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Membrane Potential as a Coupling Agent for Photophosphorylation by Bacteriorhodopsin and ATP-ase Containing Artificial Membrane

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The steady state kinetic and thermodynamic properties of a »minimal« photosynthetic system containing two different proton pumps in the planar artificial membrane have been considered. The light activated proton pump, modelled after bacteriorhodopsin (bR), and the ATP-using proton pump, modelled after *Neurospora* ATP-ase, were interacting only through the common photopotential developed by bR. In the stationary state of the illuminated coupled system the following properties were calculated by Hill's diagram method: proton flux for each macromolecule, photopotential, total effective force, efficiency of free energy storage, efficiency of light free energy utilization, entropy production, and adenylate energy charge in the internal compartment.

1. INTRODUCTION

Two electrogenic ion pumps located in the same membrane, but physically separated, can still interact through transport of the common ligand. In a highly shematic model[1] it was shown that respiratory control, i. e. the dependence of respiratory rate on adenosin diphosphate (ADP) concentration, can be understood as an example of kinetic, rather than physical interaction, between two different proton pumps. Internal proton concentration serves as the coupling agent between electron transport enzymes and adenosin triphosphatase (ATP-ase).

In this paper we examine the role of membrane potential as a coupling agent for photophosphorylation by a »minimal« photosynthetic system. Such a system, depicted in Figure 1, consists of an artificial membrane containing bacteriorhodopsin (bR) and ATP-ase. Bacteriorhodopsin is the integral membrane protein isolated from the purple membrane of *Halobacterium halobium* which acts as the light-activated proton pump[2]. There are many different types of proton pumping ATP-ases, but we shall consider here only the ATP-ase of *Neurosopora*[3]. In its usual mode of action, ATP is hydrolyzed and electrochemical proton gradient produced.

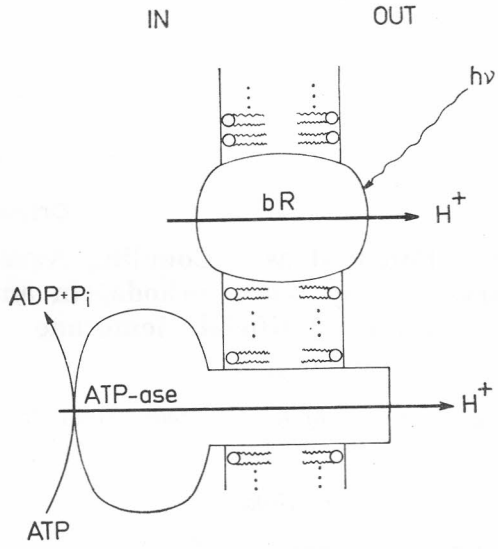

Figure 1. Artificial membrane containing bacteriorhodopsin (*bR*) and ATP-ase as »minimal« photosynthetic system. ATP synthesis becomes possible when primary transducer (*bR*) succeeds in converting photon force energy into a big enough proton electrochemical gradient to induce ATP-ase to work in reverse mode. Black lipid membrane can be used as planar artificial membrane with phospholipids in the lipid phase and proteins incorporated in appropriately established membrane.

We assume that reconstitution experiments have been successful, i. e. that both bR and ATP-ase have been incorporated in the artificial plannar lipid membrane separating compartments of known chemical composition. By means of suitable buffers and external electrodes proton concentrations can be kept constant in both compartments and transmembrane currents measured.

The mechanistic and structural details are still incopletely known for these two active transport systems, but a model can be proposed which is thought to contain some of the essential features of real proton pumps. We shall follow Laüger's approach[4], who recently discussed in considerable details the thermodynamics and kinetics of action for bR and ATP-ase proton pumps.

2. RESULTS

The orientation of both macromolecules in the membrane (Figure 1) is assumed to be such that the proton circuit is closed. Each proton pump is an energy converter in its own right. bR transforms light free energy into membrane potential, and ATP-ase (when acting in reverse mode) transforms membrane potential in Gibbs free energy of ATP hydrolysis.

For a formal description of the pumping process each proton pump may be treated as an ionic channel with multiple conformational states. In the presence of an appropriate energy source the macromolecule goes through a cycle of conformational transitions during which the potential energy barrier structure of the channel is transiently modified in order to accomplish proton translocation across the membrane.

We want to examine the nature of the steady state free energy transduction starting from the minimal kinetic model with given conformational states, transitions and rate constants. Hill's diagram method[5] is used to represent ATP-ase (Figure 2a) and bR (Figure 3a) pumping action. »Reduced« diagrams[5] (Figures 2b and 3b) are still equivalent to Laüger's reaction scheme for ATP driwen (Figure 4 in reference 4) and light driven (Figure 18 in reference 4) proton pump, respectively.

