

Coherent Properties of the Membranous Systems of Electron Transport Phosphorylation

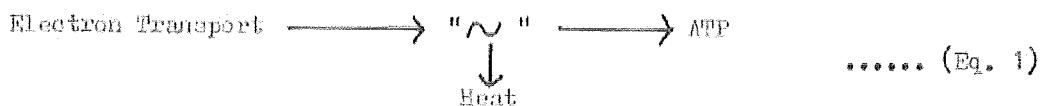
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I. INTRODUCTION AND SCOPE

"Before entering a house, it is customary to remain a while in the courtyard" – Japanese proverb.

It is now well known that the role of a universal chemical energy currency in living cells is played by the so-called high-energy compound adenosine triphosphate (ATP), whose endergonic synthesis from adenosine diphosphate (ADP) and inorganic phosphate ($\Delta G^{\circ} = + 31 \text{ kJ mol}^{-1}$) permits the cell to store free energy in a kinetically stable chemical form. One source of the free energy necessary to drive this reaction lies in processes such as oxidative metabolism or photomimetic electron flow, and the overall process of ATP synthesis coupled to electron transfer is thus referred to as electron transport phosphorylation (see e.g. Stryer, 1981, Lehninger, 1982). The question then arises as to the nature of the free energy transfer between the (exergonic) reactions of electron transport and the otherwise endergonic synthesis of ATP. It is usual to encapsulate this question in the form of a scheme (equation 1) in which a 'high energy intermediate', often denoted " \sim " ("squiggle"), constitutes the energetic link between electron transport and ATP synthesis; it is the nature of this " \sim " that forms the subject of the present considerations.



A great conceptual leap forward in the analysis of this problem was made when, in the early 1960's, Mitchell and Williams independently proposed that 'energised' protons might constitute this " \sim ", and Mitchell's proposals in particular, generally referred to collectively as the chemiosmotic coupling hypothesis, generated a number of successfully tested predictions sufficient to persuade most authorities that the essential mystery of the nature of the " \sim " had indeed been solved.

The central idea of the chemiosmotic coupling hypothesis is that the reactions of electron transport, catalysed by proteinaceous complexes embedded in the so-called coupling membrane, are more or less tightly coupled to the translocation of protons between (phases in equilibrium with) the bulk aqueous phases that the coupling membrane serves to separate. The 'energised intermediate' is then equivalent to the magnitude of the proton electrochemical potential difference

$\Delta\tilde{\mu}_{H^+}/F$ or 'protonmotive force' Δp , in electrical units (mV):

$$\Delta p = \Delta\tilde{\mu}_{H^+}/F = \Delta\Psi - 2.303RT \Delta pH/F \quad \dots \dots \text{ (Eq. 2)}$$

This protonmotive force, which, it may be noted, is of a macroscopic thermodynamic character, may act, according to the chemiosmotic model, to drive protons back across the coupling membrane via an H^+ -ATP synthase complex. So-called uncoupler molecules, which act to uncouple electron transport from phosphorylation, are taken to act as lipophilic weak acids which catalyse the return of H^+ across the coupling membrane via regions other than the H^+ -ATP synthase. These general features are diagrammed in Fig. 1. An excellent introduction to the chemiosmotic coupling hypothesis, and to some of the supporting evidence for this model, may be found in Nicholls's recent lucid monograph (Nicholls 1982), which readers lacking a background in this area are strongly urged to consult.

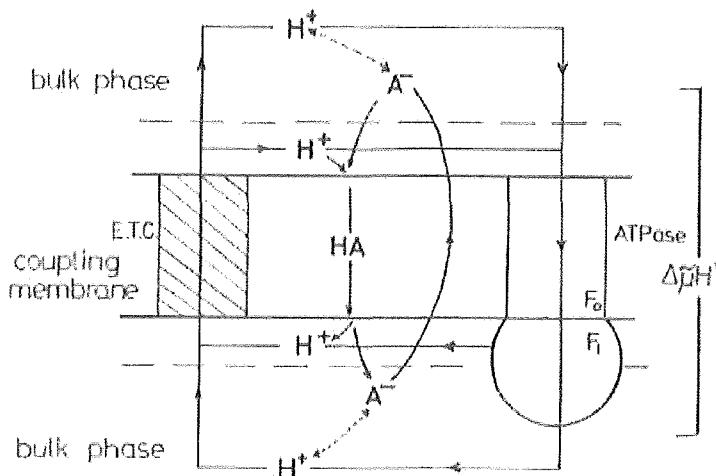


Fig. 1. Energy coupling in protonmotive systems. A coupling membrane containing spatially separate, proteinaceous electron transport (ETC) and F_0F_1 -ATP synthase (ATPase) complexes is diagrammed. A protonmotive force ($\Delta\tilde{\mu}H^+$) may be set up, between the bulk aqueous phases that the coupling membrane separates, during electron transport phosphorylation. Other proteins (not shown) may serve to carry the proton current along the membrane surfaces so that the energy coupling H^+ pathway is not in equilibrium with $\Delta\tilde{\mu}H^+$ and the 'high energy intermediate' is constituted not only by protons but by proteins (see text). In either coupling scheme, 'uncouplers' are lipophilic weak acids which can cross the membrane in both charged (A^-) and neutral (HA) form, thus returning 'energised' H^+ back across the membrane before they can pass to the ATP synthase.

