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Minireview

Kinetic Studies of ATP Synthase: The Case for the Positional Change Mechanism

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The mitochondrial ATP synthases shares many structural and kinetic properties with bacterial and chloroplast ATP synthases. These enzymes transduce the energy contained in the membrane's electrochemical proton gradients into the energy required for synthesis of high-energy phosphate bonds. The unusual three-fold symmetry of the hydrophilic domain, F_1, of all these synthases is striking. Each F_1 has three identical β subunits and three identical α subunits as well as three additional subunits present as single copies. The catalytic site for synthesis is undoubtedly contained in the β subunit or an α, β interface, and thus each enzyme appears to contain three identical catalytic sites. This review summarizes recent isotopic and kinetic evidence in favour of the concept, originally proposed by Boyer and coworkers, that energy from the proton gradient is exerted not directly for the reaction at the catalytic site, but rather to release product from a single catalytic site. A modification of this binding change hypotheses is favored by recent data which suggest that the binding change is due to a positional change in all three β subunits relative to the remaining subunits of F_1 and F_0 and that the vector of rotation is influenced by energy. The positional change, or rotation, appears to be the slow step in the process of catalysis and it is accelerated in all F_1F_0 ATPases studied by substrate binding and by the proton gradient. However, in the mammalian mitochondrial enzyme, other types of allosteric rate regulation not yet fully elucidated seem important as well.

KEY WORDS: ATP synthase; F_1F_0 ATPase; energy transduction.

INTRODUCTION

Mitochondrial ATP synthesis in eukaryotic cells depends on respiration as a driving force and is catalyzed by the proton-driven ATP synthase. This multisubunit complex can easily be split into two subcomplexes, one termed F_0, which spans the mitochondrial inner membrane and another, F_1, which is attached to F_0 by a thin stalk on the inner side of the membrane. F_1 is the site of catalysis, whereas F_0 is responsible for transducing the energy from the mitochondrial membrane's proton electrochemical potential gradient to F_1. Despite massive efforts over the last 30 years and quite detailed knowledge of the enzyme structure (Amzel and Pedersen, 1983, Lucken *et al.*, 1990), it is still unclear how the transduction machinery works. Also, attempts to understand how the enzyme controls the efficiency of the process of ATP synthesis, gearing its activity precisely to the needs of the cell for ATP, and to the supply of energy derived from electron flow, have not yielded clear answers. Nevertheless progress made in the last few years has narrowed the possibilities and sharply focused the questions asked. The purpose of this review is to describe recent advances from the particular perspective of the authors.

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CONTROL OF MITOCHONDRIAL ATP SYNTHESIS

Sensitive control of the rate of synthesis of ATP in isolated mitochondria can be demonstrated when the substrates of the synthase are varied in the physiological range (Lardy and Wellman, 1952; Chance and Williams, 1955). The substrates include not only phosphate and ADP but also the electrochemical potential gradient of protons (Mitchell, 1966). Extramitochondrial protons are substrates, while intramitochondrial protons are products of the synthase reaction. The correlation between the rates of mitochondrial ATP synthesis and ATP utilization for cellular processes in intact cells can most simply be explained by the proposal that cytosolic ADP and P_i (products of cellular utilization of ATP), regulate cellular synthesis of ATP (Lardy and Wellman, 1952; Chance and Williams, 1955). However, in many cells, and in the adult mammalian heart, in particular, it has not been possible, even with the most sophisticated tools, to detect changes in ADP or phosphate which can account quantitatively for observed changes in ATP synthesis and respiration (Katz *et al.*, 1989).

For many years, a popular theory for the control of respiration was the near equilibrium hypothesis (Wilson *et al.*, 1974). This would have explained the inadequate changes in ADP and phosphate on the basis that the ATP synthase catalyzes a reaction close to equilibrium, with the forward and back reactions very rapid relative to net flux through the system. Thus a very small change in the mass action ratio away from the equilibrium position would cause a relatively large change in the difference between forward and reverse reactions and a relatively large increase in net flux. Using isolated rat liver mitochondria, it was demonstrated by titration of the oxidative phosphorylation system with specific inhibitors that at least some components of the system operate under conditions far from equilibrium (Kuster *et al.*, 1981). The component with the high degree of control was the one which transports nucleotides across the mitochondrial membrane (Tager *et al.*, 1983; Groen *et al.*, 1982). The adenine nucleotide translocase seems to have a regulatory role (i.e., operates far from equilibrium) in isolated hepatocytes (Duszynski *et al.*, 1982).

