Journal of Bioenergetics and Biomembranes, Vol. 28, No. 5, 1996

Structural Changes in the γ and ε Subunits of the *Escherichia coli* F_1F_0-type ATPase During Energy Coupling

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Received March 19, 1996; accepted March 20, 1996

Structural changes in the *Escherichia coli* ATP synthase (ECF_1F_0) occur as part of catalysis, cooperativity and energy coupling within the complex. The γ and ε subunits, two major components of the stalk that links the F_1 and F_0 parts, are intimately involved in conformational coupling that links catalytic site events in the F_1 part with proton pumping through the membrane embedded F_0 sector. Movements of the γ subunit have been observed by electron microscopy, and by cross-linking and fluorescence studies in which reagents are bound to Cys residues introduced at selected sites by mutagenesis. Conformational changes and shifts of the ε subunit related to changes in nucleotide occupancy of catalytic sites have been followed by similar approaches.

KEY WORDS: F_1 ATPase; site-directed mutagenesis; γ and ε subunits; conformational changes.

INTRODUCTION

Conformational changes are a key part of the mechanism of action of most enzymes. In the case of the F_1F_0 ATPases, there is now evidence of major conformational changes. These include rotational movements of subunits occurring in, and responsible for, both the strong catalytic cooperativity between the three catalytic sites, and the coupling between catalytic site events (ATP hydrolysis or ATP synthesis) and translocation of protons through the F_0 part of the complex. The conformational changes involved in these two processes need not be the same, but must be linked during energy coupling by the complex. Beginning with our cryoelectron microscopy studies (Gogol *et al.*, 1990), evidence that the γ subunit rotates within the $\alpha_3\beta_3$ domain has steadily accumulated (Abrahams *et al.*, 1994; Capaldi *et al.*, 1994; Duncan *et al.*, 1995). The switching of γ (and ε) subunit(s) from one α-β pair to another during turnover must involve transient structural changes in these small subunits, which are the conformational link between the catalytic sites and proton channel. Here, we review our data on these conformational changes in the γ and ε subunits.

UPDATED STRUCTURE OF THE F_1 PART OF THE ATP SYNTHASE

Details of the structure of the $\alpha_3\beta_3$ subdomain and segments of the γ subunit of F_1 are provided by the recent X-ray structure determination of MF_1 (Abrahams *et al.*, 1994). The sectional side view in Fig. 1 shows key features of the enzyme that has been crystallized in the presence of Mg^{2+} and both AMP·PNP and ADP along with azide. The overall fold of α and β subunits is similar, with each arranged in three domains, an upper β-sheet domain, a middle nucleotide binding domain, and a lower predominantly α-helical domain. The part of the γ subunit resolved in the structure is arranged in three α-helices. There is a long C-terminal α-helix (residue 223–286 in ECF_1), which is slotted through a hydrophobic sleeve provided

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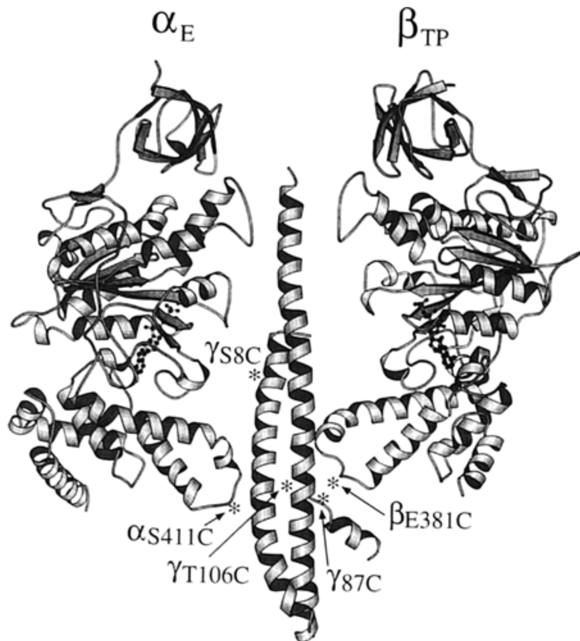

Fig. 1. Sectional view through the MF_1 structure derived from the X-ray coordinates kindly provided by Dr. J. Walker, Cambridge University, showing one α, one β, and a part of the γ subunit. Sites at which Cys residues were introduced are indicated. Also shown is the locus of residue γ 87 Cys (ECF_1).

by the upper part of the catalytic domains of the $\alpha_3\beta_3$ part. This C-terminal helix extends through the entire F_1 structure and into the stalk region. Coiled around it is a second α-helix formed by residues 1–45 (ECF_1 numbering system), which extends from the catalytic site domain into the stalk region. The third segment of γ seen in the X-ray data involves residues 82–99, and it forms a short α-helix running roughly at right angles to the other two helices of γ, lying below the F_1 and interacting with the so-called "DELSEED" region of one of the β subunits.