It is worth remarking here that this protonmotive force is widely believed to serve as an energetic intermediate between a number of different processes (in addition to electron transport phosphorylation) that are catalysed by various protonmotive complexes in biological membrane systems (see e.g. Skulachev 1980), but we shall here confine our analysis to electron transport phosphorylation, although we recognise that the type of mechanism adopted by Nature for energy and information transfer in this process is likely to be of more general occurrence.

It is fair to state at the outset that the growth of the field of the bioenergetics of protonmotive systems has been such that it has now become both extremely specialised and ramified in nature. For this reason, for limitations of space, and in the spirit of the interdisciplinary scope of this volume, what we shall here attempt is an outline of what we believe to be the salient features of current knowledge and ignorance of the behaviour of protonmotive systems, where we use the word 'protonmotive' to indicate an enzyme complex whose activity is coupled to the transport of protons across the membrane in which it is embedded.

First, we shall briefly allude to studies aimed at the measurement of the magnitude of the protonmotive force as defined above, under various experimental conditions, which lead one to suppose that the simple scheme shown in Eq. 1, with Δp fulfilling the role of " \sim ", may be inadequate.

Secondly, we shall review and present experimental data on the protonmotive activity of electron transport complexes following short bursts of electron transport, which lead one to conclude that H^+ movements observable in the bulk phase external to the membrane vesicles under study bear only a loose or indirect relationship to the protonmotive activity of various electron transport complexes.

Thirdly, we shall outline the theory and practice of the dual inhibitor titration method, the results of which lead one to take the view that in a number of simple systems the free energy released by a particular electron transport complex is, at least under the described conditions, utilizable only by a particular H^+ -ATP synthase, and is not therefore appropriately described as an intermediate with the delocalised, ensemble character of Δp .

Finally, we shall consider the extent to which the system of electron transport phosphorylation (and related processes) possesses the properties expounded in Prigogine's general theory of coherent excitations and giant dipole oscillations in biology.

2. THE PROTONMOTIVE FORCE: MEASUREMENT AND PROPERTIES

Since the notion of the protonmotive force as a kinetically and thermodynamically competent intermediate in electron transport phosphorylation is generally taken as the central tenet of the chemiosmotic coupling hypothesis, a great deal of experimental work has been directed to the measurement of, and assessment of the role of, this parameter. This work has been authoritatively reviewed several times recently

(e.g. Rottenberg 1979a, Ferguson and Sorgato 1982, Schlodder *et al.*, 1982, Azzone *et al.* 1983), and we shall therefore confine ourselves to some summary statements, illustrated by typical examples (c.f. Westerhoff *et al.* 1983a).

- (a) when measurements of the protonmotive force are performed using different methods on the same biological system, it is commonly found that the apparent degree of membrane energisation differs greatly from (and usually exceeds) the apparent value of the protonmotive force as determined from the distribution of membrane-permeable ions, acids and bases, particularly in photosynthetic systems (see e.g. Ferguson *et al.* 1979, Elema *et al.* 1979, Clark and Jackson 1981, Baccarini-Melandri *et al.* 1981, Junge, 1982).
- (b) rates of electron transport or of phosphorylation are not uniquely dependent upon the magnitude of the apparent protonmotive force measured using a given technique (e.g. Padan and Rottenberg 1973, Baccarini-Melandri *et al.* 1977, 1981, Casadio *et al.* 1978, Sorgato and Ferguson 1979, Kell *et al.* 1978a, Melandri *et al.* 1980, Zoratti *et al.* 1982, Wilson and Forman 1982).
- (c) under 'static head' conditions (for definition see e.g. Rottenberg 1978, 1979b, Westerhoff and van Dam 1979), the ratio of the free energy stored as ATP to the magnitude of the apparent protonmotive force seems to vary in an extremely arbitrary fashion (see e.g. Kell *et al.* 1978b,c, Azzone *et al.* 1978, Guffanti *et al.* 1979, 1981, Westerhoff *et al.* 1981, Wilson and Forman 1982).

Such anomalies, in terms of the scheme of equation 1 (in which Δp is taken to constitute the " \sim "), have therefore led many to take the view that (i) measurements purporting to estimate the protonmotive force as defined in equation 2 are measuring its magnitude correctly but that this protonmotive force is not the actual 'high-energy intermediate', and/or that (ii) the measurements are correctly measuring the 'high-energy intermediate' but that this is not the protonmotive force as defined in equation 2.

At this point it is worth digressing to take cognizance of the fact that an artificial protonmotive force, applied across the bulk phases that the coupling membrane separates, can induce the synthesis of ATP, although the phosphorylation rate is very finely dependent upon the magnitude of this protonmotive force, and is essentially zero if the applied protonmotive force is below approximately 150 mV (e.g. Thayer and Hinkle 1976, Mills and Mitchell 1982, Schlodder *et al.* 1982). To say the least, therefore, it is not outside the bounds of possibility, and the currently available evidence inclines us to adopt the view, that the protonmotive force as defined in equation 2 does not in fact exceed this 'threshold' value under the usual conditions of electron transport phosphorylation. This assumption would nicely account for all of the foregoing observations.

We may thus enquire as to the nature and properties of the directly observable proton-pumping activity that is in fact catalysed by electron transport complexes.