The possibility that the phosphorylation system is close to equilibrium with the electron transport chain between NADH and cytochrome oxidase has been made even less tenable in recent years. Measurements of the forward and back reaction of ATP synthesis in intact heart, kidney, and brain tissue indicate that these rates are not rapid in comparison with net mitochondrial ATP synthesis (Matthews *et al.*, 1981; Kingsley-Hickman *et al.*, 1987; Freeman *et al.*, 1983; Shoubridge *et al.*, 1982). The technique of ^{31}P-nuclear magnetic resonance saturation transfer in intact tissues reveals that the forward reaction is not rapid relative to net flux. These observations were confirmed and expanded using isolated liver and heart mitochondria (LaNoue *et al.*, 1986). Net ATP synthesis was measured over a wide range from minus 0.2 to plus 2.0 μmol/min/mg by varying the supply of ADP or by inhibiting electron flow in the presence of excess ATP. At the same time, the unidirectional forward and reverse reactions were measured isotopically using radiolabelled inorganic phosphate.

The values obtained with isolated heart mitochondria were in good agreement with the NMR measurements in intact hearts showing that the forward and reverse reactions were not rapid relative to net flux. The back reaction in particular seemed to be acutely sensitive to inhibition by ADP. Remarkably, both the forward and the back reaction were slowed when the electron transport chain was blocked in the presence of excess ATP.

To determine whether the adenine nucleotide translocase which transports ADP into the mitochondria in exchange for ATP or the ATP synthase itself was the slow step in the overall process, studies were conducted to assess the synthase flux independent of the translocase and vice versa. The back reaction of the ATP synthase was estimated during net ATP synthesis independent of the translocase by assessing ^{16}O incorporation into $^{18}O_4$-inorganic phosphate (Berkich *et al.*, 1991). Inside the mitochondria the synthase was found to have maximal capacity similar to the maximal rate of mitochondrial ATP synthesis. With saturating ADP and phosphate the synthase inside the mitochondria proceeds 90% in the forward direction.

Other studies assessed flux through the translocase independent of the synthase in intact liver mitochondria supplied with adequate levels of respiratory substrates, oxygen, and millimolar levels of external ATP and inorganic phosphate (Masiakos *et al.*, 1991). The measurement was carried out using the technique of ^{31}P-NMR saturation transfer rather than an isotopic method. The technique is better suited for measurement of rapid flux through the small, rapidly turning over intramitochondrial ATP pool than radioisotopic methods. The data indicated that flux

through the translocase is an order of magnitude faster than the ATP/P_i exchange of liver mitochondria in state 4. The V_{max} of the translocase is an order of magnitude higher than the V_{max} of the synthase but under some conditions of limiting substrate, translocase may become rate limiting. This means that the kinetic properties and maximal capacity of the ATP synthase can determine rates of ATP synthesis.

As stated above, no change in ADP or phosphate can be detected when cell respiration increases in the heart. Creatine kinase may exist in separate mitochondrial, myofibrillar, and cytosolic compartments and it may speed transmission of the signal of ATP hydrolysis from myofibrils to mitochondria (Saks *et al.*, 1978). However, it is difficult to understand how the signal could be transmitted through the bulk of the cytosol without being detected as a decrease in bulk creatine phosphate. These observations have prompted speculation that ATP synthesis may be controlled by the rate of supply of energy to the electron transport chain which in turn may be controlled by cytosolic free Ca^{2+} levels.

Since Ca^{2+} initiates many energy-requiring cellular processes such as muscle contraction, neurotransmitter secretion in nervous tissue, and gluconeogenesis in the liver, it seems an ideal intracellular signal. Ca^{2+} in the low physiological range (0.2–1.0 μM) regulates critical citric acid cycle enzymes, pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and isocitrate dehydrogenase (McCormack *et al.*, 1990; Jakobus, 1985; Gunter and Pfeiffer, 1990; Hansford, 1991). Therefore, in theory, Ca^{2+} could simultaneously stimulate the process of ATP synthesis by increasing $\Delta\mu_{H+}$ while initiating processes utilizing energy. According to this "push" hypothesis, ATP synthesis would increase in response to an increase in available energy even in the absence of increased ADP and phosphate. Evidence in favor of the "push" hypothesis has been obtained. Katz *et al.* (1987) have shown that the surface fluorescence of the intact perfused rat heart at 340 nm excitation is due to mitochondrial NADH. Calibrating this signal with an internal dye, they have found that mitochondrial NADH increases slightly with increasing work in glucose-perfused rat hearts as would be expected if increased Ca^{2+} were stimulating mitochondrial dehydrogenases. The effect, however, is rather small, subject to a variety of artifacts, and has not been confirmed.

