Local Transient Fluctuational Density as Producing Ionic Flow through Cell Membranes

The possibility of a coupling between a flow of information and a net flow of a given ionic species through cell membranes is considered. Conditions meeting the requirements imposed are discussed.

It is well known that in living systems, concentration gradients of ions and neutral molecules can be produced across the cell and organelle membrane systems. Molecular entities capable of generating such gradients are collectively known as PUMPS and have been extensively described in a variety of examples. In the present paper we analyze some hypothetical conditions in which random fluctuations of ion concentration in restricted regions might lead to transient ion flow through a cell or mitochondrial membrane and eventually produce ion concentration gradients across these structures.

The problem of generating concentration, pressure, or thermal gradients without "expenditure of energy," by simply sorting out particles of different velocities, has been extensively explored previously. These devices constitute variations of the so-called "Maxwell's demon" (1). It is generally accepted that solute flow against electrochemical gradient must be coupled to a flow of a metabolic reaction (2), which constitutes active transport. In this paper we consider the possibility of a coupling between a flow of information and a net flow of a given ionic species. Conditions meeting the requirements imposed are discussed.

We begin by considering a small volume of electrolyte solution surrounding the entrance of an ionic channel in a hypothetical membrane system, as shown in Fig. 1. The volume is small enough to allow significant fluctuations in the number of a given ionic constituting species, $n\alpha$. In order for the dissipation of such fluctuations to produce a transient flow of ions across the channel we require that: (1) They be uncorrelated with equivalent fluctuations taking place on the opposite side of the channel, and (2) they have a width comparable to the channel length (or membrane thickness), d, so that they are associated with a concentration of transitional regions having the same dimensions as d.

It is well known from the theory of fluctuations (3) that the mean square fluctuation of the number n_α of particles of type α in a small volume V of a solution containing a number N of molecules of the solvent is[1]

$$\overline{(\Delta n_\alpha)^2} = \frac{kT}{\left(\frac{\partial \mu'}{\partial n_\alpha}\right)_{P,T}}, \qquad [1]$$

where k is the Boltzmann constant and μ' is the chemical potential of the solute in the solution. The chemical potential for a one-solute system is

$$\mu' = kT \sum_\alpha \nu_\alpha \ln \frac{n_\alpha}{N} + \sum_\alpha \nu_\alpha \Psi_\alpha - \frac{e^3}{\epsilon_m^{3/2}} \left(\frac{\pi}{NvkT}\right)^{1/2} \left(\sum_\alpha \nu_\alpha z_\alpha^2\right)\left(\sum_\alpha n_\alpha z_\alpha^2\right)^{1/2}, \qquad [2]$$

where $\mu' = \sum_\alpha \nu_\alpha \mu_\alpha$, ν_α is the number of ions of type a in one molecule of the electrolyte, z_α is its corresponding valence, μ_α is the chemical potential of component α, Ψ_α are reference state chemical potentials (depending only on pressure and temperature), $v \cong V/N$, ϵ_m is the dielectric constant of the medium, e is the elementary charge (esu), and T the absolute temperature. For weak solutions, i.e., solutions in which the number of solute molecules is much less than the solvent ones, the electrostatic term can be dropped from Eq. [2], giving

$$\frac{\partial \mu'}{\partial n_\alpha} = kT \frac{\nu_\alpha}{n_\alpha}. \qquad [3]$$

This is the case, for instance, in the dissociation of water in water

$$H_2O \rightleftharpoons HO^- + H^+.$$

Consider now the addition of a symmetrical monovalent electrolite (AB) to an aqueous medium. Then, from Eq. [2] we have

$$\frac{\partial \mu'}{\partial n_{A+}} = \frac{kT}{n_{AB}} - \frac{e^3}{\epsilon_m^{3/2}} \left(\frac{\pi}{2n_{AB}VkT}\right)^{1/2}, \qquad [4]$$

where we have used $v \cong V/N$ and $n_{A^+} = n_{B^-} = n_{AB}$. The diameter D_α of the spherical volume element enclosing the fluctuation is related to the corresponding relative amplitude of the fluctuation, $\epsilon = \delta c/c = \delta n/n$, by[2]

[1] From now on we use the notation δn and δc for $\sqrt{[(\Delta n)^2]}$ and $\sqrt{[(\Delta c)^2]}$, respectively.

[2] Formulas [5] and [6] are also valid for OH^- and B^- ions, respectively.

