Journal of Bioenergetics and Biomembranes, Vol. 32, No. 5, 2000

What Is the Role of ε in the *Escherichia coli* ATP Synthase?

Steven B. Vik[1]

The ATP synthase from *Escherichia coli* is a prototype of the ATP synthases that are found in many bacteria, in the mitochondria of eukaryotes, and in the chloroplasts of plants. It contains eight different types of subunits that have traditionally been divided into F_1, a water-soluble catalytic sector, and F_o, a membrane-bound ion transporting sector. In the current rotary model for ATP synthesis, the subunits can be divided into rotor and stator subunits. Several lines of evidence indicate that ε is one of the three rotor subunits, which rotate through 360 degrees. The three-dimensional structure of ε is known and its interactions with other subunits have been explored by several approaches. In light of recent work by our group and that of others, the role of ε in the ATP synthase from *E. coli* is discussed.

KEY WORDS: ATP synthase; ATP hydrolsis; ε subunit; *Escherichia coli*; F_1-ATPase; inhibition; rotation; ATP synthesis.

INTRODUCTION

The ATP synthase (or F_1F_o-ATPase) from the bacterium *Escherichi coli* is a membrane-bound enzyme that synthesizes ATP from ADP and inorganic phosphate in response to an electrochemical proton gradient across the plasma membrane. In this regard, it is functionally similar to the enzymes found in mitochondria and chloroplasts. Under anaerobic conditions, the bacterial enzyme functions in reverse and translocates protons across the plasma membrane at the expense of ATP hydrolysis. [For recent reviews see Boyer (1997); Nakamoto *et al*. (1999).]

The ATP synthase from *E. coli* contains eight different subunits named $\alpha, \beta, \gamma, \delta, \varepsilon, a, b,$ and c in a stoichiometry of 3:3:1:1:1:1:2:9–12 (Foster and Fillingame, 1982). All of these subunits have homologs in the mitochondrial enzymes, which tend to include additional subunits as well. In the *E. coli* enzyme, all eight of the subunits are essential and a picture is developing of the role of each. Traditionally, subunits have been assigned to the F_1 or the F_o sectors, which can be generated from the intact enzyme by various *in vitro* treatments. F_1 is a water-soluble complex that can hydrolyze ATP [for recent reviews see Allison (1998); Weber and Senior (1997)]. In the *E. coli* enzyme, it is composed of $\alpha, \beta, \gamma, \delta,$ and ε. F_o is a membrane-bound complex that can transport protons [for recent reviews see Fillingame *et al*. (1998); Altendorf *et al*. (2000)]. In *E. coli*, it contains the *a*, *b*, and *c* subunits. In view of the current rotary model (Elston *et al*., 1998; Junge, 1999; Adachi *et al*., 2000) of the ATP synthase it is more appropriate to classify the subunits as belonging to the rotor or the stator.

The stator subunits are thought to include $\alpha, \beta, \delta, a,$ and *b*. The three catalytic sites for ATP synthesis are formed by the α and β subunits, which are arranged in an alternating pattern with pseudo-sixfold symmetry (Abrahams *et al*., 1994). The δ and *b* subunits interact (Häsler *et al*., 1999; McLachlin *et al*., 1998; Sawada *et al*., 1997a) and fix the $\alpha_3\beta_3$ sector (Ogilvie *et al*., 1997; Rodgers and Capaldi, 1998) to the membrane through the extended *b* subunit with its N-terminal membrane-spanning region (Dmitriev *et al*., 1999a). The *a* subunit completes the membrane anchor of the stator (Jäger *et al*., 1998; Long *et al*., 1998; Valiyaveetil and Fillingame, 1998), and also provides the functionally important interface with the *c* subunits (Jiang and Fillingame, 1998; Kaim *et al*., 1998).

The rotor subunits are thought to include γ (Noji *et al*., 1997), which fills the central cavity formed by the $\alpha_3\beta_3$ hexamer (Abrahams *et al*., 1994) and which extends to the membrane surface and *c* subunits (Hausrath *et al*., 1999). Protons are thought to be carried by a rotating oligomer of *c* subunits (Dmitriev *et al*., 1999b; Pänke *et al*.,

[1] Department of Biological Sciences, Southern Methodist University, Dallas, Texas 75275. e-mail: svik@mail.smu.edu

0145-479X/00/1000-0485$18.00/0

2000; Sambongi *et al*., 1999) that interact with the stator subunit *a* and drives the rotation of γ. The coordinated interactions between γ and the β subunits is thought to cause the binding changes at the catalytic sites that are necessary for net ATP synthesis (Oster and Wang, 2000). Thus, all subunits seem to have clear roles, with the exception of ε.

