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$1/f$ Noise in Black Lipid Membranes Induced by Ionic Channels formed by Chemically Dimerized Gramicidin A

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Summary. The noise behavior of lipid bilayer membranes, doped with a chemically dimerized gramicidin A, was investigated. In contrast to normal gramicidin A, which generates a Lorentzian type power spectrum due to the formation and disappearance of conducting dimers, the current power spectrum density $S_m(f)$ obtained with this gramicidin A derivative showed over several orders of magnitude a clear $1/f$ behavior. The intensity of this $1/f$ component was analyzed as a function of the membrane-applied voltage, membrane resistance, electrolyte concentration, and composition. The relationship between the mean-square fluctuation in current and the membrane current mean value was found to follow Hooge's equation, i.e., $\delta I^2 = \alpha I_m^2/Nf$ where N is the number of channels and α is a constant equal to 1.0×10^{-2}. It is suggested that a $1/f$ type noise was observed because the chemically dimerized form of gramicidin A produces long lasting cation selective channels.

Gramicidin A, a linear hydrophobic pentadecapeptide isolated from *Bacillus brevis*, has been used often in recent years as a model compound to study the transport of ions through hydrophilic channels as well as the mechanism of channels formation (Hladky & Haydon, 1972; for survey of literature, *see* Bamberg *et al.*, 1977). There exist so far two distinct models describing the structure of the gramicidin A channel. According to a proposal made by Urry, the gramicidin A channel is essentially a $\pi_{L,D}$ helix, formed by a head to head (formyl end to formyl end) association of two helical monomers (Urry, 1971, 1972; Glickson *et al.*, 1972). An alternative structure was more recently presented by Veatch, Fossel and Blout (1974), in which the gramicidin A channel is seen as two peptide chains coiled around a common axis. Despite basic differences, both models nevertheless agree on the existence of a narrow hydrophilic channel, 0.4 nm wide and 3 nm long, lined with the oxygen atoms of the peptide carbonyls, and oriented along the axis of a dimer

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structure. Although both proposals lead to permissible configurations, it should be mentioned, however, that the results so far obtained from electrical measurements on lipid bilayer membranes doped with negatively charged analogs of gramicidin A provide substantial support to the $\pi_{L,D}$-helix proposal alone (Apell *et al.*, 1977; Bamberg *et al.*, 1977).

In order to determine the kinetics of the dimerization process by which gramicidin A channels are formed, previous studies have used voltage jump and noise analysis techniques (Bamberg & Läuger, 1973, 1974; Zingsheim & Neher, 1974; Kolb, Läuger & Bamberg, 1975; Bamberg & Benz, 1976; Kolb & Bamberg, 1977). By applying a voltage jump across a membrane doped with gramicidin A, it is indeed possible to shift the equilibrium between nonconducting monomers and conducting dimers toward the dimers side, and thus to produce a time-dependent increase of conductance from which the kinetic parameters can be obtained. This one-to-one relationship between conducting channels and gramicidin A dimers has been in fact more directly confirmed by carrying out voltage jump and single channel experiments on lipid membranes in presence of a chemically linked form of gramicidin A (Bamberg & Janko, 1977). The main results of these experiments can be stated as follows: first the gramicidin dimers obtained by a head-to-head (formyl end to formyl end) reaction with a bifunctional reagent do not have a typical voltage-dependent channel formation process, as observed with normal gramicidin A; secondly, the mean lifetime of the channels induced by this gramicidin A derivative is increased by at least a factor 100 compared with normal gramicidin. These results led the authors to conclude that the chemically linked gramicidin forms channels *per se* without further association. One may then wonder what a noise analysis would, in this particular case, lead to since previous works have shown that the power spectrum of the current fluctuations for membranes containing gramicidin A is mainly determined by the formation and disappearance of the channels, i.e., by the fluctuation in the number of dimers (Zingsheim & Neher, 1974; Kolb, Läuger & Bamberg 1975; Kolb & Bamberg, 1977). Shall we expect with the chemically dimerized form of gramicidin A a Lorentzian type power spectrum (constant value at low frequencies, $1/f^2$ behavior for $f > f_c$, f_c being the corner frequency) as in the case of normal gramicidin A, but with a lower corner frequency, or do we have to look for a totally different noise behavior? In this regard it is worth mentioning that power spectra of the type $1/f$ have been found with systems consisting of thick artificial membranes containing long lasting aqueous pores (Green & Yafuso, 1968; Hooge, 1970; Hooge & Gaal, 1971; DeFelice

& Michalides, 1972; Dorset & Fishman, 1975; Lifson, Gavish & Reich, 1978). Since the chemically dimerized form of gramicidin A creates long lasting channels, a similar noise behavior can be expected, though the two systems are not totally equivalent; in one case we have large holes in thick membranes, whereas this gramicidin A derivative forms cation selective channels in black lipid membranes. The latter system certainly constitutes a better structural approximation of a real biological system and can thus yield new insights on the noise behavior of open channels in biological membranes, e.g., open K^+ channels in squid giant axon (Verveen & Derksen, 1968; Poussart, 1971; Fishman, 1972; Verveen & De Felice, 1974; Fishman, Moore & Poussart, 1975; Conti & Wanke, 1975).

