BRIEF COMMUNICATION

DIFFERENTIATION BETWEEN EQUILIBRIUM AND NONEQUILIBRIUM KINETIC SYSTEMS BY NOISE ANALYSIS

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ABSTRACT Methods for the differentiation between equilibrium and nonequilibrium steady-state kinetic mechanisms based on fluctuation and noise analysis are discussed. Specifically, the "sharpening" in the auto noise power spectrum is shown to be a useful indicator in identifying a nonequilibrium steady state.

INTRODUCTION

Recently noise measurements in biological systems have attracted considerable attention. A question frequently asked is: How much information on the kinetic properties of the system can one get from noise analysis? In general, if enough simultaneous independent measurements (such as conductance, optical absorbance, etc.) are available, the measured noise power spectrum matrix of the system can be used to evaluate the rate parameters of the reactions comprising the kinetic system (Chen, 1975*a*; 1977). On the other hand, even with a limited number of independent measurements (such as conductance fluctuations alone), noise data can still be used to differentiate between rival kinetic models (Chen and Hill, 1973; Chen, 1976). In this communication we present a simple method to distinguish between an equilibrium system and a nonequilibrium steady-state using the "auto" noise power spectrum of the system.

Nonequilibrium steady-state mechanisms are very common in biological systems. For example, linear models with cyclic splitting of ATP and cyclic protein conformational transformations have been proposed to explain active transport of various kinds, muscle contraction, etc. In general, a thermodynamic system must be in contact with some driving reservoir or reservoirs in order to be maintained at a nonequilibrium steady state (Hill, 1977). In biological systems, the driving free energy is usually provided by enzymatic splittings of ATP (or GTP) or concentration gradients of molecules (or electrochemical gradients of ions) across a membrane. For example, the ATP splitting provides the free energy in Na, K, and Ca active transport and in muscle contraction, while the proton (H^+) gradient across a membrane provides the free energy for phosphorylation reactions in mitochondria (Mitchill, 1961; Hill, 1977).

In contrast, an equilibrium system does not require any energy input. Thus, in any model building it is important to know whether a system is at equilibrium or not. As an example, current channel models for excitable membranes do not involve any nonequilibrium steady state, although electrochemical gradients (K^+ and Na^+) are present in these membranes. Thus, in these models it has been implicitly assumed that the kinetics of membrane channels are independent of the potassium and sodium ion gradients across the membrane. However, it has been suggested recently that the kinetics of Na^+ channels in squid axon membranes (Moore and Cox, 1976) and in *Myxicola* axon membranes (Rawlings and Neumann, 1976) may involve nonequilibrium cycling steady states, based on analyses of conductance relaxation kinetics. If this is true, it means that nonequilibrium Na^+ transport through a channel may interact with the Na^+ channel kinetics. The noise analysis described here may prove useful in resolving this problem.

The method is based on the so-called "sharpening" phenomenon in the corner frequency region of an auto noise power spectrum (see below). This phenomenon was first observed in some current noise power spectra of squid axon membranes by Fishman and his colleagues (1975, 1977). In this communication we show that this sharpening phenomenon can be used in differentiation between equilibrium and nonequilibrium systems.

ONE-TERM LORENTZIAN AND THE "SHARPENING" OF THE SPECTRUM

The (auto) noise power spectrum $G(\omega)$ of chemical reactions has a characteristic feature: it is constant at low frequencies and proportional to ω^{-2} ($\omega = 2\pi f, f =$ frequency) at high frequencies. Thus, it is always possible to define a one-term Lorentzian for any system with the two asymptotes of $G(\omega)$ at $\omega = 0$ and $\omega = \infty$ (see Fig. 1). Let us define G_0 and G_∞ as

$$G_0 = \lim_{\omega \to 0} G(\omega), \tag{1}$$

$$G_\infty = \omega^2 \lim_{\omega \to \infty} G(\omega). \tag{2}$$

The one-term Lorentzian can then be defined as

$$G_L(\omega) = G_\infty/[(G_\infty/G_0) + \omega^2]. \tag{3}$$

$G_L(\omega)$ coincides with $G(\omega)$ at $\omega = 0$ and at $\omega = \infty$, but deviates from $G(\omega)$ in the transition region. Let us further define

$$\Delta G(\omega) = G(\omega) - G_L(\omega) \tag{4}$$

Then if $\Delta G(\omega)$ is positive in the transition region, as in Fishman et al. (1975), the

