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PROTEINACEOUS PROTON PUMPS: A MINIMAL MODEL, SOME PROPERTIES, AND THEIR POSSIBLE UNIVERSALITY

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Abstract

A model is presented to describe the mechanism by which proteins embedded in biological membranes can effect the energy-linked transfer of a proton from one side of the membrane to the other. Some thermodynamic properties of an electron-transport-linked H+ pump of the present type are presented. It is suggested that the present type of model may be a general, universal feature of proton-coupled biological energy transduction. The consequence of this view is explored for several systems, and a brief review of evidence consistent with this belief is given.

1. INTRODUCTION

It is now generally believed that transmembrane proton movements are intimately associated with the transfer of electrons, synthesis of ATP and active uptake of solutes catalysed by a variety of biological membranes[1-5]. Current attention is focused on the elucidation of the molecular mechanisms by which the components of such membranes act to catalyse this protonmotive activity. In the case of the mitochondrial electron transport carriers, the original redox loop concept[6,7] by which alternating electron- and hydrogen-carriers can act to catalyse vectorial proton movements, has been criticised because it cannot adequately account for the protonmotive function of the cytochrome b-O_2 region of the respiratory chain[8-10]. Consequently, it became necessary to postulate either unidentified hydrogen carriers of appropriate redox potential (see [8]) or the operation of a protonmotive Q-cycle[11]. An alternative suggestion was that a known electron carrier possessed a protonable acidic group whose pK_a was different in the oxidised and reduced forms such that oxidation and reduction would be accompanied by the binding and release of protons, a mechanism that has been called the "membrane Bohr effect" (see [8,9,12]). However, only by invoking (a) some kind of rotation of the protein molecule and (b) a kinetic irreversibility of either proton binding or release during a cycle of oxidation and reduction is it possible, with this latter type of model, to catalyse active trans-membrane transport. Since most membrane proteins are rather high molecular weight compounds, it seems inherently unlikely that a rotating-carrier type of mechanism can account for their

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protonmotive activity, particularly since, in at least two cases[13,14], intermolecular cross-linking reagents have been found not to exert significant inhibitory effects on their activity. Further, in the case of electron transport proteins and bacteriorhodopsin, activity is retained at the temperature of liquid nitrogen, which would seem to exclude the possibility of rotation. In view of the large number of membrane proteins that appear to exhibit protonmotive conformational changes, it seems reasonable to assume that a primordial and common structure might evolutionarily have been conserved. Thus, it would be of interest to formulate a general minimal mechanism that might be required for protonmotive activity, particularly if such a mechanism might be of predictive value.

It should be mentioned, as pointed out by Tiemann et al[15] that the definitive feature of a true proton pump is that it catalyses the transfer, across the membrane dielectric, of a charged species, the proton. This is in contrast to systems[15] such as those containing quinones in which neutral H-atoms (chemically bound in redox couples) are the transmembranously-motile species.

The purpose of the present paper is to draw attention to a simple modification of the membrane Bohr model whose operation would catalyse vectorial, transmembrane proton translocation. Particular emphasis will be laid on the mitochondrial respiratory chain as an illustrative example. Certain equilibrium thermodynamic properties of electron transport carriers of this type are described. The present model economically explains a number of difficulties associated with the redox loop concept as applied to the mitochondrial electron transport chain. In particular, we would wish to promulgate the holistic view that a mechanism analogous to that described in the present model, which may be called a amphoteric relay proton pump, may be generally operative in proton-coupled biological energy transduction.

2. A MINIMAL MODEL

Figure 1 (on the opposite page) illustrates our present model for the operation of a redox-linked proton pump during a single cycle of reduction and reoxidation, using a one-electron carrier of the cytochrome type as an example. Differing electronic spin states of the Fe^{n+} moiety are not considered. Four states (I to IV) of the protein are recognised, of which two are the more stable, and in which the redox reactions of electron transfer are tightly coupled to conformational and pK changes or protonable groups A and B of the amino acid side-chains on etiher side of the membrane. Such groups have been referred to as "protodes"[5,15-17]. The protein is embedded (Fig. 1) in a phospholipid M phase, separating two aqueous phases L and R. Between the membrane and these two aqueous phases are inter-phases, which have also been included[5]. In the oxidised form of the carrier, the acidic groups AH and B are uncharged (Fig. 1, state I). Upon reduction, a conformational change takes place in the protein, leading to changes in the pK values of the protodes (state II). Rapid protonation and deprotonation reactions, leading to vectorial proton movement (state III) occur. Upon reoxidation (state IV) the acidic groups return to their original pK values. A proton migrates through a proton channel, from the positively charged BH+ group to the negatively charged A^- group via a charge-relay system of easily polarisable hydrogen bonds[5,15-18]. The