Materials and Methods

The chemically dimerized gramicidin A, referred to henceforth in this paper as malonyl-bis-desformylgramicidin, was synthesized and purified in this laboratory (Bamberg & Janko, 1977). Unless specified otherwise, the membrane-forming solution consisted of 1% 1,2-di-

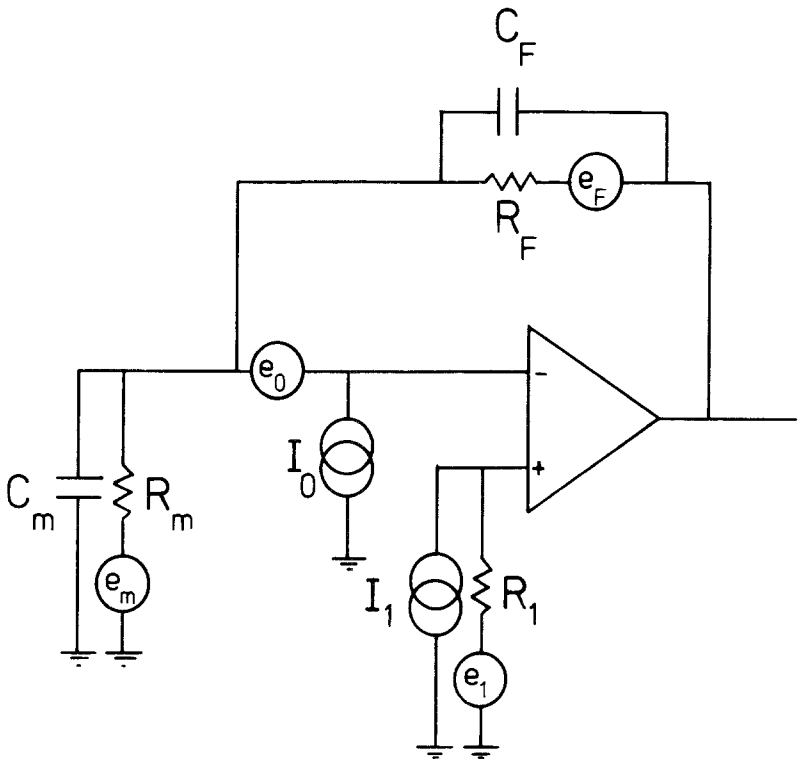

Fig. 1. Circuit diagram of the electronic setup. e_o, I_o and I_1 represent the voltage and current noise sources which take into account the noise behavior of the amplifier. e_m, e_1 and e_F are the voltage noise sources associated with R_m, R_1 and R_F, respectively

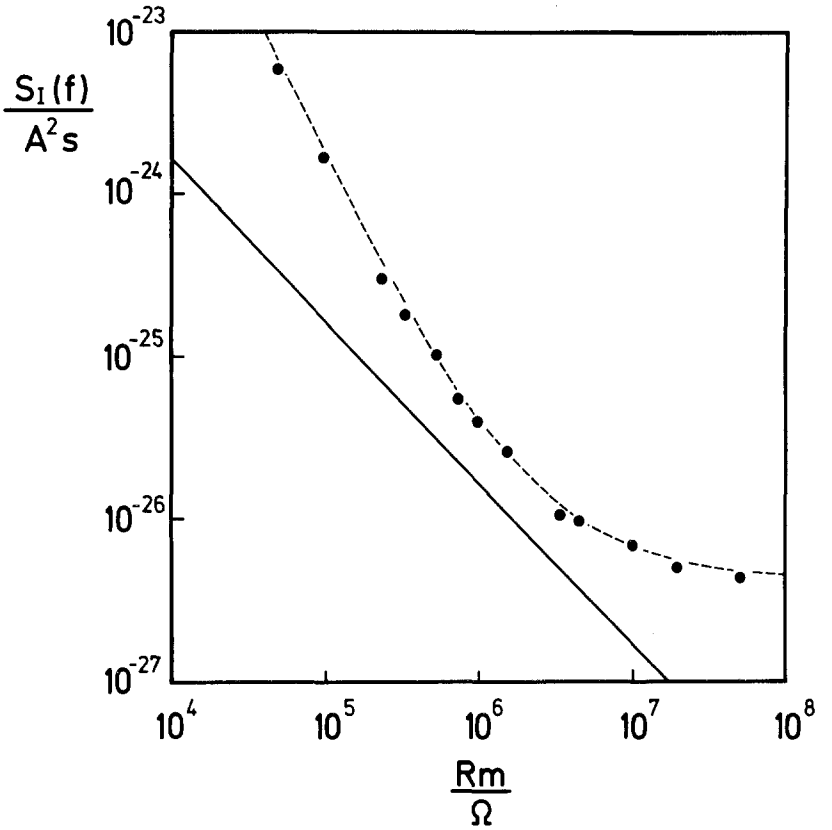

Fig. 2. (*a*): Power spectrum density $S_I(f)$ obtained with equivalent circuits as a function of the membrane resistance R_m. C_m was equal to zero and the values of $S_I(f)$ were taken at $f=200$ Hz. The dashed line represents the theoretical predictions of Eq. (2) with $S_o^o(f)=1.5\times10^{-14}$ V²/Hz. The continuous line shows the values expected from an ideal system for which $S^o(f)=0$. (*b*): Frequency behavior of the power spectrum density $S_I(f)$ obtained with equivalent circuits for various membrane resistance R_m. The dashed lines represent the theoretical predictions of Eq. (2) with $S_o^o(f)=1.5\times10^{-14}$ V²/Hz. C_m was equal to zero

phytanoyl-*sn*-glycerol-3-phosphoryl-choline (diphytanoyllecithin) in *n*-decane with 0.1% cholesterol to improve the membrane stability. The diphytanoyllecithin was synthesized by K. Janko and the *n*-decane was purchased from Merck, Darmstadt (standard for gas chromatography).

Black lipid membranes were formed the usual way in a thermostated Teflon cell filled with aqueous electrolyte solution (Läuger *et al.*, 1967). The chemically dimerized gramicidin was directly added in various amounts to the membrane-forming solution. The area of the membranes varied from approximatively 8.5×10^{-4} to 1.3×10^{-3} cm². Ag/AgCl electrodes were used, and the temperature of the system was 25 °C throughout.

