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Determining the kinetics of membrane pores from patch clamp data without measuring the open and closed times

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We present a new and very time saving way to determine kinetic rate constants from patch clamp data by using the correlation functions utilized in analyzing experiments with photon correlations.

In a patch clamp experiment a small micron sized piece of cell membrane is sealed into a micropipette. Pores in the membrane are constantly fluctuating open and closed. If there is an electrochemical gradient across the membrane, a small, but measurable current can be observed flowing through a transitorily open pore. Since only one or a few pores will be in the piece of membrane held by the pipette, these current events appear as roughly square shaped quantized pulses. Typically, a pore might open once in 100 ms for a duration of about 10 ms during which time about 100 000 ions, or about 1 pA will flow through the pore across the membrane. These techniques are comprehensively reviewed in a recent book [1].

If a pore has only one closed and one open state, then it may be represented by the reaction

$$\text{closed} \underset{k_c}{\overset{k_o}{\rightleftharpoons}} \text{open}$$

where k_o and k_c are the rate constants for opening and closing. These rate constants contain important information about the pores. For example, they can be used to determine the difference in energy ΔE between the closed and open states of the pore, namely

$$k_o/k_c = \exp(-\Delta E/RT)$$

where R is the gas constant and T the absolute temperature.

Currently, the way to determine k_o and k_c has been to examine the current as a function of time and ascertain when the pore was open and when it was closed. Then the length of each of these events is measured and a frequency histogram is plotted. It has been shown [2] that the distribution of open times of the pore is given by

$$P_o(t) = k_c \exp(-k_c t)$$

and the distribution of closed times is given by

$$P_c(t) = k_o \exp(-k_o t)$$

Thus, by fitting these functions to the frequency histogram measured from the data, k_o and k_c can be determined.

However simple in theory, this method is subject to many practical problems. The recorded current signal is often quite noisy, much work and/or complex algorithms are needed to identify the current events that correspond to the pore openings. Moreover, transitions between the open and closed states that appear as only partial due to the limited frequency response of the electronics are very difficult to differentiate from background noise, and there is a possibility that faulty interpretation of such events might affect the analysis.

We present here an entirely different way of analyzing the current signal, $F(t)$, from a patch clamp experiment. In this new method it is not necessary to reconstruct the open and closed events in the data record in order to determine k_o and k_c. Instead, the current values as a function of time, stored in the computer can themselves be analyzed. As we show below, certain statistical properties of the signal can then be used to evaluate k_o and k_c. Hence, this new method yields k_o and k_c without the judgements needed to separate square events from a complex signal. Thus, this new method cannot be biased by possible faulty interpretations from the recorded current signal.

This new method is a new adaptation of mathematical techniques previously only used to analyze photon correlations [3,4]. Let the current fluctuation around the mean, $f(t)$, be defined by $f(t) = F(t) - \langle F(t) \rangle$, where $\langle\rangle$ denotes the time average over the observation period T, that is,

$$\langle F(t) \rangle = (1/T) \int_{t=0}^{T} F(t) \mathrm{d}t.$$

The correlation functions are then given by

$$g_1(\tau) = \langle [f(t)][f(t+\tau)] \rangle / \langle [f(t)]^2 \rangle$$

and

$$g_2(\tau) = \langle [f(t)]^2 [f(t+\tau)]^2 \rangle / \langle [f(t)]^2 \rangle^2.$$

These correlation functions can be understood in the following intuitive way. The deviations $f(t)$ of the signal around its mean value are first raised to some power, n. Then $f^n(t)$ is shifted in time by a delay τ. Then at each time, the original and shifted signals are multiplied and the results of those multiplications are then summed. If the delay time τ is short compared to the pore kinetics, that is the opening and closing of the pore, then the current $f(t+\tau)$ will correlate with that at $f(t)$. Thus the average of the fluctuations $\langle f(t) f(t+\tau) \rangle$ and $\langle f^2(t) f^2(t+\tau) \rangle$ will be high and so $g_1(\tau)$ will be nearly equal to 1 and $g_2(\tau)$ will be greater than one. On the other hand, if the delay time τ is long compared to the pore kinetics, then the current $f(t+\tau)$ will correlate only weakly with that at $f(t)$. Then the fluctuations $\langle f(t) f(t+\tau) \rangle$ will average nearly to zero while the square of those fluctions $\langle f^2(t) f^2(t+\tau) \rangle$ will be nearly equal to $\langle f^2(t) \rangle \langle f^2(t) \rangle$. Hence, $g_1(\tau)$ will be nearly equal to zero while $g_2(\tau)$ will be nearly equal to 1. Thus, the change of $g_1(\tau)$ from 1 to 0, and $g_2(\tau)$ from some higher value to 1, with increasing τ provides information about the time scale of the pore kinetics.

