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Fluctuation and Relaxation Analysis of Monazomycin-Induced Conductance in Black Lipid Membranes

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Summary. Fluctuation and relaxation analyses were performed on monazomycin-induced conductance of lipid bilayer membranes. With both methods a slow (sec) and a fast (msec) current component are apparent; however, the amplitude of the slow, voltage-dependent process is greater than that of the fast component in the step relaxation experiment and less in the fluctuation experiment. The fluctuation analysis showed principally a rapid voltage-dependent process which appears to be related to the multistate character of the conducting channel. The experimental results are interpreted in terms of a simplified kinetic model which is used to calculate relaxation and noise amplitudes.

Fluctuation analysis has been used to characterize a variety of artificial and physiological membrane systems (Anderson & Stevens, 1973; Conti, De Felice & Wanke, 1975; Fishman, 1973; Fishman, Poussart & Moore, 1975; Fishman, Moore & Poussart, 1975; Katz & Miledi, 1972; Kolb, Läuger & Bamberg, 1975; Zingsheim & Neher, 1974; Wanke, De Felice & Conti, 1974). The purpose of this investigation is to characterize a voltage-dependent bilayer system using fluctuation analysis and relaxation measurements. Monazomycin and, to some extent, alamethicin are used in these experiments to produce multistate ion conducting channels which are voltage dependent and show a steep relationship between the mean conductance and the membrane potential (Muller & Finkelstein, 1972; Mauro, Nanavati & Heyer, 1972). These channels are probably formed by an aggregation reaction which is preceded by a voltage-dependent reorientation of the individual molecules (Muller & Finkelstein, 1972).

It was found that the relaxation experiments emphasize the voltage-dependent process, whereas the more rapid aggregation step is most

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easily seen with fluctuation analysis. A two-step kinetic model is shown to be sufficient to explain the amplitude differences observed in these experiments and is used to illustrate specifically how the variance of the conductance fluctuations can be calculated for a chemical system.

Materials and Methods

All bilayer membranes were made on a polypropylene support (Zingsheim & Neher, 1974) over an opening having a diameter of 250 μ. Lecithin-cholesterol membranes were formed from a *n*-decane solution containing 10 mg/ml of dioleoyl-L-α-Lecithin (Supelco, Inc., Bellefonte, Pa.) and 5 mg/ml cholesterol (Sigma Chemical Co., St. Louis, Mo.). Monazomycin, provided by Dr. Yonehara, Tokyo, was added on only one side of the membrane to the 1 M KCl bathing solution from aqueous stock solutions. Alamethicin, provided by Dr. Trauble, Göttingen, was added in the same manner. All experiments were performed at room temperature (21–23 °C).

The current measuring system (voltage clamp) was identical to that described by Zingsheim and Neher (1974). The correlation analysis was done on a Honeywell-Saicor-42 A, 100 point Analyzer. The power spectra were measured with a Honeywell-Saicor-52 B, 400 point Spectrum Analyzer. During the experiments all data were recorded with a magnetic tape system having a flat frequency response from DC to 1.25 kHz. The data were processed before the offline analysis through a 0.3 Hz high pass and a 400 Hz low pass filter.

Results

Monazomycin Experiments

Membrane conductance fluctuations in bilayers containing monazomycin are most easily seen at membrane potentials where the conductance is low. The fluctuation signals develop in proportion to the increase in conductance seen in response to a step change in the membrane potential. The upper trace of Fig. 1 illustrates such a progressive increase in conductance fluctuations as seen through a high pass filter set at 0.01 Hz. The lower trace of Fig. 1 shows, at a lower gain, the usual delayed conductance increase typical of monazomycin channels. It is apparent from Fig. 1 that the relatively rapid fluctuations of the steady state membrane conductance do not have the same time scale as the slower macroscopic conductance increase. This observation suggests that the relaxation and fluctuation experiments are measuring two different kinetic parameters. However, the two measurements are not ideally comparable since the voltage clamp step is a large perturbation from 0 to 80 mV while the conductance fluctuations occur at a constant membrane potential. Any

