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ANALYSIS OF THE IRREVERSIBLE PROCESS OF PROTON TRANSPORT IN THE PURPLE MEMBRANE OF *HALOBACTERIUM HALOBIUM*

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ABSTRACT

The proton transport across biological membrane, accompanied by energy transformation, is closely related with many basic processes involved in the maintenance of life. Active researches are carried out in this field, but so far we have not known a complete calculation.

This paper presents a model of an open and closed photon-controlled ion pore with a quantitative analysis of the irreversible process of the proton transport across the purple membrane. Upon absorbing photon by the purple membrane, the deprotonation of the Schiff base causes the ion pore to open, but it will close when it returns to bR_{570}. A set of nonlinear differential equations describing this model is given. The stability of the equations is discussed. The results of the numerical calculation for steady state are found in good agreement with the experimental data of Bakker.

I. INTRODUCTION

Halobacterium Halobium found in 1967[1] has been studied vigorously in recent years. Its function is just like an ion pump. Under the irradiation of visible light, protons are pumped from the inside to the outside of the membrane, and the concentration gradient of hydrogen ion is formed, that eventually leads to the synthesis of ATP. As to the energy transformation, the purple membrane possesses the fundamental functions of chloroplast, but its photochemical process and physical or chemical composition are far simpler. In addition, it is easy to be separated from the wall[2] and to be handled in the experiment.

The purple membrane of *Halobacterium Halobium* includes only one kind of peptide (molecular weight 25,100) and contains seven lysines. This kind of protein is called bacteriorhodopsin. Its characteristics are similar to the rhodopsin found in retina. The protein and lipid are arranged orderly and like a layer of smectic LC.

According to the characteristics mentioned above, we consider that it is the simplest biological membrane. This paper presents a model of an open and closed photon-controlled ion pore. A set of nonlinear differential equations describing the changes of conformation concentrations is given, and the stability of the equations is discussed.

The results of the numerical calculations of the steady state as compared with the experimental data of Bakker, are found in good agreement.

II. Model

According to the diffraction patterns of X-ray[3-5], neutron[6] and electron[7,8] for purple membrane of *Halobacterium Halobium*, it has been found that the membrane has a two-dimensional hexagonal structure. Its protein molecule consists of seven α-helices lying perpendicular to the membrane surfaces. The length of the helicoid rod is 40 Å. Lipid molecules are orderly arranged in the space among the proteins. Its characteristic dimension from the membrane surface is 4.6 Å (Fig. 1). The interior of such a lipid protein membrane is made up of hydrocarbon chains[9]. Probably this means that the membrane is a medium of low dielectric constant when compared to a liquid phase. Hence, the energy required to bring a small ion from the aqueous phase into the membrane will be many times the mean thermal energy. Considering the membrane as a thin homogeneous film of dielectric constant ε_m interposed between two media of dielectric constant $\varepsilon (\varepsilon > \varepsilon_m)$. Laüger[10] *et al.* have made calculations of the potential energy distribution curve, which has the shape of a symmetrical barrier with a peak in the middle of the membrane. The image force always tends to repel the ion from the membrane to the aqueous phase. So ions could only be transported across the membrane either at the location where dielectric constant increases sharply, or in combination with some carriers, thus becoming dependant on the migration of the carriers for transport. The characteristic time for rotating 180° within the membrane for protein is known to be about 0.1 ms, and that for lipid is much longer[9], whereas the characteristic time of ion passing through the membrane by irradiation is in the order of 10 μs[11]. So, it is evident that the model of carriers is not appropriate for this purpose.

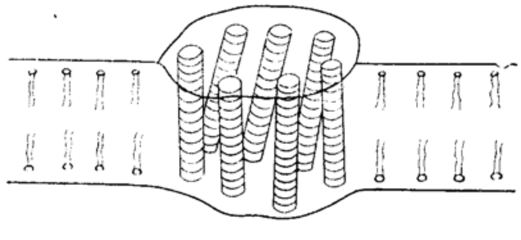

Fig. 1. Diagram illustrating bacteriorhodopsin consisting of seven α-helices.

