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PROTON-COUPLED MEMBRANE ENERGY TRANSDUCTION: PATHWAYS, MECHANISMS AND CONTROL

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INTRODUCTION

The importance of 'energised' protons as mobile coupling intermediates in many membrane energy transduction processes is now generally accepted[1-4]. Less clear cut are the nature of these energised protons and the mechanisms by which their precise vectorial transfer is achieved. We therefore first draw attention to the special thermodynamic problems inherent in the bioenergetic description of protonmotive molecular machines. We then review recent evidence that *in vivo* the energised coupling protons are caused to pass along the coupling membrane surfaces from their sources to their sinks by means of specialised H^+-transfer proteins, which act to constitute a cooperative 'protoneural' network for bioelectrochemical information transfer. Evidence is presented which suggests that in bacteria this network is the prime target of the membrane-active, but non-protonophoric, bacteriocins. Experiments are reviewed which suggest that it is the operation of this network, in addition to the build-up of a membrane potential, that accounts for the lack of appearance of protons in the bulk aqueous phase external to the cytoplasmic membrane of respiratory bacteria during 'oxygen-pulse' experiments in the absence of valinomycin or SCN^-. It is pointed out that the existence of a bulk-phase protonmotive force does not of necessity require proton current flow within the bulk aqueous phases that the coupling membrane serves to separate, and the type of model proposed here explains simply why the bulk-phase protonmotive force observed by many workers in a variety of systems is poorly coupled to membrane energy-transducing processes. Attention is drawn to the inefficiency of energy coupling exhibited by reconstituted protonmotive systems lacking the elements of the protoneural network. Finally, stress is laid upon the control possibilities inherent in mechanisms predicated upon ligand-induced changes in conformation and vectorial H^+ conductivity of the elements of the protoneural network.

THERMODYNAMICS AND PROTONMOTIVATED ENERGY COUPLING

Recognising that working protonmotive systems are not at thermodynamic equilibrium, many workers, including Azzone, van Dam, Ferguson, Hill, Rottenberg, Sorgato, Stucki, Walz, Westerhoff and their colleagues, have applied linear

irreversible thermodynamics to analysing the ability of the bulk-phase protonmotive force to act as an energetic intermediate in electron transport phosphorylation (reviews: 5-7). One important principle to emerge from this approach is that, in contrast to normal chemical systems, protonmotive systems may exhibit variable stoicheiometries and/or degrees of coupling. It is worth pointing out parenthetically that for a given electron transport pathway only conformationally-coupled H^+ pumps[8,9], and not the 'direct' chemiosmotic devices such as the redox loop[10,11], may exhibit this type of behaviour[12]. However, the general conclusion to emerge from this type of study[5,6] is that the bulk-phase protonmotive force as defined[10], and thus the macro-chemiosmotic postulate of energised proton current flow through the bulk aqueous phases, do not constitute the sole (or even major) means of protonmotivated membrane energy coupling mechanism. With what kind of energy-coupling model may we seek to replace them?

McClare[13-15] has eloquently summarised the crucial, and frequently-overlooked point that living systems which carry out useful molecular work must do so by a process of resonant energy transfer, and a number of other workers have intimated that the protonic coupling mechanism used by living systems must be thought of in terms of non-classical quantum mechanical tunnelling[5,16,17]. Now at room temperature kT is approximately equivalent in electrical terms to 26 mV, so that, to avoid exposure to energetic fluctuations amounting to a significant fraction of the free energy available at one 'site' of electron transport phosphorylation, the energy stored in the high energy intermediate of electron transport phosphorylation must not be allowed, within an interval τ, to "exchange with the translational, vibrational and rotational energies which normally constitute heat"[13]. τ is defined as the time required for the completion of 1 cycle of a molecular machine, which in the case of the protonmotive molecular machines in which our interest lies is usually in the range 1-10 ms. However, under normal buffering conditions in vivo 'normal' H^+ transfer between the surfaces of an energy coupling membrane and the adjacent bulk aqueous phase should be expected to take place far more rapidly than this[10]. Thus we are first forced to conclude that if the molecular energy-coupling machinery does not permit this energetic exchange to take place within the cycle time τ then unaided it never will. A brief thought-experiment illustrates the application of McClare's principles to bacterial chromatophores.

