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Asymmetry of the Gramicidin Channel in Bilayers of Asymmetric Lipid Composition: II. Voltage Dependence of Dimerization

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Summary. The asymmetric current-voltage relationship of gramicidin-doped asymmetric bilayers made by the Montal-Mueller technique was investigated in current relaxation experiments. It was shown that, in addition to the contribution of the asymmetric single channel conductance to the asymmetry of the steady-state current-voltage relationship, there is an asymmetric voltage dependence of the step which leads to the formation of the conducting channel. This asymmetric voltage dependence could be simulated in a model assuming a membrane-internal electrical potential drop or an equivalent potential, called asymmetry potential, which could be compensated by externally applying an offset potential. Significant asymmetry potentials were found in asymmetric bilayers made of charged lipids or only of neutral lipids. The asymmetry potential was dependent on the salt composition in the aqueous phase.

The factors responsible for the asymmetry potential do not appear to be of electrostatic origin. Several lines of evidence suggest that the dimerization step which leads to the conducting ion channel may be a complex series of reactions which are influenced by one or more membrane structural properties not yet characterized, in addition to the effects of the externally applied electric field.

The antibiotic gramicidin is believed to form ion conducting channels in membranes by a dimerization involving intermolecular hydrogen bonds between the ends of two gramicidin molecules (Urry, 1971; Urry *et al.*, 1971). After the dimer hypothesis was put forward to explain the concentration dependence of the gramicidin-induced conductance of bilayers (Tosteson *et al.*, 1968), further evidence was obtained from electrical relaxation experiments on the kinetics of channel formation (Bamberg & Läuger, 1973) which showed that the reaction leading to the

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conducting pore was a second-order process, and from membrane conductance and fluorescence studies of a bilayer doped with a fluorescent gramicidin analog (Veatch *et al.*, 1975). From spectroscopic evidence Urry and coworkers (Urry *et al.*, 1972, 1975) inferred a single stranded β (formerly π) helical structure of the channel with a pore diameter of about 4 Å. The double-stranded β helix which has also been proposed (Veatch, Fossel & Blout, 1974; Veatch & Blout, 1974) seems less likely since derivatives of gramicidin with a large polar group attached to the amino-end of the peptide chain could not form channels presumably because the end at which the two gramicidin monomers coupled was blocked and prevented from penetrating the bilayer (Apell *et al.*, 1977; Bamberg, Apell & Alpes, 1977; Bradley *et al.*, 1978).

Even though the dimerization is most probably a head-to-head association via hydrogen bonds, little is known about the exact nature of the association step. The dimerization is voltage-dependent (Bamberg & Läuger, 1973) with the dimer form favored at higher membrane potentials, and the lifetime of the channel is a function of bilayer thickness (Kolb & Bamberg, 1977). It could be shown, however, that the voltage dependence of the dimerization is not a consequence of voltage-dependent changes in the membrane thickness due to electrostriction (Bamberg & Benz, 1976). In another study, a correlation was established between the surface tension of a bilayer and the channel lifetime (Neher & Eibl, 1976).

Bamberg and Benz (1976) have demonstrated that the equilibrium between monomers and dimers depends only on the absolute value of the membrane potential but not its sign, i.e., no relaxation in membrane conductance is observed when the membrane potential is changed to a potential of opposite sign but same absolute value. This makes the effect of the electrical field in the membrane on the dipoles of the gramicidin molecules improbable as a possible mechanistic explanation (Urry, 1972) for the voltage dependence of the conductance.

This paper is a report on observations made with relaxation experiments of the gramicidin-induced conductance in artificial bilayers with asymmetric lipid compositions made according to the method of Montal and Mueller (1972). It was previously demonstrated (Fröhlich, 1979) that in an asymmetric lipid environment gramicidin exhibits an asymmetric single-channel conductance which is partly responsible for the asymmetric macroscopic conductance of gramicidin-doped asymmetric bilayers. The degree of rectification was shown to be different in measurements of the single-channel conductance and of the stationary mac-

roscopic conductance, and the difference was attributed to an asymmetric dependence of the dimerization reaction on membrane potential (Fröhlich, 1979). We describe here electrical relaxation experiments which support this notion and delineate the factors that are important in the interaction between gramidicin monomers.

Materials and Methods

The experimental conditions were the same as reported previously (Fröhlich, 1979). Briefly, the gramicidin A′ used was the commercial product from Nutritional Biochemicals (Cleveland, Ohio), which consisted of gramicidins A, B, and C (Urry *et al.*, 1975). The lipids used were either isolated[1]) (brain PS and PE) or synthesized (PC 18:1, PC 22:1 and PE 18:1) by K. Janko in this laboratory, or were obtained commercially (GMO and GME) from Nu Check Prep (Elysian, Minn.). The lipids were chromatographically pure. All other reagents were analytical grade.

Bilayers were formed according to the technique of Montal and Mueller (1972; Benz *et al.*, 1975). They were stabilized by small amounts of vaseline which was deposited around the hole in the Teflon septum previous to membrane formation. (For details, *see* Fröhlich (1979) or Benz *et al.*, 1975). The experiments were performed at room temperature.

The electrical set-up consisted of a pulse generator (Wavetek Model 115 or own design) in series with a dc voltage source (batteries with adjustable voltage divider) whose output was applied to the membrane via Ag/AgCl electrodes immersed into the aqueous phases. Membrane current was amplified with a Keithley 427 current amplifier and displayed on the screen of a Tektronix 5103 oscilloscope. The pulse generator could be programmed either to single voltage step, square pulse, or triangular pulse. Membrane formation was monitored in the triangular pulse mode at high frequency by following the rise of membrane capacitance charging currect and conductance with the current and voltage displayed on the oscilloscope screen in the x,y-mode.