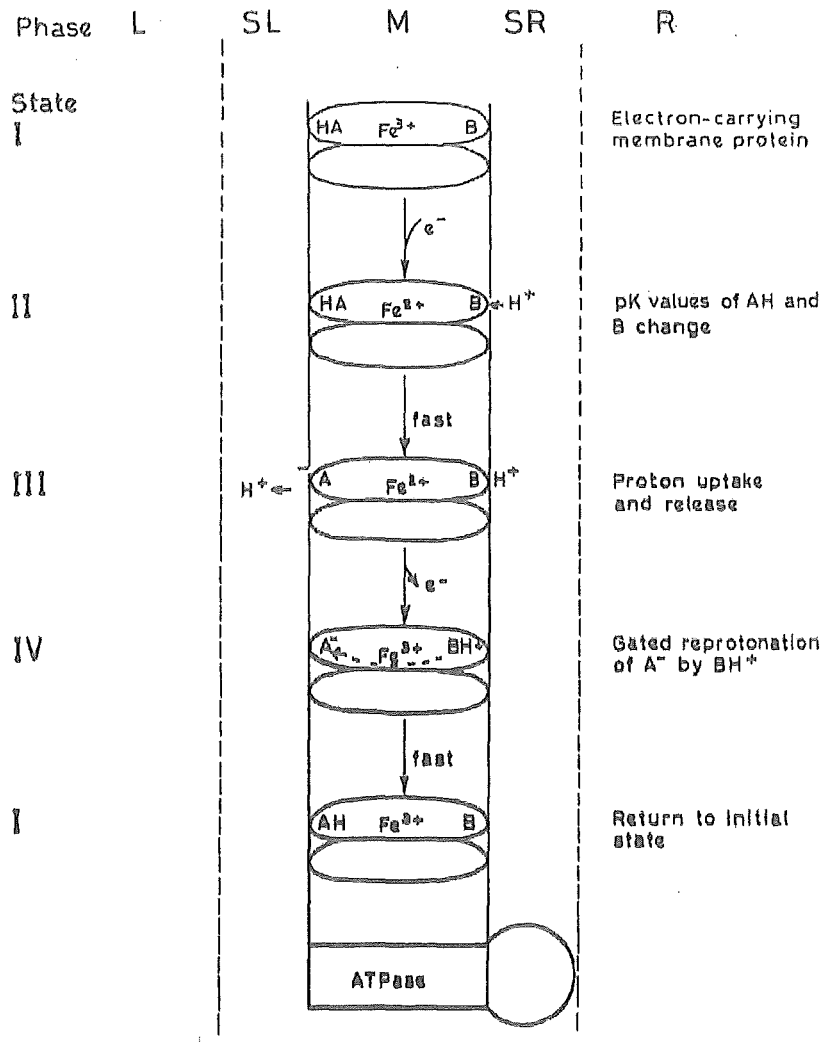

Figure 1. The operation of a transmembrane, protonmotive electron transport protein. The figure shows the four identifiable states during the operation of a protonmotive protein of the type introduced here. States I and III are the more stable states, such that the model may be rationalised as a two-state model. For other proteins the catalytic protein, represented here by the redox Fe^{n+} group, must be modified appropriately. For further discussion, see the text.

original state (I) is now regained. If x groups each of the AH and B types are present in the protein the stoichiometry of vectorial proton translocation ($\rightarrow H^+/\overline{e}$) catalysed by the protein is given by x/n for an n-electron carrier. The generation of opposite surface charges at the two faces of the membrane[5] is a necessary accompaniment to this type of mechanism (Fig. 1, state III).

It may be noted that the present model differs from those of some other workers[8,9], in that two protodes, each having access to only a single, different, membrane/solution interface, are required, instead of a single protode group which faces the two interfaces alternately. A possible experimental distinction of these models is given in the next section. In Mitchell's terminology[18a] the present model would fall into the category of an "indirect or exclusively conformational chemiosmotic type of mechanism".

