Self-similar processes and flicker noise from a fluctuating nanopore in a lipid membrane

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Stochastic properties of a fluctuating nanopore generated and sustained by an electric field in a lipid bilayer membrane are studied. It is shown that the process of voltage fluctuations, in the current clamp experiment, is a stochastic fractal with long memory, which is the main reason for its nonstationarity. The aging process contributes to the nonstationarity if molecular interactions in the membrane are weak. An attempt to classify the process reveals a non-Gaussian distribution with long tails, which contradicts the hypothesis of fractional Brownian motion, showing that stable motion may be possible. The self-similarity index, estimated by three different methods, depends on current value and membrane sensitivity to electric field in a well defined and explicable manner. The stochastic analysis provided for calculated conductance of nanopore revealed the process close to $1/f$ noise, the result observed only for the pores not exceeding 1 nm in diameter, induced in membranes with strong molecular interactions. Our results show that such a pore is the simplest biological system needed for flicker noise to occur, and the complexity of highly regulated protein channel is not a necessary factor. A case of noise $1/f^2$, observed for a pore with impeded dynamics, suggests a process without memory in such a situation. A physical interpretation is presented for some of the results.

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I. INTRODUCTION

An application of strong electric fields to lipid bilayer membranes revealed the phenomenon of electroporation [1], which could be detected due to the dramatic increase of the membrane conductance. The evidence shows [2] that the electroporation is responsible for the electropermeabilization, which stimulates the molecular transport system, and for the planar mechanical rupture of the membrane.

The phenomenon of electroporation is attracting interest because of its application to biotechnology and medicine. It is currently used as a simple and relatively nontoxic method for introducing exogenous macromolecules (such as DNA, RNA, proteins, antibodies, drugs, and fluorescent probes) into cells of various types [2]. Electroporation has also been observed as a side effect during large defibrillation shocks applied to the cardiac tissue [3,4] and may prove lethal in severe cardiac ischemia cases.

The molecular phenomena responsible for the electroporation are still poorly understood, which is a limiting factor for its wider application. We lack knowledge on the effects of electroporation parameters on molecular uptake and cell viability [5]. Not much is known on pore kinetics and molecular structural rearrangement that take place in membranes during pore formation, contraction, and disappearance. There are modeling attempts to explain some aspects of these phenomena (for example, Ref. [6]).

Most importantly, studying transport properties through the pores may improve our understanding of intriguing phenomena involved in anomalous diffusion observed in pores of nanometer size, and also nanopores induced in different membranes by other techniques. Knowledge of sensitivity of such systems to experimental conditions and transported molecules is crucial for nanosensor design (for example, Ref. [7]).

The experiments show a stochastic nature of pore behavior. Stochastic dynamics of the pore could be fully examined only when the current-clamp method was first introduced [8–13]. Unlike voltage-clamp technique, in which the membrane lifespan is very short with a high chance for an irreversible breakdown within milliseconds, the new current-clamp method is the only method in which an electroporated membrane can survive over 1 h (for high currents an average membrane lifetime decreases), and a stochastic behavior of fluctuating electropores can be observed. The reason is a feedback mechanism decreasing the transmembrane potential when pores open excessively, followed by a decrease of their size and conductance. This mechanism extends the average lifespan of membranes.

The conductance analysis of the electroporated membrane in our experiment shows opening of a singular nanopore [8,11] with average diameter ranging from 0.9 nm (for low current and high ionic strength) to 10.56 nm (for high current and low ionic strength). Interestingly, the pore size compares to the membrane thickness (~ 7 nm) and an individual molecule scale. Creating biological structures of nanosize and developing methods for their rigorous analysis contribute to development of techniques for studying single molecules. The quantitative stochastic analysis of the process may throw light on the kinetics of individual molecules, not easily accessible by means of other techniques. The results of such an analysis are even more interesting if we note that the current-clamp method, applied in this experiment, mimics the situation in a real cell with an electroporated membrane. Ion pumps of the electroporated cell tend to restore the membrane potential decreased by the ion leak through the artificial pore. In the current-clamp method ion pumps are mim-

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icked by the galvanostat action. In both natural and model systems potential changes are functions of electrical and mechanical parameters of the membrane.

In this paper, we study the stochastic fluctuations of voltage and calculated conductance by quantitative statistical methods. Until now, full study of the stochastic processes in membranes was performed only for the biological membranes with protein channels [14–17] or synthetic membranes with fabricated nanopores [18]. In the former case the protein behavior obscures the membrane contribution, and in the latter case the system is not biological. Nonetheless, these analyses showed some common features of stochastic characteristics in such nanosystems. Especially, the power-law characteristics of power spectrum density (PSD) function proved ubiquitous, and flicker noise in fluctuations of nanochannel conductivity was often observed. A comparison between these systems and nanopores in pure lipid membranes may throw some light on the molecular phenomena in biological matter.

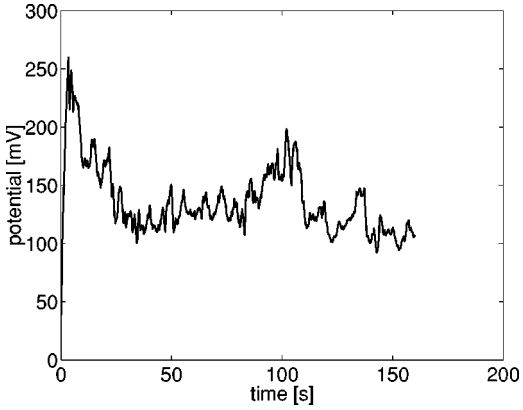

FIG. 1. A chronopotentiometric curve showing dependence of transmembrane potential fluctuations on time, under current-clamp conditions (0.4 nA). An exponential rise of the voltage is followed by rapid decrease of the potential ($2s$), which indicates the beginning of electroporation. The pore is eventually formed after 5 sec and pore fluctuations can be observed.