A bacterial chromatophore may be energised by a single turnover flash, that is to say by a flash of light which though short is bright enough to excite all the reaction centres in the chromatophore suspension simultaneously. Electron transport and proton pumping occur in all electron transport chains and energetically the net result of this activity is describable as a "$\Delta\psi$" across

the chromatophore membrane, amounting typically to approximately 90 mV (ref 18 and J.B. Jackson, personal communication). This $\Delta\psi$ is taken to exist between the bulk aqueous phases that the coupling membrane separates, and is formed across single chromatophores containing approximately 20 reaction centres[18]. Let us repeat this thought-experiment for a chromatophore containing 400 reaction centres; on this basis the single turnover flash would generate a "$\Delta\psi$" of 1.8 V, yet the energy in the absorbed photons is only approximately equal to 1.4 eV! In other words, to avoid breaking the Second Law of thermodynamics each proton pump would have to 'know' whether the other proton pumps in a chromatophore were working or not at the same time if each contributed to the same delocalised $\Delta\psi$ on the time-scale of interest. The alternative, of course, is that the energised protons generated by a given proton pump do not equilibrate with all the other energised protons generated across a given chromatophore membrane, a point of view for which there is increasing evidence[19]. A logical corollary of the adoption of McClare's definition of stored energy as applied to protonmotive systems, it therefore seems to us, is that there must be specialised devices within energy coupling membranes which act specifically to conduct energised protons between their sources and their sinks, themselves storing free energy in the form of field-induced conformational states. The best corpus of evidence presently available to support this quite necessary view is to be found amongst the bacteria, and we next outline what is known of the mechanism of action of membrane-active bacteriocins.

THE MODE OF ACTION OF MEMBRANE-ACTIVE COLICINS

Since colicins of the Ia, El and K groups are the best-understood membrane-active bacteriocins we confine attention to them, although we recognise the striking similarities of their action to that of other bacteriocins, especially to the clostridiocin butyricin 7423 presently under study in this laboratory. Briefly, for we have recently reviewed these facts in some detail with full references[20], the addition of colicin K to sensitive cells of *Escherichia coli* in amounts as low as 1 molecule/cell results in a rapid and complete uncoupling of all protonmotivated membrane energy-transducing processes. Yet these colicins, at the lowest physiologically lethal concentrations, are neither protonophoric nor (directly) ionophoric. A variety of temperature-sensitive, colicin-insensitive mutant strains exist which, on transfer to the non-permissive temperature, exhibit the physiological properties of colicin-treated cells of the parent strain; these mutations lie at genetic loci designated *ecf* and *cup*. Membrane-active colicins act to dissipate the bulk-phase $\Delta\psi$ but not the bulk-phase ΔpH. Finally, such colicins stimulate both the rate and extent of

respiration-driven H^+ translocation into the bulk phase external to suspensions of E. coli in the absence of compounds such as valinomycin or SCN^-. For this latter observation the classical chemiosmotic formulation suggests[21,22] that the appearance of H^+ in this phase during O_2-pulse experiments is due to the dissipation of a bulk-phase $\Delta\psi$ built up during the O_2 pulse, whilst the type of energy coupling model that we have sought to develop here and elsewhere[3,20] would maintain that these pumped H^+ are normally retained on the membrane surface by elements of a cooperative proton-conducting 'protoneural' network, including moieties analogous to the ecf and eup gene products, and that inhibition of the activity of these elements by membrane-active colicins allows the pumped H^+ to equilibrate with the bulk-phase H^+. Thus a simple discrimination between these views is possible; in the chemiosmotic view (a) in the absence of valinomycin, SCN^- or membrane-active colicin, protonophore-sensitive H^+ translocation should be complete when electron transport finishes, and (b) the stimulation of the apparent $\rightarrow H^+/O$ ratio by such compounds should necessarily be accompanied by an appropriate decrease in the bulk-phase $\Delta\psi$.

