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On the Coupling of Electron Transport to Phosphorylation*

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Abstract

After a general thermodynamic discussion of the coupling of oxidation to phosphorylation, quantitative treatments of free energy transduction based upon the proton gradient model, the charged membrane model and the chemical model respectively are summarized and compared with experimental data. The relationship between energy transduction and respiratory control is reexamined.

Introduction

Since the dawn of history man has been wondering—what is it that keeps him alive? His soul or his respiration or something else? Should his attempted answer be developed from the conservation of souls (reincarnation), or the conservation of mass and energy, or the non-conservation of free energy, or some other hypothesis?

The free energy liberated by respiratory or photosynthetic electron transport is often first converted to and stored in a certain form prior to the formation of ATP.[1–3] The various forms of this stored free energy suggested in the literature include energy-rich intermediates,[1] concentration gradients,[4,5] changes in macromolecular conformation and membrane structure[6,7] and electric potential energy.[8–10] In this paper, we shall develop these suggestions quantitatively and examine their validity by comparison with experimental data.

Thermodynamic Considerations

Let us consider an oxidation process represented by

$$\sum_k \nu_k A_k \rightarrow \sum_l \nu_l A_l$$

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where ν_1 moles of molecular species A_1, ν_2 moles of A_2, etc. react to form ν_n moles of A_n, ν_{n+1} moles of A_{n+1}, etc. The summation $\sum_k$ is over all reactants and the summation $\sum_l$ is over all products of this oxidation reaction. At constant temperature T and pressure P, the decrease of free energy due to this reaction is given by

$$-\Delta G_0 = \sum_k \nu_k \mu_k - \sum_l \nu_l \mu_l' \geqslant 0 \tag{1}$$

where μ_k, μ_l' represent the electrochemical potentials of A_k, A_l at their respective locations in the system, and the equality sign applies to the limiting case in which the system is already at equilibrium. If the oxidation-reduction system is not at equilibrium, the free energy released may be utilized to drive a thermodynamically unfavorable reaction represented by

$$\sum_i \nu_i A_i \rightarrow \sum_j \nu_j A_j$$

resulting in the storage of an amount of free energy equal to

$$\Delta G_s = \sum_j \nu_j \mu_j' - \sum_i \nu_i \mu_i > 0 \tag{2}$$

Thermodynamics requires

$$-\Delta G_0 > -(\Delta G_0 + \Delta G_s) \geqslant 0 \tag{3}$$

The electrochemical potential μ_i of the reactant molecular species A_i of charge number Z_i at a location with electric potential Ψ in the system may be written as

$$\mu_i = \mu_i^\circ + Z_i \mathscr{F} \Psi + RT \ln m_i + RT \ln \gamma_i \tag{4}$$

where m_i is the molal concentration, γ_i the activity coefficient of A_i, $\mathscr{F}$ the faraday, R the gas constant, and the standard potential μ_i° is characteristic of A_i but for a given set of arbitrarily chosen standard states is independent of Ψ and m_i. Likewise, the electrochemical potential μ_j' of the product molecular species A_j at another location in the system with electric potential Ψ', concentration m_j' and activity coefficient γ_j' may be written as

$$\mu_j' = \mu_j^\circ + Z_j \mathscr{F} \Psi' + RT \ln m_j' + RT \ln \gamma_j' \tag{5}$$

Substitution of equations (4) and (5) into (2) gives

$$\begin{aligned} \Delta G_s = {} & (\textstyle\sum \nu_j \mu_j^\circ - \sum \nu_i \mu_i^\circ) + \mathscr{F}(\Psi' \sum \nu_j Z_j - \Psi \sum \nu_i Z_i) \\ & + RT(\textstyle\sum \nu_j \ln m_j' - \sum \nu_i \ln m_i) \\ & + RT(\textstyle\sum \nu_j \ln \gamma_j' - \sum \nu_i \ln \gamma_i) \end{aligned} \tag{6}$$