We consider that there exists in the purple membrane an ion pore of 10 Å in diameter, surrounded by seven α-helices of bacterio-opsin. One end of the pore is open to ions and the other is controlled by the absorption and desorption of the chromophore (retinal) to make it "open" or "close" to the ions.

Different kinds of spectrotechnology[2] have proved that the retinal through a protonated Schiff base combines with an amino ε of Lys* in the bacterio-opsin. The amino acid sequence close to this Lys* is —Pro—Asp—Lys—Lys*—Phe—Tyr—. Beginning with the Pro, there are six consecutive hydrophilic amino-acids, so we can infer that here lies the joint of two helices on the boundary to the aqueous phase with retinal on the surface of one side of the membrane. Although the experiments have not yet been able to ascertain whether the retinal is located on the inner or outer side of the cell, this will not affect the calculations with the model.

From the photochemical studies, it is known[11] that after irradiation, bacteriorhodopsin bR_{570} is bleached into M_{412} (subscript denotes the wavelength of the absorption peak) and then returns to bR_{570}. From resonance Raman spectra[12], the deprotonation of the Schiff base occurs at the time of forming M_{412}. So, we may assume that after the formation of M_{412}, the ion pore opens, but closes when M_{412} returns to bR_{570}. During the "open" and "close" processes, the thermodynamic potentials of the bacteriorhodopsin changes are shown in Fig. 2. Montal's experiment[13] on the artificial membrane of lipid and rhodopsin has shown that light actually increases the permeability of the positive ion to a substantial extent.

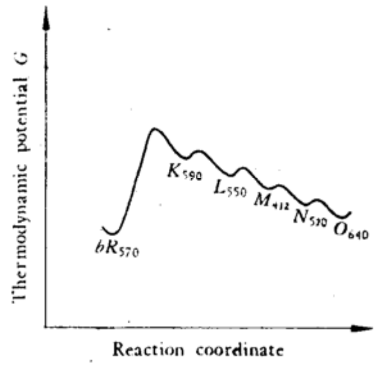

Fig. 2. Diagram illustrating the changes of the thermodynamic potential G of the bacteriorhodopsin during the "open" and "close" processes.

At the same time, apart from the above-mentioned osmosis, Mitchell[14] has shown that through the transfer of electrons and chemical groups, oxido-reduction will cause the concentration difference between the inside and outside of the membrane, whereas hydrodehydration may reduce the difference accompanied by synthesis of ATP, which completes the conversion of the biological energy. Chemosmosis is a complex process and can only take place in the red membrane having 25 different kinds of polypeptides.

Judging from the above, we suggest the following calculation model:

(1) Only the transport across the membrane is accounted. The number concentration of proton outside the membrane is denoted as A_0 and that inside of it as A_i.

(2) If the transport is to occur, protons must pass through the pore of bacterioopsin to be transported from one side of the membrane to the other. The five possible conformations are given in Fig. 3. The "closed" state corresponds to bR_{570}. Through a protonated Schiff base, the retinal located on the outside surface of membrane boundary as shown in Fig. 3 combines with bR_{570} and causes the door to close. After irradiation, the door opens and four conformations are possible. The differences of adsorption k_a, desorption k_d and permeability k_m between the two sides of the membrane have not been considered.