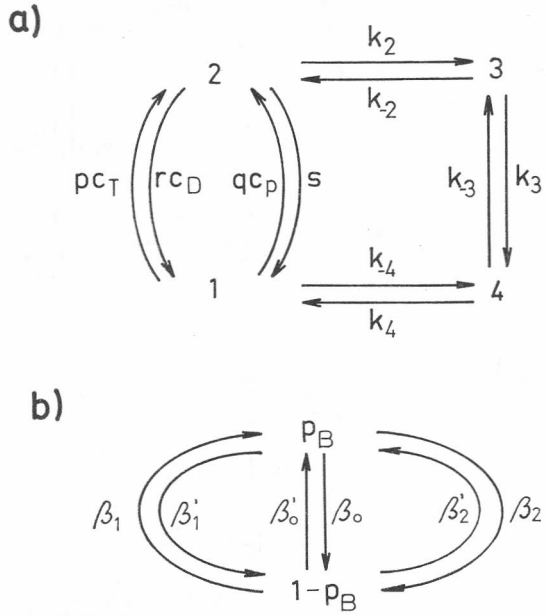

Figure 2. Model for ATP driven proton pump (macromolecule B). a) Horizontal transitions represent »fast-equilibrium« protonation or deprotonation steps. In states 2 and 3, proton binding site is exposed to the external compartment, while in states 1 and 4 it is exposed to the internal compartment. Conformational transitions occur together with phosphorylation/dephosphorylation reaction in steps $1 \rightleftarrows 2$ and $3 \rightleftarrows 4$. In external cycle $1 \rightarrow 2 \rightarrow 3 \rightarrow 4 \rightarrow 1$ one ATP molecule is hydrolyzed and one proton transported to the right compartment b) »Reduced« diagram in which fast equilibrium between states $2 \rightleftarrows 3$ and $4 \rightleftarrows 1$ has been used to reduce the number of states in the model from 4 to 2. The probability of »fast equilibrium« states is p_B and $1 - p_B$, respectively. The connection between reduced and nonreduced rate constants is given in the text.

II a) ATP-Driven Proton Pump

Phosphorylated protein in state 2 (Figure 2a) has a proton binding site exposed to the right (external) compartment, while dephosphorylated protein in state 1 accepts the proton mainly from the left (internal) compartment. During the cycle 1→2→3→4 the proton is preferentially released to the external side and another one is taken up from the internal side. Concomitantly, ATP is hydrolyzed (transition 1→2) and inorganic phosphate is released (transition 3→4). All transitions are treated as first order processes but some rate constants can be represented as $k = k^* c_s$, where k is the first order rate constant, k^* the second-order rate constant, and c_s the con-

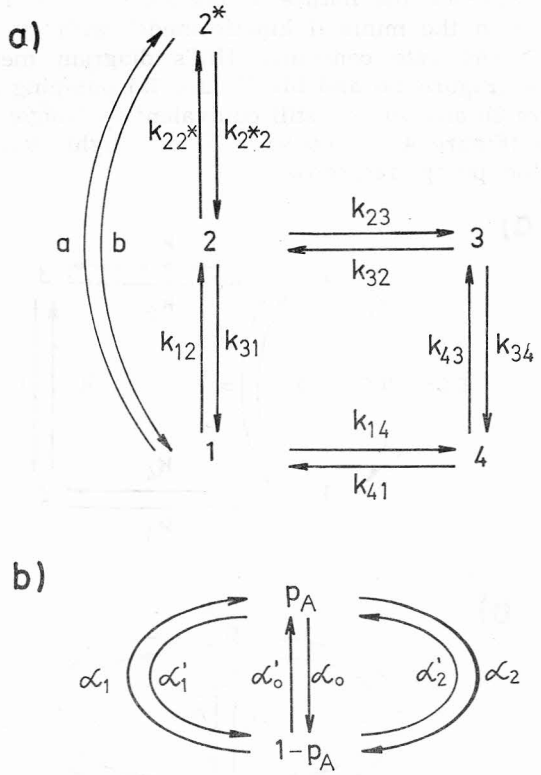

Figure 3. Model for light-driven proton pump (macromolecule A). a) Five states kinetic scheme. States 1, 2, 3 and 4 are similar to those in Figure 2a, while 2* is a short-lived excited state. Fast equilibrium is assumed between states $2^* \rightleftarrows 2$ and for protonation-deprotonation reactions $2 \rightleftarrows 3$ and $4 \rightleftarrows 1$. During the cycle $1 \rightarrow 2^* \rightarrow \rightarrow 2 \rightarrow 3 \rightarrow 4 \rightarrow 1$ one photon is absorbed and one proton transported to the external side. b) Condensation of fast equilibrium states $2^* \rightleftarrows 2 \rightleftarrows 3$ and $4 \rightleftarrows 1$ in two states reduced model with the corresponding state probabilities p_A and $1 - p_A$. The connection between rate constants in 5 and 2 state models is given in the text.

centration of the substrate. For instance $k_{-2} = k_{-2}^* c''$ and $k_4 = k_4^* c'$. Here, c' and c'' are proton concentrations in the left and right compartments, respectively. In general, all rate constants depend on temperature, membrane potential, salt concentration etc. We shall suppose that all rate constants due to conformational transitions ($1 \rightleftarrows 2$ and $3 \rightleftarrows 4$) are voltage independent. Protonation-deprotonation reactions $2 \rightleftarrows 3$ and $1 \rightleftarrows 4$ are assumed to be so fast that in effect there are only two intermediate states in the cycle. Equilibrium constants for these reactions are:

$$\frac{K_I}{c'} = \frac{k_{-4}}{k_4}, \qquad \frac{K_E}{c''} = \frac{k_2}{k_{-2}} \tag{1}$$

where indices I and E correspond to the internal and external compartments, respectively. With ion binding sites immobile during the cycle the voltage dependence of equilibrium dissociation constants becomes[4]:

