The Conformation of the $\epsilon$- and $\gamma$-Subunits within the *Escherichia coli* F$_1$ ATPase*

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F$_1$ is the water-soluble portion of the ubiquitous F$_1$F$_0$ ATP synthase. Its structure includes three $\alpha$- and three $\beta$-subunits, arranged as a hexameric disc, plus a $\gamma$-subunit that penetrates the center of the disc akin to an axle. Recently Hhausrath et al. (Hausrath, A. C., Gruber, G., Matthews, B. W., and Capaldi, R. A. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 13697–13702) obtained an electron density map of *E. coli* F$_1$ at 4.4-Å resolution in which the coiled-coil $\alpha$-helices of the $\gamma$-subunit could be seen to extend 45 Å from the base of the $\alpha_6\beta_3$ hexamer. Subsequently the structure of a truncated form of the *E. coli* $\gamma$-subunit in complex with $\epsilon$ has been described (Rodgers, A. J. W., and Wilce, M. C. J. (2000) *Nat. Struct. Biol.* 7, 1051–1054). In the present study the 4.4-Å resolution electron density map of *E. coli* F$_1$ is re-evaluated in light of the newly available data on the $\gamma$ and $\epsilon$-subunits. It is shown that the map of the F$_1$ complex is consistent with the structure of the isolated subunits. When *E. coli* F$_1$ is compared with that from beef heart, the structures of the *E. coli* $\gamma$ and $\epsilon$-subunits are seen to be generally similar to their counterparts in the bovine enzyme but to undergo major shifts in position. In particular, the two long, coiled-coil $\alpha$-helices that lie along the axis of F$_1$ both unwind and rotate. Also the $\epsilon$-subunit rotates around the axis by 81° and undergoes a net translation of about 23 Å. It is argued that these large-scale changes in conformation reflect distinct functional states that occur during the rotation of the $\gamma$-subunit within the $\alpha_6\beta_3$ hexamer.

Recently Hhausrath et al. (7) obtained an electron density map of *Escherichia coli* F$_1$ at 4.4-Å resolution in which the coiled-coil $\alpha$-helices of the $\gamma$-subunit could be seen to extend 45 Å from the base of the $\alpha_6\beta_3$ hexamer. Other rod-like features in the electron density map were interpreted as additional $\alpha$-helices of the $\gamma$-subunit.

A subsequent electron density map of bovine F$_1$ inhibited with dicyclohexylcarbodiimide (DCCD)$^1$ at 2.4-Å resolution (8) revealed details of the $\gamma$-subunit not apparent in the analysis of the non-inhibited enzyme (4). Some aspects of the structure of the bovine $\gamma$-subunit were consistent with the interpretation of Hhausrath et al. (7), but others were not. In addition, the structure of a truncated form of the *E. coli* $\gamma$-subunit (identified as $\gamma'$) in complex with $\epsilon$ has been described recently (9). This also is consistent with some but not all aspects of the interpretation of Hhausrath et al. (7).

The purpose of the present study is to re-evaluate the 4.4-Å resolution electron density map of *E. coli* F$_1$ in light of the newly available data on the $\gamma$ and $\epsilon$-subunits. It is shown that the map is consistent with the structure of the isolated $\gamma'$ complex. Some rod-like features that were previously interpreted as $\alpha$-helices are seen to correspond to regions of $\beta$-sheet.

Although the $\gamma$ and $\epsilon$ subunits can be reliably placed in the electron density map of *E. coli* F$_1$, they are seen to undergo large changes in structure relative to their counterparts in DCCD-inhibited bovine F$_1$. In particular, the two long, coiled-coil $\alpha$-helices that lie along the axis of F$_1$, substantially unwind. Also the $\epsilon$ subunit rotates around the axis by 81° and undergoes a net translation of about 23 Å. It is argued that these large-scale changes in conformation reflect distinct functional states that occur during the rotation of the $\gamma$-subunit within the $\alpha_6\beta_3$ hexamer.

**EXPERIMENTAL PROCEDURES**

**Molecular Replacement and Refinement**—Crystals were grown as described (7). Prior to freezing, they were in a mother liquor of ~100 mM Tris, pH 7.2, 15% polyethylene glycol 8000, 15% glycerol, 100 mM NaCl, 10 mM MgSO$_4$, 40 mM Li$_2$SO$_4$, 0.05% NaN$_3$, 100 $\mu$M EDTA, 5 mM AMP-PNP, and 50 $\mu$M residual nucleotide from the 100 $\mu$M ATP included in the original crystallization mixture. The $\alpha_6\beta_3$ hexamer from bovine F$_1$ (Protein Data Bank code 1BMF) was positioned in the cell of *E. coli* F$_1$ by molecular replacement using the program AMORE (10). The calculation, which included all protein atoms, gave a result essentially identical with that obtained previously based on only the backbone atoms (7). First the six individual chains and then the three domains from each chain were subject to rigid-body refinement with the program TNT (11).