Our recent biochemical studies add information on the arrangement of both the γ and ε subunits. These studies indicate that the γ subunit actually extends the full length (40–45 Å) of the stalk, to make direct contact with the ring of c subunits in the F_0 part. Residue Tyr 205 has been found to interact closely with the loop region of at least one c subunit, from our recent cross-linking experiments (Watts *et al.*, 1996). When this residue is replaced by a Cys, a disulfide bond can be formed between this site and a Cys placed at residues 39, 42, or 43 of the c subunit (Watts *et al.*, 1996). We believe that the region of the γ subunit that is missing in the X-ray data is predominantly a β-sheet structure which provides an interaction face for the ε subunit. Amino acids around residue 70, at 106, and between 202 and 223 of the γ have been identified in the ε subunit interface (Tang and Capaldi, 1996).

Our recent NMR structure determination of the ε subunit has confirmed previous conclusions that the polypeptide is organized in two domains (Kuki *et al.*, 1988; Skakoon and Dunn, 1993). The N-terminal 84 residues form a 10-stranded β sandwich structure, and the C-terminal 48 residues are arranged in an α-helix loop α-helix structure (Wilkens *et al.*, 1995). As shown in Fig. 2, the N-terminal domain of ε interacts with the γ subunit through that face of the β sandwich which includes residues 10, 38, and 43 (Aggeler *et al.*, 1992; Tang and Capaldi, 1996). A Cys at residue 10 can be cross-linked at, or close to, Tyr 228 of the γ subunit by tetrafluorophenylazide maleimides (e.g., TFPAM 3). A Cys at either residue 38 or 43 of the ε subunit forms a disulfide bond with a Cys introduced for the Tyr at residue 205 of the γ subunit (Tang, C., and Capaldi, R. A., unpublished). The ε subunit also interacts with the c subunits of the F_0 part via the bottom of the β sandwich. This interaction involves residue 31, and possibly residue 38, as shown by the cross-linking studies of Zhang and Fillingame (1995), and by our recent chemical labeling experiments (Aggeler *et al.*, 1995a). Finally, the C-terminal α-helix

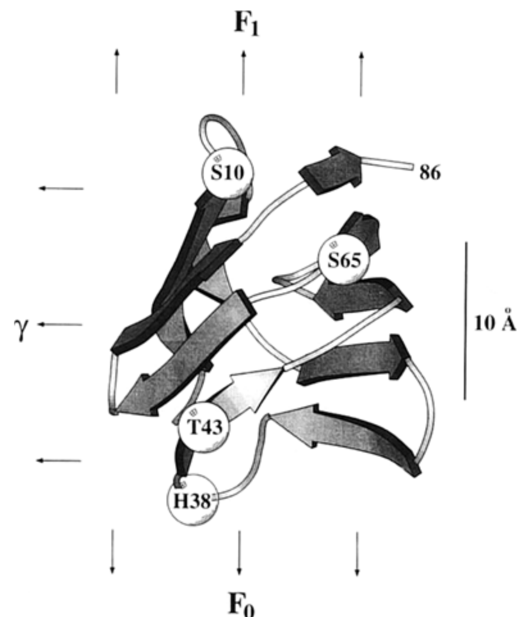

Fig. 2. Structure of the ε subunit as determined by Wilkens *et al.* (1995), indicating the interactions that this subunit makes with other subunits of the complex.

loop α-helix domain of the ε subunit lies under the F_1 part and interacts with the $\alpha_3\beta_3$ subdomain, as shown in Fig. 3 and as described in more detail later.