Whilst it will be argued that the present model is of a rather general occurrence, with the electron transfer reactions of the catalytic part of the

protein replaced by other catalytic properties in proton pumps linked to processes other than electron transfer, we will present in the next section some experimentally testable thermodynamic properties of an electron transport linked amphoteric relay proton pump.

3. SOME THERMODYNAMIC PROPERTIES OF THE PRESENT MODEL

One of the most potent methods for establishing the proton-binding roles of electron transport carriers is to study the phenomenologically-measured redox potential of such carriers under various conditions[19,20]. We therefore consider the rather interesting equilibrium thermodynamic redox properties of a relay proton pump of the type introduced above, containing x groups each of AH and B per protein molecule.

The redox potential of a simple non-proton-binding n-electron redox couple is given by the familiar Nernst equation[19-21]:

$$E_h = E'_o + \frac{RT}{nF} \ln \frac{[\text{oxidised form}]}{[\text{reduced form}]} \qquad (1)$$

in which E_h is the electrical potential exhibited by the half cell in relation to the standard hydrogen half-cell, and E'_o the midpoint potential of the redox couple under modified standard conditions of pH, temperature, etc. However, for protonmotive electron transport proteins of the type described in the previous section, which catalyse active transmembrane proton movement, the apparent, phenomenologically measured mid-point potential will be a function of the hydrogen ion activities on *both* sides of the membrane. We first consider measurements of the mid-point potential carried out under conditions in which the hydrogen ion activities on both sides of the membrane are equal. Let the acid dissociation constant K^A of the group HA [$K^A = (a_{HA}/a_{H+} \cdot a_{A-})$] in the oxidised and reduced forms of the carrier be K^A_{ox} and K^A_{red} respectively. Similarly, let the acid dissociation constants of the conjugate acid forms of group B be given by K^B_{ox} and K^B_{red} for the oxidised and reduced forms of the carrier respectively.

Thus for the acidic group we may write:

$$E_m = E_o + \frac{RT}{xF} \ln \frac{a^L_{H+} + K^A_{red}}{a^L_{H+} + K^A_{ox}} \qquad (2)$$

and for the basic group B:

$$E_m = E_o - \frac{RT}{xF} \ln \frac{a^R_{H+} + K^B_{red}}{a^R_{H+} + K^B_{ox}} \qquad (3)$$

where a^L_{H+} is the proton activity at the surface adjacent to the protode AH, and a^R_{H+} is the proton activity at the surface adjacent to B. Since the protodes occur on, and their pK values are tightly coupled to the redox state of the same protein, the midpoint potential for the carrier is given by:

$$E_m = E_o + \frac{RT}{xF} \ln \frac{a^L_{H+} + K^A_{red}}{a^L_{H+} + K^A_{ox}} - \frac{RT}{xF} \ln \frac{a^R_{H+} + K^B_{red}}{a^R_{H+} + K^B_{ox}} \quad (4)$$

or

$$E_m = E_o + \frac{60}{x} \log_{10} \left[\frac{a^L_{H+} + K^A_{red}}{a^L_{H+} + K^A_{ox}} \Big/ \frac{a^R_{H+} + K^B_{red}}{a^R_{H+} + K^B_{ox}} \right] \quad (5)$$

Now, if the effect of pH on the apparent midpoint potential is studied under (uncoupled) conditions such that $a^L_{H+} = a^R_{H+} = a_{H+}$ we may consider first the group AH. For conditions in which $a_{H+} \gg K^A_{red}$ and $K^A_{ox} \gg a_{H+}$, $E_m = E_o + 60(\log a_{H+} - \log K^A_{ox})$ such that E_m decreases by 60 mV per pH unit at 30°C. When $a_{H+} \gg K^A_{red}$ or K^A_{ox}, $E_m = E_o$, i.e. the midpoint potential is pH-independent. Applying a similar analysis to the group B, we find that when $a_{H+} \gg K^B_{red}$ and $K^B_{ox} \gg a_{H+}$, $E_m = E_o - 60(\log a_{H+} - \log K^B_{ox})$, i.e. E_m increases by 60 mV per pH unit, and when $a_{H+} \gg K^B_{ox}$ or K^B_{red} the midpoint potential becomes pH-independent. Naturally, the relative importance of these effects as a given pH are governed by the values, both relative and absolute, of K^A_{ox}, K^A_{red}, K^B_{ox} and K^B_{red}. This analysis is formally equivalent to that given by Papa[8], except that a basic group B has been introduced to meet the requirements for the present model of vectorial proton movements. It is clear that if the pH is held at the same value on both sides of the membrane, there are likely to be values of pH at which the requirements for protonation and deprotonation become conflicting, and there may be a region of pH in which changes in the measured mid-point potential are less than 60 mV/pH unit, or even pH-independent, although the protonmotive function under coupled conditions might[5] be unimpaired. To illustrate the types of behaviour which may be observed, we have plotted apparent E_m vs pH curves for a number of selected cases (see Figure 2 on the following page). The values of K^A_{ox}, K^A_{red}, K^B_{ox} and K^B_{red} used are given in Table 1 below. It may be observed that the curves are in several regards very different from those found for a simple proton-binding redox couple (case 5).

