

On the Energy Conversion by Oxidative Phosphorylation

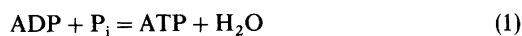
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Polyphosphates can undergo two kinds of acid-base reactions. These are considered in connection with phosphorylation. A model is suggested for the mechanism of coupling a transmembrane proton transport to the phosphorylation reaction. It is analogous to the familiar red-ox reaction at a metal electrode in an aqueous solution. This shows a possible mechanism for the vector-scalar coupling. The processes can be described by rigorous thermodynamics avoiding unmeasurable quantities such as single ion activities and local electrostatic potentials. A complete proton balance is given for the model. The consequences of recent suggested changes in the $H^+/2e^-$ and H^+/ATP ratios are discussed.

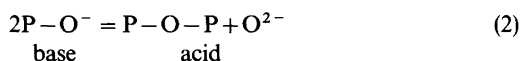
By oxidative phosphorylation in the mitochondrial membrane, the energy gained in a red-ox chain is used to convert $ADP + P_i$ to ATP , where energy is stored for later use. Several mechanisms have been suggested for this energy conversion. The most well-known ones are called the chemical coupling hypothesis originally proposed by Slater¹ and the chemiosmotic hypothesis, proposed by Mitchell.² The first is characterized by a transfer of energy *via* an unknown group of atoms in a high energy state, the second by an intermediate state in the energy conversion, where the energy is stored in the form of an electrostatic and chemical gradient over a membrane.

Operational description of the energy rich bond in ATP. The phosphorylation reaction is usually described by eqn. (1), *i.e.*, the reaction is described as a hydrolysis.

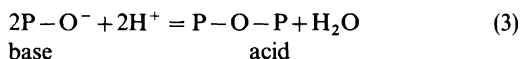


The reaction (1) may just as well be described as an acid-base reaction. It involves the transforma-

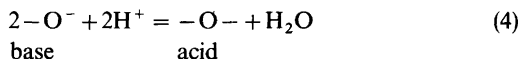
tion of the groups $P-O^- + ^-O-P$ to the group $P-O-P$ with a bridging oxygen by release of one oxygen ion. In many parts of chemistry this is described as an acid-base reaction.³



or



or in general



The position of the particular acid-base pair on an acid-base scale of course depends very much on the neighbour groups, not only on the nearest neighbour to the oxygen in the molecule and on solvent and cations present.

In addition to the above type of acid-base reaction the polyphosphate will undergo acid-base reactions of the type:



These acid-base reactions will immediately come to equilibrium with the surroundings, in contrast to reaction (3) which for polyphosphates in a neutral aqueous solution is a very slow reaction.

The "energy rich bond" can thus be described as moderately strong acid, $P-O-P$, in a medium at $pH \approx 7$, and the energy can only be released in the presence of a specific catalyst.

The synthesis of ATP from ADP and P_i . According to the above description one may consider ATP as

an acid and the pair $\text{ADP} + \text{P}_i$ as a base, and the synthesis of ATP could take place in a medium containing acids, *e.g.* in the form of H^+ in a high energy state. A mechanism on this basis was suggested by Williams in 1961.⁴

In the theory of Mitchell a pH gradient and membrane potential supplies the energy for ATP formation.

The conversion of energy in the form of gradients to energy in the form of ATP requires a coupling between a vectorial force and a scalar flux (the rate of ATP formation is a scalar flux in the continuous description of the process), and this coupling cannot take place in a homogeneous isotropic medium. When explaining a mechanism of coupling it is therefore of interest to discuss how an interface or a heterogeneity makes the scalar-vector coupling possible.

In the following a mechanism for oxidative phosphorylation will be discussed. The points we want to stress in this discussion are the following: Requirements to avoid loss of the energy in the electron circuit and the proton circuit. Requirements to create scalar energy from a vectorial force and *vice versa*. Further, a complete charge and proton balance for transports over the inner mitochondrial membrane is given. To bring out these points it is useful to consider a specific mechanism in detail.