5. PHOTONMOTIVE PROPERTIES OF ELECTRON TRANSPORT CHAINS

Following pioneering experiments with mitochondria (see Mitchell and Moyle 1967), the 'oxygen pulse' method was applied to suspensions of the respiratory microorganism *Micrococcus* (now *Paracoccus*) *denitrificans* by Scholes and Mitchell (1970). In this method (see e.g. Mitchell *et al.* 1979, Reynafarje *et al.* 1979, Wikström and Krab 1980, Nicholls 1982), a pulse of O_2 , as air-saturated saline, is added to a well-stirred, weakly-buffered suspension of the membrane vesicles of interest, and the resultant pH changes in the external aqueous phase monitored with a sensitive glass electrode system. The ratio of the number of H^+ translocated across the membrane (extrapolated to the half-time of O_2 reduction) to the number of oxygen atoms added is known as the $\rightarrow H^+/O$ ratio. It is found, in a typical experiment, that the $\rightarrow H^+/O$ ratio is greatly increased in the presence of compounds such as SCN^- or K^+ /valinomycin that are believed to cross biological membranes rapidly in a charged form.

According to the conventional chemiosmotic explanation of this behaviour (see e.g. Mitchell 1968, Scholes and Mitchell 1970, Kell 1979, Conover and Azzone 1981, Kell and Morris 1981, Kell and Hitchens 1983a), the relatively low (static) electrical capacitance of the membrane means that the electrically uncompensated transmembrane translocation of a rather small number of H^+ leads to the formation of a large membrane potential (equation 2), which drives H^+ back across the membrane before they may be detected; the method thus relies upon the fact that the magnitude of the pH gradient formed under these conditions (equation 2) is very small. K^+ /valinomycin or SCN^- act to dissipate the membrane potential and thus allow all the protons translocated into (or from) the bulk aqueous phase external to the microorganisms to remain there sufficiently long to be measured as a true, limiting stoichiometric $\rightarrow H^+/O$ ratio.

Parenthetically, we may mention that there is a lively current debate concerning the true values of the absolute $\rightarrow H^+/O$ ratios in a number of systems, and the constraints that these values place upon models of the mechanism of protonmotive activity. However, this topic is outside our present scope, and readers may gain an entry to this literature (Wikström and Krab 1980, Wikström *et al.* 1981, Wikström and Penttilä 1982, Nicholls 1982), including that containing our own prejudices (Kell *et al.* 1981b, Vignais *et al.* 1981), elsewhere.

The question to which we wish to address ourselves here concerns the pathway of H^+ that were not seen in the absence of K^+ /valinomycin or SCN^- . Did they briefly set up a large protonmotive force (as defined in equation 2), as in the conventional chemiosmotic analysis, or did they never in fact do so? Oxygen-pulse experiments in mitochondria (Archbold *et al.* 1979, Conover and Azzone 1981), in *Escherichia coli* (Could and Crummer 1977) and in *Paracoccus denitrificans* (Hitchens and Kell 1982a, Kell and Hitchens 1983) that have actually sought to answer this question show that the latter proposal must be correct, since (in the absence of K^+ /valinomycin or SCN^-)

the (submaximal) $\rightarrow H^+/O$ ratio at low oxygen concentrations is virtually independent of the size of the O_2 pulse. Such experiments have thus been interpreted by the cited authors to indicate that there must be at least 2 types of proton circuits in these membranes, only one of which, not seemingly coupled to phosphorylation (but probably serving in pH homeostasis (Padan et al 1982)), enters the bulk aqueous phase external to the membrane vesicles. This is because if the $\rightarrow H^+/O$ ratio in the absence of K^+ /valinomycin or SO_4^{2-} is 2.5 (but 7.5 in their presence) when the size of the O_2 -pulse is 10 ng atom, then the $\rightarrow H^+/O$ ratio should drop even below 2.5 when the size of the O_2 pulse is doubled, since the membrane potential generated by the reduction of an amount of O_2 corresponding to the smaller pulse should have generated a large enough membrane potential to inhibit further H^+ translocation. Since the observed $\rightarrow H^+/O$ stoichiometry is not decreased (in fact it is unchanged under the conditions described, and according to the values given, in Hitchens and Kell 1982a, Kell and Hitchens 1983), the simple conventional analysis given above must be incorrect.

The use of trains of short ($10\mu s$), saturating light flashes in chromatophores (inverted cytoplasmic membrane vesicles) from photosynthetic bacteria allows not only the number but the frequency of electron transport events to be varied. Thus the 'half-time' of the electron transfer events may be varied whilst determining the stoichiometry of proton translocation, as in the O_2 -pulse technique, by a potentiometric system. Such experiments (G.B.H. and D.B.K., unpublished) are displayed in Fig. 2, where it may be seen that, just as is found in O_2 -pulse experiments, the number of H^+ observably translocated per flash is increased in the presence of added K^+ and valinomycin. Crucially, this number is the same, for a given reaction mixture, whether the flash rate is 3 Hz or 5 Hz (Fig. 2, 3), indicating that no bulk-phase proton movements have been 'missed'. As discussed above for the case of O_2 -pulse experiments, the conventional chemiosmotic analysis ascribes the non-appearance of some of the proton movements under certain conditions to the generation of the macroscopic, delocalised membrane potential component of the protonmotive force (equation 2, and see Fig. 4). However, we again stress that this analysis would require that the $\rightarrow H^+/flash$ ratio, when submaximal (see Fig. 3(b)), should be monotonically decreasing as the flash number is raised, a type of behaviour which is not observed (Fig. 3 and see also Cogdell and Crofts 1974). We are again forced to conclude that although macroscopically observable bulk-phase proton movements are coupled to electron transport events, these H^+ are not responsible for feeding back on the electron transport chain to inhibit further proton movements to and from the bulk aqueous phases that the coupling membrane separates. Indeed, it is likely that most, if not all, of these observable H^+ that are translocated across the coupling membrane into a phase in equilibrium with the measuring electrode are translocated essentially in a fashion that is electrically compensated. On this basis, one may surmise that the membrane potential between the bulk phases under those conditions should be very small, as is observed (Vredenborg 1976).

