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Interpretation of Protein Structure and Dynamics from the Statistics of the Open and Closed Times Measured in a Single Ion-Channel Protein

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Ion channels are proteins in the lipid cell membrane. They spontaneously fluctuate between conformational shapes that are open or closed to the passage of ions. The ionic currents through an individual channel can be resolved by the patch clamp technique. Thus, the time sequence of open and closed conformational states can be measured in one channel molecule. The probability density function of the dwell times in the open and closed states displays scaling functions that may arise from: (1) a large number of conformational substates having a continuous distribution of activation energy barriers, (2) time-dependent changes in the energy barriers between states, or (3) local interactions that constrain local structures which interact hierarchically to form global structure.

KEY WORDS: Ion channels; proteins; protein dynamics; telegraph signal; neural networks; spin glasses; stochastic resonance; nonlinear dynamics.

1. INTRODUCTION

Ions in solution cannot easily cross the lipid membrane that surrounds cells. However, these ions can enter or exit the cell by passing through ion channel proteins that span the cell membrane.[1] Each channel protein can have different conformational states. The difference in energy between these states is small enough so that thermal fluctuations cause the protein to switch spontaneously from one to another. In some states, the channel has a central pore that allows ions to pass through the protein. The picoamp current through an individual open channel can be resolved by the patch

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clamp technique.[2] Thus, the sequence of the open and closed times can be measured and their statistical properties determined. This information on the fluctuations in a single protein is very valuable because other biochemical or biophysical techniques measure many molecules at once, each of which may be in a different state. We use this technique to study the properties of the ion channels in the layer of cells that lines the inside of the cornea.[3] Ion transport through this cell layer keeps the cornea dry and therefore transparent for good vision.[4]

Important information about the physical properties of the channel protein can be obtained from the probability density function of the dwell times in the open and closed states. In order to analyze these data it had been assumed that the channel protein has only a few, stable, well-defined, discrete, independent states and that the switching between these states is inherently random and thus can be described by a Markov process.[1, 2] Each transition between states produces another exponential term and the number of such terms required to fit the data was used to determined the number of discrete channel states. Since the sum of 1–6 exponential terms was required to fit the data, it was concluded that the channel protein has 1–6 stable, discrete states.

Concepts from nonlinear systems give us new ways to analyze these data that provide new insight into the physical properties of the channel protein. For example, the current through the channel is self-similar in time, namely, there are bursts within bursts within bursts of openings and closings.[3, 5, 6] The probability density functions of the dwell times measured at different time scales have similar distributions. Hence, events at one time scale are related to events at other time scales. Thus, we can fit the probability density functions of the dwell times t by a scaling function $f(t)$ that describes how the probability to change states varies with the dwell time in the open or closed state.[3, 5, 6] These scaling functions often have a power-law

$$f(t) \propto t^{-\alpha} \tag{1}$$

or stretched-exponential form

$$f(t) \propto \exp(-kt^{\alpha}), \qquad 0 < \alpha \leqslant 1 \tag{2}$$

The existence of a scaling function implies that the stable, discrete states postulated by the Markov models in order to fit the data may not exist. We describe three different physical interpretations of the origin of these scalings.

2. STRUCTURAL INTERPRETATION

The biophysical properties of proteins bound in the lipid cell membrane, such as ion channels, are technically more difficult to measure than those of soluble proteins such as globular proteins that function as enzymes inside of cells. Thus, less is known about the physical properties of membrane proteins than is known about globular proteins. However, it is likely that the physical properties of both globular and membrane proteins are similar. In globular proteins, the distribution of activation energy barriers between conformational substates can be measured from its effect on fluorescent lifetimes[7] or the time course of ligands reaching their binding site.[8] Many such experiments have shown that globular proteins have very many conformational substates and a continuous distribution of activation energy barriers between these conformational substates.

