STRUCTURAL FLUCTUATIONS AND CURRENT NOISE OF IONIC CHANNELS

P. LÄUGER
Department of Biology, University of Konstanz, D-7750 Konstanz, Federal Republic of Germany

ABSTRACT In the open state of the acetylcholine-receptor channel an increased current noise is observed, which may result from conformational fluctuations of the channel protein (Sigworth, F. J., 1985, *Biophys. J.* 47:709–720). In this study the spectrum of the current noise is analyzed assuming that low-frequency motions of structural domains of the protein give rise to conductance fluctuations. The movement of a domain is treated as the motion of an elastically bound Brownian particle, which is described by the Langevin equation. The current-noise spectrum predicted by this model is given by a sum of Lorentzians; it agrees with the observed spectrum when it is assumed that only the slowest process can be resolved in the experiment. The large value of the friction coefficient, which is obtained from the corner frequency, indicates that domain motion is restricted mainly by peptide-peptide interactions.

INTRODUCTION

Acetylcholine-activated channels exhibit an increased current noise in the open state (Sigworth, 1982, 1985; Auerbach and Sachs, 1984). The spectral intensity, $S_I(f)$, of the excess noise is proportional to the square of mean channel current and can be fitted (in the spectral range between 20 Hz and 5 kHz) by the sum of a Lorentzian and a constant term S_∞ (Sigworth, 1985):

$$S_I(f) = \frac{S_1}{1 + f^2/f_c^2} + S_\infty. \quad (1)$$

At 9°C and a membrane potential of −100 mV, the corner frequency, f_c, is ~350 Hz, corresponding to a correlation time $\frac{1}{2}\pi f_c$ of ~0.5 ms. As pointed out by Sigworth (1985), $S_I(f)$ is much larger than the intensity of shot noise, which results from the statistical nature of the discrete charge translocations in the channel (Stevens, 1972; Läuger, 1975; Frehland and Faulhaber, 1980). Moreover, from the observed amplitude distribution it appears improbable that the excess current noise is generated by unresolved transitions between two closely spaced conductance substates. A more likely explanation (Sigworth, 1985) is that the fluctuations of channel current reflect low-frequency fluctuations in the conformation of the channel protein.

It is obvious that current noise resulting from structural fluctuations contains interesting information on the dynamics of proteins. Numerous studies have shown that internal motions of proteins occur in a wide range of correlation times (Frauenfelder et al., 1979; Gurd and Rothgeb, 1979; Parak et al., 1981; Karplus and McCammon, 1983; Wagner, 1983; Petsko and Ringe, 1984). Fast motions on the subnanosecond time scale are associated with elastic properties of the protein backbone or result from rotations of single amino-acid residues (McCammon et al., 1979; Munro et al., 1979; Gô et al., 1983; Chou, 1984; Brooks and Karplus, 1983). It is clear, however, that structural fluctuations that are responsible for conductance variations in milliseconds must be different. They are likely to represent quasi-continuous collective motions within the channel protein.

X-ray analysis has shown that many proteins consist of domains, compact globular units that are only loosely connected with each other (Schulz and Schirmer, 1979; Janin and Wodak, 1983). Movements of domains relative to each other have been demonstrated for a number of enzymes such as alcohol dehydrogenase or hexokinase (Janin and Wodak, 1983). Rigid structural units that are linked by flexible segments play an important role in the function of immunoglobulins (Huber et al., 1976). In protein crystallography, domains are usually defined in terms of packing density. In the following we consider a domain simply as a dynamic unit capable of low-frequency collective motion. A dynamic unit (defined in this way) can be as small as a single α-helix or (in the case of an oligomeric channel) as large as a whole protein subunit. For convenience we shall use the term domain in all these cases.