**Real-space Search**—A real-space search was carried out to check the placement of the $\gamma'$ subunits within the *E. coli* F$_1$ electron density map. First, a "model" electron density map at 4.4 Å resolution was calculated using the program SFALL from the CCP4 package (12) based on the

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**Experimental Conditions**—

1 The abbreviations used are: DCCD, dicyclohexylcarbodiimide; AMP-PNP, adenosine 5’-(\$\beta,\gamma\$)-imidotriphosphate.

**References**—

1. This paper is available on line at http://www.jbc.org.

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coordinates of the isolated γε subunits (9). These coordinates and the associated model map were then rotated and translated through the 4.4-Å resolution map for E. coli F₁, and the point-by-point products of the respective densities were summed using MAPMASK (12), MAPMAN (13, 14), and EDPDB (15). This gave, in effect, the non-normalized correlation between the model map and the experimental F₁ density. By not normalizing the correlation, we were able to visualize the respective contributions from the γ and ε subunits (Fig. 1).

Local Correlation Analysis—To quantitate the agreement between local regions of a model and an electron density map, we calculated correlation coefficients as follows. A segment of specified length, typically 10 residues, was omitted from the model, and the remainder was used to calculate a α₃-weighted 2Fᵣ – Fᵣ “omit” map (16). This map was then subject to solvent flattening using a mask for the entire model. This allowed density to be restored for the 10 “omitted” residues. The correlation within the volume of the omitted residues was then determined between this density and “model” density based on the assumed coordinates for the 10-residue segment. The model density was obtained by calculating an “Fᵣ” map where the amplitudes were the observed structure factors and the phases were from the complete model including the 10 residues in question. The procedure was repeated for each contiguous segment in the entire structure and the correlation plotted as a function of residue number. Programs from the CCP4 suite (12) were used for mask manipulation and solvent flattening.

Other Procedures—Figures were produced with the programs Molscript (17), O (18), and Mathematica (Wolfram Research, Inc.). Superpositions were carried out using the programs EDPDB (15) and Superimpose (19). Analysis of the axial rotation was done with EDPDB and Mathematica.

RESULTS

Placement of the γ- and ε-Subunits in the E. coli F₁ Structure—The crystals of E. coli F₁ used in the present analysis contain the α₃β₃ hexamer plus subunits γ and ε. As described previously (7), and based on the presumed similarity of the E. coli and bovine α- and β-subunits, it was possible to use the coordinates of the bovine α₃β₃ hexamer (4) to position the α₃β₃ hexamer within the E. coli F₁ crystals. The prior calculation was based on just the backbone atoms in the bovine α₃β₃ hexamer. A repeated calculation, including side-chain atoms as well, gave a virtually identical result (see “Experimental Procedures”). An electron density map at 4.4-Å resolution, phased on the coordinates of the α₃β₃ hexamer and designed to show density for the γ- and ε-subunits, was also very similar to that obtained previously (cf. Fig. 2 of Ref. 7).

Visual inspection showed that it was straightforward to place the model for the isolated complex of E. coli γε (9) into this electron density map (γ is identical to γ except that the two α-helices that form the coiled-coil have been shortened to give a more compact structure). In particular, the α-helices that include residues 20–58, 91–108, and 211–248 of the γ-subunit (E. coli numbering system) could be superimposed on rods of density that had previously been labeled as helices A, C, and G (7). The positioning of γ and ε (considered as a single unit) was optimized by rigid-body refinement (see “Experimental Procedures”).

To make an independent check on the placement of the γε subunits, the coordinates were both rotated and translated away from their assumed position and the correlation calculated with the 4.4-Å resolution electron density map (see “Experimental Procedures”). Representative translational searches are shown in Fig. 1. As can be seen, the correlation falls precipitously once the γε subunits are moved as a pair away from their assumed position (Fig. 1A). Similar results are obtained when γ and ε are moved independently (Fig. 1, B and C), showing that their relative positions in the F₁ structure are the same as in the isolated γε complex.

