Discussion Letter

PROTON TRANSLOCATION MECHANISMS AND ENERGY TRANSDUCTION BY ADENOSINE TRIPHOSPHATASES: AN ANSWER TO CRITICISMS

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1. Introduction

Many of the points made by Paul Boyer [1] in his paper about my recent ATPase mechanism [2] are criticisms of his own misconceptions about my proposals rather than criticisms of the proposals themselves [2–5]. Nevertheless, my comments on these criticisms, given chronologically here, may help to shed useful sidelights on the scientific issues under discussion.

2. Discussion

2.1. *Evaluation of the proton-translocating ATPase mechanism*

I am confused by the use of the term 'proton gradient' by Boyer [1] and others, because it is not clear whether it refers, for example, to a concentration gradient, an activity gradient, a $-\log_{10}$(activity) gradient or an electrochemical potential gradient of protons; and it is most important to distinguish between such alternatives. A similar criticism applies to the term 'ion gradient'.

My recent scheme for proton-translocating ATPases [2] showed different intermediates for ATP hydrolysis and coupled synthesis for the simple reason that hydrolysis and synthesis are generally observed under different conditions. Boyer's further criticisms [1] are answered as follows:

1) Isolated F_1 has an alkaline pH optimum for ATP hydrolysis (see [6]). Thus, the activity of OH^- would not, as Boyer asserts [1], be very low. In the F_0F_1 complex, however, under conditions of ATP synthesis, the activities of H^+ and OH^- on the F_0 side of the active centre of F_1 would be very high and very low respectively, and ATP hydroxylation would not proceed. Rather, as Boyer says [1], reversible hydrolysis would occur by reaction of ATP with H_2O, as indicated by stages VII to III in fig.1 of my paper [2].

2) and 3) My scheme [2] does not assume the formation of the trinegative species $O{=}PO_3^{3-}$, as Boyer imagines [1]. He seems to have failed to read [2] that 'two of the equatorial oxygens, shown as $-O^-$ in fig.1, may be close to the Mg^{2+} that is involved in the catalysis, but this Mg^{2+} and other participating enzymic groups are not shown'. He also seems to have overlooked the penultimate paragraph [2] in which I stated explicitly, amongst other relevant things, that the P_i may be present in the active centre of the ATPase as the Mg-monoanion.

4) As indicated above, according to my formulation [2], the second protonation of the –OH of phosphate would not need to occur with an un-neutralised $-O^-$ group attached to the phosphorus atom. Boyer's suggestion [1] that the oxygen of O=P, rather than that of HO–P, might be the acceptor of the second proton would, by the subsequent elimination of H_2O, provide a feasible variation within the general class of O^{2-}-group translocation mechanism that my paper [2] sought to define.

Boyer's objection [1] that the scheme given in fig.1 of my paper [2] 'provides for use only of protons and not of a potential gradient' appears to miss the essential point that, according to the chemiosmotic hypothesis [3–5], the proton (or its chemical equivalent) is the exclusive chemical agency through which coupling is achieved. The total driving energy of the protons in the ATPase reaction catalysed by

the proton-translocating ATPase consists of the electrochemical potential difference of the protons (the protonmotive potential difference) across the enzyme system; and in this context, the electric membrane potential difference is significant only inasmuch as it defines part of the driving energy of the protons, as shown in my orignal statement of the chemiosmotic hypothesis [3] – not latterly as implied by Boyer [1].

As F_0 is continuous with the lipid membrane and has a proton-conducting channel through it [2,6], the electric membrane potential component of the protonmotive potential difference across the membrane will be converted to a pH difference down the proton-conducting channel or proton well [5]. In this way, almost the whole of the protonmotive potential difference across the membrane is expected to appear as a pH difference across the active centre of F_1. Thus, Boyer's estimate of a pH difference of some 5 units corresponds to a protonmotive potential difference of about 300 mV – in agreement with experimental data [see 7]. Electric membrane potentials of 300 to 350 mV have been measured directly for the ATPase of *Neurospora crassa* at high phosphate potential [8].

2.2. *Displacements on phosphorus and ^{18}O exchanges*

The dissociative or S_N1 mechanism, to which I assume Boyer refers in his criticism [1], and the associative or S_N2 mechanism, used as the basis of my model, are limiting cases of the class of nucleophilic displacements. In the S_N1 mechanism, the leaving nucleophile dissociates from the phosphorus centre in advance of the entering ligand, yielding the planar triply coordinate metaphosphate anion as a reactive intermediate. But, as in the S_N2 mechanism outlined in my paper [2], the entering ligand of the S_N1 mechanism may attack the phosphorus centre from the opposite side to that of the leaving group, and the fundamental O^{2-}-translocating principle of my mechanism need not be affected. However, the absolute ADP-dependence of the exchange of ^{18}O between P_i and H_2O, established by Boyer and colleagues [see 2], and other more general considerations discussed by Mildvan [9], may reasonably be taken to favour an S_N2 type of mechanism.

Boyer's contentions [1] concerning the improbability of pseudorotation are not in conflict with my ATPase model [2].

2.3. *Coupling mechanisms and catalytic conformational mobility*

It has long been recognised that group-translocation reactions, and possibly even classical group-transfer reactions, may depend upon cyclic movements or conformational changes of the proteins (and lipid) involved in the catalytic process [10]. But this general notion [see 11], that mobility of the catalytic proteins (and lipid), as well as of the substrates undergoing group transfer or translocation, can be important in the overall catalytic process, should be carefully distinguished from Boyer's conformational coupling notion [12] in which a chemical reaction in one catalytic centre is supposed to be coupled to that in another chemically separate reaction centre by the transduction of energy through the cyclic conformational changes of the intervening polypeptide system [13].

Boyer originally introduced his conformational coupling idea to explain how energy might be directly transduced from the respiratory chain to the ADP phosphorylation system in mitochondria [12]. He now seeks to adapt it [1] to explain how the reaction of proton translocation might be coupled to that of reversible ATP hydrolysis in the ATPase complex – the assumption being that conformational energy is transmitted through the polypeptide systems of the ATPase complex from sites of proton interaction to the catalytic site where ATP synthesis is driven by conformationally-linked changes in the affinity for reactants. This 'black box' type of approach is in contrast to the more practical biochemical experience [11,14,15] that coupling between one reaction and another has generally been attributable to a biochemically identifiable intermediary that is common to the two reactions – in this case, the O^{2-} group of P_i and H_2O that is common to the proton-translocation reaction and to the reversible ATP hydrolysis reaction in the F_0F_1 ATPase complex [2].

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