Hypothesis

AN EXPLANATION OF THE PROTON UPTAKE OF CHLOROPLAST MEMBRANES IN TERMS OF ASYMMETRY OF THE SURFACE CHARGES

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1. Introduction

In spite of large amounts of experimental data concerning active transport phenomena across biological membranes, there is a lack of knowledge of principles by which the active transport operates. Quite often there is a tendency to use terms such as 'ATP-driven sodium pump' or 'light-driven hydrogen pump' in order to name sophisticated lipid–protein complexes which appear to push specific ions against their electrochemical gradients. One can hardly be satisfied with the acceptance of the name of a device as a principle of its operation, hence it seems important to seek the reasons for these phenomena. The model we present here is an attempt to give a physical basis to the events associated with the ionic transport, especially through the energy transducing membranes. In the simplest words, the postulated model can be summarized as follows:

1. In equilibrium the enclosed volume and the outside solution have the same electric potential and the same concentration of ions, save for the diffuse charge layers adjacent to the membrane (fig.1a).
2. There is a difference in surface charge densities between inside and outside face of the membrane. In equilibrium this difference creates an internal electric field in the membrane.
3. On absorption of light, or due to oxido–reduction reactions, current carriers (e.g., electrons moved into an excited state by light) are created in the membrane and a current flows in the direction of the field leading to a decrease in the potential difference between the two faces of the membrane.
4. The movement of permeant ions, and other recombination processes in response to the decrease of the potential difference tend to restore the original equilibrium field. A steady state can be reached when all these fluxes are minimal. In general, the steady state depends on the permeability of the

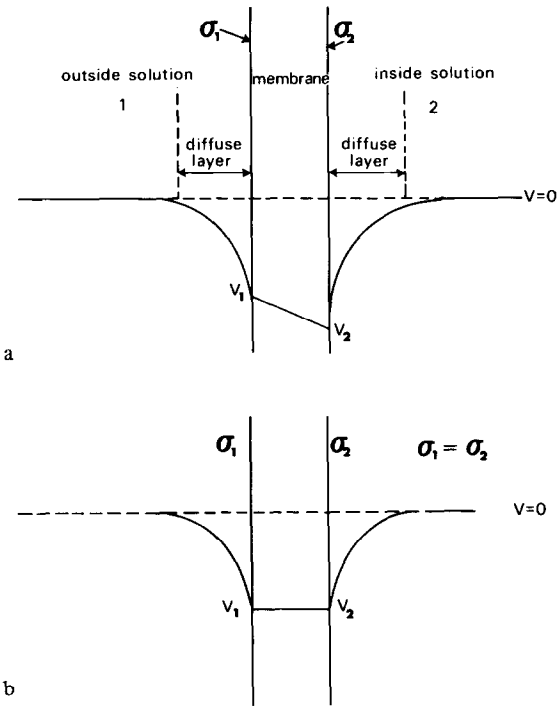

Fig.1. Potential profiles across the thylakoid membrane: σ_1, σ_2, surface charge densities; V_1, V_2, surface potentials at the external and internal side of the membrane, respectively. (a) In the dark equilibrium $|\sigma_1| < |\sigma_2|$ hence $|V_1| < |V_2|$. (b) In the light-induced steady state $\sigma_1 = \sigma_2$ hence $V_1 = V_2$.

membrane to the various ions. In chloroplast thylakoids under non-phosphorylating conditions but in the light, the steady state is characterized by the internal electric field approaching zero level within the membrane. This is a consequence of the equalization of the surface charge densities, i.e., symmetry.

The model does not pretend to explain the mechanism of generation of the current carriers in the membrane. Such a mechanism is an individual feature of each type of membrane, and is known to be complicated, especially in chloroplasts [1].

2. The model

The structure we are dealing with is the basic bioenergetic unit [2]: an enclosed aqueous region separated from the external aqueous solution by a membrane. We shall assume that the only fixed charges are on the internal and external surfaces of the membrane. Our main postulate is that there is an asymmetry of the surface charges between two sides of the membrane. This is not an established fact, though a few experimental results support this in the case of excitable membranes [3], bacterial membranes [4–6] and chloroplast thylakoids [7]. These surface charges arise out of the ionization of the surface groups such as heads of phospho- and sulpho-lipids or amino or carboxylic groups of membrane proteins. For the purpose of this discussion, we shall confine our attention to the case of acidic groups, and assume that for the chloroplast membranes the inside face is more negative than the outside. The following analysis can be logically extended to include basic groups and/or inverse polarization of the membrane.

