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# Chapter 26

# The Structure of the $CF_1$ Part of the ATP-Synthase Complex from Chloroplasts

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## Summary

The proton ATP synthase consists of a membrane integrated part, $F_o$, and a hydrophilic part, $F_1$. $F_1$ is composed of five different subunits: $\alpha$, $\beta$, $\gamma$, $\delta$ and $\varepsilon$. This chapter focuses on the chloroplast $F_1$($CF_1$) structure and discusses the overall shape and dimensions of $CF_1$, shape and size of the various subunits, subunit interactions and conformational changes in the subunit positions related to catalysis. Structural data originate mainly from X-ray diffraction and electron microscopy. Recently, the structure of $F_1$ from beef heart mitochondria has been determined at 2.8 Å and this structure, although not fully identical to $CF_1$, can be taken as a blueprint for models of the $CF_1$ structure.

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## I. Introduction

The proton ATP synthase from chloroplasts, mitochondria and eubacteria consists of a membrane-integrated part, $F_o$, which acts as a proton channel through the membrane and a hydrophilic part, $F_1$, which contains the nucleotide-binding sites. The ATP synthases from different sources have a very similar structure. This is especially the case for the $F_1$ part. The $F_1$ part from spinach chloroplasts, $CF_1$, is composed of five different subunits: $\alpha$ (55.4 kDa), $\beta$ (53.9 kDa), $\gamma$ (35.7 kDa), $\delta$ (20 kDa) and $\varepsilon$ (14.7 kDa). One $CF_1$ molecule contains three copies of the large $\alpha$ and $\beta$ subunits, and one copy each of the smaller subunits making a total mass of nearly 400 kDa. This chapter focuses on the $CF_1$ structure. The lack of a 3D structure with high resolution forces us to discuss also the $F_1$ structure from mitochondria and eubacteria. Results obtained with related $F_1$'s are also relevant for $CF_1$ structure; at the low resolution level the mitochondrial ($MF_1$) and $CF_1$ structures have a striking similarity (Boekema et al., 1990), although all 5 subunits differ in mass and amino acid sequence, resulting in a 10% smaller mass for mitochondrial $F_1$. Especially the two smallest subunits differ considerably, but the fact that the $\delta$ subunits of $MF_1$ and $CF_1$ have a very different amino acid sequence has often been overemphasized, because both $\delta$ subunits are functionally related (Engelbrecht and Junge, 1990).

There are three main methods to investigate the structure of a protein: X-ray diffraction, NMR and electron microscopy (EM). For $F_1$, the first and the third method have been widely applied. By X-ray diffraction of three-dimensional crystals the structure can be determined at atomic resolution. A structure of rat liver $MF_1$ at the sub-atomic level has been published (Bianchet et al., 1991). More recently, based on crystals from beef heart $MF_1$ (Abrahams et al., 1993) a 2.8 Å resolution structure has been determined (Abrahams et al., 1994). NMR has the same potential as X-ray diffraction but has the disadvantage that the objects studied should be smaller than 40 kDa. Thus it can only be applied to the structure elucidation of the small $F_1$ subunits. Results of such work have not yet been reported. With EM it is very difficult to determine a high-resolution structure, because it needs large two-dimensional (2D) crystals. With small 2D crystals a low-resolution 3D model can be obtained (Gogol et al., 1989a; Ishii et al., 1993). Low-resolution data can also be easily obtained with single-particle averaging (Boekema et al., 1986; Gogol et al., 1989b). In this chapter we will mainly discuss the outcomes of these techniques and, where necessary, extend them with results of biochemical investigations.

*Abbreviations:* $CF_1$ – chloroplast $F_1$; DTT – dithiothreitol; EM – electron microscopy; $MF_1$ – mitochrondrial $F_1$; NEM – N-ethylmaleimide

## II. Structural Aspects of $CF_1$

### A. Overall Shape of $CF_1$

If the electron density of a protein is solved by X-ray diffraction with a resolution of better than 3 Å, there is enough structural detail to define the amino acid chain backbone. If the resolution is lower, the ambiguity of fitting amino acids increases and above 5 Å only secondary elements, such as large $\alpha$-helices, can be recognized. First, an X-ray study on rat liver mitochondrial $F_1$ was published which did not show much of the amino acid chain backbone, although a resolution of 3.5 Å resolution was claimed (Bianchet et al., 1991). But more recently, the beef heart mitochondrial ATPase structure could be solved at 2.8 Å resolution (Abrahams et al., 1994) and this enabled the determination of the amino acid chain backbone of the $\alpha$- and $\beta$ subunits and most of the $\gamma$ subunit. We will discuss the features from both X-ray structures mainly in connection with the EM and other data on $CF_1$.