LANGEVIN TREATMENT OF STRUCTURAL FLUCTUATIONS

The movement of a domain in a channel protein is governed by strong frictional forces resulting from interactions with other parts of the protein and with the surrounding lipid and water phases. Under the influence of random thermal forces a domain carries out a kind of restricted Brownian motion in a small volume of space (Karplus and McCammon, 1981). A simple dynamical model of a domain thus consists in an elastically bound particle of mass, m, which is embedded in a viscous medium. The motion of such a particle under the influence of a statistical

force, $F(t)$, in a space coordinate x is described by the Langevin equation (Langevin, 1908; Chandrasekhar, 1943; Adelman, 1980)

$$m\ddot{x} + \beta\dot{x} + \gamma x = F(t). \tag{2}$$

$\dot{x}$ and $\ddot{x}$ are the first and second derivatives with respect to time t. The first term of the equation represents the inertial force, the second represents the frictional force, and the third the elastic restoring force (β is the frictional coefficient and γ the elastic force constant). In using Eq. 2 the implicit assumption is made that variations in $F(t)$ occur on a much shorter time scale than variations in x. The Langevin equation has recently been applied to the description of side chain rotations in proteins (McCammon et al., 1979; Swaminathan et al., 1982).

For the analysis of Eq. 2 we assume that the friction coefficient, β, is large so that the particle behaves as an overdamped oscillator (this assumption is substantiated below). As shown in the Appendix, the spectral intensity of fluctuations in domain position x is then given by ($\omega \equiv 2\pi f$)

$$S_x(\omega) = \frac{4\overline{x^2}\,\beta/\gamma}{(1 + \omega^2\tau_a^2)(1 + \omega^2\tau_b^2)} \tag{3}$$

$$\tau_a = (1 - \sqrt{1 - q})\,\beta/2\gamma \tag{4}$$

$$\tau_b = (1 + \sqrt{1 - q})\,\beta/2\gamma \tag{5}$$

$$q \equiv 4\,m\gamma/\beta^2. \tag{6}$$

The mean square of the displacement x is directly obtained from the equipartition theorem as

$$\overline{x^2} = \int_0^\infty S_x(f)df = kT/\gamma, \tag{7}$$

where k is Boltzmann's constant and T the absolute temperature. If the time constants τ_a and τ_b are to assume real values, corresponding to a nonoscillatory (overdamped) behavior, the parameter q must be <1. An upper limit for q may be estimated in the following way. The domain is assumed to be a sphere of radius $r \simeq 1$ nm and density $\rho \simeq 1$ g/cm^3 that fluctuates around $x = 0$ with a mean amplitude $\sqrt{\overline{x^2}} \simeq 0.2$ nm. This corresponds to $m \simeq 4.2 \cdot 10^{-21}$ g and $\gamma \simeq 0.1$ J/m^2. A lower limit for the friction coefficient β is obtained from the Stokes relation $\beta = 6\pi\eta r$, introducing for η the viscosity of water; this gives $\beta > 2 \cdot 10^{-11}$ J · s/m^2. (In reality, β may be much larger than this value since interactions of the domain with other parts of the protein and with the lipid matrix may result in a much higher effective viscosity). With these values of m, γ, and β an upper limit of q is given by $q < 4 \cdot 10^{-3}$.

Since $q << 1$, the time constant τ_a (Eq. 4) becomes very small, whereas τ_b may be approximated by β/γ. The spectral intensity $S_x(\omega)$ is therefore simply given by

$$S_x(\omega) = \frac{4\overline{x^2}\tau}{1 + \omega^2\tau^2} \tag{8}$$

$$\tau = \beta/\gamma. \tag{9}$$

This means that $S_x(\omega)$ becomes independent of domain mass m, an expected result in the limit of strong damping.

