Weak Nonlinearity of Current-Voltage Characteristics of Gramicidin D Channels. Experiment, Theory and Application to the Study of Transmembrane Transmission of Information

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Abstract. A new, sensitive method of measurement of current-voltage characteristics (CVC) of the type $i(U) \sim U(1 + \beta U^2)$ was applied to study ionic channels formed by gramicidin D in bilayer lipid membranes (BLM). The values of the nonlinearity coefficient β of the CVC of membranes modified by gramicidin D depended on electrolyte concentration, C, and expressed the weak nonlinearity of the CVC. In symmetric electrolyte conditions, the coefficient β_s increased with the increasing electrolyte concentration from -17 V^{-2} at 0.03 mol/l KCl to 8 V^{-2} at 3 mol/l KCl In asymmetric electrolyte conditions (different electrolyte concentrations at the two sides of the BLM) the coefficient β_A also increased with increasing C. A theory is proposed describing the weak nonlinearity of CVC of ionic channels based on electrodiffusion model. This theory assumes that the electrochemical potential $\mu(x)$ of an ion within the ion channel depends on the coordinate x along the channel axis. According to the proposed model, CVC nonlinearity is mainly determined by the ends of the ion channel. We showed that the structure of gramicidin D ionic channel is sensitive to the concentration of ions, and in fact changes at one end of the channel influence the parameters of the other channel end.

Key words: Ionic channels — Bilayer lipid membranes — Electrodiffusion model — Electrochemical potential — Macromolecular conformation changes

Introduction

The mechanism of transmission of information from the environment into the cell is

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an important problem of biophysics A particular case is transmission of information from a hormone receptor to the adenylate-cyclase system localized inside the cell It has been shown that such transmission can be realized by means of conformational reorganizations of macromolecular complexes of integral protein (Murray et al 1989) The same phenomenon could also take place for simpler integral proteins action at one terminus of the macromolecule could induce changes at the opposite terminus This phenomenon can be studied on ion channels which represent integral proteins Synthetic antibiotics may be used for this purpose as a simple model The pentadecapeptide gramicidin A and its analogs are widely used as functional units for modeling membrane ion transport (see Andersen 1983), and can promote the understanding of the mechanism of ionic transport in biomembranes (Zaciu et al 1996, Negulyaev et al 1997) According to Urry (1971), gramicidin channels are formed by a "head-to-head" dimer (formyl-NH- to formyl-NH-terminal) The secondary structure of these dimers is represented by a $\pi^6_{(LD)}$ helix with an inner channel of a diameter $\simeq 0\ 4$ nm Cifu et al (1992) showed experimentally that the dimer represents the basic conductance unit of gramicidin channels Gramicidin channel has been shown experimentally and theoretically to induce deformation of the surrounding lipid bilayer, depending on the chain length and the boundary condition (see Huang 1986, Helfrich and Jakobsson 1990, Ring 1996)

Changes of the macromolecular complex at one terminus may be transmitted to the opposite end, and influence ion transport Such changes can be studied by analyzing the properties of ionic transport Passive transport of charged particles across membranes is described by either continuous or discrete model The continuous model is based on solution to the Nernst-Planck equation using the constant field approximation The condition of the electrical field strength across the membrane being constant is frequently used (Levitt 1982, Volkenstein 1988, Chen and Eisenberg 1993) The discrete models assume that ions jump over energy barriers at the terminal and middle parts of the channel while crossing the membrane (Lauger 1973, Aiytan et al 1977, Sandblom et al 1977, Urban et al 1978, Tredgold 1979) Depending on ion concentration in the electrolyte surrounding the membrane, the ion transport can be determined by the terminal potential barriers of the channel (at relatively low ionic concentrations) and/or by the middle barrier (at relatively high ionic concentrations) (Lauger 1973)

These models need verification by comparing their predictions with the most important parameter of the ion channel, namely the shape of the current-voltage characteristics (CVC) In particular, it is important to know how the CVC of an ion channel (IC) depends on ion concentration as well as on the gradient of this concentration across the membrane

Experimental determination of the CVC of the gramicidin channel showed that CVC nonlinearity is very small (Haydon and Hladky 1972, Andersen 1983) (Nonlinearity of current-voltage characteristics can be expressed by $\imath = gU(1 +$

$f(U)$), where g is conductance and U is voltage In Ohm law, $f(U) = 0$ and CVC is linear, i e the transmembrane current depends almost linearly on the applied voltage) Therefore, the traditional method of CVC determination – by measuring transmembrane current versus applied voltage – is rather difficult for a quantitative analysis of CVC nonlinearity We developed a convenient and sensitive method for CVC determination based on measuring of the amplitude of the third harmonic of an *ac* current across the BLM arising as a result of the nonlinear current-voltage characteristic (Flerov et al 1981, Passechnik et al 1985, Hianik et al 1994) The measured parameter, nonlinearity coefficient β, was determined for identical ion concentrations, C, at both sides of the membrane (symmetric conditions) The value of β is concentration dependent However, there is no thermodynamic theory of ion transport through channels, even for symmetric ionic conditions

To check the possibility of transmission of structural changes of integral proteins from one membrane side to the other, it is important to study the behaviour of ionic channels in asymmetric ionic conditions In this case, any change at one terminus of the macromolecule can affect the ion transport at the opposite terminus, and the phenomenon can be studied by measuring CVC characteristics

The aims of the present work include (1) determination of current-voltage characteristics of ion channels formed by gramicidin D in bilayer lipid membranes in symmetric and asymmetric conditions, (2) development of a theory to explain the weak CVC nonlinearity of the channels, (3) examination of the possibility of transmolecular transmission induced by changes in electrolyte concentration at one side of the bilayer and its influence on ion movement at the opposite terminus of the channel