Precautions were taken to ensure that the asymmetries observed were due to the asymmetric lipid distribution in the bilayer and not artifactual contributions to the asymmetry from the measuring set-up (Fröhlich, 1979).

Results

Two types of relaxation experiments were utilized in this study. The first type employed a single potential jump from zero to a predetermined voltage and will subsequently be referred to as a single voltage-step experiment. In the other type, the voltage was switched from a given value different from zero to the potential of opposite sign but same magnitude. The oscilloscope traces of the latter experiments shown in the figures are sections from a continuous pulse train, i.e., the initial

[1] The following abbreviations are used: PC – phosphatidyl choline; PC 18:1 – dioleoyl-PC; PC 22:1 – dierucoyl-PC; PE – phosphatidyl ethanolamine; PE 18:1 – dioleoyl-PE; PS – phosphatidyl serine; GMO – glyceryl monooleate; GME – glyceryl monoerucate.

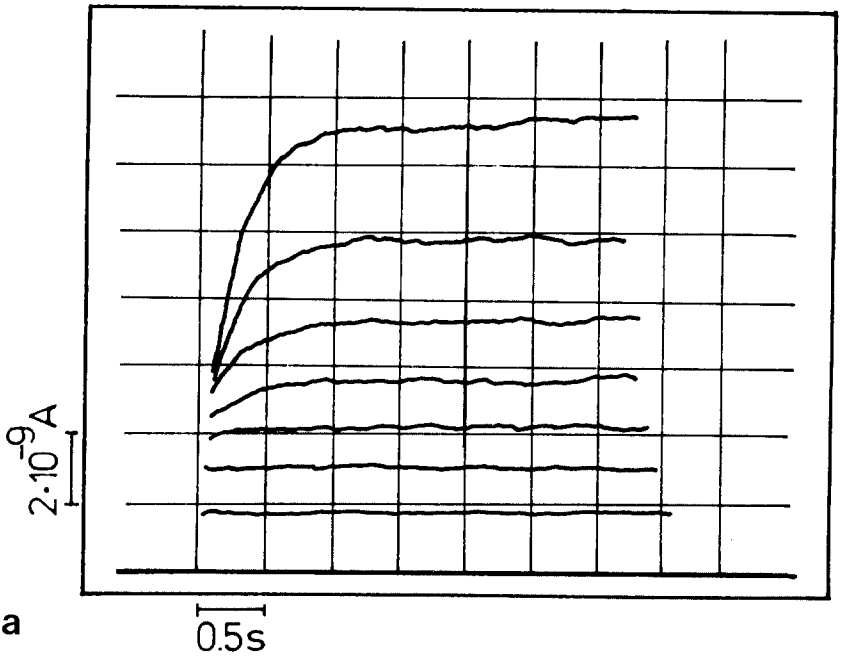
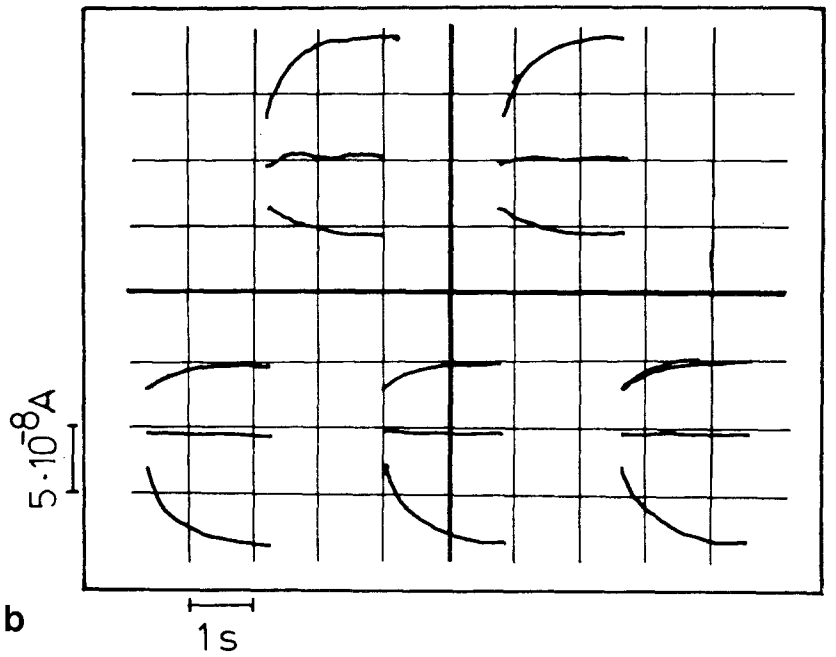


Fig. 1. (*a*): Current relaxation of a symmetrical brain-PE bilayer (Montal-Mueller type) upon applying a single voltage step from zero to 59 (bottom trace), 112, 164, 209, 255, 302, and 351 mV (top trace), respectively. Reproduction of an oscilloscope trace. $I=0$ is indicated by thick abscissa on the bottom of the grid. 10 mM NaCl was on both sides of the membrane. (*b*): Current relaxation of a symmetrical brain-PE bilayer in a continuous pulse experiment. The traces show a segment of a continuous pulse sequence. Switching voltage $V_{sw}=100$ mV. The top, middle, and bottom curves, respectively, belong together. They show the relaxations at three different offset voltages: $V_{off}=+50$ mV (top traces), 0 mV (middle traces) and -50 mV (bottom traces). $I=0$ is indicated by the thick abscissa in the middle of the grid. Symmetrical aqueous solutions contained 1 M NaCl

single step initiating this pulse train is not visible, and records were taken at least five cycles after start of the continuous pulses. We will refer to this type as a continuous pulse experiment. What will be called the switching voltage (V_{sw}) is either the potential reached in the single-step experiment or, in continuous pulse experiments, the absolute magnitude of the pulses around zero potential. In the case of asymmetric continuous pulses, i.e., when an additional offset potential (V_{off}) is applied, the switching voltage is calculated as one-half of the difference between the two voltage levels.

