used. These results suggest that uncoupled ATPase activity of the membrane-bound enzyme, like that of the isolated enzyme, does not involve the bound nucleotides, although coupled ATPase activity does involve these nucleotides in addition to the 'ATPase' site of the enzyme. By using ATP analogues, the specificity of the 'tight binding' and 'ATPase' sites can be compared with the specificity of the phosphorylation site. It is concluded that the coupled ATPase and phosphorylation utilize the same (four) nucleotide-binding sites, although the uncoupled ATPase involves only the single 'ATPase' site. It appears unlikely that phosphorylation and ATPase activity take place at entirely different sites on the enzyme, as has been suggested (Pedersen, 1975).

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Some Aspects of Adenosine Triphosphatase Mechanisms

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A good deal of evidence indicates that the energy-coupling ATPases* of the bacterial plasma membrane, the chloroplast thylakoid membrane and the inner mitochondrial membrane comprise a closely related family. These ATPases differ from the ouabain-sensitive (Na+ + K+)-dependent ATPase, the Ca2+-dependent ATPase and the myosin ATPase. Other, less well-known, ATPases may form further distinct classes, although it is of interest to note that the ATPase of the chromaffin-granule membrane may resemble the mitochondrial ATPase in some respects (Bashford et al., 1976; Casey et al., 1976).

There has always been an expectation that the energy-coupling ATPases might share common mechanistic features with the ion-transport ATPases, and this view may seem more attractive now that the energy-coupling ATPases are considered to be H+-pumping enzymes. Hence the evidence for a phosphoenzyme intermediate in (Na+ + K+)-ATPase and Ca2+-ATPase continues to generate an expectation that a similar intermediate will eventually be found for the energy-coupling ATPases (Racker, 1975). However, evidence from isotope-exchange reactions, especially the requirement of ADP for the Pi + H2O exchange in submitochondrial particles (Jones & Boyer, 1969), suggests that energy-coupling ATPases do not catalyse their reaction via a phosphoenzyme intermediate. It is pertinent though to enquire as to what advantages may accrue from an ATPase mechanism that involves a phosphoenzyme intermediate, and to ask why apparently different mechanisms for transferring energy to or from ATP have developed.

* Abbreviation: ATPase, adenosine triphosphatase.
For most enzymes in which a covalent enzyme–substrate intermediate has been identified, facilitation of catalysis may derive from the intermediate mechanism providing a pathway that splits a large activation step into two smaller ones, although Bell & Koshland (1971) in surveying this general problem did not discern a general pattern. A phosphoenzyme intermediate in an ATPase mechanism might be advantageous in that a functional group on the enzyme can act as a nucleophile towards ATP, which may obviate the need to generate high local concentrations of H+ or OH−. If though ATP hydrolysis proceeds via a dissociative mechanism:

\[
\begin{align*}
&\text{RO−P−O−} \rightarrow \text{RO−P−O−} \to \text{ROH} + \text{O=PO} \\
&\text{it is difficult to see what catalytic advantage is obtained by going via a phosphoenzyme.}
\end{align*}
\]

The difficult step in the above scheme is presumably the formation of the metaphosphate ion. As attack on this ion is facile, there seems little catalytic advantage in forming the phosphoenzyme [an acyl phosphate in (Na++K+)-ATPase and Ca2+-ATPase] from the metaphosphate. This consideration might therefore indicate that, where a phosphoenzyme intermediate is documented, ATP hydrolysis occurs via an S_n2 mechanism with a carboxyl group of the enzyme attacking the γ-phosphate group via a five co-ordinate phosphorus intermediate. An alternative role for a phosphoenzyme intermediate is that its formation is a necessary step for energy conversion rather than for catalysis. For instance a phosphorylated intermediate may provide a device for releasing energy from ATP in more than one step or may serve a temporal role if two processes dependent on energy from ATP must occur sequentially.

