Hypothesis

A CHEMIOSMOTIC MOLECULAR MECHANISM FOR PROTON-TRANSLOCATING ADENOSINE TRIPHOSPHATASES

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Received 19 April 1974

1. Introduction

Up to the present time, considerations of the possible mechanism of the reversible ATPases of mitochondria, chloroplasts and bacteria have generally depended, either on largely chemical arguments about the transitional intermediates of the reaction mechanism, or on largely thermodynamic arguments about the mechanism of reversal of the hydrolytic process [1–17]. In this paper, I have used both types of argument together, and have thus suggested a simple chemiosmotic molecular mechanism for the reversible proton-translocating ATPases. The proposed mechanism has several characteristics by which it may be recognised experimentally, and it may therefore help to stimulate further research on this subject.

2. Chemical mechanism

The purely chemical reaction catalysed by the reversible ATPases of mitochondria, chloroplasts and bacteria can be conventionally represented by the equation:

$$\mathrm{ADPOP + HOH \overset{Mg^{2+}}{\rightleftharpoons} ADPOH + POH} \qquad (1)$$

In the absence of other processes, not represented in this equation, the equilibrium lies far to the right, but the reversible arrow indicates that the enzyme facilitates rapid dehydration of ADPOH + POH under appropriate circumstances.

In 1953, by using ^{18}O-labelled water, Mildred Cohn [18] discovered that mitochondria catalyse a rapid exchange of oxygen between POH and HOH. Subsequently, it was found [3] that, when present in a coupling membrane so that the ATPase reaction could be poised towards ATP synthesis, the ATPase systems of mitochondria and chloroplasts eliminated ^{18}O from $P^{18}OH$ and not from $ADP^{18}OH$ in the reaction of eq. (1). Also it was found that a rapid exchange of ^{18}O between $H^{18}OH$ and POH occurred when the ATPase reaction was poised at an intermediate level [3,19,20]. These observations indicated that the reversible hydrolysis of ATP occurred by cleavage of the bond between the terminal oxygen bridge and the γ-phosphorus atom of the ATP, and that the OH group was (ultimately) bonded to the γ-phosphorus centre that appeared as POH, as indicated by the asterisks in eq. (2).

$$\mathrm{ADPOP + H\overset{*}{O}H \overset{Mg^{2+}}{\rightleftharpoons} ADPOH + P\overset{*}{O}H} \qquad (2)$$

At first it was thought that one or more covalent intermediates were involved in the ATPase reaction [1–3, 6–11], but this possibility became more and more unlikely as an intensive search failed to reveal any evidence for any intermediates [3,5,12–17], and, more especially, since it was observed that the ex-

Abbreviations:

POH: inorganic orthophosphate; ADPOH: adenosine diphosphate, indicating OH on β-phosphorus; ADPOP or ATP: adenosine triphosphate; $\rightarrow H^+/P$: proton translocation quotient giving number of protons translocated per ATP molecule hydrolysed; Δp: total proton motive potential difference across the coupling membrane; $\Delta G'$: increase in free energy on hydrolysis of 1 mole of ATP under given conditions.

change of ^{18}O between HOH and POH was absolutely dependent on the presence of ADP [19,20]. As recently as 1973, Cross and Boyer [21] claimed to have found a 'rapidly-phosphorylated membrane-bound mitochondrial protein', consistent with its participation as a phosphorylated intermediate; but this was later accounted for by ATP that was tightly (but not covalently) bound to the ATPase system [15,22]. It is therefore widely accepted at present that the reversible ATPase reaction does not involve covalent intermediates between POH and ATP, and that the fundamental chemical process catalysed by the reversible ATPases considered here is the interchange of the nucleophilic OH^- and $ADPO^-$ groups on the electrophilic phosphate phosphorus (or phosphorylium [23]) centre.

Nucleophilic substitution reactions at phosphate phosphorus centres are generally recognised as proceeding by way of pentavalent transitional intermediates, having trigonal bipyramidal geometry [24,25]. Classically, the attacking and leaving groups enter into and exit from the tetrahedral phosphorus centre via the long and weak apical bonds of the intermediary pentavalent trigonal bipyramid. Let us assume that the reversible ATPase reaction may proceed according to this classical scheme, as illustrated in fig. 1, where stages I to III represent ATP hydrolysis as a hydroxylation process, the H^+ of the H_2O from which the OH^- is derived in stage I being omitted for clarity. Incidentally, 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.