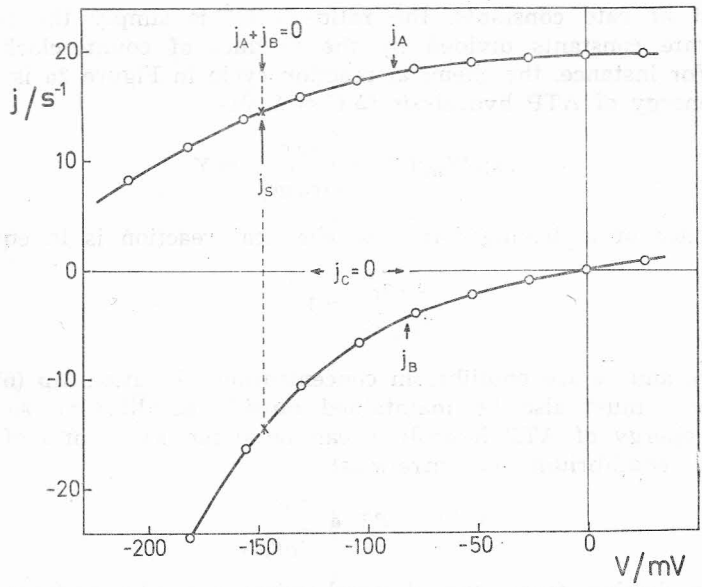

Figure 4. Stationary proton currents for bR (j_A) and for ATP-ase (j_B) have been calculated as functions of voltage $V = (RT/F)\,u$ for constant light intensity, zero ATP hydrolysis rate ($j_C = 0$) and identical proton concentrations in both compartments ($c' = c'' = c_o$). The following values for the kinetic constants were used in equations (11), (31) and (56): $a = 20$ s^{-1}, $B = 10^{-26}$, $k_{12} = 10^{-4}$ s^{-1}, $k_{21} = 10^{8}$ s^{-1}, $k_{34} = 10^{4}$ s^{-1}, $k_{43} = 1$ s^{-1}, $b = 10^{9}$ s^{-1}, $p\,c_T = 10^{3}$ s^{-1}, $q\,c_P = 10$ s^{-1}, $s = 100$ s^{-1}, $k_3 = 100$ s^{-1}, $k_{-3} = 10^{4}$ s^{-1}, $K_E/c_o = 100\,\exp(u/2)$, $K_I/c_o = 0.1\,\exp(-u/2)$, $K_E/c_o = 10^{6}\,\exp(u/2)$, $k_l/c_o = 0.01\,\exp(-u/2)$. Stable electroneutral state of coupled systems, with zero net proton current through the membrane, is reached for $j_A = -j_B = j_s$. The points in Figures 4, 5 and 6 are calculated by using a simple program in Basic on a ZX-Spectrum microcomputer.

$$K_I = \overline{K}_I \exp(-u/2), \quad \overline{K}_E = \exp(u/2) \tag{2}$$

where $\overline{K}_I$ and $\overline{K}_E$ are the values of K_I and K_E at zero voltage u. The transmembrane voltage is expressed in units of $RT/F \approx 26$ mV at 25 °C with R, T and F having their usual meaning.

Rate constants for conformational transitions ($1 \rightleftarrows 2$ and $3 \rightleftarrows 4$) are:

$$k_1 = pc_T + qc_P, \quad k_{-1} = rc_D + s, \quad k_{-3} = wc_P \tag{3}$$

where c_T, c_C and c_P are the concentrations of ATP, ADP and inorganic phosphate (P_i). Here p, q, r, s, w and k_{34} are concentration independent quantities. In particular, q and s are the rates of direct phosphorylation with inorganic phosphate or direct dephosphorylation (without ATP involvement). In the case $q = s = O$ ion transport and chemical reaction are completely coupled.

The thermodynamic driving forces are associated with closed circles in the basic diagram:

$$\exp(X/RT) = J_+/J_- \tag{4}$$

where X is the force while J_+ and J_- are one way clockwise and counterclockwise cycle fluxes[5]. Since each one way cycle flux is proportional to

the product of rate constants, the ratio in (4) is simply the product of clockwise rate constants divided by the product of counterclockwise rate constants. For instance, the chemical reaction cycle in Figure 2a is associated with free energy of ATP hydrolysis ($\Delta G = X_{B2}$):

$$\exp(X_{B2}/RT) = \frac{sp\,c_T}{rc_D\,qc_P} = Y \tag{5}$$

In the absence of a driving force the chemical reaction is in equilibrium:

$$\frac{sp\,\bar{c}_T}{r\bar{q}\bar{c}_D\,\bar{c}_P} = 1 \tag{6}$$

where $\bar{c}_T$, $\bar{c}_D$ and $\bar{c}_P$ are equilibrium concentrations. Relationship (6) between rate constants must also be maintained outside equilibrium, so that the Gibbs free energy of ATP hydrolysis can be found as a ratio of nonequilibrium and equilibrium concentrations:

$$\Delta G = -RT \ln \frac{c_T/c_D c_P}{\bar{c}_T/\bar{c}_D \bar{c}_P} \tag{7}$$

Another driving force, the electrochemical potential difference of the proton ($\Delta \mu_H = X_{B1}$), can be found from the proton translocation cycle in the basic diagram:

$$\exp(X_{B1}/RT) = \frac{K_E\,k_3\,qc_P\,c'}{K_I\,k_{-3}\,sc''} = U \tag{8}$$

Electrochemical proton gradient has electric and osmotic parts

$$\Delta \mu_H = \mu_H' - \mu_H'' = RT\,(u + \ln \frac{c'}{c''}) \tag{9}$$

From (2), (3), (8) and (9) it follows that another relationship between rate constants must always hold:

$$\frac{\bar{K}_E\,k_3\,q}{\bar{K}_I\,ws} = 1 \tag{10}$$

Using the notion of the static head state (S. H.)[6] for the steady state established by the pump when ion flow through the pump ceases, one can express the net proton flux per macromolecule B as a function of single effective force X_B:

$$j_B = A_2\,[\exp(X_B/RT) - 1] \tag{11}$$

where

$$A_2 = k_{-3}\,(K_I/c')\,(s + rc_D)/D_2 \tag{12}$$

and

$$D_2 = s + rc_D + pc_T + qc_P + \frac{K_I}{c'}\,(s + rc_D + k_{-3}) +$$

$$+ \frac{K_E}{c''}\,(pc_T + qc_P + k_3) + \frac{K_I}{c'}\,\frac{K_E}{c''}\,(k_{-3} + k_3) \tag{13}$$