All measurements were carried out at constant voltage. A period ranging from 30 to 90 min was usually needed in order to insure a stable mean membrane conductance (less than 10% deviation in 30 min). The electronics we used had already been described in detail (Kolb & Läuger, 1977). It consisted essentially of an operational amplifier (Analog Devices, Model 52K), which in our case operated in a feedback circuit containing a resistor R_F of

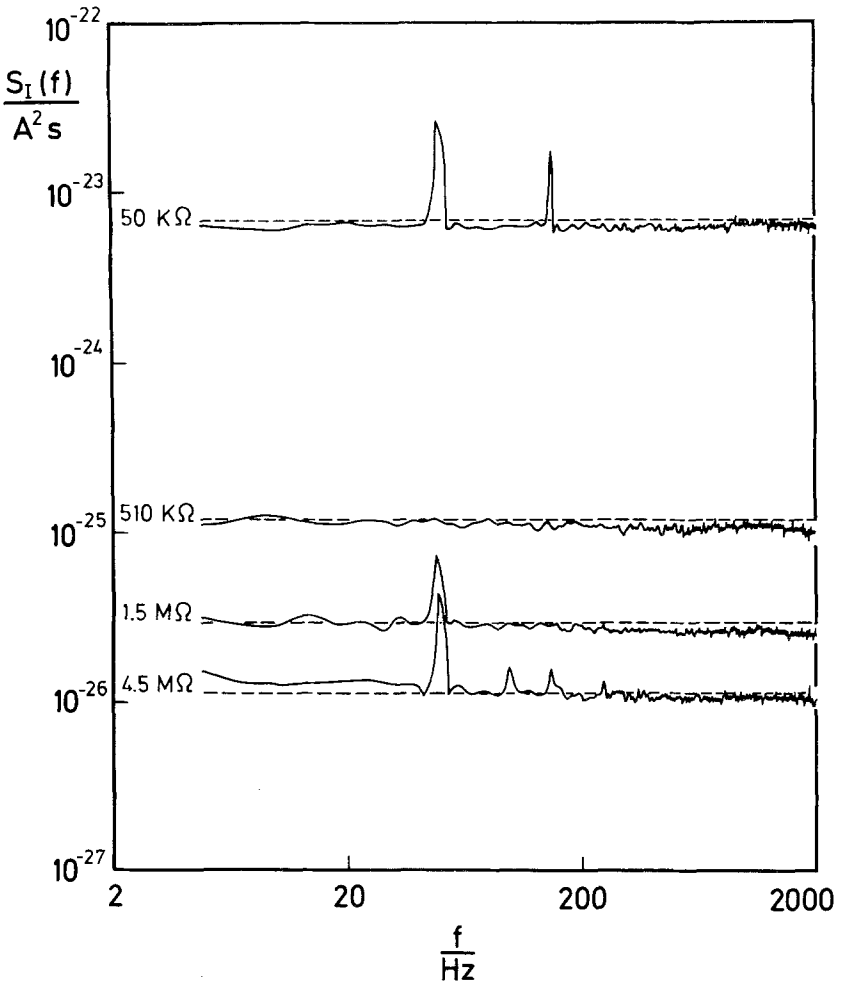

Fig. 2*b*

4.5 MΩ in parallel with a capacitor C_F of 9 pF (*see* Fig. 1). The output of the preamplifier was in turn ac coupled into a main amplifier (Princeton Applied Research, Model 113), whose low cut-off and high cut-off frequencies were set to 0.03 Hz and 30 kHz throughout. The amplified signal was finally processed by means of a Nicolet 44 A Ubiquitous spectrum analyser. (A Honeywell-Saicor 52 B real time spectrum analyzer was also utilized in some cases.) In order to determine the contribution of the electronic setup to the total noise measured in the presence of real membranes, an analysis of the power spectrum obtained with equivalent circuits was first undertaken. Ohmic resistors R_m in parallel with capacitors C_m of known values were taken as membrane substitutes. The power spectrum of the current fluctuations at the output of the preamplifier was measured for various combinations of C_m and R_m. The experimental values obtained for the current spectral intensity $S_I(f)$ were then analyzed according to the theoretical expression (*see* Fig. 1):

$$S_I(f) = S_o(f) + S_m(f) \tag{1}$$

where

$$S_o(f)=\frac{4KT}{R_F}+S_o^o(f)\left[\frac{1+\omega^2 R_F^2 C_F^2}{R_F^2}+\frac{1+\omega^2 R_m^2 C_m^2}{R_m^2}+2\frac{1+\omega^2 R_F C_F R_m C_m}{R_m R_F}\right]+S_{I_o}^o(f) \quad (2)$$

with $\omega=2\pi f$, $S_m(f)$ the spectral intensity of the current fluctuations across a membrane of dc resistance R_m, and $S_o^o(f)=4KTR_1+S_v^o(f)+R_1^2 S_{I_1}^o(f)$ in which $S_v^o(f)$, $S_{I_1}^o(f)$, and $S_{I_o}^o(f)$ are the spectral intensities of the noise sources by means of which the intrinsic noise behavior of the operational amplifier is taken into account. For a membrane represented as a simple resistor R_m in parallel to a capacitor C_m, $S_m(f)$ reduces then to $4KT/R_m$ where K is Boltzmann's constant and T is the absolute temperature. Results obtained for the special case $C_m=0$ are presented in Fig. 2*a* and *b*. Although the values of $S_v^o(f)$, $S_{I_1}^o(f)$ are known to be frequency dependent, especially within the frequency range 0.1 to 10 Hz (Analog devices, 52 K amplifier technical specifications), it should be apparent that such a frequency dependency does not affect the behavior of $S_I(f)$ appreciably. As described in Eq. (2), with a resistor R_1 equal to 918 kΩ, the sum $S_v^o(f)+R_1^2 S_{I_1}^o(f)$, which is over most of the frequency range 2–2000 Hz approximately equal to 4.0×10^{-16} V²/Hz, does not in fact contribute substantially to $S_o^o(f)$. Similarly $S_{I_o}^o(f)$, which is of the order of 2×10^{-29} A²/Hz can also be neglected compared to $4KT/R_F$. The agreement seen on Fig. 2*a* and *b* between theoretical and experimental values of $S_I(f)$ allows us to conclude that Eqs. (1) and (2) represent useful expressions from which $S_m(f)$ can be determined, though deviations may be expected as R_m decreases. All subsequent evaluation of $S_m(f)$ from $S_I(f)$ with real membrane systems was thus accomplished according to the formalism proposed in Eq. (1).