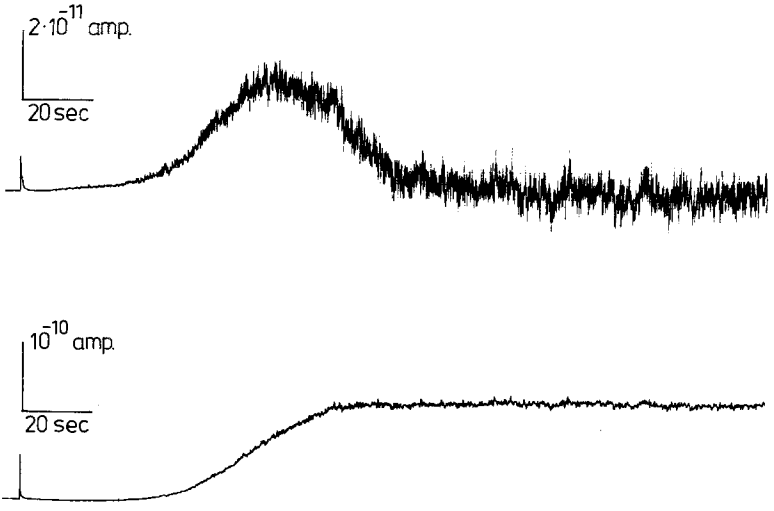

Fig. 1. Development of monazomycin-induced conductance during a step change in potential. The capacitative charging current indicates the time at which the potential was changed from 0 to 85 mV. Positive potential refers to the side of the membrane with monazomycin. The upper curve was recorded through a 0.01 Hz high pass filter. The lower curve is a conventional dc recording. The monazomycin concentration in the bathing solution on one side of the membrane was 0.3 μg/ml. The ordinate represents the membrane current and the abscissa, time in seconds

nonlinearities in the conductance change could confound the comparison of the two methods.

In order to compare more rigorously the perturbation and fluctuation experiments, the membrane was first put in a conducting state by a large membrane potential step which was then followed by a small potential perturbation for the relaxation measurement. Such small perturbations make possible the use of linear relaxation theory (Eigen & De Maeyer, 1963) to calculate relaxation and correlation times which can be compared with direct experimental measurements.

The small membrane potential perturbation seen in Fig. 2 shows a slow single exponential relaxation response without the usual delay typical of large step on-responses. The slow time constant decreased e-fold for a 15 mV increase in V_1 in a range of 65–115 mV compared to 6 mV for the corresponding conductance change (Muller & Finkelstein, 1972).

Relaxation spectra which were observed at increased time resolution showed a more complicated response containing at least two components.

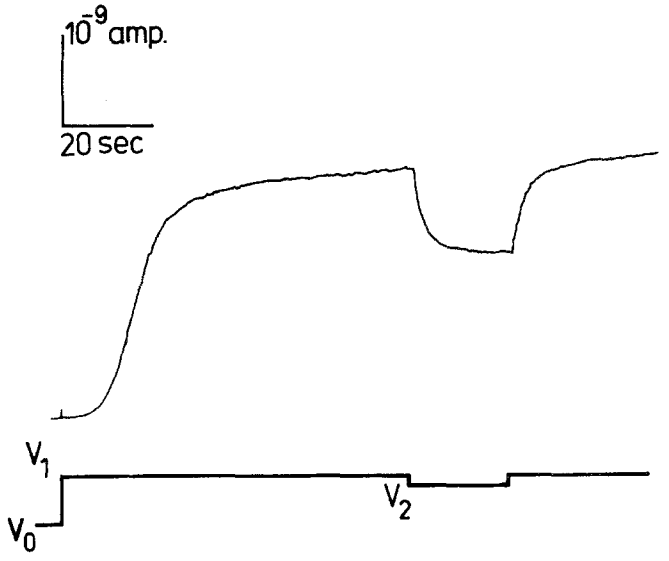

Fig. 2. Small step relaxation of monazomycin-induced conductance. The upper curve is a tracing of the membrane current during changes in the membrane potential, which are schematically shown on the lower trace as $V_o = 0$ mV, $V_1 = 80$ mV and $V_2 = 78$ mV. The monazomycin concentration in the bath was 0.4 μg/ml. The instantaneous change in current which occurred during these transients is not seen because it is too rapid to be resolved by the pen chart recorder

However, as suggested by Fig. 2, the amplitude of the more rapid component is considerably smaller than the slow component. The rapid relaxation times were analyzed by applying a short (<8 msec) voltage impulse of 40 mV magnitude and measuring the resulting relaxation. The advantage of the impulse method was to emphasize the fast component, since the short perturbing pulse did not allow time for the slow relaxation phenomena to express themselves. Fig. 3 shows an impulse relaxation time which has a half time of about 15 msec. No marked potential dependence was seen for the fast relaxation time.

The multiple component character of the kinetic process should also be seen in an analysis of the fluctuation signal. The correlation function for the steady state membrane conductance in Fig. 4 shows a fast component which has a half time of approximately 15 msec. The correlation function is clearly not a single exponential term and thus reflects the multi-step character of the presumed aggregation reaction underlying the rapid process.