EARLIER STUDIES OF THE *Escherichia coli* ε SUBUNIT

Subunit ε was isolated from the *E. coli* enzyme in 1977 (Smith and Sternweiss, 1977) and was shown to have two important properties: It inhibited ATP hydrolysis by the *E. coli* F_1-ATPase (Laget and Smith, 1979) and was essential for the binding of F_1 to F_o. In 1982, purified ε and γ subunits were shown to form a tight complex with a K_d on the order of 1 nM (Dunn, 1982). Starting in 1984 (Lötscher *et al*., 1984a), a series of cross-linking studies showed that ε occupied a central position in the ATP synthase and could be cross linked to α, β, γ, and *c* subunits (Aggeler *et al*., 1992; Dallmann *et al*., 1992; Grüber and Capaldi, 1996; Tang and Capaldi, 1996). It was shown that the conformation of ε was sensitive to nucleotide binding at the catalytic sites (Mendel-Hartvig and Capaldi, 1991a, b) and, conversely, that the presence of ε slowed the release of P_i during ATP hydrolysis at substoichiometric concentrations of ATP (so-called unisite catalysis) (Dunn *et al*., 1987). The three-dimensional structure was solved by NMR studies (Wilkens and Capaldi, 1998; Wilkens *et al*., 1995) and by X-ray crystallography (Uhlin *et al*., 1997), and shown to have two domains, a 10-stranded β sandwich and an α-helical hairpin (see Fig. 1). The similar chloroplast ε has been reviewed recently (Buckley *et al*., 1999).

In the past 2 years, evidence has been provided for the rotation of ε during ATP hydrolysis by the F_1-ATPase. Using a thermophilic bacterial F_1, Yoshida and co-workers (Kato-Yamada *et al*., 1998) showed by video microscopy that fluorescently labeled actin filaments attached to ε rotated through 360 degrees during ATP hydrolysis. The features were very similar to those when the actin filaments were attached to γ (Noji *et al*., 1997). Using the *E. coli* enzyme, Cross and co-workers were able to show that ε rotated with respect to a β subunit, in a chemical cross-linking approach (Bulygin *et al*., 1998). Junge and co-workers developed a new technique, polarized, confocal fluorometry, to show that ε (and also γ, but not δ) rotates relative to the $\alpha_3\beta_3$ hexamer (Häsler *et al*., 1998).

In 1999, a new crystal structure (Stock *et al*., 1999) of the yeast mitochondrial ATP synthase was published in which the location of ε relative to α, β, and *c* subunits was shown. (Note, in the mitochondrial nomenclature the subunit homologous to *E. coli* ε is called δ.) Since this structure was solved to a resolution of only 3.9 Å, no information is yet available about the locations of side chains. The *c* subunits were found in a ring of ten subunits, rather than the expected nine or twelve (Jones and Fillingame, 1998). As in previous structures (Abrahams *et al*., 1994; Bianchet *et al*., 1998), only part of γ was resolved. Interestingly, in another low-resolution crystal structure published in 1999 from the *E. coli* F_1-ATPase (Hausrath *et al*., 1999), γ was more completely resolved, but ε was not detected. From the point of view of the new structure (Stock *et al*., 1999), previous findings will be reviewed and the role of ε will be discussed.

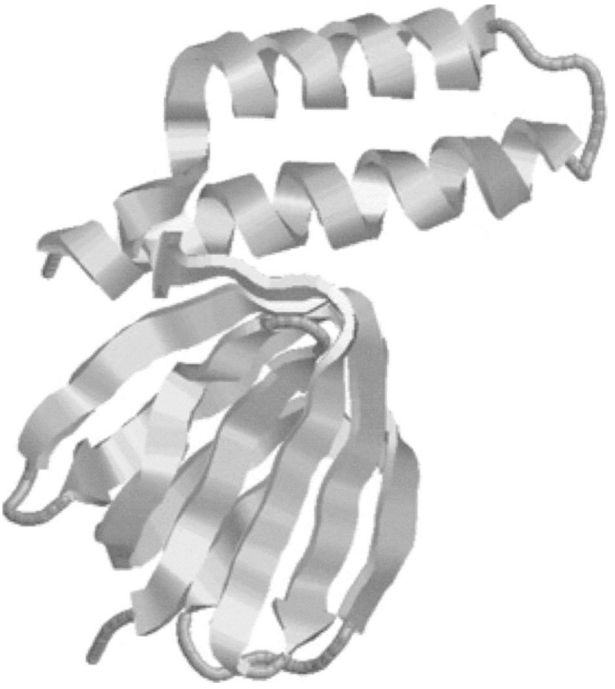

Fig. 1. The two domain structure of ε. The N-terminal 10 stranded β-sandwich domain is shown at the bottom. The C-terminal two antiparallel α-helices are shown at the top. This figure is derived from Protein Data Bank file laqt (Uhlin *et al*., 1997).