The suggested mechanism, however, should only be considered as tentative to illustrate some of the requirements for the energy conversion.

Electron and proton transport by oxidative phosphorylation. The stoichiometry of the oxidative phosphorylation is the production of 3 ATP for two electrons passing through a chain of red-ox systems ending up with reduction of oxygen. In this chain one can identify three major steps at which ATP is synthesized. At each step energy present as red-ox energy is converted to energy in the state of acid-base energy in the acid group $\text{P}-\text{O}-\text{P}$. By oxidation without phosphorylation, protons are transported out through the mitochondrial membrane to the outside solution at each of the three steps. Otherwise the membrane is impermeable for protons and for net transport of most other kinds of ions.

We may consider one of the steps in the electron transport chain, and assume that the processes taking place in the others are similar in principle (see Fig. 1).

The number of protons transported across the membrane at each step, the $\text{H}^+ / 2e^-$ ratio, and the number of protons needed for each ATP formed, the H^+ / ATP ratio, are here first considered to be equal to 2, the same number used by Mitchell in his chemiosmotic theory.

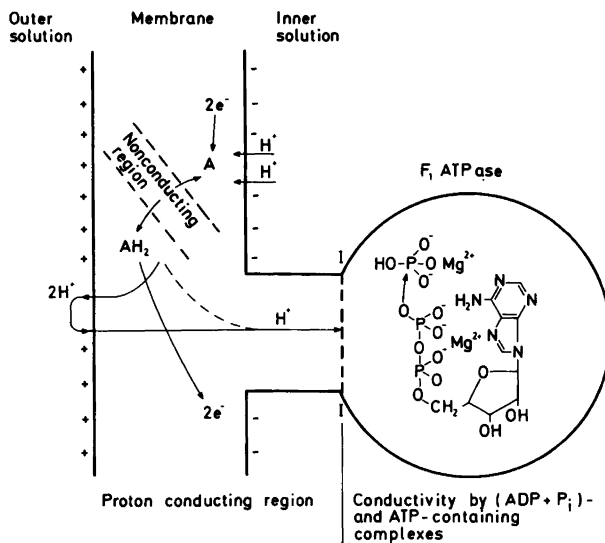
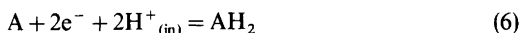


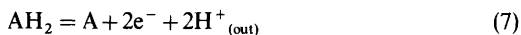
Fig. 1. Schematic picture of the mitochondrial membrane showing one possible mechanism for oxidative phosphorylation.

In the first step reduced flavin mononucleotide is formed. We may describe this reaction briefly as



where the two protons come from the inner solution.

In a later step AH_2 will donate the two electrons to the next red-ox pair in the electron transport chain and two protons are released, but now to the outer solution.



where the electrons go to a more positive potential. Thus the red-ox energy is used to transport protons across the membrane.

The two reactions (6) and (7) cannot take place in the same kind of surroundings, as this would lead to short circuit of both the electronic circuit and the proton circuit. A change in molecular arrangement has to take place in a time interval between reaction (6) and (7). The membrane must necessarily be asymmetrical. When A is converted to AH_2 , its interaction with its surroundings must change. This may lead to movements or reorientation of the molecule, or, what in this connection amounts to the same, a change in the surroundings is induced, particularly a change in electron and proton conductivity. This is a necessity to prevent loss of the red-ox energy.

In Fig. 1 a simple movement of A and of AH_2 over a nonconducting region is considered. In a later section the other alternative, a change in surroundings, is considered, described as closed or open gates.

The protons released in reaction (7) may be named "high energy protons", and they will cause the formation of ATP from ADP and P_i .

