# PROTON TRANSPORT AND CELLULAR ENERGETICS

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## INTRODUCTION

It is now widely believed that many membrane-located enzymes which transduce free energy are protonmotive. That is, the chemical, mechanical or transport reactions which they catalyse are more or less tightly coupled to the transfer of protons across the so-called "coupling membrane" in which they are embedded.

Since proton transfer reactions are amongst the most rapid reactions known, and the turnover time of enzymes such as the $F_o$-$F_1$-$H^+$-ATP synthase is relatively slow, and probably not more than $100s^{-1}$ (1), it is to be expected that the proton electrochemical potential difference across the protonmotive parts of the topologically closed coupling membrane itself should be in rapid equilibrium with that ($\Delta\mu_{H^+}$) between the 'bulk' aqueous phases that the coupling membrane serves to separate. This conception, that the so-called "protonmotive force", $\Delta\mu_{H^+}/F$, is an intermediate in processes such as electron transport phosphorylation, is usually taken as the central tenet of the body of ideas collectively known as the chemiosmotic coupling hypothesis (e.g. 2,3). Although there is much evidence that the standard chemical potential of a variety of substances in both intact cells (see e.g. Horowitz, Ling, Kellermayer, Negendank, Edelmann, Clegg, Kasturi, this volume) and bioenergetic organelles (4-6) may be significantly different from that of the external bulk aqueous phases that are typically more accessible to experimental measurement, this does not of itself affect the chemiosmotic coupling concept referred to above. What matters is that the time- and space-average proton electrochemical potential difference, measured properly, should possess the kinetic and thermodynamic competence demanded if it is to serve as an intermediate in processes such as electron transport phosphorylation in which it may be implicated.

Many attempts, reviewed recently in (7-9), have been made to assess the competence of the protonmotive force (pmf) as an intermediate in processes such as electron transport phosphorylation. However, since a signal, purporting to 'measure' the pmf, can never be assigned a causal role as an intermediate in a process such as

ATP synthesis which is driven, like it, at the same time by say electron transport, we will not here consider such measurements further, save to note that the variety of discrepancies (e.g. 7-9) found between rates and extents of reactions such as ATP synthesis and the apparent pmf values cast very serious doubt on the role of the latter parameter as an intermediate in the former.

In the present article, we will review recent work, which does not depend upon measurements of the apparent pmf, and which leads one to suppose that although the reactions of electron transport and ATP synthesis are protonmotive (as defined above), those protons coupling the two processes do not enter (come into equilibrium with those in) the bulk aqueous phases that the coupling membrane serves to separate.

## ELECTRON TRANSPORT-DRIVEN PROTON TRANSLOCATION

Upon initiation of electron transport, protons, vectorially 'pumped' across the coupling membrane, appear in (or, depending upon the polarity of the membrane system, disappear from) the bulk aqueous phase external to the coupling membrane-bounded cell, organelle or vesicle of interest. The ratio of $\underline{H}^+$ pumped to electrons ($e^-$) transferred is known as the $\xrightarrow{H^+}H^+/2e^-$ ratio. This number is greatly increased, typically 2- or 3-fold, by the presence of ions which can cross the coupling membrane in their charged form, i.e. by 'permeant' ions.

The foregoing has been known for many years. The question arises, however, as to the fate of those $H^+$ which were <u>not</u> seen in the absence of added 'permeant' ions. The conventional chemiosmotic explanation is that they set up a bulk-to-bulk phase transmembrane potential, which then drove protons, subsequently pumped, back across the coupling membrane before they could be measured. If this explanation is true, a simple prediction arises: increasing the number of electron transport events should monotonically decrease the $\longrightarrow H^+/2e^-$ <u>ratio</u> (which is already submaximal) in the absence of 'permeant' ions. This is because the supposedly large membrane potential is already maximal; otherwise there should be no constraint upon further $H^+$ ejection and measurement of something approaching the true, limiting $\longrightarrow H^+/2e^-$ ratio.

However, measurements in mitochondria (10,11), in <u>Escherichia coli</u> (12), in <u>Paracoccus denitrificans</u> (13,14) and in chromatophores of <u>Rhodopseudomonas capsulata</u>

(9) show that the conventional chemiosmotic explanation cannot be correct; changing the number of electron transport events has a negligible effect upon the $\longrightarrow H^+/2e^-$ ratio measured in a typical experiment. Thus there must be at least two types of proton circuit: one which does, and one which, for whatever reason, does not come into electrochemical equilibrium with the pmf (1,9,10-14).