Figure 1*a* and 1*b* show the relaxations of the gramicidin-induced conductance in a symmetrical bilayer made with the Montal-Mueller technique. Since the voltage-dependent thickness change of these virtually solvent-free bilayers is negligible (Benz *et al.*, 1975; Alvarez & Latorre,



1978), the relaxations shown in Fig. 1*a* are evidence that the dimerization equilibrium is dependent on factors different from the change of membrane thickness (Bamberg & Benz, 1976). Figure 1*a* gives information on both the single channel and stationary macroscopic conductance. The initial currents I_o at $t=0$, the time immediately after switching (more accurately, after the capacitive charging current has decayed), plotted as a function of the switching voltage, yield the instantaneous current-voltage curve which is identical in shape to the single-channel conductance (Fröhlich, 1979), since at $t=0$ in all cases the same number of channels are open, determined by the previous zero voltage[2].

The currents I_∞, at times long compared to the relaxation time constant, on the other hand, provide the stationary conductance which is determined by the single-channel conductance and the number of open channels which in turn depend on the membrane potential. When negative voltage steps were applied the relaxations were identical except for the opposite sign (not shown here).

Figure 1*b* shows the relaxations of the gramicidin conductance of a symmetrical bilayer in a continuous pulse experiment. The three disjointed traces are the current caused by continuous pulses with a switching

[2] Some points of Fig. 4*b* of the previous paper (Fröhlich, 1979) have been obtained by this procedure.

voltage V_{sw} of 100 mV and an additional offset voltage V_{off} of +50, 0, and −50 mV (top, middle, and bottom, respectively). In each triplet, the middle trace corresponding to symmetrical pulses around zero ($V_{off}=0$) shows no relaxation. This is in accord with the previously mentioned phenomenological description that the number of open channels depends only on the magnitude but not the sign of the applied voltage. An additional dc offset voltage which changes the originally symmetrical pulses to pulses switching between voltages of different magnitude is therefore expected to elicit relaxations towards different conductances. Pulsing between −50 and +150 mV (top trace in each triplet) causes a relaxation of the positive current to larger values and of the negative current to smaller values, and a negative offset potential (bottom traces) causes relaxations in the opposite directions.

In experiments of the type shown in Fig. 1*a* and *b*, the relaxations are all symmetrical around zero current, as expected for membranes consisting of only one lipid. This symmetry does not exist when the same experiments are perfomred on bilayers of asymmetric lipid distribution. Unlike that of the symmetrical bilayer, the single step experiment shown in Fig. 2*a* does not yield the same result with both polarities. Switching from zero to a potential positive on the PS side of a PS/PE asymmetric bilayer causes relaxations to larger conductances, and a step towards a potential of the same size but negative on the PS side decreases the conductance. The same asymmetric relaxation is observed in the continuous pulsing experiments with symmetric pulses ($V_{off}=0$) (Fig. 2). Judging from Fig. 2*a* and *b*, gramicidin in asymmetric bilayers responds to symmetrical voltage pulses as if there were an intrinisc offset potential present. We, therefore, tested to see if this membrane-intrinsic asymmetry could be counteracted by an externally applied dc offset potential. Figure 3*b* shows that applying an offset potential of the proper polarity and magnitude does abolish the relaxations and that a further increase of V_{off} causes relaxations in the opposite direction. A similar observation is made in a single voltage-step experiment (Fig. 3*a*). At small switching voltages, the relaxations are toward a smaller stationary conductance. The (normalized) relaxation amplitude $(I_\infty - I_o)/I_o$ decreases in magnitude with more negative switching voltages, is zero at a certain switching voltage, and beyond that increases again with opposite sign. The switching voltage at which the relaxation goes through zero is around −350 mV. In Fig. 4, $(I_\infty - I_o)I_o$ is plotted as a function of the offset voltage in another continuous pulse experiment; the curve crosses the voltage axis at $V_{off}=$ 120 mV (negative on the PS side).