By virtue of the fact that no membrane is completely impermeable for some ion, the equilibrium conditions require that the electric potentials and concentrations of each ion when measured sufficiently far away from the membrane on both sides are equal. Here, we are assuming that the width of the enclosed region is greater than a few Debye lengths of the bulk solution [8]. At this stage it is worthwhile to mention that, for instance, at 20 mM NaCl, 1 mM $MgCl_2$ and pH 7.0, the Debye length is around 21 Å. On the other hand the intrathylakoid space measured between the opposite lipid headgroups has the width of around 80 Å [9], thus the condition stated above appears to be satisfied. However, if it is not fulfilled, the following analysis will be subject to some quantitative changes, whilst the basic idea of the function of the equilibrium internal field in the oriented ionic transport remains the same. We shall come back to the above problem in a future communication.

The postulated asymmetry of the surface charges leads to an internal electric potential gradient across the membrane (fig.1a). Indeed, had the surface potentials on both sides been identical, the potential slopes in the diffuse layers would have been symmetric in view of the same ionic conditions on both sides far away from the membrane. As the surface charge is related to the potential gradient near the surface [8], the symmetric profiles imply that the surface charges would have been identical on both faces.

Let us now assume that by the action of external agents current carriers (e.g., electrons, cations or their transporters) are generated in the membrane. (It is instructive to realize that analogous events happen in semiconductors, where by an external force, such as light, mechanical or thermal disturbance, electrons can be moved from the valence to conduction band, thus giving rise to an increased conductivity [10].) Due to the internal electric field, a current will flow and a recombination of charges will take place at both surfaces. The decrease in the internal electric field associated with the current flow will cause a change of the surface potentials thus forcing a diffusion of ions in such a way as to attempt to restore the equilibrium internal field (according to Le Chatelier's principle). In the case of chloroplasts the internal face of the thylakoid membrane becomes more positive, which causes a diffusion of Mg^{2+} outside, and an influx of Cl^- [11]. In the presence of K^+ and valinomycin, the K^+ will respond to the increase in the internal surface potential much faster than Mg^{2+}, thus the restoration of the internal electric field is accelerated. H^+ movement on the other hand is coupled to the electron transport (through plastoquinone) and since protons are otherwise weakly permeant they accumulate inside.

The process will continue until a steady state, which is characterized by the thermodynamic law of minimum entropy production, is reached [12]. Considerations of such steady states may, in general, be quite involved. Below we discuss the case of chloro-

plast thylakoids in non-phosphorylating conditions under steady saturating light. To a first approximation the thylakoid membrane under such circumstances is impermeable to hydrogen ions thus the concentrations of protons in the diffuse layers on both sides of the membrane can be quite different without a significant contribution to the entropy production in the system (the entropy production is related to the diffusion fluxes of ions across the membrane). On the other hand, a diffusion equilibrium is set up for permeant ions and the internal electric field vanishes since the membrane is still conducting. In order to satisfy the last two conditions, the potentials and concentrations of ions (other than H^+) near the surfaces on both sides must be equal. In view of the experimental finding that the free aqueous H^+ concentration inside is $< 10^{-4}$ M, while the concentrations of other ions such as Mg^{2+}, K^+ and Cl^- greatly exceed this amount [13, 15], it follows that the potential profile will be determined by the latter species. Thus, in the non-phosphorylating steady state in the light the electric potential is symmetric (fig.1b) and the surface charges on both faces are identical. This can take place when the internal aqueous hydrogen concentration is sufficiently high to allow protonation of the internal excess negative charges.