## LOCALISATION OF ENERGISED MEMBRANE STATES

The pmf is a macroscopic variable. Implicitly and explicitly, this is taken to mean that in electron transport phosphorylation the free energy released by a particular electron transport-linked $H^+$ pump is freely available to all $H^+$-ATP synthases in the same coupling membrane. Thus, the sensitivity of the overall flux (rate of phosphorylation) to an electron transport inhibitor should be diminished if the "control" rate of phosphorylation is decreased by a specific $H^+$-ATP synthases inhibitor. However, experiments in a variety of systems (9, 15-20) indicate that electron transport and $H^+$-ATP synthases complexes behave as 'units'; the free energy released by a particular electron transport chain is not freely available to all $H^+$-ATP synthases in the same coupling membrane, and cannot, therefore, be passing in an intermediate form between the two types of reaction as a delocalised pmf.

It may be mentioned that although a "delocalised" coupling scheme could accommodate this type of observation if the pmf were decreased during the inhibition of electron transport, such a decrease is not observed, as is to be expected given the (crucially important) independence between the $P/2e^-$ ratio and the rate of electron transport (see (9)).

A second type of "dual inhibitor titration" experiment studies the effect of so-called uncoupler molecules on the rate of phosphorylation as a function of the number of active $H^+$-ATP synthases. A "delocalised" coupling scheme would predict that the titre of uncoupler necessary fully to inhibit phosphorylation should be independent of the number of enzymes with access to "the" pool of high-energy intermediate. However, if free energy transfer is localised at a level more microscopic than that of the entire coupling membrane, then inhibiting some of the $H^+$-ATP synthases can remove some of the otherwise potentially available free energy; since an uncoupler can (evidently) only uncouple a potentially coupled reaction, the titre of uncoupler

necessary fully to inhibit phosphorylation may be lowered by inhibiting some of the $H^+$-ATP synthases. In practice (9,16,21,22), the latter behaviour is found. Thus, the "energised state" of coupling membranes of the type presently under consideration is more microscopic than is implicit in the "delocalised" chemiosmotic coupling hypothesis (see also e.g.(23)).

## SOME COMMENTS ON "ACID BATH" EXPERIMENTS

Following the remarkable observations of Jagendorf and Uribe (24), a number of workers (e.g. 25-28), but cf.29) have found that an artificially imposed pmf ("acid bath") can lead to the net synthesis of ATP in a variety of systems. Typically, one measures the relationship between the applied pmf and the quasi-steady-state rate of phosphorylation ($J_p$). This is invariably found to be highly non-linear; a threshold, typically 150 mV, exists, below which $J_p$ is negligible, whilst above this threshold there is a steep dependence of $J_p$ upon the magnitude of the (clamped) pmf.

Although, at very high values of the applied pmf, $J_p$ values as great as those driven by electron transport may be observed (e.g. (30)), it is a jump in logic to state that this alone indicates that a pmf is an intermediate in electron transport phosphorylation. To show this, it is necessary, at the very least, to show that rates of phosphorylation driven either by electron transport or by an artificially imposed pmf depend unequivocally, and to an equal extent, on the magnitude of the pmf under the different conditions. Not only has this not been done to date, but, as noted above (7-9), net electron transport phosphorylation can take place at values of the apparent pmf far below the 'threshold' observed in acid-bath experiments.

Further, it should be mentioned that although in many cases the <u>total yield</u> of ATP following a given applied pmf is essentially independent of the relative magnitudes of its membrane potential and pH gradient components, this result is not consistent with the view that the applied pmf dissipates across the coupling membrane in concert with the phosphorylative activity (31). Additionally, the effect of osmolarity upon the total ATP yield (31) reinforces the view that it does not in fact do so.

It may therefore be concluded that these very elegant experiments, whilst seductive by their beauty, must be reflecting a type of behaviour that is not occurring during electron transport phosphorylation <u>in vivo</u>.

## PROTONMOTIVE SYSTEMS IN BIOLOGY

From the foregoing (and many other) considerations, we find ourselves forced to accept that although electron transport chain and ATP synthase protein complexes are protonmotive, their mutual activities are not coupled via a delocalised pmf. The available evidence to date, whilst partly circumstantial, has led us to the view (see e.g. 1,9,32) that these energy coupling membranes normally contain proteins, which are separate from those in the normally recognized proton pumps, and whose role, for want of a better word, is to "channel" free energy quanta between the protonmotive sources and sinks. We have called the transient networks of communication which they form "protonneural networks" (1,9,32). Now, because of the history of this subject, our collective attention has been concentrated upon membranous systems. However, if we now accept that it is the membrane proteins, and not the aqueous phases, which become energised (see above), then a number of very exciting and heretofore unexpected possibilities arise.

For our present purposes, we wish only to draw attention to the possibility (see 33-36) that a variety of other, non-membrane-integrated enzyme systems may in fact be functionally linked to active protonmotive systems. One important consequence of this (33-36) is that measured intracellular or intra-organellar metabolite concentrations would bear only an indirect relationship to their true activities in vivo. It does not, therefore, seem unreasonable to end on a SOMEWHAT SPECULATIVE NOTE: those enzymes presently regarded as coupled, directly or indirectly, to proton pump activity are probably only the first few to be so regarded during our long, yet fascinating, path to an integrated understanding of cellular organisation and biophysics.

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