The first through the fourth terms on the righthand side of equation (6) have been used loosely to represent the free energies converted by

the energy transduction process and stored in the form of energy-rich chemical intermediates, electric potential energy, concentration gradients and additional molecular interactions respectively. This arbitrary representation is misleading, because in a biological system each of these four effects contributes to two or more terms on the righthand side of equation (6). For example, the formation of chemical intermediates may affect all four terms on the righthand side of equation (6) for the following reasons: (a) Molecules in general react at concentrations m_i, m_j' ..., with activity coefficients γ_i, γ_j'..., instead of at their standard states; (b) it is well-known since Volta's experiments in 1800 and Faraday's experiments in 1833 that chemical reactions may cause electric potential changes and local concentration changes. Therefore the experimental detection of concentration gradients generated by energy transducing processes in chloroplasts[3,11–13] and mitochondria[14] does not support the proton gradient or chemiosmotic model in preference to the chemical intermediates hypothesis. For the same reason, the observation of membrane electric potential and structure changes does not necessarily support the charged membrane model and the conformation change model respectively in preference to the competing hypotheses. On the other side, the detection of a phosphorylated intermediate[15] does not necessarily support the chemical coupling mechanism in preference to the chemiosmotic and electric potential models either, because it is thermodynamically possible to drive the generation of chemical intermediates with the free energy stored in concentration gradients or electrically charged membranes.

The successful coupling of phosphorylation to electron transfer in homogeneous model systems[16,17] does demonstrate the feasibility and potential efficiency of the chemical coupling mechanism, because macroscopic homogeneity precludes concentration gradient, electric potential gradient and membrane structural changes. But results obtained from artificial model systems cannot be used as direct experimental evidence for the coupling mechanism in chloropolasts and mitochondria.

Proton Gradient Models

The central assumption of proton gradient models[4,5] is that the first two terms on the righthand side of equation (6) are negligible and hence the free energy stored prior to ATP synthesis is given by

$$\Delta G_s \approx RT(\sum \nu_j \ln m_j' - \sum \nu_i \ln m_i) + RT(\sum \nu_j \ln \gamma_j' - \sum \nu_i \ln \gamma_i) \quad (7)$$

To simplify the theoretical treatment, let us avoid pH changes due to net oxidation–reduction reactions and consider the efficiency of photosynthetic energy conversion under conditions of cyclic electron transport, as measured by Z^* or X_E, by a chloroplast in a

medium buffered by $B^- + BH$. If the ionic strength of the medium is maintained constant by a higher concentration of sodium chloride, the free energy increase due to the light-driven transfer of δ mole of H^+ (or H_3O^+), with accompanying counter ions, from Side 1 to Side 2 of the chloroplast membrane at constant temperature is given by

$$\Delta G_s = RT \int_0^{\delta} \ln\left(\frac{[H^+]_2}{[H^+]_1}\right) d\delta \tag{8}$$

where $[H^+]_1$ and $[H^+]_2$ represent the concentration of H^+ (or H_3O^+) on Side 1 and Side 2 of the membrane respectively. Let us assume that the chloroplast has been immersed in the buffered medium in the dark for a long time so that

$$[BH]_1/[B^-]_1 = r = [BH]_2/[B^-]_2 \tag{9}$$

According to the proton gradient models, the number of moles of H^+ translocated is proportional to the number of moles of electrons transported. By using intense light for a very short illumination period, it is possible to translocate a sufficiently large number, δ, of moles of H^+ from one side of the chloroplast membrane to the other without appreciable amounts of B^- and BH diffusing across simultaneously. Under such experimental conditions, the concentrations of H^+ on the two sides immediately after the light-driven proton translocation are given by

$$[H^+]_2 = K'_a\left\{\frac{[BH]_2}{[B^-]_2}\right\} = K'_a\left\{\frac{a_2 r/(1+r) + \delta}{a_2/(1+r) - \delta}\right\}, \tag{10}$$

