RATE-AMPLITUDE CORRELATION FROM SINGLE-CHANNEL RECORDS

A Hidden Structure in Ion Channel Gating Kinetics?

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ABSTRACT Lifetime probability density functions from single-channel recording are usually assumed to follow a multiexponential form. The amplitudes and rate constants for each expoenential component are presumed to be independent. We have explored this assumption and have found a correlation between the amplitudes and the rate constants in certain cases. We examine this correlation and the possibility that other functional forms may also properly describe these distribution functions.

Single-channel recording provides us with a unique view of the unimolecular dynamics of ion channel proteins. By counting the number of channel events (i.e., openings or closings) of similar duration and plotting that count versus duration time, one can construct a histogram that is an approximate representation of the probability density function (pdf), $f(t)$, for the lifetime of a particular conducting state (Sakmann and Neher, 1983; Millhauser and Oswald, 1988). The usual assumption is that channels can exist in a small number of conformational states and that the transitions among the states are Markovian in nature. In such cases $f(t)$ will be multiexponential. Data from these lifetime histograms, therefore, are fit to an n-component pdf of the form

$$f(t) = a_1 k_1 e^{-k_1 t} + a_2 k_2 e^{-k_2 t} + \ldots + a_n k_n e^{-k_n t} \qquad (1)$$

and the amplitudes, a_i, and the associated rate constants (or more generally, the decay constants), k_i, are presumed to be independent. (For the following discussion we assume the rate constants are ordered such that $k_i > k_{i+1}$.) Along with such a presumption, the rate constants are usually interpreted to be the eigenvalues of a kinetic matrix. Likewise, each amplitude, a_i, is interpreted as an area which is proportional to the number of events with an average lifetime k_i^{-1} (Sakmann and Neher, 1983). Such interpretations cannot assume any *apriori* relationship between these rate constants and amplitudes because simple kinetic models constructed from n states can be made to fit any distribution described by an arbitrary set of n positive a_i's and k_i's.

By examining the relationship between the amplitudes and rate constants from a range of systems reported in the literature, we have found that these quantities are often not independent. In a number of cases they appear to follow a relationship of the form

$$a_i \propto k_i^p, \qquad (2)$$

where $p \approx \frac{1}{2}$ for closed time distributions. This empirical rate-amplitude correlation suggests an underlying dynamic structure to ion channel gating.

We focus our attention on the closed time distributions. The rich structure of closed time pfd's often require several components (large n) for accurate fitting. In our literature search we studied examples where three or more components were required for fitting. Furthermore, in the case of ligand gated channels, we limited our examinations to cases where the channels were exposed to saturating quantities of agonist so that the pdf was mostly controlled by channel gating dynamics and not ligand binding kinetics. First we consider the fast chloride channel (Blatz and Magleby, 1986). To our knowledge this is one of the most well resolved distributions in the literature—the experimental pdf is constructed from over 40,000 events. The data range over four orders of magnitude in time and the authors fit the distribution to a sum of five exponential functions ($n = 5$). In Fig. 1 *a* we show a logarithmic plot of the reported amplitudes versus rate constants for this system. The linear relationship is clear with $p = 0.65$ thus

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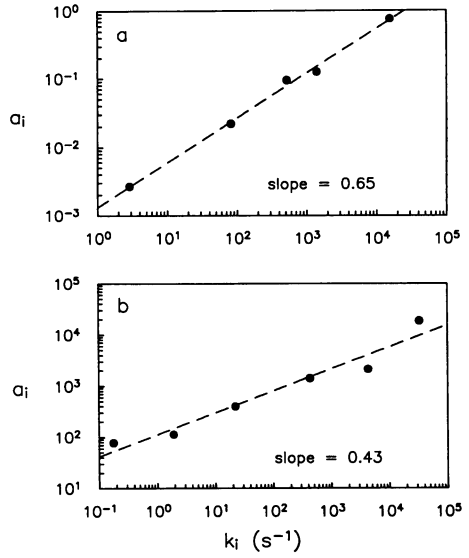

FIGURE 1 Rate-amplitude correlations for (*a*) the fast Cl^- channel from Blatz and Magleby, 1986 and (*b*) the acetylcholine receptor with [ACh] = 1.0 mM from Sine and Steinbach, 1987. The quantities a_i and k_i, which are defined by Eq. 1, are the values reported by these authors. The linear nature of these plots is consistent with the hypothesis of Eq. 2. A linear least-squares fit, represented by the dashed line, yields exponents of 0.65 and 0.43, respectively.