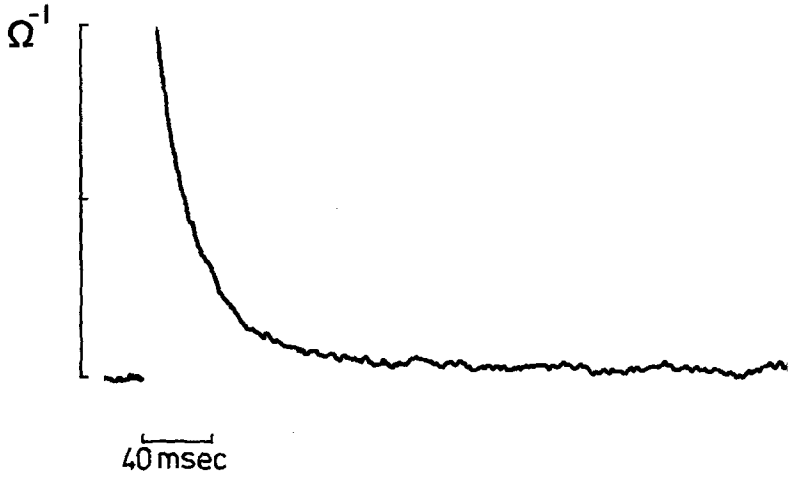

Fig. 3. Impulse response of monazomycin-induced conductance. At the break in the conductance curve a 40 mV pulse (<8 msec) was superimposed on a preceding potential step of 80 mV. The membrane current preceding the short impulse was 10^{-9} amperes. The ordinate is the membrane conductance in arbitrary units and the abscissa, time in msec. The monazomycin concentration was 0.4 μg/ml

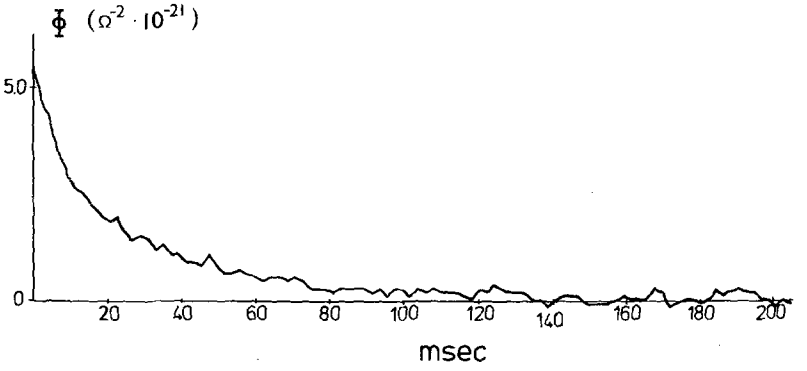

Fig. 4. Correlation function of monazomycin-induced conductance. The membrane potential was 95 mV and the monazomycin concentration was 0.2 μg/ml. The ordinate is the correlation function, $\Phi(\tau)$, of the conductance; and the abscissa, time. The correlation is an average of 32,768 summations. Note that this analysis was done on data processed through a high pass filter of 0.3 Hz

There is also a slow component (sec) in the correlation function, not seen in Fig. 4, whose amplitude is at most 30% of that of the fast component and appears to have a time constant in the same range as the slow relaxation time constant. It was not possible to accurately measure the

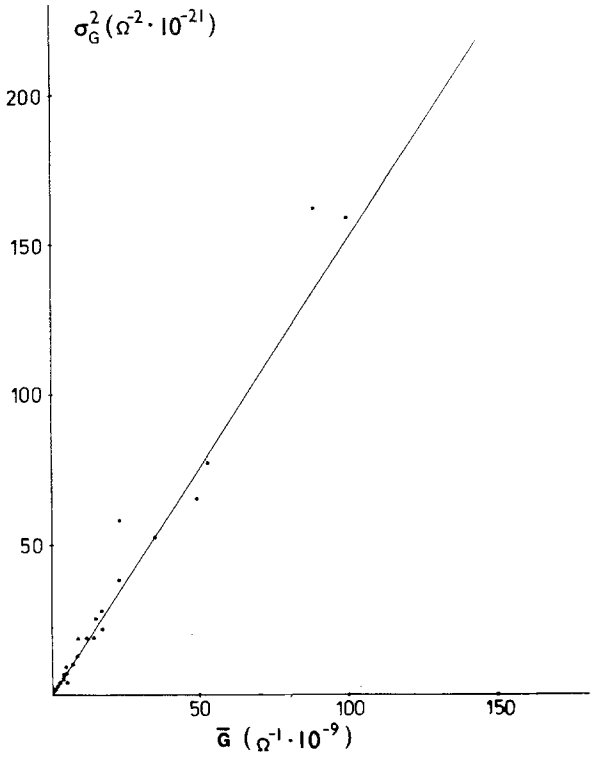

Fig. 5. Variance of monazomycin-induced conductance fluctuations *vs.* the mean conductance. A straight line is drawn through the data. The ordinate is the value of the correlation function of the fast component at zero time (σ_G^2) measured for different membrane potentials (50–110 mV) and monazomycin concentrations (0.2–0.4 μg/ml); the abscissa is the corresponding conductance ($\bar{G}$)

slow, low amplitude component of the correlation function due to artifacts resulting from membrane instability and uncontrollable environmental factors such as air currents, temperature fluctuations, and vibrations.