THE PATHWAY OF H^+ TRANSFER DURING SHORT BURSTS OF ELECTRON TRANSPORT

There are at least 4 published studies[23-26] in which the O_2-pulse method in intact bacteria has been applied in the absence of so-called 'permeant' ions. However, in each case the $t_{\frac{1}{2}}$ of measured H^+ ejection is very much (>10x) greater than the time taken for the reduction of the O_2 in the pulse. It would seem that the H^+ measured in the bulk phase under these conditions are thus essentially irrelevant to the energy-coupling process. What is the effect of compounds such as SCN^- on the apparent $\rightarrow H^+/O$ ratio and the $\Delta\psi$ developed during the O_2 pulse? Since calculations showed[3] that the role of SCN^- in stimulating apparent $\rightarrow H^+/O$ ratios could not mainly lie in collapsing a bulk-phase $\Delta\psi$ it is unfortunate that no critical experiments designed to test the kinetic adequacy of the electrogenic role of SCN^- in stimulating H^+ ejection into the bulk aqueous phase during O_2-pulse experiments have so far been reported. Indeed, we are not aware of any evidence whatsoever that at the minimum concentration of SCN^- necessary to provoke maximum stimulation of the apparent $\rightarrow H^+/O$ ratio this compound acts by collapsing a bulk-phase $\Delta\psi$. Thus, by implication, the view ostensibly obtainable from O_2-pulse experiments that the in vivo pathway of H^+ current flow is within the bulk aqueous phases that the coupling membrane serves to separate must also be deemed unproven.

ENERGY COUPLING IN RECONSTITUTED LIPOSOMAL SYSTEMS

It is well known that the synthesis of ATP may be driven at apparently high rates in reconstituted liposomal systems whose only protein components of any quantitative significance are a light-or electron transport-driven H^+ pump and an F_0F_1-H^+-ATPase of appropriate polarity[27-29]. In fact these rates appear reasonably high only when expressed in terms of nmol ATP/min/mg protein. Since these reconstituted systems are highly enriched in the supposed sole energy-coupling proteins, however, this is an inappropriate means of relating the observed rates of ATP synthesis to those in more native preparations, and it is of interest to enquire as to the rates of ATP synthesis observed based on the turnover numbers of the reconstituted ATPase enzymes. This calculation has recently been performed by Hauska and colleagues[29], who find that in their reconstituted systems of photophosphorylation the turnover numbers of the ATPase enzymes are at best only 0.4% of those observed in chloroplast thylakoids, and point out that this type of discrepancy is quite comparable with that observed in the reconstituted systems of all other workers. It should be mentioned that the apparent protonmotive force generated in their liposomes was equal to that observable in the thylakoids from which the reconstituted proteins were prepared. Even in the simple case of the H^+ channel of an F_0-ATPase[28] a turnover number of only $6H^+$/s/100 mV was obtained whilst the in vivo rates, based on an $\rightarrow H^+$/ATP ratio of 2.5, a very conservative ATPase turnover number of 100/s[30-32] and a $\Delta\tilde{\mu}_{H^+}$ of 200 mV[32], are approximately 140 H^+/s/100 mV. Thus we are forced to conclude that for rapid and effective protonmotivated energy coupling something else is required in addition to the protonmotive sources and sinks and an intact insulating coupling membrane; we believe that the available evidence is best viewed as supporting the notion that additional, specialised proton-conducting proteins, distinct from the proton pumps, are an important feature of protonmotivated energy coupling, and it is of interest to enquire further as to their nature and mechanism. In passing we may also draw attention to the recent genetic sequencing of the mitochondrial genome in which eight different proteinaceous gene products remain unidentified[33].

THE ENERGY-COUPLING PROCESS

It was pointed out earlier that protonmotive molecular machines must operate by a process of resonant energy transfer. Now the key feature of this type of quantum mechanical energy transfer is that the energy levels of the donor and acceptor states must be identical for it to take place, and in the absence of this condition being met energy coupling is impossible. It would seem then that this type of resonant energy transfer must be exploited by protonmotive molecular machines (Fig. 1), and that reconstituted systems, lacking a device for