The idea of high energy protons as an intermediate in oxidative phosphorylation was first introduced by Williams.^{4,5} The energy of the protons released depends on the red-ox potential of the pair AH_2/A and on the red-ox potential of the acceptor pair for the electrons. After a stationary state is attained, transport of protons from the membrane to the outer solution cannot occur unless the charge transport is compensated for. Whether the protons go directly (follow the dotted line in Fig. 1) to the site of the ATP-ase as suggested by Williams, or to the outer solution and back through the membrane at the site of ATP-ase as suggested

by Mitchell, is not important for the principle of the reaction mechanism.

In the chemiosmotic theory the energy is stored intermediately as an electrochemical potential difference for protons $\Delta\tilde{\mu}_H$. This energy is considered to consist of two parts, a difference in chemical potential $\Delta\mu_H$ (or ΔpH) and a membrane potential $\Delta\psi$.⁶

It is impossible to distinguish between these energy contributions by thermodynamic measurements, as one cannot separate an electrochemical potential of an ion into a chemical potential of the ion and an electrostatic potential by rigorous thermodynamics.⁷ Thermodynamic calculations deal with processes where the *total* transfer always adds up to transfer of neutral components giving no electric charge accumulation. The thermodynamic determination of an electric potential difference always includes electrodes with defined electrode reactions. Further, for reactions involving stationary state condition, there is no change in local charge separation and thus no change in electrostatic energy. Therefore electrostatic potential gradients are not needed in calculations of the energy conversion.

The coupling of vectorial force and scalar energy. In the chemiosmotic theory a vectorial force is described as the cause of the production of ATP, a scalar Gibbs energy. This energy conversion cannot take place in a homogeneous isotropic medium.

In the following section we will suggest a model with analogy to simple electrode reactions, which shows a possible mechanism for vector-scalar coupling.

It is known that the membrane is permeable to protons if the F_1 -ATPase is removed.⁸ Thus charge transfer by protons can take place up to a line I-I indicated in Fig. 1. Beyond this line, through the nonpolar F_1 -ATPase phase, charge transfer must take place by other ions or complexes of ions. The $P-O^-$ groups of ADP or P_i cannot capture protons at this interphase, as this would result in proton transport across F_1 -ATPase and no phosphorylation. Further, only a limited number of groups in ATP may capture protons to account for the H^+/ATP ratio.

Thus the reactants $ADP + P_i$ are assumed to be in a complex (e.g., with Mg^{2+} ions) blocking the $P-O^-$ groups from capturing protons according to reaction (5), and after the proton uptake at the interface I-I, ATP forms a complex blocking most of its basic groups, (they may be blocked by protons

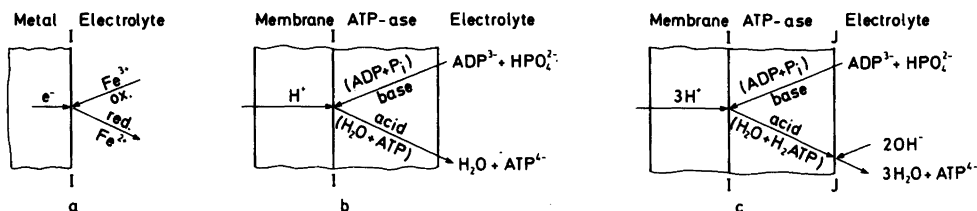


Fig. 2. Vectorial force-scalar energy coupling at interphases. a. Red-ox reaction at a metal electrode–electrolyte interphase. b. Acid-base reaction at the membrane–ATPase interphase for the ratio $H^+/ATP=2$. c. Acid base reaction at the membrane–ATPase interphase for the ratio $H^+/ATP=4$.

in hydrogen bonds or by cation forming strong bonds). As the complexes are soluble in the nonpolar F_1 -ATPase phase, they are expected to have a low charge.

The main point in our description, however, is that the species for transport of charge change abruptly at the I–I phase boundary. To transport protons or positive charges from the outside to the inside, a chemical reaction has to take place at the phase boundary. Thus we give one possible explanation of how an inhomogeneity can make the vector-scalar coupling possible. For the conversion to take place without significant energy losses, the inhomogeneity should be an abrupt change.