II. MATERIALS AND METHODS

A. Chemicals

Egg yolk phosphatidylcholine PC was purchased from Fluka (Buchs, Switzerland), *n*-decane from Aldrich (Gillinghem-Dorset). Analytical KCl was obtained from POCh (Gliwice, Poland). Forming solution for bilayer membranes contained lipids (20 mg/ml) dissolved in *n*-decane. The electrolytes ($2M$ KCl and $0.1M$ KCl) were buffered with Hepes (Aldrich, Gillinghem-Dorset) to $p\mathrm{H}=7.0$. Ultrapure water was prepared with a Milli-Q system (Millipore).

B. Measurements

Experiments were performed on planar bilayer membranes formed in an aperture of septum separating two electrolyte solutions. The membranes were formed by the Mueller-Rudin method [19] in a vessel made of one-piece Teflon, which consisted of two chambers, 10 cm^3 volume each. The septum between the chambers was 0.3 mm thick and the aperture diameter was 1 mm. The process of spontaneous membrane formation was monitored by the membrane capacitance recording and by visual observation of transmitted light. The following membranes were used: (a) PC membrane in $2M$ KCl (reference), (b) PC membrane in $0.1M$ KCl (influence of ionic strength tested), and (c) PC and cholesterol membrane at the molar ratio about 1:1 in $0.1M$ KCl (influence of cholesterol). The experiments were performed at temperature 19–22 °C. We were also very careful so as not to introduce any ionophore into the membrane by an accidental contamination.

Chronopotentiometry measurements were performed with four-electrode potentiostatgalvanostat described in the earlier paper [20]. The measuring system is fully controlled by a computer and software working in Windows TM (Microsoft) environment. The system uses two pairs of Ag/AgCl electrodes. One of these pairs applies direct constant intensity current. They are connected to the current supply. Specialized software, designed by one of the authors for the purpose of this measurement method, controls current supply connected to 12 bit digital-to-analog converter. Two other electrodes connected to the amplifier of high input resistance measure the transmembrane voltage, which is converted by the 12 bit analog-to-digital converter. No filtering was introduced at this stage. Input resistance of the amplifier is higher than $10^{12}\Omega$ and input offset current is lower than 0.5 pA.

C. Data acquisition

Data of 16 000 points in each series were taken at the sampling frequency $f_s=100$ Hz, and for a comparison at 10, 500, and 625 Hz. The most interesting frequency range was covered by the experiment at 100 Hz of the sampling frequency. First 500–2500 points of each series were cut off to allow time for a pore formation or the system to reach the equilibrium if the membrane already had a pore. The applied current values ranged from 0.1 nA to 5 nA. Some measurements were conducted at the same current value until irreversible breakdown of a membrane, which provided us with relatively long time series. Other results were taken in several stages for different current values, in increasing and decreasing sequences, for the same membrane. Our objective was a comparative analysis performed on the data from the same membranes, without averaging over stochastic properties of the membrane [21–23].

III. EXPERIMENTAL RESULTS

A typical chronopotentiometric curve is shown in Fig. 1. The curve begins with an exponential rise of the voltage, for which membrane capacitance is responsible. Then, a sudden decrease of the voltage can be observed. This effect, combined with the optical observation of the membrane light transmittance, reveals a pore formation. Estimation of the pore diameter [11] indicates that usually a single nanopore is formed, and short breaks in current supply do not let the pore reseal. The pore diameter ranged from 0.93 nm (0.2 nA) to 4.2 nm (5 nA) for PC membranes in $2M$ KCl; from 3.44 nm

(0.2 nA) to 10.56 nm (1 nA) for PC membranes in 0.1*M* KCl. Adding cholesterol slightly decreased the pore size (10.5 nm in 2 nA). The diameter was calculated with the simplifying assumption of a cylindrical pore shape.

The stage of pore formation is followed by stochastic voltage oscillations. The curve shape depends on the current value. For low current values no electroporation takes place and the voltage rises exponentially to a constant value. It should be noted that results similar to those received for membranes at very low current values (no electroporation) were obtained for passive RC circuit, with electric parameters corresponding to the typical planar bilayer membrane formed in our laboratory, for low and high current values. This result reassured us that the registered voltage fluctuations originate in the electroporated membrane processes.

A preliminary spectral analysis of the process, by means of fast Fourier transform, shows that low frequencies strongly dominate. No specific frequency component indicating a particular membrane process was observed.

IV. STATIONARITY ANALYSIS

Stationarity of a time series, which in general means that statistical properties of a stochastic process are constant in time, is a very desirable feature assuring that standard analytical methods can be applied. For example, the calculation of the power spectrum from the correlation function is correct for stationary processes only (Wiener-Khinchin theorem).

However, nanopore voltage fluctuations are nonstationary. The analysis based on two-point correlation function, which proved time dependent [13], confirmed this hypothesis. Still, the nature of the nonstationarity was not revealed nor a method to remove it was proposed. Therefore, no systematic study of the process could have been performed.

An informative way to test the stationarity is the quantile line method [14], which can detect not only nonstationarity but also indicate its source. For each quantile order a function of time (quantile line) is determined. When only one realization of a process is available, quantile lines can be received by cutting a series into intervals, and the quantile for each interval calculated. For stationary processes quantile lines of various quantile orders are parallel to the time axis. Otherwise, the process is nonstationary. Lines parallel to each other but not to the time axis indicate a process with a constant variance and a variable mean (or a variable median for processes without a finite mean).

The quantile line analysis of the electroporation process showed that lines of different order are not parallel to the time axis nor to each other [Fig. 2(a)]. The process is obviously nonstationary and a changeable variance is very probable.