The function $g_1(\tau)$ is a normalized version of the autocorrelation function which is often used in signal analysis [5]. The Fourier transform of the autocorrelation function is the power spectrum, the amount of power in the signal at each frequency. Thus, $g_1(\tau)$ contains similar information to that found in the power spectrum, that is, it is sensitive to the power of the fluctuations of the current at different frequencies. On the other hand, the function $g_2(\tau)$, used in photon correlations to measure the light intensity, is here sensitive to the statistical distribution of those fluctuations. For example, if the signal has fluctuations that have a Gaussian distribution, that is, if the probability of observing any signal level f is given by

$$P(f) = \left(1/\sqrt{2\pi\langle [f(t)]^2 \rangle}\right) \exp\left(-f^2/2\langle [f(t)]^2 \rangle\right)$$

then it can be shown [6,7] that $g_2(\tau) = 1 + 2[g_1(\tau)]^2$. If that condition is met, then $g_2(\tau)$ and all the higher correlation moments are known functions of the mean and $g_1(\tau)$; thus, $g_n(\tau)$ for $n \geq 2$ do not provide additional informationabout the statistical properties of the signal that is not already contained in the autocorrelation function $g_1(\tau)$. However, the $f(t)$ of a membrane pore has a distribution of current fluctuations that is highly non-Gaussian; the current through the pore is either zero (pore closed) or equal to its full channel conductance level (pore open). Thus $g_2(\tau)$ is not equal to $1 + 2[g_1(\tau)]^2$, and so $g_2(\tau)$ can yield very important additional information about the kinetics of the pores.

To learn what $g_1(\tau)$ and $g_2(\tau)$ might tell about the pore kinetics we simulated the action of the pores. A fluctuating pore was modeled by a finite difference calculation evolved in time steps $\Delta t = 1$ ms. At each time step we chose a random number R on the inveral $0 < R < 1$. If the pore was closed and $R < k_o \Delta t$, we open the pore. If the pore was open and $R < k_c \Delta t$, we close the pore. By varying

k_o and k_c we found that: (1) the slope of $\ln[g_1(\tau)]$ vs. τ is equal to $-(k_o + k_c)$ and (2) that $g_2(0)$ is approximately equal to k_c/k_o (for $k_o < k_c$) or k_o/k_c (for $k_o > k_c$). These numerical results are consistent with analytical results. Several authors [8–10] have shown that

$$\langle f(t)f(t+\tau)\rangle = \langle f^2(t)\rangle \exp[-(k_o + k_c)\tau]$$

and in the appendix here we show that

$$g_2(0) = \langle f^4(t)\rangle / \langle f^2(t)\rangle^2$$

$$= \left[k_c/k_o + 1/(k_c/k_o)^2\right] / [1 + 1/(k_c/k_o)]$$

which is approximately equal to k_c/k_o (for $k_o < k_c$) or k_o/k_c (for $k_o > k_c$).

Thus, both from our numerical pore simulation and from analytic derivations we found that $g_1(\tau)$ and $g_2(\tau)$ yield two independent equations for $k_o + k_c$ and k_c/k_o. Hence, knowing $g_1(\tau)$ and $g_2(\tau)$, one can solve for k_o and k_c. The procedure is first to determine, for example by least squares, the slope S of $\ln[g_1(\tau)]$ vs. τ. As shown above $S = -(k_o + k_c)$. Second, since $g_2(0) = (B + 1/B^2)/(1 + 1/B)$ where $B = k_c/k_o$, this equation can be solved to find B as a function of $g_2(0)$. This is most easily done by iterating $B_{k+1} = g_2(0)(1 + 1/B_k) - 1/B_k^2$ starting with the initial $B_0 = g_2(0)$ and continuing until $|B_{k+1} - B_k|$ is within the desired accuracy, for example, less than 10^{-5}. These results can be combined to find $k_o = -S/(1 + B)$ and $k_c = Bk_o$, assuming $k_o < k_c$. (If the pore is more often open than closed; that is if $k_o > k_c$, then $k_c = -S/(1 + B)$ and $k_o = Bk_c$.)

