The inhibitory effect of $\epsilon$ subunit in F$_1$-ATPase from thermophilic Bacillus PS3 was examined focusing on the structure-function relationship. For this purpose, we designed a mutant for $\epsilon$ subunit similar to the one constructed by Schulenberg and Capaldi (Schulenberg, B., and Capaldi, R. A. (1999) J. Biol. Chem. 274, 28351–28355). We introduced two cysteine residues at the interface of N-terminal $\beta$-sandwich domain (S48C) and C-terminal $\alpha$-helical domain (N125C) of $\epsilon$ subunit. The $\alpha_3$$\beta_3$$\gamma_3$ complex containing the reduced form of this mutant $\epsilon$ subunit showed suppressed ATPase activity and gradual activation during the measurement. This activation pattern was similar to the complex with the wild type $\epsilon$ subunit. The conformation of the mutant $\epsilon$ subunit must be fixed and similar to the reported three-dimensional structure of the isolated $\epsilon$ subunit, when the intramolecular disulfide bridge was formed on this subunit by oxidation. This oxidized mutant $\epsilon$ subunit could form the $\alpha_3$$\beta_3$$\gamma_3$ complex but did not show any inhibitory effect. The complex was converted to the activated state, and the cross-link in the mutant $\epsilon$ subunit in the complex was efficiently formed in the presence of ATP-Mg, whereas no cross-link was observed without ATP-Mg, suggesting the conformation of the oxidized mutant $\epsilon$ subunit must be similar to that in the activated state complex. A non-hydrolyzable analog of ATP, 5'-adenylyl-$\beta$-$\gamma$-imidodiphosphate, could stimulate the formation of the cross-link on the $\epsilon$ subunit. Furthermore, the cross-link formation was stimulated by nucleotides even when this mutant $\epsilon$ subunit was assembled with a mutant $\alpha_3$$\beta_3$$\gamma_3$ complex lacking non-catalytic sites. These results indicate that binding of ATP to the catalytic sites induces a conformational change in the $\epsilon$ subunit and triggers transition of the complex from the suppressed state to the activated state.

F$_3$F$_1$-ATP synthase catalyzes ATP synthesis coupled with proton flow across energy-transducing membranes such as the bacterial plasma membrane, mitochondrial inner membrane, and chloroplast thylakoid membrane (1–4). F$_1$-ATPase is the water-soluble portion of F$_0$F$_1$-ATP synthase and contains a catalytic core for ATP synthesis and hydrolysis. The F$_3$-ATPase consists of five kinds of subunits with a stoichiometry of $\alpha_3$$\beta_3$$\gamma_3$ of $\gamma_1$$\delta_1$$\epsilon_1$. The catalytic sites of ATP synthesis and hydrolysis are located mainly on the $\beta$ subunits, and non-catalytic nucleotide-binding sites are located mainly on the $\epsilon$ subunits (5). The $\alpha_3$$\beta_3$$\gamma_3$ subcomplex of F$_1$-ATPase is regarded as a minimum stable complex that has catalytic features similar to F$_1$-ATPase (6–8). Three catalytic sites of F$_1$-ATPase exhibit strong negative cooperativity in ATP binding and positive cooperativity in ATP hydrolysis. Recently, the rotation of the $\gamma$ and $\epsilon$ subunits in the $\alpha_3$$\beta_3$$\gamma_3$ hexagon during the catalysis has been confirmed (9–16). Although the rotation of the $\epsilon$ subunit ring accompanied with the rotation of $\gamma$ and $\epsilon$ subunits has been also suggested (17–19), it is not proven definitely (20).