The scaling functions seen in the ion channel data may arise from the distribution of activation energy barriers among the conformational substates. Dewey and Spencer[9] called this a "structural model" because in this model the energy structure of the protein is fixed. Transition state theory[10] predicts that the kinetic rate constant k for a transition over an activation energy barrier ΔE is given by

$$k = \frac{k_B T}{h} \exp\left(\frac{-\Delta E}{k_B T}\right) \tag{3}$$

where k_B is the Boltzmann constant, T is the absolute temperature, and h is the Planck constant. Thus, the distribution of activation energy barriers $g(\Delta E)$ can be equivalently expressed in terms of the distribution of rate constants $g(k)$ of the transitions between the conformational substates. The cumulative probability $P(t)$ that the time spent in a state is greater than t is given by the relationship[8]

$$P(t) = \int_0^\infty g(k) e^{-kt}\, dk \tag{4}$$

The functional form of $P(t)$ depends on the width of the $g(k)$ distribution. When the activation energy barrier distribution is very narrow, that is, when $g(k) = \delta(k - k_0)$, then $P(t) = \exp(-k_0 t)$. When the activation energy barrier distribution is very broad, that is, when $g(k) = \text{const}$, then $P(t) \propto t^{-1}$. The intermediate forms of $P(t)$ connecting these two extremes can be parametrized by the stretched exponential

$$P(t) = \exp(-at^b) \qquad \text{where} \quad 0 < b \leqslant 1 \tag{5}$$

and $g(k)$, evaluated from the inverse Laplace transform of Eq. (4),[11] is given by

$$g(k) = \frac{1}{\pi} \int_0^\infty \{\exp[-kx - ax^b \cos(b\pi)]\} \sin[ax^b \sin(b\pi)]\, dx \qquad (6)$$

Other scaling functions $P(t)$ can be represented by other $g(k)$ distributions, such as those with high- or low-energy cutoffs.[11]

Different ion channels have different structure. The scaling functions of some channels are power laws,[5, 12] which implies that there are many similar conformational substates, many pathways between them, and a thus broad distribution of activation energy barriers. In other channels, the scaling functions are exponentials,[5] which implies that there are few similar conformational substates, few pathways between them, and thus a narrow distribution of activation energy barriers. Some channels have different characteristics at different time scales, which implies that different processes, having different widths of activation energy barrier distributions, operate at different time scales.

3. DYNAMIC INTERPRETATION

Many different experimental methods[13, 14] and molecular dynamic simulations[14, 15] have shown that globular proteins have time-dependent energy barriers. Often, the reaction mechanisms of enzymes are hard to understand in terms of the time-averaged position of the atoms because the reaction occurs when the protein very briefly fluctuates into a structure where the reaction rates are exponentially faster. That is, it is important to remember that the net reaction rates are proportional to $\langle \exp(-\Delta E/k_B T) \rangle$ rather than $\exp\langle -\Delta E/k_B T \rangle$, where the brackets denote the time-averaged quantities.

The scaling functions seen in the ion channel data may therefore arise from time-dependent changes in the activation energy barriers. Dewey and Spencer[9] called this a "dynamic model." The transitions over a sequence of different energy barriers may be represented by transitions over one barrier that is time dependent. For example, the stretched-exponential scaling of Eq. (5) can also result from a time-dependent rate constant $k(t)$ of the form[3]

$$k(t) \propto t^{b-1}$$

Thus, structural and dynamic models are related. However, in some cases, time-dependent experiments may be able to differentiate between these two types of models.[16]

It had always been assumed that the switching of a channel protein from one state to another is an inherently random process. However, the output of a deterministic nonlinear system may be so complex that it mimics random behavior, a phenomenon now called chaos. Dynamic models based on iterative maps[17] and modified Duffing equations[18] can also produce some of the statistical properties of the scaling functions of the ion channel data. In principle, the dimensionality of the phase-space sets constructed from the time series of the currents measured through the channels could determine if the switching between the open and closed states was driven by random or deterministic processes. However, this analysis cannot be done with the patch clamp data because the time series of those data is self-similar and thus not differentiable. The embedding theorems require that the time series be differentiable in order to construct the phase space set.[17]

The physical interpretation of chaotic ion channel models is that the channel protein functions as a few independent pieces that interact with each other. The channel should then be thought of as a nonlinear mechanical oscillator that can organize nonperiodic thermal fluctuations in its structure into coherent motion to drive itself from one conformational state to another. This is analogous to the failure of the Tacoma Narrows Bridge, where a mechanical structure organized the nonperiodic motion of the wind into destructive coherent oscillations.[19]

However, the simplest chaotic models do not yield all the scaling functions found in all the channel data. The additional complexity that needs to be added to some chaotic models to produce power law scalings suggests that if the channel acts as a deterministic system, it actually consists of many small locally interacting functional pieces, rather than a few large ones.