The maximal diameter of $CF_1$ parallel to the plane of the membrane is close to 110 Å, because this is the repeating distance of $CF_1$ in two-dimensional crystals (Boekema and Böttcher, 1992). In crystals the repeat can be determined by EM with an error of only a few Å, even if the resolution of the image features is much lower. The diameter of bovine heart $MF_1$ is 100 Å (Abrahams et al., 1994). This is somewhat in discrepancy with the 120 Å diameter from the rat liver $MF_1$ model (Bianchet et al., 1991). The height of $CF_1$ vertical to the membrane plane is about 83 Å. A similar height has been found for mitochondrial $F_1$ (Boekema et al., 1988; Abrahams et al., 1994) and *E. coli* $F_1$ (90 Å; Lücken et al., 1990). Average side views of $F_1$ complexes in $CF_oF_1$ (Fig. 1) and *E. coli* $F_oF_1$ (Lücken et al., 1990), recorded by EM, show a trapezoid form for symmetrical views (Fig. 3) of $F_1$.

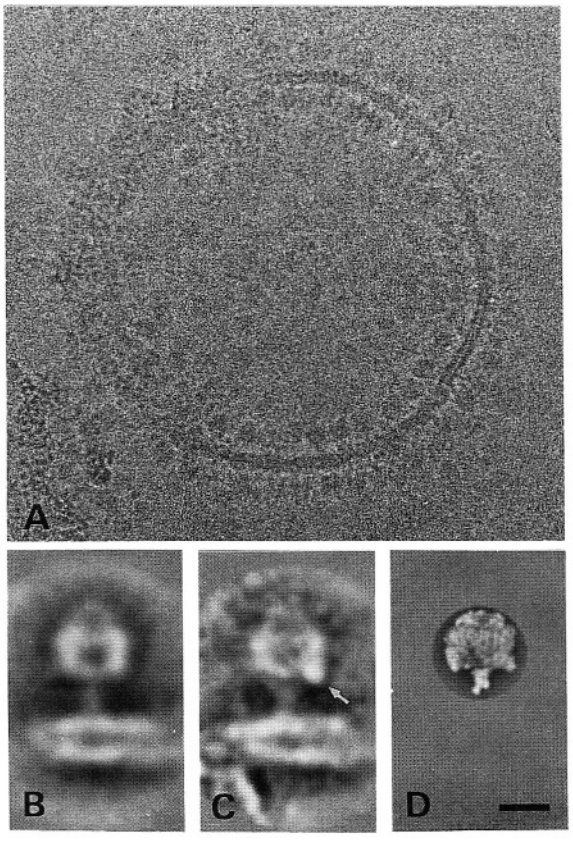


*Fig. 1.* Cryo-electron microscopy and image analysis of unstained $F_oF_1$ ATPase from spinach chloroplasts. (A) EM of $F_oF_1$ ATPase reconstituted in lipid vesicles (B) average image of reconstituted $F_oF_1$ particles (C) average image of $F_oF_1$ particles labeled with a gold cluster, which binds specifically to the S-S disulfide in the loop of the $\gamma$ subunit. Procedure: the -SH groups in $CF_1$ were blocked with NEM, the S-S bond was reduced with DTT and the reduced -SH groups labeled with a monomaleimidogold label. The S-S group is in the periphery of $CF_1$ (position marked by an arrow), in accordance with the fluorescence energy transfer data (see chapter 23). (D) structure of the bovine heart $MF_1$ (Abrahams et al., 1993) shown on the same scale. For representation and comparison the electron density was low-pass filtered to enhance the low-resolution features of the map. The bar represents 100 Å.

## B. Structure of the Individual Subunits

### 1. Shape of the α- and β-Subunits

The 2.8 Å map of $MF_1$ (Abrahams et al., 1994) shows that the $\alpha$- and $\beta$ subunits are almost identically folded: each consists of N-terminal six-stranded $\beta$-barrel, a central $\alpha$-$\beta$ domain containing the nucleotide-binding site and a C-terminal bundle of 7 and 6 helices, respectively. The X-ray model confirms and extends all previous work on the structure of the large subunits, such as the fact that the distribution of 21 protease-cleaved sites in the isolated $\alpha$ subunit is similar to that of the $\beta$ subunit, thus providing experimental evidence for a similar folding topology of the two subunits (Tozawa et al., 1993).