$$[H^+]_1 = K'_a\left\{\frac{[BH]_1}{[B^-]_1}\right\} = K'_a\left\{\frac{a_1 r/(1+r) - \delta}{a_1/(1+r) + \delta}\right\} \tag{11}$$

where a_2 and a_1 represent the total number of moles of buffer, B^- plus BH, on Side 2 and Side 1 of the membrane respectively, $K'_a = [B^-][H^+]/[BH]$, and the illumination is sufficiently short so that $\delta < a_2 r/(1+r) < a_1 r/(1+r)$ and $\delta < a_2/(1+r) < a_1/(1+r)$.

Substituting equations (10) and (11) into equation (8) and integrating, we get[18]

$$\Delta G_s \approx \frac{(1+r)^2}{2r}\left(\frac{1}{a_1} + \frac{1}{a_2}\right) RT\delta^2 \tag{12}$$

Equation (12) shows that for a given number, δ, of moles of proton translocated, the free energy stored should decrease as the buffer concentration is increased. Since experimental data[18] clearly show that ΔG_s increases rapidly as the buffer concentration is raised, we can only conclude that most of the Z^* or X_E produced by photosynthetic energy conversion under conditions of cyclic electron transport is not of proton gradient nature.

Attempts have been made to improve the proton gradient theory by postulating that protons are not translocated from Side 1 to Side 2, but from the exterior to the interior of the chloroplast membrane. This type of proton translocation would produce a much larger proton gradient for the same number of protons translocated. But the improved theory also contradicts the observed rapid increase of ΔG_s as the buffer concentration is raised.

Charged Membrane Models

These models assume that the free energy of electron transport is first used to charge the thylakoid or inner mitochondrial membrane and stored as electric potential energy. This energy is later utilized to translocate ions and phosphorylate ADP.[8–10] Accordingly, Z^* or X_E is equal to the work required to charge a membrane condenser by redistributing the ions in the chloroplastic or mitochondrial system. The work required to charge a condenser of constant capacity C to a potential V is equal to $CV^2/2$. But since practically all the electric charges in a biological system reside on ions and since the capacity of a membrane condenser also depends on the distribution of ions, we expect the free energy stored by charging such a membrane condenser to vary with the concentration of the principal ionic species, say Na^+ and Cl^-, but to be practically independent of the concentration of the dilute buffer, $[B^-] + [BH]$, which does not contribute significantly to the total ionic strength.

For simplicity, let us consider the free energy stored by charging an infinite planar membrane by bringing sodium ions to it from the sodium chloride solution of molar concentration C_s on one side of the membrane until the membrane potential becomes Ψ_0. For $C_s < 0{\cdot}05$ M and $\Psi_0 < 1$ V, theoretical considerations[18] show that the free energy stored per unit area of the membrane is equal to

$$\Delta G_s = \sqrt{\frac{2DC_sRT}{1000\pi}}\left\{\Psi_0 \sinh\left(\frac{\mathscr{F}\Psi_0}{2RT}\right) - \frac{2RT}{Z\mathscr{F}}\left[\cosh\left(\frac{\mathscr{F}\Psi_0}{2RT}\right) - 1\right]\right\} \quad (13)$$

where D is the dielectric constant of the solvent. Therefore in the range of experimental conditions for which equation (13) is applicable, ΔG_s at a given sodium chloride concentration should be independent of buffer concentration which does not contribute significantly to the total ionic strength.

Since this deduction contradicts the experimental observation[18] that at approximately constant ionic strength ΔG_s increases rapidly as the buffer concentration is raised and that at a given buffer concentration ΔG_s does not increase as C_s changes from 10 to 50 mM, we conclude that most of the Z^* or X_E produced by photosynthetic energy conversion under conditions of cyclic electron transport is not in the

form of electrically charged thylakoid membrane. The experimental observation also precludes a combination of proton gradient and electric potential energy as the principal form in which ΔG_s is stored.