In an effort to clarify the situation, our laboratory (Wan *et al.*, 1992) has recently measured the electrical component of $\Delta\mu_{H+}$ ($\Delta\Psi$) in intact perfused rat hearts using three different substrates at three workloads, causing respiration to change over a fourfold range.

We found, as others have (From *et al.*, 1990), that phosphate was lower and creatine phosphate and ATP higher in pyruvate-perfused hearts than in glucose-perfused hearts. The measured values of $\Delta\Psi$ were also higher in the pyruvate-perfused hearts. The values of $\Delta\Psi$ were estimated from kinetic estimates of the equilibrium distribution of the lipophillic cation tetraphenylphosphonium (TPP^+) between the perfusate and the cardiac tissue. Increased ATP utilization was accompanied by no change in high-energy phosphate levels but a steady decline in $\Delta\Psi$ with respiration rate. This is in agreement with previous observations (Kauppinen, 1983). This renders the push hypothesis unlikely. It is an especially intriguing dilemma since the ^{18}O isotope exchange data suggest that the capacity for flux through the synthase is low, making it, rather than the translocase, the rate-limiting step. Therefore, previously unappreciated nonsubstrate factors may control the synthase and these may be of prime physiological importance in the control of respiration, especially in adult mammalian heart. The possibility that these factors are small regulatory proteins influenced by Ca^{2+} is supported by data reviewed by Harris and Das (1991). These recent findings emphasize the importance of understanding how the machinery of the ATP synthase works, how its structure relates to function, and which of the steps in the process of ATP synthesis are slow and influenced directly by $\Delta\mu_{H+}$.

RELATIONSHIP BETWEEN STRUCTURE AND FUNCTION OF THE MITOCHONDRIAL ATP SYNTHASE

The details of the multisubunit structure of various ATP synthase isozymes from plants and bacteria to mammals has been reviewed in this journal (Pedersen, 1992) and elsewhere (Lucken *et al.*, 1990; Tiedge and Schafer, 1989; Graber *et al.*, 1988; Amzel and Pedersen, 1983). The hydrophylic catalytic domain, F_1, is composed of three large β subunits, three large α subunits, and single copies of subunits γ, δ, and ε. The significance of the vectorial orientation of the synthase through the mitochondrial membranes was

not fully appreciated until about 15 years ago. The award of the Nobel Prize to Peter Mitchell marked general acceptance of the notation that energy is supplied to the synthase in the form of an electrochemical potential gradient of protons. When the gradient is high (150–200 mV), ATP is synthesized but when it is low, ATP is hydrolyzed, building $\Delta\mu_{H+}$.