To remove the nonstationarity classical decomposition method was applied in which a nonstationary process X_t can be decomposed into slowly varying trend m_t, seasonal component s_t (if is exists), and a stationary random noise component Y_t.

$$X_t = m_t + s_t + Y_t. \tag{1}$$

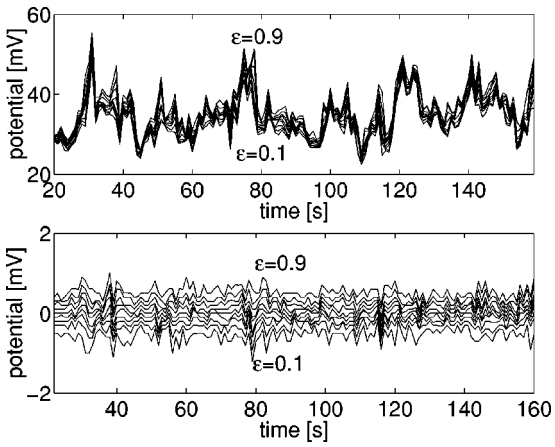

FIG. 2. (a) Quantile lines of the raw series showing nonstationary nature of the process (lines are not parallel to the time axis). Order of the bottom quantile line is $\varepsilon = 0.1$, top line $\varepsilon = 0.9$, with interval between lines $\triangle\varepsilon = 0.1$. (b) Quantile lines for the differenced series. Fluctuations are partly due to the finite length of the series.

The difficulty with the classical decomposition model is to find an effective filter for trend removal. Four filtering methods were tested on raw chronopotentiometric time series in order to extract a stationary component: polynomial estimation by least squares (appropriate for deterministic trends), moving average, exponential filter of infinite impulse response type, and differencing (stochastic trends). Only the differencing transform ∇ defined by

$$\nabla^j X_t = X_t - X_{t-j} \tag{2}$$

provided the expected result, which is consistent with a stochastic nature of the trend [24].

Differencing with step 2 applied for a series measured at $f_s = 100$ Hz removed successfully the trend and a small deterministic artifact of 50 Hz from energetic network. Quantile lines for the differenced process are presented in Fig. 2(b). Fluctuations of the lines are partly due to the finite length of the series and they do not exceed 15% of the differenced series amplitude (90% for the raw series).

Further analysis was provided on the stationary differenced series. Generally, a differenced process is deprived of the low frequency component (stochastic trend) and reflects dynamics of the phenomenon only. However, in case of a self-similar process with long correlations full characteristics of the original process can be acquired from its stationary increments.

V. MEMORY OF THE PROCESS

An informative method to characterize a stochastic process is analyzing the nature of its correlations, which defines the memory. Information on memory is essential for statistical analysis, especially if methods assuming data independence are to be employed. On the other hand, weak dependence between observations is negligible for practical purposes. In many cases quantitative information can be obtained this way. If a correlated process has short memory it

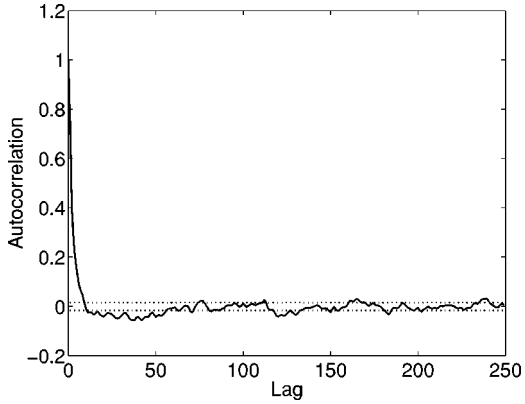

FIG. 3. Autocorrelation function (solid line) for increments at $I=0.4$ nA ($2M$ KCl) and Bartlett's limits (dotted line).

could be modeled by linear ARMA (or ARIMA) model [25].

Time series X_t is an ARMA (p,q) process if X_t is stationary and if for every t it holds

$$X_t-\phi_1X_{t-1}-\cdots-\phi_pX_{t-p}=Z_t+\theta_1Z_{t-1}+\cdots+\theta_pZ_{t-p}, \tag{3}$$

where $\{Z_t\}$ represents white noise with mean 0 and variance σ^2, and the polynomials $(1-\phi_1z-\cdots-\phi_pz^p)$ and $(1+\theta_1z+\cdots+\theta_pz^q)$ have no common factors. ARIMA is a nonstationary process whose increments can be modeled by an ARMA process.

Estimation of the linear model order is possible by evaluation of orders of MA and AR components on the basis of the range of autocorrelation and partial autocorrelation functions, reflecting memory of the process. Autocorrelation and partial autocorrelation range can be derived from Bartlett's formula. The large sample of size n is from an MA(q) process if autocorrelation function $\rho(h)$ estimated by the sample autocorrelation function is negligible for $h>q$, i.e., with probability 0.95 it falls between the bounds $\pm1.96\sqrt{[1+2\rho^2(1)+\cdots+2\rho^2(q)]/n}$. In practice $\pm1.96/\sqrt{n}$ bounds can be used. Similarly, the sample of size n is from an AR(p) process if the partial autocorrelation function estimated by the sample partial autocorrelation function is negligible for $h>q$.

It was observed that for voltage fluctuations neither correlation nor partial autocorrelation function of the differenced fluctuation process falls into the Bartlett's range completely (Fig. 3). It means that electropore voltage fluctuations cannot be modeled by means of a linear ARIMA process of a reasonably low order. For both functions, however, there are lag values above which correlation and partial autocorrelation functions oscillate very closely around bounds of the negligibility range.

The analysis of ARIMA models applied for stochastic voltage fluctuations showed that the process could not be approximated by a short-memory linear model or other models with short correlations such as Markov processes. However, the analysis did not bring a definite answer on the nature of correlations. The question if the fluctuations can be qualified as a long-memory stochastic process should be addressed by means of other methods [14–17,26].

VI. SELF-SIMILAR NATURE OF VOLTAGE FLUCTUATIONS

Power spectrum analysis applied for a stationary stochastic processes is an essential tool for detecting long-memory and self-similar properties of the process. A signal whose PSD takes the power-law form

$$s(f)\sim f^{-\beta}, \quad \beta\neq0, \tag{4}$$

is a stochastic process with long memory. Moreover, if increments of a nonstationary process are stationary with a power-law PSD function then the process can be classified as self-similar (fractal) and shows mathematically interesting scaling properties [27].