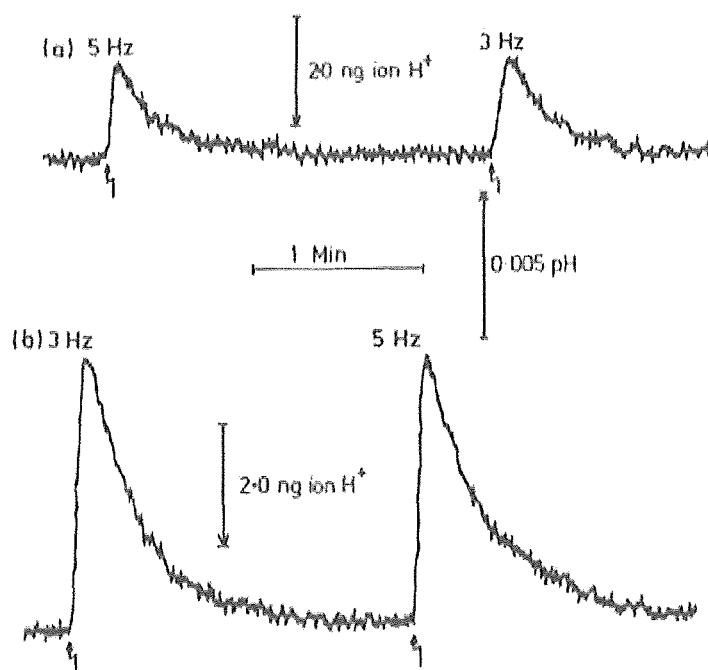


Fig. 2. Light-induced H^+ uptake by *Rhodopseudomonas capsulata* chromatophores. Chromatophores were prepared as described (Hitchens and Kell 1982b), resuspended and stored in 30 mM K_2SO_4 /10 mM $MgSO_4$, adjusted to pH 6.2. Illumination, at the frequencies indicated, was via a FS16 stroboscope (Pro-plan systems, Salisbury; 10 μ s flashes, 2.1J/flash). Light was filtered, and H^+ movements were measured in a stirred 6 ml reaction cell, as described (*ibidem*). Bacteriochlorophyll concentration was 10 μ M, and control experiments ensured that the illumination was saturating. Signals from the pH electrode were amplified by an ISEAMP 200 (bandwidth 15 Hz) designed and constructed by Dulas Engineering, Llwyngwern Quarry, Machynlleth. 20 flashes were given in each case at the points arrowed. In (a) 100 mM KCl was also present, whilst in (b) valinomycin (1 μ g/ml) was further added. No H^+ movements were seen in the presence of 1 μ M carbonyl cyanide p-trifluoromethoxy phenylhydrazone (not shown).

Thus the conclusion from the foregoing type of experiment is that the observable protonmotive events measured using conventional techniques are not adequately accommodated in a simple scheme such as that of Equation 1 and in which " \curvearrowright " is represented by Δp . For reasons such as those alluded to in the foregoing, therefore, we and others have sought an experimental approach to the problem of electron transport phosphorylation that is independent of the measurement either of bulk-phase proton movements or of the protonmotive force as defined in equation 2. One such approach forms the subject of the following section.

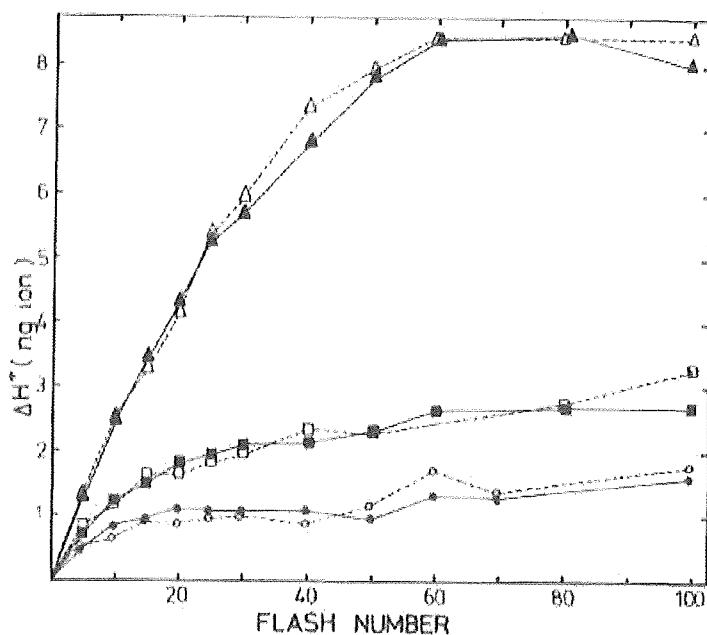


Fig. 3. Effect of KCl, valinomycin and flash number on light-induced H^+ uptake by *Rb. capsulata* chromatophores. Light-induced H^+ uptake was measured as described in the legend to Fig. 2 at frequencies of 3 Hz (open symbols) and 5 Hz (closed symbols). The basic reaction medium ($30 \text{ mM } K_2SO_4/10 \text{ mM } MgSO_4$) (\square , \bullet) was supplemented with $100 \text{ mM } KCl$ (\square , \blacksquare) or with $100 \text{ mM } KCl$ plus valinomycin ($1 \mu\text{g/ml}$) (Δ , \blacktriangle) as indicated, and the figure includes the data of Fig. 2.