The present suggestion of vector-scalar coupling is analogous to the more familiar red-ox reaction taking place at a metal electrode surface (see Fig. 2 a and b).

Just as an electrolyte cannot dissolve electrons and a chemical reaction is enforced by charge transfer, the ATPase cannot dissolve protons and the phosphorylation reaction is enforced by charge transfer. The vector-scalar coupling which occurs at this interface has some feature in common with active transport.⁹

Thermodynamic description of oxidative phosphorylation using a cell analogue. We will now describe the process by rigorous thermodynamic terms and avoid concepts like high energy protons, chemical potential of ions and electrostatic potential differences, which are not measured and are not needed for a thermodynamic analysis.

We also have to take into account two other transport processes closely associated with oxidative phosphorylation:

The inorganic phosphate, P_i , is transported into the inner solution by exchange diffusion, where $H_2PO_4^-$ is transported inwards and OH^- is transported out in a 1:1 ratio. The transport is thus

charge neutral.

The other important transport is the exchange diffusion bringing ADP^{3-} inwards and ATP^{4-} out. As this transport is not charge neutral, it has to be directly coupled to another charge transfer process to maintain electroneutrality inside the membrane. We will first consider the stoichiometry used in the chemiosmotic theory: $H^+/2\bar{e}=2$ and $H^+/ATP=2$. The oxidation of AH_2 releases two positive charges ($2H^+$) to the outer solution. As the $ADP^{3-} - ATP^{4-}$ exchange diffusion consumes one of the positive charges, only one H^+ is left for the phosphorylation reaction.

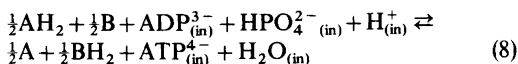
To make it easier to understand the thermodynamic description, let us picture the essential parts of the membrane as a galvanic cell, and even include a potentiometer, which of course should be short-circuited to resemble the membrane system (see Fig. 3).

The cell has two electrodes, the A/ AH_2 electrode (in a position where protons can only be released to the outer solution) and a B/ BH_2 electrode, which is schematically the next major step on the red-ox chain (in a position where the protons captured come from the inner solution).

To resemble a galvanic cell the two red-ox pairs A/ AH_2 and B/ BH_2 are removed from the membrane and placed as electrodes in the two solutions.

The cell shown in Fig. 3 consists of two cells in parallel. The current passes partly through the ATPase and partly through the site of $ADP-ATP$ exchange diffusion. The potentials over the two cells must necessarily be the same.

The cell reaction in the upper part of the cell will be



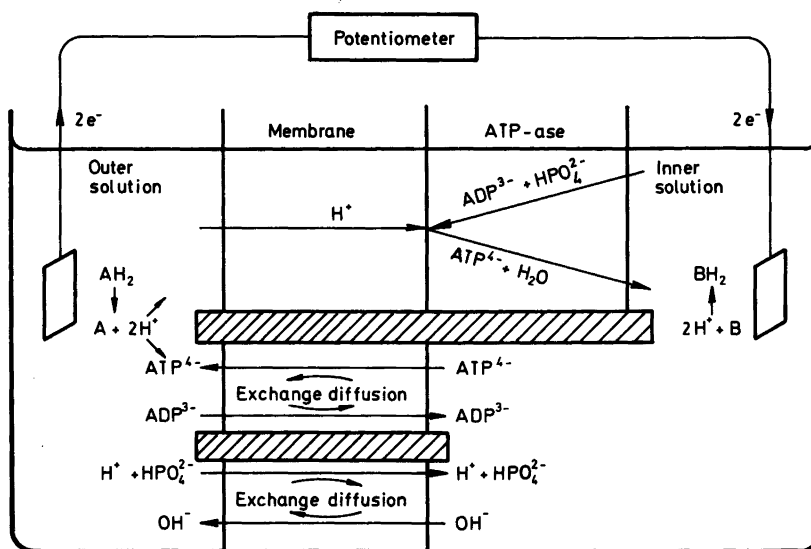
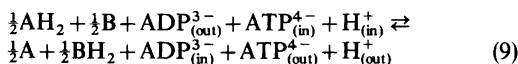


Fig. 3. A cell analogue of reactions and transport taking place by oxidative phosphorylation.