INTERACTIONS BETWEEN γ AND ε

The surface of ε that interacts with γ is thought to be framed by three residues, S10, H38, and T43 (Aggeler *et al*., 1992; Tang and Capaldi, 1996). After mutagenesis of these residues to Cys and reaction of the ATP synthase with the cross linker TFPAM [*N*-(4-azido-2,3,5,6-tetrafluorobenzyl)-3-maleimidopropionamide], ε was covalently cross linked to γ. As shown in Fig. 2A, these

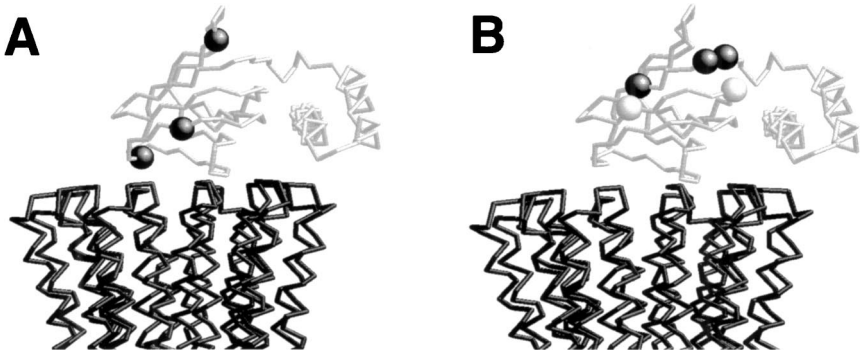

Fig. 2. Surface of ε proposed to interact with γ. The top half of the 10 *c* subunits and the ε subunit are shown. This figure is derived from Protein Data Bank file 1 qo 1 (Stock *et al*., 1999). (A) Residues in ε that when changed to Cys can be cross linked to γ are shown as black spheres. These residues, from left to right are H38, T43, and S10. (B) Residues in ε that when changed to Ala result in altered affinity for F_1 binding are shown as spheres. Those in black, from left to right, are T77, D81, and T82. These mutants have lower affinity. Those in white, from left to right, are E70 and S65. These residues have increased affinity.

residues form a narrow triangle on one surface of ε that is roughly perpendicular to the cytoplasmic surface of the membrane (or the *c* subunits). These residues are facing the interior of the ring of *c* subunits, a likely location for the γ subunit. Similar cross links have also been found from ε to γ in the chloroplast enzyme (Schulenberg *et al*., 1997).

The sites of five mutations that were constructed and analyzed in our group are indicated in Fig. 2B (Xiong and Vik, 1995; Xiong *et al*., 1998). These residues are found on two consecutive β-strands (8 and 9) that lie just between the β-strands that contain S10 and T43 (Fig. 2A). When these residues were mutated to Ala, the resulting ε subunits were shown to have altered affinity toward ε-free F_1. Thus, both structural and functional evidence places them at the γ-binding site. What is especially interesting is that those residues shown in black, T77, D81, and T82, all resulted in lower affinities when mutated to Ala, while those shown in white, E70 and S65, had higher affinities than the wild type. General polarity or charge effects would seem to be ruled out since each group contained residues with side chains of both carboxyl and hydroxyl groups. Similar results were obtained by Kanazawa and co-workers (Sawada *et al*., 1997b) when they subjected γ to random mutagenesis. Using a yeast two-hybrid system, they identified mutants with both higher and lower affinities toward ε. When analyzed in the context of the entire ATP synthase, the mutant with higher affinity γ-G85D caused lower ATPase activity in membrane preparations, while those with lower affinity, γ-M102T and γ-D141V, caused higher ATPase activity.

While it is possible that Nature has overlooked an opportunity to maximize the binding between ε and γ, other explanations might exist to explain this. In the three-dimensional structure of ε residue, S65 is very close to residues D81 and T82 and residue E70 is very close to T77 (Fig. 2A). Our results indicate that the most favorable interactions are mediated through residues 77, 81, and 82. When E77 and S65 are changed to Ala, the interactions are stronger. This is consistent with an electrostatic interaction becoming stronger in a more hydrophobic environment. The crystal structure (Uhlin *et al*., 1997) of ε also reveals several bound water molecules in the vicinity of S65, which would likely be lost in the S65A mutant.

One possibility for the suboptimal binding between ε and γ is that during ATP synthesis slightly different conformations of ε or γ might occur as a consequence of interactions with F_o subunits that are rotating (Pänke *et al*., 2000) or changing in conformation (Rastogi and Girvin, 1999). Under these transient conditions, E77 and S65 might become important for maintaining proper interactions between ε and γ. Conformational changes in ε are likely to occur if trypsin sensitivity changes are to be explained. Isolated ε is known to be rather resistant to trypsin digestion (Wilkens and Capaldi, 1998), as is ε in an F_1 or F_1F_o complex, if ATP + Mg^{2+} is present. However, if trypsin digestion occurs in the presence of AMP–PNP or ATP + EDTA, proteolysis of ε proceeds more rapidly (Mendel-Hartvig and Capaldi, 1991a, b). The sites of proteolysis are thought to be in the α-helical regions at the C-terminus of ε. The efficiency of cross linking from ε to other subunits is also sensitive to the same nucleotides (Aggeler *et al*., 1992).