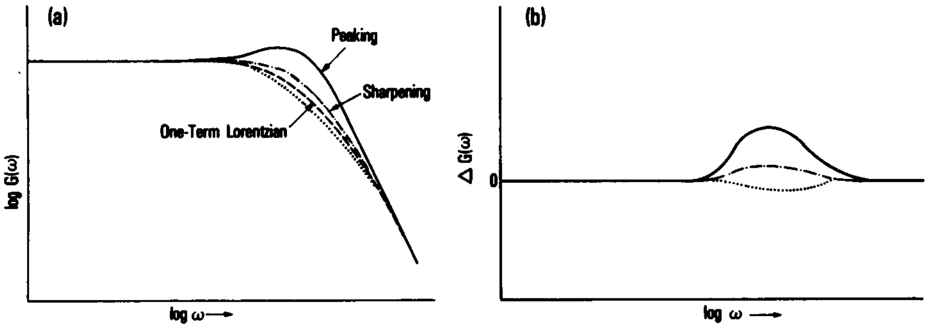

FIGURE 1 Schematic $G(\omega)$ showing the peaking, the sharpening, and the flattening in the transition region (a) and corresponding $\Delta G(\omega)$ values (b).

$G(\omega)$ is said to be sharper than its corresponding one-term Lorentzian $G_L(\omega)$ (see Fig. 1). In recent papers (Chen, 1975*a*, *b*; 1977) we have demonstrated that a "peaking" (local maximum in $G(\omega)$ in the transition region) can be used to identify a nonequilibrium system. In this communication, we show that sharpening can serve the same purpose. One must note that a sharpening does not necessarily mean that a local maximum (peaking) is present in the transition region. But, a peaking spectrum is of course sharper than its corresponding one-term Lorentzian. Thus, for practical model differentiation, sharpening is more useful than peaking.

The basic principle for differentiating between equilibrium and nonequilibrium systems is contained in the following theorem.

Theorem

For any equilibrium system, $\Delta G(\omega)$ in Eq. 4 is always negative or zero at all ω values, while it may be positive for some nonequilibrium steady-state systems.

Thus, by examining the differences between a noise power spectrum and its corresponding one-term Lorentzian, one may be able to differentiate between a nonequilibrium steady state and an equilibrium system. The proof of this theorem is as follows.

In a recent paper (Chen, 1975*b*) we demonstrated that cycling steady-state systems could have a peaking spectrum (therefore $\Delta G(\omega)$ is positive), if the rate constants of the system are properly chosen. Therefore we have only to prove the first part of this theorem.

Recently we have proved that for linear systems at equilibrium the auto noise power spectrum can be expressed as a sum of simple positive Lorentzians (see Eqs. 17 and 37 of Chen and Hill, 1973):

$$G(\omega) = \sum_{l=1}^{x} a_l/(b_l + \omega^2), \tag{5}$$

where a_l and b_l are positive constants and x is the number of independent variables of the system. In another communication (Chen, 1978), we show that Eq. 5 is also applicable to equilibrium systems with nonlinear chemical reactions. Thus, for any equilibrium system, the corresponding one-term Lorentzian becomes (see Eqs. 1, 2, and 3)

$$G_L(\omega) = \frac{\sum_{l=1}^{x} a_l}{\left(\sum_{l=1}^{x} a_l \Big/ \sum_{l=1}^{x} \frac{a_l}{b_l}\right) + \omega^2}. \tag{6}$$

We want to show that $G_L(\omega)$ in Eq. 6 is larger than $G(\omega)$ in Eq. 5 at all ω values for cases with $x > 1$. If $x = 1$, $G(\omega)$ and $G_L(\omega)$ are identical and therefore $\Delta G(\omega) = 0$. We consider here the case $x = 2$. The case of an arbitrary x value is discussed in the Appendix.

