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Minireview

Structure of the *Escherichia coli* ATP Synthase and Role of the γ and ε Subunits in Coupling Catalytic Site and Proton Channeling Functions

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The structure of the *Escherichia coli* ATP synthase has been studied by electron microscopy and a model developed in which the α and β subunits of the F_1 part are arranged hexagonally (in top view) alternating with one another and surrounding a central cavity of around 35 Å at its widest point. The α and β subunits are interdigitated in side view for around 60 Å of the 90 Å length of the molecule. The F_1 narrows and has three-fold symmetry at the end furthest from the F_0 part. The F_1 is linked to F_0 by a stalk approximately 45 Å long and 25–30 Å in diameter. The F_0 part is mostly buried in the lipid bilayer. The γ subunit provides a domain that extends into the central cavity of the F_1 part. The γ and ε subunits are in a different conformation when ATP + Mg^{2+} are present in catalytic sites than when ATP + EDTA are present. This is consistent with these two small subunits switching conformations as a function of whether or not phosphate is bound to the enzyme at the position of the γ phosphate of ATP. We suggest that this switching is the key to the coupling of catalytic site events with proton translocation in the F_0 part of the complex.

KEY WORDS: ATP synthase; ECF, F_0; cryoelectronmicroscopy; subunit arrangements; nucleotide-dependent conformational changes.

INTRODUCTION

An F_1F_0-type ATPase is present in the plasma membrane of bacteria, the thylakoid membrane of chloroplasts, and the inner membrane of mitochondria. It functions both to synthesize ATP from ADP + Pi in the presence of a light-driven or respiratory chain-generated proton gradient, and as an ATPase, using the hydrolysis of ATP to establish a pH gradient for subsequent use in ion or substrate transport processes. We are studying the structure of the F_1F_0-type ATPase from *Escherichia coli*, which is made up of eight different subunits. The F_1 part contains five subunits, α, β, γ, δ, and ε, in the molar ratio 3:3:1:1:1. The F_0 part contains three subunits, a, b, and c, in the molar ratio $1:2:\simeq 12$ (Cross, 1988; Senior, 1988; Futai *et al.*, 1989). In this brief review we describe our structural analyses of both the F_1 part (ECF_1) and the intact ATP synthase (ECF_1F_0) using electron microscopy. We then review recent work on the structure of the γ and ε subunits, and on the conformational changes in these subunits, which we believe play a critical role in coupling catalytic site events with proton pumping in the enzyme complex.

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STRUCTURE OF THE F_1 PART

The structure of F_1 has been actively investigated for 20 years using a variety of physical techniques.

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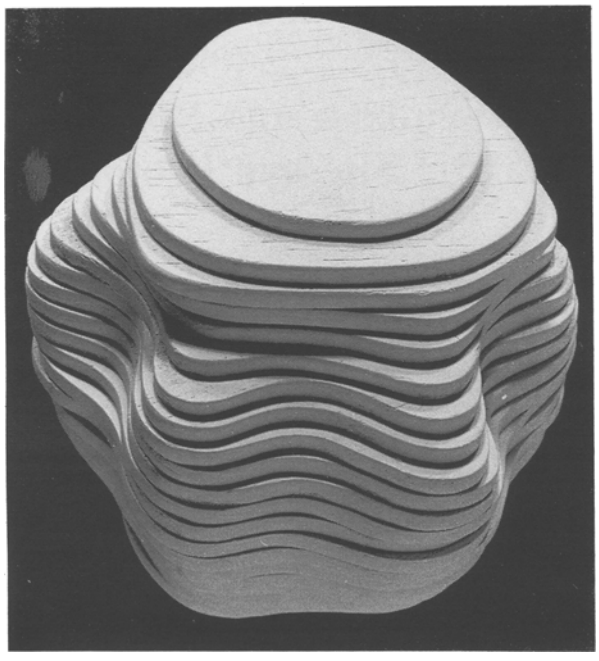

Fig. 1. A balsa wood model of the ECF_1 complex derived from analysis of two-dimensional crystals of the protein stained with phosphotungstate (taken from Gogol *et al.*, 1989a with permission).