Any conformational changes that occur within ε must be rather limited, according to recent work (Schulenberg and Capaldi, 1999). They constructed four double mutants in ε, each with two Cys residues that could form internal disulfides. These disulfides were designed to occur between the two terminal α-helices or between an

α-helix and the β-sandwich domain. None had a significant effect on ATP synthesis or ATP-driven proton translocation. The largest changes seen were in the specific activities of ATP hydrolysis by F_1F_o preparations. One mutant, A94C/L128C, showed approximate twofold increased activity relative to the wild type, whether or not the disulfide had formed. A second mutant, M49C/A126C, also showed approximate twofold increased activity relative to the wild type, but only after the disulfide had formed. This increase in activity could be related to the role of the C-terminal region of ε in inhibition of ATPase activity.

It was reported by Kuki *et al.* (1988) that the C-terminal half of ε was not necessary for the active ATP synthase from *E. coli*. Amino-terminal fragments with as few as 78 of the full-length 138 amino acids were shown to be capable of forming membrane-bound ATPase activity and ATP synthesis, as judged by allowing growth on succinate-minimal medium. ATP-driven proton pumping was also shown to be at wild-type levels. However, when F_1 was purified from such membranes, no ε was found, and accordingly, no concentration-dependence of ATPase activity was seen.

Slightly longer amino-terminal fragments of 80 and 93 amino acids were seen to purify with F_1 at substoichiometric fractions of 0.8 and 0.2, respectively. Only the larger fragment, 93 amino acids, showed concentration-dependent inhibition of ATP hydrolysis, but it was not to the same extent as wild type. From these experiments it was not possible to determine to what extent this truncated ε subunit could inhibit ATP hydrolysis at full occupancy.

In 1998, this question was reexamined by our group (Xiong *et al.*, 1998) by purification of three truncated forms of ε containing 80, 94, and 117 amino acids. The shortest fragment showed no inhibitory effects on ATP hydrolysis when added to ε-free F_1. We could not rule out altered folding of the purified protein. However, no growth on succinate-minimal medium was observed, indicating a nonfunctional ε *in vivo*. The fragments of 94 and 117 amino acids showed about 50 and 80% of the wild-type inhibition, respectively, but only when assayed at 300 nM, about 100 x the apparent wild-type K_d. In our expression system, these truncations were also not without effect on growth yield, but that was probably a consequence of reduced levels of membrane-bound ATPase. The longer mutants, 94 and 117, were capable of near-normal ATP-driven proton pumping.

In summary, the loss of the two α-helical segments at the C-terminus of ε, beginning at about residue 90, weakens the binding of ε to F_1. In the context of F_1F_o, the ε binding might be enhanced relative to F_1 due to interactions between γ, ε, and *c* subunits. If so, truncated ε might remain bound *in vivo*, but be lost during F_1 isolation. The C-terminal α-helices seem to be directly involved in the inhibitory property toward F_1-ATPase, but their role in ATP synthase function is less clear. One potential difficulty is that assays of ATP-driven proton translocation using acridine dyes are not strictly linear, so that differences from wild type might be over- or underestimated using a particular set of conditions. Such limitations may also apply to assays of ATP synthesis.

The question of whether the membrane-bound ATP synthase is inhibited by ε has been discussed recently (Capaldi and Schulenberg, 2000; Kato-Yamada *et al.*, 1999; Nakamoto *et al.*, 2000). It seems that it is inhibited about twofold, based on the observed stimulation of three- to fourfold by LDAO (lauryl dimethylamine oxide) treatment (Lötscher *et al.*, 1984b; Xiong and Vik, 1995), and in consideration of the results discussed above. The ATPase activities of membranes containing the two truncated forms of ε (94 and 117) are stimulated less than twofold by LDAO (Xiong *et al.*, 1998), indicating a lack of inhibition by ε (Dunn *et al.*, 1990). Difficulty in resolving this issue results from the situation that without ε there is no binding of the F_1 to F_o, in the case of the *E. coli* enzyme. Therefore, while some inhibition does occurs, it may not be a direct consequence of ε, but rather be due to F_1–F_o interactions.

Another reason to consider the role of the C-terminal α-helices in inhibition of the F_1-ATPase is the number of cross links that have been detected between residues in ε and the α and β subunits. In Fig. 3, the recent crystal structure of the ATP synthase is shown with the corresponding *E. coli* residues α-S411 and ε-S108 marked. When mutated to Cys, these residues can form a disulfide bond (Aggeler and Capaldi, 1996). Similarly, ε-S108 can be cross linked to β-E381 (Aggeler *et al.*, 1995). The residue ε-135 is also marked to show the C-terminus of ε. The actual C-terminal residue M138 when changed to Cys can be cross linked to the β subunit by a TFPAM (Tang and Capaldi, 1996). Figure 3 is designed to show the distances involved in forming these cross links. If ε remains in contact with the *c* subunits, as indicated by the ε-E31C cross link to *c*-Q42C (in a special double-sized *c* subunit), as shown recently (Schulenberg *et al.*, 1999), and if the C-terminal α-helices do not undergo large conformational changes, then other mechanisms must be considered to explain the close proximity that will be necessary between ε and α or β. One possibility is that during ATP synthesis or hydrolysis there is considerable twisting and bending of *b* and γ subunits that brings α and β close to ε. Recent work by Cain and co-workers indicates that the *b* subunits can be shortened (Sorgen *et al.*, 1998) or lengthened (Sorgen *et al.*, 1999) by 10–14 residues without great effect. Flexibility in γ can only be inferred by the difficulties in finding parts of it in the various crystal structures