Table 1

List of values for the pK's of the reduced and oxidised forms of AH and B used for the plots in Figure 2

Case	1	2	3	4	5	6
pK^A_{red}	7	7	9	11	7	6
pK^A_{ox}	4	4	6	8	7	3
pK^B_{red}	8	6	4	4	8	8
pK^B_{ox}	11	9	7	7	11	11

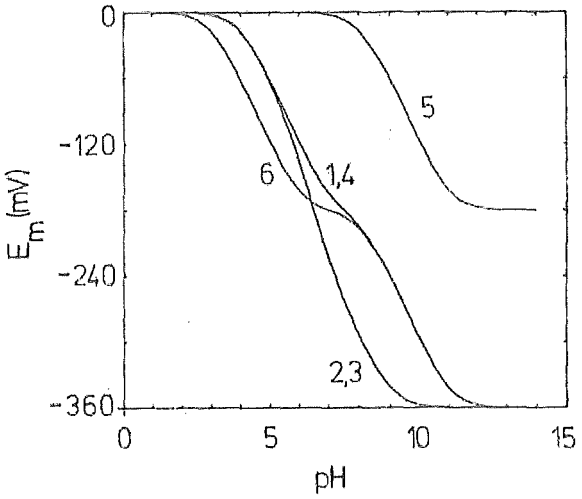

Figure 2. E_m vs pH plots for a truly protonmotive electron transport protein of the present type. Values of the change in E_m as a function of pH were plotted assuming an E_0 value of O mV, using equation (5). The values used for the six selected cases are given in Table 1. Case 5 is that of a protein containing only one protode since only one of the groups AH and B alters its pK during the protonmotive redox cycle. Whether this protein is protonmotive or not depends upon whether the protode alters the interface with which it exchanges protons during its redox cycle[9]. The other cases are chosen to illustrate the rather subtle E_m/pH effects which accompany the present type of model and distinguish it from the more traditional proton-binding redox carrier. Plots were obtained directed on a Hewlett-Packard Plotter driven by a PDP 11/60 computer, using appropriate values in equation (5). The programme was written in Pascal.

It should be pointed out that study of the E_m vs pH properties should be carried out over at least five pH units (with points at least every 0.2 pH units) to distinguish the two types of mechanism (see Fig. 2). It is also useful to point out that the maximum change in E_m as the pH is altered is $60 \log_{10} [10 (pK^A_{red} - pK^A_{ox} - pK^B_{red} + pK^B_{ox})]$. Further, the free energy storage capacity of such a protonmotive machine is

$$G = -\frac{2.3RT}{F} (\Delta pK^A_{red\text{-}ox} + \Delta pK^B_{ox\text{-}red}) \text{ kJ.mol}^{-1}$$

(see, for example, [22,23]). Further, values of (a) K^A_{red} and K^B_{ox}, and (b) K^B_{ox} and K^A_{red} (see cases 1 and 4, Fig. 2) are interchangeable. In order to distinguish the different values it is necessary to measure E_m/pH values when $A^L_{H+} \neq a^R_{H+}$. This type of experiment has previously been performed to give further intimation that cytochrome oxidase contains a proton pump[24]. Measurement of E_m vs pH relationships in the presence of ATP, i.e. under "energised" conditions, has also been shown to cause sizeable translational shifts in such plots, and a number of authors (for example, [8,10,19,25,26]) have pointed out that this type of behaviour may be diagnostic of protonmotive proteinaceous electron carriers.

An alternative, and potentially useful, experimental tool for the analysis of this type of model is the ability to alter the surface charge of biological membranes on either side using non-permeating, charged, lipophilic substances.

Under equilibrium conditions such substances will have the effect of altering the local pH at one side of the membrane. Such substances would include biguanides[17], salicylates[28] and ethidium bromide[29].