The fluctuation analysis and the small step relaxation experiments thus show similar spectra, although the ease of measurement and relative amplitudes of the fast and slow processes differ for the two methods. The kinetics of the rapid component are easily observed in the fluctuation spectra, whereas the behavior of the slow component is more pronounced in the relaxation experiment. The large amplitude response of the slow component in the relaxation measurement compared to the noise analysis is related to the strong dependence of the conductance on the membrane potential.

The value of the correlation function at time zero, $\Phi(0)$, or the variance of the fluctuation, σ_G^2, in conjunction with the mean conductance $\bar{G}$, can be used to estimate a unit conductance step (Rice, 1944; Zingsheim & Neher, 1974; Kolb *et al.*, 1975). This estimate, $\Lambda_{est} = \sigma_G^2/\bar{G}$, calculated as a linear regression of σ_G^2 versus $\bar{G}$ for only the fast process was $1.55 \times 10^{-12}\ \Omega^{-1}$. The correlation coefficient between these two quantities was 0.98. The data of Fig. 5 indicate that Λ_{est} is independent of the membrane potential in the range 50–110 mV and the monazomycin concentrations from 0.2 to 0.4 μg/ml. This finding is consistent with a multistate model for channel formation which has a voltage-dependent step for the insertion of ionophores into the membrane and a subsequent series of voltage-independent aggregation steps to form the conducting channel (Muller & Finkelstein, 1972).

Comparison of Monazomycin and Alamethicin-Induced Conductances

Since the evidence for voltage-independent aggregation of alamethicin is based on unit channel measurements (Boheim, 1974; Cherry, Chapman & Graham, 1972; Eisenberg, Hall & Mead, 1973; Gordon & Haydon, 1972), it is useful to compare results of a correlation analysis of alamethicin

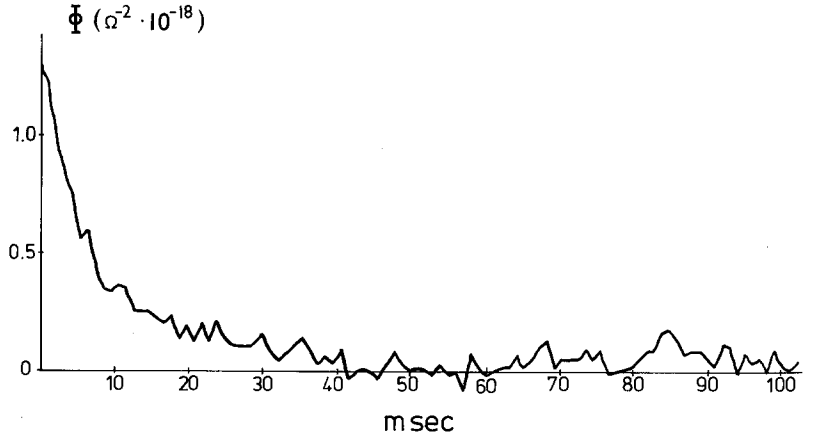

Fig. 6. Correlation function of alamethicin-induced conductance. The membrane potential was 50 mV and the alamethicin concentration was 10^{-8} M. The ordinate is the correlation function, $\Phi(\tau)$, of the conductance; and the abscissa, time. The correlation function is an average of 131,072 summations

channels with the above findings for monazomycin. Fig. 6 shows a correlation function for alamethicin-induced channels showing a more rapid time course than seen for monazomycin in Fig. 4.

The average value of Λ_{est} for the alamethicin channel calculated as above was $8.8 \times 10^{-10}\ \Omega^{-1}$. The correlation coefficient between σ_G^2 and $\bar{G}$ was 0.99. Direct measurements of the alamethicin unit channel conductance range from $0.4\text{–}20 \times 10^{-10}\ \Omega^{-1}$ depending on the open channel state (Boheim, 1974; Eisenberg *et al.*, 1973). This range includes the value of Λ_{est} reported above, suggesting that Λ_{est} is a weighted average of the multiple conductance levels.

In general, the supposed multiple state conductance system for monazomycin and alamethicin channels should show a complicated relaxation spectrum, or correspondingly, a multi-exponential correlation function. The correlation functions observed in these experiments appear to represent a spectrum of channel conducting states whose duration varies about some mean value. Thus, by analogy to the alamethicin data where unit levels have similar durations (Boheim, 1974; Eisenberg *et al.*, 1973), it is