In early formulations of the chemiosmotic hypothesis, Mitchell proposed that the proton gradient was sensed directly by the catalytic site in F_1, that the site was in a "proton hole" where the imposed proton gradient could facilitate removal of the elements of water (H^+ and OH^-) in opposite directions across the membrane, and away from ADP and phosphate to form ATP (Mitchell, 1966, 1974, 1979, 1985). The formulation required that the stoichiometry of protons to ATP synthesized be 2. It was soon clear, however, that at least 3 H^+'s are transported for each ATP synthesized (Alexandre *et al.*, 1978). Originally, Boyer and coworkers (1975a,b) were the most vigorous opponents of the concept that the $\Delta\mu_{H+}$ interacts directly with F_1 at the catalytic site. They proposed an alternate scheme whereby the input of energy did not facilitate formation of the covalent P–O bond but rather caused a change in the binding constant of ATP (Boyer *et al.*, 1973). The binding change mechanism is supported by impressive experimental evidence which continues to accumulate (Cross *et al.*, 1982; Adolfsen and Moudrianakis, 1976; Labahn *et al.*, 1990). The original hypothesis was formulated on the basis that the exchange of water oxygen with ^{18}O-labelled phosphate catalyzed by the ATP synthase is far less sensitive to decreases of $\Delta\mu_{H+}$ than the isotopic exchange of medium ATP and phosphate (Rosing *et al.*, 1977). Both processes are dependent on the making and breaking of the ATP γ-phosphate–oxygen covalent bond, but the ATP/P_i exchange is additionally dependent on release of ATP from the tight catalytic site. Another piece of compelling evidence in favor of the binding change was the observation that although at least six binding sites for adenine nucleotides exist on F_1, incubation of submitochondrial particles or of isolated F_1 with ADP and phosphate results in formation of ATP at a single tight site (Fromme and Graber, 1990). This bound ATP cannot be removed by incubation with a hexokinase trapping system or by extensive washing. Its formation does not require energy. Subsequent studies by Smith *et al.* (1976), Penefsky (1985), as well as others have confirmed and expanded these observations showing importantly that the tight site is catalytically competent (Grubmeyer and Penefsky, 1981; Kozlov *et al.*, 1985) unless jammed with ADP and Mg^{++} in the absence of phosphate (Kohlbrenner and Cross, 1978).

Kinetic studies of the details of the binding change mechanism have provided new constraints on the mechanism of catalysis and the involvement of $\Delta\mu_H{}^+$ in the process of ATP synthesis. In investigating the dependence of the phosphate/water exchange in submitochondrial particles on the concentration of external nucleotides, some revealing observations were made. The release of ATP from the catalytic site following exchange with water oxygens is dependent not only on the presence of a continuously regenerated $\Delta\mu_{H+}$ but also on the presence of external ADP and phosphate (Hackney *et al.*, 1979). Conversely, the release of phosphate from the tight site following phosphate/water exchange does not require $\Delta\mu_{H+}$ but does require the presence of external ATP (Hackney, 1984; Berkich *et al.*, 1991). This is a remarkable observation since phosphate/H_2O exchange should, in principle, require only bound nucleotide. By a variety of techniques it could be demonstrated that external nucleotides induce the required conformational changes when they bind to the enzyme at a site separate from the one which contains the very tightly bound nucleotides (Grubmeyer and Penefsky, 1981; Cross *et al.*, 1982). The dependence of the release of phosphate on external ATP concentrations suggested the presence of one low K_m process and a higher K_m process (O'Neal and Boyer, 1984; Rosing *et al.*, 1977). Simultaneous measurements of the incorporation of water oxygens into the released phosphate showed that the release of phosphate from the catalytic site depended on the higher ATP K_m, but the rate of breaking and formation of the covalent bonds within the tight site depends only on entry of ATP into the catalytic site, which is dependent on the lower ATP K_m.

These data may be viewed in two ways; one suggests that the nucleotides which promote release of products from the catalytic sites are bound at noncatalytic regulatory sites. The other suggests that the binding site which promotes release subsequently becomes the catalytic site. The beta subunit of F_1 (or an α–β interface) encompasses the active catalytic site and since the α and β subunits are present on F_1 in three identical copies, it stands to reason there are three catalytic sites, all of which have at least latent capacity for activity.

According to this alternating catalytic site hypothesis, one beta subunit contains the occluded (or very

tight-binding site) where synthesis or hydrolysis of ATP takes place. Binding of nucleotide to one of the more exposed sites then promotes release of product from the tight site, and the subunit with the newly bound nucleotide then becomes the tight site. Recent reports, however, that recombinant alpha and beta subunits from thermophilic bacteria can be reconstituted as alpha/beta heterodimers and that these exhibit high ATPase catalytic activity argue against the alternating catalytic site model (Harada *et al.*, 1991). Nevertheless, further studies with this reconstituted heterodimer need to be carried out to demonstrate that the mode of catalysis is similar to that seen in normal F_1 and to confirm that the presumed alpha/beta heterodimer had not polymerized to the more stable α_3, β_3 hexamer (Kagawa *et al.*, 1989; Miwa and Yoshida, 1989).