Since we have suggested in the introduction that the present type of protonmotive proteinaceous electron carrier may be a general feature of biological energy transduction, it is appropriate to review briefly the types of evidence which are consistent with this contention. It may be noted that in the present model only a *single* redox carrier need be regarded as a "site" of energy transduction[29a] in contrast to the more traditional belief that it is necessary to specify a *pair* of redox couples to define a "site".

4. THE MITOCHONDRIAL RESPIRATORY CHAIN

ATP-dependent shifts in mid-point potential have been reported for Fe-S Centre N2[30], for cytochrome b_{566}[19], and for cytochrome oxidase[31]. Of particular interest and significance to the present considerations is the observation[32] that loss of Fe-S centre N2 *alone* is sufficient to cause a loss in free-energy-conserving capacity at site 1 of the respiratory chain of the mitochondrion-like bacterium *Paracoccus denitrificans*. The sensitivity of the E_m of cytochrome oxidase to the pH on either side of the mitochondrial membrane[24], and its protonmotive activity[9,10,33,34] (but cf.[35]), are also indicative that a mechanism of the type described here may be operative at each of the traditional "sites" of the mitochondrial electron transport chain. Notably, each of these proteins spans the mitochondrial membrane[25], and there is evidence that at least cytochrome oxidase contains a proton channel of the type required for the state IV → I-transition of Fig. 1. The ability of cytochrome oxidase to drive alanine uptake into vesicles containing only this protein and the alanine carrier from a thermophilic bacterium, with reducing power provided by cytochrome c and ascorbate[36], as well as the light-induced inhibition of respiration observed in liposomes containing only cytochrome oxidase and bacteriorhodopsin[37] are most economically explained by the view that cytochrome oxidase is indeed a transmembrane proteinaceous proton pump. Finally, the ability of purified cytochrome oxidase to generate an electrical potential when incorporated into artificial systems, in the same manner as the protonmotive ATPase and bacteriorhodopsin[38] would argue for a protonmotive function for this protein, particularly as it is known[39] that the two haems of the cytochrome aa_3 complex are parallel to each other and perpendicular to the plane of the membrane, and thus cannot bring about transmembrane electronic charge transfer. The evidence that pyridine nucleotide transhydrogenase pumps protons in a manner similar to that described here has been lucidly reviewed by Rydstrom[40]. The possible universality of the mitochondrial electron transport-linked H+ pump has recently been emphasised by Pozzan et al[40a].

5. THE RESPIRATORY CHAIN IN CHROMATOPHORES OF PHOTOSYNTHETIC BACTERIA

Whereas the evidence that we have briefly alluded to above for the protonmotive activity of four proteinaceous electron transport complexes of the mitochondrial respiratory chain may be regarded as being rather suggestive of

the generality of the operation of the present type of model, the situation in chromatophores is far less clear-cut. It is widely believed by the authorities in this field that protonmotive electron transport in these systems may be catalysed via a purely direct chemiosmotic system based on a redox loop/Q cycle type of mechanism, although the molecular mechanism must be regarded as being by no means resolved (see, for example, [41-43]). Therefore, we restrict our treatment in this section to emphasising the possibility that (a) the primary electron acceptor[44] and (b) a b-type cytochrome (b_{50}) may be protonmotive. Circumstantial evidence for this view comes from the pH-dependence of their mid-point potentials (see, for example, [19,41-47]), the ability of the former to exhibit non-electronic charge transfer across phospholipid membranes[45] and the effects of uncouplers on the electrogenic reactions monitored by means of the "carotenoid shift" (see [41,42]).

We are not aware of any experiments which have so far addressed themselves to the question of the ATP-dependence (or otherwise) of the mid-point potentials of these components, or of the effect of changing the pH on a single side of the membrane on this parameter, but it would seem to be of great importance to test such effects. Whilst we are of course aware that the quinone moieties, both free and bound, can act as hydrogen carriers, we are not aware of any evidence that they actually carry protons from one side of the chromatophore membrane to the other (see Introduction). It seems more likely that they bind protons at one side of the membrane and donate both an electron and a proton to the protonmotive b cytochrome, according to our present model.

