Biochimica et Biophysica Acta, 642 (1981) 196—202
© Elsevier/North-Holland Biomedical Press

BBA 79164

THE DEPENDENCE OF THE CONDUCTANCE AND LIFETIME OF GRAMICIDIN CHANNELS ON THE THICKNESS AND TENSION OF LIPID BILAYERS

V.S. RUDNEV, L.N. ERMISHKIN, L.A. FONINA and Yu.G. ROVIN

Institute of Chemistry, Academy of Sciences U.S.S.R., Vladivostok, Institute of Biological Physics, Academy of Sciences U.S.S.R., Pushchino and Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences U.S.S.R., Moscow (U.S.S.R.)

(Received August 13th, 1980)
(Revised manuscript received December 3rd, 1980)

Key words: Gramicidin channel; Lipid bilayer

Summary

The lifetimes of channels formed by natural gramicidin and its dimeric analog in monoglyceride lipid bilayers of various compositions were investigated. The bilayer surface tension was altered by changing the length of the monoglycerides' fatty acid chain or the chain length of hydrocarbon solvent by isomerization or saturation of the lipid, by varying the amount of solvent in the bilayer, and by changing the salt composition of the aqueous solutions. The logarithms of mean channel lifetimes were found to be proportional to the surface tension of the membrane irrespective of how the surface tension was changed. In contrast, no simple relationship between channel conductance and surface tension or bilayer thickness was found.

Many authors [1—6] have shown that the parameters of gramicidin A channels depend on the lipid bilayer composition. In bilayers made from monoglycerides of various chain lengths, the channel conductance decreases with the thickness of the membrane [5]. However, if the change of the membrane thickness is achieved by the use of various *n*-alkanes as solvents, the channel conductance does not depend on the membrane thickness [1—5]. The lifetime (τ) of gramicidin channels is also dependent on the membrane composition. Hladky and Haydon [1] have demonstrated that in bilayers formed from the same lipid but with various solvents, τ increases as the membrane thickness decreases. However, according to Neher and Eibl [4], in membranes made from synthetic lipids which differ in polar groups, τ increases with the bilayer thickness. They believe that the only parameter that affects τ is the coefficient of surface tension.

This paper attempts to examine correlations (if any) between the channel parameters and the thickness and tension of the bilayer. Membranes were used which differed not only in the length of the lipid chains and the solvent, as in the above-mentioned works, but also in the amount of the solvent (partially dried bilayers), the type of isomerization, and the degree of saturation of the lipid. The channels were formed by natural gramicidin (a mixture of gramicidins A, B and C) and its dimeric analog, bisglutaryldesformylgramicidin. The main finding is that the logarithm of the mean channel lifetime is proportional to the surface tension coefficient, irrespective of the composition of the hydrophobic part of the membrane.

Materials and Methods

Commercial gramicidin (Sigma, U.S.A.) was used as a starting product for the synthesis of the gramicidin derivative. This product contains approx. 72% gramicidin A, 9% gramicidin B and 19% gramicidin C. Desformylgramicidin was prepared by treating gramicidin with a solution of HCl in CH_3OH (5.6 M solution, 20° C, 3 h); purification was performed by ion-exchange chromatography on Dowex 50X2 in a pH gradient. Bisglutaryldesformylgramicidin was formed by reacting desformylgramicidin with the dipentafluorophenyl ester of glutaric acid (dioxane, 25° C, 48 h). The bis-derivative was separated from ionogenic contaminants on an ion-exchange resin and was further purified by preparative thin-layer chromatography. Bisglutaryldesformylgramicidin was tested by thin-layer chromatography on silica gel in the system $CHCl_3/CH_3OH/H_2O$ (1.3 : 0.45 : 0.04, v/v), (R_f 0.38) as well as by circular dichroic and infrared spectroscopy. Its dimeric nature was confirmed by gel filtration through silanized silica gel.