Results

Fig. 3 shows the power spectrum obtained from a malonyl-bis-desformylgramicidin doped membrane in contact with a 1 M KCl solution. It should be apparent that, as the voltage across the membrane is increased, a new component of the form $1/f^n$ ($n\simeq1.06$) emerges from the background noise. The values of R_m range in this particular case from $7.8\times10^5\,\Omega$ with $V_m=164$ mV to $9.6\times10^5\,\Omega$ when $V_m=32$ mV. In order to insure that the observed excess noise was directly related to the presence in the membrane of malonyl-bis-desformylgramicidin molecules, the following control experiments were carried out. Undoped membranes of the same lipid composition as those used for the malonyl-bis-desformylgramicidin experiments were formed, parallel to which a resistance of 1 MΩ, representing the change in conductance due to the chemically dimerized gramicidin A, was placed. Fig. 4*a* clearly shows that no excess noise can be observed even at membrane potentials of 160 mV. It may thus be concluded that, under the same conditions of membrane dc resistance and lipid content, a membrane without malonyl-bis-desformylgramicidin does not *per se* lead to a $1/f$ power spectrum. It is known, furthermore, that gramicidin A, and consequently the chemically dimerized form of gramicidin A, is impermeable to divalent cations such as Ca^{++} (Bamberg & Läuger, 1977). The power spectrum of doped membranes in contact

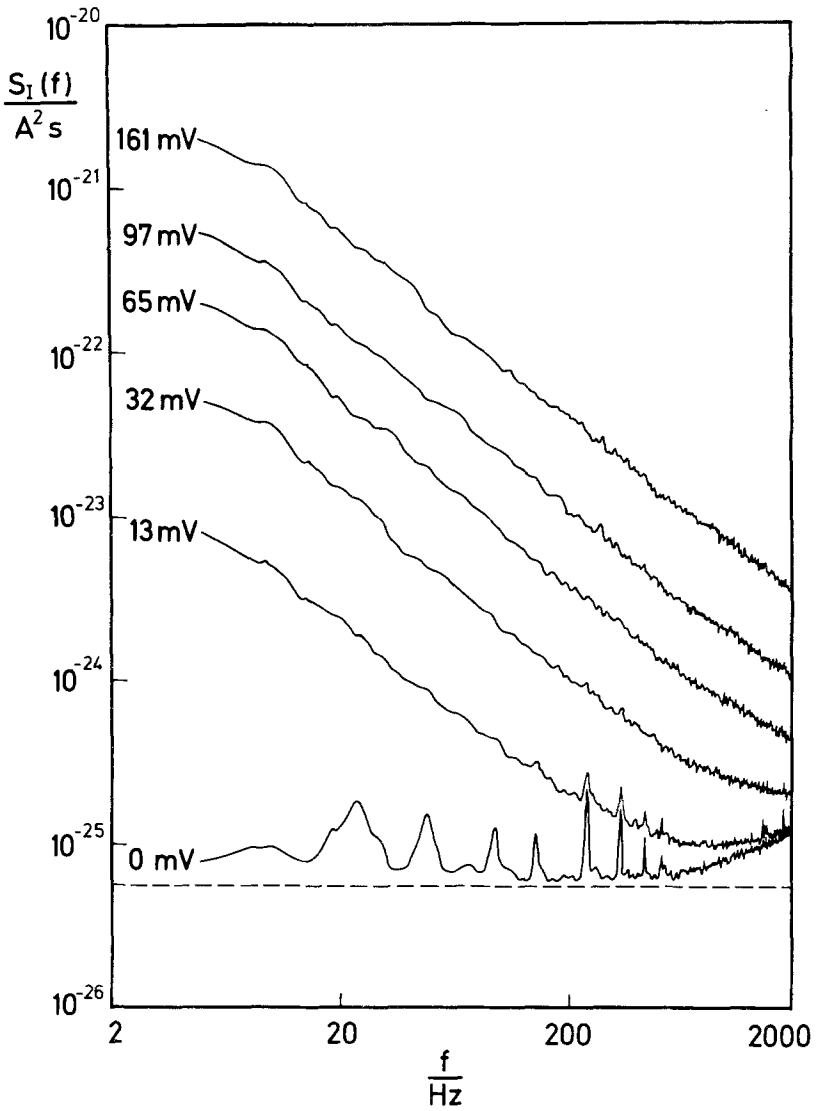

Fig. 3. Power spectrum density $S_I(f)$ obtained from a malonyl-bis-desformylgramicidin doped membrane. Lipid: PC 16:4 CH_3/cholesterol 1.0%/0.1%/*n*-decane (w/v); electrolyte: 1 M KCl, membrane resistance $7.2 \times 10^5\ \Omega$ for $V_m = 161$ mV, $8.40 \times 10^5\ \Omega$ for $V_m = 97$ mV, $1.10 \times 10^5\ \Omega$ for $V_m = 65$ mV, and $9.6 \times 10^5\ \Omega$ $V_m = 32$ mV. The dashed line represents the expected value of $S_I(f)$, assuming $C_m = 0$; $R_m = 850$ KΩ. $T = 25$ °C

with a 2 M $CaCl_2$ solution was thus measured, an example of which is shown in Fig. 4*b*. As seen again, no $1/f$ component is present, which leads us to conclude that the $1/f$ power spectrum obtained previously was not caused by unspecific membrane leaks due to the perturbation of the lipid structure by the channel forming molecules.