Recent data from our own laboratory have used highly coupled intact rat heart mitochondria to test the alternating catalytic site hypothesis. Since our previous data had indicated that the translocase does not influence the rate of exchange of ATP and P_i in intact mitochondria, we were able to carry out a number of experiments in which the rates of the partial reactions of ATP synthesis and hydrolysis were measured as a function of the concentrations of ADP, Pi, ATP, and $\Delta\mu_{H+}$ (Berkich *et al.*, 1991). The magnitude of $\Delta\mu_{H+}$ could be varied over a much wider range in these experiments than in previous studies using submitochondrial particles. Definitions of these partial reactions and the methods used to make the measurements have been published in detail. Briefly, the rates of incorporation of ^{32}P radiolabelled P_i into ATP (V_1), incorporation of gamma ^{32}P-ATP into P_i (V_2), release of phosphate from the ATP synthase catalytic site as measured by incorporation of water oxygen into phosphate (V_3) and the rate of formation of the covalent P–O bond in the catalytic site (V_4) have been measured. Also, in as yet unpublished work, we have assessed V_5, the release of ATP from the catalytic site as measured by the rate of disappearance of the $^{18}O_4$ isotopomer of the γ phosphate of ATP from the reaction media.

The data taken as a whole demonstrate that regulation of product release by nucleotide binding is due to binding at a catalytic site, not a regulatory site. The pertinent observations are as follows. The rate of ATP/P_i exchange (V_1) measured in the absence of added ADP is dependent on external concentrations of ATP and P_i, but follows a strict 1 : 2 stoichiometry with P_i/HOH exchange (V_3).

There is no *a priori* reason why, if exchange occurs with participation of only a single catalytic site, the concentration of ATP should affect the P_i/H_2O exchange since the nucleotide, which participates in the reaction, can remain bound to the enzyme. P_i/H_2O exchange at a single site is theoretically independent of ATP binding and release. If P_i release is dependent on ATP binding at a regulatory site, the dependence of P_i release on ATP should be different from the dependence of ATP/P_i exchange on ATP. We observed a constant 1 : 2 ratio of ATP/P_i exchange to the rate of disappearance of the $^{18}O_4$–P_i isotopomer from the media while media ATP and phosphate concentrations were varied widely and when temperature changed from 37 to 15°C. The dependence of phosphate release on ATP binding at a second catalytic site was even more strikingly illustrated in a recent but as yet unpublished experiment using rotenone-inhibited mitochondria. It had been shown previously that rotenone-inhibited mitochondria incubated with 5 mM ATP and 5 mM phosphate have a slightly lower $\Delta\mu_{H+}$ but much lower ATP/P_i exchange than mitochondria in state 4 (without rotenone and with glutamate) (LaNoue *et al.*, 1986). The partial reactions were recently assessed in this situation to determine the site of inhibition of ATP/P_i exchange when electron flow is low. The rate of phosphate entry into the catalytic site was the same as in state 4; the rate of P_i release as measured by the disappearance of $^{18}O_4$—isotopomer of phosphate was slightly faster. Importantly, the rate of ATP entry into the catalytic site (assessed from ATP/HOH exchange) was identical to the rate of P_i release as judged from V_3, but both these partial reactions were approximately three times faster than the rate of ATP/P_i exchange. Explaining these data in terms of a single site would be very cumbersome.

Another very revealing feature of the data obtained with intact mitochondria was the constancy of the rate of catalysis in the tight site (V_4), the rate of covalent phosphate bond turnover in the catalytic site. At a fixed temperature of 37°C, this rate was independent of the external ratio of ATP/ADP, of net flux through the system, and also of the value of $\Delta\mu_{H+}$.

When the mitochondria were incubated with saturating ADP and phosphate, and a hexokinase trap was employed to lower media ATP levels and measure the ATP produced, V_3, the release of P_i from the catalytic site was very slow. When various concentrations of dinitrophenol were used to lower $\Delta\mu_{H+}$ stepwise from 117 to 50 mV, net ATP synthesis decreased

