Effect of the Dipole Potential of a Bilayer Lipid Membrane on Gramicidin Channel Dissociation Kinetics

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ABSTRACT
A technique of measuring of the light-induced transients of the gramicidin-mediated electric current across a membrane in the presence of a photosensitizer has been applied for the study of the effect of agents modifying the dipole potential of a bilayer lipid membrane (phloretin, 6-ketocholestanol, and RH421) on the processes of the gramicidin channel dissociation and formation. It is shown that phloretin, known to lower the dipole potential, decelerates the flash-induced decrease in the current, whereas 6-ketocholestanol and RH421, known to raise the dipole potential, accelerate the current decrease. It is revealed that the addition of phloretin leads to a decrease in the dissociation rate constant, whereas addition of either 6-ketocholestanol or RH421 causes an increase in this constant. Single-channel data show that phloretin brings about an increase in the lifetime of the gramicidin channels, whereas RH421 produces a more complicated effect. It is concluded that the dipole potential affects the process of channel dissociation, presumably via the influence on the movement of the dipoles of gramicidin molecules through the layer of the dipole potential drop near the membrane-water interface.

INTRODUCTION
The pentadecapeptide antibiotic gramicidin A represents an interesting model for studying interaction of ionic channels with membrane environment and membrane-solution interfaces. It is generally accepted that gramicidin channels are dimers formed by the transmembrane association of monomers (Hladky and Haydon, 1984; Andersen and Koepppe, 1992; Killian, 1992; Busath, 1993; Koepppe and Andersen, 1996). The indole dipoles of their tryptophan residues were suggested to interact with the interfacial potential (the so-called membrane dipole potential) (Seoh and Busath, 1995). According to the literature (Bamberg et al., 1976; Andersen, 1978; Providence et al., 1995), the dipole potential does affect the ionic conductance of the gramicidin channel, but this effect is much less than that observed in the case of the carrier-mediated cation transport (Hladky and Haydon, 1973; Andersen et al., 1976; Andersen, 1978). These data are in agreement with a theoretical study by Jordan (1983), which showed substantial shielding of the dipole potential in the interior of a membrane pore. In line with earlier data on the effect of phloretin on the average channel duration (Andersen, 1978), recent studies have indicated that gramicidin channel lifetimes differ considerably for membranes formed from ether or ester lipids (Providence et al., 1995; Seoh and Busath, 1995), which may be associated with variations in interfacial dipole potential. It is interesting to study the influence of agents modifying the dipole potential on the lifetime and the rate of formation of gramicidin channels. We have shown recently that the rate constants of gramicidin channel formation and dissociation can be determined from the kinetics of the flash-induced inactivation of gramicidin-mediated conductance of planar lipid bilayers in the presence of a photosensitizer (Rokitskaya et al., 1996). Here we present the results of studying the effect of agents modifying the dipole potential of a bilayer lipid membrane (BLM) on the gramicidin channel association and dissociation rate constants obtained by the photoinactivation technique, and compare these data with single-channel measurements.

MATERIALS AND METHODS
BLMs are formed from a solution of 2% diphytanoylphosphatidylcholine (Avanti Polar Lipids, Birmingham, AL) in n-decane (unless otherwise stated) by the brush technique (Mueller et al., 1963) on a 0.55-mm-diameter hole in a Teflon partition separating two aqueous compartments. The bathing aqueous solutions in the cell contain 1 M KCl, 10 mM 2-N-morpholinoethanesulfonic acid (MES), 10 mM Tris, 10 mM β-alanine, pH 7.0. Gramicidin (Sigma, St. Louis, MO) is added from a stock solution in ethanol to the bathing solutions at both sides of the BLM. Aluminum tetrasulfophthalocyanine (AlPcS₄) was kindly provided by Dr. M. G. Galpern (Research Institute of Organic Intermediates and Dyes, Moscow, Russia). AlPcS₄ is added to the bathing solution at the trans side (the cis side is the front side with respect to the flash lamp).

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N-(4-Sulfobutyl)-4-[4-[p-(dipentylamino)phenyl]butadienyl]-pyridinium
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inner salt (RH421) was obtained from Molecular Probes (Eugene, OR). The origins of the various reagents used were Fluka (Buchs, Switzerland) for KCl and phloretin; Sigma for Tris- (hydroxymethyl)aminomethane, 6-ketocholestanol, and MES; and Merck (Darmstadt, Germany) for β-alanine.