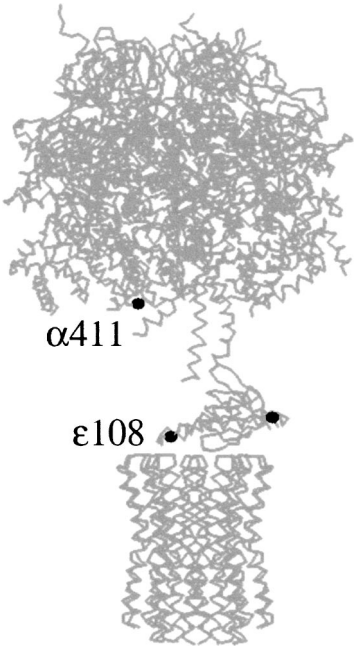

Fig. 3. Overall view of the ATP synthase. The residues labeled have been shown to form a disulfide bond after mutation of both to Cys. The unlabeled black sphere at the right represents the C-terminus of ε. This residue can also be cross linked to the β subunit. It can be seen that these distances cannot be bridged as a result of small conformational changes. This image is derived from Protein Data Bank file 1qo1 (Stock *et al*., 1999).

published so far (Abrahams *et al*., 1994; Bianchet *et al*., 1998; Stock *et al*., 1999; Hausrath *et al*., 1999). The role of elasticity in F_1 has been considered in several models recently (Cherepanov *et al*., 1999; Pänke and Rumberg, 1999).

INTERACTIONS BETWEEN F_o AND ε

A specific interaction between ε and *c* subunits was first indicated by the discovery (Zhang *et al*., 1994) of second–site suppressors in ε (E31L,V) of a *c* mutation (Q42E). After mutagenesis to Cys, these subunits could be cross linked by a disulfide bond (Zhang and Fillingame, 1995). Later, it was shown (Hermolin *et al*., 1999) that six other residues in ε were capable of forming disulfides with one or more residues in the *c* subunit (40, 42, or 44). These ε residues are highlighted in Fig. 4B, C, using the recently reported ATP synthase structure. In the top view (4B) it can be seen that these residues in ε are clustered above the inner ring of α-helices from the *c* subunits and all are in close proximity to two or three different *c* subunits. It is also clear that the entire ε subunit lies over approximately one-half of the *c* subunits, as was predicted from labeling (Watts and Capaldi, 1997) and accessibility (Birkenhager *et al*., 1999) experiments. In the side view (Fig. 4C) it is clear that all of the residues in ε that formed disulfides with *c* subunits are found at the interface of the two subunits. It should now be possible to predict other residues in ε that could also form disulfide bonds with particular residues in *c* subunits.

The crystal structure seems to show a rather extensive interface between ε and *c* subunits, although without seeing the side chains, the strength of the interaction cannot be estimated. What else is known about this interaction? As mentioned previously, recent work (Schulenberg *et al*., 1999) has shown that a disulfide bond between ε-E31C and *c*-Q42C does not impair ATP synthase function. This indicates that ε need not move away from the *c* subunits during ATP synthesis hydrolysis. On the other hand, we have discovered several mutants in ε that impair the N,N', dicyclohexylcarbodiimide (DCCD), sensitivity of ATP hydrolysis by the membrane-bound enzyme (Xiong and Vik, 1995; Xiong *et al*., 1998). Since DCCD reacts specifically with the *c* subunit under these conditions, this implies disruption in F_1–F_o coupling. The sites of these mutations are shown in Fig. 4A. The residue closest to the ring of *c* subunits, E31A, has 76% of the wild-type sensitivity to DCCD, while the more distantly located E70A and S65A have only about 63% of the wild-type sensitivity. Thus, disruption of the interface between ε and γ may cause a greater loss of DCCD sensitivity than disruption of the interface between ε and *c* subunits. These results suggest that the direct interactions between ε and *c* subunits are not as strong as those between γ and *c* subunits (Ketchum and Nakamoto, 1998; Schulenberg *et al*., 1999). There may also be interactions between ε and the *a* subunit, although direct evidence has not yet been provided (Gardner and Cain, 1999).

In summary, the role of ε has not yet been clarified. It appears that the N-terminal domain makes extensive contacts with the γ subunit and with several *c* subunits. The C-terminal α-helices make transient contact with the α and β subunits. It is likely that the contacts between ε and γ are also not entirely fixed. The effect of subtle mutations in ε on DCCD sensitivity suggests that one role of ε is to strengthen the interactions between γ and the *c* subunits. The transient nature of the interactions between ε and the α and β subunits suggests that ε might also be involved in the conversion of the smaller rotational steps by *c* subunits into a 120-degree step by γ (Sabbert and Junge, 1996; Yasuda *et al*., 1998).