Lipid membranes were made from chromatographically pure (more than 99%) α-monoglycerides of fatty acids: monopalmitolein (C16 : 1), monoolein (C18 : 1), monoerucin (C22 : 1), monoelaidin (C18 : 1, *trans* isomer) and monolaurin (C12 : 0). The monolaurin membranes were made from a 1 : 1 (w/w) mixture of the monoglyceride with cholesterol. *n*-Heptane, octane, decane and *n*-octane/dioxane were used as solvents. The solvents were purified on a column with activated Al_2O_3. The membrane-forming solutions contained 0.5% lipid. All experiments were performed at 20—22° C and pH 6.0—7.0, at a membrane potential of 100 mV. Techniques of single-channel measurements were the same as those given in Ref. 7. The most probable conductance and mean lifetimes of the channels were determined from the graphs of the corresponding distribution functions. Not less than 350 channels in the case of gramicidin and 150 channels in the case of the bis-derivative were used to construct the graphs. The membrane thickness was calculated from the specific capacity, with $\epsilon = 2.1$ for all the membranes. The surface tension coefficient, γ, was determined from the tension, σ, of the interface between a hydrocarbon solution of lipids and water. It is clear that $\gamma = 2\sigma \cdot \cos\theta$, where θ is the contact angle between the lipid bilayer and the torus. The θ values do not exceed 2.5° [7] for all monoglycerides and solvents studied. Therefore, $\gamma = 2\sigma$ with good accuracy. σ was measured by weighing a platinum frame, placed on the interface, with and without a film [9]. Tension values using this method for a

monooleate solution in dodecane on 0.1 M NaCl are in good agreement with the data by White [10] who used the lying drop method for determination of σ.

Results and Discussion

Fig. 1 shows the distribution functions of the conductance and lifetime of channels, formed by natural gramicidin and the covalent dimer, in membranes formed from monoolein in heptane. The most probable conductance (Λ) of gramicidin channels (in 1 M KCl) is 44 pS; the lifetime distribution is single exponential with an average of 0.31 s. These values of Λ and τ are in agreement with those obtained by others [1,3]. The distribution of the conductance values for channels formed by the covalent dimer is much wider, with no pronounced maximum (Fig. 1B). Therefore, for all the membranes studied, we estimated the mean conductance values of the channels, $\bar{\Lambda}$. On the monoolein in heptane membranes, $\bar{\Lambda}$ was found to be 23.8 pS. A record of covalent dimer channels is shown in Fig. 2. Lifetimes of these channels are much longer than those of gramicidin. During its lifetime a channel frequently switches off for short periods of time. Bamberg and Janko [11] observed the same behavior of channels formed by the other covalent dimer, bismalonyldesformylgramicidin.

Table I presents data for channels formed by gramicidin and the covalent dimer in membranes of various compositions. The conductance of gramicidin channels in bilayers made from monoolein in various solvents is practically the same. In membranes made from monoelaidin, which is a *trans* isomer of monoolein, the channel conductance is the same and does not depend on

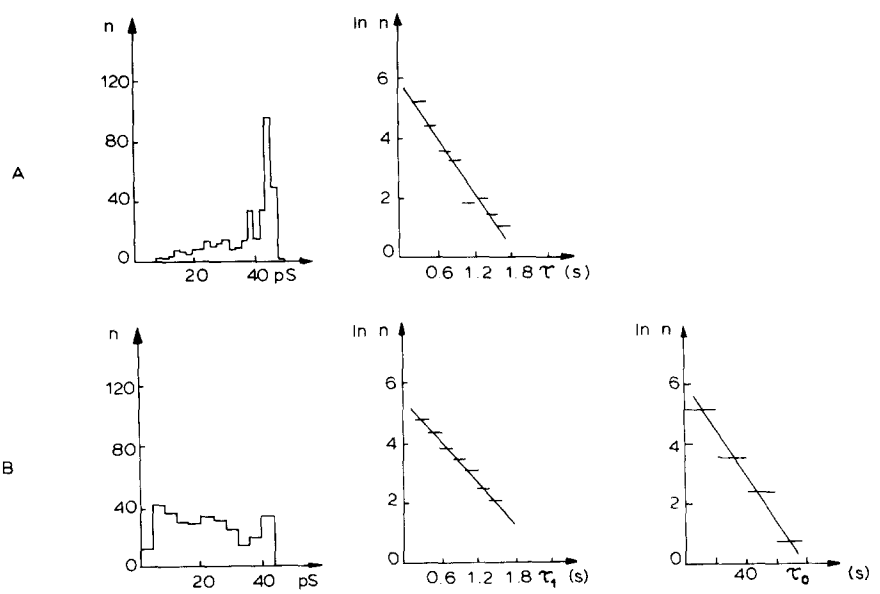

Fig. 1. Distribution functions of conductance (left) and of lifetime (right) in bilayers formed from glycerol monooleate and *n*-heptane in 1 M KCl, 100 mV; pH 6—7, 20—22° C. A, natural gramicidin; B, covalent dimer.