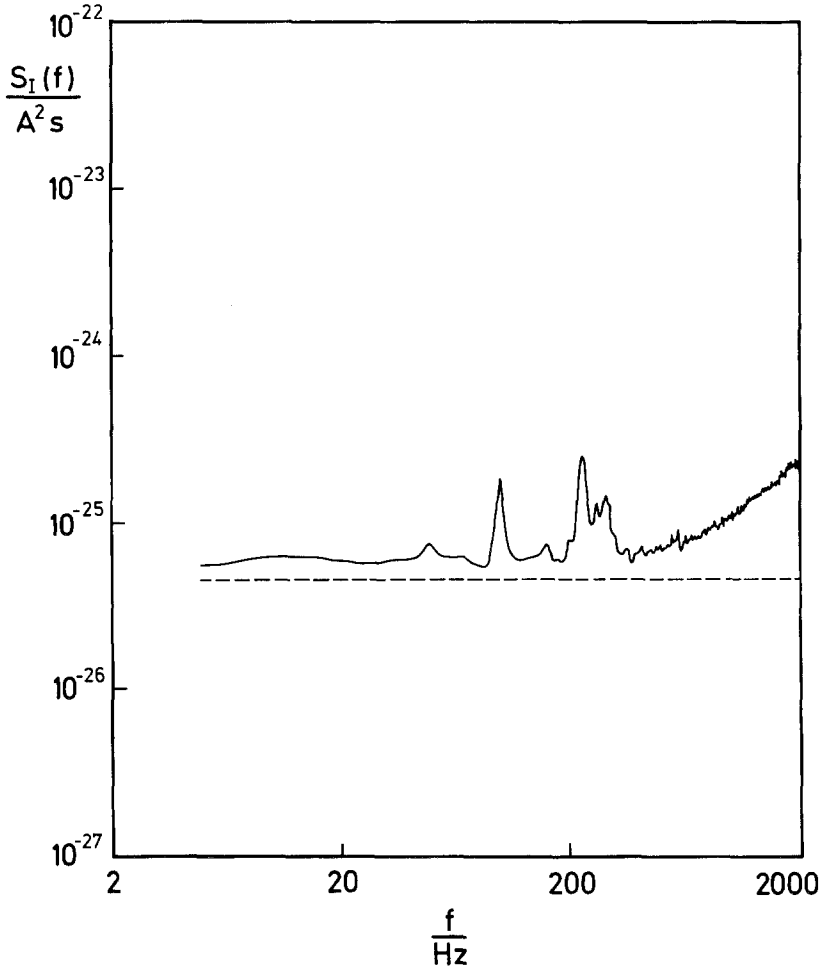

Fig. 4. (*a*): Power spectrum density $S_I(f)$ obtained with an undoped membrane having in parallel a 1-MΩ resistance. The dashed line represents the expected value of $S_I(f)$, assuming $C_m=0$. Lipid: PC 16:4 CH_3/cholesterol 1.0%/0.1%/*n*-decane (w/v). Electrolyte: 1 M KCl. $V_m=160$ mV. Irregularities are due to acoustic vibrations centered at 250 Hz. (*b*): Power spectrum density $S_I(f)$ obtained from a doped membrane in contact with a 2-M $CaCl_2$ solution. Lipid: PC 16:4 CH_3/cholesterol 1.0%/0.1%/*n*-decane (w/v). $V_m=160$ mV. Irregularities are due to acoustic vibrations centered at 250 Hz

The effect of the membrane-applied voltage and membrane conductance on the amplitude of the $1/f$ component of $S_I(f)$ is illustrated in Fig. 5. The calculations were carried out on the basis that the membrane noise source could be described as

$$S_m(f)=\frac{4KT}{R_m}+\frac{A}{f^n}. \tag{3}$$

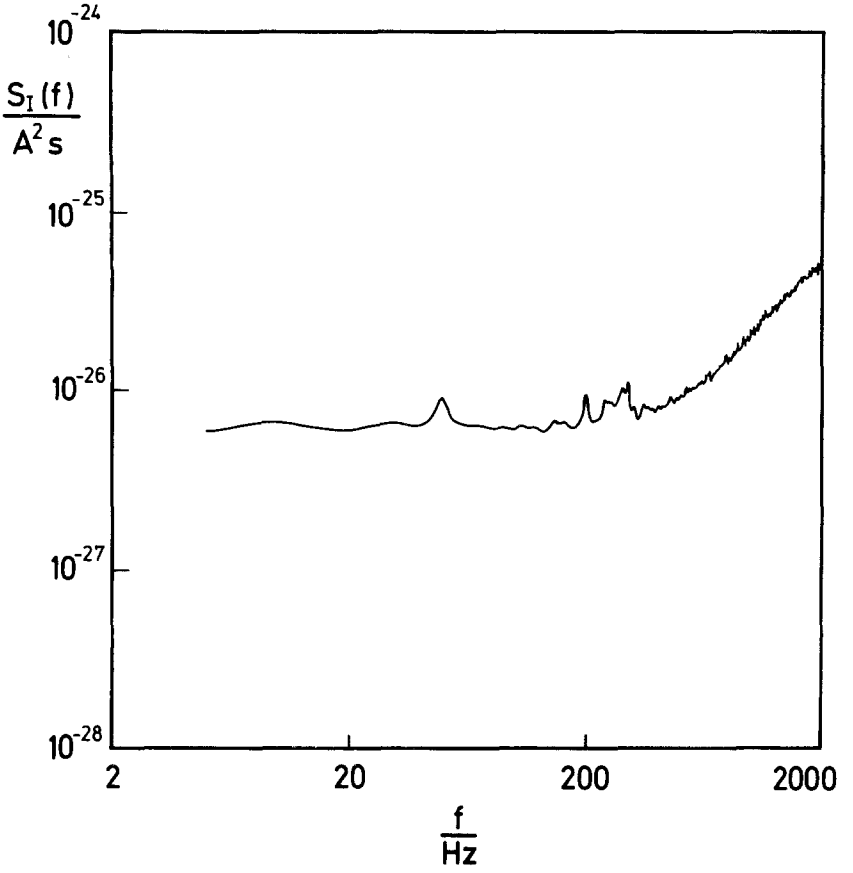

Fig. 4*b*

The values of A, n and C_m were adjusted using a least squares fitting procedure. The main results of these calculations can be stated as follows: $n=1.03$ with a SD of 0.06 (average over 23 membranes and 62 power spectra), and the amplitude A depends upon R_m and I_m, the membrane mean current, according to