Chemical Coupling Mechanisms

Chemical mechanisms include all four terms on the righthand side of equation (6) and hence are thermocynamidally more general than the proton gradient and charged membrane theories.

For the convenience of discussion, let us tentatively adopt as a working hypothesis the radical coupling mechanism discovered in model systems[16, 17] for the cyclic photophosphorylation in chloroplasts, and assume that under illumination an imidazole group of a cytochrome molecule is first photo-oxidized to a substituted imidazolyl radical. If this substituted imidazolyl radical is adjacent to a suitable phospholipid or phosphoprotein, $(RO)(R'O)PO_2^-$, of the chloroplast membrane, it can rapidly react with the latter to form a substituted phosphoimidazolyl radical which can subsequently be reduced to substituted orthophosphoimidazole. The unstable substituted orthophosphoimidazole can either decompose to the original reactants or capture a proton to form water and substituted 1-phosphoimidazole. The latter can then transfer its substituted phosphoryl group to an acceptor group A^- or AH on the membrane to form the intermediate (RO)(R'O)POA. Both the substituted 1-phosphoimidazole and (RO)(R'O)POA are "non-phosphorylated" energy-rich intermediates[19] (customarily represented by $X \sim I$, Z^* or X_E) and either of them can subsequently react with inorganic phosphate P_i and ADP to form ATP and regenerate the original reactants.

For short illumination periods, an approximate steady-state treatment of photosynthetic energy conversion by this radical coupling mechanism under conditions of cyclic electron transport gives the following result[18]:

$$\Delta G_s \approx B\left\{st - \frac{1}{4}\left[1 + \frac{2m(1+r)^2 a}{(1+m)rc}\right]s^2 t^2\right\} \tag{14}$$

where $c = [B^-] + [BH] \neq 0$, $r = [BH]/[B^-]$ before the illumination, t is the illumination time, and B, s, m, a are constants. Although the washed chloroplasts must still contain a small amount of endogenous buffer, we expect from equation (14) that the additional free energy stored due to the added buffer will increase with the buffer concentration c at low c when

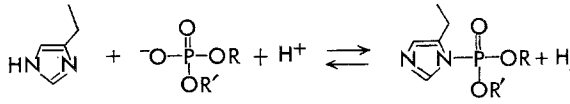
$$\frac{2m(1+r)^2}{(1+m)rc} \geqslant 1, \qquad c \neq 0.$$

But at very high c, we expect this additional ΔG_s to become independent of c when

$$\frac{2m(1+r)^2 a}{(1+m)rc} \ll 1.$$

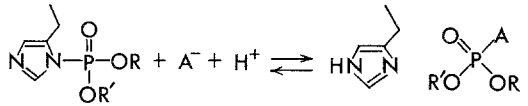
The available experimental data[18] are consistent with this inference at low c. At $c = 10$ mM, the yield of $X \sim I$ has not yet reached its saturation value.

The net reactions leading to $X \sim I$ in this particular chemical coupling mechanism are



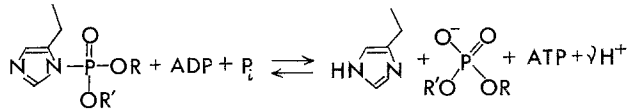
(15)

and



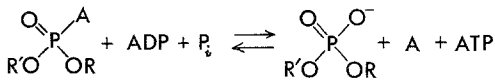
(16)

The net reactions for the formation of ATP from $X \sim I$ are



(17)

or



(18)

