An instrument has been built to measure the kinetics of subunit self-association reactions by monitoring the intensity of light scattered at 90° (proportional to weight-average molecular weight). Although relative volume changes are small (change in partial specific volume is typically 10^{-3} ml/g), the relevant thermodynamic parameter is the difference between the molar volumes of products and reactants. These differences tend to be large (50–100 ml/mol of subunit), making this experimental approach feasible. Sinusoidal pressure perturbation of 3 atm amplitude is achieved with a stack of piezoelectric ceramic elements. Lamp noise and static light scattering are removed by subtracting the output from a beam splitter-reference photomultiplier tube combination. The signal is then processed by a home-built phase-sensitive detector and gated integrator. The instrument displays the real and quadrature components of the light-scattering signal as the frequency is varied from 0.0005 ($\tau \simeq 300$ s) to 30 kHz ($\tau \simeq 5\,\mu$s). Frequencies above 150 Hz ($\tau < 1$ ms) are not currently accessible.

Measurements on the kinetics of the indefinite linear self-association of bovine liver glutamate dehydrogenase will be compared with literature values

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CREATION OF A NONEQUILIBRIUM STATE IN SODIUM CHANNELS BY A STEP CHANGE IN ELECTRIC FIELD

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Voltage clamp of electrically excitable membranes can be considered an E jump, in which the macromolecules associated with the specific ion conductance changes are subjected to a sudden change in electric field of up to approximately 10^7 V/m. If the conductance and activatability of the channels are taken as measures of the populations of different states of the gating molecules, then at any constant voltage the potassium conductance gates are distributed between two states (conducting and nonconducting), while the sodium conductance gates are distributed among three states (conducting, activatable, and inactivated). Kinetic analysis of the sodium conductance changes, taking into account the phenomena of inactivation shift and τ_c-τ_h separation (anomalous with respect to the Hodgkin-Huxley model), suggests that a fourth state, which has no population at rest, may be the direct precursor of the conducting state (1). In this paper a physical model of the fourth state is suggested. The fourth state may consist of a non-Boltzmann distribution of gating molecule conformations and orientations relative to the energy minima that characterize the steady state at any particular value of average electric field. This state might be created when the electric field changes too rapidly for gating molecule orientation and conformation to

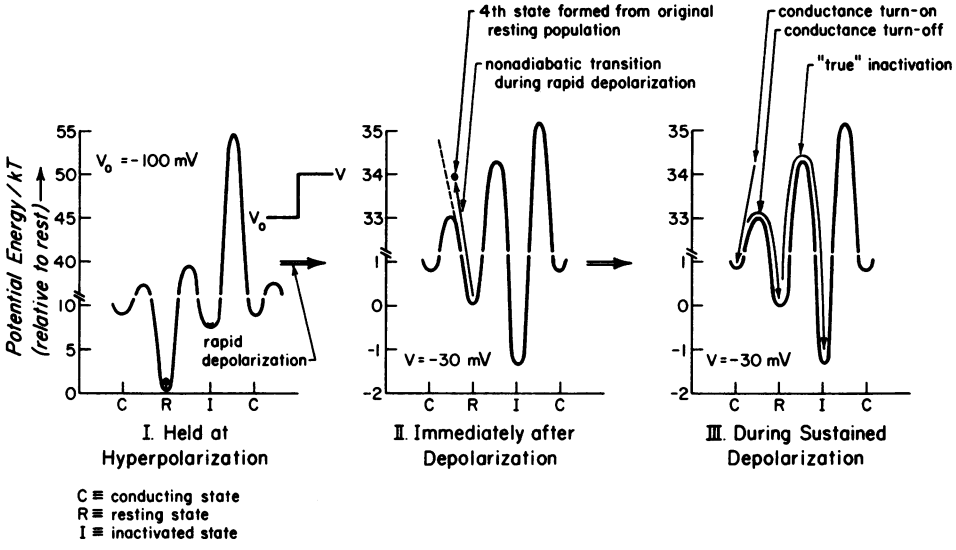

FIGURE 1 Energy-level diagram showing heights of potential barriers, depths of potential wells, and dominant transitions (nonadiabatic during rapid voltage change and adiabatic at constant voltage), hypothesized to be associated with the response of an axonal membrane to a step voltage depolarization.

follow, and would be similar to the state of "nonequilibrium dielectric polarization" postulated to be involved in electron-transfer reactions (2). Carrying the analogy with the existing electron-transfer formalism a step further, the creation of the fourth state is hypothesized to be a "nonadiabatic transition," which occurs during the brief time that the electric field is changing very rapidly.

Fig. 1 illustrates by energy level diagrams the sequence of events hypothesized in the gating mechanism of an ensemble of sodium channels in response to a depolarizing voltage step from −100 to −30 mV. The heights of the energy barriers and depths of the energy wells are consistent with rate constants fit to the kinetics of giant axon sodium channels (1), together with the observation that the Q_{10} of sodium channel kinetics is about three (3).

