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BIOENERGETICS AND CHARGE TRANSFER ACROSS LIQUID-LIQUID INTERFACES

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Abstract. The current understanding of charge transfer processes across the interface between two immiscible electrolyte solutions (ITIES) is used to explain ion and electron transfer across biological membranes. Coupled ion and electron transfer, proton "uncouplers", proton pumps, and reverse electron transfer in bioenergetics are discussed in terms of charge transfer across the ITIES.

Keywords: bioenergetics, biological membranes, charge transfer, liquid-liquid interfaces, ITIES, chemiosmotic hypothesis, proton uncouplers, proton pumps, reverse electron transfer.

In accepting the invitation to submit an article - emphasizing our work on the electrochemistry at liquid-liquid interfaces - to commemorate the tenth anniversary of the *Bulletin of the Chemical Society of Ethiopia*, I had to chose between two alternatives: (1) give a summary or overview of our contributions towards understanding charge transfer processes across liquid-liquid interfaces, or (2) write a general paper explaining the relevance of studies of ion and electron transfer across the interface between two immiscible electrolyte solutions to bioenergetics, and thereby explain what some of the reasons are for our interest in such investigations. The former, I felt, would be a review of what has already been published and would be of interest only to the specialist, whereas the latter could be addressed to a wider audience and at the same time include topics that could provoke further dialogue and discussion on some important themes in bioenergetics. In particular, I thought it was about time that we took stock of our current understanding of the electrochemistry at the ITIES and see to what extent it can explain certain themes (some of which are still controversial) in bioenergetics. I believe that we have gone a long way in understanding charge transfer across biological membranes; however, there still remain quite a lot to unravel. I therefore felt that it would be a good opportunity to present how we view certain processes and phenomena in bioenergetics from the point of view of the electrochemistry at the ITIES. The present contribution is a modest attempt towards this objective. Readers interested in some general aspects of bioelectrochemistry are referred to the article by Koryta that appeared in the *Bulletin* a few years ago [1].

INTRODUCTION

Cells are the fundamental units of life and are the building blocks from which all organisms are made. They are enclosed by a plasma membrane and they contain several organelles that perform specific functions such as regulating the exchange of materials between the cell and its external environment,

carrying out reactions which are utilized for the synthesis and breakdown of organic molecules in the process known as metabolism, etc. The organelle that is involved in metabolism is the membrane enclosed mitochondrion, which is situated in the soluble aqueous phase of the cytoplasm (cytosol). The mitochondrion consists of an outer membrane and an inner membrane separated by an intermembrane space. The inner membrane encloses the gel-like matrix, which is the interior of the mitochondrion. The electron-transferring molecules of the respiratory chain and the enzyme molecules that synthesize ATP from ADP and phosphate are mostly embedded in the inner membrane. The components of the respiratory chain are: (a) pyridine nucleotides, (b) flavoproteins, (c) ubiquinone, or coenzyme Q, (d) cytochromes, and (e) iron-sulfur proteins. These electron carriers are organized sequentially as shown in Figure 1. The NADH-oxidizing site of Complex I is on the matrix-side (M-side) of the inner membrane; ubiquinone is believed to be located in the interior of the inner membrane; Complex III includes cytochromes b, c_1 and Fe-S protein which are located in the middle region of the membrane bilayer; cytochrome c is a peripheral protein associated with the cytosol-side (C-side) of the inner membrane, whereas Complex IV is an integral protein of the inner membrane. The topology of these electron carriers (i.e. their asymmetric orientation in the inner membrane) is believed to be significant in understanding the vectorial translocation of protons from the inner (matrix) side to the outer (cytosol) region of the membrane. It is clear from the brief description above that electron transfer in biological membranes occurs in media of different polarity (i.e. on the M- and C-side of the inner membrane, as well as inside the inner membrane). Hence, an understanding of the mechanism of ion and electron transfer across the ITIES may shed some light on the mitochondrial process of oxidative phosphorylation.