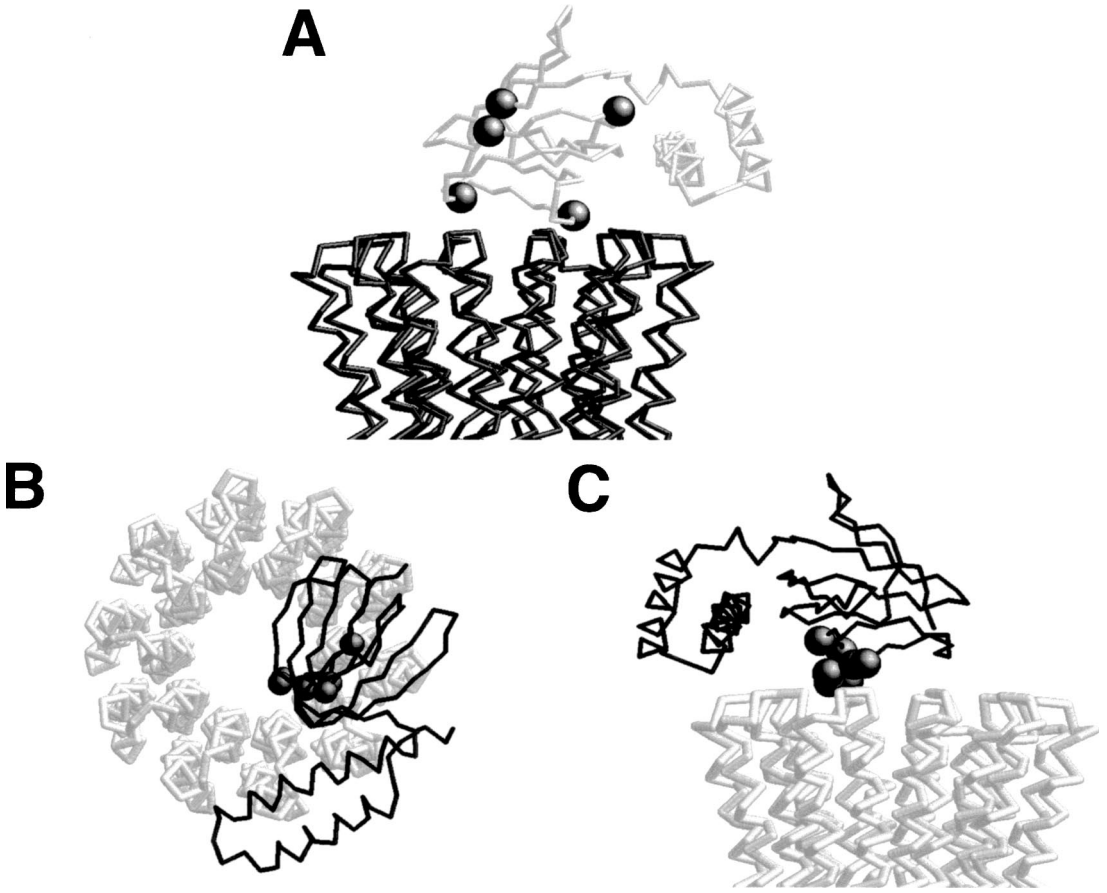

Fig. 4. Interactions between ε and F_o subunits. This figure is derived from Protein Data Bank file 1qo1 (Stock *et al*., 1999). (A) The top half of the 10 *c* subunits and the ε subunit are shown. Residues in ε that when changed to Ala result in lowered sensitivity of the membrane-bound ATPase activity to DCCD are shown as black spheres. The lower two residues, from left to right, are H38 and E31. The upper three residues, from left to right, are E70, T77, and S65. (B) Top view of the 10 *c* subunits with ε in black wire frame. The residues in ε that when changed to Cys can form disulfide bonds to various *c* subunit mutants are shown as black spheres. The *c* subunits contained Cys substitutions at positions 40, 42, or 44, in the polar loop. (C) Side view of the image in Fig. 4B.

ACKNOWLEDGMENT

This work was supported by NIH grant GM40508.

REFERENCES

Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994). *Nature (London)* **370**, 621–628.

Adachi, K., Yasuda, R., Noji, H., Itoh, H., Harada, Y., Yoshida, M., and Kinosita, K. Jr. (2000). *Proc. Natl. Acad. Sci. USA* **97**, 7243–7247.

Aggeler, R., and Capaldi, R. A. (1996). *J. Biol. Chem.* **271**, 13888–13891.

Aggeler, R., Chicas-Cruz, K., Cai, S. X., Keana, J. F., and Capaldi, R. A. (1992). *Biochemistry* **31**, 2956–61.

Aggeler, R., Haughton, M. A., and Capaldi, R. A. (1995). *J. Biol. Chem.* **270**, 9185–9191.