Materials and Methods

Method and instrumentation

Current-voltage characteristics were determined using the method described in detail by Passechnik et al (1985) The method employed for the direct determination of the current-voltage characteristics of modified membranes is based on the recording of the third current harmonic generated in BLM having a nonlinear current voltage relationship

$$i = gU(1 + \beta U^2) \tag{1}$$

where g is conductance and U is voltage The coefficient of nonlinearity, β, is given by

$$\beta = -4A_3(1 + rg)^3/U_0^2\, A_1 \tag{2}$$

where A_1, A_3 are the amplitudes of the first (fundamental) and third current harmonics, respectively, r is the combined resistance of the electrodes and the elec-

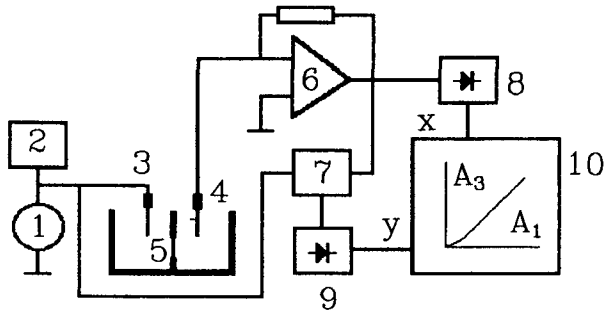

Figure 1. A block diagram of the apparatus for the measurement of the nonlinearity coefficient β For explanation, see the text

trolyte, $g = A_1/(U_0 - rA_1)$, and U_0 is the amplitude of the alternating voltage applied to the electrodes – electrolyte – membrane system The determination of coefficient β is based on the measurement of relative changes of conductance, and it therefore does not depend on the concentration of gramicidin D dimers The sign of β was determined by measuring the phase shift, φ, of A_3 relative to A_1 Values of A_1, A_3, and φ can be determined using common electronic components, resonance amplifiers, and a phase meter (see Passechnik et al 1985) The block diagram of the instrumentation needed to measure nonlinearity coefficient β is shown in Fig 1 The alternating voltage (frequency $f = \omega/2\pi$, measured using a frequency meter (2)) was supplied from generator (1) with a low coefficient of harmonic distortion through electrodes (3) and (4) to the membrane (5) The total current through the membrane was amplified by amplifier (6), rectified by detector (8), and also fed to the resonance amplifier (7) (tuned to frequency 3ω) and, after rectification by detector (9) applied to input Y of the plotter (10), which recorded A_3 as a function of A_1 The built-in oscillographic indicator of resonance amplifier (7) was synchronized by the voltage from the generator (1) Based on Lissageous figures the phase of A_3 could be determined with respect to the applied voltage The noise of the device corresponded to $|\beta| < 0\,5\ \mathrm{V}^{-2}$, using other resonance amplifiers it is, however, possible to improve the parameters of the device quite easily (no measurement of smaller β values was required in our experiments) (see Passechnik et al 1985 and Hianik and Passechnik 1995 for more detail) In our experiments, alternating voltage with amplitude $U_0 = 100$ mV and a low frequency $f = 40$ Hz (to eliminate capacitance current) was employed This frequency is sufficient to measure CVC in order to avoid influence of voltage on the process of the formation of gramicidin channels This was checked earlier by two methods (see Passechnik et al 1985 for more detail) a) We applied to gramicidin modified BLM a sawtooth wave with different rates of voltage change We have shown that at rates higher

than 750 mV/s, the shape of the dependence of $i(U)$ remains unchanged Thus, if voltage changes occur with characteristic times shorter than 200 ms, the opening of ionic channels does not affect the membrane CVC, which is then effectively determined by the CVC shape of the ionic channels only Therefore, to measure the CVC nonlinearity coefficient frequencies higher than 5 10 Hz should be used ($2\pi/\omega \ll 200$ ms, where $\omega = 2\pi f$ is the circular frequency) Consequently, the frequency of 40 Hz used in our experiments is sufficiently high to avoid the influence of the dimerization process of the ionic channels b) Also, we measured the value of β of gramicidin modified BLM using the third harmonic method at different frequencies of applied sinusoidal voltage The same β values were obtained in the frequency range of 20–1000 Hz, i e , the results obtained by our method under the conditions used were not affected by the formation of new ionic channels

The coefficient β was determined for various KCl electrolyte concentrations ranging between 0 01–3 mol/l For the symmetric conditions the measurements of CVC were conducted on BLM prepared according to Mueller et al (1962) on a circular hole, approx 0 5 mm in diameter, in the wall of a teflon cup The volume of the inner and the outer chamber was 3 ml each The electrolyte concentration was changed in a stepwise way, for selected electrolyte concentrations Average values and standard errors of β obtained from 10 consecutively formed membranes were used to represent the dependence β ([KCl]) For measurements in asymmetric conditions the concentration was changed continuously by perfusion on one side of the membrane, while it was kept constant for the other side In this case the membranes were formed on a circular hole (diameter 0 5 mm) in the wall of a PVC syringe, the syringe being equipped with a float that kept the depth of immersion of the syringe in a cuvette filled with 1 mol/l KCl constant (Kruglyakov and Rovin 1978) This layout prevented changes to occur in hydrostatic pressure that could have caused the BLM to bulge The electrolyte concentration in the cup was changed by slow addition of distilled water through a hole in the upper part of the cup The same volume of electrolyte flowed out through another hole situated at the same level as the first one During the dilution the electrolyte was stirred with a magnetic stirrer The electrolyte concentration on the opposite side of the BLM was kept constant at 1 mol/l KCl The electrolyte concentration was measured with a K^+- selective electrode (Crytur) with an accuracy of approximately 10^{-4} mol/l