$\epsilon$ subunit of F$_1$-ATPase is known as an endogenous inhibitor (21–23). In the case of F$_0$F$_1$ complex from eukaryotic organella, several regulation systems are known (24–27). For example, mitochondrial F$_0$F$_1$ has a specific ATPase inhibitor protein and its cooperative proteins (24, 25). When the activity of respiratory chain decreases, these proteins prevent ATP synthesis/hydrolysis activity of F$_0$F$_1$, and thus maintain both the electrochemical potential across the membrane and the ATP level in the cell (26). In chloroplast F$_0$F$_1$, the activity is controlled both by the electrochemical potential of protons which activates the enzyme and by the redox state in the chloroplast which controls the activity of the enzyme through the formation and the cleavage of a disulfide bond on the $\gamma$ subunit (reviewed in Ref. 27). In Escherichia coli F$_0$F$_1$, $\epsilon$ subunit is a unique endogenous inhibitor of the ATPase activity (22), and the function is more obvious in the partial complex, F$_1$ (23).

Recently, we found that the $\epsilon$ subunit of thermophilic F$_1$ (TF$_1$) modulates the activity of TF$_1$ (28) and further the holoenzyme, TF$_r$F$_r$ (29). From these results, we concluded that TF$_r$F$_r$ has two different states that are mutually convertible by a function and/or a certain conformational change of the $\epsilon$ subunit. This finding led us to propose the possible role of the $\epsilon$ subunit in a regulatory system of F$_0$F$_1$. However, there is no direct evidence for the movement or the conformational change in the $\epsilon$ subunit in the complex accompanying this conversion, and the molecular mechanism of the inhibition by the $\epsilon$ subunit is still unclear.

The structure of the isolated $\epsilon$ subunit of E. coli F$_1$-ATPase has been solved both by NMR and by x-ray crystallography (30–32). Schulenberg and Capaldi (33) have suggested from the biochemical studies that the structure of the $\epsilon$ subunit in F$_0$F$_1$ is essentially the same as that of the isolated $\epsilon$ subunit. In the

---

*The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

§ To whom correspondence should be addressed: Chemical Resources Laboratory, R-1, Tokyo Institute of Technology, 4259 Nagatsuta, Yokohama, 226-8503, Japan. Tel.: 81-45-924-5234; Fax.: 81-45-924-5277; E-mail: thisabor@res.titech.ac.jp.

1 The abbreviations used are: TF$_r$, F$_r$-ATPase from thermophilic Bacillus PS3, a soluble portion of F$_3$F$_1$-ATP synthase and contains a catalytic core for ATP synthesis and hydrolysis. The F$_3$-ATPase consists of five kinds of subunits with a stoichiometry of $\alpha_3$$\beta_3$$\gamma_3$ of $\gamma_1$$\delta_1$$\epsilon_1$. The catalytic sites of ATP synthesis and hydrolysis are located mainly on the $\beta$ subunits, and non-catalytic nucleotide-binding sites are located mainly on the $\epsilon$ subunits (5). The $\alpha_3$$\beta_3$$\gamma_3$ subcomplex of F$_1$-ATPase is regarded as a minimum stable complex that has catalytic features similar to F$_1$-ATPase (6–8). Three catalytic sites of F$_1$-ATPase exhibit strong negative cooperativity in ATP binding and positive cooperativity in ATP hydrolysis. Recently, the rotation of the $\gamma$ and $\epsilon$ subunits in the $\alpha_3$$\beta_3$$\gamma_3$ hexagon during the catalysis has been confirmed (9–16). Although the rotation of the $\epsilon$ subunit ring accompanied with the rotation of $\gamma$ and $\epsilon$ subunits has been also suggested (17–19), it is not proven definitely (20).

$\epsilon$ subunit of F$_1$-ATPase is known as an endogenous inhibitor (21–23). In the case of F$_0$F$_1$ complex from eukaryotic organella, several regulation systems are known (24–27). For example, mitochondrial F$_0$F$_1$ has a specific ATPase inhibitor protein and its cooperative proteins (24, 25). When the activity of respiratory chain decreases, these proteins prevent ATP synthesis/hydrolysis activity of F$_0$F$_1$, and thus maintain both the electrochemical potential across the membrane and the ATP level in the cell (26). In chloroplast F$_0$F$_1$, the activity is controlled both by the electrochemical potential of protons which activates the enzyme and by the redox state in the chloroplast which controls the activity of the enzyme through the formation and the cleavage of a disulfide bond on the $\gamma$ subunit (reviewed in Ref. 27). In Escherichia coli F$_0$F$_1$, $\epsilon$ subunit is a unique endogenous inhibitor of the ATPase activity (22), and the function is more obvious in the partial complex, F$_1$ (23).