The $\Delta G(\omega)$ for $x = 2$ is

$$\Delta G(\omega) = a_1/(b_1 + \omega^2) + a_2/(b_2 + \omega^2) - A/[(A/B) + \omega^2], \tag{7}$$

where

$$\begin{aligned} A &= a_1 + a_2 \\ B &= a_1/b_1 + a_2/b_2. \end{aligned} \tag{8}$$

After rearrangement, Eq. 7 becomes

$$\Delta G(\omega) = \frac{1}{(b_1 + \omega^2)(b_2 + \omega^2)[(A/B) + \omega^2]} \tag{9}$$
$$\cdot \{a_1(b_2 + \omega^2)[(A/B) + \omega^2] + a_2(b_1 + \omega^2)[(A/B) + \omega^2] - A(b_1 + \omega^2)(b_2 + \omega^2)\}.$$

Since a_1, a_2, b_1, b_2 and therefore A and B are all positive numbers, the denominator in Eq. 9 is always positive at all ω values. As a result, we have to only look at the quantity inside the curly brackets in Eq. 9.

This quantity is a polynomial in ω^2. By examining each term in the polynomial separately and using Eq. 8, we find:

Coefficient of ω^4: $a_1 + a_2 - A = 0.$

Coefficient of ω^2: $a_1 b_2 + a_1 A/B + a_2 A/B - A b_1 - A b_2$
$= -a_1 a_2 (b_1 - b_2)^2/[a_1 b_2 + a_2 b_1].$

Coefficient of ω^0: $a_1 b_2 A/B + a_2 b_1 A/B - A b_1 b_2 = 0.$

The only nonzero term is that in ω^2. The coefficient for this term is always negative since the a's and the b's are positive numbers. Thus, the quantity inside curly brackets in Eq. 9 is always negative for all ω values, and we have proved the theorem for $x = 2$.

DISCUSSION

The analysis in the previous section does not depend on any particular reaction mechanism or any particular measurement. Therefore the theorem is very general in that it applies to nonlinear as well as linear kinetic systems and to any kind of measurement. In other words, if the system is at equilibrium, the auto noise power spectrum of the system will always be less than or equal to the corresponding one-term Lorentzian, whether one is measuring the total conductance, the optical absorbance, or any other quantity.

One must note that, as stated implicitly in the Theorem, steady-state is only a necessary condition (not sufficient) for a kinetic system to have sharpening in its noise power spectra. Therefore, if a spectrum is less sharp (flattened) than the corresponding one-term Lorentzian, it is impossible to tell whether the system is at equilibrium or not. However, if the spectrum is sharper than the corresponding one-term Lorentzian, the system is definitely not at equilibrium.

Fishman and his colleagues (1975) have observed that the conductance noise power spectrum of potassium channels calculated with the Hodgkin-Huxley formalism is always less sharp than the corresponding one-term Lorentzian. This is true because the model is equivalent to a kinetic system consisting of four linear consecutive reactions at equilibrium (Hill and Chen, 1971). As long as the equilibrium condition is maintained, no variation of the Hodgkin-Huxley model, such as the multiconductance κ model of Hill and Chen (1972), will eliminate this "flattening" phenomenon. On the other hand, Fishman and his colleagues (1975) have recently observed the peaking and sharpening phenomena in some current noise power spectra of squid axon membranes. If these phenomena are really derived from the conductance kinetics of potassium (or sodium) channels, the usual Hodgkin-Huxley model will have to be modified to include some steady-state mechanism.