Chemicals

Membranes were prepared from egg phosphatidylcholine (egg PC) (Plant of Chemical Preparations, Kharkov, Ukraine), egg PC + cholesterol (33 mol%) (Fluka Buchs, Switzerland) and 1 glycerolmonooleate (GMO) (P-L Biochemical, USA) in n heptane (Kodak, USA) (20 mg/ml) KCl in distilled water was used as the electrolyte (pH 5–5 5) Commercially available gramicidin D (GRD) (P L Biochemical USA) (a mixture of gramicidin A, B and C at approximate ratios 8 1 2) was dis-

solved in ethanol and added to the electrolyte on both sides of the membrane to a concentration of 10^{-7} mol/l The ethanol concentration in the electrolyte did not exceed 0 7%

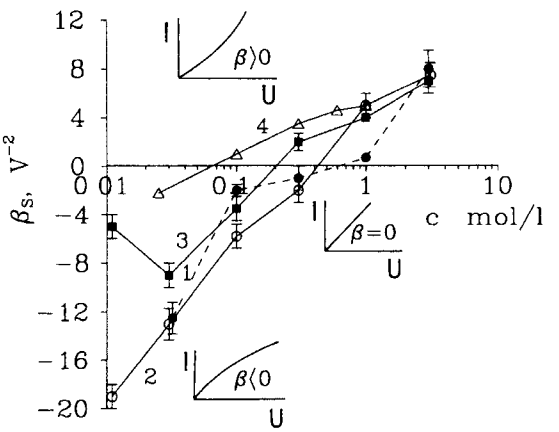

Figure 2. The dependence of the nonlinearity coefficient, β, on the BLM composition and electrolyte concentration, c, in symmetric (β_S) (curves 1 3) and asymmetric (β_A) (curve 4) ionic conditions 1 GMO, 2 – egg PC (0), 3, 4 egg PC + 33 mol% cholesterol (3 symmetric 4 asymmetric ionic conditions) The insets present the shape of CVC for $\beta < 0$ $\beta = 0$, and $\beta > 0$ Each point on curves 1–3 represents mean ± S E for 10–12 membranes Electrolyte KCl, $U_0 = 100$ mV, frequency $f = 40$ Hz

Results

Fig 2 shows the dependence of coefficient β_S on electrolyte concentration under symmetric condition (identical KCl concentrations at both sides of BLM) for BLM of different lipid composition It is seen that β_S increased in parallel with the electrolyte concentration C For curves 1-3, we have $\beta_S \sim \log(C)$ At low electrolyte concentrations the CVC was sublinear (current increased more slowly than voltage, $\beta_S < 0$) At concentrations $C_R = 0\,02 - 1$ mol/l (depending on BLM lipid composition) the CVC was linear ($\beta_S \cong 0$) and the sign of β_S reversed For higher electrolyte concentrations CVC was superlinear, i e the current increased faster than voltage ($\beta_S > 0$) (see insets, Fig 2) It is obvious from the Figure that, depending on the lipid composition of the BLM, the $\beta_S(C)$ curves (1–3) have different slopes and different values of C_R

For measurements of the value of β_A under asymmetrical condition the electrolyte concentration on one side of the membrane was kept constant (1 mol/l) while it was changed on the other side in the interval from 1 to 0 02 mol/l within

5 min The corresponding plot of β_A for egg PC + 33 mol% cholesterol is shown in Fig 2 (curve 4) It is obvious that the values of β_A are intermediate to those for symmetric electrolyte conditions

Thus, the values of nonlinearity coefficients β_A and β_S depend on electrolyte concentration and change in the range from -19 V^{-2} at $C = 0\,02$ mol/l to 8 V^{-2} at $C = 3$ mol/l KCl This implies that at $U_0 = 0\,1$ V the deviation of CVC shape from linearity does not exceed 20%, i e nonlinearity of CVC is weak To analyse the reasons underlying the weak nonlinearity it is necessary to make a theoretical analysis of the mechanism of ion transport through the channel

The mechanism of the weak CVC nonlinearity

The analysis of the well-known models of ionic transport across IC reveals that none of them can explain the dependence of β_S on the logarithm of concentration in symmetric ionic conditions (Passechnik et al 1985) In all cases the following contradictions are present if a substantial nonlinearity of CVC exists at low values of U, then CVC saturates at high voltages, if nonlinearity exists at high voltages ($U \sim 300$ mV), then at low voltages one cannot obtain large values of β_S ($\sim$ 20 V^{-2}) This is not in harmony with our experimental results presented above Therefore, a mechanism has to be proposed for the weak CVC nonlinearity In addition, the CVC shape obtained under both symmetric and asymmetric ionic conditions has to be explained

The model

As a basis for our theoretical analysis we took the well known continuous electrodiffusion model of ion transport (Markin and Chizmadzhev 1974, Aivtan et al 1977) which has been widely used (see Chen and Eisenberg 1993, equation (6))

We modified the model (Markin and Chizmadzhev 1974, Aiytan et al 1977) by excluding the discrete stage of sorption-desorption of ions at the entrance and at the exit part of the IC We assume that all processes of the ion transport, beginning from the entrance of the ions into the IC up to their exit from the IC can be described using the electrochemical potential $\mu(x)$, which is a function of the coordinate x along the IC ($0 \le x \le l$)