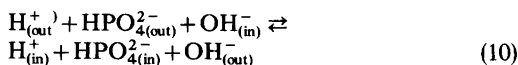
The subscript (in) means inner solution. The cell reaction can also be described in terms of neutral components by including the cations present in the solution. The chemical potential of reactants and products will then be well-defined thermodynamic quantities. This is of particular importance if one will describe the dynamic process by the formalism of irreversible thermodynamics.⁹

The cell reaction causing the ADP-ATP exchange diffusion is:



where subscript (out) means outside solution.

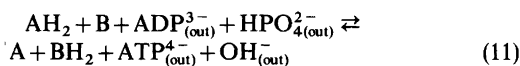
Indirectly coupled to the two cell reactions is the $\text{H}_2\text{PO}_4^- - \text{OH}^-$ exchange diffusion:



The transport of OH^- out is equivalent to transport of H_2O out and H^+ inwards. This means that when this exchange is balanced, the chemical potential of H_3PO_4 must be the same on both sides. (Assuming no change in chemical potential for H_2O). As the acid will dissociate to $2\text{H}^+ + \text{HPO}_4^{2-}$, the ΔpH over the membrane is not expected to be

large. Analysis of the chemical potential of phosphate complexes would be useful to obtain information about differences in pH on the two sides of the membrane.

Summing up all reactions (8), (9) and (10) gives the total reaction:



We see that in total there is no change in the inner solution, in particular there is no change in the content of H^+ and OH^- . As the volume of the inner solution is small compared to the surface area where reactions take place, any suggested mechanism will not seem likely if it leads to accumulation of H^+ or OH^- .

The electrochemical potential difference over the membrane for protons, $\Delta\tilde{\mu}_{\text{H}^+}$, can now be correlated to measurable quantities using the cell description.

The operational definition of $\Delta\tilde{\mu}_{\text{H}^+}$ is the electrical potential measured between two similar electrodes reversible to hydrogen ions. Adopting the ratio $\text{H}^+/2\text{e}^- = 2$ we would thus obtain $\Delta\tilde{\mu}_{\text{H}^+}$ by measuring the balanced emf of a cell having a B/BH_2 electrode on each side of the membrane. The emf of this cell, $\Delta\phi_1$, is related to $\Delta\tilde{\mu}_{\text{H}^+}$ by

$$\Delta\phi_1 - \Delta\tilde{\mu}_{H^+} = 0 \quad (12)$$

On the other hand $\Delta\phi_1$ is related to the potential difference between a B/BH₂-electrode and an A/AH₂-electrode, $\Delta\phi_2$, placed in the same electrolyte, *e.g.* both on the left hand side of the membrane, which is about 300 mV:

$$\Delta\phi_1 + \Delta\phi_2 = 0 \quad (13)$$

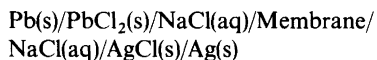
which makes

$$\Delta\tilde{\mu}_{H^+} = -\Delta\phi_2 \quad (14)$$

for the stoichiometry used in the chemiosmotic model, $H^+/2e^- = 2$.

In the cell formalism one would not divide the process into one step creating an electrochemical potential difference for protons, which in the next step created ATP. The processes (eqns. (8), (9) and (10)) are *coupled* and the processes take place simultaneously. The "high energy" intermediate state *cannot be separated from the red-ox process*.

This point may appear more clear if we consider an analogous and more well-known case, *e.g.* the cell:



where the sodium chloride concentration is the same on both sides. If the cell is short-circuited and the membrane is permeable to chloride ions, these ions will be forced to migrate from right to left through the membrane.