The electric current (I) is recorded under voltage-clamp conditions. The currents are measured by means of a home-made amplifier, digitized by DT2814 (Data Translation, Marlborough, MA) and analyzed by a personal computer. Ag-AgCl electrodes are put directly into the cell, and a voltage of 65 mV is applied to the BLM. In single-channel experiments, fluctuations of the current are monitored by a recorder. BLMs are illuminated by single flashes produced by a xenon lamp with a flash energy of ~0.3 J and a flash duration of <3 ms. A glass filter, which cuts off light with the wavelengths of <500 nm is placed in front of the flash lamp.
RESULTS

It has been shown previously that irradiation of a bilayer lipid membrane with visible light in the presence of a photosensitizer leads to suppression of gramicidin-mediated ionic conductance, which is associated with interaction of gramicidin molecules with reactive oxygen species generated upon excitation of a photosensitizer (Strässle and Stark, 1992; Rokitskaya et al., 1993; Kunz et al., 1995). Examination of the kinetics of sensitized photoinactivation of gramicidin channels induced by single flashes of visible light and their analysis according to the formulae derived by Bamberg and Laeuger (1973) allowed us to determine the rate constants of channel formation and termination (Rokitskaya et al., 1996) ascribed to reactions of gramicidin dimerization and dissociation, respectively.

Fig. 1 shows the time courses of the decrease in the gramicidin-mediated electric current through BLM after the flash in the presence of phthalocyanine in controls (curve 1) and upon the addition of phloretin (curve 2) or RH421 (curve 3). It is seen that phloretin, which is known to decrease the dipole potential (Andersen et al., 1976; Melnik et al., 1977; Pohl et al., 1997), markedly decelerates the kinetics, whereas RH421, which is known to increase the dipole potential (Malkov and Sokolov, 1996), considerably accelerates it. Curve 4 in Fig. 1 illustrates the kinetics of gramicidin-mediated current through BLM formed from the decane solution of diphytanoylphosphatidylcholine and 6-ketocholestanol (2:1 by weight), which is known to increase the dipole potential (Franklin and Cafiso, 1993). It is seen that in the latter case the kinetics is markedly accelerated compared to the control. As seen from the inset in Fig. 1, all of the observed kinetics are well approximated by single exponentials.

In agreement with these results, the addition of phloretin and RH421 also leads to deceleration and acceleration, correspondingly, of the kinetics of relaxation of the gramicidin-mediated current after a sudden change in the applied voltage (data not shown) in voltage-jump experiments according to the procedure of Bamberg and Laeuger (1973).

As seen from Fig. 2 A, the characteristic time of photoinactivation (\(\tau\)) increases gradually, as the phloretin concentration increases. In contrast \(\tau\) diminishes gradually, as the concentration of RH421 is increased (Fig. 2 B). At high concentrations of RH421, the relative amplitude of photoinactivation decreases considerably (Fig. 1, curve 3, and Fig. 2 B, inset), which is probably due to quenching of reactive oxygen species by RH421.

Fig. 3 illustrates the dependences of the reciprocal of the characteristic time of photoinactivation on the square root of the BLM conductance (\(\lambda\)) in controls (curve 1) and in the presence of phloretin (curve 2) or RH421 (curve 3). Table 1 shows the values of \(k_R\) and \(k_D\) calculated from a series of such dependences according to the formulae derived by Bamberg and Laeuger (1973). It is seen that \(k_D\) decreases upon the addition of phloretin and increases upon the addition of either RH421 or 6-ketocholestanol. The value of \(k_R\) rises in the presence of any of these agents.

Fig. 4 A shows records of the current through BLM made at low gramicidin concentrations to observe single channels. It is seen that the addition of phloretin leads to a considerable increase in the channel duration: its average value changes from 2.1 ± 0.4 s to 20 ± 3 s (mean ± SE). The effect of RH421 on the gramicidin channel behavior is more complicated. Short-lived channels (average lifetime 0.67 ± 0.15 s) occur along with channels, the duration of which is even longer than that of the control channels. Under our
dependence of current on the concentration of experimental agents. The data on the effect of phloretin on the single-channel conductance and duration are in agreement with earlier results obtained by Andersen (1978).

**DISCUSSION**

The data presented in this paper demonstrate that the agents known to modify the membrane dipole potential produce marked effects on the kinetics of the sensitized photoinactivation. It can be concluded that these agents alter the rate constants of gramicidin dissociation and association, i.e., the open-channel lifetime and the formation rate constant, because the dissociation rate constant is assumed to be the reciprocal of the channel lifetime (Koepp and Andersen, 1996).