In the case of one penetrating ion, Theorell's equation gives the current density j as $j = -z(FD/\mathrm{R}T)c(x)\ \frac{\mathrm{d}}{\mathrm{d}x}(M(x) + zF\phi(x))$ where $M(x)$ denotes the standard chemical potential (SCP) of the ions, $\phi(x)$ is the electric potential inside the membrane, z is the valence of the penetrating ions (charge in proton units), F is the Faraday constant, $c(x)$ denotes the local ion concentration, D is the diffusion coefficient of the ion, R is the gas constant, and T is absolute temperature In the framework of the electrodiffusion approach several different factors could affect the nonlinearity coefficient β, such as a variation of either the diffusion coefficient or the electric field strength along the channel (e g Chen and Eisenberg 1993) For

simplicity, we shall consider D = const and will use the constant field approximation, i.e. electric field strength $E = -\mathrm{d}\phi(x)/\mathrm{d}x$ = const inside the membrane. Under these assumptions the transmembrane current density is given by

$$j = \frac{DzF[C_0 - C_l \exp(zFU/\mathrm{R}T)]}{\int_0^l \exp[(M(x) + zFU(x/l))/\mathrm{R}T]\mathrm{d}x} \tag{3}$$

where C_0 and C_l are ion concentrations in the solutions adjacent to the membrane at $x = 0$ and $x = l$, respectively; U denotes the transmembrane potential ($U = \phi(l) - \phi(0)$). In the first approximation, the chemical potential of the ion in the solution is the sum of two terms: standard chemical potential $M(x)$ and $\mathrm{R}T \ln C$. The first term denotes formally the free energy of one mol of ions for 1 mol/l concentration. It depends on the ion surroundings, i.e. on the energy of interaction with the neighboring particles. The value of $M(x)$ can be counted from the value of electrolyte solution near the membrane surface. The standard chemical potential of the ion in the channel has the meaning of the height or depth of the potential barriers. We shall set this value to zero outside the membrane, as is the electric field ($E = 0$), so we can use here the border condition

$$M(0) = M(l) = 0 \tag{4}$$

The term of the chemical potential depending on concentration, C, needs special determination for the case of ion channels because there is a limited space inside the channel. As a matter of fact, the same situation takes place also in real solutions. Let us consider a small volume in the solution. The ion flowing through this part of the membrane volume can be either located in this volume or absent. Therefore, in reality ion concentration in a small volume of solution is either a time-averaged or space-averaged value. Thus, we shall consider local co-ordinate dependent ion concentration inside the channel.

Equation (3) can be simplified for voltages $U \sim 0.1$ V which were used in our experiments. In order to obtain the approximate formulae let us assume that the standard chemical potential $M(x)$ of the ion inside the membrane consists of two terms, a constant, μ_0, plus a small term $\mu(x)$ varying with distance x

$$M(x) = \mu_0 + \mu(x) \qquad 0 < x < l \tag{5}$$

The term $\mu(x)$ represents a small contribution to the standard chemical potential, which reflects the changes of the potential barriers inside the channel over co-ordinate x. As will be shown below, comparing experimental data with the theory, the interaction of the ion with the channel interior results in very small changes of the potential barriers of the channel only, i.e. $\mu(x)/\mathrm{R}T \ll 1$. The two terms in (5) can be separated by the additional normalisation condition

$$\int_0^l \mu(x)\mathrm{d}x = 0 \tag{6}$$

The expression (6) shows that along the ion moving across the membrane, the area under potential profile equals zero, i e any increase of the height of the potential profile is connected with potential reduction at other sites of the ion channel Expanding the exponential factor in the denominator in a series, the following equation is obtained from (3)

$$J \cong \frac{(D/l)\exp(-\mu_0/\mathrm{R}T)\ (F^2z^2/\mathrm{R}T)U[C_0 - C_l\exp(zFU/\mathrm{R}T)]}{(\exp[zFU/\mathrm{R}T] - 1) + (zFU/\mathrm{R}Tl)\int_0^l(\mu(x)/\mathrm{R}T\ \ \exp[zFU(x/l))/\mathrm{R}T]\mathrm{d}x} \tag{7}$$

If the standard chemical potential is constant inside the membrane, then $\mu(x) = 0$ and equation (7) is the standard one for ion current density in the constant field approximation The terms D/l and $\exp(-\mu_0/\mathrm{R}T)$ are the permeability coefficient and the distribution coefficient, respectively

It can be shown that if $\mu(x) \neq 0$ the main contribution to the integral of equation (7) is produced by the regions located near the membrane borders To prove this statement one uses coordinates symmetric with respect to the medial plane of the membrane ($x = l/2$), and expands the exponential in the integral into a series If, for symmetric electrolyte concentrations, the nonlinearity coefficient β can be written as $\beta \sim -\int_{l/2}^{l}[\mu(x) + \mu(l-x)](x - l/2)^2\mathrm{d}x$, the main contribution being for $x \cong l$

As we have no detailed information about the function $\mu(x)$ we assume a step wise dependence of the SCP on the x coordinate satisfying condition (6), namely

$$\mu(x) = -[\mu(0) + \mu(l)](L/l) + \begin{cases} \mu(0) & 0 < x \leq L, \\ 0 & L < x < l - L \\ \mu(l) & 1 - L \leq x < l \end{cases} \tag{8}$$

where $\mu(0)$ and $\mu(l)$ are the deviations of the SCP from the mean value of $\mu(x) = -[\mu(0) + \mu(l)](L/l)$ inside the membrane, L is the width of the potential barriers (or wells) (Fig 3*a*)

By expressing exponentials as hyperbolic functions, e g $\mathrm{e}^x = \mathrm{sh}\,x + \mathrm{ch}\,x$, expanding the latters in a series, and retaining only the cubic term (equation (1)), the coefficient of nonlinearity β is given by

$$\begin{aligned}\beta = &-(1/6)(L/l)(zF/\mathrm{R}T)^2\ \ \{[C_0/(C_0 + C_l)]\ \ (\mu(0)/\mathrm{R}T) + \\ &+ [C_l/(C_0 + C_l)]\ \ (\mu(l)/\mathrm{R}T)\}\end{aligned} \tag{9}$$

