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Subunit Movement During Catalysis by F_1-F_o-ATP Synthases

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The catalytic portion (F_1) of ATP synthases have the subunit composition α_3, β_3, γ, δ, ε. This composition imparts structural asymmetry to the entire complex that results in differences in nucleotide binding affinity among the six binding sites. Evidence that two or more sites participate in catalysis, alternating their properties, led to the notion that the interactions of individual $\alpha\beta$ pairs with the small subunits must change as binding site properties alternate. A rotation of the γ subunit within the $\alpha_3\beta_3$ hexamer has been proposed as a means of alternating the properties of catalytic sites. Evidence argues that the rotation of the complete γ subunit during ATP hydrolysis is not mandatory for activity. The γ subunit of chloroplast F_1 may be cleaved into three large fragments that remain bound to F_1. This cleavage enhances ATPase activity without loss of evidence of site-site interactions. Complexes of $\alpha_3\beta_3$ have been shown to have significant ATPase activity in the absence of γ. Mg^{2+}ATP affects the interaction of γ with the different β subunits, and induces other changes in F_1, but whether these changes are induced by catalysis, or are fast enough to be involved in the catalytic turnover of the enzyme has not been established. Likewise, changes in structure and in binding site properties induced in thylakoid membrane bound CF_1 by formation of an electrochemical proton gradient may activate the enzyme rather than be apart of catalysis. Mechanisms other than rotary catalysis should be considered.

KEY WORDS: ATP synthase; ATPase; rotational catalysis; site–site interactions; asymmetry.

INTRODUCTION

All F_1s[2] of ATP synthases so far isolated have the polypeptide composition of $\alpha_3\beta_3\gamma\delta\varepsilon$, in which the larger α and β polypeptides assume a heterohexameric structure of alternating α and β subunits. The structure of F_1, and therefore F_1-F_o, is asymmetric. Much evidence exists for this asymmetry. The lack of three-fold symmetry of the γ, δ, and ε subunits indicates that interactions among the small subunits and individual α/β pairs cannot be equivalent. This asymmetry may also contribute to the inability of x-ray crystallographic methods to resolve the smaller F_1 subunits (Bianchet *et al.*, 1991; Abrahams *et al.*, 1994). The β subunits, which are chemically identical, have different reactivities with protein modifiers and with the small subunits (Haughton and Capaldi, 1995). The α subunits also display different reactivities (Nalin *et al.*, 1985; Turina *et al.*, 1993).

Evidence for site-site interactions during catalysis has given rise to the binding-change mechanism, in which at least two sites participate in catalysis, alternating their properties as the enzyme turns over (reviewed in Boyer, 1989). To break the asymmetry of F_1 catalytic sites during catalysis, as the binding change mechanism mandates, alterations in the way in which α/β pairs interact with the single copy F_1 subunits, especially γ, must occur. In this article we review the evidence in support of movement of the F_1 subunits during catalysis. Because of the brevity of this article,

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[2] Abbreviations used: F_1, catalytic portion of the ATP Synthase; F_o, membrane-associated, proton-translocating portion of the ATP Synthase.

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we are restrained with respect to the number of citations and we apologize for omissions.

ROTATION AS A MODEL FOR CATALYSIS

The concept of alternating catalytic sites is appealing in its seeming simplicity. Yet, in view of the structural asymmetry of ATP synthases, this concept has implications that place rigorous demands on structural changes during catalysis. If three catalytic sites operate as suggested in the alternating sites mechanism, each of these sites must become equivalent during catalysis despite the asymmetry induced by the small subunits. With a turnover time in the msec range, rapid subunit rearrangements must take place. To preserve credence in the binding change mechanism, it has been proposed that the γ subunit rotates with respect to the α/β heterohexamer during catalysis (reviewed in Cross, 1992). Should rotation occur, it would certainly satisfy the requirement that the α/β contacts with γ become randomized (i.e., equivalent) during catalysis.

Much has recently been made of the "rotary mechanism" of F_1. The evidence in its support is weak at best, despite the claims. The remarkable achievement of Walker and his colleagues in obtaining a partial structure of a form of beef heart F_1 by x-ray crystallographic techniques at a resolution of 2.9 Å has shed considerable light on the nucleotide binding sites of the enzyme. Based on a single, static structure of an inhibited form of F_1, Abrahams *et al.* (1994) attempted to predict dynamic mechanistic features of the enzyme. A potentially unfortunate consequence of this attempt is that the "hard" structural data could obscure the fact that the structural information *per se* contributes little new information about rotary catalysis.