The value of exponent β is a quantitative way of estimating the rate of high to low frequency component. A stationary process with PSD function of the form (4) and $\beta<1$ shows weak persistence of its memory. Such a process is classified as fractional Gaussian noise. A nonstationary process obeying Eq. (4) would have $\beta>1$, and due to the nonstationarity it would be more difficult to analyze. However, if the increments of such process are stationary then the original process can be analyzed indirectly, by means of the differenced process, and the most essential properties of the original process would be acquired. Among others, the value of β exponent can be estimated from the relation

$$\beta_{or}=\beta_{inc}+2, \tag{5}$$

where β_{or} denotes power-law exponent of the original process and β_{inc} of increments. The relation (5) does not apply to the highest frequencies, where PSD may be deformed [28]. When $\beta=1$ we observe $1/f$ (flicker) noise; for white noise process with no memory $\beta=0$.

A self-similar process $X(t)$ is statistically indistinguishable from $X(at)$ if its amplitude is rescaled by a certain factor a^H,

$$X(at)\overset{d}{=}a^HX(t), \tag{6}$$

where H is a positive real number called a self-similarity index, $a\geqslant0$, and $\overset{d}{=}$ means equality by distribution.

Self-similarity includes also independence of the frequency scale. If the frequency of a self-similar stochastic process is multiplied by a certain factor, the PSD function diminishes by the same fraction, regardless of the frequency value. The self-similarity index H from Eq. (6) gives a quantitative information on the process. It can be estimated by several methods such as rescaled range analysis invented by Hurst, Orey index method, detrended fluctuation analysis (DFA), or directly from the value of exponent β in PSD function. The optimal method depends on mathematical properties of the process, especially its Gaussianity or non-Gaussianity [29,30].

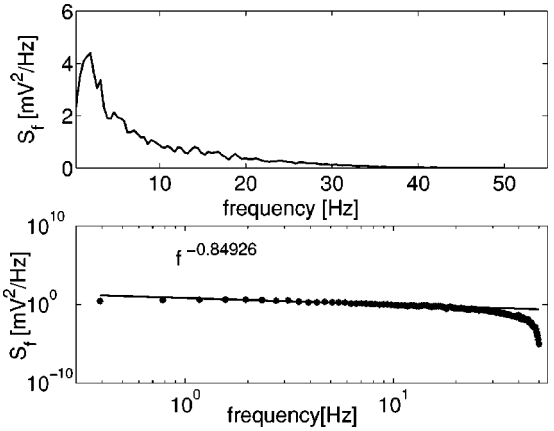

FIG. 4. Amplitude of power spectrum density (PSD) function, for $I=0.4$ nA, in linear and logarithmic scale. A power-law dependence indicates $f^{-\beta}$ noise.

The best known nonstationary self-similar process is fractional Brownian motion, observed if the process of increments is stationary and Gaussian. Another interesting class of self-similar processes is stable motion [30] when increments are non-Gaussian with a long-tailed distribution.

PSD function of the differenced voltage fluctuations in electroporated lipid membranes was estimated by periodogram. The periodogram for frequencies below 50 Hz showed power-law dependence (Fig. 4). A sudden decrease of PSD in 50 Hz is caused by selection of the differencing step to remove artifacts from energetic network. At higher frequencies damping effect of membrane capacitance obscures other physical phenomena. This result indicates that the original nonstationary process can be classified as self-similar with a strong long-memory dependence.

It was tested how β value is affected by the current I. The exponent β proved sensitive, showing a clear and distinct characteristic $\beta(I)$ decreasing as the current increased (Fig. 5). Explanation of this result comes from electrical properties of the lipid membrane with the pore. This system can be represented by membrane capacitance C_m, pore conductance G_p, and negligible membrane conductance G_m, all connected into parallel circuit. Therefore, dynamic properties of the membrane are described by the relation

$$\frac{dU}{dt}=\frac{i_c}{C_m}=\frac{I-i_p}{C}, \tag{7}$$

where i_c denotes the capacitance charging/discharging current, I the total current (applied), and i_p the pore current.

An increase of high frequency component (decrease of β) observed experimentally in voltage fluctuations for bigger currents, and expected from Eq. (7), can be attributed to faster charging and discharging processes. The value of β estimates this change quantitatively. This result contradicts the hypothesis [13,31] that flicker noise with $\beta=1$, observed in voltage fluctuations for a specific current value, is a universal phenomenon. Rather, it seems a special case of the self-similar stochastic process in which membrane charging and discharging processes play a significant role. Analyzing nanopore conductance could be more informative (discussed in the following section).

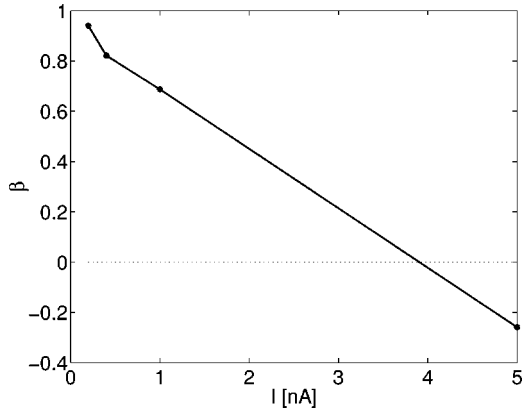

FIG. 5. Dependence of PSD exponent β on the current value for voltage increments.

However, a situation when exponent β does not depend on current value is also possible. According to Eq. (7) it could be observed when pore resistance ($R_p=1/G_p$) is linearly dependent on voltage U. The decrease of $\beta(I)$ in the experiment with $2M$ KCl showed that the pore conductance was not very sensitive to voltage rise. Membranes in lower ionic strength are expected to be more sensitive. This hypothesis was confirmed experimentally on membranes in $0.1M$ KCl (due to the aging only the initial sequences of the series were analyzed). PC membranes with cholesterol showed almost constant $\beta(I)$ for $I>0.4$ nA (Fig. 6), while pure PC membranes in $0.1M$ KCl proved to be the most sensitive to voltage with slightly rising $\beta(I)$ for $I>0.4$ nA. Therefore, the value of β depends also on the sensitivity of the particular membrane to the electric field (voltage).