From EM of negatively stained specimens followed by computer analysis (Boekema et al., 1992) it could be concluded that the overall shape of the isolated $\beta$ subunit of spinach $CF_1$ is also elongated. The $\beta$ subunit has dimensions of about 66–69 × 42–44 Å. This indicates that the packing of the large subunits in $CF_1$ and $MF_1$ could have a similar folding. Two independent low-resolution 3D reconstructions of bacterial $F_1$ from EM images also indicate that the $\alpha$- and $\beta$ subunits are elongated. In a model of Gogol et al. (1989a) these subunits are very elongated, e.g. 90 × 30 Å. In the model of Ishii et al., 1993 they are shown to be 70 x 30 Å.

### 2. Shape of the Small Subunits γ, δ and ε

By analytical ultracentrifugation experiments it was found that subunit $\gamma$ from *E. coli* $F_1$ is rather elongated (Dunn, 1982). EM analysis of a $CF_1$ particle, from which subunits $\delta$ and $\varepsilon$ were removed, showed that the diameter of the central mass was 25–28 Å, much smaller than the projected diameter of the large subunits (Fig. 2). Considering the mass of $\gamma$, 35.7 kDa, this indicates that it is an elongated subunit with a calculated length of 50 Å (Boekema et al., 1990). The X-ray map of bovine mitochondrial $F_1$ shows that the $\gamma$ subunit is even much longer: a 90 Å long $\alpha$-helix forms the center of the $MF_1$ structure, a second $\alpha$-helix of 60 Å forms a left-handed antiparallel coiled coil with the first helix (Abrahams et al., 1994).

The chloroplast $\gamma$ subunit has an interesting component, which is a flexible loop that includes a disulfide bond (McCarty and Hammes, 1987). The distance of this disulfide bond to two other sulfhydryl groups on $\gamma$, determined by fluorescence resonance energy transfer, was 44 and 47 Å, or about 38 Å, if projected in a plane parallel to the membrane. Thus this loop, which is important for ATPase activity, is really a loop protruding from the rest of the $\gamma$ subunit. The position of the S-S disulfide was also investigated by cryo-EM (U. Lücken, unpublished data) using a monomaleimidogold label (Wilkens and Capaldi, 1992). Results indicate that the S-S disulfide bridge is indeed extending far from the center of $CF_1$ (Fig. 1).

Unfortunately, the atomic structure of $\delta$ and $\varepsilon$

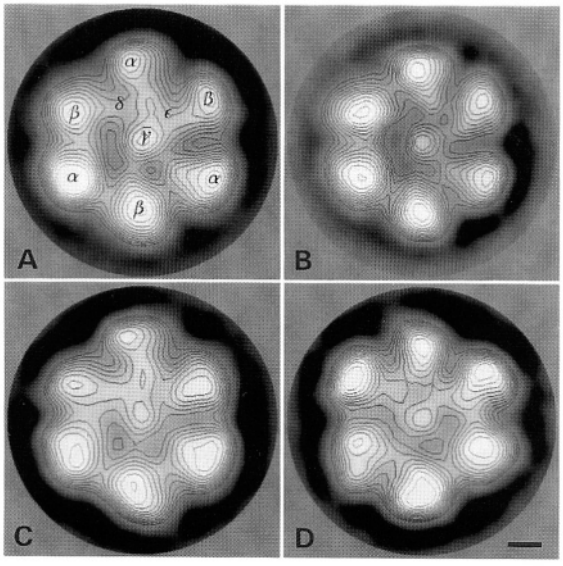


*Fig. 2.* Electron microscopy of negatively stained $CF_1$. (A) average image of holo-enzyme $CF_1$ in the hexagonal view (modified from Boekema et al., 1990); (B) average image of $CF_1$ depleted of subunits $\delta$ and $\varepsilon$ (modified from Boekema et al., 1990); (C) and (D) average images of projections from $CF_1$ particles arranged upside- up and upside-down on the carbon support. In general, in negatively stained samples the lower half of the particle is more strongly embedded in the stain layer and this leads to a stronger contrast of the features of this part in the projection. In the predominant view (A and the similar view C) the $\alpha$- and $\beta$ subunits that are close to $\delta$ appear less pronounced, because the structure is slightly lifted up by $\delta$. The $\alpha$- and $\beta$ subunits become more evenly embedded in the stain layer when the $\delta$ subunit is absent, as in Fig. B, or when $\delta$ is away from the carbon support in upside-down attached particles, as in D. The bar represents 20 Å.