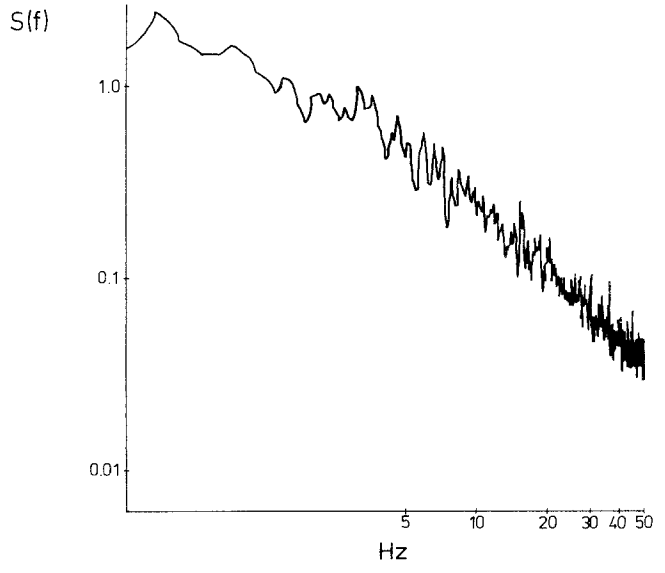

Fig. 7. Spectral analysis of monazomycin-induced conductance fluctuation under voltage clamp conditions. The power spectrum, $S(f)$, in arbitrary units, was measured on the same data used in Fig. 4. The power spectrum shown is an average of 16 spectra

reasonable to assume that the multiple states of monazomycin channels show an average duration of approximately 20 msec.

Power Spectral Analysis

In using noise analysis to characterize a system, it is of interest to separate processes which show definite multiple relaxations from those which show $1/f$ noise over a limited frequency range. For membrane systems this distinction is important, since $1/f$ noise can be observed in essentially all permeability barriers as a manifestation of nonspecific ion channels (Dorset & Fishman, 1975; Verveen & De Felice, 1974).

The power spectra shown in Figs. 7 and 8 demonstrate that the fluctuation phenomena discussed above are not of the $1/f$ type and are not described by a single Lorentzian function. These power spectra are consistent with the correlation function measured for the monazomycin and alamethicin channels. Similar power spectra for monazomycin were recently reported by Wanke (1975).

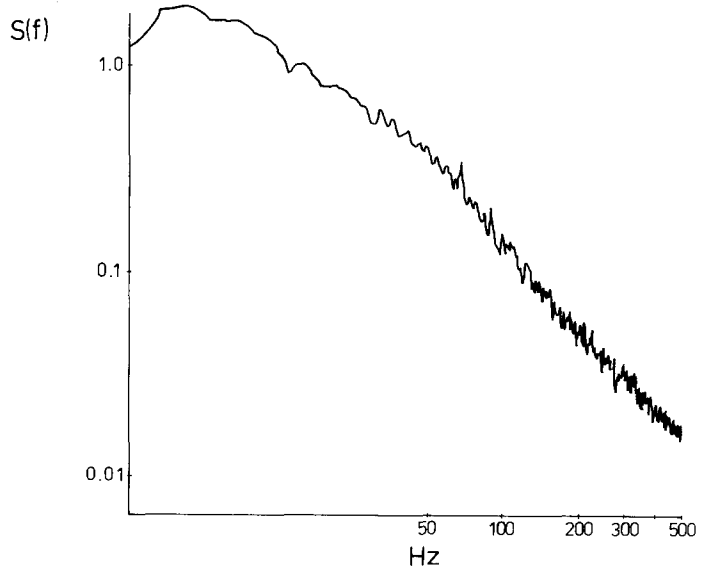

Fig. 8. Spectral analysis of alamethicin-induced conductance fluctuations under voltage clamp conditions. The power spectrum, $S(f)$, in arbitrary units, was measured on the same data as Fig. 6. The power spectrum shown is an average of 64 spectra

Discussion

The equivalence of fluctuation analysis and small step relaxation experiments has been shown for gramicidin channels, which are presumed to have only one conducting state (Bamberg & Läuger, 1973; Kolb *et al.*, 1975; Zingsheim & Neher, 1974). The experiments described in this paper generally confirm this conclusion, but, in addition, show that the amplitudes of the components of a multi-step process can be different for fluctuation *vs.* relaxation analysis.

The fluctuations of multistate processes arise from changes in the number of channels as well as variations in the channel conductance itself (Eisenberg *et al.*, 1973). Assuming statistical independence of the channels, it has been shown that the variance in the total conductance, σ_G^2, is given by Eisenberg *et al.* (1973)

$$\sigma_G^2 = (\bar{\gamma} + \sigma_\gamma^2/\bar{\gamma}) \cdot \bar{G} \tag{1}$$

where $\bar{\gamma}$ is the mean conductance of a single channel, σ_γ^2 is the variance of the single channel conductance, and $\bar{G}$ is the mean conductance. Fluctuations arising from alamethicin channels (Eisenberg *et al.*, 1973) suggest that $\sigma_\gamma^2/\bar{\gamma} < \bar{\gamma}$.

Interpreting the monazomycin data in terms of Eq. 1 leads to the conclusion that $\sigma_\gamma^2/\bar{\gamma} > \bar{\gamma}$ where $\sigma_\gamma^2/\bar{\gamma}$ is attributed to the fast fluctuation resulting from switching between different conductance levels and $\bar{\gamma}$ refers to the slower formation and dissolution of channels.