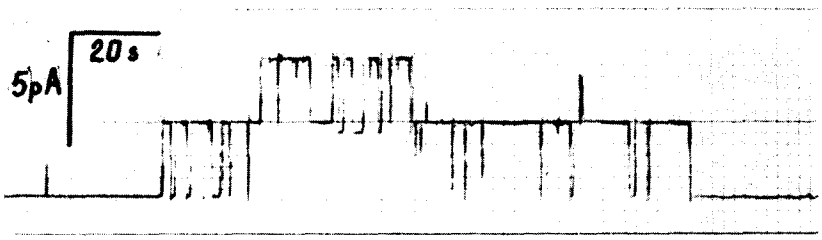

Fig. 2. Current fluctuations in a membrane containing two channels formed by the covalent dimer of gramicidin A. 1 M KCl, 22°C, 100 mV, pH 6—7. Membrane formed from glycerol monooleate and decane.

the type of *n*-alkane. There is a considerable difference, however, of channel conductance in membranes made from monoerucin (C22 : 1) compared to membranes made from monoolein (C18 : 1). With the increase in the acyl chain length, i.e., with the increase in the bilayer thickness, the channel conductance decreases as described in Ref. 5.

It is seen from Table I that the mean conductance of the covalent dimer channel is similarly dependent on the lipid chain length, and for all the systems studied it is approx. 1.8 times less than the most probable conductance of the gramicidin channel.

Due to the solubility of dioxane in water, the use of alkane/dioxane mixtures as solvents allows the formation of the membranes with variable thickness and alkane content up to bilayers obtained from lipid monolayers [12]. Table II presents the parameters of the bilayer and channels in such partially dried bilayers. The thickness of the bilayer formed from monoelaidin in *n*-octane and dioxane decreases from 49 to 31.0 Å with the increase in dioxane content in the membrane-forming solution. In this case, the channel conductance is only slightly changed. Similar results were obtained for other monoglycerides. This suggests that with the same lipid, the thickness has only a slight

TABLE I

SPECIFIC CAPACITANCE (C), CALCULATED THICKNESS (h), AND SURFACE TENSION COEFFICIENT (γ) OF MEMBRANES OF DIFFERENT COMPOSITION, AND PARAMETERS OF CHANNELS FORMED BY NATURAL GRAMICIDIN AND THE COVALENT DIMER OF GRAMICIDIN A

τ is the mean lifetime of gramicidin channels. τ_0 is the total lifetime of dimer channels. τ_1 is the mean lifetime between short-time closures of dimer channels. Λ is the most probable conductance of gramicidin channels; $\overline{\Lambda}$ is the mean conductance of dimer channels. 1 M KCl, 100 mV, pH 6.7, 20—22°C.

Membrane composition	C (μF/cm^2)	h (Å)	γ (dyne/cm)	Monomer		Dimer		
				Λ (pS)	τ (s)	$\overline{\Lambda}$ (pS)	τ_1 (s)	τ_0 (s)
$C_{18:1}$ + heptane	0.38	49	5.1	44	0.31	23.8	0.44	15
$C_{18:1}$ + octane	0.38	49	4.9	44	0.41	24.5	24.5	20
$C_{18:1}$ + decane	0.38	49	4.7	40	0.50	23.8	0.56	26
$C_{18:1}$ (*trans*) + heptane	0.38	49	6.9	44	0.09	22.0	0.08	8
$C_{18:1}$ (*trans*) + octane	0.38	49	5.9	44	0.20	23.3	0.20	10
$C_{16:1}$ + heptane	0.42	44	4.8	54	0.42	—	—	—
$C_{22:1}$ + heptane	0.31	60	9.1	9	0.03	5.10	—	1.8

TABLE II

CAPACITANCE AND THICKNESS OF PARTIALLY DRIED BILAYERS FROM MONOGLYCERIDE SOLUTIONS IN MIXTURES OF OCTANE AND DIOXANE

τ and Λ are mean lifetimes and the most probable conductance, respectively, of gramicidin channels. 1 M KCl, 100 mV, pH 6—7, 20—22°C.