Here

$$X_B = RT \ln \frac{U}{U_B} \tag{14}$$

and

$$U_B = \frac{C_B + 1}{C_B + Y}, \quad C_B = \frac{s}{rc_D} \tag{15}$$

In analogy with eqs. (11)—(15) »chemical flux« (C) per macromolecule is given by:

$$j_C = A_3 [\exp (X_C/RT) - 1] \tag{16}$$

where

$$A_3 = rc_D (qc_P + k_{-3} \frac{K_I}{c'})/D_2 \tag{17}$$

and

$$X_C = RT \ln \frac{Y}{Y_{S.H.}} \tag{18}$$

with

$$Y_{S.H.} = \frac{C_C + 1}{C_C + U}, \quad C_C = \frac{qc'}{wK_I} \tag{19}$$

The static head state in which ATP hydrolysis ceases ($X_C = j_C = 0$) is, with the exclusion of trivial equilibrium state, different from the static head state in which the net proton flux ceases ($X_B = j_B = 0$). This is due to the incomplete coupling between the chemical reaction and proton translocation. In fact, ATP hydrolysis ($j_C > 0$) still proceeds for $j_B = 0$, and net ATP synthesis ($j_C < 0$) is only possible for the net proton flux directed (by some external force) toward the internal compartment ($j_B < 0$). In other words, acting alone, macromolecules of type B (ATP-ases) can never accomplish ATP synthesis. However, and that is the actual job of *Neurospora* ATP-ase, nonequilibrium electroneutral state with $U = U_B$ can be maintained with a constant ATP hydrolisis rate.

II b) Light-Driven Proton Pump

Light activated bacteriorhodopsin (bR) dissipates photon energy $h\nu$ through pathway $2^* \rightarrow 2 \rightarrow 1$ or $2^* \rightarrow 2 \rightarrow 3 \rightarrow 4 \rightarrow 1$ (Figure 3a). Proton is released to the external (right) compartment if pathway $2 \rightarrow 3 \rightarrow 4 \rightarrow 1$ is used. It is thought that the proton release (to the external side) step takes place between L_{550} and M_{412} intermediate and the proton association step between O_{640} and bR_{570} intermediate in the bR photocycle[7]. States 2, 3, 4 and 1 (Figure 3a) can be tentatively assigned to L_{550}, M_{412}, O_{640} and bR_{570} intermediates.

The rate constants have the following significance. The overall rate constant (a) for transition $1 \rightarrow 2^*$ contains contribution from radiationless transitions: a_o, and absorption rate $\sigma (J_s + J)$ which always predominate:

$$a = a_o + \sigma (J_s + J) \approx \sigma J_s \tag{20}$$

where σ is the absorption cross-section of bR in state 1 for radiation of frequency ν, J_s is the flux density of quanta emitted by the radiation source

and impinging on the surface of the bR containing membrane, and J is the total flux density at ambient temperature T which reaches the membrane at the same temperature T. We have used here the Planck's[8] idea that any radiation of a given wavelength and intensity may be related to the black body radiation temperature T_s. With $T_s \gg T$ it usually follows that $J_s \gg J$ as was already assumed in (20). The rate constant (b) for $2^* \rightarrow 1$ transition contains the contribution from the spontaneous emission of photons b_f, for radiationless process b_o and for induced emission $\sigma (J_s + J)$ which can be neglected in the expression for b at normal light intensities:

$$b = b_o + b_f + \sigma (J_s = J) \approx b_o + b_f \tag{21}$$

In the thermodynamic equilibrium at ambient temperature T:

$$Q = a_o/b_o = \exp (-h\nu/KT) \tag{22}$$

In the case of isotropic source radiation and when all nonradiative transitions between states $2^* \rightleftarrows 1$ and $2 \rightleftarrows 1$ can be neglected ($a_o = b_o = k_{12} = k_{21} = 0$) the proton pump becomes completely coupled, i. e. light free energy can be transformed with maximal energy storage efficiency into electric and osmotic energy.

Steady state kinetics is simplified by the assumption of fast equilibrium between states $2^* \rightleftarrows 2$, $2 \rightleftarrows 3$ and $4 \rightleftarrows 1$ with the corresponding equilibrium constants:

$$B = \frac{k_{22^*}}{k_{2^*2}}, \quad \frac{k_I}{c'} = \frac{k_{14}}{k_{41}}, \quad \frac{k_E}{c''} = \frac{k_{23}}{k_{32}} \tag{23}$$

As before, we shall assume that proton dissociation constants k_I and k_E are voltage dependent:

$$k_I = k_I \exp (-u/2), \quad k_E = k_E \exp (u/2) \tag{24}$$

The thermodynamic driving forces associated with photon absorption and proton translocation can be found from the rate constants in the left and right cycles of Figure 3a:

$$\exp (X_{A1}/RT) = \frac{a}{bQ} = X \tag{25}$$

$$\exp (X_{A2}/RT) = \frac{k_E k_{34} k_{12} c'}{k_I k_{43} k_{21} c''} = U \tag{26}$$

It can be easily shown that $X = J_s/J^4$.

We have used (4) to calculate the »light force«. However, (4) is the part of Hill's formalism that breaks down in the case of light absorbing system. The justification for using (4) is that the diagram method can be extended in the case of nondissipative transitions involving only the elementary process of emission or absorption of radiation[9]. When the much more complex formula for thermodynamic force from reference 9 is applied, the result is not very different from (25). In any event, $\Delta G_I = X_{A1}$ is

considerably smaller than $N_o h\nu$ at moderate light intensities (when $a \ll b$). N_o is Avogadro's number.