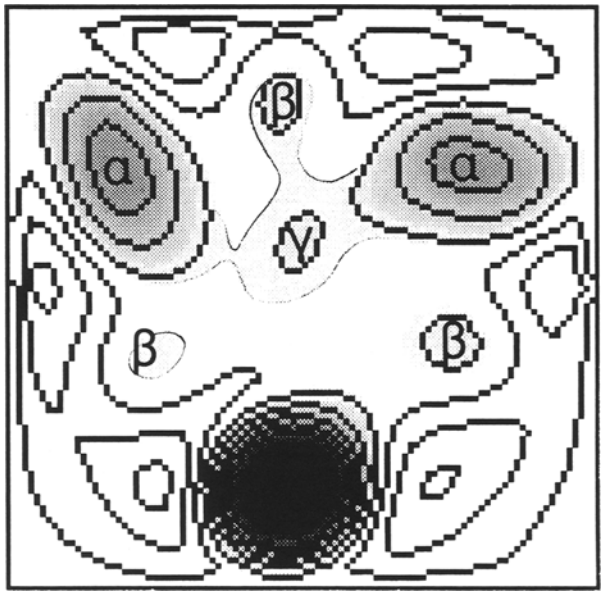

Fig. 2. An average of 100 particles of ECF_1 reacted with the gold maleimide under conditions that react a cysteine in the α subunit with subsequent labeling of the complex with anti-α Fabs to orient the complex in the hexagonal view. The gold particle is visualized as an 18 Å diameter density.

Early models based on X-ray diffraction of 3D crystals of mitochondrial F_1 (MF_1) to 9 Å resolution (Amzel *et al.*, 1982), and examination of negatively stained single particles of MF_1, ECF_1, and chloroplast F_1 (CF_1) by electron microscopy (Akey *et al.*, 1983; Tiedge *et al.*, 1985; Tsuprun *et al.*, 1984; Boekema *et al.*, 1986, 1988), showed a hexagonal arrangement of subunits. The six large masses, assumed to be α and β subunits, were proposed to occur in two tiers. From electron microscopy in uranyl acetate, dimensions of the F_1 molecule of 105–115 Å in the hexagonal dimension (top view) and 60 Å in the side view, were calculated (Tiedge *et al.*, 1985; Boekema *et al.*, 1988).

We have examined the gross structure of the ECF_1 complex by electron microscopy and image reconstruction approaches, using two-dimensional arrays of the enzyme stained with phosphotungstate (Gogol *et al.*, 1989a). Cryoelectron microscopy of single particles of ECF_1 embedded in a thin layer of amorphous ice has also been used to examine the structure of the complex in a more native state (Gogol *et al.*, 1989a). The shape of the ECF_1 molecule based on a 3D reconstruction from images of the negatively stained enzyme is shown in Fig. 1. The ECF_1 complex is hexagonal in top view, while in side view (perpendicular to the hexagonal view), the six large subunits are interdigitated for around 60Å, with $\simeq$ 15 Å of three-fold symmetric structure at one end, and $\simeq$ 10 Å of undefined arrangement of protein at the other end.

The balsa wood model shown in Fig. 1 was built from the contour density data to accommodate a volume of the ECF_1 molecule equivalent to the calculated molecular weight of the complex (385,000), without consideration of internal cavities in the protein. This underestimates the size of the complex because cryoelectron micrographs, as well as uranyl acetate-stained single molecules, show a large cavity in the center of the ECF_1, which was not penetrated by phosphotungstate in the crystals. Increasing the volume of the structure to allow space for the internal cavity would increase the diameter more than the length (to around 100 Å diameter, 95 Å length), but does not alter the considerable interdigitation of the large subunits, or the overall spherical appearance of the protein complex.

ECF_1 embedded in ice showed the six α and β subunits surrounding a central cavity of diameter around 35 Å. A seventh mass was seen, placed asymmetrically and extending into the central cavity. This central mass was still present in preparations from

which the δ and ε subunits had been removed by proteolysis (Gogol *et al.*, 1989b), indicating that it consists primarily of the γ subunit. Immuno decoration of ECF_1 with Fab′ fragments derived from monoclonal antibodies (mAbs) to the α subunit showed that the α and β subunits alternate around the periphery of the complex (Gogol *et al.*, 1989b). With this same technique, accessible epitopes on the δ and ε subunits were found to be located close to β subunits. A mAb against the C-terminal third of the γ subunit also bound close to a β subunit and near the periphery.

In ongoing studies, we are adding cysteine residues into the ECF_1 and ECF_0 subunits by site-directed mutagenesis and using these sites to react (maleimide-linked) heavy-atom derivatives for electron microscopy studies, crosslinkers for determining near neighbors, and fluorescent probes for studying the dynamics of the conformational change essential for enzyme function. Figure 2 shows the result of incorporating a maleimide-linked gold cluster into a unique cysteine of the α subunit which is reactive in only one of the three copies of this subunit per complex (Wilkens, S., and Capaldi, R. A., unpublished data). The gold particle can be clearly visualized against the background of the F_1 and associated anti-α Fabs used to orient the particles in the hexagonal view. Labeling of a mutant γS8C places a gold particle on the central mass, confirming that this is the γ subunit (Wilkens, S., and Capaldi, R. A., unpublished).