CONFORMATIONAL REARRANGEMENTS OF THE γ SUBUNIT

The observations above, which show that both the γ and ε subunits interact with catalytic site-containing β subunits at one end and the proton-conducting c subunits at the other end, make these subunits obvious candidates for the conformational energy transduction pathway within the ATP synthase. Both show conformational changes with each enzyme turnover that occur during, and is in some cases in addition to, the overall rotational movements between α-β pairs.

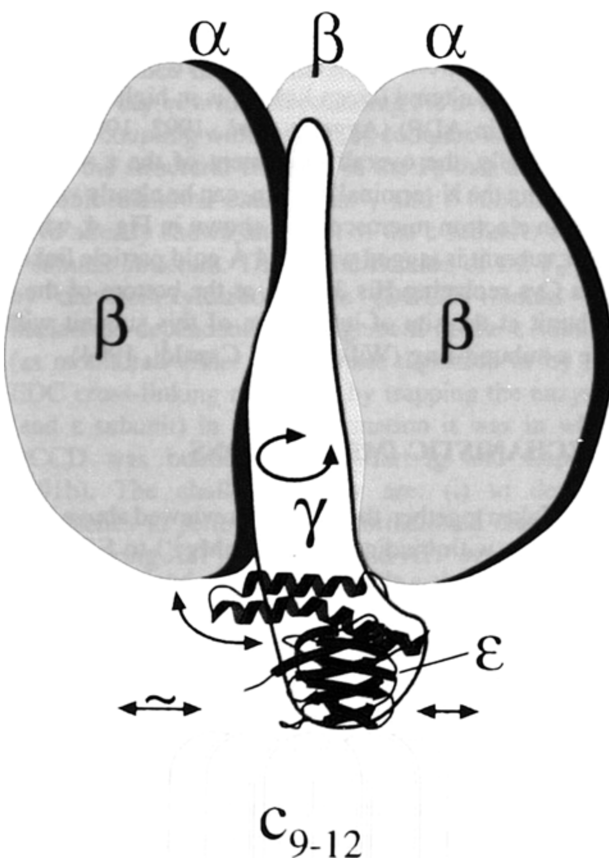

Fig. 3. Schematic of the arrangement of the ε subunit within the stalk region of ECF_1F_0.

As one approach to studying the nucleotide-dependent conformational change in the γ subunit, we have bound the fluorescent reagent N-[4-[7-(diethylamino)-4-methyl] coumarin-3-yl] maleimide (CM) at a Cys residue introduced at positions 8 and 106 (also at position 108 of the ε subunit), and examined the fluorescence changes occurring during both unisite and multisite (i.e., cooperative) ATP hydrolysis (Turina and Capaldi, 1994a, b). ATP binding caused an enhancement of the fluorescence of CM, which was followed by fluorescence decay as the ATP was converted to ADP+P_i. This fluorescence decay has been shown to occur at rates corresponding to the bond cleavage reaction under unisite conditions. Other evidence of nucleotide-dependent changes in the γ subunit comes from cross-linking experiments with TFPAMs attached to the Cys at residue 8 (Aggeler and Capaldi, 1993; Aggeler *et al.*, 1993). Additionally, there are differences in the yield of disulfide bond formation between Cys 87 of γ and the Cys introduced for Glu 381 in the DELSEED region of β, depending on whether ATP or ADP are bound in catalytic sites (Aggeler *et al.* 1995b). Finally, we have recently found that the intrinsic Cys 87 of γ, which is buried in wild-type ECF_1 or ECF_1F_0, becomes available for reaction with maleimides when Glu 381 is converted to an Ala or a Cys (Feng, Y., Aggeler, R., Haughton, M. A., and Capaldi, R. A., submitted). The reactivity of Cys 87 in these mutants is highly nucleotide dependent. This site reacts readily with maleimides such as N-ethylmaleimide (NEM) or CM when ATP+Mg^{2+} is bound in catalytic sites, but it is shielded from reaction with ADP+Mg^{2+}-bound.

Taken together, these data suggest that ATP binding rearranges the γ subunit, an effect which probably requires ATP binding in one (the high-affinity) catalytic site only. The rearrangement appears to involve loosening of the interaction of the N-terminal α-helix and the short central α-helix from partner β subunits to initiate the rotation of the γ subunit. At the same time, there may be shifts of the N and C terminal helices relative to one another (controlled by binding of the ε subunit: see below), which alter the interaction of γ with the c subunits of F_0. Catalytic conversion of ATP to ADP in turn reverses the conformational changes in the γ subunit, leading to a return of the tight binding of this subunit at the next α and β subunits pair.