In the derivation of (9) we neglect the terms of order higher than (L/l)

Equations (3), (8), (9) enable the analysis for various electrolyte distributions We will consider the case of a symmetric electrolyte distribution first

In this case, the concentrations at both borders are equal, i e $C_0 = C_l$ The gramicidin dimer is symmetric with respect to the center of the bilayer, so the

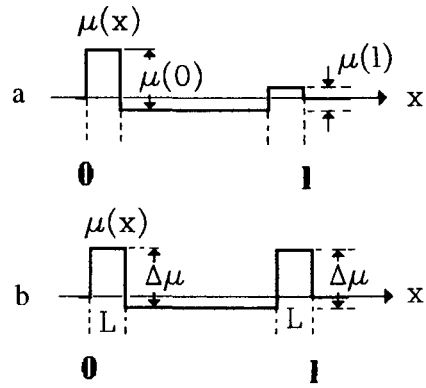

Figure 3. *a*. SCP profile as two barriers with the heights $\mu(0)$ and $\mu(l)$, measured from the mean value, and with the width L. *b*. The same for symmetric electrolyte conditions $\mu(0) = \mu(l) = \Delta\mu$.

SCP profile should be symmetric, and the assumption $\mu(0) = \mu(l) = \Delta\mu$ should be valid. To calculate current-voltage characteristics for this case even at wide range of voltages it is necessary to use the exact equation (3) instead of the approximate one (7). From equation (5) and (8), the symmetric SCP profile is (Fig. 3*b*)

$$M(x) = \mu_0 - 2\Delta\mu\ (L/l) + \begin{cases} \mu(0) & 0 < x \leq L, \\ 0 & L < x < l - L \\ \mu(l) & l - L \leq x < l \end{cases} \tag{10}$$

Current-voltage characteristics are plotted in Fig. 4 for values of $\Delta\mu/RT = -1$; 0; 1 (curves 1–3, respectively). The curves were normalized to the same membrane conductance at $U = 0$, and the sign of the current density was changed in order to compare these curves with those derived from equation (1). One can see that for $\Delta\mu/RT > 1$, the CVC nonlinearity is only observed for low membrane voltages not exceeding 0.1–0.2 V. For higher voltages, the curves are practically linear. This shape of the curve coincides excellently with the experimental data for large transmembrane voltages (Flerov et al. 1981). It follows from Fig. 4 that the measurements of the nonlinearity coefficient β should be performed at low voltages (about 0.1 V), as was used in our study.

From comparison of curves (1) and (3) one can see that the sign of the nonlinearity coefficient in symmetric condition, β_S, depends on the sign of $\Delta\mu$ at the terminus of the IC. A positive barrier ($\Delta\mu > 0$) yields a sublinear CVC ($\beta_S < 0$, curve 3), and a well at the terminus yields a superlinear CVC ($\beta_S > 0$) (curve 1).

The combination of the parameters of the barriers (wells) can be calculated

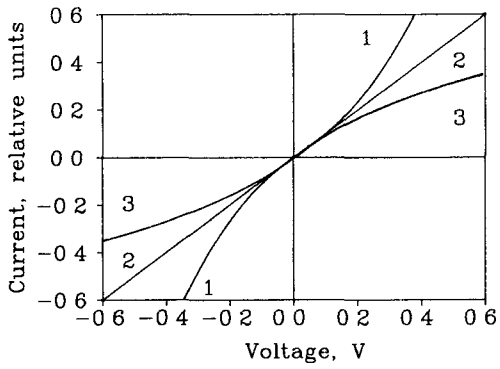

Figure 4. The normalized current-voltage characteristic in symmetric ionic conditions for the SCP profile shown in Fig 3 and containing two barriers (or wells) with height $\Delta\mu$ and width L The relative width of the barriers is $L/l = 0\ 2$ $\Delta\mu/RT = -1$ (curve 1), 0 (curve 2), 1 (curve 3)

from the value of β_S. It follows from (9) that

$$\beta_S = -(1/6)(L/l)(zF/RT)^2 \cdot (\Delta\mu/RT), \tag{11}$$

where $\Delta\mu$ is defined as in Fig. 3*b*. One can see from (11) that at a first approximation, the nonlinearity is determined by the product of the barrier height and its relative width $\Delta\mu(L/l)$. To obtain a numerical estimate we note that for $T \cong 300$ K, the ratio $RT/zF = 0.025$ V, so at $L/l = 0.1$ the relative height of the barrier is $\Delta\mu/RT = -0.0375\ \beta_S$. For $|\beta_S| \sim [10 \div 20]$ V^{-2} the changes of the SCP profile $\Delta\mu$ lie between $(0.3 \div 0.64)\ RT$.

Thus, the proposed model furnishes an explanation of the experimentally determined CVC profile and yields the parameters of the SCP profile inside the ion channel under symmetric ion conditions. These results now allow us to consider the case of an asymmetric electrolyte distribution. The experimental curve of the nonlinearity coefficient under asymmetric conditions, β_A, shown in Fig. 2 is intermediate to the curves corresponding to the nonlinearity coefficients for the two ion concentrations measured under symmetric conditions, β_S. Let us analyze the possible mechanisms of this phenomenon.