Crosslinking experiments using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) and novel bifunctional reagents, tetrafluorophenylazide maleimides (TFPAMs), have shown that the γ, δ, and ε subunits each interact with both (an) α and β subunit, and therefore, that all three must bind near the interfaces between the two large subunits (Mendel-Hartvig and Capaldi, 1991a; Aggeler and Capaldi, 1992; and unpublished). The ε subunit also interacts with the γ subunit based on crosslinking experiments (Aggeler *et al.*, 1992) and as demonstrated in direct binding studies (Dunn, 1982). Figure 3 summarizes these data in a low-resolution topographical model of ECF_1.

The shape and interdigitation of the subunits in our model of F_1 contrasts with arrangements proposed earlier (see above). Published objections raised to this model (Boekema *et al.*, 1990) are based on an unfortunate misunderstanding of the procedures used in 3D reconstruction and their effects on the resulting structure determination. Thus, Boekema *et al.* (1990)

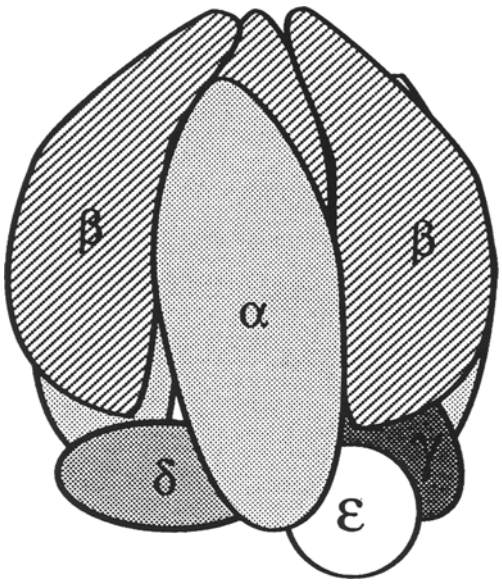

Fig. 3. A schematic to show the arrangement of subunits in the ECF_1 complex.

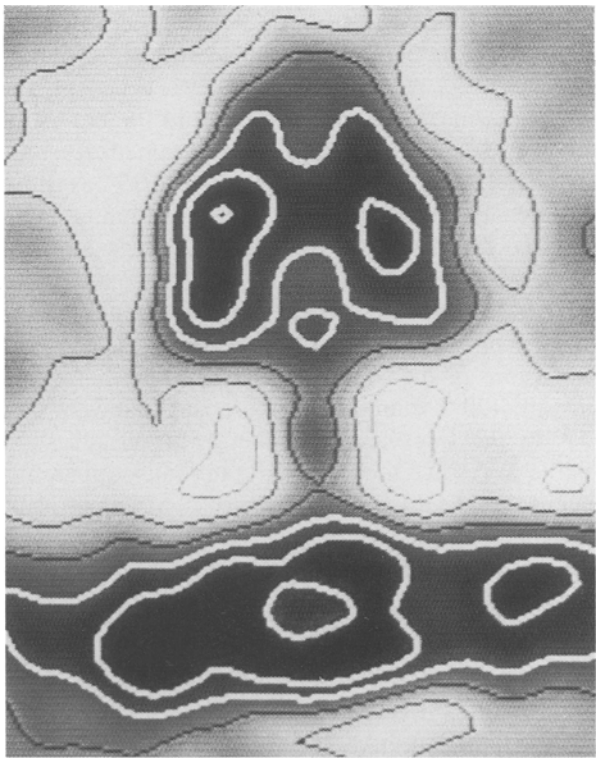

Fig. 4. An average of side views of ECF_1F_0 showing the three domains of the complex, the F_1 part, a stalk, and the F_0 part embedded in the membrane.

Table I. Summary of Evidence for Nucleotide Dependent Conformational Changes in the γ and ε Subunits of ECF_1

ATP + Mg^{2+} or ADP + Mg^{2+} + Pi	ATP + EDTA	Reference
The γ and ε subunits are insensitive to trypsin cleavage	The γ and ε subunits are rapidly cleaved by trypsin	Bragg and Hou (1987); Mendel-Hartvig and Capaldi (1991a)
The γ subunit is crosslinked to a β subunit in high yield by TFPAMs through Cys 8 in the mutant γS8-C	The yield of crosslinking of γ to β via Cys 8 is lower and the $M_{r\,app}$ of the major product of these two subunits is different from that obtained in ATP + Mg^{2+}	Aggeler, R., and Capaldi, R. A., unpublished data
The ε subunit is crosslinked to a β subunit in high yield by EDC	The yield of crosslinking of ε to β by EDC is low	Mendel-Hartvig and Capaldi (1991a)
The ε subunit is crosslinked to an α subunit in high yield by TFPAMs in a mutant εS108-C, while crosslinking of ε to γ in a mutant εS10-C is in low yield with the same crosslinkers	The yield of crosslinking of ε to α via Cys 108 is low while the yield of crosslinking of ε to γ via Cys 10 is high	Aggeler *et al.* (1992)
The γ subunit (central mass) associates preferentially with that $\alpha\beta$ subunit pair to which the ε subunit is attached, based on cryoelectron microscopy studies	The γ subunit is not associated with any preference for that $\alpha\beta$ pair to which the ε subunit is bound	Gogol *et al.* (1990)