The process could be described in two steps: First the creation of an electrochemical potential difference for chloride ions over the membrane, $\Delta\tilde{\mu}_{Cl^-}$, then in the next step $\Delta\tilde{\mu}_{Cl^-}$ causes the flux of chloride ions through the membrane.

It would be simpler, however, to consider the difference in standard potential for the two electrodes as a driving force. The concept "high energy Cl⁻ ions" would then be unnecessary.

In the theory of Williams, however, where protons are created in a hydrophobic medium inside the membrane and do not enter the outside electrolyte, the term high energy protons is more useful.

In the present cell description, with two cells parallel and the ratio $H^+/ATP = 2$, the Gibbs energy supplied would be equally shared between the one

giving phosphorylation and the one giving $ADP^{3-} - ATP^{4-}$ exchange diffusion. This would give a very low concentration of ATP in the inner solution. Transport by diffusion at low concentrations must necessarily give large losses in Gibbs energy.

Consequences of recently suggested changes in stoichiometry. Recently strong experimental support has appeared for a different stoichiometry for the reactions involved in oxidative phosphorylation (see, *e.g.*, review article by Chance¹⁰ and Refs. 11 and 12). The $H^+/2e^-$ ratio at each site is likely to be 3 or 4. Thermodynamic analysis of reversed and forward electron transport at the first phosphorylation site in submitochondrial particles by Rottenberg and Gutman¹¹ gives an $ATP/2e^-$ ratio of 4/3. With $H^+/2e^- = 4$ this gives $H^+/ATP = 3$. The same ratio is found for the phosphorylation reaction in spinach chloroplast thylakoids by McCarty and Portis.¹²

The ratio $H^+/ATP = 3$ in submitochondrial particles would give $H^+/ATP = 4$ when one positive charge must be supplied for the $ADP^{3-} - ATP^{4-}$ exchange diffusion in mitochondria.

Using the model of two parallel cells, this would mean that 1/4 of the available energy is spent on the $ADP^{3-} - ATP^{4-}$ exchange diffusion and 3/4 of the energy is spent on the phosphorylation reaction. This would give a higher equilibrium concentration of ATP inside the membrane and decrease the above-mentioned Gibbs energy loss for any transport by diffusion.

It has been emphasized by Williams¹³ that the processes taking place in mitochondria are so complex that one should be careful in using any fixed stoichiometry. It is, however, of interest to consider some of the thermodynamic consequences and to see how the model suggested here would have to be changed when the stoichiometry is changed.

The ratio $H^+/ATP = 4$ would mean that the reaction at the interface I-I involves the transference of 3 positive charges as shown on Fig. 2c. The two additional protons would most likely be used for hydrogen bonds in the ATP-ATPase complex.

According to this model the removal of ATP from the ATPase will be enhanced by a basic solution on the right hand side. If H_2ATP^{2-} is strongly binded to ATPase and its removal is a significant part of the phosphorylation energy,¹⁴ then the reaction at the interphase J-J of the two protons with hydroxyl ions in a basic solution will not represent an energy

loss, but an energy coupled to phosphorylation. This may to some extent explain the formation of ATP in chloroplast observed by Jagendorf and Uribe.¹⁵ In their experiment a difference in pH, 4 on the left hand side and 8.5 on the right hand side, caused the formation of ATP from ADP + P_i.

The ratio $H^+/2e^- = 4$ instead of 2 would also mean a change in the calculated $\Delta\tilde{\mu}_{H^+}$ from -300 mV to -150 mV as the total energy converted is fixed.

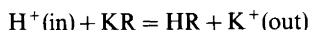
The mechanism of transporting 4 protons instead of 2 from right to left needs comment. As mentioned above, instead of moving the electron carrier A across a nonconducting region to avoid short-circuiting the cell, the alternative would be a shift in the surroundings, which will be discussed in more detail. The shift in surroundings can be described as a set of gates which can open or close according to the state of the electron carrier A, a process which must involve energy changes.