could not be determined in the 2.8 Å density map (Abrahams et al., 1994). The chloroplast $\delta$ subunit has an elongated shape (Wagner et al., 1988). It is predicted to be an $\alpha$-helical protein with only limited $\beta$-structure. Conserved amino acids are found in the N-terminal start of a putative amphipathic $\alpha$-helix (Hoesche and Berzborn, 1993). The shape of the chloroplast $\varepsilon$ subunit is not known, $\varepsilon$ from *E. coli* has been crystallized (Codd et al., 1992), but the structure has not yet been reported.

### *C. Positions and Interactions of the Subunits*

From thermal denaturation experiments it was found that the major forces that stabilize $CF_1$ must be between the $\alpha$, $\beta$ and $\gamma$ subunits; subunits $\delta$ and $\varepsilon$ have little effect on the stability (Wang et al., 1993). Thus the three largest subunits with a total of 7 copies define the shape of $CF_1$. Nevertheless, the smallest subunits are not totally unimportant for the overall shape. It was demonstrated that the positions of the $\alpha$ subunits in $CF_1$ changed when $\delta$ or $\delta$ plus $\varepsilon$ were removed (Boekema and Böttcher, 1992).

Although not all interesting questions concerning the detailed structure can be answered, several conclusions concerning the arrangement of the large subunits and small subunits can be drawn:

1. The overall shape of $CF_1$ is asymmetric. This means that all three $\alpha$- and all three $\beta$ subunits 'see' a different surrounding environment. Several lines of evidence support this conclusion. For instance; the three $\beta$-$\beta$ center-to-center average distances in $CF_1$ are 79, 77 and 73 Å (Boekema and Böttcher, 1992). Similar asymmetrical distances were found for the $\beta$ subunits in *E. coli* $F_1$ (Wilkens and Capaldi, 1994). The 2.8 Å resolution structure of $MF_1$ (Abrahams et al., 1994) shows the asymmetry of the overall structure in a very detailed way. The main reason why the other X-ray map presents a symmetric $F_1$ structure (Bianchet et al., 1991) is the fact that a polypeptide chain can be better fitted if noisy data are 3-fold averaged. The large subunits not only 'see' a different surrounding but also have a slightly different conformation. For example: only one of the three $\alpha$ subunits of $CF_1$ reacts with Lucifer yellow which modifies lysine 378 (McCarty and Hammes, 1987). Similarly, Wilkens and Capaldi (1992) found that only one of the $\alpha$ subunits in *E. coli* $F_1$ has a cysteine group that reacts with maleimides. The inequivalent catalytic sites in $CF_1$ originate, of course, also from a structural difference (see chapters 24 and 25).

2. In the plane parallel to the membrane, the six copies of the large subunits alternate in position. This was first shown unambiguously by immuno-electron microscopy with monoclonal antibodies (Gogol et al., 1989b).

3. There is some evidence that the $\alpha$- and $\beta$ subunits in $CF_1$ are not exactly at the same height as shown in Fig 3. The $MF_1$ model from Bianchet et al. (1991) shows two slightly offset, interdigitated layers of large subunits. At the top, the $\beta$ subunits protrude about 15 Å higher than the $\alpha$ subunits. The $CF_1$ data indicate a similar arrangement

(Boekema et al., 1992). However, the 2.8 Å resolution structure of $MF_1$ shows that only one $\beta$ subunit ($\beta_{DP}$) is at a substantially higher level in the structure, whereas two $\alpha$ subunits are extending slightly more to the base of $F_1$.

4. A seventh mass seen in the center of the $\alpha_3\beta_3$ configuration (Fig. 2) is formed by the small subunits and consists mainly of subunit $\gamma$ (Boekema et al., 1990; Wilkens and Capaldi, 1992).

5. The small subunits are positioned in the lower half of $F_1$, which is the part of $F_1$ closest to the membrane. This was already inferred based on biochemical evidence, but recently Ishii et al (1993) directly showed that the central mass is in the lower half of $F_1$.