In order to test whether $g_1(\tau)$ and $g_2(\tau)$ evaluated from experimental data can indeed reliably determine k_o and k_c, we used the above numerical simulation to provide test data where k_o and k_c were known. Then we analyzed these data with the new method to see of we could recover the correct values for k_o and k_c. The table shows a comparison of the values of k_o and k_c used in generating the simulated $f(t)$ with the values of k_o and k_c calculated from the functions $g_1(\tau)$ and $g_2(\tau)$. In these examples, the integrals were evaluated over 20000 time steps, or $T = 20$ s, using an extended Simpson's Rule [11].

We also tested this new procedure on data from patch clamp experiments that we have performed on the rabbit corneal endothelium [12], the single layer of epithelial cells that line the inside of the cornea. Seals were obtained using Boralex pipettes and the currents measured with a Dagan 8900 patch clamp amplifier. The data were recorded on an FM tape recorder and digitized at 5 kHz. The computations were done on a PDP 11/34. The rate constants were evaluated by: (1) manually measuring the open and closed times and calculated the frequency histograms (which took most of one day to do), and (2) by this new method (which took 6 min). From method 1 we found that $k_o = 13\ \mathrm{s}^{-1}$ and $k_c = 130\ \mathrm{s}^{-1}$, while method 2 yielded $k_o = 13\ \mathrm{s}^{-1}$ and $k_c = 107\ \mathrm{s}^{-1}$.

Any data analysis technique has advantages and disadvantages. We discuss first some limitations and provide some warnings about the use of this new technique: (1) Many data points must be used to evaluate $g_1(\tau)$ and $g_2(\tau)$ in order to obtain sufficient accuracy in k_o and k_c. The fractional error decreases slowly, only in inverse proportion to the square root of the number of points used. For 20000 points of current data, as can be seen from Table I, the errors are less than about 5%. (2) Since $g_2(0)$ has the same value when k_c/k_o is replaced by k_o/k_c, one must know if k_o is less than or greater than k_c in order to unambiguously determine which is which. This can be done by inspecting the original data to see of the pore is mostly open ($k_o > k_c$) or mostly closed ($k_o < k_c$). (3) The analysis presented here is limited to patches with one pore. For patches with two or more pores the relationship between $g_2(\tau)$ and the rate constants k_o and k_c is more complex, and in the limit of a large number of pores, as the signal becomes more Gaussian, $g_2(0)$ approaches 3 and is not a function of either k_o or k_c. (4) We have assumed that the pore has only two states with a single conductance level when open. Many pores are indeed more complex than this simple picture. We believe our method can be extended to include such cases, although it will become algebraically more complicated. However, any new method needs some starting point. To develop and test a new method the best place to start if at the simpliest and clearest problem, which is what we have done here. What is encouraging, is that even at this stage of development, this new method is useful in analyzing data from a two state pore or

TABLE I

VALUES OF k_o AND k_c (in s^{-1}) FOR 12 DIFFERENT SIMULATIONS USED TO GENERATE TEST DATA (I) AND VALUES OF k_o AND k_c (in s^{-1}) FOR THE 12 CASES IN (I) CALCULATED[a] FROM $g_1(\tau)$ AND $g_2(\tau)$ AS EVALUATED FROM THE TEST DATA (II).

		I	II
1	k_o	10	10
	k_c	20	20
2	k_o	10	11
	k_c	50	50
3	k_o	10	11
	k_c	100	107
4	k_o	10	12
	k_c	200	216
5	k_o	20	20
	k_c	20	20
6	k_o	20	21
	k_c	50	51
7	k_0	20	21
	k_c	100	105
8	k_o	20	22
	k_c	200	206
9	k_o	50	51
	k_c	20	21
10	k_o	50	49
	k_c	50	57
11	k_0	50	56
	k_c	100	108
12	k_o	50	56
	k_c	200	223