from 3.2 μmol/min/mg of mitochondrial protein to one-tenth of this rate. However, at each concentration of dinitrophenol studied the rate of formation of the high-energy phosphate bond within the catalytic site was constant at about 11 μmol/min/mg. Also, the rate of P_i released from the catalytic site remained constant and slow (0.3 μmol/min/mg). These data are also very difficult to explain assuming a single catalytic site. However, as noted by others, the binding change catalytic model also falls short of adequately explaining the influence of $\Delta\mu_{H^+}$. In the presence of a high $\Delta\mu_{H^+}$ and adequate ADP and phosphate, the synthase binds ADP and P_i to the exposed catalytic site clearly in preference to ATP, and releases ATP from the occluded site. If ADP binding to the exposed catalytic site simply opens the occluded site, why is only ATP released, not phosphate and ADP, when the occluded site holds both in about equal amounts as shown by Grubmeyer *et al.* (1982). Conversely, at low $\Delta\mu_{H^+}$, ATP binds and opens the catalytic site releasing only ADP and P_i. A simple mechanical model can be drawn which will account for these data as shown in Fig. 1.

The model is in most respects similar to the rotational catalytic model suggested by Boyer (1989) in a recent review. However, our model emphasizes more strongly than previous models the likelihood that the position of the three subunits relative to the α's and to the single copy γ, δ, and ε subunits determines their kinetic characteristics. Therefore, we have renamed this the positional change mechanism. In the model shown, the occluded site is drawn not as a change in conformation of the β subunit when the first nucleotide binds, but rather as preexisting because of the special environment of one of three β's which shelters the catalytic site from the external media. The shelter drawn in blue in the cartoon is not part of the β subunit, but is envisioned as provided in some way by the environment. The "shelter" could be due to a Hatispecial interaction between an α and a β subunit. Nucleotides can only enter the occluded site through special entryways after they bind to exposed sites on one of the two exposed β's. These two exposed β's are not identical because they are also exposed to different environments. As drawn, ADP and P_i can enter the occluded site after binding to the subunit to the right of the occluded site, whereas ATP can only enter after binding to the one on the left. Exit from the occluded site is similarly constrained. Formation and hydrolysis of the phosphate bond, occurring exclusively in the occluded site, is represented schematically as rotation of a "turntable." Each 180 degree turn represents either formation or breakage of the covalent bond. The data show that it turns at a constant rate at a given temperature. Figure 1 also illustrates why decreases in $\Delta\mu_{H^+}$ do not increase the K_m for ADP and phosphate (Matsuno-Yagi and Hatefi, 1986) nor decrease the K_i of ATP for ATP synthesis (Perez and Ferguson, 1990) as one might anticipate from a binding change mechanism. Energy may not change the binding constants of the nucleotides for the exposed subunit sites, but instead $\Delta\mu_{H^+}$ may facilitate the change of position of the β subunits, relative to their environment.

Input of energy is only needed to move the β subunits in the counterclockwise direction; no energy is needed to rotate the catalytic site turntable. This model explains the following observations. Only external ADP and P_i promote the release of ATP from the catalytic site. Only ATP promotes release of ADP and P_i. Neither the rate of formation nor the rate of hydrolysis of the phosphate bond in the catalytic site is affected by concentrations of external substrates. However, the concentrations of external substrates including $\Delta\mu_{H^+}$ determine direction and rate of the overall reaction. The suggestion has been made, based on the fact that some inhibitors can affect hydrolysis more profoundly than synthesis, that separate catalytic sites exist for these two processes (Schwerzmann and Pedersen, 1986; Yamada and Huzel, 1989). Subsequent studies showed, however, that synthesis and hydrolysis follow a single catalytic pathway (Stroop and Boyer, 1985; O'Neal and Boyer, 1984; Berkich *et al.*, 1991), but most probably the binding site of ATP for ATP hydrolysis is distinct from the site of ADP, for ATP synthesis.

An alternative scheme which may fit the present data is one in which the asymmetry of the β subunits is imposed by binding the first nucleotide. Then binding to the second subunit might induce the release of nucleotides from the first. One would have to build into this model two different modes of product release from the occluded site, one involving use of the protonmotive force, binding ADP and P_i at a second catalytic site and inducing ATP release. This should occur without affecting the rate of ATP synthesis relative to breakdown within the catalytic site. The second mode would involve generation of $\Delta\mu_{H^+}$, ATP binding to a catalytic site followed by ADP and P_i release at another. Although it is possible to build a model (or models) with these constraints, the design is clearly more cumbersome than the positional change mechanism depicted in Fig. 1 where simple conversion

