Discussion Letter

PROTON-DRIVEN PHOSPHORYLATION REACTIONS IN MITOCHONDRIAL AND CHLOROPLAST MEMBRANES

Comment upon an article written by Dr P. Mitchell, FEBS Letters, 1974, 43, 189–194

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In a recent article Mitchell [1] has redeveloped certain hypotheses concerning the formation of ATP in biological membranes. His stated objective is to explain how reaction (1) can be driven backwards.

$$ATPO^{2-} + H_2O \longleftrightarrow ADPO^- + PO^- \quad (1)$$

The postulate which is advanced is that *protonation of phosphate* (specifically, see later) *in the membrane* (the F_1 complex of the ATP-ase) drives the reaction written as (2).

$$POH^+ + ADPO^- \rightarrow ATPO + H_2O \quad (2)$$

In this paper I shall indicate why I believe that this reaction is a most curious proposal but before doing so I must re-introduce my own views [2,3] of how reaction (1) can be driven backwards by 'protons produced in high *local* concentration *in the region* of the ATPase' [2]. This re-introduction is necessary as Mitchell [1] has misrepresented my views.

Mitchell [1] writes in his introductory remarks "A lowering of pH in equilibrium with the reaction domain would not, as originally suggested by Williams [2] cause ATP synthesis because, as subsequently discussed by Williams [3] protonation would inhibit the attack by $ADPO^-$ as much as it would promote the withdrawal of OH^-; and this is confirmed by the well-known fact that the free energy of hydrolysis is virtually pH-independent below pH 6 [4]."

This sentence completely misunderstands my views. To clarify the position it is important to distinguish first kinetic steady states from equilibrium conditions. The formation of ATP *in a membrane* has to be described in terms of a steady state to which energy, redox energy, is being applied. It follows that the considerations of Lipmann [4], to which Mitchell refers, concerning the equilibrium position of reaction (1) and its pH dependence *in free aqueous solution* are not relevant although one can ask what is the equivalent pH at which the formation of ATP could be driven in free aqueous solution. I made this distinction carefully in [3] and I re-affirmed that a *high local concentration of protons in a membrane* would imply a higher 'effective pH' than 0.0 if it was to represent the energy required to drive ATP formation. Thus reference [3] only amplified reference [2] and in no way changed my proposals.

In view of the statements in reference [1] I wish also to state why it was necessary to amplify in [3] what I had written in [2]. In a letter to me early in 1961 Mitchell asked me to explain the meaning of *a high local concentration of protons.* In my subsequent correspondence with him I realised that what I had written in my first article [2] could be difficult to understand. Therefore both in my replies to Mitchell and in [3] I tried to elaborate upon my remarks and I went out of my way to thank Mitchell [3] p.222 for directing my attention to what could be taken to be lack of clarity. In the course of our exchange and the writing of [3] I made it clear that *protons in the membrane* rather than an *osmotic trans-membrane gradient* of protons were required to drive ATP formation. All the letters and both articles

[2,3] were written before the paper on the chemi-osmotic hypothesis [7] appeared. It is important now to see how an apparent extension to a theory based on osmotic energies [1] has come to resemble through equation (2) a theory based upon a high local concentration of protons in a membrane, for throughout the debate I have pointed out that even if osmosis were involved it is necessary to pass the proton at high effective pH back through the ATP-ase.

In the reaction (1) the equilibrium will be driven to the left (to ATP formation) if in the region of space which contains the ATP-ase, water can be removed. Coupling to redox energy and the nature of the redox reactions, they involve the generation of protonic charge, suggests that ATP formation could be driven if the water was removed through the need to hydrate these protons. It is well established experimentally that pyrophosphate can be made from phosphate by dehydration brought about by strong acids. Clearly in a membrane of a biological system the reaction could only go if the redox energy which drives proton formation is not dissipated by allowing free diffusion of the proton into water or elsewhere. It therefore was necessary in my opinion to develop a high non-equilibrium proton concentration locally but nowhere do I refer to 'equilibrium with the reaction domain' [1]. (In Mitchell's chemi-osmotic sense the reaction domain must be the whole cell or organelle and its surrounding media. The dissipation of energy in such an equilibration is quite foreign to my way of thinking and I have not seen an explanation of these energy losses in the chemi-osmotic hypothesis which from its creation has required such an equilibration).