To calculate the spectral intensity $S_I(\omega)$ of the current, we introduce the parameter w, which describes the dependence of single-channel conductance, Λ, on displacement x:

$$w \equiv \left(\frac{d\Lambda}{dx}\right)_{x=0}. \tag{10}$$

If V is the total driving force expressed as a voltage, the amplitude δI of current fluctuations may be expressed by the displacement x according to $\delta I = V\,\delta\Lambda = wVx$. Thus,

$$S_I(\omega) = w^2V^2S_x(\omega). \tag{11}$$

In general, the movements of several domains may contribute to fluctuations of I. Assuming, to a first approximation, that the movement of each domain is independent of the motions of the other domains, the fluctuation of channel current may be expressed by

$$\delta I = V\sum_i w_i x_i. \tag{12}$$

The parameters w_i are defined in analogy to Eq. 10, and the summation is carried out over all domains of the channel protein. The directions of the coordinate axis x_i are chosen in such a way that $d\Lambda/dx_i$ represents the maximum contribution of domain i to the conductance fluctuation; in this approximate treatment motions of the domain in other space directions are neglected. Thus, in analogy to Eqs. 7–9 and 11 one finally obtains

$$S_I(\omega) = 4V^2\sum_i \frac{\overline{x_i^2}\,\tau_i w_i^2}{1 + \omega^2\tau_i^2} \tag{13}$$

$$\tau_i = \beta_i/\gamma_i; \quad \overline{x_i^2} = kT/\gamma_i. \tag{14}$$

According to Eq. 13, the spectrum of current fluctuations consists of a sum of Lorentzians with corner frequencies $f_i = 1/2\pi\tau_i$. In the experiments of Sigworth (1982, 1985) only one Lorentzian with $\tau_1 \simeq 0.5$ ms is observed, whereas $S_I(\omega)$ approaches a finite value at higher frequencies (Fig. 1). On the basis of Eq. 13 this finding may be explained assuming that in the experimentally accessible frequency range all terms $\omega^2\tau_i^2$ are small compared with 1 for i = 2, 3, ... In this case Eq. 13 reduces to

$$S_I(\omega) = \frac{S_1}{1 + \omega^2\tau_1^2} + S_\infty \tag{15}$$

$$S_1 = 4V^2\overline{x_1^2}\tau_1 w_1^2 \tag{16}$$

$$S_\infty = 4V^2\sum_{i>1}\overline{x_i^2}\tau_i w_i^2. \tag{17}$$

The form of the spectrum predicted by Eq. 15 is identical with the experimentally observed spectrum (Eq. 1).

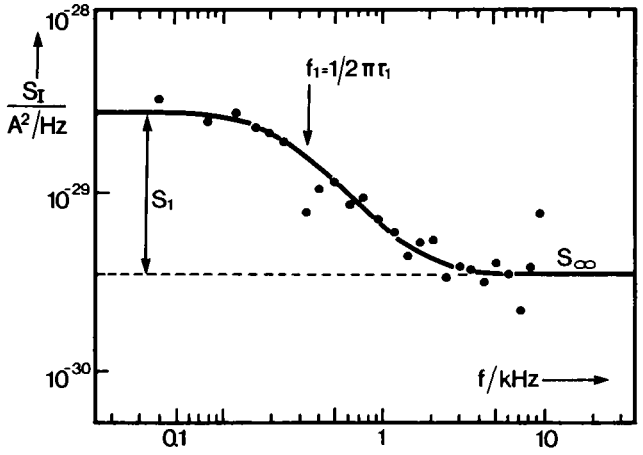

FIGURE 1 Spectral intensity of current fluctuations in the open state of the acetylcholine-activated channel. The experimental points have been taken from Sigworth (1985, Fig. 3 *C*), the line represents the spectrum predicted by Eq. 15 (or Eq. 1) with $f_1 = f_c = 350$ Hz, $S_1 = 2.36 \cdot 10^{-29} A^2/Hz$, $S_\infty = 3.7 \cdot 10^{-30} A^2/Hz$.

DISCUSSION

In this study a semiphenomenological treatment of current noise in the open state of ionic channels has been given. The analysis has been based on the assumption that the movements of structural domains of the channel protein may be described as the motions of independent, elastically bound Brownian particles. The current-noise spectrum predicted for such a system is represented by a sum of Lorentzians with corner frequencies that are determined by the friction coefficients β_i and the elastic force constants γ_i of the domains.