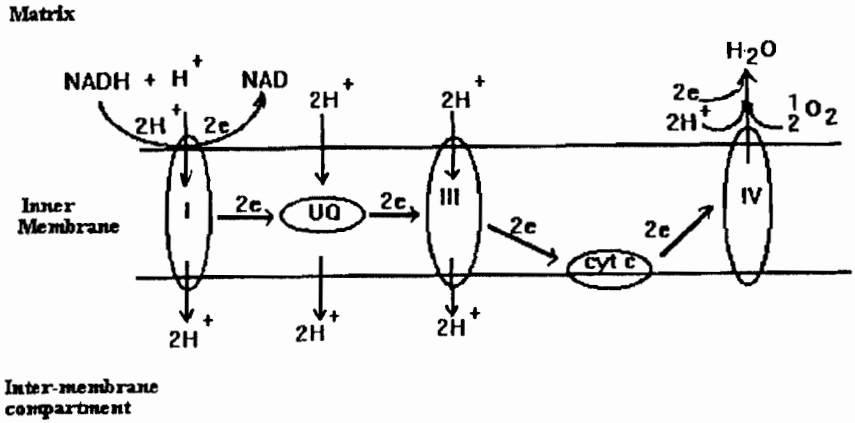

Figure 1. Model of the mitochondrial inner membrane showing the topology of the electron carriers. (I, III, IV: Complexes I, III and IV; UQ: Ubiquinone; cyt c: cytochrome c).

The *chemiosmotic hypothesis* [2] states that the electron transfer chains of mitochondria are coupled to ATP synthesis by a proton electrochemical potential gradient across the energy transducing

membrane. One of the major tenets of this hypothesis is that proton gradients are *generated by* electron transport.

The purpose of this communication is to discuss some aspects of bioenergetics, with particular emphasis on the chemiosmotic hypothesis, from the viewpoint of our current understanding of charge transfer processes across the ITIES.

ION TRANSFER COUPLED TO ELECTRON TRANSFER

Since proton transfer is intimately linked to the electron transport chain in biological membranes, two issues (both as they relate to the ITIES) will be addressed in this section:

1. electron transfer induced by ion transfer, and
2. ion transfer induced by electron transfer.

The first process was experimentally demonstrated by Schiffrin *et al* [3] in the paper on the role of phase transfer catalysis in two-phase redox reactions. In this pioneering paper, it was shown that it is possible to induce an electron transfer across redox couples placed one in each of two immiscible electrolyte solutions by generating an interfacial galvani potential difference across the interface through the use of potential-determining ions (or phase transfer catalysts). In other words, electron transfer can be induced by ion transfer. It was earlier shown [4, 5] that no electron transfer between such redox couples would occur in the absence of either an externally imposed galvani potential difference $\Delta\varphi$ or a $\Delta\varphi$ imposed internally through potential determining ions. Samec *et al* [6] had also shown, using the ferricyanide system in water and ferrocene in nitrobenzene, that the heterogeneous redox reaction has a positive ΔG^o of 13.1 kJ.mol^{-1} whereas the homogeneous reaction is spontaneous with a ΔG^o of -1.3 kJ.mol^{-1}.

The theoretical basis for electron transfer across the ITIES explains the fundamental questions: *what drives the two-phase electron transfer reaction? Is a difference in the standard reduction potential of the redox components of the electron transfer chain a* ***sufficient*** *condition for the spontaneity of the redox reaction?*

These questions may be answered as follows. Consider an aqueous (w) redox couple

$$O_1(w) + n_1 e = R_1(w), \quad E_1^o \tag{1}$$

with standard reduction potential E_i^o (with respect to the SHE), and a redox couple in an immiscible organic solvent (o):

$$O_2(o) + n_2 e = R_2(o), \quad E_2^o \tag{2}$$

with a standard reduction potential of E_2^o (*measured in the same organic solvent, but referred to the SHE*). The heterogeneous redox reaction is thus:

$$n_2 O_1(w) + n_1 R_2(o) = n_2 R_1(w) + n_1 O_2(o) \quad (3)$$

An assumption is made that none of the components of the redox couple partition into the adjoining phase. The Gibbs energy change ΔG_{het} for this redox reaction is given by the difference in electrochemical potential of products and reactants, where the electrochemical potential μ_i^α of a component I in phase α is:

$$\mu_i^\alpha = \mu_i^{o(\alpha)} + RT \ln a_i^{(\alpha)} + z_i F \varphi^{(\alpha)} \quad (4)$$

$\mu_i^{o(\alpha)}$ is the standard chemical potential of component I in phase α and $\varphi^{(\alpha)}$ is the galvani potential of phase α. It can easily be shown that ΔG_{het} is given by [3]:

$$\Delta G_{het} = n_1 n_2 F(E_2^o - E_1^o) + RT \ln \frac{a_{R_1}^{n_2} a_{O_2}^{n_1}}{a_{O_1}^{n_2} a_{R_2}^{n_1}} + n_1 n_2 F(\varphi^{(o)} - \varphi^{(w)}) \quad (5)$$