6. Subunit $\alpha$ interacts with an $\alpha$ subunit and with subunit I, a $F_o$ subunit, which contributes to the stalk connecting $F_1$ to $F_o$ (Beckers et al., 1992). Although subunit $\delta$ is partly hidden within $CF_1$, it must also be involved in the stalk, because it forms a protrusion on $F_1$. In original EM images the protrusion was barely visible, but the large subunits around $\delta$ look differently embedded in projections of negatively stained specimens (Fig. 2). The protrusion is clearly visible in the structure of bovine heart $MF_1$ (Abrahams et al., 1993), where it consists of two $\alpha$-helices in a coiled-coil making a 40 Å stem (Fig. 1D). But it needs to be confirmed if the entire protrusion consists of only the $\delta$ subunit.

7. In $CF_1$ the subunits $\gamma$ and $\varepsilon$ are close and interact with each other (McCarty and Hammes, 1987). Interestingly, a complex of $\gamma$ and $\varepsilon$ was purified and crystallized from *E. coli* (Cox et al., 1993).

8. According to Fig. 3, subunit $\gamma$ is the central $CF_1$ subunit that interacts with all other $F_1$ subunits and with the stalk formed by $F_o$. Because it protrudes from $MF_1$, it is very likely that conformational changes occurring in the stalk of $F_oF_1$ can be of catalytic relevance if mediated by subunit $\gamma$. But also other $CF_1$ subunits may interact with the stalk and be directly influenced by changes occurring in the stalk. In *E. coli* $F_1$ a $\beta$ subunit, different from the one which interacts with the $\varepsilon$ subunit, binds to the b subunit of the $F_o$ part (Wilkens et al., 1994).

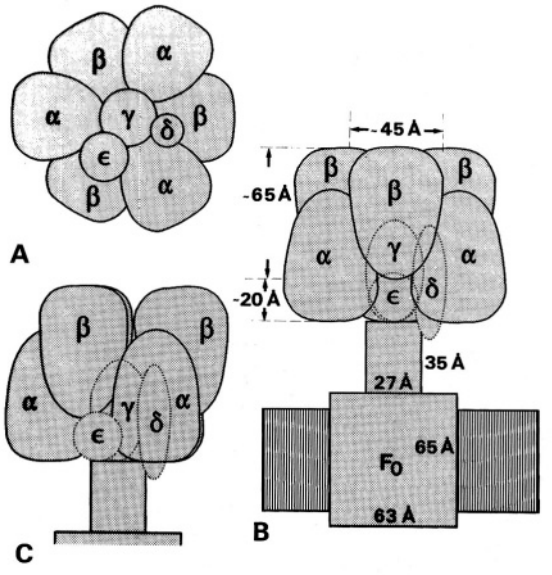


*Fig. 3.* Views of a model for $CF_1$ ATP synthase. A. The model in the hexagonal projection, parallel to the plane of the membrane, seen from the membrane. B. The model seen from aside in a 'symmetrical view'. C. The model seen from aside in an 'asymmetrical view'. The positions of 3B and 3C differ by 30°. The dimensions of the $F_o$ part and the stalk, symbolized as cylinders, have been determined, but the positions of the subunits within the membrane are still not fully clear.

### *D. Conformational Changes in Subunits and Subunit Positions*

It has been shown that there is evidence for movements of subunit $\varepsilon$ that could play a role in the catalytic mechanism and regulation of ATP synthesis/hydrolysis (Gogol et al., 1990; Boekema and Böttcher, 1992). Wilkens and Capaldi (1994) showed that both $\gamma$ and $\varepsilon$ subunits are mobile, probably by moving as a single domain through distances as great as 10–20 Å.

## III. Concluding Remarks

Further structural data are in demand, even after the determination of the $MF_1$ structure from beef heart mitochondria, which is a breakthrough for the field of bioenergetics. For the precise mechanism of ATP synthesis in chloroplasts it will also be necessary to solve the $CF_1$ structure at atomic resolution by X-ray diffraction. Only based on such a structure will all the biochemical data attain their full significance. But $F_1$ is not only a fixed structure, as seen by X-rays

in a 3D crystal. Large rearrangements, such as the movement of the $\gamma$ and $\varepsilon$ subunit cannot be easily studied by X-ray diffraction if the conformation is unstable. Here, cryo-EM, which usually results in a much lower resolution than X-ray diffraction, will continue to be of value.

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