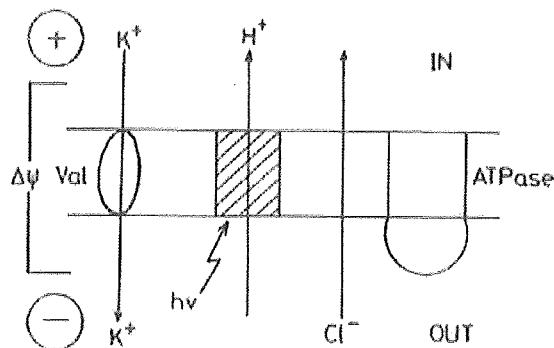
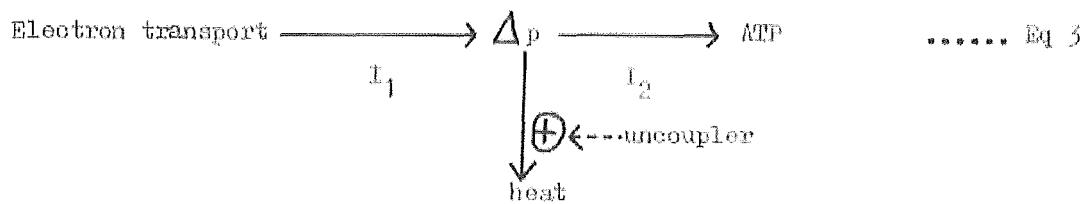


Fig. 4. Conventional chemiosmotic explanation of light-induced H^+ uptake by bacterial chromatophores. The protonmotive segment of the photosynthetic electron transport chain (shaded) pumps a proton from the external bulk phase which sets up a delocalised membrane potential $\Delta\psi$, which acts to inhibit further protonmotive activity on subsequent flashes. Uniport of Cl^- or K^+ (in the presence of valinomycin) decrease $\Delta\psi$ and increase the $\rightarrow H^+$ /flash ratio. For further discussion, see text.

4. DUAL-INHIBITOR TITRATIONS DETERMINE THE DEGREE OF COHERENCE BETWEEN INDIVIDUAL MOLECULAR STEPS IN A SEQUENCE OF REACTIONS.

The idea behind the dual-inhibitor titration approach was first expounded, at least in the context of electron transport phosphorylation, by Baum and colleagues (Baum *et al* 1971, Baum 1978), and may be expressed as follows. If we have available tight-binding and specific inhibitors of electron transport and of the H^+ -ATP synthase, we may modify the delocalised chemiosmotic scheme conforming to that of equation 1 thus:



so that the sensitivity of the overall pathway flux (rate of phosphorylation) to I_1 (electron transport inhibitor) will be decreased by the presence of a partially inhibitory titre of I_2 (H^+ -ATP synthase inhibitor) if Δp as defined in equation 2 does serve as a delocalised coupling intermediate. By 'delocalised', we mean the concept that any free energy released (say as Δp) by a particular electron transport chain is available to any H^+ -ATP synthase in the membrane vesicle. If energy coupling (or for that matter a metabolic pathway) is microscopic in nature, in the sense that quanta of free energy or material interacting with individual enzymes in the system are not available to other enzyme molecules of the same type in contact with the same aqueous compartment, then the presence of I_2 will have no effect upon the titration of the observed pathway flux with I_1 and *vice versa*. In practice, experiments of the I_1/I_2 type demonstrate, at first sight unequivocally, that the latter behaviour is observed in a number of systems (Fig. 5; Baum *et al* 1971, Baum 1978, Hitchens and Kell, 1982b,c, Westerhoff *et al* 1982a,b, 1983a,b).

Now, as pointed out for instance by Parsonage and Ferguson (1982), such behaviour could also be accommodated in a delocalised, chemiosmotic coupling scheme of the type shown in equations 1 and 3 if the initial portions of the two titration curves were accompanied by a decrease (of the same magnitude) in the value of the proton-motive force. However, a variety of measurements of the proton-motive force indicate that even quite severe inhibition of electron transport by an I_1 -type inhibitor do not decrease the proton-motive force (e.g. Kell *et al* 1978a, Songato and Ferguson 1979), in particular when bacterial chromatophores are titrated with antimycin A (Venturoli and Melandri 1982) as in Fig. 5. Wikström (see Westerhoff *et al* 1983b) has raised the interesting possibility that the binding of such inhibitors might be an energy-dependent process (i.e. dependent in some fashion upon the magnitude of the steady-state Δp), and that this behaviour (if true) could lead to an artefactual