Analysis of the model for asymmetric ion distribution

1. The simplest possible scheme for the influence of the two terminals of the macromolecule on CVC nonlinearity is an additive one. One can assume that the SCP profiles at the two ends of the macromolecule are only determined by local electrolyte concentrations in the adjacent electrolyte solutions, i.e. the ends of the gramicidin molecule are independent of each other. Then, from (9) it follows that the value of β_A for aqueous concentrations C_0 and C_l is determined by the values of β_S (C_0) and β_S (C_l) measured in symmetric conditions for concentrations C_0 and C_l according to equation

$$\beta_A(C_0, C_l) = (C_l/(C_0 + C_l))\beta_S(C_l) + (C_0/(C_0 + C_l))\beta_S(C_0) \tag{12}$$

Equation (12) predicts that if the ion concentration at one end of the gramicidin molecule, e.g. C_0, exceeds substantially the concentration at the opposite end ($C_0 \gg C_l$), the overall nonlinearity coefficient is determined by the nonlinearity coefficient measured in symmetric conditions for the higher electrolyte concentration, β_S (C_0). In order to calculate the values of β_A according to equation (12) we used the experimental results of measurement β_S in symmetric conditions for BLM of egg PC with 33 mol% cholesterol. The results of the calculation (curve 3) as well as the experimentally obtained results of the dependence of β_A (curve 1) and β_S (curve 2) on electrolyte concentration for BLM of egg PC with 33 mol% cholesterol are presented in Fig. 5. We can see that the theoretical curve (curve 3) does not coincide with the experimental curve 1, and approaches the asymptotic value of $\beta_S = \beta_S$ (1 mol/l) if C_l decreases down to 0.01 mol/l, whereas the experimental curve 1 decreases monotonically.

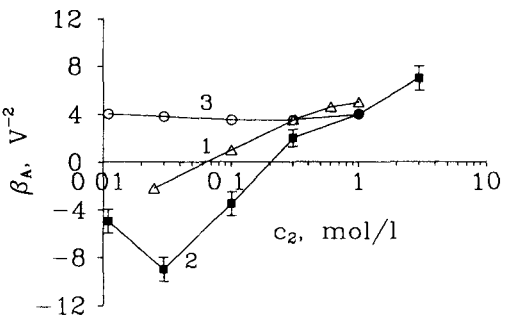

Figure 5. The dependence of nonlinearity coefficient β_A in asymmetric ionic conditions 1 – experimental curve for the dependence of β_A on concentration c_2 for BLM of egg PC (33 mol% of cholesterol), the concentration C_1 being 1 mol/l, 2 – experimental dependence of β_S for BLM of the same composition ($C_1 = C_2$), 3 – theoretical curve for β_A constructed for the additive scheme (equation (12))

In the framework of the electrodiffusion theory this implies that the nonlinearity coefficient determined by one end of the macromolecule β_S (C_0) depends on the properties of the other end, i.e. β_S (C_0) is a function of C_l and at the same time $\beta_S(C_0) = \beta_S(C_0, C_l)$. The latter can be measured experimentally because from (12), $\beta_S(C_0, C_l) \cong \beta_A(C_0, C_l)$ for $C_0 \gg C_l$. Since the nonlinearity coefficient is determined by the profile of the standard chemical potential, the result obtained above means that, in the framework of this model, the SCP at one terminus depends on the SCP at the opposite terminus.

The change of the SCP profile at either terminus at varying electrolyte concentrations at the other terminus can be considered as the result of some driving force

transmitted through the macromolecule. As a measure of this force we take, at a first approximation, the difference of SCP's, $\Delta\bar{\mu}(L/l)|_{dr}\ (C_0, C_l)$ at point $x = 0$ as follows:

$$\Delta\bar{\mu}(L/l)|_{dr}(C_0, C_l) = \mu(0)(L/l)|(C_l, C_l) - \mu(0)(L/l)|(C_0, C_0) \tag{13}$$

where concentrations in the brackets show successively the ion concentrations at the two sides of the membrane (at $x = 0$ and at $x = l$). The values $\mu(0)(L/l)|(C_i, C_i)$ are the heights of the SCP profile barriers calculated from the values β_S measured under symmetric conditions for the two concentrations $C_i = C_0$ and $C_i = C_l$.

The result of the influence of side i on side k is manifested as the change in height of the SCP profile, $\Delta\bar{\mu}(L/l)|_{as}(C_0, C_l)$, under asymmetric conditions at point $x = 0$ when the electrolyte concentrations at point $x = 0$ and at the opposite terminus are different (C_0 and C_l, respectively):

$$\Delta\bar{\mu}(L/l)|_{as}(C_0, C_l) = \mu(0)(L/l)|(C_l, C_l) - \mu(0)(L/l)|(C_0, C_l) \tag{14}$$

The values of $\mu(0)(L/l)|(C_i, C_k)$ in equations (13,14) are calculated in RT units by means of the following formulae

$$\mu(0)(L/l)|(C_i, C_i) \cong -\mathrm{R}T[6(\mathrm{R}T/zF)^2\beta_S(C_i)] \quad i = k \tag{15}$$

$$\mu(0)(L/l)|(C_i, C_k) \cong -\mathrm{R}T[6(\mathrm{R}T/zF)^2\beta_A(C_i, C_k)] \quad i \neq k \tag{16}$$

The plot $\Delta\bar{\mu}(L/l)|_{as}(C_0, C_l)$ vs. $\Delta\bar{\mu}(L/l)|_{dr}(C_0, C_l)$ is a straight line having a slope of about 0.5 in the concentration range $0.03 \leq C_l \leq 1$ mol/l at $C_0 = 1$ mol/l (Fig. 6). This again demonstrates that the influence of the structural change at one terminus of the macromolecule is transmitted to the opposite one, and has a significant effect on the SCP.