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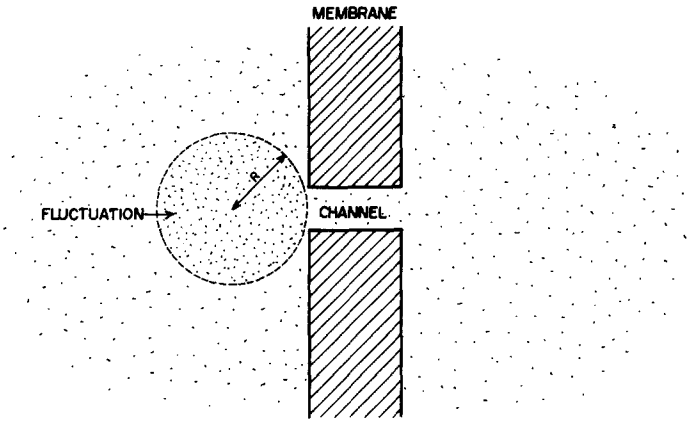

FIG. 1. Ionic fluctuation close to a membrane channel.

$$D_{H^+} = \left(\frac{A}{c_{H^+}\epsilon^2}\right)^{1/3} \quad [5]$$

$$D_{A^+} = \left(\frac{A}{c_{AB}\epsilon^2}\right)^{1/3} \Big/ \left[1 - \frac{e^3}{\epsilon_m^{3/2}}\left(\frac{\pi N_A}{2(kT)^3}\right)^{1/2} c_{AB}^{1/2}\right]^{1/3}, \quad [6]$$

where $A = 6/\pi N = 3.17 \times 10^{-24}$, $n = cVN_A$, c is the concentration in moles/m^3, and N_A is the Avogadro number.

The magnitude of the diffusional force $\mathbf{F}_\alpha$ (per unit of volume) acting on the component α during a short time interval (equivalent to the relaxation time of the fluctuation) is given by

$$\mathbf{F}_\alpha = -c_\alpha \frac{n_\alpha}{M_\alpha} \nabla\mu'_\alpha, \quad [7]$$

where M_α is the molecular weight of component α for weak solutions and from Eq. [2], can be written for the χ component of the force (4)

$$F_{\alpha x} = -RT \frac{\nu_\alpha}{M_\alpha} \frac{\partial c_\alpha}{\partial x}, \quad [8]$$

Figure 2(a, b) shows D_α as a function of ϵ for typical values of c_α of biological systems. We observe from Fig. 2(a, b) that the larger the required ϵ, the smaller the corresponding volume enclosing the fluctuation. It is apparent that very large relative fluctuations in the solute concentration take place in volumes which are negligibly small compared with the membrane thickness. On the other hand, for large enclosing volumes, the relative amplitude of the fluctuations are small. However, there is a broad range of concentrations for which significant fluctuations of c take place in enclosing volumes having diameters comparable to the membrane thickness. In these cases, the dissipation of a fluctuation close to the membrane could result in a transient net flow of the corresponding ion through the channel.

In the case of protons (Figure 2b), the fluctuation in concentration should have considerable significance due to the very low basal H^+ concentration. For example, in a volume of 1 μm^3 there are about 60 free protons at pH 7 and this number should fluctuate between 52 and 68, which should produce significant changes in the local pH. Such fluctuations could, in principle, be detected by an idealized pH microelectrode of sufficiently fast response, having a 1-μm tip diameter.

In cell organelles with lesser volumes, such as mitochondria, fluctuations in proton density should be even larger and the associated transmembrane proton gradients correspondingly so. Since many molecular processes are fast in comparison with the relaxation times of these protonic fluctuations, the very meaning of local pH in such small systems warrants revision.

Since the fluctuations in the concentration of a given species α take place with equal frequency on either side of a hypothetical channel (we assume equal solutions on both sides) they are not associated with a net stationary flow of α across the channel. The relatively recent identification of many types of gating mechanisms in ionic channels (5) gives however a novel dimension to this problem. Many mechanisms have been proposed to account for the gating property of ion channels (5). In some examples, gates interact with transmitter molecules, as in the case of the opening of end-plate channels by acetylcholine molecules. Rapid exchange of information in the form of conformational changes are expected to occur in this case. In other examples, gates are described as fluctuating entities sharing their time between open and closed configurations, randomly.

Ions are known to induce important conformational changes in many types of channels (7) and the binding of certain ions to specific sites in channel-like carrier systems possibly produces the necessary changes in conformation required to conform to affinity changes.