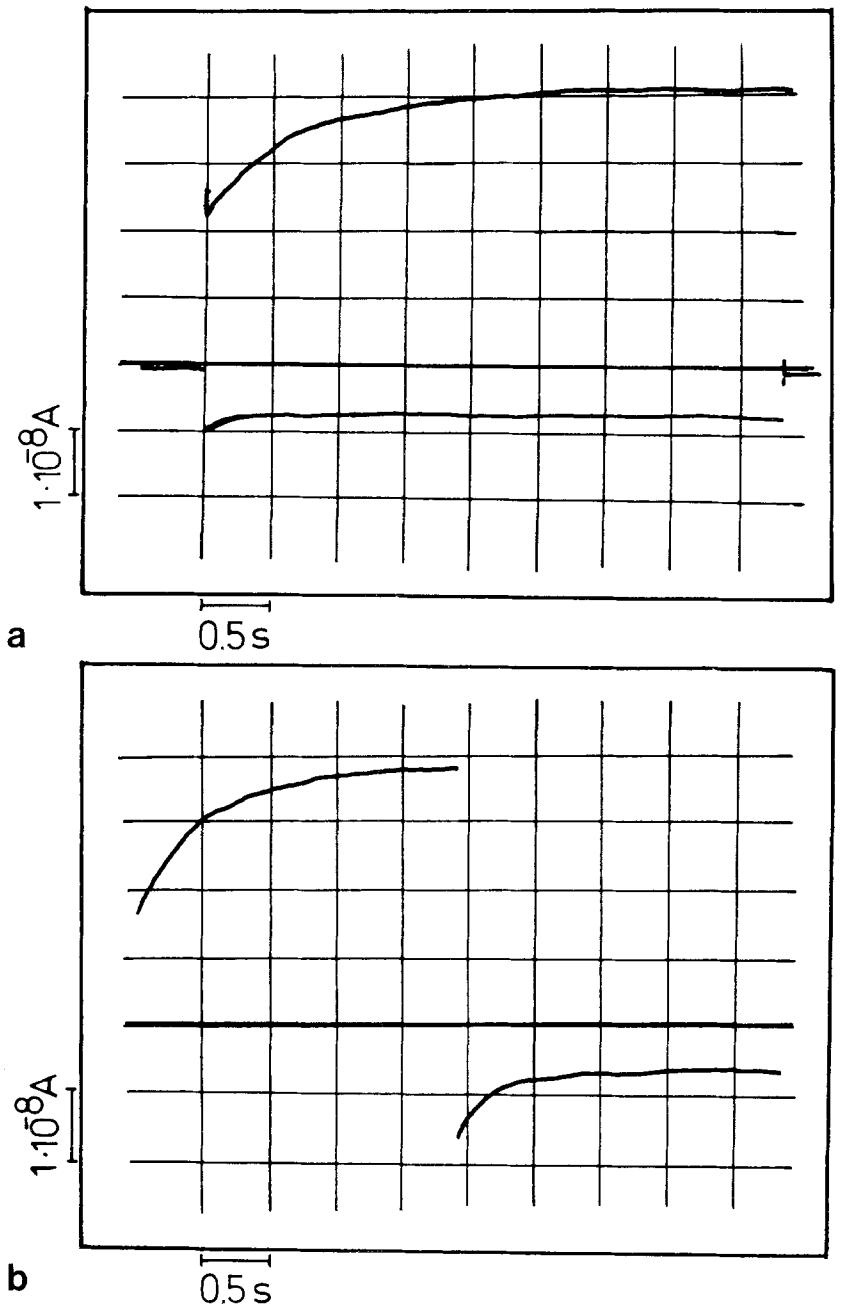


Fig. 2. (*a*): Current relaxation of an asymmetric brain-PE/PS bilayer upon applying a single voltage step from zero to + and −102 mV. The positive current corresponds to the flux of cations from the PS side to the PE side. $I=0$ is indicated by the thick abscissa in the middle of the grid. Symmetrical aqueous solutions contained 10 mM NaCl. (*b*): Current relaxation of an asymmetric brain-PE/PS bilayer in a continuous pulse experiment at $V_{sw}=103$ mV. Positive current corresponds to flux of cations from PS side to PE side. $I=0$ is indicated by thick abscissa. Symmetrical aqueous solutions contained 10 mM NaCl