These findings suggest that the estimated unit conductance, Λ_{est}, is predominately a function of the multiplicity of states and that the mean unit conductance of a monazomycin channel may be smaller than the estimated value. Alternatively, the assumption of channel independence in the monazomycin experiments may not be valid, since the reported measurements were made at high channel densities in order to observe fluctuations. This experimental condition contrasts with that obtained during measurements of alamethicin-induced single channels.

The kinetic data obtained in these experiments are consistent with a model similar to that proposed by others for both monazomycin and alamethicin (Bauman & Mueller, 1974; Boheim, 1974; Eisenberg *et al.*, 1973; Muller & Finkelstein, 1972). More specifically, the mechanism discussed below is the simplest model found to be consistent with the following experimental findings:

Relaxation Results. (a) a slow conductance relaxation in response to a voltage step with a voltage-dependent time constant; and

(b) a voltage-independent fast relaxation time constant with a relatively small contribution to the overall relaxation amplitude.

Fluctuation Results. (a) a predominantly fast fluctuation with voltage-independent kinetics; and

(b) a relatively small amplitude for slow fluctuations.

An additional purpose of the model described below is to show the effect of frequently used simplifying assumptions for the calculation of both noise amplitudes and relaxation spectra.

It is assumed that the potential-dependent step is an orientation of monazomycin monomers as follows:

$$A \underset{k_2(V)}{\overset{k_1(V)}{\rightleftarrows}} B \tag{2}$$

where A is the initial monomer state, B is a secondary monomer state, and $k_1(V)$ and $k_2(V)$ are voltage (V) dependent rate constants. The second step in the proposed model is a relatively fast, potential-independent, aggregation reaction,

$$nB \underset{k_4}{\overset{k_3}{\rightleftarrows}} C \tag{3}$$

where n monomers form a conducting channel, C. The measured conductance is taken to be proportional to C.

The reaction scheme of Eqs. 2 and 3 can be linearized and solved for C to give a relaxation response of the form

$$C = C_1 + C_2\, e^{-t/\tau_1} + C_3\, e^{-t/\tau_2} \tag{4}$$

where C_1, C_2 and C_3 are constants and τ_1 and τ_2 are the relaxation times of the slow and fast processes, respectively. According to the fluctuation dissipation theorem (Kubo, 1957), the autocorrelation function can be expressed in the same form as Eq. 4 with identical time constants but, in general, different amplitudes.

Calculation of the Relaxation Response

In order to simplify the analysis, the initial slower reaction will be treated for the condition of a rapid equilibrium between B and C, thus

$$c_C = K_2 \cdot c_B^n \tag{5}$$

$$n\frac{dc_C}{dt}+\frac{dc_B}{dt}=k_1 c_A-k_2 c_B \tag{6}$$

where $K_2=k_3/k_4$ and c_A, c_B and c_C are concentrations of A, B and C. For small voltage steps and the stoichiometric condition

$$\delta c_A=-\delta c_B-n\delta c_C \tag{7}$$

and

$$\delta c_C=K_2 n c_B^{n-1}\delta c_B$$

Eqs. 5 and 6 can be solved for δc_C to give a single exponential function whose time constant is

$$\tau_1^{-1}=k_1+k_2\Big/\left[1+n^2\cdot\frac{c_C}{c_B}\right]. \tag{8}$$

The experimental result that τ_1^{-1} increases with voltage is consistent with the conclusion following Eq. 11 that $n^2 c_C/c_B \ll 1$. The increase in τ_1^{-1} can be accounted for by a voltage dependent k_1 and k_2.

Similarly, the second, fast reaction can be analyzed for the condition of negligible concentration change in c_A, thus,

$$\delta c_B=-n\delta c_C \tag{9}$$

where

$$dc_C/dt=k_3 c_B^n-k_4 c_C \tag{10}$$

and after linearization

$$\tau_2^{-1}=n^2 k_3 c_B^{(n-1)}+k_4=k_4(1+n^2 c_C/c_B). \tag{11}$$

Since the fast time constant is relatively independent of voltage, the term, $n^2 c_C/c_B \ll 1$. This is equivalent to the statement that the equilibrium lies toward the monomer side.

It is evident from Eqs. 5 and 9 that the amplitude of the fast relaxation is determined only by the voltage dependence of K_2. Thus, in the limiting case of the proposed model the relaxation amplitude of the fast reaction would be zero. Correspondingly, the equilibrium condition for the first step depends on k_1 and k_2 which are voltage dependent. Thus, any ratio between the amplitudes C_2 and C_3, of Eq. 4 can be obtained by assigning the proper voltage dependence to the equilibrium constants.