In the idiom of fig. 1, the fact that the hydrolysis equilibrium of eq. (1) normally lies far to the right is accounted for by the rule that, at electrophilic phosphorus centres, electron-releasing groups, such as OH^-, tend to form short strong bonds, whereas electron-withdrawing groups, such as $ADPO^-$, tend to form long weak bonds [25,26]. In this context it is particularly noteworthy that oxonium groups ($-OH_2^+$) are in the latter category, and are very good leaving groups.

It is one of our main objects to explain how the reaction of eq. (1) and fig. 1 may be poised towards ATP synthesis. A lowering of the pH in equilibrium with the reaction domain would not, as originally suggested by Williams [28], cause ATP synthesis be-

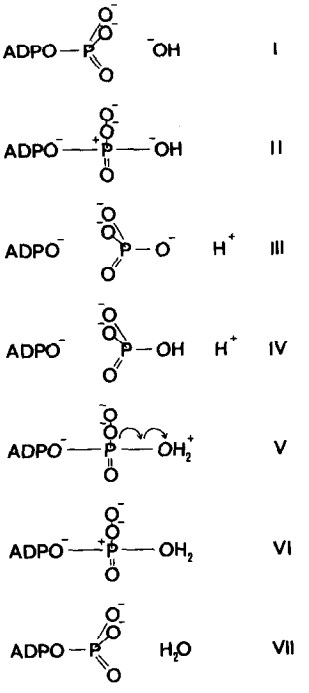


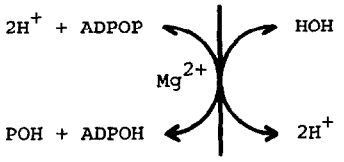
Fig. 1. Illustration of classical nucleophilic substitution mechanism for ATP hydrolysis (stages I to III) and ADP phosphorylation (stages III to VII), via the trigonal bipyramidal pentavalent transitional phosphorus configurations represented by stages II, V and VI. Stages III to IV and IV to V represent two specifically orientated protonations from a high total potential of H^+ on the right, while the left side of the reaction centre remains effectively in equilibrium with a relatively low potential of H^+. This proton motive field across the reaction centre favours ATP synthesis as indicated in stages V to VII and as explained in more detail in the text.

cause, as discussed subsequently by Williams [29], 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 of ATP is virtually pH-independent below pH 6 [23]. However, if, as illustrated in fig. 1, stages III to VII, the side of approach of the $ADPO^-$ (the left) were basic and electrically negative, while the other side of the phosphorus centre (the right) were acidic and electrically positive, the nucleophilic attacking capability of the unprotonated $ADPO^-$ group would be preserved, while the leaving capability of the $-O^-$ group would be enhanced by conversion to an oxonium group (stage V), and there would thus be

a poising effect in the direction of ATP synthesis. This effect may most simply be described as being due to a proton motive field across the reaction centre in the ATPase, positive on the right according to the conventions of fig. 1. In theory, the magnitude of the forces on the $ADPO^-$, OH^- and $P^+O_3^{2-}$ groups, tending to promote ATP synthesis in the ATPase, could presumably be given in terms of the components of the proton motive field strength. It is essential to note, however, that the poising effect of the proton motive field depends quantitatively on the total work done (reversibly) as the groups involved in the reaction migrate through the field along specifically determined pathways. Thus, our analysis of the ATPase mechanism must embrace osmotic as well as chemical specifications.