Lastly, it is of great significance that Cogdell and Crofts[46] found that the primary acceptor in *Rhodopseudomonas viridis* chromatophores exhibited an E_m/pH relation with a slope of 30 mV per pH unit. Whilst, as they pointed out, this behaviour is not explicable in terms of a mere proton-*binding* carrier, it may be easily accommodated in our present model, where such a slope may be demonstrated (Fig. 2, cases 1 and 3, pH circa 6.5 to 8.5) under appropriate conditions.

6. CHLOROPLAST THYLAKOIDS

Just as current models of the protonmotive activity of chromatophore electron transport carriers lean heavily on the redox loop type of mechanism (see above), so too do those of the chloroplast thylakoid electron transport chain[15,47-49], although[41] "the sceptical will be inclined to point out that the evidence for such a detailed molecular mechanism is at present weak". However, in keeping with our suggestion that proteinaceous molecules are responsible for the protonmotive activity of these types of systems, we find interesting circumstantial evidence that cytochrome b_{559} exhibits redox properties consistent with the type of mechanism outlined here. Several authors have enjoined the view that cytochrome b_{559} is intimately involved in free energy conservation[50,51], and the recent demonstration of a "third site" of free energy conservation in chloroplast thylakoids[52] would also be suggestive of such a role for this cytochrome. Nor should we omit from consideration the possibility that the O_2-evolving reaction itself is protonmotive, possibly via the primary acceptor protein complex of photosystem II[53].

7. BACTERIORHODOPSIN

It is widely accepted that the protonmotive "purple membrane protein" from *Halobacterium halobium*, i.e. bacteriorhodopsin (reviews[54-56]) constitutes the simplest, and experimentally most accessible, biological proton pump. It is now virtually certain[23,26,57,58] that it operates via the type of mechanism outlined here, possessing all the necessary features of the present model. Thus it contains two "protodes", it spans the membrane in which it is embedded, possesses a proton-conducting channel, and undergoes conformational changes linked to pK changes during its photocycle, although it is likely that one of its protodes is the retinaldehyde residue linked to one of the protein's lysine residues, rather than an acidic amino-acid side-chain *per se*.

8. OTHER MEMBRANE-BOUND PROTEINS

A great many other membrane proteins are believed to exhibit protonmotive activity, and space does not permit even an outline discussion of these varied systems. It is, however, germane to draw attention to the evidence, reviewed by Lo[59], that at least one proton-symporter, the dicarboxylic cid transporter in *Escherichia coli*, appears to function in the way described here. In this case, of course, the proton channel is a "dicarboxylate channel" as well. Recent evidence[59a] suggests that the mitochondrial adenine nucleotide translocase also contains a "substrate channel". The conformational changes undergone by proton-symporting transport proteins have been well characterised[60]. The functioning of the protonmotive ATPase has also recently been reviewed in detail[61,62] and a mechanism of the present type (cf. [63]) seems favourably supported by the available evidence. Lastly, we would point out that it has been shown[64] that what was previously thought to be perhaps the best example of a direct chemiosmotic, redox loop system, the *E. coli* nitrate reductase[65] must pump protons in a different manner. We would favour the unifying view that a b cytochrome acts as a proton pump in this system too.

9. CONCLUDING REMARKS

Whilst some of the notions put forward here have necessarily been speculative in nature, we believe that the predictive aspects of the present model will be of help in resolving the current difficulties and controversies accompanying the process of bettering our understanding of the molecular mechanics of protonmotive machines. To what extent such a unifying view can be sustained remains to be seen.

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Reviewer Comment

I found this a very interesting and carefully constructed paper. At the moment there are a number of ideas on how protons are pumped across membranes, but little evidence for any of them. The ideas put forward in this paper are another serious attempt to unify the current theories.

Haemoglobin is a well known protein which changes its conformation and emits a proton when reacted on by an oxygen molecule. If held across a membrane it could emit the proton in an uni-directional manner. This is the kind of mechanism D.B. Kell et al. are proposing.

Note Added in Proof

Since the submission of this paper a number of important contributions have appeared. Space does not permit a detailed discussion, but we should like to draw the attention of readers to Walz's perspicacious review[66] of the application of non-equilibrium thermodynamics to electron transport-linked proton pumps, to two marvellously comprehensive reviews by Wikstrom and colleagues[67,68], to the hypothesis of von Jagow and Engel on cytochrome b[69] and to the demonstration of proton pumping at each mitochondrial "site" by Tu and co-workers[70]. These reviews give further credence to the suggestions herein that a unifying view of the nature and existence of the present type of proton pump may indeed by sustainable.

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