$$A=\gamma\, I_m^2 R_m \tag{4}$$

where $\gamma=(1.8\pm0.5)\times10^{-13}$ S (*see* Fig. 5). If the membrane resistance R_m is expressed as $1/NA$, where N is the number of malonyl-bis-desformyl-gramicidin channels and A the single channel conductance [$(17.0\pm2)\times 10^{-12}$ S; Bamberg & Janko, 1977], the equality Eq. (4) then becomes

$$A=\frac{\alpha\, I_m^2}{N} \tag{5}$$

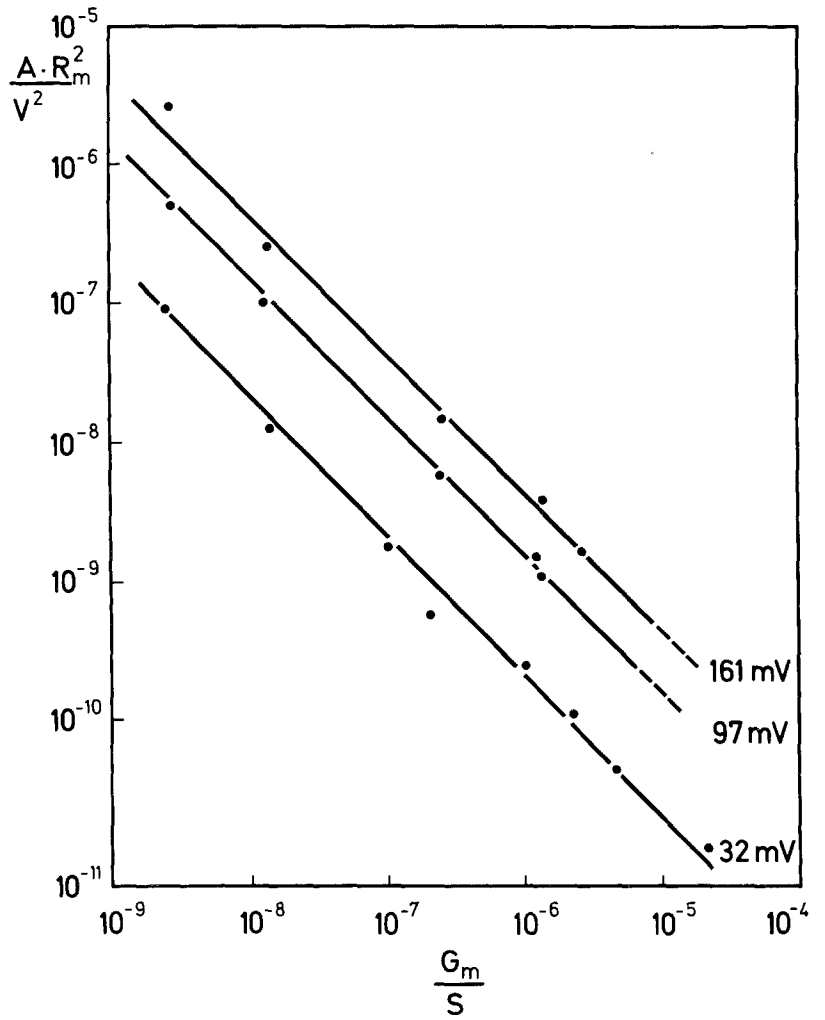

Fig. 5. Amplitude A of the $1/f$ component of $S_I(f)$ as a function of membrane conductance $G_m = 1/R_m$ and applied voltage V_m. Lipid: PC 16:4 CH_3/cholesterol 1.0%/0.1%/*n*-decane (w/v); electrolyte: 1 M KCl; $T = 25$ °C

where $\alpha = 1.0 \pm 0.4 \times 10^{-2}$. This equation is similar to the empirical formula, but forward by Hooge and his co-workers (Hooge, 1969; Hooge & Hoppenbrouwers, 1969*a*, 1969*b*; Hooge, 1970; Hooge, 1972) stated that the amplitude of a $1/f$ power spectrum due to voltage fluctuations should be proportional to the square of the dc applied voltage and inversely proportional to the number of charge carriers in the system. However, according to their study, the constant of proportionality α should be for a membrane in contact with a 1 M monovalent ion salt solution approximately equal to 15. Such a value is far above the actual

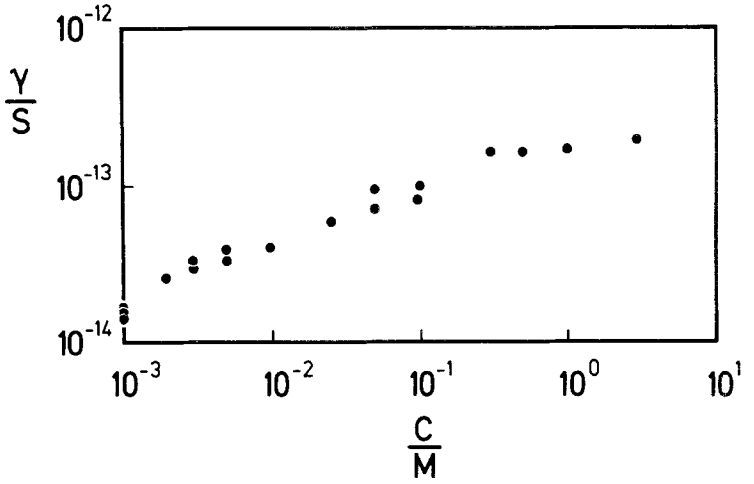

Fig. 6. Variation of γ as a function of the bulk concentration of KCl. Lipid: PC 16:4 CH_3/cholesterol 1.0%/0.1%/*n*-decane. (w/v). Applied voltage $V_m = 97$ mV. $T = 25$ °C

estimate obtained with malonyl-bis-desformylgramicidin channels, though a direct comparison between Hooge's equation and the present results remains questionable. However, the above discrepancy seems to indicate that, despite a formalism which appears to be totally valid, Hooge's equation cannot be applied without restrictions to any kind of membrane system, unless a clearer physical interpretation of the parameter α is provided (Stevens, 1972; Dorset & Fishman, 1975; DeGoede & Verveen, 1977; Van den Berg *et al.*, 1977). Results on the dependency of γ [*see* Eq. (4)] upon the concentration of permeable ions in the bathing solution are shown in Fig. 6. It is observed that γ increases sharply at low K^+ concentrations, but saturates as the concentration exceeds 1 M. The finding of an asymptotic value is interesting, since it indicates that at high concentrations of the permeable ion the amplitude of the $1/f$ power spectrum becomes, for a given value of R_m and I_m, independent of the number of permeable ions in the bulk solution.