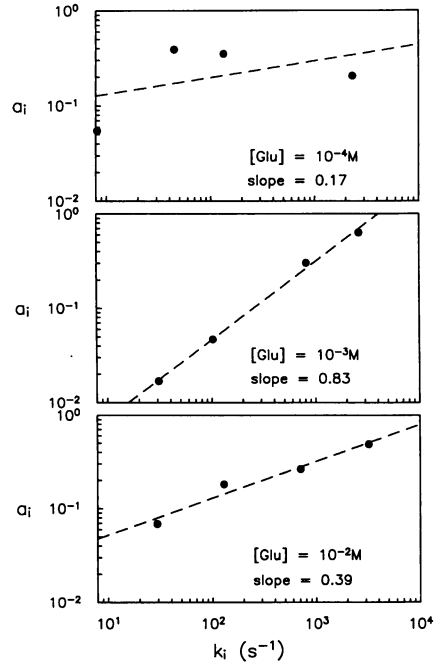

FIGURE 2 Rate-amplitude correlation for the glutamate receptor as a function of [Glu] from Kerry et al., 1988. The correlation only emerges at higher concentrations. This is also the case for the other ligand-gated channels considered here.

establishing the correlation between a_i and k_i as expressed in Eq. 2. A second example is provided by studies on the nicotinic acetylcholine receptor (AChR) at high concentrations of acetylcholine (ACh) (Sine and Steinbach, 1987). In this case the temporal range was five orders of magnitude and the data were fit to six exponentials. Fig. 1 *b* is the logarithmic a_i versus k_i plot for the case of [ACh] = 1.0 mM. Again we find a strong correlation with $p = 0.43$. These data and the results for two other ligand-gated channels are summarized in Table I. It is interesting to note that the various values for p seem to be clustered around ½. In cases where good data exist as a function of agonist concentration, a relationship of the form of Eq. 2 is a good fit to the data only for large concentrations. At low concentrations of agonist the fit is worse and the mean value of p can be appreciably larger or smaller than ½ (see Fig. 2).

TABLE I
EXPONENTS FROM THE RATE-AMPLITUDE CORRELATION FOR SEVERAL ION CHANNEL SYSTEMS

Channel type	p
Fast Cl^- channel*	0.65
Acetylcholine receptor‡	0.43
Ca^{++}-activated K^+ channel§	0.71
Glutamate receptor‖	0.39

*Blatz and Magleby, 1986.
‡Sine and Steinbach, 1987 with [Ach] = 1.0 mM.
§Magleby and Pallotta, 1983 with [Ca^{++}] = 0.75 μM.
‖Kerry et al., 1988 with [Glu] = 10.0 mM.

A correspondence exists between the rate-amplitude correlation described above for the different types of channels and approximate power law distributions. Fig. 3 *a* shows how such an approximate power law distribution can be constructed from a sum of regularly spaced exponential functions. For any value of t, the amplitude of $f(t)$ is mainly determined from the ith exponential component such that k_i^{-1} is of order t. As this ith exponential component decays (i.e., when $t > k_i^{-1}$), the corresponding loss in amplitude of $f(t)$ is $a_i k_i = k_i^{1+p}$ by Eq. 2. After this analysis, the relationship expressed in Eq. 2 leads to a different form for the pdf which is

$$f(t) \propto t^{-(1+p)} \tag{3}$$

in cases where the spacing of rate constants, k_i, is sufficiently small, the total number, n, of rate constants is sufficiently large and $t > k_1^{-1}$. For the fast chloride channel our fits to p should give $f(t) \propto t^{-1.65}$ and, for the AChR, $f(t) \propto t^{-1.43}$. The other channels shown in Table I exhibit similar closed time behavior.

Given a multiexponential form for $f(t)$ as in Eq. 1, with the relationship in Eq. 2 satisfied, the deviations from a power law form, as expressed in Eq. 3, depend on the spacing of the rate constants k_i. We express these deviations in a phenomenological function $g(t)$, which is $f(t)$ with the power law trend divided out