Since Reactions 15 and 16 involve the uptake of protons whereas Reactions 17 and 18 involve the release of protons, we can drive the first two reactions to the right by lowering the pH and drive the last two reactions to the right by raising the pH. Consequently according to this chemical coupling mechanism it should be possible to make ATP from ADP and P_i by incubating the mixture with chloroplasts in the dark at low pH and then suddenly raise the pH to a high value, but it should not be possible to do the same by incubating with chloroplasts in the dark at high pH and then suddenly decrease the pH to a low value. These inferences are quite consistent with the well-known experimental work of Jagendorf and Uribe.[11] Similarly when a chloroplast suspension is illuminated under the conditions of

cyclic electron transport in the absence of ADP and P_i, the external pH is expected to rise initially due to Reactions 15 and 16, and gradually to reach a steady-state value when the rate of production of $X \sim I$ equals the rate of its hydrolysis. After the light is turned off, a drop in external pH should follow the hydrolysis of $X \sim I$. Whether or not this radical coupling mechanism is applicable to mitochondria and chloroplasts has yet to be shown. But the above discussion shows that all chemical mechanisms which involve proton uptake during the formation of $X \sim I$ and proton release during the reaction of $X \sim I$ with ADP and P_i to form ATP are consistent with these experimental data.

The formation of $X \sim I$ may cause conformation changes in either or both of the macromolecular moieties (X or I) and consequently trigger changes in the gross structure of the membrane.[20] These conformational and membrane structural changes consume some of the converted free energy, since they follow spontaneously the formation of the energy-rich bond. (If these secondary processes had not taken place, the energy-rich bond may be energy-richer, although its formation would be slower for the same amount of free energy input from electron transport.) Although these conformational and membrane structural changes do not by themselves represent free energy storage, they may be very important to the related physiological functions of the organelle, such as permeability regulation and respiratory control.

The observed dependence of respiration rate on the concentrations of P_i, ADP and ATP[21,22] has been attributed to the effect of free energy storage on the midpoint reduction potentials of the electron carriers at the control sites.[23,20] Qualitatively this mechanism of respiratory control may be described as follows. At steady-state, the rate of oxidation of the reduced form of every carrier in an electron transport chain is equal to the rate of reduction of its oxidized form. Therefore in order for the electron transport chain to function efficiently in the kinetic sense, every carrier must maintain a healthy ratio of its oxidized and reduced forms. If the midpoint reduction potential of a particular carrier is raised substantially as a result of the energy storage prior to the formation of ATP, then a very large fraction of this carrier will automatically go to its reduced form. Consequently, the steady-state rate of electron transfer to it from its neighboring carriers on the substrate side of the respiratory chain will become very slow and hence rate-limiting. Conversely, if the midpoint reduction potential of this carrier is lowered substantially as a result of the energy storage, a very large fraction of this carrier will automatically go to its oxidized form. Consequently, the steady-state rate of electron transfer from it to its neighboring carrier on the oxygen

side of the respiratory chain will become very slow and hence rate-limiting. Therefore unless the primary form of stored free energy is being continually used up for the phosphorylation of ADP or discharged by the action of uncouplers etc., electron transport in the normal direction will be severely retarded.

Evidence of quite large changes of the *in vivo* midpoint reduction potentials of cytochrome b_T and of heme a_3 in cytochrome oxidase by free energy storage has been obtained from spectroscopic data.[24]

In recent years a number of investigators have treated the coupling of electron transport to phosphorylation as an ideal, thermodynamically reversible process. Indeed, by completely inhibiting cytochrome oxidase with CN^-, it is even possible to use ATP to reverse the direction of electron transport through a part of the respiratory chain.[25,26] But inasmuch as no one has yet succeeded in preparing the ideal mitochondria which utilize the hydrolysis free energy of ATP to sustain the steady-state oxidation of water by $NADP^+$, the basic assumption of this convenient approach is open to question.

The observed coupling of electron transfer to phosphorylation in homogeneous solutions[16,17] shows clearly that in general respiratory control is not necessary for energy transduction, although energy transduction is necessary for respiratory control. For these reasons, it may be more realistic conceptually and more fruitful experimentally to treat the coupling of electron transport to phosphorylation as a steady-state rate process with optimum characteristics than as an ideal, thermodynamically reversible process. After all, life itself is irreversible!

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