Our aim was to obtain an electron density map that was of as high quality as possible and, at the same time, could be used to compare in an objective fashion the placement of the γε subunits described above with the partial model for γ described by Hausrath et al. (7). To do so, we calculated a map that was phased with the α- and β-subunits and was subject to solvent flattening using an all inclusive mask that included the α₃β₃ hexamer, the γε pair, and the partial γ model. The resultant map is shown in Fig. 2. In general there is good agreement with the helical regions of both γ and ε (but see the following section). The density in the vicinity of the β-sheet regions is not
as clear and could not be interpreted without knowledge of the β-subunit structure.

Conformation of the ε-Subunit—It was shown previously that the structure of the isolated E. coli ε-subunit (20, 21) differed from that in the γε complex (9). This subunit consists of an N-terminal β-sandwich domain of about 80 residues plus a C-terminal α-helical region of about 50 residues. In the isolated form, the C-terminal region forms an antiparallel helical hairpin that folds compactly against the β-sandwich (we will refer to this as the “down” conformation). In the γε complex, the two α-helices extend away from the N-terminal domain in an “up” configuration. The structure of the analogous β-subunit in the yeast and bovine structures resembles the down form of ε.

To search for either of these two possible conformers in the E. coli F₁ structure, and to do so without bias, we constructed a solvent-flattened map, phased on the αεβ₃ hexamer, with a mask that included both possible structures for ε. The up structure was represented by ε from the γε complex and the down structure by the β-subunit from yeast F₁F₀ (22). The resulting map (Fig. 3A) has continuous density for the first α-helix in the “extended” conformation (helix ε–α₁ of Ref. 9), consistent with the conformation observed in the γε complex. However, only weak, discontinuous density can be seen for the second helix (ε–α₂). The electron density in the region corresponding to the helical hairpin in the yeast β-structure is also weak and discontinuous (Fig. 3A). Therefore, the ε-subunit in the crystal structure of E. coli F₁ is inferred to have a conformation that is akin to that seen in the γε complex, except that the α-helix at the extreme C terminus is either disordered or has a different conformation. Indeed, if the C-terminal α-helix of ε were to have the same conformation as in γε it would clash sterically with the closest β-subunit.

As a further test to differentiate between the two different conformations of the ε-subunit we evaluated a series of omit maps for each of the alternative models in which 10 residues at a time were omitted sequentially (see “Experimental Procedures”). Within the N-terminal β-sheet sandwich, the correlation of the up and the down models with the observed map is roughly the same (Fig. 3B). This is as expected because the up and down models are essentially the same in this region. Within α-helix ε–α₁, however, the correlation is much higher for the up than for the down conformation. At the extreme C terminus (helix ε–α₂) the correlation coefficients for both structures are low because the electron density does not support either.

Conformational Differences between E. coli and Bovine F₁—One of the most striking features of the present analysis is that it reveals a major structural change between the structure of E. coli F₁ and that of the bovine enzyme (Fig. 4).

To compare the two structures, the respective αεβ₃ hexamers were superimposed and the discrepancies calculated between the corresponding backbone atoms of the other subunits. For the γε-subunits the root-mean-square difference in the backbone of the 152 residues that are structurally most similar is 5.6 Å. Comparing the β-sandwich domain of the ε-subunit with the β-sandwich of the corresponding β-subunit in bovine F₁, the root-mean-square difference in backbone is 25.5 Å (Fig. 4). In contrast, optimal superposition of these subunits, considered in isolation, yields root-mean-square deviations of 1.7 and 1.5 Å, respectively. Thus, the differences between the stalk subunits of E. coli and DCCD-inhibited bovine F₁ consist largely, although not exclusively, of rigid-body movements. The center of mass of the β-sandwich in ε compared with its counterpart in bovine F₁ has shifted by 23.0 Å. There is also a radial displacement of 3.5 Å and a significant reorientation. That part of the
Fig. 4. Comparison of the structure of E. coli F1 (A) (this work) and the DCCD-inhibited bovine enzyme (B) (8). The direction of view is the same as in Fig. 2. The α- and β-subunits are shown in green, the γ-subunits in red, and the ε-subunit (E. coli) and its ε-counterpart in beef are in yellow. The two C-terminal helices of the E. coli ε-subunit are shown as in the isolated γε structure (9), although density for the second of these helices (ε-c2) is not seen in the F1 density map. The α- and β-subunits of the E. coli and beef enzymes are in essentially identical locations. The γ-subunit and ε-subunit of the E. coli enzyme have rotated substantially relative to their counterparts in the bovine enzyme. Also, the C-terminal helices of the E. coli ε-subunit extend away from the N-terminal domain toward one of the β-subunits, whereas the corresponding α-helices in beef heart F1 remain in the down configuration, packed against the N-terminal domain.