Analysis of a variety of isotope-exchange reactions is a major technique in the study of energy-coupling ATPases. It is useful to note that these exchange reactions, which are particularly associated with the ATPase in submitochondrial particles, may be quite different from the usual type of enzymic partial reaction. The exchange reactions in submitochondrial particles may represent activities of individual ATPase molecules or they may reflect an activity that is associated with all the ATPase molecules in a membrane. For example the rate of exchanges might depend on the magnitude of the delocalized protonmotive force across the membrane, and if the exchanges are measured under conditions of net ATP hydrolysis then all the ATPases on a given membrane particle will be involved in generating the protonmotive force. An important development has come from the work of Rosing et al. (1977), who have shown that an uncoupler-insensitive component of the H218O exchange in submitochondrial particles is an exchange at the active site (intermediate exchange), and therefore indicative that the condensation of ADP and P_i at the active site is not dependent on energy input from the membrane. This would be analogous to the myosin ATPase, in which ATP, ADP and P_i are close to equilibrium at the enzyme active site (Trentham et al., 1976). A curious feature of the isolated mitochondrial ATPase, which is contrary to what might be expected if this exchange is diagnostic of an essentially energy-independent catalytic step in the enzyme reaction. A further intriguing point about isotope-exchange reactions is that they are much less prominent in the chloroplast and some bacterial systems (Shavit et al., 1967; Ferguson et al., 1975a,b, 1976b). This may indicate that the ATPase in these systems is less freely reversible than the mitochondrial ATPase as discussed elsewhere (Ferguson, 1977).

Several pieces of evidence now indicate (but do not prove) that the energy-coupling ATPases may function via a 'half (or fraction of) of the sites' mechanism in which there is a compulsory relationship between two or more catalytic centres in an enzyme, so that both centres cannot be catalysing the same part of a catalytic sequence simultaneously (Levitski & Koshland, 1976). Ferguson et al. (1975a,b, 1976b) showed that modification of a single tyrosine or lysine residue in both the isolated and membrane-bound
mitochondrial ATPase was sufficient to abolish activity. This indicates that there is
asymmetry of behaviour between otherwise identical polypeptide chains of the enzyme
(Ferguson et al., 1975b). Kayalar et al. (1977) have proposed a dual catalytic-site sequence
for the ATPases as a result of their studies on substrate-binding patterns and exchange-
reaction inhibition. Adolfsen & Moudrianakis (1976) also suggested a flip-flop model
on the basis of a study of nucleotide binding to the ATPase. The advantage to an enzyme
of a half of the sites mechanism is not always clear, but in the case of energy-coupling
ATPases the following suggestions may be made. Repke & Schon (1974) have advocated
a half of the sites model seemingly on the basis that such a mechanism would tend to
minimize the free-energy changes at each stage of a coupling reaction and thus promote
efficient energy coupling. An alternative view (Ferguson et al., 1975c) suggested that a
half of the sites mechanism would allow the ATPase to dissipate an energy pressure
continuously, in contrast with enzyme with independent catalytic sites which would be
unable to operate in this way. Similarly Kayalar et al. (1977) have pointed to the
attractive feature of a symmetry of catalytic sites than can be obtained with a half of
the sites mechanism. A further important feature of the model of Kayalar et al. (1977)
is that it allows a single step of energy input to accomplish changes in affinities of all
substrates.

In conclusion one might speculate that in the energy-coupling ATPases intersubunit
interactions play a role in synchronizing energy coupling, whereas in the ion-transport
ATPases a phosphoenzyme intermediate is important in this respect.

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The Role of Lipoic Acid in Adenosine Triphosphatase Synthases

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A variety of ATP synthase complexes have been prepared from mitochondrial bacterial
and chloroplast preparations. These have been labelled as F₁–F₀ complexes (based on the
terminology of Racker, 1970) composed of the dissociable F₁-ATPase* and the mem-
brane-bound component, F₀.

* Abbreviation: ATPase, adenosine triphosphatase.

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