Evidence that the above-described movements of the γ subunit are coupling events, and not just conformational changes as part of catalytic site cooperativity, comes from studies on ε-free F1 ATPase. The nucleo-

tide-dependent conformational and positional changes of the γ subunit described above are lost when the ε subunit is removed. Nevertheless, ε-free ECF_1 is a highly cooperative ATPase.

It is also important to note that all the conformational changes of γ (and the ε subunit, as discussed later) that we have described in ECF_1 have also been seen in ECF_1F_0, ruling out that they are artifactually related to separation of the catalytic F_1 part from its membrane sector.

CONFORMATIONAL REARRANGEMENTS OF THE ε SUBUNIT

With ADP bound to the enzyme, the C-terminal α-helix loop α-helix domain of the ε subunit lies under the F_1 part (as shown in Fig. 2) and, as described above, there is interaction of the loop region with the DELSEED region of one β subunit, as evidenced by 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide (EDC) cross-linking of Ser 108 of ε to Glu 381 of the β subunit (Mendel-Hartvig and Capaldi, 1991a, b; Dallmann *et al.*, 1992), and by disulfide bond formation between a Cys at position 108 of ε and a Cys at position 381 of β (Aggeler *et al.*, 1995b). In this state, the C-terminal part of ε appears to span two β subunits, based on experiments with a mutant of ECF_1 (εM138C). In this mutant, it has proved possible to generate a β-ε-β cross-linked product in high yield with one β linked to ε via the EDC-catalyzed reaction of Ser 108 and Glu 381 of β, the second via the TFPAM-modified Cys at position 138 (Wilkens, S., and Capaldi, R. A., unpublished). This could only occur if the C-terminal part of the ε subunit lies roughly parallel to the bottom of the F_1. In this position, with ADP in catalytic sites, the ε subunit is cleaved only slowly by trypsin in ECF_1 and is resistant to proteolysis in F_1F_0 (Mendel-Hartvig and Capaldi, 1991a, b).

The close interaction of the C-terminal α-helix loop α-helix region of the ε subunit with the β subunit is lost when ATP is bound. There is no cross-linking of ε Ser 108 to β Glu 381 catalyzed by EDC (Mendel-Hartvig and Capaldi, 1991a, b), while disulfide bond formation between a Cys in ε at position 108 and the Cys at β position 381 is very slow, and obtained only in low yield (Aggeler *et al.*, 1995b). Also, cross-linking from the Cys at ε position 138 to a β subunit via TFPAM is minimal (Tang and Capaldi, 1996).

In ATP, the region around Ser 108 moves from close to the β to close to an α subunit, allowing disulfide bond formation between a Cys at position 108 of ε and Cys introduced for Ser 411 in the α subunit (Ser 411 in α is at the same position as Glu 381 of the β subunit) (Aggeler and Capaldi, 1996). This switching of the ε subunit between the α and β subunits is clearly demonstrated by the nucleotide dependence of disulfide bond formation in the mutant αS411C: βE381C:εS108C (see Fig. 3 of Aggeler and Capaldi, 1996). In the ATP state, the C-terminal domain of ε becomes highly protease sensitive in ECF_1 at sites within the C-terminal helix, suggesting some relaxation of the structure when ATP is bound (Mendel-Hartvig and Capaldi, 1991a). In ECF_1F_0, cleavage by trypsin is more limited, with clipping only of the very C-terminal end (Mendel-Hartvig and Capaldi, 1991b). In addition to changes in the C-terminal domain, there are differences in the interaction of the N-terminal domain with the γ subunit when the structure of ECF_1 or ECF_1F_0 in ADP versus ATP is compared. In ATP, cross-linking from a Cys at position 10 of the ε to the γ subunit is altered (cross-linking is in higher yield in ATP than in ADP) (Aggeler *et al.*, 1992, 1995a).

Finally, the overall movement of the ε subunit, including the N-terminal domain, can be clearly visualized in electron microscopy as shown in Fig. 4, where the ε subunit is tagged with a 14 Å gold particle linked at a Cys replacing His 38, i.e., at the bottom of the ε subunit at the site of interaction of this subunit with the c subunit ring (Wilkens and Capaldi, 1994).