Thus the channel lifetime increases, as the dipole potential is lowered upon the addition of phloretin. In line with this, the lifetime decreases, as the dipole potential is raised upon the addition of RH421 or 6-ketocholestanol. Unlike the changes in the channel lifetime, variations in the formation rate constant do not correlate with alterations of the dipole potential. The increase in $k_R$ produced either by RH421 or by 6-ketocholestanol is even greater than the increase produced by phloretin.

The results of the single-channel measurements of the action of phloretin are in agreement with the data on the effect of phloretin on the channel lifetime obtained from the photoinactivation experiments. A comparison of the top and middle records of Fig. 4 A shows that the addition of phloretin considerably lengthens the duration of the open state of the gramicidin channel. In contrast with the effect of phloretin, the action of RH421 cannot be interpreted simply as a decrease in the channel lifetime. Fig. 4 A (bottom

**TABLE 1** The effect of the agents modifying the dipole potential of bilayer lipid membranes on the association ($k_a$) and dissociation ($k_d$) rate constants of gramicidin A

<table>
<thead>
<tr>
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<th>$k_a$ (s$^{-1}$) mean ± SE</th>
<th>$k_d$ (s$^{-1}$mole$^{-1}$cm$^2$) mean ± SE</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.31 ± 0.03</td>
<td>$(6 ± 1) \times 10^{12}$</td>
</tr>
<tr>
<td>10 μM phloretin</td>
<td>0.043 ± 0.014</td>
<td>$(2.3 ± 0.8) \times 10^{13}$</td>
</tr>
<tr>
<td>1 μM RH421</td>
<td>0.71 ± 0.05</td>
<td>$(4.3 ± 0.4) \times 10^{13}$</td>
</tr>
<tr>
<td>6-Ketocholestanol*</td>
<td>0.73 ± 0.09</td>
<td>$(2.5 ± 1.1) \times 10^{13}$</td>
</tr>
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</table>

*1% by weight in the membrane-forming solution.
record) shows considerable variation of the open channel duration, which can hardly be explained by statistical distribution. It may be supposed that some portion of gramicidin molecules forming the channels interact directly with RH421, which leads to alteration of the single-channel properties. Another possibility is a phase separation induced by RH421. In the latter case, the two channel populations might reflect the channel behavior in two different structural phases of the membrane.

Nevertheless, the complex pattern of the single-channel data in the presence of RH421 does not argue against the effect of the RH421-induced increase in the dipole potential on the channel dynamics. Moreover, it is seen from Fig. 4 that the single-channel conductance is reduced upon the addition of RH421, as is expected when the dipole potential increases. Furthermore, the kinetics of photoinactivation is accelerated and remains monoexponential after the addition of RH421. This fact indicates that the influence of RH421 on the photoinactivation kinetics is associated mainly with the effect of the dye on the dipole potential of the BLM.

Otherwise, the time course of photoinactivation would become multiexponential after the addition of RH421, because of the appearance of the different populations of gramicidin channels.

Changes in \( k_R \) caused by the addition of RH421 and 6-ketocholestanol probably reflect the sensitivity of the channel formation process, not to the dipole potential, but to some other properties of the BLM.

Concerning the mechanism of the effect of the dipole potential on the channel lifetime, it should be noted that processes of gramicidin channel formation and dissociation obviously include movement of dipoles (most likely the indole dipoles of tryptophan residues; Hu and Cross, 1995) through the membrane interface, which should be regulated by the membrane dipole potential (Franklin and Cafiso, 1993; Brockman, 1994). Similar regulation has been found for carrier-mediated ion transport. It has been shown that nigericin-induced ion fluxes are sensitive to changes in the dipole potential (Antonenko and Bulychev, 1991), presumably because the transfer of the electroneutral ion-carrier...
complex causes dipoles to move through the membrane interface.

Another mechanism of the effects of phloretin and RH421 on the gramicidin channel lifetime cannot be excluded: the effect of a change in the surface tension caused by adsorption of these agents on BLM. A series of studies have shown that the average lifetime of gramicidin channels essentially depends on bilayer surface tension (see Elliott et al., 1983, and references therein), which has been explained theoretically (Ring, 1996). However, we have not found in the literature any data concerning the influence of phloretin, ketocholestanol, and RH421 on the bilayer tension.

Recently Lundbæk et al. (1996) suggested alterations in the stiffness of lipid bilayers as a general mechanism for modulation of membrane protein function. Apparently this mechanism is not relevant to the effects presented here, because it has been shown that phloretin and 6-ketocholestanol produce negligible changes in the lipid membrane structure (Brockman, 1994).

REFERENCES