3. Osmotic mechanism

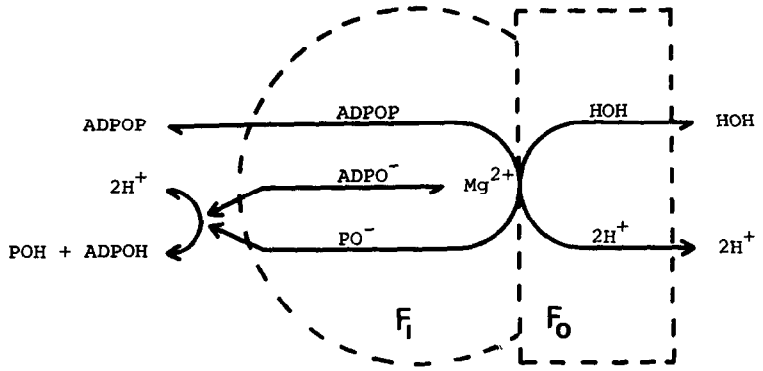
The complete ATPase systems of mitochondria, chloroplasts and bacteria have been found to catalyse a chemiosmotic reaction in which protons are translocated across the coupling membrane stoicheiometrically with ATP hydrolysis. The overall chemical and osmotic changes are conveniently represented by a two-dimensional type of equation in which the vertical line represents the membrane, and the double barb arrow heads represent the (purely formal) forward direction of the reaction, as follows:



(3)

The polarity of proton translocation relative to substrate accessibility, and the stoichiometry of 2 H^+ translocated per ATP hydrolysed, represented in eq. (3), have been observed experimentally in the ATPases from rat liver mitochondria, from ox heart mitochondria and from spinach leaf chloroplasts [30–33]; but some experimental data [34–36] indicate that the $\rightarrow H^+/P$ quotient for the ATPase from spinach leaf chloroplasts might be 3 or 4 rather than 2. The same ATPases have also been shown to catalyse ADP phosphorylation when proton translocation through them is caused by a proton motive potential difference of appropriate polarity [36–40], and this reversibility is indicated by the single barbs on the arrows in eq. (3).

In view of the lack of evidence for covalent intermediates, discussed above, it has been suggested that the osmotic process of proton translocation may be integral with the chemical process of group translocation by which ATP is reversibly hydrolysed in the ATPase system [14]. Thus, the specific osmotic pathway for proton translocation across the enzyme system may coincide (at least in part) with the cyclic translocation of one or more of the chemical groups involved in the reversible hydrolytic process. Of a



(4)

number of possible schemes that have been considered, the one represented in eq. (4) is especially interesting because it is compatible with the classical chemical mechanism of fig. 1.

This scheme is effectively that of eq. (3) at higher magnification, the membrane, shown as the vertical line in eq. (3), being represented by the ATPase components F_0 and F_1 in eq. (4). The lipophilic F_0 component, which is continuous with the lipid phase of the coupling membrane, has a specific proton-conducting pathway through it that can be blocked by oligomycin or dicyclohexylcarbodiimide, as discussed previously [14]. We assume that H_2O can also migrate through this or through a neighbouring pathway across F_0. The component F_1, which catalyses the hydrolysis of ATP, is known to close the proton-conducting pathway through F_0 in the intact F_0F_1 ATPase complex (see [14]). We assume, therefore, that the active centre is situated in the region of the interface between F_0 and F_1 so that the γ-phosphorus centre of ATP in the ATP-saturated enzyme (or the phosphorus centre of inorganic phosphate in the inorganic phosphate-saturated enzyme) becomes specifically accessible on the right side (but not on the left side) to the hydrogen ions that are at a relatively high potential in the aqueous phase on the right when an ATP-poising proton motive potential difference is applied across the coupling membrane.

According to eq. (4), the mechanism of ATP synthesis (in the direction of the single barbs) is conceived as follows: Inorganic phosphate (POH) and adenosine diphosphate (ADPOH), in a specific state of deprotonation corresponding at least to the monoanion forms represented by PO^- and $ADPO^-$, enter into a complex with F_1. After arrival at the active centre, the PO^- is protonated from the right side to give successively POH and POH_2^+, the reaction proceeding as in stages III to VII of fig. 1 to give ADPOP which is released from the F_1 in a specific state of deprotonation and salt formation carrying the same net cationic complement as the $PO^- + ADPO^-$ which entered the reaction (but with two less negative charges, as indicated). It is noteworthy that the translocation of $2\ H^+$ from the right to the left aqueous phase is achieved by the net transfer of O^{2-} from left to right across the ATPase, and it is this bivalent oxygen atom, derived from H_2O, that accounts for the two extra net negative charges in $PO^- + ADPO^-$ compared with ADPOP.