The galvani potential difference $(\varphi^o - \varphi^w)$ may be controlled by making the interface permeable to ions or by allowing ions to cross the interface. Under such conditions, $\Delta\varphi$ is given by the Nernst-Donnan equation:

$$\Delta_o^w \varphi = \varphi^w - \varphi^o = \varphi_i^o + \frac{RT}{z_i F} \ln \frac{a_i(o)}{a_i(w)} \quad (6)$$

where the a_i's are the corresponding activities of the ions in each phase.

The spontaneity of a two-phase redox reaction is thus governed by all three terms in equation 5. In other words, the difference (E_2^o - E_1^o) is *not* a sufficient condition for the heterogeneous electron transfer reaction to take place. The relative magnitudes of the three terms in equation 5 is such that the first and last terms are more significant than the second.

In transposing these considerations to the inner membrane/matrix interface, at which the first electron transfer step in the respiratory chain takes place, namely the oxidation of NADH, the galvani potential difference between the inner membrane and the matrix must be such that, together with the difference in standard reduction potential (E_2^o - E_1^o) between the NADH/NAD redox couple in the matrix and the redox couple in the inner layer, the ΔG_{het} is rendered negative for the redox reaction to be spontaneous. In other words, in order to utilize the Gibbs energy released due to the

oxidation of NADH the inner membrane/matrix interface must be permeable to an ion (lipophilic?) present in both phases, such that the $\Delta\varphi$ thereby generated contributes in making the ΔG negative, hence the reaction spontaneous. *An important assumption is made here that the inner membrane and the matrix are "immiscible", or of different "phase".* Subsequent electron-transfer processes in the respiratory chain take place in the inner membrane of the mitochondrion. Since there is no galvani potential difference within a homogeneous phase, homogeneous redox reactions are governed only by the difference in the standard reduction potential of the redox couples. The redox reaction between cytochrome c and oxygen, mediated by Complex IV, again involve species in different media and hence the same considerations apply as for the first step. Once ΔG_{het} is rendered negative, the redox reaction would take place. This is accompanied by a translocation of protons from the matrix side to the cytosol side of the membrane. This translocation generates a proton electrochemical potential gradient $\Delta\mu_{H^+}$ consisting of both a proton concentration gradient ΔpH as well as a membrane potential $\Delta\psi$ *across both sides of the inner membrane.*

$$\Delta\mu_{H^+} = F\Delta\psi - RT\Delta pH = \Delta G \qquad (7)$$

This Gibbs energy content ΔG is sufficient for the synthesis of ATP as protons now flow down their electrochemical potential gradient (from the cytosol side to the matrix side of the inner membrane).

We now return to the second question posed at the beginning of this section, namely, *is it possible to have ion transfer **induced or driven by** electron transfer?* As shown above, from the ITIES point of view, this is not in line with the thermodynamic formalism of two-phase redox reactions. In this connection, reference is made to the works of Maeda *et al* [7] and to that of Kakiuchi [8] who claim that ion transfer can be induced by electron transfer. For the reasons mentioned above, it is difficult to accept the results in these papers. An alternative viewpoint is the following.

In bioenergetics, the inner membrane is normally impermeable to protons. There must therefore be a driving force to pump protons through the inner membrane. The chemiosmotic hypothesis proposes that it is the Gibbs energy of electron flow that provides the driving force. For a charged species such as the proton to transfer from one phase to another, the required Gibbs energy can be expressed in terms of a difference in the galvani potential of the two phases. Since there must exist a sufficient galvani potential difference across the inner membrane for electron transfer itself to occur, it is therefore logical to speculate whether this galvani potential difference could also simultaneously pump protons across the membrane.

A consideration of ion transfer across the ITIES provides a very simple answer. Ions can be made to cross the ITIES if a sufficient galvani potential difference is imposed across the interface. This potential difference is related to the Gibbs energy of solvation of the ion in the two phases. Every ion

has its own specific value of galvani potential difference at which it crosses a particular interface. It is therefore quite possible that the galvani potential difference imposed across an interface is of sufficient magnitude to drive both two-phase redox reactions or even drive specific ions across the interface. The same consideration could therefore apply for proton transfer across the inner membrane, if the galvani potential difference that must exist for the two-phase redox reactions of the respiratory chain is of sufficient magnitude for protons to transfer cross the inner membrane. This would be equivalent to a *simultaneous* ion and electron transfer, rather than ion transfer induced by electron transfer.