$$g(t) \equiv f(t)\, t^{(1+p)}. \tag{4}$$

With large but fairly uniform spacing (on a logarithmic

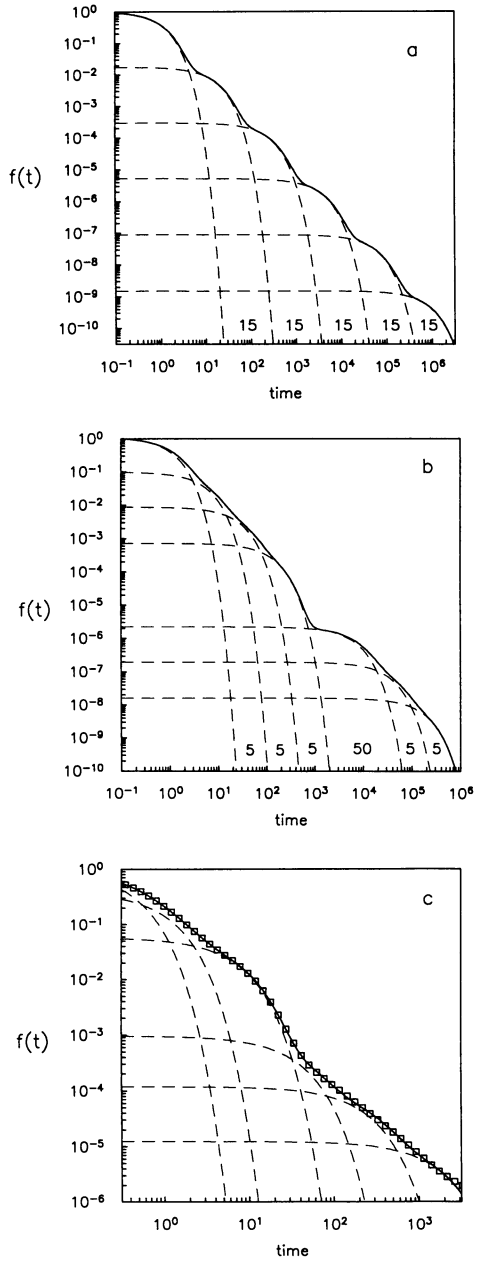

FIGURE 3 A demonstration of how the rate-amplitude correlation of Eq. 2 leads to an approximate power law of the form $f(t) \propto t^{-(1+p)}$ (Eq. 3). The dashed lines in each figure represent each individual exponential component of Eq. 1. (*a*) The rate constants are chosen so the ratio $k_i/k_{i+1} = 15$ which gives equal spacing between the components on this logarithmic plot. The number of glitches is approximately equal to the number of exponential components. (*b*) The ratio of sequential rate constants varies as indicated. When $k_i/k_{i+1} = 5$, $g(t)$ (see Eq. 4) is nearly constant. In this distribution, the only deviation in $g(t)$ is when the ratio changes to 50. (*c*) A simulation of the diffusion model with a single constriction proposed by Millhauser et al., 1988 (*symbols*) and the best fit to Eq. 1 with $n = 6$. Such a model is also well described by a power law multiplied by phenomenological $g(t)$ containing one glitch.

scale), such as $k_i/k_{i+1} \approx 10$ or greater, as many scallops or glitches are evident in $g(t)$ as there are constants k_i. An illustration can be seen in Fig. 3 *a*. In such cases the number of parameters, a_i and k_i, needed for a purely empirical multiexponential fit, of the form of Eq. 1, is probably as small or smaller than for other analytic forms. By contrast, Fig. 3 *b* illustrates a case where $k_i/k_{i+1} = 5$ for a number of intervals, $i \rightarrow i + 1$, with an intermediate break where this ratio is ten times larger. In such a case, $g(t)$ is almost constant for large (logarithmic) intervals of time with only an occasional scallop or glitch. For such cases, a purely empirical fitting formula to $g(t)$ can probably be found with fewer parameters needed than for Eq. 1.

We have recently suggested a diffusion model for channel gating (Millhauser et al., 1988) which, in its simplest form with uniform rate constants, gives $g(t)$ constant (except that $g(t) \rightarrow 0$ as $t \rightarrow 0$). Such a model predicts

$$f(t) \approx (1 + 2kt)^{-1.5}, \tag{5}$$

where k is a rate constant sequentially connecting a large number of states all of similar energy. (We further postulated that k^{-1} is smaller than the patch clamp time-scale so that for all measurable times $kt \gg 1$ and thus $f(t) \propto t^{-1.5}$). This model, modified with an occasional constriction in the coefficients, will give a $g(t)$ with corresponding glitches. In Fig. 3 *c* we show a numerical simulation of this case with a single constriction. The entire distribution is parametrized by three terms: the intrinsic rate constant (k), the location of the constriction, and the change of the rate constant just at the constriction. In contrast, fitting this distribution to Eq. 1 requires an n of six.

It is clear that the closed time distributions considered here exhibit the rate-amplitude correlation of Eq. 2. Such relationships are not predicted by unconstrained models constructed from arbitrary rate constants. It is striking to note that when the rate-amplitude correlation applies, as in the case of the ligand-saturated channels and the chloride channel considered here, the exponent, p, has very similar values which give an exponent (i.e., $1 + p$) similar to that in Eq. 5. The existence of this relationship is strong evidence for a specific underlying structure to the ion channel gating process. An understanding of this relationship, and the power law trend, may provide a physical basis for channel gating and a universal structure for the gating mechanisms among disparate classes of channel proteins. The physical basis may be related to the α-helix melting transition which can be described as a one-dimensional diffusion process in the configuration space of hydrogen bonds. It is also interesting that fractal-time processes used to describe nonexponential relaxation in dielectrics yield relaxation profiles similar to Eq. 1 with the added constraint of Eq. 2 (Shlesinger, 1984). Likewise, the recently proposed Fractal Model (Leibovitch et al., 1987) will yield power law behavior when the fractal dimension approaches an upper limit ($D \rightarrow 2$) although the actual exponent is not

specified from their theory. From a somewhat different experimental perspective, similar power law relaxation profiles have also been observed in the recombination of CO with myoglobin at low temperatures (Ansari et al., 1985, and references therein).

We are grateful to Dr. Karl Magleby for a number of helpful comments. We are also grateful to Ms. Alison Carter for proofreading the manuscript and for helpful comments.

This work was supported by National Institutes of Health grants 1 F32 NS 07871-03 (received by Dr. Millhauser) and 1 R01 NS 18660-04 NEUB and the Muscular Dystrophy Association (received by Dr. Oswald).

Received for publication 17 June 1988 and in final form 15 August 1988.

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