Membrane composition	C (μF/cm^2)	h (Å)	Λ (pS)	τ (s)
$C_{18:1}^{(trans)}$ + octane	0.38	49	44	0.2
$C_{18:1}^{(trans)}$ + octane + dioxane (1 : 11)	0.38	49	44	0.21
$C_{18:1}^{(trans)}$ + octane + dioxane (1 : 11)	0.47	39.5	42	0.22
$C_{18:1}^{(trans)}$ + octane + dioxane (1 : 19)	0.53	35.1	42	0.26
$C_{18:1}^{(trans)}$ + octane + dioxane (1 : 49)	0.60	31	40	0.38
$C_{16:1}$ + octane + dioxane (1 : 49)	0.59	31.5	50	1.9
$C_{18:1}$ + octane + dioxane (1 : 49)	0.52	35.7	42	0.62
$C_{12:0}$ + cholesterol (1 : 1) + octane + dioxane (1 : 49)	0.71	26.2	33	0.19

effect on the channel conductance. In the membranes made from the short, saturated-chain monolaurin (C12 : 0) plus cholesterol (1 : 1), the channel conductance is less than in thicker membranes made from unsaturated lipids.

Thus, the data in Tables I and II indicate that the conductance of gramicidin channels is determined by the length and degree of saturation of the fatty acid chain. The conductance is not affected by either isomerization of acyl chains or by the amount and length of the hydrocarbon solvent. No direct relationship between bilayer thickness and channel conductance was found.

The mean lifetime of gramicidin channels strongly depends on the bilayer composition. The relationship between the bilayer thickness and the lifetime, noted in Ref. 1, is observed only with changes of thickness resulting from changes in the lipid chain length (from C16 and C22 in Table I), but not from an increase in the amount of solvent in the bilayer (Table II). There is no simple relationship between the bilayer thickness and the lifetime of channels; in bilayers of the same thickness (monoolein and monoelaidin with various solvents), τ varies greatly; in bilayers of 26.2 Å thickness formed from monolaurin plus cholesterol, the value of τ is considerably less than in thicker membranes made from unsaturated monoglycerides.

For all membranes considered in Table I, τ correlates with the bilayer tension as Neher and Eibl [4] earlier supposed. With tension decrease, τ increases, irrespective of the bilayer composition. Fig. 3 shows the dependence of ln τ on the surface tension coefficient γ. It is seen that all the points are in a good agreement with the equation:

$$\tau = A \exp(-\gamma B)$$

where A = 10.3 s and B = 0.65 cm/dyne.

The mean times, τ_0 and τ_1, of channels formed by the covalent dimer are also γ-dependent. Here, τ_0 is the mean lifetime of dimer channels and τ_1 is the mean

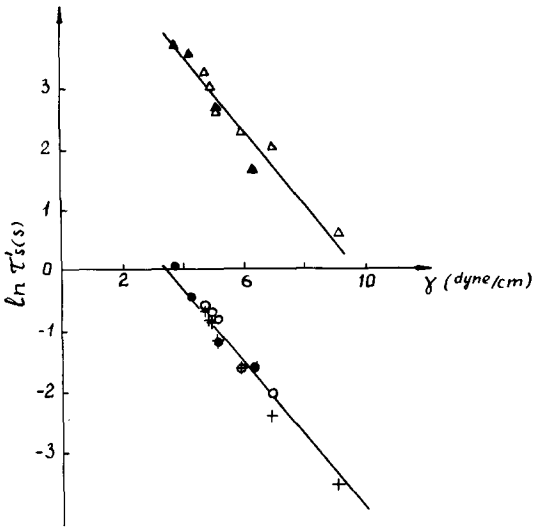

Fig. 3. Dependence of the logarithm of the τ values (τ, τ_1, τ_0) upon the surface tension coefficient γ (the data are from Tables I and II). +, τ; ○ and ●, τ_1; △ and ▲, τ_0. The crosses and the open symbols were obtained in 1 M KCl with different membrane-forming solutions; the closed symbols were obtained on membranes formed from glycerol monooleate and decane with different salts (see Table III). Note that τ and τ_1 points fall on the same line.