A schematic picture of a set of four gates surrounding electron carrier A is shown on Fig. 4 to illustrate a model which does not violate physical chemical laws.

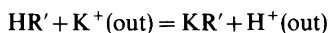
In the figure a double bar \parallel is a closed proton gate and a horizontal line $-$ is a closed electron gate. The figures from *a* to *f* represent a complete cycle of states for A. To avoid local charges, cations, e.g. $2 K^+$, are allowed to leave and enter the membrane on one of the sides, a process which in total would not contribute to energy change.

The crucial point in this description is that the transfer of two electrons from A to B must release four protons. It seems reasonable that two protons

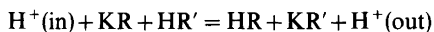
are released on the left hand side, when the electron carrier A increases its charge by two. If two more protons are to be released in exchange for two K^+ ions, this means that the dissociation constant, $K_{(diss)}$ for two acid groups in A (or, in a complex of A and surrounding molecules) is increased when A increases its state of oxidation. This may further be enhanced by a change in the ability to form a complex with a cation, expressed as $K_{(complex)}$. If R represents an acid group in A in the reduced state and R' is the group when A is in the oxidized state, the acid-base and complex reaction can be written as



and



with the total reaction



With $\Delta\tilde{\mu}_{H^+}/(2.303 RT/F) = 2.5$ one finds that under equilibrium conditions the changes in the dissociation and the complex constant would be given by:

$$\Delta \log K_{(diss)} + \Delta \log K_{(complex)} = 2.5 \quad (15)$$

by change in the state of oxidation of A.

The new stoichiometry would not change the proton balance, as the formation of AH_4 instead of AH_2 would remove two more protons from the

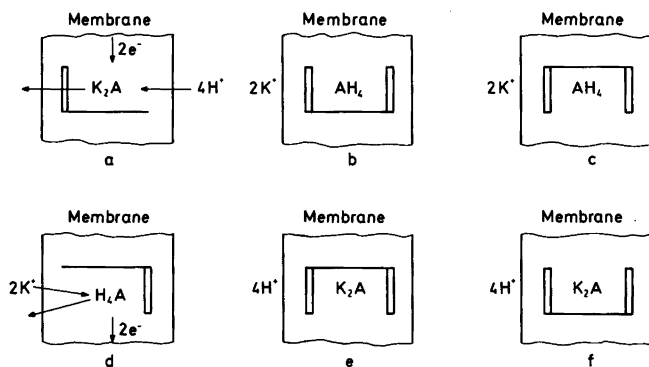


Fig. 4. Changes in state and surroundings of the electron carrier A by transfer of two electrons and four protons. A double bar is a closed proton gate, a single horizontal line is a closed electron gate.

inner solution, and the formation of H_2ATP^{2-} instead of ATP^{4-} would bring two more protons back.

There are two more major steps in the red-ox chain which the electron pair goes through. We assume that the reactions connected to phosphorylation are the same as for the first step.

In addition to the reactions discussed we have all the transport and the reactions taking place in the inner solution prior to the formation of the reduced flavin mononucleotide. It is of interest to study the total proton balance of all these transports and reactions.

CONCLUSION

Oxidative phosphorylation is described by a cell model with emphasis on the following points:

1. The circuits of proton and electron transport should avoid significant loss of energy, as most of the steps in the process are likely to be reversible.

2. The ATP formation is connected to a vectorial charge transfer. The coupling: vectorial force — scalar energy is assumed to occur at an interface where the transport species and solubilities change abruptly. The analogy to the familiar red-ox electrode is demonstrated.

3. The three coupled reactions and transport processes composing oxidative phosphorylation can be described by rigorous thermodynamics involving chemical potentials of neutral components.

4. The combined charge and mass balance for transport over the inner mitochondrial membrane gives no accumulation of protons or of hydroxyl ions in the inner solution for the present model.

5. The model can be adopted to recently suggested changes in the $H^+/2e^-$ and H^+/ATP ratios.

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