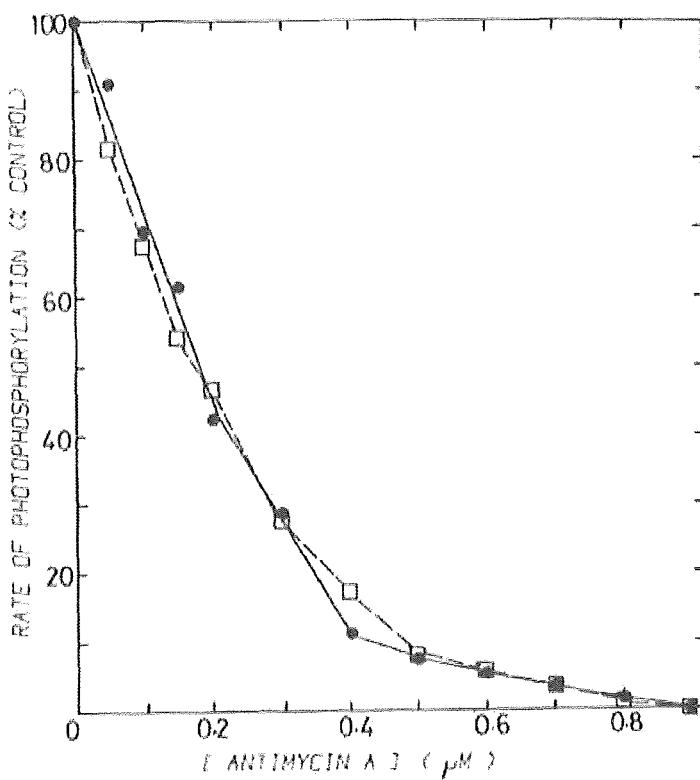


Fig. 5. Effect of dicyclohexyl carbodiimide (DCCD) and antimycin A on phosphorylation by *Rps. capsulata* chromatophores. The rate of phosphorylation was titrated with the electron transport inhibitor antimycin A (●, □). In one case (□) chromatophores were preincubated with a concentration ($50 \mu\text{M}$) of the covalent H^+-ATP synthase inhibitor DCCD sufficient to reduce the rate of phosphorylation in the absence of antimycin by 50%. Control rates were 1.18 and $0.58 \mu\text{mol ATP}/\text{min/mg bacterio-chlorophyll}$. Were energy couplings effected via a delocalised intermediate, such as Δp , the second titration (□) should initially have had a lesser slope than the first; it is evident that the 2 titration curves are virtually identical. Data after Hitchens and Kell (1982b).

interpretation of this type of experiment. At least two lines of reasoning serve to exclude this criticism: (a) I_1/I_2 titrations are symmetrical, in the sense that neither of the pair decreases the inhibitory properties of the other (see e.g. Hitchens and Kell 1982c), and since the inhibitors act on different sides of the 'high energy intermediate' they would be expected to behave differently depending upon which way the experiment is performed (i.e. which inhibitor concentration forms the abscissa in experiments of the type in Fig. 5) if energy-dependent binding effects might be of importance to the analysis; (b) the use of a covalent H^+-ATP synthase inhibitor in concert with trains of saturating light flashes of different frequency (i.e. conditions in which no energy-dependent inhibitor binding may be invoked) demonstrates that the same type of localised energy coupling is observed in bacterial chromatophores (Venturoli and Melandri 1982).

It should be stressed that the number of ATP molecules synthesised per pair of electrons passing down an electron transport chain is, for a well-coupled system as isolated, in the absence of added uncoupler molecules, independent of the rate of

electron transport over a fairly wide range (van Dam and Tsou 1970, Ernster and Nordenbrand 1974, Ferguson *et al.* 1976, Gruber and Witt 1976, Jackson *et al.* 1981). This shows that any macroscopic native 'energy leak' in the system, loosely shown as 'heat' in the schemes of equations 1 and 3, is insignificant under these conditions. Further, an I_2 -type inhibitor cannot be expected to lower the value of a delocalised intermediate such as Δp . Thus, whatever the putative relationship between Δp and the rate of phosphorylation in a scheme such as that of equation 3, the titre of uncoupler required for full uncoupling cannot be decreased by an I_2 -type inhibitor, since equation 3 supposes that uncouplers act only by decreasing Δp . The remarkable fact is, however, that a variety of uncouplers and ionophores act more potently in bacterial chromatophores (Hitchens and Kell 1982a, 1983a,b) and in submitochondrial particles (Westerhoff *et al.* 1982a, 1983a,b) when the reaction supposedly driven by Δp is partially restricted by an I_2 -type inhibitor. In terms of the 'localised coupling' alluded to above, however, this result is indeed to be expected, since the concept of localised coupling implies, in this context, that inhibition of an H^+ -ATP synthase molecule abolishes the utilisability elsewhere of a quantum of free energy from 'its' electron transport chain complexes. Thus uncouplers only seek out coupling units in which the whole unit is potentially active, and will work better when this number is decreased by an I_2 -type inhibitor, as observed. Thus the free energy transfer is not, in this type of system, a stochastic process.

It is germane to point out here that the foregoing, rather qualitative, expose has a quantitative counterpart, often termed 'metabolic control theory', that has been applied to metabolic pathways (most recently reviewed in Groen *et al.* 1982). This latter formalism is itself a 'delocalised' (macroscopic) one in the sense alluded to above, and, in terms of this latter formalism, it would be found experimentally that the sum of the 'control strengths' of the individual steps of electron transport phosphorylation in the experiments alluded to here exceeds 1. As pointed out (Groen *et al.* 1982), such a general finding poses formidable problems for metabolic control theories of this type, and, we would aver, provides a powerful experimental approach to the testing of the important concepts of a rather more sophisticated cellular organisation than those that are commonly held (see e.g. Welch 1977, Kell 1979, Berry 1981, Clegg 1981 and this volume; Welch and Berry, this volume).