Estimates of Model Parameters

To obtain an estimate of the parameters β_1, γ_1, and w_1 of Eqs. 15 and 16, we assume that the acetylcholine-receptor channel consists of wide, water-filled entrance pores in series with a narrow part acting as a selectivity filter (Kistler et al., 1982). We further assume that structural fluctuations responsible for the current noise occur in the region of the wide access pores where domains may be more loosely bound than in the rest of the channel.

Fluctuations of domain position may affect the electrical series resistance represented by the access pores and/or may be mechanically transmitted to the selectivity filter. For an order-of-magnitude estimate of the force constant γ we choose the amplitude of displacement to be $\sqrt{\overline{x_1^2}} \simeq 0.2$ nm, which yields $\gamma_1 = kT/\overline{x_1^2} \simeq 0.1$ J/m^2 (see above). With the experimental value $\tau_1 = \beta_1/\gamma_1 \simeq 0.45$ nm (corresponding to a corner frequency $f_1 = 1/2\pi\tau_1 \simeq 350$ Hz) one obtains for the friction coefficient $\beta_1 \simeq 5 \cdot 10^{-5}$ J $\cdot$ s/m^2. It is instructive to translate this value of β_1 into an equivalent viscosity η using Stokes' law. With a domain radius of $r \simeq 1$ nm one finds $\eta = \beta_1/6\pi r \simeq 2 \cdot 10^3$ J $\cdot$ s/m$^3 \simeq 2 \cdot 10^4$ poise. This value is by four orders of magnitude larger than the effective viscosity of a lipid membrane, which is in the range of 1-10 poise (Quinn, 1981). It is therefore likely to assume that the friction experienced by the moving domain is mainly determined by peptide-peptide interactions, which make a much larger contribution to β_1 than peptide-lipid or peptide-water contacts.

Is this large value of the friction coefficient physically reasonable? So far it is difficult to compare the value of β_1 with other experimental parameters of proteins. From the observation that small molecules such as O_2 can easily move within a protein (Lakowicz and Weber, 1973), it has sometimes been inferred that the interior of a protein is analogous to a liquid of low viscosity. But this comparison is misleading. For instance, a small molecule may diffuse through a gel at a high rate, although the viscosity of the gel is virtually infinite. The motion of a domain within a protein involves a large number of simultaneous conformational rearrangements, some of which may have a high activation energy. Brownian motion of the domain may therefore be visualized as the motion of a large particle in a viscoelastic polymer with a viscosity that may be many orders of magnitude higher than the viscosity of an ordinary liquid (Ferry, 1970).

With the experimental value $S_1 \simeq 2.4 \cdot 10^{-29}$ A^2/Hz at $V = -100$ mV (Sigworth, 1985), the coefficient $w_1 = d\Lambda/dx_1$ is estimated from Eq. 16 to be ~5 pS/nm, which corresponds (with $\Lambda \simeq 30$ pS) to a relative conductance variation of 0.17 nm^{-1}. This value (which depends on the choice of $\overline{x_1^2}$) is not unreasonable if the conductance fluctuations result from large-scale domain motions in the entrance region of the channel.

For the experimentally observed voltage dependence of the corner frequency (Sigworth, 1985) a straightforward interpretation cannot be given so far. According to Eq. 14, the corner frequency $f_1 = 1/2\pi\tau_1$ is equal to $\gamma_1/2\pi\beta_1$. Since an electric field in the membrane may change the equilibrium positions of the domains, both the friction coefficient β_1 as well as the elastic force constant γ_1 may be influenced by a variation of voltage.