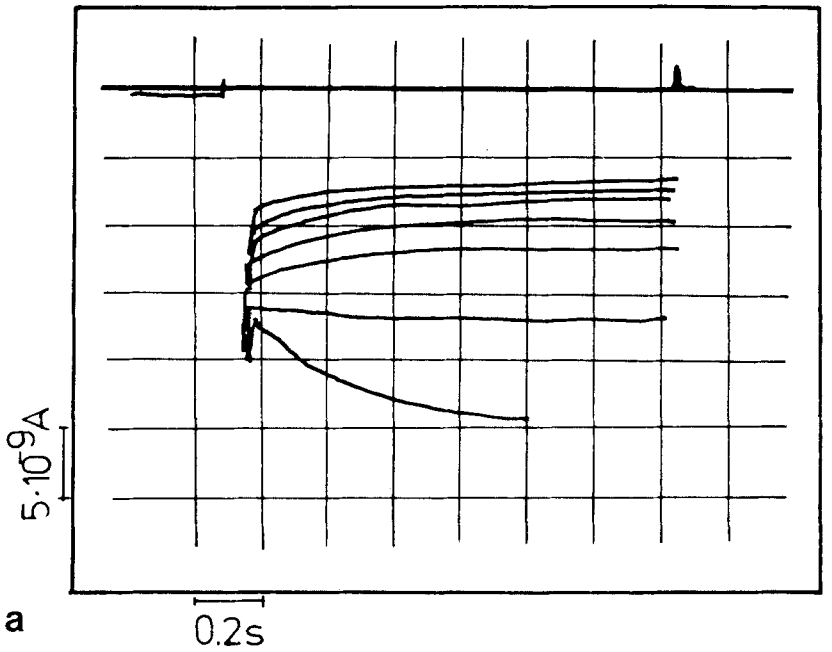


Fig. 3. (*a*): Reversal of direction of relaxation of brain-PE/PS bilayer conductance in a single voltage-step experiment with increasing V_{sw}. The curves show the relaxations at $V_{sw} = -102$ (top trace), -130, -155, -212, -268, -361, and -440 mV (bottom trace). Reversal of direction occurs around -350 mV which corresponds to an asymmetry potential of $+175$ mV (for details, *see* text and *Discussion*). The negative current corresponds to a flux of cations from the PE side to the PS side. Symmetrical aqueous solutions contained 10 mM NaCl. The spike at the onset of the relaxation is due to the membrane capacitance charging current, which is not fully resolved on this time scale. (*b*): Reversal of direction of relaxation of brain-PE/PS conductance in a continuous pulse experiment with increasing offset voltage. $V_{sw} = 50$ mV. The top curves on the left and right sides correspond to $V_{off} = 0$ mV. The second to the top curves on the left and right correspond to $V_{off} = -55$ mV. The third, fourth, and bottom curve correspond to $V_{off} = -108$, -165, and -218 mV, respectively. Positive current corresponds to flux of cations from the PS side to the PE side. Symmetrical aqueous solutions contained 10 mM KCl

All these experiments can be explained by assuming a membrane-internal electrical field which can be counteracted by an externally applied offset potential (for a more detailed description *see* Fig. 6 and *Discussion*). When $V_{off} = 0$, i.e., when the voltage pulses are symmetrical around zero, this intrinsic component causes the gramicidin to behave in the same way as if an offset potential was applied in a symmetrical bilayer (Figs. 1*b* and 3*a*). Regardless of its precise physical nature, we therefore will call this voltage-equivalent component "asymmetry potential" (V_{asym}) and define it as the negative of V_{off} at which the relaxation amplitude

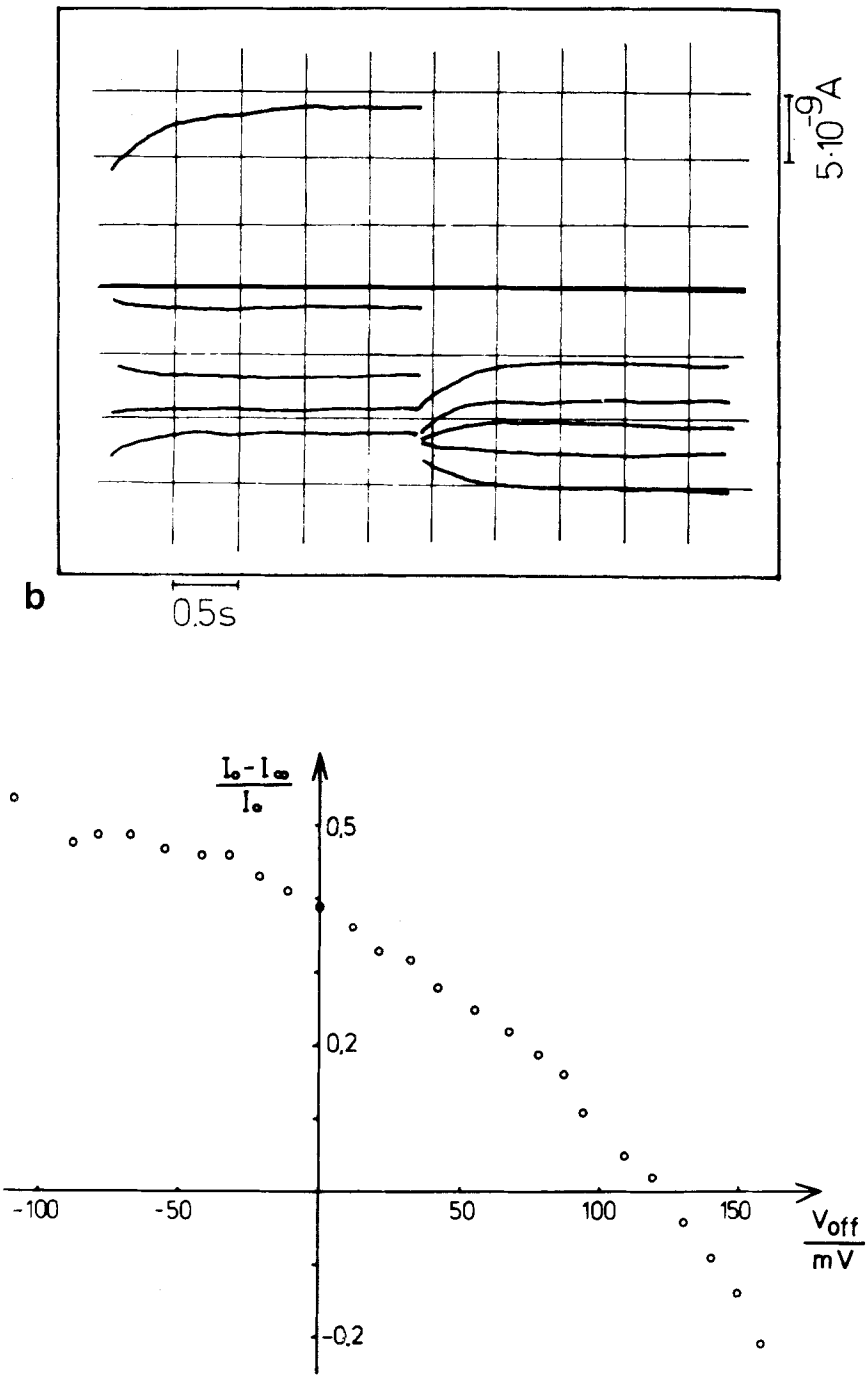


Fig. 4. Relaxation amplitude $(I_o - I_\infty)/I_o$ as a function of offset voltage in a brain-PE/PS bilayer. $V_{sw} = 100$ mV. Symmetrical aqueous solutions contained 500 mM NaCl. The intersection of the curve with the abscissa gives an asymmetry potential $V_{asym} = 125$ mV positive on the PS side