In the paper by Duncan *et al.* (1995), it is emphatically stated that, in the rotary mechanism, *all* of the γ subunit must rotate. This possibility seems unlikely. The γ subunit of chloroplast F_1 may be cleaved by trypsin into pieces of 8, 10, and 15 kDa that remain firmly associated with the α/β core (Hightower and McCarty, 1996a). The trypsin-cleaved enzyme is very active and is as stable to thermal denaturation as CF_1 with intact γ (Hightower and McCarty, 1996b). The strength of the interaction of even fragmented γ with the α and β subunits as well as the stability of the enzyme that contains the cleaved γ suggest that rotation of the entire γ subunit is not an obligatory part of catalysis. Although trypsin treatment affects more than just the γ subunit, the trypsinized enzyme shows many of the same characteristics as intact chloroplast F_1, including tentoxin sensitivity. In addition, the exchange of bound nucleotide for nucleotide in the medium is much faster than the release of bound nucleotide in the absence of nucleotide in the medium, suggesting that site-site interactions are conserved.

The γ subunit is not required for ATP hydrolysis. Complexes of $\alpha_3\beta_3\delta$ and $\alpha_3\beta_3$ of the F_1 of thermophilic bacterium PS3 have been prepared that have Mg^{2+}ATPase activities up to 20% that of $\alpha_3\beta_3\gamma$ (Yokoyama *et al.*, 1989; Miwa and Yoshida, 1989). An α/β preparation from chloroplast F_1 also has significant ATPase activity, especially in the presence of tentoxin that appears to stabilize the α/β hexamer (Sokolov and Gromet-Elhanan, 1996). The γ subunit does, however, stabilize the α/β hexamer in both PS3 and chloroplast F_1.

There is mounting evidence that suggests that the region of γ around Cys-87 (*E. coli* numbering) is in contact with the β subunit in the region of β known by its sequence as DELSEED. The introduction of a Cys residue to this region of β allowed the facile formation of γ-β crosslinks upon oxidation (Aggeler *et al.*, 1995). In a remarkable series of experiments, it was shown that the exposure of a reconstituted enzyme to Mg^{2+}ATP altered subsequent γ-β interactions (Duncan *et al.*, 1995). However, rotation was not shown explicitly. Also, whether catalytic turnover is required for the alteration of γ-β interactions is unknown. These studies did not address the issue of kinetic competence. Whether the rate of formation of new γ-β crosslinks is fast enough to be related to the catalytic mechanism is unknown.

There is evidence of movement of the small subunits elicited by the electrochemical proton potential. For example, the establishment by light-dependent electron flow of an electrochemical proton potential across the thylakoid membrane markedly enhances the accessibility of the γ subunit of chloroplast F_1 to attack by reagents, including enzymes, in the medium. An alteration of the chloroplast F_1 ε subunit also occurs during illumination. These effects of illumination (and therefore, of electron transport) are abolished by reagents that dissipate the proton gradient. These changes are profoundly influenced by the presence of ATP or ADP in the medium (See McCarty *et al.*, 1988 for review).

During the initial stages of illumination, CF_1 is converted from an inactive to an active form. The activation has been correlated with an energy-depen-

dent release of ADP from the enzyme (Graber *et al.*, 1977). The rebinding of ADP to CF_1 in the dark has in turn been correlated to the conversion of the enzyme back to its inactive form (Strotmann and Bickel-Sandkotter, 1984). This and other changes in the structure of F_1 caused by illumination may in fact be part of the activation of the enzyme, rather than a direct part of catalysis.