In order to determine to what extent a change in single channel conductance may influence the parameter γ, experiments were also carried out in which various amounts of $CaCl_2$ were added to regular 1 M KCl aqueous phase solutions. The malonyl-bis-desformylgramicidin channels are then gradually blocked while the number of permeable ions in the bulk solution remains unchanged (Bamberg & Läuger, 1977). The results shown in Fig. 7 clearly indicate that γ decreases drastically as a function of the bulk Ca^{++} concentration, especially for $[Ca^{++}] \leqq 0.3$ M, but reaches a saturation level roughly equal to 5.0×10^{-14} S as $[Ca^{++}]$ increases.

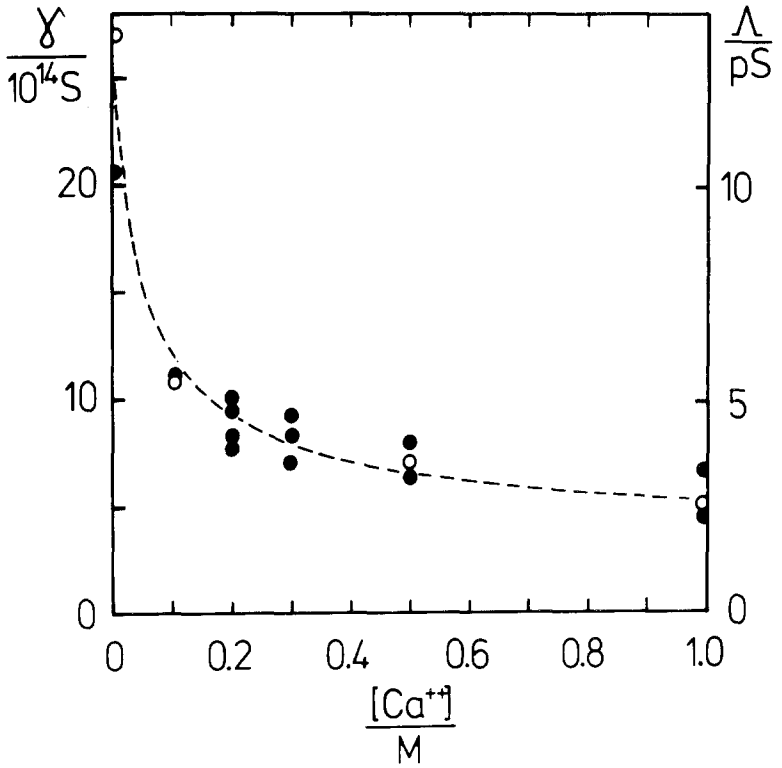

Fig. 7. Variation of γ as a function of the bulk concentration of $CaCl_2$. Lipid: PC 16:4 CH_3/cholesterol 10%/0.1%/*n*-decane. (w/v). Electrolyte: 1 M KCl + various amounts of $CaCl_2$. $V_m = 97.2$ mV. $T = 25$ °C. Filled dots: results obtained from noise analysis. Open dots: results obtained from single channel experiments

These results were compared with those coming from single channel experiments. As seen in Fig. 7, both γ and Λ are found to follow the same $[Ca^{++}]$ dependency.

Discussion

Let us briefly summarize the main findings of the present study. It was first established that malonyl-bis-desformylgramicidin yields a current power spectrum of the $1/f$ form. This $1/f$ component was shown to be directly related to the presence of the chemically dimerized gramicidin A in the membrane phase, and to require the presence of a permeable ionic species, such as K^+, in the bathing solution. It was also found that the intensity of the $1/f$ component of $S_I(f)$ is proportional to the product $R_m \cdot I_m^2$, where I_m is the mean membrane current and R_m the dc membrane

resistance. Finally, the constant of proportionality γ was shown to saturate at high concentration of permeable ions and to decrease by adding divalent cations, such as Ca^{++}, to the bathing solution. It is first interesting to note that, under similar experimental conditions, the current power spectra obtained with malonyl-bis-desformylgramicidin A differs substantially from the Lorentzian type power spectra observed with regular gramicidin A. The typical $1/f^2$ behavior of $S_I(f)$ coming from the current fluctuations due to opening and closing of the gramicidin A channels has been replaced by a strong $1/f$ component. It is worth recalling in this regard that single channel studies have clearly established that one of the main differences between the malonyl-bis-desformylgramicidin and the gramicidin A channel stands in their respective mean lifetimes, i.e., more than 2 min for chemically dimerized gramicidin and 0.1 to 1 sec for normal gramicidin (Hladky & Haydon, 1972; Bamberg *et al.*, 1976; Bamberg & Janko, 1977). However, such a difference seems to be appreciable enough to yield, in the case of long lasting channels, $1/f$ type power spectra and not merely new Lorentzian curves of lower corner frequencies. There are, nevertheless, similarities between the power spectra produced by gramicidin A and those obtained with malonyl-bis-desformylgramicidin. In the case of gramicidin A, for instance, one may show that for frequencies larger than the corner frequency f_c the spectral intensity $S_m(f)$ can be written as (Kolb & Bamberg, 1977).