The relationship between rate constants analogous to (10) must always hold because the rate constants of each cycle must be consistent with the thermodynamic forces in the cycle:

$$\frac{\bar{k}_E k_{34} k_{12}}{k_I k_{43} k_{21}} = 1 \tag{27}$$

Net absorption rate of light quanta (L) per macromolecule A in the steady state will be:

$$j_L = \frac{bB}{D_1} \left(k_{43} \frac{k_I}{c'} + k_{12}\right) \frac{X}{X_{S.H.}} - 1 \tag{28}$$

with

$$D_1 = (a + k_{12})(1 + B) + k_{21} + bB + \frac{k_I}{c'} (k_{21} + bB + k_{43}(1 + B)) +$$

$$+ \frac{K_E}{c''} (k_{12} + a + k_{34}) + \frac{k_I}{c'} \frac{k_E}{c''} (k_{43} + k_{34}) \tag{29}$$

and

$$X_{S.H.} = \frac{C_L + 1}{C_L + U}, \quad C_L = \frac{k_{12}'}{k_{43}k} \tag{30}$$

Proton transport rate in the steady state will be:

$$J_A = A_1 [\exp (X_A/RT) - 1] \tag{31}$$

where

$$A_1 = k_{43} (k_{21} + bB) (k_I/c')/D_1 \tag{32}$$

and

$$X_A = RT \ln \frac{U}{U_A} \tag{33}$$

with

$$U_A = \frac{C_A + 1}{C_A + X}, \quad C_A = \frac{k_{21}}{bB} \tag{34}$$

For the light activated proton pump only the static head state with $X_A = j_A = 0$ is of practical interest. In theory, the net rate of photon absorption will also cease for $X_L = RT \ln (X/X_{S.H.}) = 0$. However, the membrane potential needed to make $X = X_{S.H.}$, for any normal range of light intensities, is so large (negative) that dielectric breakdown of the membrane should occur sooner than such reversal potential is even approached. Left alone the bR proton pump will use the net rate of photon absorption $j_L > 0$ to maintain the nonequilibrium electroneutral state characterized by $U = U_A$. Energy is stored in this state with energy storage efficiency

$$\eta_A = - \frac{X_{A2}}{X_{A1}} \tag{35}$$

II c) Coupling of the Proton Pumps Through the Steady State Membrane Potential

In considering the time evolution and steady state properties of the coupled system, it is convenient to use the method of diagram reduction[5]. For ATP-ase the probabilities of finding macromolecules in two »fast equilibrium« states ($2 \rightleftarrows 3$ and $1 \rightleftarrows 4$) are

$$p_B = p_2 + p_3, \qquad 1 - p_B = p_1 + p_4 \tag{36}$$

where $p_i\,(i = 1, \ldots 4)$ is the ratio of the number of macromolecules in state $i : N_i$, to the total number N_B of the macromolecules of type B. The connection between the rate constants in »reduced« (Figure 2b) and »nonreduced« diagram (Figure 2a) can be easily found:

$$\beta_o = s\Theta'', \qquad \beta_1 = pc_T\Theta', \qquad \beta_2 = k_3\,(1 - \Theta'') \tag{37}$$

$$\beta_o' = qc_P\Theta', \qquad \beta_1' = rc_D\Theta'', \qquad \beta_2' = k_{-3}\,(1 - \Theta') \tag{38}$$

$$\Theta' = (1 + K_I/c')^{-1}, \qquad \Theta'' = (1 + K_E/c'')^{-1} \tag{39}$$

In the case of bR, the assumed fast equilibrium for protonation-deprotonation reactions ($2 \rightleftarrows 3$ and $1 \rightleftarrows 4$) and for relaxation from a short-lived excited state ($2^* \rightleftarrows 2$) has the effect of reducing 5 states in the model (Figure 3a) to a »reduced« diagram (Figure 3b) with only two states with the corresponding probabilities

$$p_A = p_2^* + p_2 + p_3, \qquad 1 - p_A = p_1 + p_4 \tag{40}$$

The connection between »reduced« and »nonreduced« rate constants are:

$$\alpha_0 = k_{12}\,\vartheta'', \qquad \alpha_1 = a\,\vartheta', \qquad \alpha_2 = k_{34}\,\frac{k_E}{c''}\,\vartheta'' \tag{41}$$

$$\alpha_0' = k_2\,\vartheta', \qquad \alpha_1' = bB\,\vartheta'', \qquad \alpha_2' = k_{43}\,(1 - \vartheta') \tag{42}$$

$$\vartheta' = (1 + k_I/c')^{-1}, \qquad \vartheta'' = (1 + B + k_E/c'')^{-1} \tag{43}$$

In the situations of biological interest, ADP concentration is much more sensitive to the change in external conditions than c_T or c_P. For simplicity we assume that ATP and inorganic phosphate concentrations are maintained at a constant level in the interior compartment. With proton concentrations also constant in both compartments, time evolution in the coupled system is possible for ADP concentration: c_D, for membrane potential u, and for the probabilities p_A and p_B. Differential equations for c_D, p_A and p_B can be found directly from the reduced diagrams:

$$\frac{dp_A}{dt} = (1 - p_A)\,(\alpha_1 + \alpha_o' + \alpha_2') - p_A\,(\alpha_1' + \alpha_o + \alpha_2) \tag{44}$$

$$\frac{dp_B}{dt} = (1 - p_B)\,(\beta_1 + \beta_o' + \beta_2') - p_B\,(\beta_1' + \beta_o + \beta_2) \tag{45}$$

$$\frac{V}{N_B}\,\frac{dc_D}{dt} = j_C = \beta_1\,(1 - p_B) - \beta_1'\,p_B \tag{46}$$

where V is the volume of the internal compartment.