Recently, we found that the $\epsilon$ subunit of thermophilic F$_1$ (TF$_1$) modulates the activity of TF$_1$ (28) and further the holoenzyme, TF$_r$F$_r$ (29). From these results, we concluded that TF$_r$F$_r$ has two different states that are mutually convertible by a function and/or a certain conformational change of the $\epsilon$ subunit. This finding led us to propose the possible role of the $\epsilon$ subunit in a regulatory system of F$_0$F$_1$. However, there is no direct evidence for the movement or the conformational change in the $\epsilon$ subunit in the complex accompanying this conversion, and the molecular mechanism of the inhibition by the $\epsilon$ subunit is still unclear.

The structure of the isolated $\epsilon$ subunit of E. coli F$_1$-ATPase has been solved both by NMR and by x-ray crystallography (30–32). Schulenberg and Capaldi (33) have suggested from the biochemical studies that the structure of the $\epsilon$ subunit in F$_0$F$_1$ is essentially the same as that of the isolated $\epsilon$ subunit. In the
recently reported partial structural analysis of $F_{0}F_{1}$ from yeast mitochondria (34), the $\beta$ subunit (equivalent to the $\epsilon$ subunit of bacterial $F_{0}F_{1}$) lies on the top of the 10 $\epsilon$ subunit ring which is far from the bottom part of the $\alpha_3\beta_3\gamma$ ring including the DELSEED region of the $\beta$ subunit. Although the resolution of this structure is limited to 3.9 Å, the electron density from the $\delta$ subunit fits well with the reported structure of the isolated $\epsilon$ subunit from E. coli $F_{1}$-ATPase (30–32). On the contrary, the interaction between DELSEED region of $\beta$ subunit and C-terminal domain of $\epsilon$ subunit has been suggested from cross-linking studies (21, 32, 35, 36). Taken together, a rearrangement of the $\epsilon$ subunit in the complex and/or drastic conformational changes in $F_{0}F_{1}$ or $\epsilon$ subunit itself is supposed to occur during the conversion from the suppressed state to the activated state. Actually, the change in the position of the $\epsilon$ subunit in the complex and the conformational change in this subunit itself during the catalytic turnover are also suggested (21, 32, 37).

In order to investigate a conformational change in the $\epsilon$ subunit on the occasion of the conversion from the suppressed state to the activated state of the complex, we introduced two cysteines each into the C-terminal helical domain and the faced region in the N-terminal $\beta$-barrel domain of the $\epsilon$ subunit of TF$_1$. Similar mutant $\epsilon$ subunits of E. coli $F_1$ were already constructed by Schellenberg and Capaldi (33). By using these mutants, they have concluded that the structure of the $\epsilon$ subunit in the intact $F_{0}F_{1}$ should resemble that determined for the isolated $\epsilon$ subunit. They also discussed that the relative movement of the domains in the $\epsilon$ subunit is not required for the functioning of $F_{0}F_{1}$, although they have pointed out the modulation of the activity of $F_{0}F_{1}$ by the $\epsilon$ subunit.

Our results indicate that the $\epsilon$ subunit should be in different conformation from the reported structure (30–32) when it works as an inhibitor and that the C-terminal helical domain moves against the N-terminal domain when switching from the suppressed state to the activated state. The latter must be similar to that of the isolated $\epsilon$ subunit. Furthermore, we conclude that the ATP binding to the catalytic site(s) is a trigger for the transition from the suppressed state to the activated state.