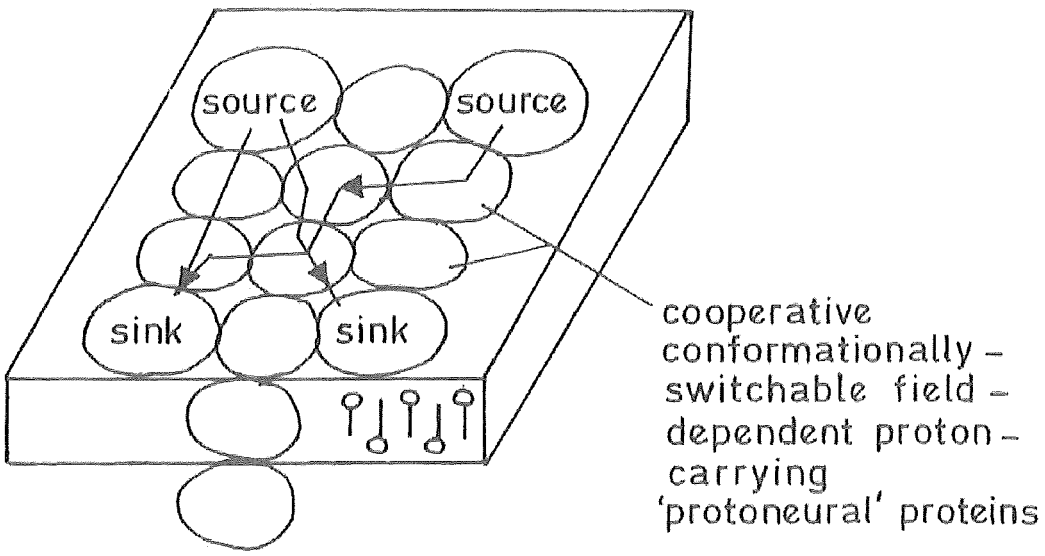

Fig. 1. A model of membrane energy coupling by surface proton conduction

The figure shows in diagrammatic representation the type of model of proton-motivated membrane energy coupling that is outlined herein. The important feature is the inclusion of proton-carrying proteins distinct from the proton-motive sources and sinks which act, in a variably-cooperative fashion, to conduct energised protons along the surfaces of the coupling membrane between their sources and their sinks. Such 'protoneural' proteins are envisaged as exhibiting field-dependent changes in conformation and proton-conducting capacity, and in certain cases may also be switched on by ligand-induced conformational changes, for instance by methylation during information transfer to bacterial flagellar motors. The conformational flexibility inherent in this type of system allows for energetic matching between 'energised' protons and their source- and sink-protodes. ⟶ proton current pathway.

presenting the H^+ in the required energetic form, are inevitably inefficient in terms of rates of energy coupling. It may therefore be suggested that, in addition to conducting the H^+ between their sources and their sinks, the elements of the protoneural network play another role: that of matching the energy of the energised protons to that of their desired destination sites. In this regard it is possible to envisage a very subtle type of control of membrane energy processes - proton sinks such as active transport carriers and flagellar motors may be activated both by ligand- (e.g. H^+-) induced changes in their own conformation but also by quite specific and arcane ligand-induced changes (e.g. methylation[24]) in the conformation of the elements of protoneural network, thereby simply effecting a change in the energy input to the desired process.

It has not escaped our attention that this type of energy coupling differs fundamentally from that[10,21] based on the diffusion of free ions down their concentration gradients. In this latter type of system the bulk aqueous phases,

at distances from a surface that are large compared with the Debye length during the steady state, are essentially electrically isoenergetic; in the present type they are not necessarily. It is therefore of great interest to note the variety of careful but inexplicable observations confirming the existence of this phenomenon of field gradients through a bulk aqueous phase in a number of systems (e.g. refs. 35-38), which may perhaps be fruitfully reanalysed from this standpoint.

SUMMARISING REMARKS

In the foregoing we have endeavoured to set out the necessary coupling properties of protonmotive machines, both from the standpoint of theory and in the way that current experimental data suggest that they do indeed function. Because of the molecular nature of these systems, the introduction of time into the second law of thermodynamics is a necessary adjunct to their bioenergetic description, and makes it apparent that they operate by a process of resonant energy transfer. With the realisation that quite specialised surface H^+ conduction pathways, along which H^+ flow is catalysed by cooperative conformational changes in proteins distinct from the H^+ pumps, are a feature of protonmotive energy coupling, it is pointed out that 'energisation'-dependent changes in the electrical field in the bulk aqueous phases that the coupling membrane serves to separate may be induced without any significant H^+ current flowing in them.

The precise energetic matching required for H^+ transfer by quantum mechanical tunnelling explains the gross inefficiency (rate of free energy conservation) exhibited by reconstituted systems lacking the elements of the protoneural network, and indicates that a very subtle channelling of energised H^+ *in vivo* may be simply achieved by conformational changes in proteins distinct from the protonmotive sources and sinks. Evidence is summarised which suggests that the network of these latter proteins may indeed be the target of the membrane-active bacteriocins.

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