In further reference to my papers Mitchell [1] states as my view that "protonation would inhibit the attack by $ADPO^-$ on PO^- as much as it would promote the withdrawal of OH^-." I can not find this remark in my writings for I have not discussed the attack in this language. However it comes extraordinarily close to the hypothesis which he now promotes [1]. This is
(a) PO^- and $ADPO^-$ enter the F_1 complex
(b) PO^- is protonated to POH and then POH_2^+
(c) ADPOP leaves together with water.
(a) and (c) are accepted. In (b) protonation occurs at a site where there is both PO^- and $ADPO^-$ but, while $ADPO^-$ is left as it is, PO^- becomes POH_2^+. Thus the production of POH_2^+ generates an attack on $ADPO^-$ without being inhibited by attack of protons on $ADPO^-$! Even if we let this reaction be, what is the effective pH at the ATP-ase site of such a protonation? The pK_a in water to give POH_2^+ from POH can be estimated as -3.0. To use the language of reference [2] this is a very high local concentration of protons in the ATP-ase, though it is not directly what was envisaged in [2].

But it can be asked why is POH_2^+ postulated? It is clear enough that in this way a stoicheiometry of two protons per ATP formed can be generated. However Mitchell also states that he can account for 3–4 protons per ATP, as could well be required in some chloroplasts according to Witt [5]. He does so by allowing the ADPO and PO to enter at a lower initial state of protonation than in say mitochondria. As all the protons come from the same place in chemi-osmotic theory this must mean that the energy lost is $\frac{3-4}{2}$ H^+ (from the gradient) per ATP. Another way of putting this is to state that ADP and P are 50% uncouplers in chloroplasts!

I wish to end this paper on a very different note. It is clear that the areas of agreement between Mitchell and myself are quite considerable. Unknown to one another we both [2,6] put forward views about ATP formation by a means which does not require a chemical intermediate. Since the publication of those first papers we have both propagated the idea that the ATP formation was proton driven and an exchange between us has developed those ideas [3,7] though on somewhat different lines. We have agreed that it is the spatial separation of charge which is the initial energy store, that proton gradients can be coupled to other reactions than to ATP formation, that protonation of the phosphates in the active site of the ATP-ase could occur. However our language is often very different. Thus Mitchell refers to *bulk* protomotive forces and membrane potentials brought about by translocation, while I refer to the energies of *local* charges generated by the separation of protons from electrons by dislocated reactions. I picture strictly localised charge densities, which is the conventional approach of a chemist, while he describes potentials on a membrane much as on a condenser plate, which is the conventional approach of the physicist to the same phenomena. We differ in the role which we suppose the bulk solvents to play for chemi-osmotic theory of necessity equilibrates membrane

produced energies with all the surrounding spaces. I have consistently rejected this and kept non-equilibrium localised charges. I think there is one other area where we fail one another – that is in our abilities to describe or appreciate one another's points of view.

Reference

[1] Mitchell, P. (1974) FEBS Lett. 43, 189–194.
[2] Williams, R. J. P. (1961) J. Theoret. Biol. 1, 1–17.
[3] Williams, R. J. P. (1962) J. Theoret. Biol. 3, 209–229.
[4] Lipmann, F. (1960) in: Molecular Biology (Nachmansohn, D., ed.) pp 37–47 Academic Press, New York.
[5] Witt, H. T. (1974) in: Bioenergetics of Photosynthesis (Gorindjee, R. ed.) Academic Press, New York, in press.
[6] Mitchell, P. (1961) in: Biological Structure and Function (Goodwin and Linberg, eds.) Vol.II, pp 595–597, Academic Press, New York.
[7] Mitchell, P. (1961) Nature 191, 144–145.

Additional Note.

Since this article was submitted two additional papers have appeared in FEBS Letters: P.D. Boyer, FEBS Lett. (1975) 50, 91, and P. Mitchell, FEBS Lett. (1975) 50, 95. Boyer, like myself, points out how inherently improbable the proposals of Mitchell are. Others can judge for themselves the merits of Mitchell's reply which in my opinion removes none of the objections to them (which have been made repeatedly). In this exchange a different approach is mentioned by Boyer – the dehydrating action of the proton could occur through the generation of conformational changes. I consider that the proposal that conformational changes, which are inevitable in the redox chain of chloroplasts and mitochondria (see R. J. P. Williams, (1969) in: Electron Transport and Energy Conservation (J. M. Tager, S. Papa, E. Quagliariello and E. C. Slater, eds.) p.7 Adriatica Editrice, Bari) play a role in the transmission of energy from the site at which charge is generated to the site at which ATP is formed as entirely plausible. A discussion of this very point is to be found in R. J. P. Williams, (1972) in: Membrane Structure and Mechanism of Biological Energy Transduction (J. Avery, ed.) p.81 Plenum Press, London, using the co-operative effects of protonation and oxygen uptake by haemoglobins as an illustration. However the introduction of transmission via conformational change does not remove the essential feature of the discussion that *increase of charge flux in the membrane* can drive ATP formation, and that the charges should not be allowed to leave the membrane to be diluted in the aqueous environment. If charge is thrown out into the medium, as in osmotic theories, then we face the problem of equilibration of the energy of a single cell on its outer side with the whole of the volume in which it is suspended, say the Pacific Ocean.