Calculation of Fluctuation Amplitudes

The object of this section is to calculate the amplitudes of the correlation function (Lax, 1960) for the slow and fast processes in the above reaction

scheme. Since the time scales of the fast and the slow process are very different, their fluctuations can be measured separately by selecting appropriate filters. In principle a single exponential term should be obtained in both cases where the amplitudes are the corresponding variances. These variances can be calculated in a simple manner by making assumptions which are equivalent to the above filters. The starting point for this treatment is an extension of the Boltzman Law to macroscopic systems (Landau & Lifschitz, 1958) which, under the conditions of these experiments, states that the probability of finding a chemical system in a state, characterized by the number of moles, n_A, n_B, and n_C, is proportional to

$$e^{-\Delta G(n_A, n_B, n_C)/kT}$$

where ΔG is the difference in the Gibbs free energy between the given state and the system's equilibrium state. Considering small fluctuations of n_C around its equilibrium value, ΔG can be expanded into a Taylor series with vanishing first order terms. This leads to a probability, $p(\Delta n_C)$ of finding the system in a state which is characterized by a fluctuation in n_C of the size, Δn_C:

$$p(\Delta n_C) \propto \exp[-(d^2 G/dn_C^2)/(2kT)]. \tag{12}$$

Eq. 12 describes a Gaussian distribution where

$$\sigma_{n_C}^2 = kT/(d^2 G/dn_C^2). \tag{12a}$$

An alternative derivation of Eq. 12a by Einstein can be found in Tolman (1938).

Thus, for the above reaction scheme

$$dG = \mu_A dn_A + \mu_B dn_B + \mu_C dn_C \tag{13}$$

where μ_A, μ_B, and μ_C are the chemical potentials of A, B, and C.

The term, $\dfrac{d^2 G}{dn_C^2}$ can be calculated from Eq. 13 as follows:

$$\frac{d^2 G}{dn_C^2} = \mu_A \frac{d^2 n_A}{dn_C^2} + \left(\frac{d\mu_A}{dn_C}\right)\left(\frac{dn_A}{dn_C}\right) + \mu_B \frac{d^2 n_B}{dn_C^2} + \left(\frac{d\mu_B}{dc_C}\right)\left(\frac{dn_B}{dn_C}\right) + \frac{d\mu_C}{dn_C}. \tag{14}$$

For the slow reaction where Eqs. 5 and 7 apply, then

$$dn_C = n n_B^{n-1} K_2 dn_B$$

$$\frac{dn_B}{dn_C} = \frac{1}{n n_B^{n-1} K_2}, \qquad \frac{dn_A}{dn_C} = -n - \frac{1}{n n_B^{n-1} K_2}.$$

Using $\frac{d\mu_A}{dn_C}=\left(\frac{RT}{n_A}\right)\left(\frac{dn_A}{dn_C}\right)$, $\frac{d\mu_B}{dn_C}=\left(\frac{RT}{n_B}\right)\left(\frac{dn_B}{dn_C}\right)$ and the equilibrium condition that $\mu_A=\mu_B$, it follows that

$$\frac{d^2G}{dn_C^2}=\frac{RT}{n_C}+\frac{RT}{n_A}\left[n+\frac{1}{nn_B^{n-1}K_2}\right]^2+\frac{RT}{n_B}\left[\frac{1}{nn_B^{n-1}K_2}\right]^2. \tag{15}$$

Using Eq. 5 and $K_1=n_B/n_A$

$$\frac{d^2G}{dn_C^2}=RT\left[\frac{1}{n_C}+\frac{(K_1+1)n_B}{n^2n_C^2}+\frac{K_1n^2}{n_B}+\frac{2K_1}{n_C}\right] \tag{16}$$

and

$$\frac{n_C}{\sigma_{n_C}^2}=N_L\left[1+\frac{K_1+1}{n^2}\cdot\frac{n_B}{n_C}+K_1n^2\cdot\frac{n_C}{n_B}+2\cdot K_1\right] \tag{17}$$

where N_L is Avogodro's number.

Correspondingly, for the fast reaction, where Eq. 9 applies and $dn_A=0$, it follows that

$$\frac{d^2G}{dn_C^2}=n^2\frac{d\mu_B}{dn_B}+\frac{d\mu_C}{dn_C} \tag{18}$$

and

$$\frac{n_C}{\sigma_{n_C}^2}=N_L\cdot\left[1+n^2\frac{n_C}{n_B}\right]. \tag{19}$$

Using the expressions

$$\bar{G}=N_Ln_C\gamma \quad \text{and} \quad \sigma_{\bar{G}}^2=N_L^2\gamma^2\sigma_{n_C}^2$$

Eqs. 17 and 19 become

$$\sigma_{G_s}^2=\bar{G}\gamma[1+2K_1+(1+K_1)/(n^2n_C/n_B)+n^2K_1(n_C/n_B)]^{-1} \tag{20}$$

$$\cong\bar{G}\gamma[1+2K_1+(1+K_1)/(n^2n_C/n_B)]^{-1} \tag{20a}$$

and

$$\sigma_{G_f}^2=\bar{G}\gamma[1+n^2n_C/n_B]^{-1} \tag{21}$$

$$\cong\bar{G}\gamma \tag{21a}$$

where $n^2n_C/n_B\ll 1$. The terms, $\sigma_{G_s}^2$ and $\sigma_{G_f}^2$, refer to the slow and fast processes respectively.