We are primarily interested in energy transduction properties of a coupled system in stationary state, i. e. when the net proton current through the membrane vanishes:

$$N_A [\alpha_2 p_A - \alpha_2' (1 - p_A)] + N_B [\beta_2 p_B - \beta_2' (1 - p)] = 0 \tag{47}$$

Inserting stationary values for p_A and p_B from eqs, (44) and (45) and assuming for simplicity that $N_A = N_B$, eq. (47) becomes:

$$j_A + j_B = 0 \tag{48}$$

where j_A and j_B are given by

$$j_A = \frac{-\alpha_2' (\alpha_o + \alpha_1') + \alpha_2 (\alpha_1 + \alpha_o')}{\alpha_o + \alpha_1 + \alpha_2 + \alpha_o' + \alpha_1' + \alpha_2'} \tag{49}$$

$$j_B = \frac{\beta_1 (\beta_o + \beta_2) - \beta_1' (\beta_o' + \beta_2')}{\beta_o + \beta_1 + \beta_2 + \beta_o' + \beta_1' + \beta_2'} \tag{50}$$

which becomes identical to the corresponding expressions (31) and (11) when relationships (37—39) and (41—43) between »reduced« and »nonreduced« rate constants are taken into account.

In a coupled system one can define the single effective force X_{AB} by:

$$j_A + j_B = (A_1 + A_2) [\exp (X_{AB}/RT) - 1] \tag{51}$$

Here

$$X_{AB} = RT \ln \frac{U}{U_s} \tag{52}$$

by

$$\frac{1}{U_s} = \frac{\nu_1}{U_A} + \frac{\nu_2}{U_B} \tag{53}$$

and

$$\nu_1 = \frac{A_1}{A_1 + A_2}, \qquad \nu_2 = \frac{A_2}{A_1 + A_2} \tag{54}$$

In the stationary state:

$$X_{AB} = 0, \qquad U = U_s \tag{55}$$

Stationary ADP concentration is reached when $j_C = 0$, which is equivalent to $Y = Y_{S.H.}$ condition. Using (5) and (19) one can find stationary ADP concentration:

$$r c_D^s = p c_T \frac{s + k_3 K_E/c''}{q c_P + k_{-3} K_I/c'} \tag{56}$$

Inserting (56) either in (48) or in (55) stationary membrane potential $V_s = (RT/F) u_s$, is found for any given light intensity.

Let us examine in more detail the simplest case when our system is initially in equilibrium state of zero membrane potential ($u = 0$) and zero proton gradient ($c' = c''$). In that case $Y_{S.H.} = 1$ and from $j_C = 0$ also $Y = 1$. In other words both thermodynamic forces operating on macromolecule B

vanish: $X_{B1} = X_{B2} = 0$, so that the proton flux j_B must also vanish. Illumination creates the maximal value of net proton current through macromolecule A ($j_A > 0$) before membrane potential has had time to adjust itself to the appearance of the new force. Such level flow state[6] is unstable until the condition of zero net current through the membrane (48) is reached (Figure 4). In our example $V_s = -148$ mV. More negative stationary membrane potential cannot be reached spontaneously with this choice of kinetic constants (choosen numerical values of all parameters can be found in the legend to Figure 4).

Efficiency of the overall process can be discussed either as a ratio of work obtained to the free energy input, or as a ratio of secondary to primary forces, when secondary forces created by constant primary forces have reached maximal values (in the static head state). Energy storage efficiency of our system is simply the product of the corresponding storage efficiencies for each macromolecule

$$\eta_{st} = \eta_A \eta_B = \left(-\frac{X_{A2}}{X_{A1}}\right)\left(-\frac{X_{B2}}{X_{B1}}\right) \tag{57}$$

Taking into account that the proton electrochemical gradient must be the same for both macromolecules, when they are located in the same membrane, ($X_{A2} = X_{B1}$) (57) reduces to:

$$\eta_{st} = \frac{X_{B2}}{X_{A1}} \tag{58}$$

The expression for the total entropy production of the coupled system (macromolecules in the membrane plus both compartments):

$$T\,\frac{d_i S}{dt} = j_L X_{A1} + j_A X_{A2} + j_B X_{B1} + j_c X_{B2} \tag{59}$$

must have a net positive sign as required by the second law of thermodynamics. Some summands in (59) can still have a negative sign representing the rate of free energy increase rather than free energy dissipation. For instance, $j_A X_{A2}$ is negative because of the negative membrane potential developed as a result of light activated net proton transport rate j_A. It is also always smaller in absolute value than the primary dissipation $j_L X_{A1}$ associated with light absorption.

If we restrict our attention to the steady state in which the net chemical flux has ceased ($j_C = 0$), the efficiency of utilization of light free energy can be defined as

$$\eta_t = -\frac{j_A X_{A2}}{j_L X_{A1}} \tag{60}$$

Efficiencies η_t and η_{st} are plotted in Figure 5 as functions of membrane potential $V = (RT/F)\,u$. For a small membrane potential η_t has a similar value as η_{st}. In the limit of small membrane potential the coupling ratio for bR: j_A/j_L is close to one, i. e. one proton is transported per each absorbed photon. On the other hand, η_B is also close to one for any choice of para-