The question which appears naturally is whether the observed process of voltage fluctuations in electroporated membrane can be classified as fractional Brownian motion. To ensure the positive answer the histogram of increments

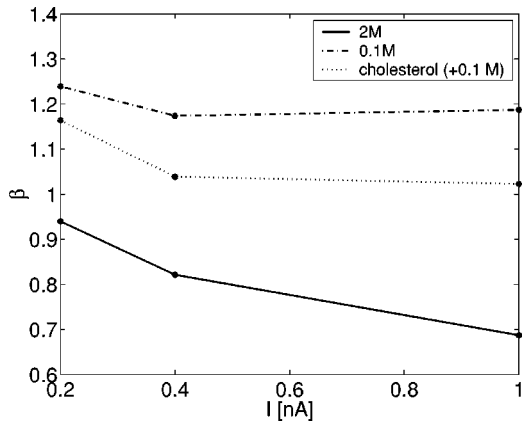

FIG. 6. Dependence of PSD exponent β at the beginning of the process compared for PC membrane in $2M$ KCl (solid line), PC membrane in $0.1M$ KCl (broken line), PC + cholesterol membrane in $0.1M$ KCl (dotted line).

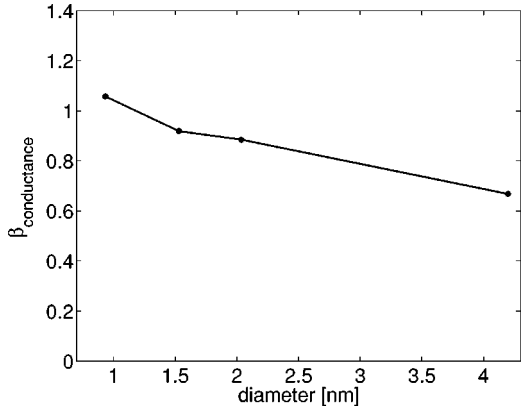

FIG. 7. Pore conductance—dependence of PSD exponent β on the pore diameter ($2M$ KCl) for the pore conductance.

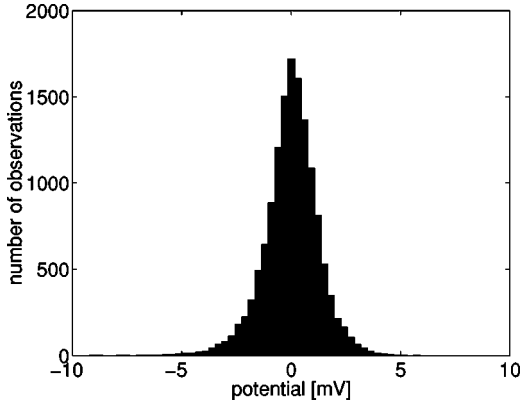

FIG. 8. Estimation of the probability density function of voltage increments by means of the histogram for 14 000 points and $I=0.4$ nA. Outstanding observations form two asymmetrical tails.

(Fig. 7) should represent a Gaussian distribution. However, additional statistical tests [for example, Lilliefors (0.02)] rejected the hypothesis on the Gaussianity (test significance level showed in the brackets). Although the data in long-memory processes are not independent, which may affect the test results, it seems there are too many outstanding values in the series for the Gaussian distribution. The extreme data form left and right tails of the probability distribution function (PDF).

The PDF shape changes with the current value. As the current value rises the right tail builds up (not shown). This phenomenon can be interpreted physically. An increase of the right tail for high currents means that the process of charging the membrane capacitance C_m, by the charging capacitance current i_c, grows faster (positive voltage increments become larger). Note that the charging process occurs when the pore is closing. Voltage dynamics dU/dt described by Eq. (7) rises since the pore current i_p is very small and the total current I increases. Therefore, the rise of the right tail with the current value is natural. The left tail is a consequence of discharging the membrane capacitance when the pore is opening. We observe some elongation of the left tail as the current rises, however not as significant as for the right tail. At low current values the left tail is much better pronounced than the right tail. At high currents the left tail dominance disappears and the PDF becomes more symmetrical.

The statistical and physical analysis shows that we do not observe a Gaussian process. Further investigation is needed to test if the distribution can be qualified as a typical heavy-tailed stable distribution, which may indicate a self-similar stable motion.

VII. FLICKER NOISE IN ELECTROPORE CONDUCTANCE

The spectral analysis was also provided for calculated membrane conductance. The method to calculate the electropore conductance, based on preelectroporation and postelectroporation characteristics of the membrane, was fully presented in Ref. [8]. It gives an approximation of electropore conductance where the actual nanopore behavior is not obscured by charging/discharging process of the membrane capacitance. Although dynamical properties of the system may be partly altered or averaged by the calculation method, so the first part of our analysis was conducted on voltage fluctuations (a typical approach for current-clamp experiments [13]), the conductance analysis is necessary to compare our results with the behavior of different nanochannels for which a voltage-clamp experiment was possible. The result of such an analysis reveals the real pore dynamics.

The conductance time series showed much better stationarity than voltage fluctuations. The stationarity decreased for higher currents so the analysis was performed for the raw series and the series differenced with step 2 (similarly as for the voltage fluctuations). Cutting series into shorter sequences was also applied to avoid aging effect. The results were compared, Eq. (5), and the exponent β of the conductance series calculated.

The PSD analysis on calculated conductance series from experiments in $2M$ KCl showed a power-law characteristics of the process with the exponent very close to $\beta=1$ (flicker noise). For high currents some decrease of β was observed (Fig. 8). This result indicates that the increase of high frequency component observed in voltage fluctuations is not only due to the membrane capacitance but also due to the pore dynamics.

A spectral response close to $1/f$ noise can be observed in many complex systems. Although the phenomenon is well known for macrosystems, recently it has been also found in synthetic membrane with fluctuating nanopore [18] and biological protein channels [33]. Flicker noise seems natural in systems which are distributed and loosely coupled. However, there is no generally accepted rigorous theory explaining the mechanism of this phenomenon. With regard to conducting nanochannels, a hypothesis that flicker noise could result from random transitions between various conductivity states of the channel is considered [18,33]. However, a question arises as to the extent to which the noise characteristic depend on transport properties of the channel or complexity of its structure.