3. Some conclusions from the model

3.1. *Internal hydrogen concentration*

As a first application of the model let us calculate the concentration of H^+ near the internal surface as a function of external pH for chloroplasts. Denote by r_1 and r_2 the number of acidic surface groups per unit area on the external and internal face of the membrane, respectively. For chloroplasts, according to the model, we have $r_2 > r_1$, since the electron flow is directed from inside. Let K_1 and K_2 be dissociation constants of the corresponding groups and $[H_1^+]$ and $[H_2^+]$ the concentrations of aqueous H^+ near surface 1 and 2, respectively. The surface charge density on each side is given by:

$$\sigma_i = -er_i/(1+[H_i^+]/K_i),\ i = 1,2 \qquad (1)$$

where e is the electron charge.

In the non-phosphorylating steady state but in light, we have $\sigma_1 = \sigma_2$, that is:

$$r_1/(1+[H_1^+]/K_1) = r_2/(1+[H_2^+]/K_2) \qquad (2)$$

Hence the ratio of the H^+ concentrations on both sides can be expressed as:

$$[H_2^+]/[H_1^+] = (r_2/r_1 - 1)\,(K_2/[H_1^+]) + (r_2/r_1)\,(K_2/K_1) \qquad (3)$$

An approximation of eq. (3) can be made when the first term on the right handside of eq. (3) is much larger than the second one. The estimate of pK values by Åkerlund et al. [7] gives $pK_2 = 4.1$ and $pK_1 = 4.4$. Assuming that the surface density asymmetry r_2/r_1 is, say, > 1.1 we can neglect the second term when the pH_1 is > 6.75 and the resulting error will be $< 10\%$.

Equation (3) is then simplified to:

$$[H_2^+] = (r_2/r_1 - 1)\,K_2 \qquad (4)$$

which means that the steady state concentration of H^+ near the internal surface is independent of outside pH, for $pH_1 > 6.75$.

As a consequence one can expect a linear dependence of experimentally measured ΔpH on the pH in the bulk solution, provided the latter is sufficiently high. Indeed, experiments results of Pick et al. [14] and Chow [15] confirm this for pH 7–9. At pH < 7 there is a decline from the linear behaviour, which indicates that full eq. (3) should be used instead.

3.2. *Effects of K^+ and valinomycin*

The light-induced electron flow in chloroplasts involves several intermediate carriers [1] of which at least one, plastoquinone, is mobile. A recombination at the external surface may be achieved by protonation of negatively charged PQ^{2-} to neutral PQH_2, which is believed to be hydrophobic [16] thus able to move within the membrane.

We propose that the re-cycling of plastoquinone may occur, through two alternative processes:

1. By giving up protons in phosphorylation process as suggested by Robertson and Boardman [16];
2. By giving up protons at the inside surface of the

membrane when the internal electric field is sufficiently strong.

In normal conditions it appears that the rate limiting step in the establishment of the steady state is the diffusion of ions across the membrane. As we have mentioned above, the movement of ions is, according to Le Chatelier's principle, trying to restore the internal electric field, which has been decreased through the fast electron transfer across the membrane. Thus the oxidation of PQH_2 will relate to the phosphorylation process. However, if K^+–valinomycin is added, the restoration of the internal electric field is accelerated, forcing PQH_2 to give up H^+ at the inside face of the membrane. The pool of PQH_2 is diminished and phosphorylation cannot proceed until a pH gradient between two surfaces rises to such value that a new pathway of protons is established.

The above mechanism would explain experimental observations of the time lags in photophosphorylation upon addition of K^+–valinomycin to the system [17–19]. It is worthwhile to note that a similar reasoning can be applied to explain the effects of nigericin on ATP synthesis in chloroplasts [18].

It has not escaped our notice that the induced change of membrane surface charge densities, asymmetry to symmetry, provides a physical basis for the vectorial direction of H^+ in the Mitchell chemiosmotic hypothesis. However further analysis is necessary to decide whether the H^+ directly active in photophosphorylation are either the minor uptake of aqueous H^+ in the osmotic volume or the possible internal membrane protonated carriers, or the vast majority uptake of H^+ bound to surface groups which possess a lateral mobility along the fluid membrane surface, all three induced pools being in equilibrium with each other.

Further work is also needed to tie up the surface potential difference with fluorescence and absorbance changes which may serve as useful indicators of the electrical phenomena in the membrane [20–23].

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