Another function capable of differentiating between equilibrium and nonequilibrium systems is the time correlation matrix $\mathbf{C}(t)$ which is obtained by using two (at least) or more independent measurements simultaneously (see Chen, 1977). In general, the "cross" time correlation functions for a pair (i, j) of simultaneous measurements (such as conductance and optical absorbancy, for example), $C_{ij}(t)$ and $C_{ji}(t)$, are defined as

$$
\begin{aligned}
C_{ij}(t) &= <(X_i(t) - \overline{X}_i)(X_j(0) - \overline{X}_j)>, \\
C_{ji}(t) &= <(X_j(t) - \overline{X}_j)(X_i(0) - \overline{X}_i)>, \qquad (10)
\end{aligned}
$$

where $< >$ means ensemble average and $X_i(t)$ is the macroscopic value of the measurement i at time t after some initial time zero. Experimentally, $C_{ij}(t)$ can be obtained by averaging the quantity in the brackets in Eq. 10 over a long period of time. Generally, $C_{ij}(t) \neq C_{ji}(t)$ for $t > 0$ unless the system is at equilibrium (Chen, 1977). Therefore, by examining the symmetry of $\mathbf{C}(t)$ $(t > 0)$, one is able to differentiate between equilibrium and nonequilibrium systems.

In conclusion, our main purpose of this paper is to point out a practical criterion to unambiguously identify certain nonequilibrium chemical systems. If only a single measurement is available, the criterion is the peaking or the sharpening in the corner frequency region of the auto noise power spectrum. If more than one simultaneous measurement is available, the symmetry of the time correlation function matrix can serve the same purpose.

APPENDIX

The following (revised) general proof of the theorem was given by Dr. Bruce Knight.

From Eqs. 1 and 5, we may rewrite $G(\omega)$ as

$$G(\omega)/G_0 = <1/[1 + \omega^2/b_l]>, \tag{A-1}$$

where the average is taken over the weights

$$W_l = (a_l/b_l)\Big/\sum_{m=1}^{x} (a_m/b_m). \tag{A-2}$$

Similarly, from Eqs. 2 and 5,

$$G_\infty/G_0 = <b_l>. \tag{A-3}$$

Thus, according to Eq. 3, the one-term Lorentzian becomes

$$G_L(\omega)/G_0 = 1/[1 + \omega^2/<b_l>]. \tag{A-4}$$

Let us define the function $\gamma(\omega)$ by

$$G(\omega)/G_0 = <1/(1 + \omega^2/b_l)> = 1/(1 + \gamma\omega^2). \tag{A-5}$$

Then, it is easy to show that

$$\gamma(\infty) = 1/<b_l>. \tag{A-6}$$

With the use of Eqs. A-4, A-5, and A-6, the $\Delta G(\omega)$ in Eq. 4 becomes

$$\Delta G(\omega)/G_0 = 1/(1 + \gamma\omega^2) - 1/(1 + \gamma(\infty)\omega^2). \tag{A-7}$$

Since G_0 is always positive, the theorem $\Delta G(\omega) \leq 0$ is proven if we can show that the condition

$$\gamma(\omega) \geq \gamma(\infty) \tag{A-8}$$

holds for all positive ω values. To show this, let us solve Eq. A-5 for $\gamma(\omega)$:

$$\gamma(\omega) = \frac{1}{\omega^2 < 1/(1 + \omega^2/b_l)>} - 1/\omega^2. \tag{A-9}$$

Now, by elementary calculus,

$$\frac{d\gamma}{d\omega} = \frac{2[(<B_l>)^2 - <B_l^2>]}{\omega^3(<B_l>)^2}, \tag{A-10}$$

where

$$B_l \equiv 1/(1 + \omega^2/b_l).$$

Since the arithmetic mean of a square always exceeds the square of the corresponding arithmetic mean, the quantity in the bracket of Eq. A-10 is always negative. This means $d\gamma/d\omega$ is negative for positive ω values and $\gamma(\omega)$ is a monotonically decreasing function of ω. As a result, we have proved Eq. A-8 and the theorem.

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