Another issue worthy of consideration is the values of the standard reduction potentials. The values normally quoted in the literature for the redox couples in the respiratory chain are for the species in aqueous medium. In the analysis above for two-phase redox reactions (across the ITIES), the standard reduction potential for the couple in the organic solvent was the value in the organic solvent, but referred to the (aqueous) SHE. It is important to take this point into consideration since the environment in which the redox couple is present greatly affects the redox potentials, as pointed out recently for the case of ubiquinone [9]. The standard reduction potentials of the species in the hydrophobic inner membrane may therefore not follow the same sequence as in aqueous solution.

UNCOUPLERS OR PROTON TRANSLOCATORS

It is known that the presence in the inner membrane of certain ionophores (uncouplers or proton translocators) that facilitate the transfer of protons down their electrochemical potential gradient (from the cytososl to the matrix side of the inner membrane) inhibits the synthesis of ATP without, however, affecting the electron transfer process. This "uncoupling" of the two processes is believed to be due to the fact that the ionophores dissipate the electrochemical potential gradient which then becomes insufficient for ATP synthesis. Mechanistically, a typical "uncoupler" such as 2,4-dinitrophenol is a lipophilic weak acid which, in its deprotonated form, moves towards the cytosol (proton-rich) side of the inner membrane, becomes protonated, and shuttles back to the matrix (or proton-poor) side of the inner membrane, where it delivers the proton, thereby vectorially translocating protons in a direction opposite to their movement during electron transfer. This translocation would take place with less energy than the alternative route of a protein-catalysed H^+ transport that leads to the synthesis of ATP.

In ITIES terminology, this is equivalent to stating that the presence of the ionophores shifts the galvani potential difference for proton transfer to a lower value (it "facilitates" the transfer), such that the electrochemical potential gradient that would normally have been generated is reduced. Effectively, the $\Delta\mu_{H^+}$ is "short-circuited" or dissipated.

The transfer of several ions, facilitated by ionophores, has been extensively studied in the electrochemistry at the ITIES [10] and references cited therein). Ions that would normally require very large potential differences to transfer across the water-organic solvent interface have been made to transfer at much lower potential differences through the use of ligands and complexing agents.

PROTON PUMP

A proton pump brings about a vectorial translocation of protons from one side of a biological membrane to another. Such a vectorial proton transfer can, in principle, be constructed at the ITIES. A lipophilic anion (such as tetraphenylborate, TPB^-) - present in both an aqueous (w) phase and in an adjoining immiscible organic (o) solvent - may, by thereby generating a galvani potential difference according to Eq. (6), (with the water phase having a positive galvani potential with respect to the organic phase) induce the transfer of H^+ from (w) to (o). A lipophilic cation (such as tetraphenylarsonium, $TPAs^+$) - present both in the organic phase and an adjoining aqueous phase - may similarly (by making the water phase have a galvani potential negative with respect to the organic phase) induce the transfer of H^+ from (o) to (w), thereby effectively constituting a proton pump (see Figure 2 below). Depending on the nature of the lipophilic ions, the magnitude of the galvani potential difference across the (w)/(o) interface may be such that not only protons, but also other ions may be simultaneously transferred across the interface. In biological terms, this process would be termed *co-transport* or *symport*.

REVERSE ELECTRON TRANSFER

The term "reverse electron transfer" is used in bioenergetics to describe a redox reaction in which an electron is transferred "uphill" from a couple with the more positive standard reduction potential to one with a lesser reduction potential. The term is also used to describe a situation whereby ATP hydrolysis leads to an establishment of proton gradients and an induction of electron flow in the reverse direction, or where artificially produced proton gradients induce reverse electron flow [11].