[a] S, the slope of $\ln[g_1(\tau)]$ vs. τ was determined by a least-squares fit from $\tau = 0$ to $\tau = 5$ ms, and $B = \max(k_c/k_o, k_o/k_c)$, was solved by iterating $B_{k+1} = g_2(0)[1 + 1/B_k] - 1/B_k^2$.
If $k_o < k_c$, $k_o = -S/(1+B)$ and $k_c = Bk_o$.
If $k_o > k_c$, $k_c = -S/(1+B)$ and $k_o = Bk_c$.

from a multistate pore where the rate constants are well separated. For example, if the graph of $\ln[g_1(\tau)]$ vs. τ is not a straight line it indicates that the pore has more than one open and one closed state. However, if the initial slope from $\tau = 0$ is sufficiently linear before the graph becomes curved, then if the analysis is limited to small τ, the rate constants of the faster reaction can be determined.

(5) We must always remember that we ask Nature, we don't tell her. The existence of an automated algorithm never absolves one from examining the raw data to be sure that the data does indeed match the model assumptions of the data analysis. One should be careful to check for novel features in the data such as sublevels, brief shuttings, multiple pore types etc. before committing it to any data analysis.

On the other hand, the very significant advantage of this new method is that the pore transitions do not have to be reconstructed out of the usually noisy signal and it is not necessary to measure and calculate frequency histograms in order to obtain the kinetic rate constants. Thus, this new method represents a substantial saving of human effort and machine computation time.

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Appendix

If the pore has two states, c = closed and o = open, then the current I flowing through the pore will be $I_c = 0$ and $I_o = I_0$. The probability that the pore is closed $P_c = P(I=0) = k_c/(k_o + k_c)$ and the probability that the pore is open $P_o = P(I = I_o) = k_o/(k_o + k_c)$. (See, for example, Ref. 13.) The average current is then the expectation value $\langle I \rangle = I_c P_c + I_o P_o = I_o/(1 + B)$ where $B = k_c/k_o$. The current fluctuations f with respect to the mean current are then $f_c = I_c - \langle I \rangle = -I_o/(1 + B)$ when the pore is closed, and $f_o = I_o - \langle I \rangle = I_o B/(1 + B)$ when the pore is open. The moments can then be evaluated: $\langle f^2 \rangle = f_c^2 P_c + f_o^2 P_o = I_o^2 B/(1 + B)^2$ and $\langle f^4 \rangle = f_c^4 P_c + f_o^4 P_o = I_o^4 B(1 + B^3)/(1 + B)^5$. Thus, $g_2(0) = \langle f^4 \rangle / \langle f^2 \rangle^2 = (B + 1/B^2)/(1 + 1/B)$.

References

1 Sakmann, B. and Neher, E. (eds.) (1983) Single Channel Recording, Plenum Press, New York

2 Colquhoun, D. and Hawkes, A.G. (1983) in Single Channel Recording (Sakmann, B. and Neher, E., eds.), pp. 135–175, Plenum Press, New York
3 Pusey, P.N. (1977) in Photon Correlation Spectroscopy and Velocimetry (Cummins, H.Z. and Pike, E.R., eds.), pp. 45–141, Plenum Press, New York
4 Oliver, C.J. (1980) in Scattering Techniques Applied to Supramolecular and Non-equilibrium Systems (Chen, S.-H., Chu, B. and Nossal, R., eds.), pp. 87–160, Plenum Press, New York
5 Fundamentals of Signal Analysis (1981) Hewlett Packard Applications Note 243, HP
6 Rice, S.O. (1954) Mathematical analysis of random noise in Selected Papers on Noise and Stochastic Processes (Wax, N., ed.), p. 227, Dover, New York
7 Saleh, B. (1978) Photoelectron Statistics, p. 133, Springer-Verlag, New York
8 Stevens, C. (1972) Biophys. J. 12, 1028–1047
9 Verveen, A.A. and DeFelice, L.J. (1974) Prog. Biophys. Mol. Biol. 28, 189–265
10 Conti, F. and Wanke, E. (1975) Q. Rev. Biophys. 8, 451–506
11 Abramowitz, M. and Stegun, I. (eds.) (1964) Handbook of Mathematical Functions, eq. 25.4.6, GPO, Washington, DC
12 Koniarek, J., Todorova, I., Fischbarg, J. and Liebovitch, L.S. (1985) in preparation
13 DeFelice, L.J. (1981) Introduction to Membrane Noise, Plenum Press, New York