Table 1. Asymmetry potential and rectification of gramicidin conductance in asymmetric bilayers[a]

Membrane left/right[b]	Rectification ratio r_{100}	Asymmetry potential V_{asym}
Brain-PS/brain-PE	2.3	145
Brain-PE/PC 18:1	1.7	90
Brain-PE/PC 22:1	2.6	75
Brain-PE/PE 18:1	1.4	50–100
GME/brain-PE	1.6	35–115

[a] r_{100} is the ratio of the instantaneous currents at ± 100 mV. 10 mM KCl was the bathing solution in all experiments.

[b] The lipid combination is arranged such that the conductance (at ± 100 mV) from the left lipid to the side of the right lipid is larger than in the opposite direction. At the same time, the asymmetry potential is positive on the side of the lipid on the left.

is zero. For example, the asymmetry potential of the PS/PE asymmetric bilayer in Fig. 4 was 120 mV, the PS side being positive.

Table 1 lists five asymmetric bilayer systems. It shows the asymmetry potential (measured at $V_{sw} = 100$ mV) along with the rectification ratio r_{100} of the instantaneous macroscopic conductance. r_{100} is the ratio of the instantaneous current values at + and -100 mV as measured

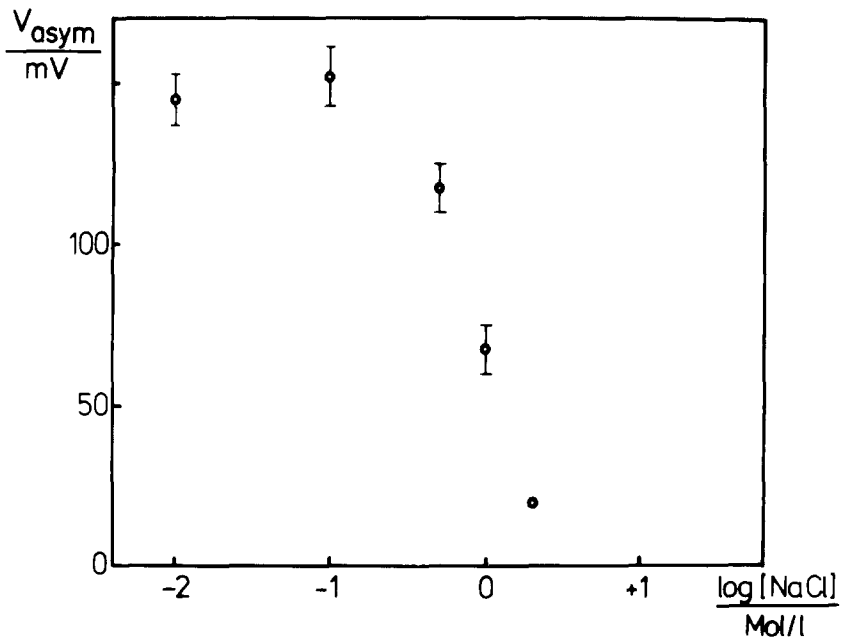


Fig. 5. Dependence of asymmetry potential V_{asym} of a brain-PS/PE bilayer on salt concentration in aqueous phase. V_{asym} is positive on PS side of bilayer

from continuous pulse records. It has previously been shown that the r values of the instantaneous macroscopic conductance and of the single-channel conductance are identical (Fröhlich, 1979). It is evident from Table 1 that rectification and asymmetry potential are significant in both charged/neutral and neutral/neutral asymmetric bilayers.

Like the channel rectification, the asymmetry is a function of the salt concentration in the aqueous phase. At low concentrations (10–100 mM), V_{asym} values of a brain PE/PS bilayer lie on a plateau, but above 100 mV V_{asym} falls off rapidly (Fig.5). A similar concentration dependence was found with PS/PC 22:1 bilayers. $V_{asym} = 90$ mV (PS side positive) in 10 mM NaCl, and at concentrations above 30 mM V_{asym} decreases with increasing concentration (not shown).

Discussion

When gramicidin is introduced into a bilayer of asymmetric lipid composition the membrane conductance is different when potentials of the same magnitude but of opposite polarity are applied, i.e., the current-voltage curve is asymmetric. It was shown in the previous paper (Fröhlich, 1979) that this asymmetry is composed of two components: (i) a rectifying single-channel conductance and (ii) the asymmetric voltage dependence of the dimerization step which leads to the formation of a conducting pore. The asymmetric single-channel conductance has been discussed previously (Fröhlich, 1979), and in this paper we deal with the voltage dependence of the macroscopic conductance.