Allison, W. S. (1998). *Acc. Chem. Res*. **31**, 819–826.

Altendorf, K., Stalz, W., Greie, J., and Deckers-Hebestreit, G. (2000). *J. Exp. Biol*. **203**, 19–28.

Bianchet, M. A., Hullihen, J., Pedersen, P. L., and Amzel, L. M. (1998). *Proc. Natl. Acad. Sci. USA* **95**, 11065–11070.

Birkenhager, R., Greie, J. C., Altendorf, K., and Deckers-Hebestreit, G. (1999). *Eur. J. Biochem*. **264**, 385–396.

Boyer, P. D. (1997). *Annu. Rev. Biochem*. **66**, 717–749.

Buckley, N. M., Cruz, J. A., Cohen, W. S., and McCarty, R. E. (1999). *Photosynth. Res*. **59**, 137–145.

Bulygin, V. V., Duncan, T. M., and Cross, R. L. (1998). *J. Biol. Chem*. **273**, 31765–31769.

Capaldi, R. A., and Schulenberg, B. (2000). *Biochim. Biophys. Acta* **1458**, 263–269.

Cherepanov, D. A., Mulkidjanian, A. Y., and Junge, W. (1999). *FEBS Lett*. **449**, 1–6.

Dallmann, H. G., Flynn, T. G., and Dunn, S. D. (1992). *J. Biol. Chem*. **267**, 18953–60.

Dmitriev, O., Jones, P. C., Jiang, W. P., and Fillingame, R. H. (1999a). *J. Biol. Chem*. **274**, 15598–15604.

Dmitriev, O. Y., Jones, P. C., and Fillingame, R. H. (1999b). *Proc. Natl. Acad. Sci. USA* **96**, 7785–7790.

Dunn, S. D. (1982). *J. Biol. Chem*. **257**, 7354–7359.

Dunn, S. D., Zardorozny, V. D., Tozer, R. G., and Orr, L. E. (1987). *Biochemistry* **26**, 4488–4493.