Experiments on nanopores in synthetic membranes showed that complexity of highly regulated biological chan-

nels is not a necessary condition for flicker noise to appear. Nevertheless, there has been no information on the behavior of corresponding biological system with fluid lipid membrane whose dynamical properties are different. This work shows that the simplest biological system needed for flicker noise to appear is a pore of the size not exceeding 1 nm in its diameter. For larger pores we observed a decreasing β value and a departure from flicker noise. It should be noted, however, that larger pores are induced by bigger current. Therefore, an intriguing question appears if the result $\beta<1$ is due to the bigger pores or stronger electric field.

Somehow different results were obtained from experiments in lower ionic strength. Spectral analysis carried out on calculated conductance fluctuations at $2M$ KCl shows an increase of high:low frequency rate as the current rises (decrease of β value). No such trend was observed for the membranes in $0.1M$ KCl. What is more important is that the values of β were randomly scattered around $\beta=1$, with a large deviation approaching $\Delta\beta=0.4$. Therefore, the occurrence of $1/f$ noise in $0.1M$ KCl seems ambiguous. In lower ionic strength membrane molecules are more loosely coupled and interactions are supposed to be weaker, showing a higher ionic mobility. This hypothesis was tested by computer modeling [34] and supported by our experiment, in which nanopores in lower ionic concentration displayed a greater variation of their diameter ($\Delta d=1.84$ nm, $0.1M$ KCl) than in higher ionic strength ($\Delta d=0.707$ nm, $2M$ KCl). The question appears if these can be the key factors affecting the occurrence of $1/f$ noise and what kind of interactions between molecules of the membrane are necessary for the flicker noise to occur.

Another interesting observation was recorded when a pore with very slow dynamics formed. The exponent β, in this case, assumed the value $\beta=1.9$, so the noise close to f^{-2} was observed. One possible explanation is that the pore may have not been able to close completely during its minor fluctuations. The hypothesis that f^{-2} noise occurs if a nanoscale conducting pathway stays permanently open during fluctuations (in contrast to pores showing $1/f$ noise) was presented in Ref. [18] and justified by experiments on synthetic nanopores fabricated in polyimide foil. This type of noise indicates a regular exponential relaxation with no memory. A further investigation into the electroporated lipid membranes with f^{-2} is needed to see what phenomena underlie the observed result in our experiment and what is the actual behavior of the pores in this case.

VIII. SELF-SIMILARITY INDEX OF VOLTAGE FLUCTUATIONS

The self-similarity index H is important for two reasons. It gives quantitative information on the scaling properties of the process. At the same time it defines the exact characteristics of the system memory. For uncorrelated increments $H=0.5$, positive correlations in the process with long memory (persistent process) are indicated by $H>0.5$, and negative by $H<0.5$

The most straightforward method to estimate the index H is based on β exponent:

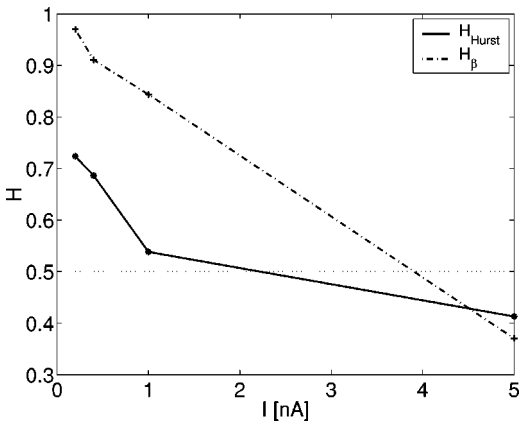

FIG. 9. Dependence of the self-similarity index on the current value. Comparison between index estimation by Hurst method (solid line) with the method based on PSD exponent (dash-dot line), ($I=0.4$ nA, $2M$ KCl).

$$H=\frac{1+\beta}{2}. \tag{8}$$

The most popular rigorous method to estimate the index is the rescaled range analysis (R/S) invented by Hurst [32]. Among other methods there is DFA. Both estimators of the self-similarity index H_{Hurst} and H_{DFA} can be applied for a wide class of stochastic processes. They yield correct results also for a non-Gaussian process. Although their idea is based on a variance or standard deviation of the process or its increments, both can be also applied for processes with heavy tails, which do not have a finite variance.

As discussed previously, autocorrelation function, calculated for the increments of voltage fluctuations in electroporated membrane, is oscillating around the bounds of negligibility up to a very large lag. Therefore, we could not model the process with any linear ARIMA process. On the other hand, the departure from the Bartlett's limits seemed rather minor so the behavior of the autocorrelation function did not bring a definite answer to how strong and significant are the long-range correlations. Also, this analysis did not allow us to compare the memory properties for different experimental conditions quantitatively. Estimation of the self-similarity index may give us a definite answer on the correlation nature.

The Hurst analysis performed on the increments of voltage fluctuations showed decreasing characteristics of the self-similarity index H versus current (Fig. 9, solid line).

For low currents ($I>2$ nA) Hurst exponent assumes the value from the range $0.5<H<0.75$. The result $H>0.5$ means a persistent process with long-memory. Since the long memory property is confirmed for a differenced process, it can be stated that the original process of voltage fluctuations has a strong long-memory characteristic. A closer look at the sequence of incremental values confirms that clusters of data with the same sign can be observed.

For currents $I>2$ nA the Hurst exponent assumes values $H<0.5$, decreasing to $H=0.3$. It can be concluded that the process becomes antipersistent for higher currents and the

characteristics of increments is rough. It means shortening the memory of the original process.

Another interesting feature was observed for Hurst analysis at high currents. The R/S characteristic is more likely to show two separate frequency regions with two different exponents H for low and high frequencies. Interpretation of such a characteristic is still not clear, it may suggest that different phenomena predominate at higher frequencies. In such a case the Hurst exponent is calculated as the limit value for large lags [27].

The DFA analysis estimated the self-similarity index very close to the Hurst exponent (not shown). The differences were usually about 1% (an outstanding result reached 15%). On the other hand, the estimation by R/S and DFA did not compare equally well with the self-similarity index calculated from PSD exponent (8) (Fig. 9, dash-dot line). It should be noted, however, that all three methods are not very exact, all of them rely heavily on the linear regression analysis and the choice of points selected to calculate the exponent. Also, the Hurst exponent should be calculated as a limit value for sufficiently large lags and it is very problematic to decide which lag should be the starting point.