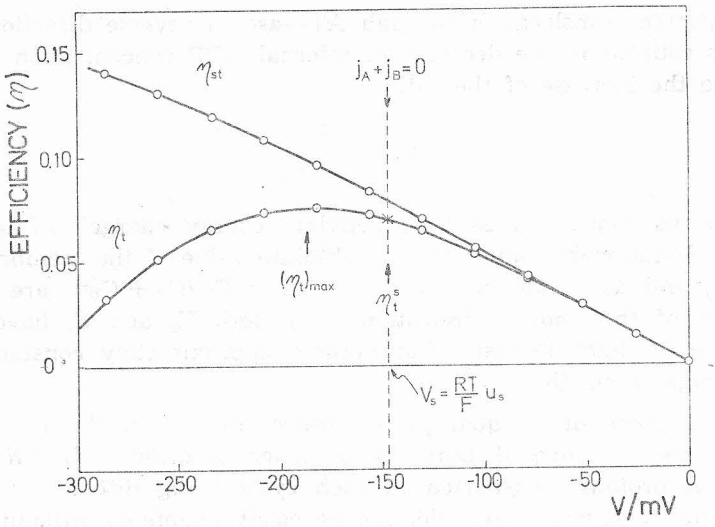

Figure 5. Energy storage efficiency η_{st} and efficiency of utilization of light free energy η_t as functions of membrane potential V. The conditions and rate constants are the same as described in the legend to Figure 4. While η_{st} increases, with more negative membrane potentials, the coupling ratio for bR decreases, and as a result η_t passes through the maximal value for the optimal membrane potential. In our example the maximal η_t is quite modest (0.074). The stationary value of $\eta_t : \eta_t^s$, for zero net proton current through the membrane, is in general lower than $(\eta_t)_{max}$.

meters. From (57) $\eta_A \approx \eta_{st}$ and from (60) $\eta_t \approx \eta_{st}$. Since η_t is the product of the coupling ratio and energy storage efficiency η_A, the decrease in the coupling ratio for a more negative membrane potential and the corresponding increase in the energy storage produces maximal value of η_t for the unique optimal membrane potential.

Notice that in (60) ATP-ase kinetic constants are absent, i. e. efficiency η_t is independent of secondary energy transformer characteristics. This is not so for the relationship between the maximal value of η_t and stationary value η_t^s in the electroneutral state of a coupled system with both proton pumps present. In general, $(\eta_t)_{max}$ does not coincide with η_t^s. However, in the coupled system, it is always possible to find the optimal light intensity for which the stationary membrane potential V_s becomes the optimal membrane potential (giving maximal η_t) as well. Optimal light intensity also depends on ATP-ase characteristics.

Assuming that the static head state of zero net proton current through the membrane and zero net chemical flux ($j_C = 0$) have been reached, entropy production expression (59) reduces to

$$TP = j_L X_{A1} \tag{61}$$

Expression (61) is formally identical with the entropy production of bR molecules alone in the static head state ($j_A = 0$)[9]. Nevertheless, its value is different (greater) because it has to be evaluated at the membrane potential which is considerably less negative (—148 mV instead of —317 mV in our numerical example).

The proton translocation through ATP-ase in reverse direction (outside--inside) is coupled to the decrease of internal ADP concentration and consequently to the increase of the ratio

$$E = \frac{c_T}{c_T + c_D} \tag{62}$$

which can be considered as the adenylate energy charge[10] of our system. In Figure 6 stationary values for the absolute value of the membrane potential ($|V_s|$) and adenylate energy charge ($E_s = C_T/(C_T + C_D^s)$) are plotted as a function of the photon absorption rate. Both V_s and E_s have a strong dependence on light intensity but become approximately constant for sufficiently high intensities.

The influence of unequal proton concentrations in the left and right compartments, of unequal numbers of macromolecules ($N_A \neq N_B$), and of numbers of protons transported in each cycle being different from 1 and different for each macromolecule, can be easily examined without any basic change in the model.

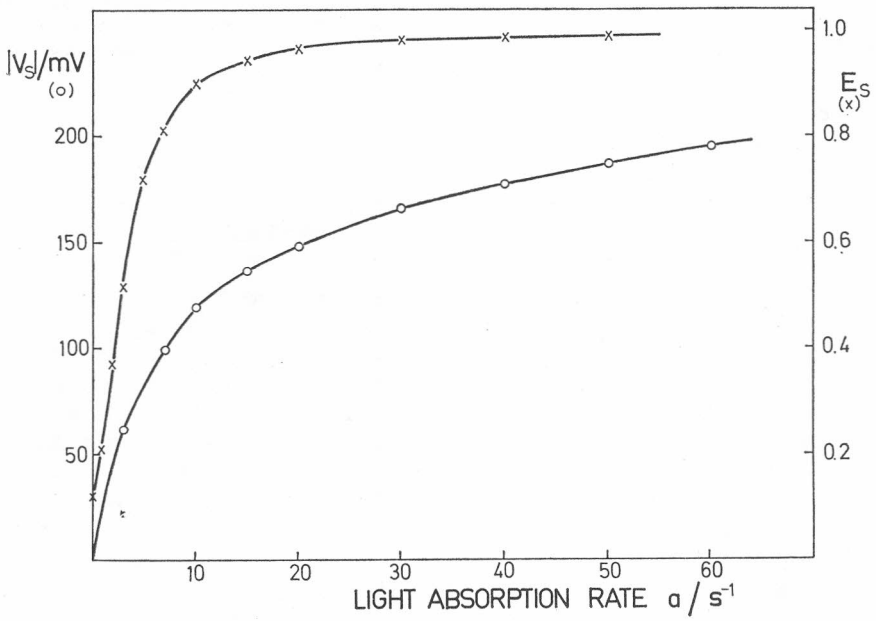

Figure 6. Stationary membrane potential V_s and adenylate energy charge E_s have been calculated for different electroneutral states with zero net chemical flux ($j_A + j_B = j_C = 0$) by varying the photon absorption rate (a), i. e. for different light intensities. Cells with photosynthetic abilities want to keep their crucial photosynthetic parameters V_s and E_s in the desired optimal range. This can be accomphished by achieving high values for V_s and E_s even for low light intensities and by controlling these parameters by the use of different dissipative pathways such as photorespiration. Our »minimal« photosynthetic model reproduces only the fast rise of V_s and E_s by increasing the light intensities.