A possible mechanism for CVC modification, and the influence of one terminus on the other one

Before attempting to explain the influence of the ion concentration near one terminus of the ion channel on the CVC determined at the opposite terminus of the channel, we need a mechanism for the nonlinearity of CVC formally represented as the dependence of $\mu(x)$ on coordinate C.

From the preceding discussion it is clear that the interpretation of β values by means of the SCP profile $\mu(x)$ is not unambiguous. In the framework of the electrodiffusion approach, several different factors may affect the nonlinearity coefficient β, such as variation of either the diffusion coefficient or the electric field strength along the channel (Chen and Eisenberg 1993).

Considering that the lipid composition of the membrane is the main factor determining changes of β, we assume that the influence of field strength along the

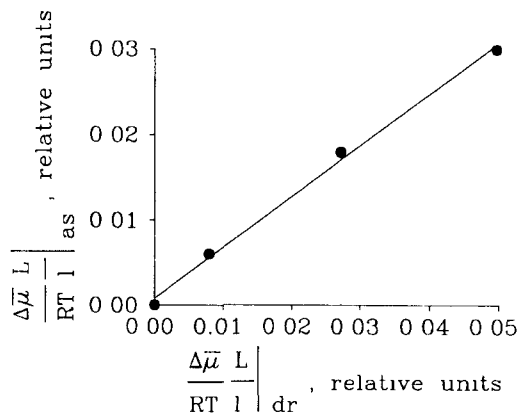

Figure 6. The plot of the change of SCP $(\Delta\overline{\mu}/RT)(L/RTl)|_{as}$ (C_1, C_2) at point $x = 0$ for asymmetric condition as a function of the driving force $(\Delta\overline{\mu}/RT)(L/RTl)|_{dr}$ (C_1, C_2) The electrolyte concentration C_1 at point $x = 0$ is constant $C_1 = 1$ mol/l

channel is not dominant. On the other hand, it is difficult to develop an electric model which could be able to explain this result. Furthermore, as changes in the ion mobility inside the channel cannot be distinguished from changes of standard chemical potential of the ion, we shall consider the CVC nonlinearity to be the result of the dependence of the overall standard chemical potential $M(x)$ on coordinate x. This nonlinearity could be induced by electrostatic forces as a result of a local change of either dielectric permittivity or geometry of the channel. Presently, we have no theoretical approach to describe the role of permittivity. The second possibility can be considered quantitatively, however, an example of this kind has already been presented. A change in the geometry of IC was analyzed earlier and shown to explain the logarithmic dependence of β_S for some lipids (Hianik at al. 1994). A correlation has also been found between the value of β and the order parameter of the bilayer as influenced by the addition of cholesterol to the membrane solution (Hianik at al. 1994).

Let us consider in what way the electrostatic component of the ion SCP inside the membrane is determined by IC electric permittivity and geometry. Pores with high permittivity considerably decrease the charge energy inside membranes (Volkenstein 1988). The gramicidin channel is filled with water, which is probably in a very similar state to that of bulk water. Let us estimate the ion distribution coefficient $\gamma = \exp(-\mu_0/RT)$, from the ion conductance λ_0 of a unit channel formed by gramicidin A. The value of λ_0 is about 40 pS in 1 mol/l KCl (Haydon and

Hladky 1972) It follows from formula (3) that

$$\lambda_0 = 10^3 F^2 Dc\gamma s/lRT \tag{17}$$

where $s = 4 \times 10^{-20}$ m^2 is the cross sectional area of the conducting pore, and the multiplier 1000 is conversion factor from mol/l to mol/m^3 If one assumes the same value of the diffusion coefficient as in water ($D = 2 \times 10^{-9}$ m^2/s), the value of γ calculated from (17) is $\simeq$ 0 3 If the value of D is smaller, the distribution coefficient is of the order of unity Thus, the pore formed by the gramicidin D (or gramicidin A) molecule is similar to water and should have a high dielectric permittivity

In this case, the electrostatic contribution, M_E, of the ion to the SCP inside a membrane is determined by the external dimension of the channel as follows (Volkenstein 1988)

$$M_E \approx e^2/(8\pi\varepsilon_0\varepsilon b) \tag{18}$$

where $\varepsilon_0 = 8\ 85\ 10^{-12}\ F/m$ is the dielectric permittivity of free space, $\varepsilon \approx 2$ is the relative dielectric permittivity of the hydrophobic part of the bilayer, and b is the outer radius of the macromolecule for $b = 1$ nm we have $M_E \approx 15$ RT Applying (18) we get

$$\Delta b = -b\ \ (\Delta\overline{\mu}(L/l)|_{as}\ (C_0, C_L)/M_E), \tag{19}$$

from which we see that a change of Δb by only about 0 1 nm will change the SCP ($\Delta\overline{\mu}(L/l)|_{as}(C_0, C_l)$) by R$T$ (note that an increase in b decreases the value of M_E)

This results enables us to give a purely mechanical interpretation of the changes in the structure of the ion channel resulting from the interaction of the macromolecule with electrolyte adjacent to the terminus In this scheme, the radius of this region is changed with respect to the mean radius of the macromolecule the larger the radius the smaller the contribution of $\Delta\mu$ to the standard chemical potential, and the higher the nonlinearity coefficient (Fig 7, columns a and b) For symmetric electrolyte concentrations, the increase of $C_0 = C_L$ will result in an increase in the radius of the terminus of the channel (Fig 7a, b) Thus, changes of electrolyte concentration influence the standard chemical potential indirectly changes of the concentration of ions at one terminus of the ionic channel lead to changes of the channel structure The changed channel structure is characterized by changed potential profiles and, hence, by new value of μ_0