EFFECT OF NUCLEOTIDES

The evidence that the presence of nucleotide in the medium affects F_1 structure is overwhelming. ADP and ATP, for example, slow the loss of ATPase activity of F_1 at cold temperatures, very likely by preventing at least a partial dissociation of the $\alpha_3\beta_3$ heterohexamer (Hightower, 1996). Data from Capaldi's laboratory with *Escherichia coli* F_1 support the concept that the presence of ADP influences the interaction of the smaller subunits with the remainder of F_1 in a manner different from that of ATP. For example, Aggeler *et al.* (1995) generated mutants of *E. coli* F_1 in which Cys residues were introduced into β (at positions 381 or 383). Cross-linking by Cu^{2+} oxidation of the β cysteine residue to the γCys-87 was observed. The yield of the β-γ cross-link was greater in the presence of ATP than in the presence of ADP. Addition of ATP [or the nonhydrolyzable analogue adenylyl imidodiphosphate (AMP-PNP)] caused changes in the fluorescence of coumarin-phenylmaleimide attached to some specific Cys residues in γ of *E. coli* F_1 (Turina and Capaldi, 1994).

The experiments in support of a binding change or an alternating site mechanism are based in part on the kinetic analysis of "unisite" vs. "multisite" catalysis. Unisite catalysis is defined as turnover at an F_1-to-ATP molar ratio of less than one. Multisite catalysis, which occurs with substrate levels of nucleotide, showed markedly enhanced rates of hydrolysis as well as product release, indicating cooperativity between sites (Cross *et al.*, 1982).

The exchange of nucleotide bound tightly (i.e., very slow rate of release) to F_1 with nucleotide in the medium likely occurs via a mechanism in which the binding of nucleotide to a loose, dissociable site promotes the release of tightly bound nucleotide (Bruist and Hammes, 1981). As such, these site-site interactions are likely to involve binding-induced changes in the structure of the enzyme, especially near a tight-binding site.

Chloroplast F_1, when exposed to Mg^{2+}-ATP, undergoes a phenomenon known as "site switching." Two sites switch their properties, tight-binding for loose-binding, during incubation of CF_1 with Mg^{2+}-ATP (Shapiro and McCarty, 1990). These effects were monitored by fluorescence resonance energy transfer using Lucifer-yellow VS which binds to Lys-378 of only one of three α subunits. Catalysis is not required for site switching, which can be induced by incubation with Mg^{2+}-AMP-PNP.

All of these observations have been used to support the binding-change mechanism in which nucleotide binding to a dissociable site promotes product release from a tight site in part by causing the binding affinities of the two sites to switch. This cycle is then repeated, with sites alternating between two or three sites depending on the number of assumed catalytic sites. However, only the exchange of tightly bound nucleotide for nucleotide in the medium has been shown to be kinetically competent as part of the catalytic mechanism (Leckband and Hammes, 1987). Site-switching, like the γ-β crosslinking, changes, has not been observed as a function of time. Instead, the data show only different possible conformations giving no hint as to the time required for the changes to take place.

ALTERNATIVE MECHANISMS

Mechanisms that do not involve rotation should be considered. A mechanism in which site-site interactions are between catalytic and regulatory sites may involve less extensive structural alterations during catalysis. For example, rotation of γ with respect to the $\alpha_3\beta_3$ hexamer would not be required.

There are six nucleotide binding sites on F_1-F_o located at the α-β interfaces. To sort out the function of each of these sites has been and will continue to be very difficult. Regulatory and structural roles for the nucleotide binding sites have been established, but, which of the sites are truly involved in catalysis is much more difficult to prove. Can the supposition that one (or perhaps, two) catalytic site(s) is under the control of one (or more) regulatory site to be excluded? For example, whether the exchange of bound nucleotide with that in the medium is initiated by binding of nucleotide in the medium to a regulatory or a catalytic site is not clear.

The evidence that various F_1s undergo structural changes as a result of the formation of an electrochemi-

cal proton potential is very strong. In most cases, however, there is no unequivocal evidence that relates the structural changes to catalysis. The best correlation, as shown in particular for chloroplast F_1, is for structural changes related to energy-dependent activation of the complex.

Virtually all of the work on the mechanism has been done with isolated F_1 in solution, and for ATP hydrolysis rather than ATP synthesis. The roles of the electrochemical proton potential, and the association of F_1 to F_o, are neglected by this approach. F_1 subunit interactions during catalysis are, however, almost certainly affected by the proton potential and by F_0.

Given the fact that there are pronounced interactions between at least two nucleotide binding sites on F_1, structural changes during catalysis are inevitable. If, as seems likely, the rotary mechanism as currently proposed is untenable, more subtle changes in $(\alpha\beta)$-γ interactions would have to occur. New approaches are required to investigate these structural perturbations and to show their relevance to catalysis.

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