From the relaxation experiments shown in Figs. 2 and 3 it seems that the gramicidin molecule senses a membrane-intrinsic property which has the same effect on gramicidin dimerization as an externally applied electrical field. Although it is not certain that the membrane asymmetry sensed by the gramicidin is an electric field, it is useful for a more quantitative description of the observed asymmetry to employ the phenomenological concept of an equivalent electric potential, which we call the asymmetry potential. All our experiments so far can be explained in this framework, and the possible physical nature of the asymmetry potential will be discussed below. Figure 6 shows how the asymmetry potential can be envisioned to influence the equilibrium between the non-conducting monomer and conducting gramicidin dimer. Somewhere in the membrane interior where the potential-sensing part of the pore is located, the electric field is composed of two components, the potential

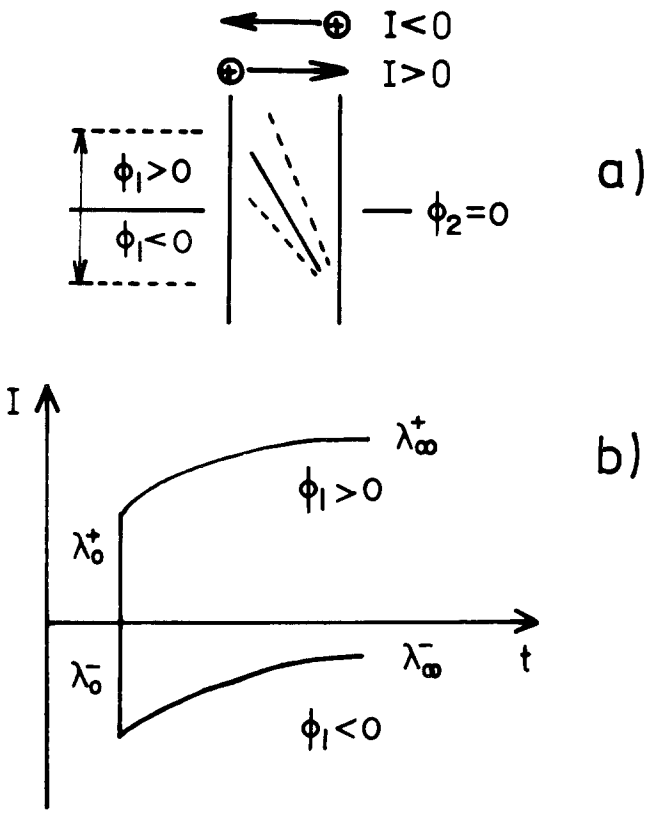


Fig. 6. Schematic representation of the electrostatic concept of an asymmetry potential. (*a*): The electric field in the membrane in the absence of an externally applied potential ($\phi_1=\phi_2=0$) is given by the solid line. Applying an external potential increases (with $\phi_1>0$) or decreases (with $\phi_1<0$) the magnitude of the internal field (dahed lines). (*b*): The curves qualitatively show the expected current relaxations after positive ($\phi_1>0$) and negative ($\phi_1<0$) voltage steps. λ_o denotes the instantaneous conductance obtained from the initial current after the voltage step (neglecting the membrane capacitance charging current). λ_∞ denotes the stationary conductance obtained after the relaxation has resided. In this representation the single-channel conductance was arbitrarily assumed to be symmetrical ($\lambda_o^+=\lambda_o^-$)

drop due to an applied voltage and the component due to the asymmetry potential. In the absence of an asymmetry potential, a single voltage step from $\phi_1=0$ to either $\phi_1>0$ or $\phi_1<0$ causes the same relaxation amplitude, because in both cases the change of the field in the membrane has the same magnitude (not shown here). However, in a membrane with an intrinsic asymmetry potential positive on the left side, the effective field from an imposed positive voltage step, ϕ_1, will be increased to a larger value and the magnitude of the effective field from an imposed

negative voltage step ($\phi_1 < 0$) will be decreased. In a single voltage-step experiment, therefore, the positive current due to $\phi_1 > 0$ relaxes to larger values and the negative current relaxes to smaller values. Similar relaxations are expected in a continuous pulse experiment. If an offset potential is applied which cancels the asymmetry potential, the electric field change in the membrane caused by the imposed voltage steps becomes symmetrical again even though the imposed potential steps between the aqueous phases are not.

This scheme can also be used to explain the single pulse experiment of Fig. 3*b*. A small switching voltage negative on the PS side of the bilayer causes a decrease of the magnitude of the electrical field and, therefore, the relaxation goes towards a smaller conductance (note that V_{asym} is positive on the PS side). With increasing switching voltage the electric field component due to the applied potential becomes more negative and decreases the absolute value of the potential drop, which the gramicidin senses. At a switching voltage twice as large as the asymmetry potential, only the sign but not the absolute value of the field is changed. As a consequence, no relaxation is observed. At still larger switching voltages, the direction of the relaxation reverses because now the pulse causes larger field strengths within the membrane. The asymmetry potential of ~ 350 mV/2 = 175 mV from Fig. 3*b* is reasonably close to the asymmetry potential of 150 mV obtained in the continuous pulsing experiment of Fig. 3*a*.

The asymmetry potential is only a phenomenological explanation of the voltage-dependent macroscopic conductance, without a physical description of the underlying process. The asymmetry potential clearly is not due to the surface charge on the membrane because neutral/neutral asymmetric bilayers which have zero surface charge on both sides also exhibit an asymmetry potential. In addition, the asymmetry potential of a PS/PE bilayer is opposite in sign to what would be expected from the negative surface charges due to the PS.

