of the tryptophan residues through the membrane interface. The opening/closure of the SRE channel may similarly include the movement of the channel-forming dipoles and/or specific charged groups (SRE and/or lipid molecules[29]) through the membrane interface that, in turn, is sensitive to φ_d. The pronounced dependence of the channel open time on the membrane dipole potential allows us to suggest that the polar groups involved in the SRE channel gating are located closer to the hydrophobic part of the channel or a membrane.

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