In summary, we may state that, although even more subtle and arcane interactions almost certainly take place in energy coupling membranes (Kell *et al.* 1981a), the approach outlined in this section shows that in a number of systems the energy coupling events within the separate molecular complexes of electron transport phosphorylation may be said to possess the property of coherence in that the successful transfer of free energy between electron transport and H^+ -ATP synthase complexes occurs strictly at the level of the individual molecular complexes. Thus

free energy can only be transferred by a particular electron transport complex in a form suitable for driving the synthesis of ATP under the conditions described if a particular H⁺-ATP synthase is potentially active. We shall now explore this concept in more detail.

5. COHERENCE IN THE PROCESS OF ELECTRON TRANSPORT PHOSPHORYLATION

5.1. The Fröhlich theory of long-range coherent oscillations in biology

Over the last fifteen years or so, H. Fröhlich has developed, and stimulated experimental study upon, a theory of long-range coherent excitations in biology that we think is likely to be of the greatest relevance to the problem of free-energy transfer in electron transport phosphorylation. Since a recent review is available (Fröhlich 1980), we shall discuss only what, according to our reading of the model, seem to be its salient features for our present purposes:

- (a) one or more polar modes of the constituents of biomembranes may be coherently excited through energy supplied from metabolic processes, provided that the rate of energy supply exceeds a critical, threshold value; the oscillations are then condensed into a single, lower frequency mode, a phase transition analogous to the Einstein condensation of a Bose gas and to the many other types of phase transition summarised by Haken (1977);
- (b) such states may be stabilised by non-linear interactions between the electrical and vibrational modes of the charged membranes and its heat bath, where the word 'membrane' is taken to include both the lipid and protein constituents and the adsorbed ions and water molecules. Under appropriate conditions, this mode possesses the properties of a relatively long-lived (metastable) ferroelectric state (Bilz *et al* 1981, Kaiser 1981);
- (c) externally applied electromagnetic energy may feed into the system to raise it into, or destroy, the metastable state;
- (d) free energy may be transferred over relatively long distances as a wave of polarisation (Fröhlich 1968). The ferroelectric state should be experimentally observable by dielectric measurements (Fröhlich 1975) as a giant, oscillating dipole.

We shall therefore discuss these points, from a bioenergeticist's standpoint, in terms of what is known of the process of electron transport phosphorylation, and may begin by remarking upon the striking similarities between the Fröhlich model and those invoked by us, for quite different reasons, in this context (Kell *et al* 1981a, Kell and Morris 1981).

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5.2. 'Metabolic pumping' by protonmotive systems

It is now axiomatic that a variety of membrane-located metabolic enzymes are protonmotive, as discussed above; indeed, one is inclined to remark on the fortuitous semantic coincidence in the use of the term 'pump' by the physicists and biologists in this context. As discussed above, therefore, both the requirement for a threshold value of the free energy supply and the pumping of protons across the dielectric barrier of coupling membranes are well-recognized features of the process of electron transport phosphorylation.

5.3. The existence of non-linear interactions in energy coupling membranes

As discussed in section 4, it is an experimental observation that individual, spatially separate redox and ATP synthase proton pumps exhibit a very strict coupling relationship. This of itself requires a highly non-linear interaction. The electrical and elastic properties of biomembranes are not usually considered from the standpoint of the type of idea explicit in the Fröhlich model, though recent reviews that perhaps most closely approach this analysis may be consulted (Evans and Skalak 1980, Miller 1981). From the bioenergetic viewpoint, one of the particularly important virtues of the Fröhlich model is the demonstration that such non-linear interactions leading to a stabilisation of one or more coherent modes may exist, and should therefore be directly sought experimentally.

5.4. Absorption and emission of electromagnetic energy by protonmotive systems

One of the especially noteworthy features of the Fröhlich model is the prediction that very weak electromagnetic radiation of frequency ca. 100 GHz may be expected to change the properties of the types of system under discussion by purely non-thermal means. A number of very striking observations (e.g. Grundler and Keilmann 1976, Grundler *et al.*, Kremer, Nintz, this volume) have shown that this behaviour can indeed be observed in intact living cells. The suggestions concerning the importance of the above (microwave) frequency range were prompted by a consideration of the relationship between the thickness of biomembranes (10^{-8} m) and the likely speed of sound (phonons) therein (1000 ms^{-1}). However (Fröhlich 1980), many types of factor may serve to lower the frequency range in which weak external electromagnetic fields may affect biomembrane systems. One should of course note, in this context, the fact that large external electric field pulses may in fact be used to drive ATP synthesis, just as may large pH jumps (e.g. Teissie *et al.* 1981, Gräber *et al.* 1982, Hamamoto *et al.* 1982, Schlodder *et al.* 1982) although the exact mechanisms at work remain far from clear (Vinkler and Korenstein 1981, Vinkler *et al.* 1982). Weak microwaves have been reported not to affect oxidative phosphorylation in isolated rat-liver mitochondria (Elder and Ali 1975), but irradiation for 15 minutes at 20 kHz did seem to inhibit this process by about 20% (Straub and Garver 1975). Since any such effects are expected to be highly frequency-dependent, a more systematic study than seems so far

to have been attempted, using isolated protonmotive systems, seems warranted. Similarly, it is not possible for us at this stage to determine whether the very exciting observations of enhanced anti-Stokes Raman scattering from a number of active microorganisms (e.g. Drissler and Macfarlane 1978, Webb 1980, Del Guidice *et al.* 1982, Drissler, this volume) may be due to energised membrane states involved in electron transport phosphorylation. The emission of electromagnetic energy from a number of cells that may be detected by microdielectrophoresis (Pohl 1981 and this volume) or by the vibrating probe method (Jaffe 1981) does, however, seem to be best viewed as a membrane phenomenon.