MECHANISTIC IMPLICATIONS

Taken together, the evidence reviewed above indicates that with binding of ATP($+Mg^{2+}$) to ECF_1 and ECF_1F_0, there is a shifting of the ε subunit, alterations in the interaction of the γ and ε subunits with the $\alpha_3\beta_3$ domain, and changes of association of the γ and ε subunits with each other. Bond cleavage reverses these conformational changes while rotating the mobile γ domain 120° to interact with a different α-β pair.

Although direct evidence is lacking, it is reasonable to believe that these movements of γ and ε include alterations in the interaction of these subunits with the ring of c subunits, and that this couples catalytic site events to proton pumping. In the direction of ATP hydrolysis, movements of the γ and ε subunits would open the proton channel for proton translocation. In the direction of ATP synthesis, the buildup of the proton

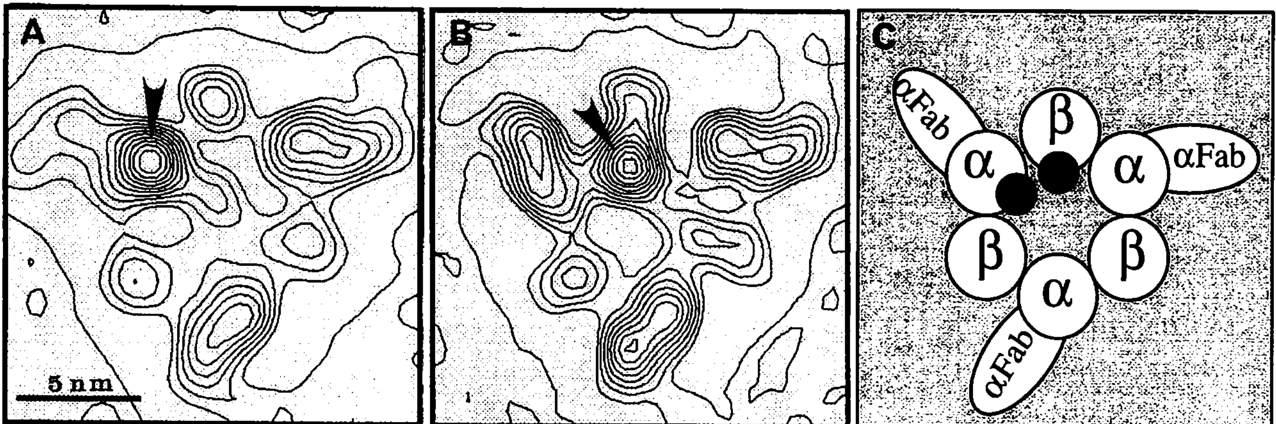

Fig. 4. Movements of the ε subunit indicated by positions of this subunit observed in cryoelectron microscopy, using ε labeled with a 14 Å gold particle attached at a Cys replacing His 38. See Wilkens and Capaldi (1994) for details.

gradient would drive structural changes in the F_0 which move the γ and ε subunits, and alter the interaction of these with the $\alpha_3\beta_3$ domain, thereby providing the energy for phosphate binding and subsequent release of ATP formed in catalytic sites.

The key now to understanding the overall mechanism of coupling within the F_1F_0 complex is to characterize the structural changes in the F_0 that are linked to conformational changes in γ and ε subunits. We have already shown an effect of the c subunits on the ε subunit structure. Thus, modification of the F_0 part by dicyclohexylcarbodiimide (DCCD) blocks the nucleotide-dependent rearrangement of the ε subunit (as monitored either by protease digestion or by the EDC cross-linking reaction), by trapping the enzyme (and ε subunit) in the conformation it was in when DCCD was bound (Mendel-Hartvig and Capaldi, 1991b). The challenges now are: (i) to develop approaches to follow the conformational changes in the F_0 during ATP hydrolysis and ATP synthesis, and (ii) to develop a system of reconstituted F_1F_0 in which the proton gradient-driven ATP synthesis occurs for a sufficient length of time to allow biochemical and biophysical measurements of the coupled conformational changes in the F_1 part. Such experiments are ongoing in our laboratory.

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