Fig 7c, d shows the change of the radius of the terminus contacting the solution with concentration C_0 = const when concentration C_L is changed We have no experimental data on either the SCP profile (Fig 7c) or terminus diameter (Fig 7d) for the second terminus in asymmetric conditions, so the data for the symmetric case were used to construct the plot

As depicted in Fig 7d, a decrease in the ion concentration at the right terminus is accompanied by a constriction of that terminus When this constriction

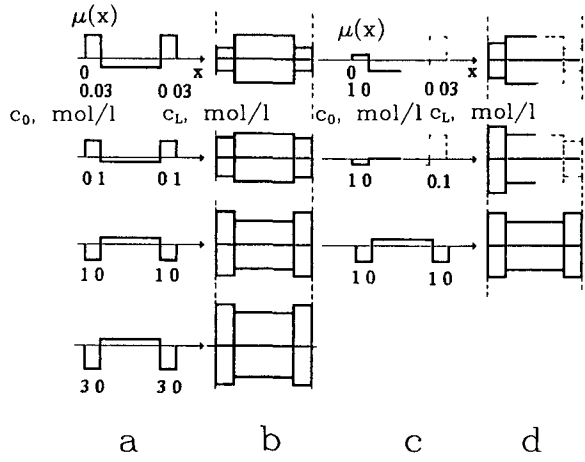

Figure 7. Schematic representations of the SCP profile $\mu(z)$ (a, c) and the corresponding shapes of the channel macromolecules (b, d) for various electrolyte concentrations (the values indicated under the corresponding part of the $\mu(x)$ profile) a and b symmetric ionic conditions $(C_0 = C_1)$ c and d asymmetric conditions $(C_0 \neq C_1)$ In b) and d), the vertical dotted lines show the border of the membrane Changes of macromolecule geometry are out of scale The terminal pieces are shown in solid lines in those cases where the $\mu(x)$ profile was experimentally accessible For comparison, the data for the corresponding barriers are shown for the terminal at the lower concentration (light lines) in d

is transmitted to the opposite (left) terminus, a corresponding decrease of its diameter is induced. It should be mentioned once more that the scheme proposed above illustrates the new mechanism of influence of ion concentration upon the chemical potential of the ion inside the channel. A change of ion concentration at one terminus of the ion channel has an indirect influence through changing the ion channel structure, and thus by through changing the standard chemical potential, on the opposite terminus of the channel.

Discussion

In this report, the inference about transmembrane transmission of changes of macromolecular properties is based on indirect data concerning the ion transport through ion channels Let us consider the reliability of the assumptions used. The experimental data concerning CVC nonlinearity were measured using a new physical method. We consider our experimental method based on measurements of the third harmonic of the transmembrane current to be very reliable. The method and the possible difficulties which could be involved have been studied for many years (Passechnik et al. 1985; Hianik and Passechnik 1995). The theoretical consideration

of the channel behaviour was made in three steps First, the theory was derived to approximate the process of ion transfer across the membrane Second, we demonstrated that for the asymmetric case, i e different ion concentrations at the two sides of the membrane, the ion transport is actually only determined by the potential barrier located at the membrane side with the higher ion concentration Third, we showed that, in the asymmetric case, the nonlinearity coefficient which is determined by the side at the higher electrolyte concentration varies when the lower electrolyte concentration at the opposite terminus of the macromolecule is varied

The theory is based on the model of ion transport in ion channels (Markin and Chizmadzhev 1974) but differs from it in some aspects In some papers, the ion transport is analyzed in the framework of the three-barrier model (Aiytan et al 1977) The transport is considered as a sequence of the following events jump of the ion from the electrolyte into the membrane over the first barrier (transfer rate constant k_1), transfer of the ion along the SCP profile into the internal part of IC (at least a jump of the ion over a barrier with the height D/l), and finally, passage of the ion from IC into the solution across a third barrier (transfer rate constant k_2) Constants k_1 and k_2 are not considered to depend on the electric field strength The shortcoming of this model is the prediction of CVC saturation at potentials of about $0\,1 \div 0\,2$ V under symmetrical conditions, which is inconsistent with experimental data (Flerov at al 1981, Andersen 1983) Our variant of the electrodiffusion theory, on the other hand, approximates CVC for all transmembrane voltages in the symmetric case This consistency of theory and experimental data enabled us to derive equation (12) for the asymmetric case which can be used to analyze the transmission of conformation change from one terminus of the macromolecule to the other one

The "driving force" of this transmission used in our study, the difference of the heights of the SCP profiles for the two ion concentrations at opposite termini, is of course a purely qualitative approach Instead of the parameter which is the direct characteristic of the conformation of the macromolecule we used an indirect one which varies due to the conformational change However, this approach enabled us to measure the phenomenon for membrane proteins The calculations of the changes of the potential barriers as well as of the shape of the macromolecule is only one possible interpretation Another approach would be the calculation of the free energy barrier inside a single file ion channel by the Monte-Carlo analysis, taking the contribution of water and carbonyl groups inside the file into consideration (Dorman et al 1995)

Thus, we showed that the structure of ionic channel of gramicidin D is sensitive to the concentration of ions, and in fact the changes in one end of the channel influence the parameters of second end of the channel Probably, the concentration of ions changes the construction of the system by means of changes in the height

of the potential barriers For example, increasing of concentration of ions at one or both sides of the channel leads to an increase of their sorption on the functional groups of the channel This induces changes of potential barriers of the channel This approach is principally different from that used in chemical kinetics In the former case, changes in concentration do not change the construction of the system, and the influence of the concentration on the parameters of the corresponding reactions is considered by means of adding the term $RT \ln C$

The physical mechanism of transmembrane transmission of conformational change by means of the macromolecule needs further study

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