4. Thermodynamic balance of chemiosmotic forces

There are two obvious stages in the scheme of eq. (4) at which (following the direction of the single barbed arrows) the osmotic work available from the proton motive potential difference is transformed into the chemical work available from the phosphoryl group potential difference, according to the mechanism of fig. 1. One is the first stage of protonation of PO^- (fig. 1, stage IV) by which POH is osmotically trapped at an effectively high concentration in the active centre of the ATPase, whence it must not be able to escape in this protonated state. The other is the second stage of protonation (fig. 1, stage V), in which the tendency of the $-OH$ group to escape from the phosphoryl group is enhanced by its conversion to an oxonium group, $-OH_2^+$. As, under conditions of chemiosmotic equilibrium, the free energy changes are given by the products of the forces and co-linear displacements of the particles, integrated along the specific translocational pathways, this simple type of mechanism allows us to conceive the balance of osmotic and chemical forces that corresponds to the more abstract thermodynamic energy balance described by the equation:

$$2\,\Delta p + \Delta G' = 0 \qquad (5)$$

where Δp stands for the total proton motive potential difference across the coupling membrane, positive on the right, in eq. (3) or (4), and $\Delta G'$ stands for the free energy of hydrolysis of ATP, under given (not standard) conditions.

5. Concluding remarks

The discussion of the proton-translocating ATPases in this paper is primarily intended to define the general principle of a feasible type of molecular mechanism. Certain variations or sophistications of this type of mechanism are obviously possible. The following are three examples. If the passage of inorganic phosphate into and out of the F_1 complex were specific for a more deprotonated species, represented by PO^{2-} or PO^{3-} in the symbolism of eq. (4), a $\rightarrow H^+/P$ quotient of 3 or 4 instead of 2 might be accounted for. In the mechanism of ATP synthesis represented by eq. (4), the second stage of protonation (fig. 1, stage V) might

effectively occur by the escape of OH^- in a process corresponding to stages II and I, respectively, of fig. 1, followed by protonation of the OH^- ion after leaving the phosphorus centre. The relatively rapid ^{18}O exchange observed between POH and HOH, when the ATPase reaction is poised, might imply that the hydrolytic process involved a mechanism including a pseudorotation about the phosphorus centre, as suggested by Korman and McLick [13]. Pseudorotation could occur without change in the fundamental mechanism of interaction between the chemical and osmotic forces acting along the specific pathways through the anisotropic active centre, as considered in this paper. The only differences might be in the relative directions of the pathways of the attacking and leaving groups at the phosphorus centre and in the number of –OH groups susceptible to protonation in equilibrium with the phase on the right, according to the formality of eq. (4).

It is noteworthy that the symbols ADPOP, $ADPO^-$ and PO^- in eq. (4) represent the adenine nucleotides and inorganic phosphate in specific states of protonation and salt formation obligatory for entry to and exit from the active centre of the ATPase. However, the symbols do not show what these states are, but only that there is one more oxygen atom and two more electrons in $ADPO^- + PO^-$ than in ADPOP. Thus, for example, it is conceivable that ADPOP, $ADPO^-$ and PO^- might travel as the electrically neutral K_2Mg-salt, the K_2-monoanion and the Mg-monoanion respectively. Ionic groups that are components of the ATPase protein or lipid might also or alternatively be involved in the species represented by ADPOP, $ADPO^-$ and PO^-

Apart from its intrinsic value as a basis for understanding and correlating various aspects of the present knowledge of the proton-translocating ATPases, the type of theoretical view of the ATPase mechanism described in this paper provides a useful basis for further experimental exploration. For example: In the absence of ATPase activity, information about the ATPase mechanism may be obtained by observing cation, acid/base and electric charge entry or exit across the membrane in concert with movements of inorganic phosphate and ATP or their analogues into or out of the active centre of the ATPase. Since the ATPase exhibits a considerably higher apparent affinity for inorganic phosphate during ATP synthesis in the poised state than during ATP hydrolysis in the unpoised state, a study of the apparent phosphate binding coefficient as a function of Δp and of the pH values on either side of the membrane should help to shed light on the phosphorylation mechanism.

Acknowledgements

I would like to thank my research colleague Dr. Jennifer Moyle for helpful discussion, and Mrs. Stephanie Key and Mr. Robert Harper for expert assitance in preparing the manuscript and figures. I am also indebted to Glynn Research Ltd for general financial support.

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