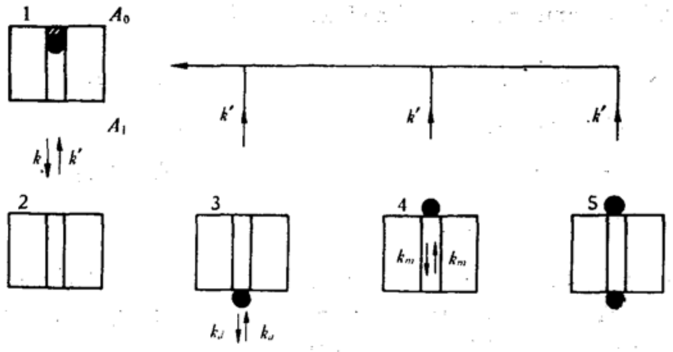

Fig. 3. Five conformations of bacterio-opsin.

● Proton.

(3) Chemosmosis takes place in the red membrane. Ion concentrations on the two sides of the membrane are homogeneous. Proton flux of chemosmosis $\dot{m}_{ch}$ represents the net proton numbers transported from the outside to the inside of the membrane by chemical reaction. The synthesis rate of ATP should be proportional to $\dot{m}_{ch}$. Under the condition of oxygen deficiency, the fully dark-adapted state corresponds to $\dot{m}_{ch} = 0$. The heavy dots shown in Fig. 5 are derived from the experimental results of Bakker. The relation of $\dot{m}_{ch}$ to A_0, A_i could not be deduced from the theory. According to the experimental results[17], we resort to the empirical formula,

$$\dot{m}_{ch} = K \left\{ \log \frac{A_0}{A_i} - 4.6 \left[\left(\frac{A_0}{A_0^*} \right)^{0.08} - 1 \right]^2 - 0.176 \right\}^n, \tag{1}$$

where A_0^* is the concentration of hydrogen ion when $pH_{out} = 8$. In case of not far diverging from zero steady state, we can assume $n = 1$ as a linear approximation.

As to the conformations in Fig. 3, the rate equations are

$$x_1 + x_2 + x_3 + x_4 + x_5 = 1, \tag{2}$$

$$\frac{dx_1}{dt} = -kx_1 + k'(x_2 + x_3 + x_4 + x_5), \tag{3}$$

$$\frac{dx_3}{dt} = -k'x_3 + k_a(A_i x_2 - A_0 x_3) + k_d(x_5 - x_3) + k_m(x_4 - x_3), \tag{4}$$

$$\frac{dx_4}{dt} = -k'x_4 + k_a(A_0 x_2 - A_i x_4) + k_d(x_5 - x_4) + k_m(x_3 - x_4), \tag{5}$$

$$\frac{dx_5}{dt} = -k'x_5 + k_a(A_i x_4 + A_0 x_3) - 2k_d x_5, \tag{6}$$

$$\frac{dA_0}{dt} = Nk_d(x_4 + x_5) - Nk_a A_0(x_2 + x_3) - k'Nx_2 + kNx_1 - \dot{m}_{ch}, \tag{7}$$

$$\frac{dA_i}{dt} = Nk_d(x_3 + x_5) - Nk_a A_i(x_2 + x_4) + k'Nx_5 + \dot{m}_{ch}, \tag{8}$$

where $x_i = N_i/N$ is the relative concentration of ith conformation, and N is the number concentration of bacterio-opsin.

The dimensionless numbers are defined as follows:

$$j = k'/k_d, \qquad a_i = A_i k_a/k_d,$$
$$m = k_m/k_d, \qquad k = k/Nk_d,$$
$$\langle r \rangle = x_2 + x_3 + x_4 + x_5, \qquad J_{\text{ch}} = \dot{m}_{\text{ch}}/Nk_d,$$
$$a_0 = A_0 k_a/k_d,$$

where $\langle r \rangle$ is the percentage of M_{412}, and j is the ratio of the "closed" rate constant to the desorption coefficient. These two numbers should both be less than 1 and $\langle r \rangle$ decreases with the reduction of light intensity, while in fully dark state $\langle r \rangle = 0$. k/k' is a constant related to $\langle r \rangle$. Because the period of the transport process is much longer than the process of desorption, generally m should be less than 0.01. a_0 and a_i are the dimensionless proton concentrations inside and outside of the membrane respectively.