The differences between the γ-subunits of the E. coli and bovine enzymes, although at first sight less obvious, are also substantive. Fig. 5 shows the position of the coiled-coil of the γ-subunit of the bovine enzyme relative to the electron density of the stalk in the E. coli enzyme. The figure is drawn such that the αβ3 hexamers of the respective enzymes superimpose. At the top of the coiled-coil, the N- and C termini of γ superimpose well. At the bottom, however, the coiled-coil has partially unwound, contributing to the 81° rotation of the ε-subunit.

As noted above, there are also large changes in the conformation of the helical C-terminal region of ε. In bovine F1 this region forms a compact helical hairpin, analogous to that seen in the structure of the isolated E. coli ε-subunit (8, 20, 21). In contrast, in the E. coli structure this helical region appears to be extended, with a conformation similar to that seen in the structure of the γε complex (Fig. 3A) (9). As a result of these structural changes, the helical region of the C terminus of the ε-subunit of the F1 enzyme extends upward and contacts the catalytic subunit, βTP (Fig. 4). In contrast, in the DCCD-inhibited form of bovine F1, the corresponding α-helical region is located at the base of the stalk complex, approximately in the plane of the membrane (8).

Comparison with Yeast F1F0—A composite model has been described for the αβεεεε subcomplex of yeast F1F0 (22). To compare this model with E. coli F1, the structures of the respective αβ3 hexamers were superimposed and the discrepancy determined between the bacterial ε-subunit and the analogous δ-subunit in yeast. For the 81 Co carbons of the β-sandwich domain, the root-mean-square difference is 6.5 Å, and the centers of mass differ by 5.1 Å. When considered in isolation the root-mean-square difference is 0.7 Å. However, in the yeast composite model, the free E. coli ε-subunit was used to represent the yeast δ-subunit (22), and so this value may be artificially low. A comparison of the relative distance between the centers of mass indicates that the position of the N-terminal domain of the ε-subunit in E. coli F1 is much more similar to the position of the corresponding δ-subunit in the yeast structure (distance of 5.1 Å) than the position of the same subunit in the DCCD-inhibited bovine structure (distance of 23.0 Å). Note, however, that in the yeast F1F0, structure the C-terminal domain of ε is in the down conformation as in DCCD-inhibited bovine F1 and in contrast to the up arrangement in E. coli F1. The differences are summarized in Table I.

Discussion

Re-evaluation of the 4.4-Å resolution electron density map of E. coli F1 (7) shows that it is consistent with the 2.1-Å resolution crystal structure of the isolated γε complex (9). As shown in Fig. 2, the structure of the latter complex satisfactorily accounts for the observed electron density. Rod-like electron density features seen in the map were previously interpreted as α-helical regions in the γ-subunit. Some of these are confirmed, but others are not. In particular, the extension of the long coiled-coil α-helices that constitute the “axle” of the F1 particle is seen in the structure of isolated γε as is the helix that includes residues 88–103. On the other hand, other density features putatively identified as α-helices are now seen to correspond to regions of β-sheet.
Conformations of the stalk subunits in bovine, E. coli, and yeast F1

The table summarizes the differences between the overall conformations of the stalk subunits as seen in DCCD-inhibited bovine F1 (8), E. coli F1 (this work), and yeast F1F0 (22). In the “unwound” state, the coiled-coil α-helices of γ are unwound (Fig. 5), and the subunit as a whole is also rotated. In the up state, the two α-helices in the C-terminal domain of ε (or δ for bovine and yeast) extend toward the αβ3 hexamer, whereas in the down state they remain packed against the N-terminal domain (Figs. 3A and 4).

| Structure       | Conformational state |
|-----------------|----------------------|
| Bovine F1       | Wound                |
| E. coli F1      | Zero rotation        |
| Yeast F1F0      | Unwound              |
| **γ-Subunit**   | ~90° rotation        |
| **ε- or δ-Subunit** | ~90° rotationa    |
| C-terminal      | Down                 |
| domain of ε or δ | Up                   |

* The quoted rotation of 37° (8) is about an axis remote from the center of F1.

Do the Structures of E. coli and Bovine F1 Represent Distinct Functional States?—As shown in Figs. 4–6 and in Table I, when the αβ3 hexamers of E. coli and DCCD-inhibited bovine F1 are superimposed, the “stalk” subunits (γ and ε in E. coli) differ substantially. Relative to the bovine enzyme, the two long, coiled-coil α-helices of γ that form the axle of the F1 particle unwind and rotate substantially (Fig. 5) rotating the β-sandwich domain of the ε-subunit around the central axis by 81° compared with its counterpart in the bovine enzyme (Fig. 6C). There is also a radial displacement and significant reorientation of the ε-subunit.