Dunn, S. D., Tozer, R. G., and Zadorozny, V. D. (1990). *Biochemistry* **29**, 4335–4340.
Elston, T., Wang, H. Y., and Oster, G. (1998). *Nature (London)* **391**, 510–513.
Fillingame, R. H., Jones, P. C., Jiang, W., Valiyaveetil, F. I., and Dmitriev, O. Y. (1998). *Biochim. Biophys. Acta* **1365**, 135–142.
Foster, D. L., and Fillingame, R. H. (1982). *J. Biol. Chem*. **257**, 2009–2015.
Gardner, J. L., and Cain, B. D. (1999). *Arch. Biochem. Biophys*. **361**, 302–308.
Grüber, G., and Capaldi, R. A. (1996). *Biochemistry* **35**, 3875–3879.
Häsler, K., Engelbrecht, S., and Junge, W. (1998). *FEBS Lett*. **426**, 301–304.
Häsler, K., Pänke, O., and Junge, W. (1999). *Biochemistry* **38**, 13759–13765.
Hausrath, A. C., Grüber, G., Matthews, B. W., and Capaldi, R. A. (1999). *Proc. Natl. Acad. Sci. USA* **96**, 13697–13702.
Hermolin, J., Dmitriev, O. Y., Zhang, Y., and Fillingame, R. H. (1999). *J. Biol. Chem*. **274**, 17011–17016.
Jäger, H., Birkenhäger, R., Stalz, W. D., Altendorf, K., and Deckers-Hebestreit, G. (1998). *Euro. J. Biochem*. **251**, 122–132.
Jiang, W. P., and Fillingame, R. H. (1998). *Proc. Natl. Acad. Sci. USA* **95**, 6607–6612.
Jones, P. C., and Fillingame, R. H. (1998). *J. Biol. Chem*. **273**, 29701–29705.
Junge, W. (1999). *Proc. Natl. Acad. Sci. USA* **96**, 4735–4737.
Kaim, G., Matthey, U., and Dimroth, P. (1998). *EMBO J*. **17**, 688–695.
Kato-Yamada, Y., Noji, H., Yasuda, R., Kinosita, K., Jr., and Yoshida, M. (1998). *J. Biol. Chem*. **273**, 19375–19377.
Kato-Yamada, Bald, Y., D., Koike, M., Motohashi, K., Hisabori, T., and Yoshida, M. (1999). *J. Biol. Chem*. **274**, 33991–33994.
Ketchum, C. J., and Nakamoto, R. K. (1998). *J. Biol. Chem*. **273**, 22292–22297.
Kuki, M., Noumi, T., Maeda, M., Amemura, A., and Futai, M. (1988). *J. Biol. Chem*. **263,** 17437–17442.
Laget, P. P., and Smith, J. B. (1979). *Arch. Biochem. Biophys*. **197**, 83–89.
Long, J. C., Wang, S., and Vik, S. B. (1998). *J. Biol. Chem*. **273**, 16235–16240.
Lötscher, H. R., deJong, C., and Capaldi, R. A. (1984a). *Biochemistry* **23**, 4134–4140.
Lötscher, H. R., deJong, C., and Capaldi, R. A. (1984b). *Biochemistry* **23**, 4140–4143.
McLachlin, D. T., Bestard, J. A., and Dunn, S. D. (1998). *J. Biol. Chem*. **273**, 15162–15168.
Mendel-Hartvig, J., and Capaldi, R. A. (1991a). *Biochemistry* **30**, 1278–1284.
Mendel-Hartvig, J., and Capaldi, R. A. (1991b). *Biochemistry* **30**, 10987–10991.
Nakamoto, R. K., Ketchum, C. J., and Al-Shawi, M. K. (1999). *Annu. Rev. Biophys. Biomol. Struct* **28**, 205–234.
Nakamoto, R. K., Ketchum, C. J., Kuo, P. H., Peskova, Y. B., and Al-Shawi, M. K. (2000). *Biochim. Biophys. Acta* **1458**, 289–299.
Ogilvie, I., Aggeler, R., and Capaldi, R. A. (1997). *J. Biol. Chem*. **272**, 16652–16656.
Oster, G., and Wang, H. (2000). *Biochim. Biophys. Acta* **1458**, 482–510.
Pänke, O., Gumbiowski, K., Junge, W., and Engelbrecht, S. (2000). *FEBS Lett*. **472**, 34–38.
Pänke, O., and Rumberg, B. (1999). *Biochim. Biophys. Acta* **1412**, 118–128.
Rastogi, V. K., and Girvin, M. E. (1999). *Nature (London)* **402**, 263–268.
Rodgers, A. J. W., and Capaldi, R. A. (1998). *J. Biol. Chem*. **273**, 29406–29410.
Sabbert, D., and Junge, W. (1996). *Proc. Natl. Acad. Sci. USA* **94**, 2312–2317.
Sambongi, Y., Iko, Y., Tanabe, M., Omote, H., Iwamoto-Kihara, A., Ueda, I., Yanagida, T., Wada, Y., and Futai, M. (1999). *Science* **286**, 1722–1724.
Sawada, K., Kuroda, N., Watanabe, H., Moritani-Otsuka, C., and Kanazawa, H. (1997a). *J. Biol. Chem*. **272**, 30047–30053.
Sawada, K., Watanabe, H., Moritani-Otsuka, C., and Kanazawa, H. (1997b). *Arch. Biochem. Biophys*. **348**, 183–189.
Schulenberg, B., and Capaldi, R. A. (1999). *J. Biol. Chem*. **274**, 28351–28355.
Schulenberg, B., Wellmer, F., Lill, H., Junge, W., and Engelbrecht, S. (1997). *Eur. J. Biochem*. **249**, 134–141.
Schulenberg, B., Aggeler, R., Murray, J., and Capaldi, R. A. (1999). *J. Biol. Chem*. **274**, 34233–34237.
Smith, J. B., and Sternweiss, P. C. (1977). *Biochemistry* **16**, 306–311.
Sorgen, P. L., Bubb, M. R., and Cain, B. D. (1999). *J. Biol. Chem*. **274**, 36261–36266.
Sorgen, P. L., Caviston, T. L., Perry, R. C., and Cain, B. D. (1998). *J. Biol. Chem*. **273**, 27873–27878.
Stock, D., Leslie, A. G. W., and Walker, J. E. (1999). *Science* **286**, 1700–1705.
Tang, C. L., and Capaldi, R. A. (1996). *J. Biol. Chem*. **271**, 3018–3024.
Uhlin, U., Cox, G. B., and Guss, J. M. (1997). *Structure* **5**, 1219–1230.
Valiyaveetil, F. I., and Fillingame, R. H. (1998). *J. Biol. Chem*. **273**, 16241–16247.
Watts, S. D., and Capaldi, R. A. (1997). *J. Biol. Chem*. **272**, 15065–15068.
Weber, J., and Senior, A. E. (1997). *Biochim. Biophys Acta* **1319**, 19–58.
Wilkens, S., and Capaldi, R. A. (1998). *J. Biol. Chem*. **273**, 26645–26651.
Wilkens, S., Dahlquist, F. W., McIntosh, L. P., Donaldson, L. W., and Capaldi, R. A. (1995). *Natur. Struct. Biol*. **2**, 961–967.
Xiong, H., and Vik, S. B. (1995). *J. Biol. Chem*. **270**, 23300–23304.
Xiong, H., Zhang, D., and Vik, S. B. (1998). *Biochemistry* **37**, 16423–16429.
Yasuda, R., Noji, H., Kinosita, K., Jr., and Yoshida, M. (1998). *Cell* **93**, 1117–1124.
Zhang, Y., and Fillingame, R. H. (1995). *J. Biol. Chem*. **270**, 24609–24614.
Zhang, Y., Oldenburg, M., and Fillingame, R. H. (1994). *J. Biol. Chem*. **269**, 10221–4.