The arrows in sections II and III of Fig. 1 show the dominant transitions during rapid voltage change, and during a constant voltage after a sudden depolarization, respectively. The kinetic properties of the fourth state (i.e., the rate constants for transitions from it) will be a function of both V_0 and V for the voltage step shown above. The following differential equations include terms for other transitions between states in addition to the dominant ones represented in Fig. 1, but neglect transitions within the R-state:

$$dC/dt = -(k_{CI} + k_{CR})C + k_{IC}I + k_{R(V_0)C}R(V_0) + k_{R(V)C}R(V), \quad (1)$$

$$dI/dt = -(k_{IC} + k_{IR})I + k_{CI}C + k_{RI}R(V), \quad (2)$$

$$dR(V_0)/dt = -k_{R(V_0)C}R(V_0), \tag{3}$$

$$dR(V)/dt = k_{CR}C + k_{IR}I - (k_{RI} + k_{R(V)C}R(V). \tag{4}$$

Where all the k's are voltage-dependent rate constants, and the $R(V_0)$ and $R(V)$ denote those populations of the resting state that have appropriate resting conformations for V_0 and V, respectively. (The excited state at V is presumed to have the same conformation as the resting state at V_0.) For a single voltage step, in which the voltage change may be assumed to be instantaneous (i.e., fast compared to any other processes), the kinetic description of the system by Eqs. 1–4 is (relatively) simple. On the other hand, if the voltage is changing continuously (as it does in the normal functioning of the animal) then we have a continuous distribution of conformations of the R-state (i.e., a continuous distribution of "fourth states"), with each element of the distribution having its own rate constant for emptying. To write the equations for the case of continuously varying voltage so as to be amenable to numerical computation, it is necessary to divide the continuous distribution of R-state conformations into a finite number of sections. If we label each element of the R-state according to the voltage for which it has the steady-state minimum energy conformation, then we can create the discrete spectrum of R-states by:

$$R'(V') = \int_{V=V'-\Delta/2}^{V=V'+\Delta/2} dV\, R(V), \tag{5}$$

where Δ is some small increment.

The number of $R'(V')$'s depends on the membrane history and the size of Δ. For example, if Δ = 1 mV and the membrane potential has varied from −70 to +40 mV, then there will be 110 values of $R'(V')$, one for each integral value of V' from −70 to +40.

The differential equations describing time courses in the continuously varying voltage case are:

$$dR'(V')/dt = -(k_{R(V')C} + k_{RR})R'(V') + k_{RR}R'(V' + \Delta\,|\,V - V'\,|/(V - V')) + \int_{V'=V-\Delta/2}^{V'=V+\Delta/2} dV'\delta(V - V')(k_{CR}C + k_{IR}I + (k_{RR} - k_{RI})R'(V')), \tag{6}$$

$$dC/dt = k_{R(V')C}R(V') + k_{IC}I - (k_{CR} + k_{CI})C, \tag{7}$$

$$dI/dt = -(k_{IC} + k_{RC})I + k_{CI}C + \int_{V'=V-\Delta/2}^{V=-V+\Delta/2} k_{RI}R'(V'), \tag{8}$$

where $\delta(V - V')$ is the Dirac delta function and k_{RR} is the rate constant for relaxation *within* the R-state, such that after a long time at one voltage the R-state population becomes concentrated at $V = V'$.

It should be noted that Eq. 6 in practice is not one differential equation, but rather an algorithm for writing $(V_{max} - V_{min})/\Delta$ simultaneous differential equations, where V_{max} and V_{min} are the range of voltages the membrane has experienced. Thus descrip-

tion of the sodium gating by this formalism involves the numerical integration of many more simultaneous equations than do other models used to describe this process. The need for the extra equations comes directly from the hypothesis of nonequilibrium dielectric polarization, the need for which hypothesis in turn comes directly from the observation of the τ_c-τ_h separation.

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HIGH-FREQUENCY DIELECTRIC SPECTROSCOPY OF CONCENTRATED MEMBRANE SUSPENSIONS

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The interfaces between biological membranes and their aqueous environments are of special interest to biology. This is because the many substances that bind to membranes, react with membranes, or pass through membranes must first enter and interact with these interfaces. We investigated the physiological chemistry and physics of the membrane-aqueous interface by high-frequency dielectric spectroscopy of concentrated suspensions of membranes. We adapted the recently developed "time-domain" technique for dielectric measurements (1). We observed a previously unreported dielectric absorption in concentrated suspensions of membranes obtained from either the outer segments of rod photoreceptor cells of the retina or blood erythrocytes.

We observed an anomalous dielectric absorption in these membranes having a dielectric constant of about 1,000 and a characteristic frequency, f_c, of about 170 MHz. It is a discreet absorption, well separated from the closest characteristic frequencies, 1 and 20,000 MHz, previously reported in tissue or cell suspensions (2). The discreet nature of the absorption ruled out the possibility that this absorption is the tail end of one of the neighboring absorptions. It also ruled out an artifact of the conductivity contribution to dielectric loss (3).