IX. STOCHASTIC CHARACTERISTICS SHOW AGING EFFECTS IN LOWER IONIC STRENGTH

Three sets of experimental conditions were arranged. PC membranes formed in $2M$ KCl, which are resistant to strong electrical field, were compared with membranes in more physiological $0.1M$ concentration of KCl electrolyte. These membranes, which do not sustain strong electric fields easily (no experiment at $I>1$ nA was possible), were in turn compared with much stronger lipid membranes with cholesterol added ($0.1M$ KCl) [12]. The biggest electropores formed in PC membranes in $0.1M$ KCl. Adding cholesterol changes the membrane structure and slightly decreases electropore radius. Different properties of nanopores result from a diverse molecular arrangement of the membranes. The distance between lecitine molecules is decreased in high ionic strength due to the stronger attraction between charged phosphate and choline groups. This accounts for a smaller pore diameter whose creation proceeds at higher energetical expense. Cholesterol, in turn, increases stability of the membrane suppressing molecular mobility. Cholesterol molecules build into the curvature of the pore stabilizing it.

We tested how PSD and self-similarity index of the process change in time. The values were estimated for a sequence of several series (15 000 points each) from the same membrane. The analysis for a lipid membrane in $2M$ KCl did not show any significant change of the value (an exemplary membrane shown in Fig. 10, solid line). It can be concluded that all stochastic properties are constant in time and the processes that change the membrane configuration are not strong enough to affect the mathematical characteristics of the process. Therefore, in this case, the nonstationarity of the original process is a consequence of the cumulative, self-similar nature of the process only.

Different results were obtained for membranes in $0.1M$ KCl. The value of exponent β calculated for a sequence of time series from the same membrane showed decreasing time characteristics. The aging membrane has a stronger high frequency component. Insignificant increase of electropore conductance and subsequent decrease of the charging current I_c should result in insignificantly lower rate of high frequency component. The reverse result shows that some other phenomena take place and high frequency rate becomes more pronounced as the electroporated membrane ages. In contrast to membranes in high ionic strength, physical properties of the membranes in $0.1M$ KCl change significantly. The effect is more pronounced at high current values.

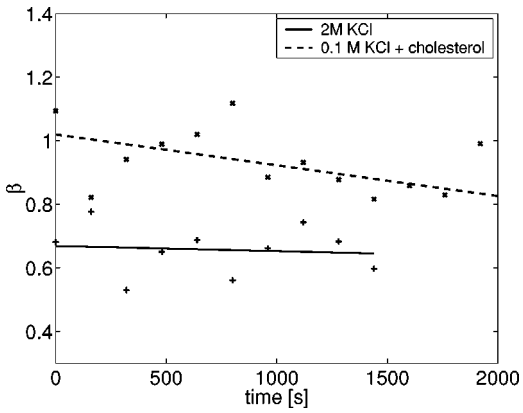

FIG. 10. A negligible trend in PSD exponent β in high ionic strength ($I=1$ nA, $2M$ KCl, solid line and crosses). The membrane does not change its stochastic characteristics over time, even for higher currents. It contrasts with a significant change of PSD exponent β in lower ionic strength ($I=1$ nA, cholesterol $+0.1M$ KCl, dotted line and stars). Calculations on voltage increments.

The stationarity of membranes with cholesterol slightly improves, however not very significantly. Although the range of conductance changes is almost constant in time (the rise is much smaller than for pure membranes in $0.1M$ KCl) the high frequency component, expressed by the change of β exponent value in PSD function, increases in time (Fig. 10, dotted line). It is also more pronounced for higher currents. This increase is slightly less significant than for PC membranes in $0.1M$ KCl.

Thus, higher frequency molecular phenomena appear in membranes formed in lower ionic strength as the membranes age. The aging seems somehow related to the pore radius. The result indicates that time characteristics of the membranes formed in electrolytes of low ionic strength is not constant in time. It is a very undesirable feature with regard to the potential use of such membranes in sensor design.

X. DISCUSSION AND CONCLUSIONS

In this paper we presented the stochastic analysis of nanopore generated and sustained by electric field in the current-clamp experiment on planar lipid bilayer membranes. The experiment showed stochastic fluctuations of the measured voltage values, resulting partly from charging/discharging processes on membrane capacitance but also from random transitions between different conductivity states of the pore.

First part of the analysis was devoted to the process of

voltage fluctuations. It threw some light on the origin of the observed nonstationarity, which turned out to result mainly from regular properties of self-similar stochastic process. Therefore, the nonstationarity can be easily removed, allowing for standard analytical methods designed for stationary series. Moreover, the analysis on the series, processed accordingly, provides full information on the original nonstationary process. It also turned out that the aging process contributes to the nonstationarity only if molecular interactions in the membrane are very weak (low ionic strength); this kind of nonstationarity is very difficult to remove due to its irregularity. Improving the mechanical stability of the membrane by adding cholesterol does not reduce the aging process significantly.

The analysis demonstrated that the process cannot be approximated by a linear ARIMA or other process with short memory, and long correlations were hinted. Spectral analysis of the correlation nature, by means of PSD function, revealed that voltage fluctuations can be classified as a self-similar stochastic process with long correlations. The process of increments can be modeled by means of fractional Gaussian noise. However, the non-Gaussian characteristics with long tails do not confirm that the original process is the fractional Brownian motion and indicate that a stable motion may be possible. The self-similarity index, estimated by three different methods, allows for a quantitative analysis of persistent or antipersistent nature of the self-similar process, and is related to underlying physical phenomena.

The decrease of exponent β and self-similarity index H observed in $1/f^{\beta}$ noise was predicted analytically and confirmed experimentally. It contradicts the hypothesis formulated by some authors that flicker noise in voltage fluctuations is the universal phenomenon, regardless of the current value. Rather, it is a special case of the long-memory process in which the value of β is current dependent. A situation when β is constant can be observed only if pore resistance is linearly dependent on the voltage (observed for PC membranes with cholesterol in 0.1M KCl). Therefore, the membrane sensitivity to the electric field is a key factor, too.