3. DISCUSSION

The central point of this paper is that energy transducers of type B (secondary transducers) when coupled to energy transducers of type A (primary transducers) are capable of transforming light free energy into chemical free energy. This is a new property of the artificial membrane system containing both types of macromolecules, which appears as a consequence of kinetic and thermodynamic coupling of macromolecules in the steady state through a common membrane potential and/or proton gradient.

In our model only the common membrane potential connects two energy transducing units. This situation can be regarded as a weak coupling limit in the chemi-osmotic theory[11] as opposed to strong coupling when protons, as free-energy coupling intermediates, exhibit fast concentration changes in one (small volume) compartment[12]. Neither of the two macromolecules considered here: bR and ATP-ase, has photosynthetic ability per se, but when coupled through the steady-state membrane potential, they are capable of synthesizing ATP. The ATP synthesis was indeed detected in the illuminated artificial vesicular system containing these two macromolecules[13]. Experiments on chloroplasts and plant cells show that dark-light transition causes a sudden increase in adenylate energy charge[14]. This is in accord with our results (Figure 6).

With possible exception of phototactic responses, for photosynthetic cells it is not an advantage to act as light free energy transducers in the region where E_s and V_s have a strong dependence on the light intensity, because they do not have efficient mechanisms for the control of light intensity. Rather, the capability to open new dissipative leak pathways will provide the cells with the possibility of controlling their most important parameters like E_s and V_s while operating in the specific region of saturating light intensities. One such pathway, commonly used by plants, is photorespiration[15].

Our purpose in introducing expressions (14), (33) and (52) for an effective single force operating on the proton current of macromolecule A, B and coupled system, respectively, was twofold. In all these expressions, the non-equilibrium static head state is the reference state, in which effective forces vanish. Further, we wanted to point out the essential nonlinear nature of the flux — force relationships (11), (31) and (51). Determination of the effective force in each particular case is equivalent to definition of the static head state and also to measurement of the system's displacement from that reference state. For each macromolecule that transforms primary force X_{I} into secondary force X_{II} the relationship between effective force X_{eff} and X_{I}, X_{II} appears to have the general form

$$X_{\mathrm{eff}} = X_{\mathrm{II}} + RT \ln \frac{C + \exp(X_{\mathrm{I}}/RT)}{C + 1} \tag{63}$$

In our models $C = C_{\mathrm{B}}$, $X_{\mathrm{I}} = X_{\mathrm{B2}}$, $X_{\mathrm{II}} = X_{\mathrm{B1}}$, $X_{\mathrm{eff}} = X_{\mathrm{B}}$ for ATP-ase and $C = C_{\mathrm{A}}$, $X_{\mathrm{I}} = X_{\mathrm{A1}}$, $X_{\mathrm{II}} = X_{\mathrm{A2}}$, $X_{\mathrm{eff}} = X_{\mathrm{A}}$ for the bR macromolecule. In a coupled system two effective forces are operating, X_{A} and X_{B}, with the total effective force being equal to

$$X_{\mathrm{AB}} = X_{\mathrm{A}} + X_{\mathrm{B}} + RT \ln (\nu_1/\nu_2) \tag{64}$$

where ν_1 and ν_2 are essentially fractional conductances for macromolecules A and B, respectively (expressions (54) in our model).

When the static head state of a coupled system is defined as the state of zero net proton current through the membrane, and zero net ATP hydrolysis rate, only the light absorption current j_L has nonzero value, which can be compared with its value in the static head state created by bacteriorhodopsin alone. The result is that j_L and entropy production $j_L X_{A1}$ have a higher value in the presence of interaction between macromolecules, i. e. in the static head state of the coupled system (C. S.). The ratio of the corresponding entropy productions: $(j_L X_{A1})_{c.s.}/(j_L X_{A1})_{bR}$ is always greater than 1 and keeps increasing with the increase in light intensity. Another quantity of interest, the efficiency of utilization of light free energy (60), raises from zero (S. H. state of *bR*) to near maximal value (S. H. state of C. S.) when ATP-ase »leak« is added. Free energy stored in the nonequilibrium state, by transduction of light free energy into proton electrochemical gradient, has a natural tendency to decrease. Evolution has created the hierarchy of energy converters which satisfy this tendency by increasing both the entropy production and efficiency of the potential utilization of light free energy by energy coverters further down the hierarchical ladder.

The model can be easily extended for the case when the net ATP synthesis rate has the constant value different from zero, for instance by adding a third energy converter or leak responsible for ATP removal. The efficiency of utilization of ATP free energy can be defined in a manner analogous to (60). It becomes greater than zero when light intensity and the corresponding absolute value of the stationary membrane potential exceed certain minimal values (results not shown).

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SAŽETAK

Kinetička i termodinamička svojstva fotosintetičkog sistema

D. Juretić i F. Sokolić

Razmatrana su kinetička i termodinamička svojstva »minimalnog« fotosintetičkog sistema koji sadrži dvije različite protonske crpke u planarnoj umjetnoj membrani. Protonska pumpa aktivirana svjetlošću (model za bakteriorodopsin, bR) i protonska pumpa koja koristi ATP (model za ATP-azu iz *Neurospore*) međudjeluju preko zajedničkog fotopotencijala što ga stvara bR. Metodom Hillovih dijagrama izračunana su slijedeća svojstva osvijetljenoga vezanog sistema u stacionarnom stanju: protonska struja za svaku makromolekulu, fotopotencijal, ukupna efektivna sila, efikasnost pohranjivanja slobodne energije, efikasnost upotrebe slobodne energije, produkcija entropije i adenozinski energijski naboj u unutrašnjem odjeljku.