Amplitude Distribution of Current Fluctuations

The mechanism discussed here leads to a Gaussian (normal) distribution of current amplitudes, in agreement with the experimental results (Sigworth, 1985). This may be shown in the following way. The probability of finding domain i between x_i and $x_i + dx_i$ is equal to $W(x_i)dx_i$, where $W(x_i)$ is given by Boltzmann's equation

$$W(x_i) = C \cdot \exp(-\gamma_i x_i^2/2kT) = C \cdot \exp(-x_i^2/2\overline{x_i^2}). \quad (18)$$

$\gamma_i x_i^2/2$ is the potential energy associated with a displacement x_i and C is a constant. According to Eq. 12 the current fluctuations δI are linear combinations of the x_i. Since the variables x_i have a Gaussian distribution (Eq. 18) and are independent (according to the assumption introduced above), the distribution of current amplitudes δI is

also Gaussian (Walpole and Myers, 1972)

$$W(\delta I) = \frac{\exp\left[-(\delta I)^2/2\overline{(\delta I)^2}\right]}{\sqrt{2\pi\overline{(\delta I)^2}}}. \quad (19)$$

The variance of the distribution, $\overline{(\delta I)^2}$, is related to the spectral parameters of Eq. 13

$$\overline{(\delta I)^2} = \sum_i \frac{S_i}{4\tau_i}; \quad S_i \equiv 4V^2\overline{x_i^2}\tau_i w_i^2. \quad (20)$$

For a quantitative comparison of Eq. 19 with the experimental values of $W(\delta I)$, the background noise, which tends to broaden the distribution, has to be taken into account.

CONCLUSION

In the foregoing analysis the process leading to low-frequency current noise in the acetylcholine-receptor channel has been treated as a quasi-continuous collective motion of parts of the channel protein. This treatment is different from the more familiar description of open-channel conductance fluctuations based on transitions between discrete conformational states (Läuger, 1985). The difference is more of a formal nature, however. In fact, a system carrying out transitions between many closely spaced states approaches a quasi-continuous behavior. The main advantage of the (semiphenomenological) Langevin treatment is its simplicity. Whereas the Langevin analysis contains only three model parameters (β_1, γ_1, and w_1), a treatment based on transitions between several discrete states involves many more parameters (rate constants and conductance levels) that are not directly accessible from the experiment.

APPENDIX

Derivation of Eq. 3

The motion of an elastically bound Brownian particle is described by the Langevin equation (Eq. 2). In the following we consider the overdamped case in which $4\ m\gamma/\beta^2 < 1$. The average position x at time t of a particle that had position x_0 at $t = 0$ is given by (Chandrashekar, 1943)

$$\langle x(t)\rangle_{x(0)=x_0} = x_0 Q(t) \quad (A1)$$

$$Q(t) \equiv \exp\left(-\frac{\beta t}{2m}\right)\cdot\left[\cosh\left(\frac{\beta s}{2m}t\right) + \frac{1}{s}\sinh\left(\frac{\beta s}{2m}t\right)\right] \quad (A2)$$

$$= \frac{1}{2s}\left[(s-1)\exp\left(-t/\tau_a\right) + (s+1)\exp\left(-t/\tau_b\right)\right] \quad (A3)$$

$$s \equiv \sqrt{1 - 4\ m\gamma/\beta^2} \quad (A4)$$

$$\tau_a \equiv \frac{\beta}{2\gamma}(1-s); \quad \tau_b \equiv \frac{\beta}{2\gamma}(1+s) \quad (A5)$$

The autocorrelation function $C_x(t) \equiv \langle x(0)x(t)\rangle$ may be directly obtained from Eq. A1 by averaging over all positions x_0:

$$C_x(t) = \langle x_0 \cdot \langle x(t)\rangle_{x(0)=x_0}\rangle = \overline{x^2}\,Q(t). \quad (A6)$$

The spectral intensity $S_x(\omega)$ is related to $C_x(t$ by the Wiener-Khintchine theorem (van der Ziel, 1970)

$$S_x(\omega) = 4\int_0^\infty C_x(t)\cos\omega t dt. \quad (A7)$$

Carrying out the integration yields

$$S_x(\omega) = \frac{4\overline{x^2}}{\tau_b - \tau_a}\left(\frac{\tau_b^2}{1+\omega^2\tau_b^2} - \frac{\tau_a^2}{1+\omega^2\tau_a^2}\right). \quad (A8)$$

This proves Eq. 3.

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