As indicated above, perhaps the least equivocal technique that might be applied, in the present context, to biological systems generally, and protonmotive systems in particular, is to compare their dielectric properties under active (working) and inactive (dormant or equilibrium) conditions (Fröhlich 1975). Unfortunately, we are not aware of any experiments that have so far sought differences in the dielectric properties of biological systems in different, identified metabolic or catalytic states. This current experimental lacuna may be ascribed to the lack, until recently, of instrumentation of a rapidity adequate to measure these properties accurately under steady-state, non-equilibrium conditions. However, the theories and data available to date on dormant or equilibrium systems do enable us to introduce some considerations that will permit us to adopt the optimistic conclusion that the extension of dielectric spectroscopy to working biochemical systems, especially protonmotive ones, is likely to prove of value. Since high-frequency work is discussed elsewhere (Genzel, this volume) we confine ourselves to work below 100 MHz.

5.5. Conformational dynamics and the dielectric properties of biochemical systems

In the case of enzymes, including those of electron transport phosphorylation, which typically have a turnover number in the range 1–10 ms, a plethora of experimental approaches have demonstrated that isolated proteins exhibit a variety of conformational fluctuations in a frequency range of ca. 10^{-5} Hz to 10^{12} Hz. The view has evolved, therefore, that an isolated, substrateless enzyme is, in Weber's (1975) evocative phrase, 'a kicking and screaming stochastic molecule'.

We may then ask the question "to what extent do the high frequency motions of active and inactive membranes and proteins bear a causal relationship to each other and to those of lower frequency?"; two marvellous recent reviews (Careri *et al.* 1979, Welch *et al.* 1981) have summarised the idea that a correlational or causal relationship between the various fluctuational properties of proteins is indeed crucial to enzymatic function. Are these properties perhaps observable by dielectric spectroscopy in proteins generally? Although, we may reiterate, changes in dielectric properties during enzyme activity have not so far been sought successfully, the available evidence is usually interpreted to favour two main mechanisms causing dielectric dispersions in isolated (inert) globular proteins (e.g. Takashima and Minakata 1975, Grant *et al.*

1978, Pethig 1979, Gascoyne et al 1981 and references therein; see also Hasted, this volume):

- (a) Debye-like rotation of the entire protein molecule due to its possession of a permanent dipole moment;
- (b) ion, and especially proton, movement to and from, and between, groups on the protein surface. (We do not here discuss the relaxation of protein-bound water).

Actually, the overwhelming majority of models assume an independence from each other of the elementary processes leading to the observed, macroscopic dielectric dispersions, but, as indicated above, there are reasons to suppose that this is likely to turn out to be something of an oversimplification. The fit between theory and experiment found in even the deepest studies (e.g. South and Grant 1972, Petersen and Cone 1975) leaves abundant scope to invoke factors other than rotation of the whole protein or protonic fluctuations. We would suggest, therefore, that correlated conformational fluctuations in different regions of the tertiary structure of the protein backbone itself may constitute an additional important mechanism of dielectric dispersion (c.f. White and Slutsky 1972). Formally, they would appear as a non-additive superposition of the small changes in (static and mean square) dipole moment caused by the individual conformational fluctuations of charged and polar groups of interest.

As far as non-metabolising, membrane-bound cells and vesicles are concerned (e.g. Schwan 1957, Schanne and Ceretti 1978, Pethig 1979, Stoy *et al* 1982 and references therein), a large body of dielectric measurements by a number of workers indicates that the presence of the membrane gives rise to two main dispersions; the β -dispersion occurs in the radio-frequency range, and is mostly ascribable to a Maxwell-Wagner effect at the interface between the aqueous phases and the poorly-conducting cytoplasmic membrane. Interestingly, however, concentrations of uncoupler sufficient to release maximally the respiratory control of intact Paracoccus denitrificans cells have only a marginal effect on the β -dispersion in this organism (G.D.H. and D.B.K., in preparation). The α -dispersion is seen in the audio-frequency range, and seems to be dominated by the relaxation of bound ions tangential to charged surfaces (Schwarz 1962, Dukhin and Shilov 1974).

Since, from the earlier discussion, we may expect 'energised' coupling membranes to possess non-equilibrium surface charge distributions, we may anticipate striking, and possibly resonant, dielectric properties to be observed in the audio-frequency range under appropriate conditions. Such a search is in progress.

In summary, the available dielectric data on biological systems in states of electrochemical equilibrium allow one to conclude that if the types of electrical states envisaged in the Fröhlich model are formed during electron transport phosphorylation, they should indeed be amenable to experimental observation by dielectric spectroscopy, as suggested (Fröhlich 1975).

6. CONCLUDING REMARKS

The foregoing outline of electron transport phosphorylation may be summarised briefly as follows:

- (a) there is abundant evidence that proteinaceous complexes in electron transport phosphorylation are protonmotive;
- (b) it is not easy to understand their properties in terms of macroscopic coupling theories which invoke a delocalised proton electrochemical potential (or other delocalised, macroscopic thermodynamic force) as 'the' intermediate of electron transport phosphorylation;
- (c) the conception of the 'energised state' of coupling membranes as a giant oscillating dipole with non-linearly coupled electrical and vibrational modes, as described in Fröhlich's theory of long-range interactions in biology, is consistent with the available experimental data on these systems, and suggests some extremely interesting experimental tests of, in bioenergetic terms, a novel character.

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