Rewrite Eqs. (7) and (8) into dimensionless forms,

$$\frac{1}{Nk_a}\frac{da_0}{dt} = x_4 + x_5 - a_0(x_2 + x_3) - jx_2 + \frac{k}{k'}x_1 - J_{\text{ch}}, \tag{9}$$

$$\frac{1}{Nk_a}\frac{da_i}{dt} = x_3 + x_5 - a_i(x_2 + x_4) + jx_5 + J_{\text{ch}}. \tag{10}$$

When k', $k_d > N \cdot k_a$, it can be assumed that steady state between the conformations will be readily reached, so that Eqs. (2)—(6) become

$$\left.\begin{aligned} & x + \langle r \rangle = 1, \\ & \frac{k}{k'}x_1 = \langle r \rangle, \\ & x_3(j + 1 + a_0 + m) = a_i x_2 + m x_4, \\ & x_4(j + 1 + a_i + m) = a_0 x_2 + m x_3 + x_5, \\ & x_5(2 + j) = a_0 x_3 + a_i x_4. \end{aligned}\right\} \tag{11}$$

Deducing x_1 to x_5 from Eq. (11) and substituting them into Eqs. (9) and (10), we obtain

$$\frac{1}{k_a N}\frac{da_0}{dt} = J_{\text{p}} - J_{\text{ch}}, \tag{12}$$

$$\frac{1}{k_a N}\frac{da_i}{dt} = -J_{\text{p}} + J_{\text{ch}}. \tag{13}$$

$$J_{\text{p}} = \langle r \rangle \frac{\alpha\delta(m + j)a_i^2 - \alpha m\delta a_0^2 + (\alpha m + j)\delta a_0 a_i + (j\alpha + 2m)a_i - m a_0}{\delta a_0 a_i(a_0 + a_i) + \delta\theta a_0 a_i + \delta(m + \alpha)(a_i^2 + a_0^2) + \lambda(a_0 + a_i) + \xi}, \tag{14}$$

$$J_{\text{ch}} = k\left\{\log\frac{a_0}{a_i} - 4.6\left[\left(\frac{a_0}{a_0^*}\right)^{0.08} - 1\right]^2 - 0.176\right\}, \tag{15}$$

where δ, α, θ, ξ, λ are the constants depending on j and m.

$$\left.\begin{aligned} \delta &= \frac{1}{2+j}, \\ \alpha &= j+1, \\ \theta &= 3j+2m+4, \\ \xi &= (j+1)(j+2m+1), \\ \lambda &= j+1+2m+\frac{j^2+2j+1+jm}{j+2}. \end{aligned}\right\} \tag{16}$$

Two variable nonlinear differential Eqs. (12) and (13) are dynamic equations, defining the transport process. J_p shows the dimensionless proton flux photo-pumping from the inside to the outside of the membrane.

Eqs. (14) and (15) apparently express the relation of the "flux" to "force" for ion pump and transducer in Fig. 4. We can use them and general theory[15,16] to discuss the stability of steady state and dissipative structure.

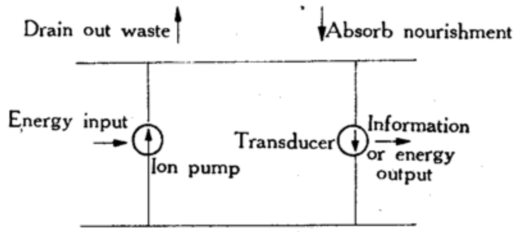

Fig. 4. The function of membrane.

III. Discussion

The difference between Eqs. (12) and (13) is only a negative symbol, so their integral curves are a set of straight lines $a_0 + a_i = c$, where c is given by the initial conditions. The result of straight lines reflects the fact that only physical and chemical transports are concerned and the creation and annihilation of the proton by chemical processes are not accounted. It shows the restraint of conservation of the whole proton numbers on both sides of the membrane.