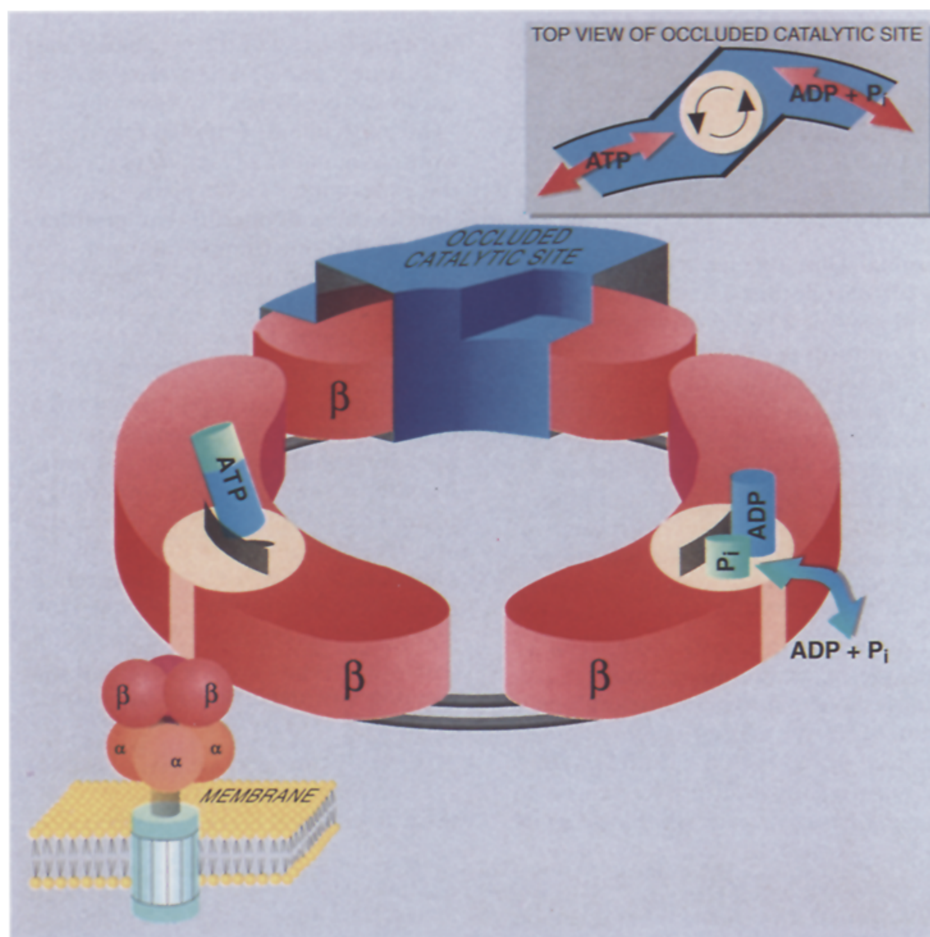

Fig. 1 Schematic representation of the position change mechanism of ATP synthase. In the lower left corner is shown the arrangement of the subunits of chloroplast ATP synthase with suggested subunits sizes based on electron microscopy peformed by Graber *et al.* (1988). The cartoon in the middle is meant to illustrate features of the mechanism discussed in the text. The diagram in the upper right is a top view down through the "blue" occlusion.

of the vectorial protonmotive force to a vectorial movement of β subunits relative to the α, γ, δ, and ε subunits produce the media ATP. Definitive solutions, however, to the problem of the relationship of structure to function will depend finally on high-resolution three-dimensional analysis. Data obtained recently (Gogol *et al.*, 1990) using the *E. coli* enzyme indicate that an asymmetric structure observed in F_1 may rotate during catalysis. After reaction with fragments of monoclonal antibodies to α and ε subunits, the γ subunit can also be identified as a central density using the technique of cryoelectron microscopy. These workers found that the observed spatial relationship of the γ subunit to the β subunits was altered after initiation of catalytic activity (ATP hydrolysis).

Therefore, the final solution to the long-standing problem of the molecular mechanism of the synthase seems imminent. Although kinetic analyses can never prove structure-function relationships nor provide final proof of any model, recent kinetic studies have been especially useful in providing restraints within which models can be built. It should soon be possible to say how the substrates and products of the synthase are bound and interconverted. As discussed in the earlier part of this review, however, it is a troubling, largely ignored, possibility that substrates do not control rates of ATP synthesis in the intact cell.

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