$$S_m(f) = \frac{R_m I_m^2 (k_D \Lambda_G / \pi^2)}{f^2} \tag{6}$$

where Λ_G is the single channel conductance of the gramicidin A channel and k_D the dissociation constant in the dimerization reaction of gramicidin

$$G + G \underset{k_D}{\overset{k_r}{\rightleftharpoons}} G_2. \tag{7}$$

There is an obvious similarity between the amplitude of the $1/f^2$ power spectrum of gramicidin A at high frequency and the empirical relationship found for the amplitude A [*see* Eq. (4)] with malonyl-bis-desformylgramicidin. In both cases the amplitude of the spectral intensity $S_m(f)$ is seen to be directly proportional to the product $I_m^2 R_m$. Interestingly enough, the constant of proportionality γ is found in the case of gramicidin A channels to be solely dependent upon Λ_G and k_D. It thus follows that a decrease of the rate of dissociation k_D automatically implies a decrease of the spectral intensity $S_m(f)$, independently, of the rate of association k_r. Although there exists no comparable explicit

definition of γ in the case of malonyl-bis-desformylgramicidin channels, one may also tentatively propose an equation of the form

$$\gamma = \alpha \Lambda, \tag{8}$$

where α is to be considered a constant independent of Λ, i.e., γ directly proportional to Λ. It ought to be stressed in this regard that our experimental results are totally compatible with such formalism. It is known, for instance, that Ca^{++} ions remarkably lower the single channel conductance of gramicidin A channels (Bamberg & Läuger, 1977). A comparison of the effect of Ca^{++} on γ and Λ constitutes, therefore, a valuable test to determine if the relationship between γ and Λ can be expressed as $\gamma = \alpha \Lambda$. As seen in Fig. 7, the experimental results obtained from noise analysis and single channel measurements support, within experimental error, the equality proposed in Eq. (8). The finding that γ tends towards an asymptotic value as the concentration of permeable ions increases, is also compatible with the experimental results obtained on Λ. Though such findings do not constitute a formal proof that the equality [Eq. (8)] holds in the case of malonyl-bis-desformylgramicidin, they nevertheless provide a serious ground upon which such particular formalism can be justified. The physical meaning of the constant α remains, however, to be determined.

In view of the results obtained with malonyl-bis-desformylgramicidin, one may wonder about a possible $1/f$ component in the power spectra produced by gamicidin A. In several instances, slight deviations of the typical $1/f^2$ behavior, other than the usual effect due to the background noise, were observed at high frequencies, i.e., $f \geqq 50 f_c$. It was, however, impossible to explicitely assign those deviations to a $1/f$ component whose amplitude A would be given by Eq. (5). The predicted value for A exceeded by at least a factor of 2 the value needed to make this hypothetical $1/f$ component compatible with the experimental observations. Several explanations can be put forward. There is, for example, no real evidence that the molecular mechanism at the origin of the $1/f^2$ component of the spectral intensity $S_m(f)$ does not also influence the $1/f$ behavior of the system. The equality derived in Eq. (5) would in such conditions become inapplicable. Without a detailed knowledge of the physical parameters which determine the value α, no real estimate of a possible $1/f$ contribution to the power spectrum of gramicidin A can for the moment be proposed. It may, nevertheless, be concluded that, if such a contribution is indeed present, its intensity is certainly less than what the results obtained with malonyl-bis-desformylgramicidin A would tend to imply. Let us also

briefly mention that several experiments, in which the constant of dissociation k_D was reduced by decreasing the temperature of the surrounding medium, were carried out. However, the results obtained so far did not yield new information on a possible $1/f$ produced by gramicidin A. The details of such experiments will be presented in a forthcoming work.

Another conclusion, which may be inferred from the present noise analysis of the malonyl-bis-desformylgramicidin system, is that no detectable association-dissociation process is in this particular case taking place. A simple association-dissociation mechanism would have yielded a Lorentzian type power spectrum, but such an additional component was never observed within a frequency range of 2–2000 Hz. The chemically dimerized form of gramicidin A seems, therefore, to constitute an ionic channel *per se* without any further molecular association. This conclusion is also largely confirmed by the finding of a $1/f$ power spectrum with malonyl-bis-desformylgramicidin, as in systems consisting of large holes in thick artificial membranes (DeFelice & Michalides, 1972; Dorset & Fishman, 1975). The failure to detect intermolecular associations among malonyl-bis-desformylgramicidin molecules is particularly important, since it substantiates the argumentation recently proposed by Bamberg, Apell and Alpes (1977) in favor of Urry's model for the molecular structure of the gramicidin A channel. Our results undoubtedly support the proposal of a gramicidin A channel consisting of a dimer which is formed by head-to-head (formyl end to formyl end) association of two gramicidin A monomers. Since open channels can be obtained from single malonyl-bis-desformylgramicidin molecules, there is no reason to believe that the nonpermanent association of two gramicidin A monomers would behave differently.

Although the present study showed that a $1/f$ power spectrum can be obtained with long lasting cation selective channels, nothing concerning the molecular mechanism by means of which the $1/f$ noise is generated can be inferred. However, by comparing our results to those obtained with large holes in thick membranes, it seems clear that the physical entities which are responsible for the transport of ions across the membrane phase are not the determining factors. The transport of ions through a 0.4 nm wide long lasting channel is certainly not equivalent to a simple nonselective constrained flow of ions through a large aqueous pore; nevertheless, both cases lead to a $1/f$ power spectrum (Green & Yafuso, 1968; Hooge, 1970; Hooge & Gaal, 1971; DeFelice & Michalides, 1972; Dorset & Fishman, 1975; Lifson *et al.*, 1978). Our study indicates that the intensity of the $1/f$ component of $S_m(f)$ depends to a large extent

upon membrane parameters such as R_m, I_m and α, though the exact nature of α needs to be determined. One may thus conclude that the source of the $1/f$ noise is more related to the phenomenon of ion transport than to the actual mechanism of ion transport across the membrane. The latter may, however, be important in determining the intensity with which the $1/f$ appears.

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