Because of this restraint, the solution of steady state is not confined to some isolated singular points on a_0, a_i plane; it is a curve described by

$$J_p - J_{ch} = 0. \tag{17}$$

J_p is related with the dimensionless parameters $\langle r \rangle$, j and m. The j and m cannot be chosen arbitrarily, but must be in accord with general facts. From Eq (17), it can be seen that variation in $k/\langle r \rangle$ will only change the vertical position of the curve, but not its shape. It can also be seen from Fig. 5 that the results of calculation with $a_0^* = 0.03$ and $k/r = 0.004$ are in good agreement with the experimental data by Bakker. The a_0, a_i plane is divided into three parts by the dark-adapted and light-adapted lines. In parts I and II, $J_p > 0$; in part III, $J_p < 0$. In part II, $J_p > J_{ch}$ is

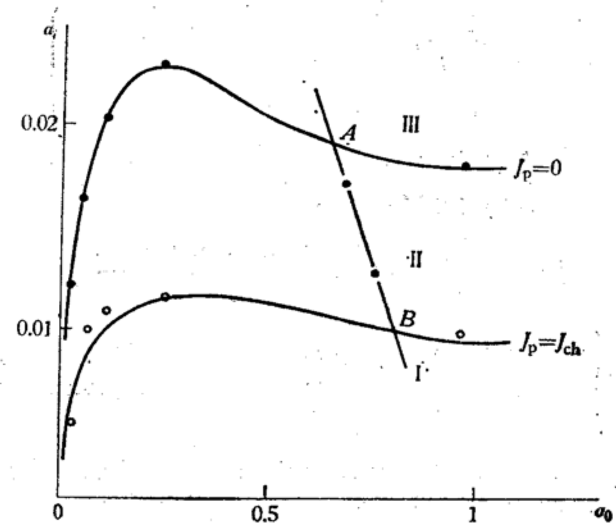

Fig. 5. Diagram showing the singular curve on a_0, a_i plane. In parts I and II, $J_p > 0$; in part III, $J_p < 0$; in part II, $J_p > J_{ch} > 0$; in part I, $J_{ch} > J_p > 0$.

● dark; ○ light.

satisfied and in part I, $J_{ch} > J_p$ is satisfied. According to Eqs. (12) and (13), in mathematical sense the singular curve which corresponds to the case of strong light is a sink line. So long as the intensity is sufficient to make $J_p > 0$, there will be a state corresponding to dark-adapted case A along with the straight line AB for transition from A to B. The B point is steady. Bakker[17] and Danon[18] both have proved by experiments that after the membrane is suddenly exposed to the light, the transition is steady, and no concentration vibration phenomena such as limit-cycle will occur. According to thermodynamics it is reasonable, because the main function of purple membrane is the energy transfomation, but not produces information. If the dissipative structure does not occur, the process would maintain a less irreversibility and has a rather high efficiency[15].

It has been further found in experiments by Bakker and Danon that when purple membrane is irradiated in weak light intensity, a_0 sometimes will be less than that in dark-adapted case. It is known as an acidification and can also be explained by Eq. (14), and the condition of $J_p > 0$ can be deduced to,

$$j(j+1) + 2m + \frac{j}{j+1} a_0 + \frac{j+1}{j+2} m a_0 (1 - a_i) + \frac{j+1}{j+2} (m + j) a_i > \frac{a_0}{a_i} m. \tag{18}$$

Therefore, if the light intensity is not sufficient enough to maintain inequality (18), irradiation will cause acidification. Critical value j_c is defined when inequality (18) changes to equality, and is related to light intensity and a_0. When the light intensity exceeds the critical value, alkalization occurs instead of acidification. We are studying further to judge if this kind of sudden change is a dissipative structure. In principle, the calculations presented here may be applicable to other kinds of membrane as well.

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