The nucleotide content of the DCCD-treated enzyme has been reported (8). Mg-AMP-PNP is bound in two β-subunits, whereas the catalytic site in the third is empty. Unfortunately, the level of resolution obtained so far for the crystals of E. coli F1 do not allow identification of nucleotide occupancy. The enzyme was crystallized in the presence of Mg-AMP-PNP and MgATP, along with azide and it is likely that some catalytic sites contain AMP-PNP or other ligands resulting from the expected hydrolysis of AMP-PNP and ATP during crystallization. Likewise, the nucleotide content of catalytic sites in the yeast F1F0 structure remains undefined, although crystallization conditions were more similar to those of the ECF1 than the DCCD-MF1 structure. Without the detailed understanding of catalytic site occupancy in the three crystal forms, it is not possible to relate the movements of γ and ε with individual steps in catalysis. However, we believe that the different states are physiologically relevant structures because such movements of the γ- and ε-subunits have been seen before within the E. coli F1, and shown to be nucleotide-dependent. For example, Wilkens and Capaldi (23) used cryoelectron microscopy to examine the position of the ε-subunit within the αβ3 hexagon. The ε-subunit was specifically labeled with a small (14 Å) gold particle at residue 38. A corresponding electron-dense feature was found close to a β-subunit with uncleaved ATP trapped in the enzyme (by rapid freezing) but moved along with the γ-subunit counterclockwise to the adjacent α-subunit when ATP was allowed to turn over before freezing (Fig. 6, A and B). This represents a movement of the ε-subunit of ~20 Å. As shown in Fig. 6, the 81° rotation of ε suggests by the respective crystal structures is somewhat greater than that suggested in the electron micrographs. The overall 20-Å movement of ε shown by the electron microscopy is, however, remarkably close to the 23-Å difference in the positioning of ε in the two crystal structures, emphasizing the essential concordance of the two very different structural approaches.

Also, a nucleotide-dependent shift in the position of Glu318 in the ε-subunit has been observed in cross-linking experiments (24, 25). In the presence of ATP, a cysteine at this site can be cross-linked to an α-subunit, whereas in ADP + Mg2+ + Pγ, the cysteine can be covalently linked to residue 381 at the bottom of a β-subunit. Finally, Yasuda and co-workers (26, 27) have recently followed the rotation of the γ-subunit in the αβ3 subcomplex of Bacillus PS3 F1. They were able to dissect the 120° rotation that occurs with each ATP hydrolyzed into two steps, one of ~90° and the second of ~30°. They show that the +90° rotation is dependent on ATP binding, whereas the 30° step is independent of ATP binding, and they propose that this is due to product release. By reference to the structures described here, the rotation step of ~90° could correspond to the partial unwinding and rotation of the α-helical coiled-coil that is a distinctive feature of the ε-subunit.

Different Arrangements of the C-terminal Domain of the ε-Subunit—The position of the C-terminal α-helical domain of ε is radically different between E. coli F1 and both the DCCD-inhibited MF1, and the yeast F1F0 structures (Table I). In the E. coli F1 structure, these two α-helices are separated and stretch upwards to contact the αβ3 hexamer. In the other two structures the two α-helices are in a hairpin structure compactly folded against the β-sandwich and located toward the F0 in the
intact ATP synthase. It has recently been shown that both positions of the C-terminal domain can exist in E. coli F₁F₀ (28). It proved possible to cross-link a cysteine placed at position 118 of ε to a cysteine at position 99 of γ, as predicted by the model of the isolated γ and ε subunits reported by Rodgers and Wilce (9). This initial evidence that the purified complex of γ and ε is organized as in the intact enzyme is now strongly confirmed here. What remains to be established is the precise location of the very C-terminal helix 110–113, which is not well represented by density in the 4.4-Å resolution map of E. coli (Fig. 3A).

As the E. coli ATP synthase is fully functional and therefore must work as a rotary motor in mutants missing the C-terminal domain of ε (28), the movements of this domain described here are not tied compulsorily to the unwinding and rotation of the γ- and ε-subunits. As discussed already, the β-sandwich and C-terminal domains of ε appear to have separate functions. The up arrangement of the α-helices of ε causes inhibition of ATPase activity (but not ATP synthesis) when ADP is trapped in the enzyme (28). There remains a debate about whether this inhibitory ADP is in a catalytic or noncatalytic site(s).

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