Consideration of Eqs. 20 and 21 shows that in both cases the maximum relative fluctuation, $\sigma_{\bar{G}}^2=\bar{G}\gamma$, is the limiting case for independent fluctuations. All additional terms in these equations are positive and act to reduce $\sigma_{\bar{G}}^2$. In the case of the fast process the reduction is negligible since $n^2n_C/n_B\ll 1$. This was the requirement for the fast time constant to be voltage independent.

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References

Anderson, C.R., Stevens, C.F. 1973. Voltage clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junction. *J. Physiol.* **235**:655

Bamberg, E., Läuger, P. 1973. Channel formation kinetics of gramicidin A in lipid bilayer membranes. *J. Membrane Biol.* **11**:177

Baumann, G., Mueller, P. 1974. A molecular model of membrane excitability. *J. Supramol. Struct.* **2**:538

Boheim, G. 1974. Statistical analysis of alamethicin channels in black lipid membranes. *J. Membrane Biol.* **19**:277

Cherry, R.J., Chapman, D., Graham, D.E. 1972. Studies of the conductance changes induced in bimolecular lipid membranes by alamethicin. *J. Membrane Biol.* **7**:325

Conti, F., DeFelice, L.J., Wanke, E. 1975. Potassium and sodium ion current noise in the membrane of the squid giant axon. *J. Physiol.* **248**:45

Dorset, D.L., Fishman, H.M. 1975. Excess electrical noise during current flow through porous membranes separating ionic solutions. *J. Membrane Biol.* **21**:291

Eigen, M., De Maeyer, L. 1963. Relaxation methods. *In:* Techniques of Organic Chemistry. A. Weissberger, editor. Vol. 8, p. 895. Interscience, New York

Eisenberg, M., Hall, J.E., Mead, C.A. 1973. The nature of the voltage-dependent conductance induced by alamethicin in black lipid membranes. *J. Membrane Biol.* **14**:143

Fishman, H.M. 1973. Relaxation spectra of potassium channel noise from squid membranes. *Proc. Nat. Acad. Sci. USA* **70**:876

Fishman, H.M., Moore, L.E., Poussart, D.J.M. 1975. Potassium-ion conduction noise in squid axon membrane. *J. Membrane Biol.* **24**:305

Fishman, H.M., Poussart, D.J.M., Moore, L.E. 1975. Noise measurements in squid axon membrane. *J. Membrane Biol.* **24**:281

Gordon, L.G.M., Haydon, D.A. 1972. The unit conductance channel of alamethicin. *Biochim. Biophys. Acta* **255**:1014

Katz, B., Miledi, R. 1972. The statistical nature of the acetylcholine potential and its molecular components. *J. Physiol.* **224**:665

Kolb, H.A., Läuger, P., Bamberg, E. 1975. Correlation analysis of electrical noise in lipid bilayer membranes. Kinetics of gramicidin A channels. *J. Membrane Biol.* **20**:133

Kubo, R. 1957. Statistical mechanical theory of irreversible processes. I. General theory and simple applications to magnetic and conduction problems. *J. Phys. Soc. Jpn.* **12**:570

Landau, L.D., Lifschitz, E.M. 1958. Statistical Physics, p. 350, Pergamon Press, London

Lax, M. 1960. Fluctuations from the nonequilibrium steady state. *Rev. Mod. Phys.* **32**:25

Mauro, A., Nanavati, R.P., Heyer, E. 1972. Time variant conductance of bilayer membranes treated with monazomycin and alamethicin. *Proc. Nat. Acad. Sci. USA* **69**:3742

Muller, R.U., Finkelstein, A. 1972. Voltage-dependent conductance induces in thin lipid membranes by monazomycin. *J. Gen. Physiol.* **60**:263

Rice, S.O. 1944. Mathematical analysis of random noise. *Bell Syst. Tech. J.* **23**:282

Tolman, R.C. 1938. The Principles of Statistical Mechanics, p. 636. Oxford University Press, New York

Verveen, A.A., DeFelice, L.J. 1974. Membrane noise. *Prog. Biophys. Mol. Biol.* **28**:189

Wanke, E. 1975. Monazomycin and nystatin channel noise. *Abst. Int. Biophys. Congr.*, p. 368

Wanke, E., DeFelice, L.J., Conti, F. 1974. Voltage noise, current noise and impedance in space clamped giant axons. *Pfluegers Arch.* **341**:63

Zingsheim, H.P., Neher, E. 1974. The equivalence of fluctuation analysis and chemical relaxation measurements: A kinetic study of ion pore formation in thin lipid membranes. *Biophys. Chem.* **2**:197