Perhaps a more reasonable explanation is to invoke the effect of the dipole moment of the lipid molecules on the potential profile inside the bilayer. Since the lipid molecules are oriented in the membrane, any bond of the molecule having a dipole moment with a component normal to the plane of the bilayer contributes to the dipole potential in the membrane. The effect of this dipole potential has been well documented by using carriers and lipophilic ions as conductance probes (Hladky & Haydon, 1973; Szabo, 1975; Anderson *et al.*, 1976; Latorre & Hall, 1976). The data presented here, however, are not reconcil-

able with this notion because (i) the asymmetry potential depends on the ionic strength of the medium (Fig. 5), but the dipole potential is believed to be unaffected by it (Latorre and Hall, 1976) and (ii) the asymmetry potential of an asymmetric bilayer made of monoglycerides and phospholipids (e.g. GME/PE, Table 1; or GMO/PC 22:1, not shown) is positive on the side of the monoglyceride, but experiments with lipid monolayers and with conductance probes in bilayers indicate that the dipole potential of phospholipid is about 150 mV more positive than the monoglyceride (Hladky & Haydon, 1973).

There is an additional feature of the asymmetry potential which is difficult to explain in terms of asymmetric dipole or surface potentials. Knowing the asymmetry potential of a PE/PS (+145 mV on the PS side) and a PC 22:1/PS bilayer (+90 mV on the PS side) one should be able to predict the asymmetry potential of the combination PE/PC 22:1. The experimental result of +75 mV on the PE side, however, is in sharp contrast with the predicted value of about 55 mV, negative on the PE side. This indicates that a more complex mechanism than merely electrostatic effects is responsible for the asymmetry potential.

Surface charges and lipid dipoles so far are the only membrane properties known to contribute to the electrical potential profile inside a membrane. Since they cannot account for the effects observed here, the question has to be raised if the asymmetry potential is of electrostatic origin at all. It is evident that the macroscopic conductance is a function of the membrane potential (Bamberg & Läuger, 1973; Fröhlich, 1979; this work). This does not necessarily mean, however, that the potential affects the gramicidin dimerization directly, e.g., through a group of dipoles (Urry, 1972) or a polarizable region in the molecule.

At least two possible explanations can be devised to explain how an electric field could indirectly affect the channel formation. Either the binding of ions could change the conformation of gramicidin such that the dimerization is favored, and it is the binding of the ion which is potential dependent; or the electrical field could exert its effect via a membrane lipid structural property. First, the binding site for ions is believed to be situated at few angstroms away from the channel mouth (Levitt, 1978). Binding, therefore, could be potential-dependent as assumed by Hladky *et al.* (1978) in their kinetic model of gramicidin conductance.

Second, the electrical field could affect a membrane structural property which in turn has an effect on the gramicidin. Bamberg and Benz (1976) already have excluded the possible explanation that the thinning of the

membrane as a consequence of an applied potential is responsible for the voltage-dependent conductance. Neher and Eibl (1977) found that the channel lifetime as well as the single-channel conductance of the gramicidin pore can be correlated with the surface tension of the bilayer. It is known that the membrane surface tension is a function of the potential because of the parallel plate condensor properties of the bilayer (Requena, Haydon & Hladky, 1975), and qualitatively the correlation between the channel lifetime (and therefore the dimer equilibrium) and the surface tension of bilayers of different composition is consistent with the dependence of the macroscopic conductance on the membrane potential and its associated change in surface tension (Neher & Eibl, 1977). It is understood, however, that a correlation between the two quantities does not necessarily imply a direct causal relationship.

It is interesting to note here that bilayers made of two related phospholipids—the naturally occuring PE from beef brain and the synthetic dioleoyl PE—exhibited an asymmetric single channel conductance as well as an asymmetry potential (Table 1). There are several differences between these two lipids: (i) the naturally occurring PE consists to a certain percentage of the plasmalogen anlog in which the ester linkage between the fatty acid and the glycerol is replaced by an ether bond; (ii) the two lipids have different fatty acid compositions; and (iii) the synthetic PE was a racemic mixture of the D and L isomers, and the brain PE was the naturally occuring L isomer. Lecithins and their ether analogs have a greatly different dipole potential; as determined from lipid monolayers, the dipole potential of the either lipid is about 200 mV more negative than the dipole potential of the ester lecithin (Paltauf, Hauser & Phillips, 1971). Since the asymmetry potential is positive on the side of the natural PE, this further substantiates the above conclusion that the dipole potential plays no decisive role in determining the dimerization equilibrium. As to the difference in the fatty acid composition, it is conceivable that a higher fluidity of the lipids could destabilize the dimer pore. How this is regulated in an asymmetric bilayer, however, remains even more speculative. The stereospecificity difference does not lead to any conclusion because nothing is known about the role of stereospecificity in the interaction among lipid molecules or between lipids and polypeptides.

A strict correlation exists between the direction of single-channel asymmetry and the sign of the asymmetry potential (Table 1). In an asymmetric bilayer the side on which the asymmetry potential is positive is also the side governing the single-channel conductance, or alternatively,

the conductance in the direction away from the side of the positive V_{asym} is always larger than the conductance in the opposite direction. Apparently, rectification of the channel conductance is related to voltage dependence of the dimer equilibrium. This indicates that the influence of the lipid matrix on the gramicidin may be to hold the peptide in a certain overall configuration rather than to affect the molecule locally either at its coupling site with another molecule or at the channel mouth where the ion binding site is located.

In summary, these observations together strongly suggest that the gramicidin relaxations involve a complex series of reactions. At this point it is impossible to identify which are the principle controlling factors. It is evident that the lipid surrounding the pore affects its configuration, but it remains unknown to what extent the responses to an electric field and to solution ions are direct on the gramicidin or indirect through the surrounding lipid. We can, on the other hand, eliminate surface charges and membrane dipoles as the main physical factors behind the asymmetry potential.

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