lifetime between their short-time closures. For all systems studied, τ_1 is close to the mean lifetime τ of gramicidin channels and τ_0 is approx. 50-times greater. The slope of the curves $\ln \tau_0$ vs. γ and $\ln \tau_1$ vs. γ is approximately the same. This may be explained by assuming the following formal scheme of transition for these channels:

$$O \underset{K_2}{\overset{K_1}{\rightleftharpoons}} C_1 \underset{K_4}{\overset{K_3}{\rightleftharpoons}} C_2$$

where O is open and C_1 and C_2 are closed states. This scheme can describe the observed behavior of a single channel if $K_2 >> K_1 >> K_3 >> K_4$. The relationships between τ and K values are: $\tau_1 = 1/K_1$ and $\tau_0 = K_2/K_1K_2$. Thus, to explain similar τ_0 and τ_1 dependences on γ it sufficed to assume that only K_1 is γ-dependent and $K_2/K_3 = 50$. Gramicidin channels may also be described by

TABLE III

MEAN CONDUCTANCE ($\overline{\Lambda}$), MEAN TOTAL LIFETIME (τ_0) AND MEAN LIFETIME BETWEEN SHORT-TIME CLOSINGS (τ_1) OF THE DIMER CHANNELS IN MEMBRANES FORMED FROM GLYCEROL MONOOLEATE AND OCTANE IN SOLUTIONS OF DIFFERENT SALT COMPOSITIONS

Parameters	0.1 M KCl	0.5 M KCl	1. M KCl	1 M CsCl	1 M KNO_3
γ (dyne/cm)	6.3	5.1	4.9	4.2	3.7
τ_1 (s)	0.2	0.3	0.5	0.65	1.1
τ_0 (s)	5.1	14.0	20.0	34.0	35.0
$\overline{\Lambda}$ (pS)	6.5	13.0	24.5	31.3	21.1

this scheme with the same K_1 but $K_2 << K_1$ and $K_3 = 0$. In this case, $\tau = \tau_1 = 1/K_1$.

Table III lists the values $\bar{\Lambda}$, τ_1, τ_0 and γ for bilayers made from monoolein and octane in solutions of various salt compositions. A similar dependence of gramicidin channel lifetimes on salt solution was observed by Kolb and Bamberg [5]. These authors attributed the effect of salt to electrostatic stabilization of the channel. Our results indicate that the salt dependence of τ and τ_1 is explained by the effect of salt solution on surface tension as is clearly shown in Fig. 3. No separate electrostatic mechanism need be invoked.

References

1 Hladky, S.B. and Haydon, D.A. (1972) Biochim. Biophys. Acta 274, 294—312
2 Goodall, M.C. (1971) Arch. Biochem. Biophys. 147, 129—135
3 Bamberg, E., Noda, K., Gross, E. and Lauger, P. (1976) Biochim. Biophys. Acta 419, 223—228
4 Neher, E. and Eibl, H. (1977) Biochim. Biophys. Acta 464, 37—44
5 Kolb, H.-A. and Bamberg, E. (1977) Biochim. Biophys. Acta 464, 127—141
6 Hendry, B.M., Urban, B.W. and Haydon, D.A. (1978) Biochim. Biophys. Acta 513, 106—116
7 Ermishkin, L., Kasumov, Kh., Potseluyev, V. (1977) Biochim. Biophys. Acta, 470, 357—367
8 Requena, J. (1974) Ph.D. dissertation, Cambridge
9 Rovin, Yu.A. (1976) Biofizika 20, 118—127
10 White, S.H. (1975) Biophys. J. 15, 95—117
11 Bamberg, E. and Janko, K. (1977) Biochim. Biophys. Acta 464, 486—499
12 Rudnev, V.S., Bagaveyev, Y.A. and Rovin, Yu.A. (1981) in Membrane Biophysics (Tverdislov, V., ed.), Moscow, Nauka, in the press