erroneously calculate the resolution of our 3D map as 37 Å, rather than the correct value of 24 Å (i.e., the highest-order diffraction spot is the 3,1).

A structure of F_1 from rat liver has been presented very recently that is based on X-ray diffraction data collected to 3.5 Å (Bianchet *et al.*, 1991). This picture of the enzyme, developed prior to full tracing of the polypeptide chains, has many of the same features as our model. Bianchet *et al.* (1991) describe F_1 as three-fold symmetric with the α and β subunits interdigitated for around 50 Å, with the three α subunits extending from one end where they become closely opposed, and with the three β subunits extending from the opposite end and widely separated. The overall dimensions of the complex based on these X-ray studies are 120 Å diameter in the hexagonal view and 75 Å in the side view. Bianchet and colleagues are unable to resolve the γ, δ, and ε subunits, apparently because these subunits are arranged nonsymmetrically around the crystallographic three-fold axis, which they explain as due to disordering of these small polypeptides in the F_1 crystals.

ORIENTATION OF F_1 ON THE F_0 PART

We have collected side views of ECF_1 by cryoelectron microscopy of single particles of both ECF_1 (Gogol *et al.*, 1989b) and ECF_1F_0 in membrane vesicles (Lucken *et al.*, 1990). These studies confirmed that the F_1 part contains a channel running perpendicular to the hexagonal view, and indicated that the hexagonal view is the top view of ECF_1 when oriented on F_0 in the membrane (Fig. 4). The narrowest part of the ECF_1 is uppermost and the widest part closest to the membrane. Side views of ECF_1F_0 revealed that the F_1 part is separated from the F_0 part by a stalk of around 45 Å length and 25–30 Å diameter. Different side views of the ECF_1F_0 molecule all gave the same dimensions of the stalk, indicating that it must be roughly symmetrical. A stalk of the dimensions shown in Fig. 4 could accommodate five to seven closely packed α-helices. Candidate subunits to contribute to the stalk are the δ subunit of ECF_1 (Mendel-Hartvig and Capaldi, 1991b) and the b subunit of the F_0 part. The F_0 part of the complex was seen to extend only a small way from the bilayer on the F_1 side, while there is little or no protein extending from the membrane on the opposite side.

STRUCTURAL CHANGES IN THE γ AND ε SUBUNITS DURING ENZYME FUNCTIONING

In terms of the functioning of the ECF_1F_0 complex, the key issue is how events at the three catalytic sites, now established to be at α–β interfaces (reviewed in Cross, 1988; Senior, 1988; Futai *et al.*, 1989), are coupled to proton channeling through the F_0 part, reactions separated by at least the length of the stalk

and possibly by as much as 100 Å. We have provided several different lines of evidence that the γ and ε subunits change conformation, and/or their interaction with α and β subunits, as a function of which nucleotides are in the catalytic site. A summary of these date is presented in Table I. The effects of the different nucleotide conditions indicate that the arrangement of the γ and ε subunits is determined by the presence of phosphate at the position of the γ phosphate of ATP in catalytic sites.

Important evidence for structural changes in the ε subunit during ATP synthesis has come from the work of Richter and McCarty (1987) which showed that this subunit is reactive to antibodies in thylakoid membranes during illumination (i.e., during phosphorylation) but not in the dark. We have found that the nucleotide dependence of trypsin sensitivity of the ε subunit in ECF_1F_0 is altered by binding of dicyclohexylcarbodiimide (DCCD) to the F_0 part (Mendel-Hartvig and Capaldi, 1991c), providing additional evidence that the ε subunit senses changes in the structure of the proton channel.

The observations that both nucleotide binding changes in the F_1 part and proton translocation through the F_0 part can change the arrangement of the γ and/or ε subunit, focuses attention on these two subunits as important participants in coupling ATP hydrolysis and ATP synthesis with proton translocation. Ongoing work in our laboratory is directed at determining the interactions between the γ and ε subunits and those subunits making up the F_0 part of the ATP synthase.

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