Motions in small molecules are driven by thermal fluctuations. Motions in macroscopic objects are driven by mechanical forces. It is not clear if channel proteins are large enough to behave partially as true mechanical systems. It is also not clear if channel proteins are in local thermodynamic equilibrium. If proteins dissipate energy through the radiation emitted by accelerated charges or by inelastic collisions, then they may be in a steady state rather than in equilibrium with the environment. If that is true, then there may be long-lived states that correspond to dynamic resonances rather than to local minima in the potential energy function. Even if the energy dissipated is much less than $k_B T$, the existence of any dissipation can break symmetries and thus generate structures in space or time.

4. NEURAL NETWORK INTERPRETATION

Proteins share many properties in common with spin glasses and neural networks.[20–23] Proteins do not have global potential energy minima, because of local steric conflicts. This property is called "frustration." In order to pass from one local minimum to another may therefore require that the potential energy increase before it decreases. That is, the folded protein structure may need to unfold slightly before it can refold in a slightly different way. This property is called "ultrametricity." Thus, a protein has many nearly identical energy minima. These properties are also common to spin glasses and neural networks, which suggests that it may be helpful to think about structural or dynamic "states" in a different way.

We now transform the description of a channel protein switching between open and closed conformational states from one based on the positions of its atoms in physical space to a symbolic space resembling a neural network. Consider a neural network consisting of nodes and connections between them. Each node corresponds to one physical unit of the channel protein such as one atom. If the position in physical space of an atom corresponds to that it would have if the channel protein was closed, the value of that node will be nearly -1. If the position in physical space of an atom corresponds to that it would have if the channel protein was open, then the value of that node will be nearly $+1$. At each time step, the value at each node is determined from a function of the connections into that node. As the calculation is advanced in time, the values of the nodes will change.

Important qualitative features arise from this representation. First, there are no "states." Although many nodes may have values near one extreme, there will always be some nodes with the complementary value. Although we could enumerate all possible combinations of the values of all the nodes and call them "states," there would be so many of them that this is not a useful way to think about what is happening. A more useful approach is to think of the channel protein as being approximately open or closed, although many of its atoms may be in the "wrong" condition.

Local constraints between nearby atoms are quite strong and thus there are highly ordered structures within local regions. However, these local structures may conflict with each other. A useful analogy is a neural network that solves a visual illusion called the Necker cube.[24] The Necker cube has 8 vertices that can be connected by edges in different ways that correspond to two different global patterns. Each vertex is represented by one node in the network. In one pattern one particular face of the cube is in the front of the object, while in the other pattern it is in the back of the object. When a vertex would have a physical position corresponding to one

pattern its corresponding node has a value near -1 and when it would have a physical position corresponding to the other pattern its correspond node has a value near $+1$. The network has an energy function with two global minima that correspond to the two global patterns. There are also local minima that correspond to some vertices in one pattern and other vertices in the complementary pattern. Local constraints near each corner are strong and global constraints much weaker. Thus, the energy function of the network can evolve into a local minimum where the diagonally opposite corners have each satisfied their local constraints, but in contradictory ways that prevent the formation of a consistent global pattern.

When the channel is closed most of the nodes have values corresponding to the closed conformation. Since the channel protein is quite large, there are many local regions each of which has been consistently ordered into the closed conformation by strong local interactions. Thermal fluctuations will be able to flip some local regions to their values in the open conformation which will be locally consistent and in conflict with their adjacent regions. The structure of the channel protein is always bulging out the wrong way in local regions. It is not useful to think in terms of "substates" because it is not that the system is at a local energy minimum and then shifting to another energy minimum, but rather that it is always exploring the barriers as well as the minima. Eventually enough local regions will have thermally flipped into the open conformation so that they will begin to interact with each other to constrain ever larger regions into the open conformation. Larger and larger regions will interact, in a hierarchical manner, until most of the atoms will have switched to their values associated with the open conformation. Thus the channel protein will have switched from closed to open. The hierarchical nature of the dynamics will lead to distributions of dwell times with stretched-exponential or power-law behavior.

We are now beginning to test the validity of this qualitative picture by formulating neural networks where the connections between nodes corresponds to the pairwise forces between atoms in proteins. The dynamics of the switching between global conformational shapes predicted by these networks will be compared to that measured experimentally and that predicted by molecular dynamics simulations.

5. CONCLUSIONS

The patch clamp technique provides the amazing ability for us to follow the fluctuations of an individual ion channel protein between its open and closed conformational states. The probability density of the open and closed dwell time distributions provides important information on the

structure and motion within the channel protein that causes it to open and close. We presented three approaches to interpret that data in ways that shed light on channel structure and that are consistent with the known properties of other proteins.

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