Reverse electron transfer may be explained from the ITIES point of view by considering equations 3 and 5. For equation 3 to take place in the forward direction $(E_1^o > E_2^o)$, $\Delta_o^w\varphi$ has to be positive (for then $\Delta G < 0$). For the reaction to take place in reverse ("reverse electron transfer") one must set $\Delta_o^w\varphi$ such that it is more negative than $(E_2^o - E_1^o)$. As explained earlier, it

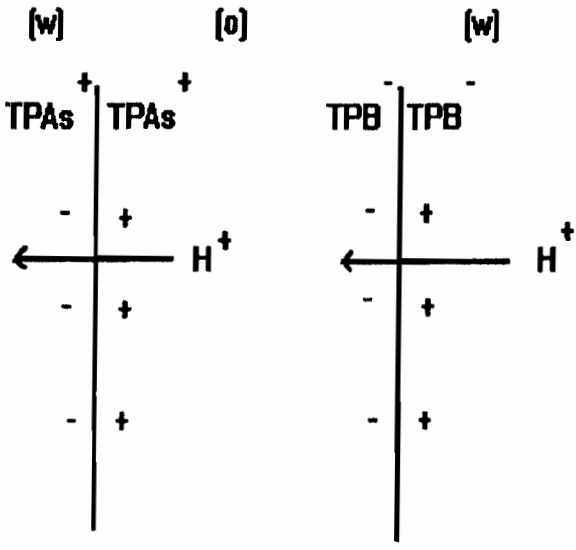

Figure 2. A hypothetical proton pump at the ITIES.

is possible to vary at will the magnitude of $\Delta_o^w\varphi$ using potential determining ions. Reverse electron transfer at the ITIES induced by potential determining ions has recently been demonstrated experimentally [12].

CONCLUSIONS

An attempt has been made to explain some aspects of the chemiosmotic hypothesis from the point of view of the electrochemistry at the ITIES. Several conclusions of relevance to bioenergetics emerge from the presently accumulated data on charge transfer at the ITIES.

1. Two-phase redox reactions can only take place if a sufficient galvani potential difference exists at the interface. This galvani potential difference could be of a magnitude that also pumps protons across the interface. Ion transport *accompanied by* (rather than induced by) electron transfer can only be explained from this perspective; it is otherwise incompatible with the thermodynamic formalism of heterogeneous electron transfer.

2. The mechanism of action of "uncouplers" can be understood in terms of a facilitated proton transfer across liquid-liquid interfaces.

3. A proton pump (and indeed any ion pump) can, in principle, be constructed at the liquid/liquid interface, and this may provide further insight into the role and mechanism of action of proton pumps or, in general, of ion transport across energy-transducing membranes.

4. Reverse electron transfer at the ITIES can be induced by generating an ion electrochemical potential gradient. This would be similar to the situation where artificially generated proton electrochemical potential gradient induces reverse electron transfer across biological membranes

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RESONANCE ENERGY TRANSFER FOR THE DONOR-ACCEPTOR PAIR CURCUMINE-METHYLENE BLUE AND SINGLET OXYGEN SENSITIZATION

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p. 9 (Abstract) line 4, and p 14, line 15

... rate constant of (k_{et}) of 7.11×10^{11} L mol^{-1} s^{-1}(not 7.11×10^{-11})

p. 9, Abstract, line 5-6, and p 15, line 13

in cellulose acetate polymer films, the rate constant of energy transfer was 4.5×10^{10} L mol^{-1} s^{-1} (not 4.5×10^{-10})

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KINETICS OF ETHANE HYDROGENOLYSIS ON $Os_3(CO)_{12}/Al_2O_3$ CATALYST

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p 147.

Table 2. Steady state kinetic models for ethane hydrogenolysis.

Mode	Rate =	Comments
PL	$kP_E^{x}p^{y}_H$	Power law rate
SS1	$\frac{kKP_E/P_H^{(a-1)}}{1 + K_EP_E/p_H^{a_H}}$	C_2H_6 adsorption equilibrium; rds involves H_2 gas
SS2	$\frac{K_1P_E}{1 + bP_H^{(a-f)}}$	C_2H_6 adsorption nonequilibrium; rds involves H_2 gas
SS3	$\frac{kK_EP_EP_H}{[P_H^{a}K_HP_H)^{1/2} + K_EP_E + P_H^{a}]}$	Competitive H_2 adsorption; H_2 gas in rds
SS4	$\frac{kK_EK_H^{1/2}P_E/P_H^{(a-1/2)}}{[(K_HP_H)^{1/2}+K_EP_E+1]^2}$	Competitive H_2 adsorption vacant site (*) in rds
SS5	$\frac{kK_H^{1/2}P_E/P_H^{(a-1/2)}}{[(K_HP_H)^{1/2}+K_EP_E/(P_H^{a}+1)]^2}$	Competitive H_2 adsorption adsorbed H in rds