The problem of coupling the action of a microscopic

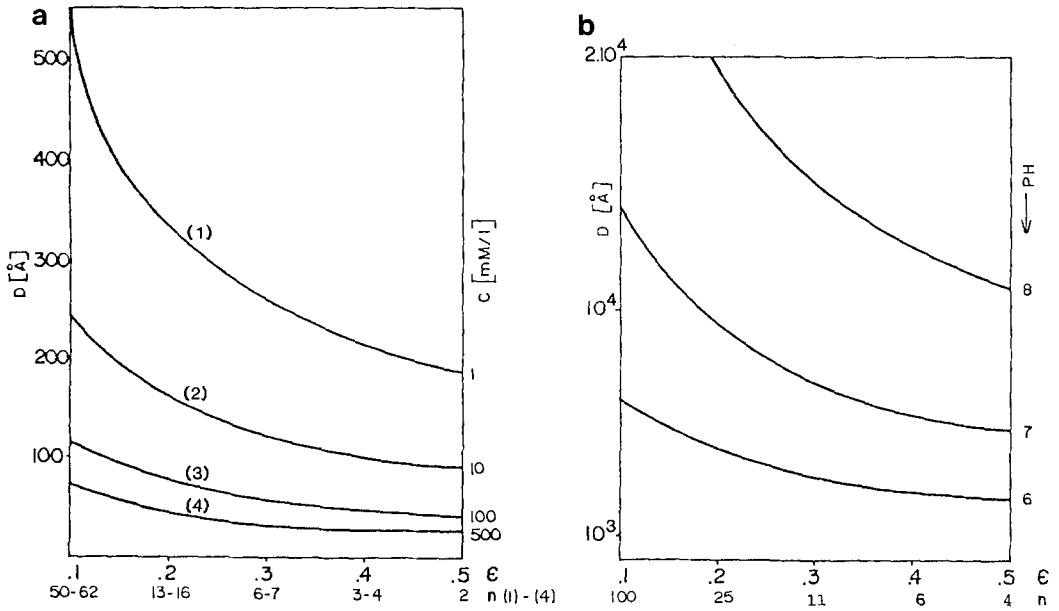

FIG. 2. Fluctuation size (D) versus its relative amplitude (ϵ) and number of the particles envolved (n) for typical values of concentrations (c) or pH of biological systems. (a) Ionic fluctuations in general. (b) Protonic fluctuations.

entity to the surrounding fluctuations of density, pressure, or temperature has been well explored since the last century (1, 6, 8). To enable a channel to correlate its state of aperture to the phase of a nearby ion density fluctuation, a flow of information is required by the second law of thermodynamics.

Also, so that random fluctuations of the concentration of an ionic species α could give rise to a net flow of α through a hypothetical channel, a close coupling between fluctuations and gating events is required. This requires a causal relationship between these two phenomena. Also, to produce a net flow of species α in a given direction, say $1 \rightarrow 2$, the channel is required to respond asymmetrically to ionic fluctuations on sides 1 and 2, namely, going to an open conformation each time it senses a local increase of c_α on side 1, and going to a closed conformation when local increments of c_α occur at side 2. Another requirement is that the conformational changes inside the channel, associated with state transitions, occur within a time shorter than the typical relaxation time of the fluctuation, given by

$$\tau_\alpha = \frac{d^2}{2D_\alpha}, \qquad [9]$$

where D_α is the diffusion coefficient of α. In the case of Na^+ ions, τ is of the order 10^{-8} s for a fluctuation spanning a region about the width of the membrane thickness.

Studies employing different techniques have shown recently (7) that channel proteins experience important conformational changes in response to binding or proximity of ions. Internal motions in proteins may involve individual atoms, groups of atoms, or whole sections of a molecule. The time scales of structural fluctuations extend from fractions of picoseconds for atomic vibrations, to nanoseconds, microseconds, or even milliseconds for larger scale conformational changes. According to a recent review (7), computer simulations of internal motions of proteins can be carried out for time periods of the order of a few hundred picoseconds. Such calculations have shown that fluctuations in the picosecond time scale consist basically of local oscillations superimposed upon collective motions of larger groups of atoms. These fast fluctuations are probably important for the much slower transitions involved in many biochemical reactions of proteins, such as enzymatic catalysis. Larger scale conformational changes, such as those required to make a channel go from a closed to an open configuration, must overcome high potential energy barriers caused by steric interactions. Fast fluctuations on the atomic scale can however provide a mechanism by which these energy barriers may be transiently lowered (7). The effects of conformational fluctuations on ion transport depend, according to Lauger (7), on the time scale of structural transitions in the channel protein: "When backbone motions are fast compared with the jumping frequency of ions in the channel, the ligand system may change its conformation many times during the dwell time of the ion in the energy well."

The above considerations suggest that it is possible, at least in principle, that transitions between open and closed channel states do occur faster than 10^{-8} s or so. Conse-

quently, it seems reasonable to consider that biological channels of cell and subcellular membrane systems display many of the necessary molecular devices required to recognize a fluctuating local-ion density and sincronously respond to it so as to create a net flow of a given ionic species. The main distinction between such a mechanism and well established active transport is that in active transport a flow of metabolic reaction is closely coupled to that of a chemical species, whereas in the mechanism proposed above, an ion flow is coupled with a flow of information. Both ultimately require expenditure of metabolic energy, be it to restore an enzyme to a previous affinity pattern (as in an ATPase for example) or to erase molecular memory of previous information content (6) (as in the present proposal).

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