However, the more informative analysis on estimated conductance fluctuations where the actual nanopore behavior was not obscured by charging and discharging processes on the membrane capacitance, showed $1/f^{\beta}$ noise where β is close to 1 (flicker noise). This result was obtained only for nanopores whose diameter did not exceed 1 nm, induced in lipid membranes with strong molecular interactions (high ionic strength of the electrolyte). Bigger pores produced a noise with lower value of the exponent. A question appears if $\beta<1$ is due to the bigger size of the pore or stronger electric field, which induces the bigger pore.

Comparable results from biological channels, nanopores in synthetic membranes, and nanopores in lipid membranes may suggest that it is a pore size and fluctuating dynamics rather than transport properties or nanochannel structure that underlies a stochastic response close to $1/f$ noise. Nevertheless, our study shows that the membrane structure cannot be neglected, as seen from our experiments on lipid membranes with weak molecular interactions (low ionic strength), where occurrence of flicker noise was ambiguous. It seems that a certain level of intermolecular interactions, contributing to the membrane stability, is necessary for flicker noise to occur. Another observation, concerning a pore with impeded dynamics, showed that a noise close to f^{-2} may appear in such a situation. This fact agrees with results obtained for nanopores in plastic membranes, where a nanopore was not able to close completely, and had no memory.

The observation of flicker noise in the classical model lipid membrane shows that the simplest almost-biological system capable of producing flicker noise is a nanopore of small enough diameter induced in lipid membrane with strong molecular interactions.

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[1] J.C. Weaver and Y.A. Chizmadzhev, Bioelectrochem. Bioenerg. **41**, 135 (1996).
[2] A. Chang and C. Donald, *Guide to Electroporation and Electrofusion* (Academic Press, New York, 1992).
[3] F. Aguel *et al.*, J. Cardiovasc. Electrophysiol. **10**, 701 (1999).
[4] A. Al-Khadra, V. Nikolski, and I.R. Efimov, Circ. Res. **87**, 797 (2000).
[5] P.J. Canatella, J.F. Karr, J.A. Petros, and M.R. Prausnitz, Biophys. J. **80**, 755 (2001).
[6] J.C. Neu and W. Krassowska, Phys. Rev. E **67**, 021915 (2002).
[7] R. Metzler and J. Klafter, Biophys. J. **85**, 2776 (2003).
[8] S. Kalinowski, G. Ibron, K. Bryl, and Z. Figaszewski, Biochim. Biophys. Acta **1369**, 204 (1998).
[9] S. Koronkiewicz and K. Bryl, Cell. Mol. Biol. Lett. **4**, 567 (1999).
[10] S. Koronkiewicz, S. Kalinowski, and K. Bryl, BBA **1510**, 300 (2001).
[11] S. Koronkiewicz, S. Kalinowski, and K. Bryl, BBA **1561**, 223 (2002).
[12] M. Kotulska, S. Koronkiewicz, and S. Kalinowski, Acta Phys. Pol. B **33**, 1115 (2002).
[13] A. Ridi, E. Scalas, and A. Gliozzi, Eur. Phys. J. E **2**, 161 (2000).
[14] S. Mercik and K. Weron, Phys. Rev. E **63**, 051910 (2001).
[15] Z. Siwy, S. Mercik, K. Weron, and M. Ausloos, Physica A **297**, 79 (2001).
[16] Z. Siwy, M. Ausloos, and K. Ivanova, Phys. Rev. E **65**, 031907 (2002).
[17] A. Fulinski, Z. Grzywna, I. Mellor, Z. Siwy, and P.N.R. Usherwood, Phys. Rev. E **58**, 919 (1998).
[18] Z. Siwy and A. Fulinski, Phys. Rev. Lett. **89**, 158101 (2002).
[19] P. Mueller, D.O. Rudin, H.T. Tien, and W.C. Wescott, J. Phys. Chem. **67**, 534 (1963).
[20] S. Kalinowski and Z. Figaszewski, Meas. Sci. Technol. **6**, 1050 (1995).

[21] M. Kotulska and A. Jurlewicz, Acta Phys. Pol. B **31**, 1085 (2000).
[22] M. Kotulska, Cell. Mol. Biol. Lett. **4**, 105 (1999).
[23] M. Kotulska and K. Weron, Proc. SPIE **37DP**, 56 (1998).
[24] P. J. Brockwell and R. A. Davis, *Introduction to Time Series and Forecasting* (Springer, Berlin, 1987).
[25] G. E. P. Box and G. M. Jenkins, *Time Series Analysis: Forecasting and Control* (Holden-Day, 1970).
[26] A. Wolf, Z. Siwy, Y. E Korchev, N. Reber, and R. Spohr, Cell. Mol. Biol. Lett. **4**, 553 (1999).
[27] J. Beran, *Statistics for Long-Memory Processes* (Chapman and Hall, 1994).
[28] A. Eke, P. Herman, J. Bassingthwaighte, G. Raymond, D. Percival, and M. Cannon, Eur. J. Physiol. **439**, 403 (2002).
[29] S. Mercik, K. Weron, K. Burnecki, and A. Weron, Acta Phys. Pol. B **34**, 3773 (2003).
[30] K. Burnecki, J. Rosiński, and A. Weron, in *Stochastic Processes and Related Topics*, edited by I. Karatzas, B. Rajput, and M. Taqqu (Birkhäuser, Boston, 1998), pp. 1–14.
[31] S. De, R. Basu, and P. Nandy, Phys. Rev. B **61**, 6689 (2000).
[32] H.E. Hurst, Trans. Am. Soc. Civ. Eng. **116**, 770 (1951).
[33] S.M. Bezrukov and M. Winterhalter, Phys. Rev. Lett. **85**, 202 (2000).
[34] R.A. Böckman and A. Hac, T. Heimburg, and H. Grubmüller, Biophys. J. **85**, 1 (2003).