**Experimental Procedures**

Recombinant Proteins—The mutant $\alpha_3\beta_3\gamma$ complex (ac193S and a4W63F) of TF$_1$, whose characteristics are almost identical to the wild type complex was prepared as described previously (7). This mutant $\alpha_3\beta_3\gamma$ complex is referred as the wild type in this paper. The non-catalytic site-deficient mutant ($\Delta$NC) of the $\alpha_3\beta_3\gamma$ complex was prepared as described (7, 38). The expression plasmid for the mutant $\epsilon$ subunit of which Ser$_{48}$ and Asn$_{125}$ (Fig. 1) were substituted to Cys, respectively ($\epsilon^{NC}$), was prepared by the method of Kunkel et al. (39, 40). Two primers were applied simultaneously to a single-strand DNA of the expression plasmid for the wild type $\epsilon$ subunit, pTE28 (28). One primer contained a substitution of Ser$_{48}$ to Cys and a new BglII site (5’-GCGGGCGCCACAGATCTCCAGGGCGCCGG-3’), and the other contained a substitution of Asn$_{125}$ to Cys and a new SalI site (5’-GCAAGCTTACGGACCATGCGCCGGT-3’). The mutations were screened by the digestion with the respective restriction enzymes and confirmed by DNA sequencing. The resulting plasmid was introduced into E. coli BL21(DE3). The $\epsilon^{NC}$ was expressed and purified as described (40) except all buffers during the purification contained 1 mM DTT to avoid the intramolecular cross-link formation.

Isolation of $\alpha_3\beta_3\gamma\epsilon$ Complex Containing Reduced and Oxidized $\epsilon^{NC}$—For reduction, $\epsilon^{NC}$ as ammonium sulfate suspension was incubated with 10 mM DTT overnight at 4 °C. The sample was then collected by centrifugation, dissolved in 50 mM Tris-HCl (pH 8.0) containing 100 mM NaCl, and desalted by a Sephadex G-25 (Amersham Pharmacia Biotech) column equilibrated with the same buffer. For complete oxidation of the mutant $\epsilon$ subunit, purified $\epsilon^{NC}$ was incubated with 10 $\mu$m CuCl$_2$ for 1 h at room temperature in 50 mM Tris-HCl (pH 8.0), and 100 mM NaCl. The reaction was terminated by the addition of 10 mM EDTA. The oxidized $\epsilon$ subunit ($\epsilon^{NC-OX}$) or the reduced one ($\epsilon^{NC-RED}$) were reconstituted with the $\alpha_3\beta_3\gamma$ complex as described previously (28).

Detection of the Intramolecular Disulfide Bridge Formation—The formation of the intramolecular disulfide bridge in the $\epsilon^{NC}$ was confirmed by the modification of cysteine residues with tetrathydrophosphamine-5-maleimide (TMR maleimide) (Molecular Probes, Eugene, OR). The isolated $\alpha_{3}\beta_{3}\gamma^{NC-OX}$ and $\alpha_{3}\beta_{3}\gamma^{NC-RED}$ complexes (0.5 mg/ml) were denatured by 0.1% (w/v) SDS and reacted with 25 $\mu$m TMR maleimide in 50 mM TES-NaOH (pH 7.0), and 100 mM NaCl for 10 min at room temperature. The reaction was quenched by the addition of 10 mM N-ethylmaleimide. Then the samples were subjected onto a 15% (w/v) polyacrylamide gel electrophoresis in the presence of 0.1% (w/v) SDS (SDS-PAGE) without reducing reagent (41). The incorporation of TMR was visualized by illumination with an UV transilluminator (254 nm), and the image was photographed by an instant camera, RB67 (Mamiya, Japan). Then, the protein bands in the gel were stained with Coomassie Brilliant Blue R-250. Non-denaturing PAGE was also carried out to confirm whether $\epsilon^{NC-OX}$ and $\epsilon^{NC-RED}$ can form the $\alpha_{3}\beta_{3}\gamma$ complex, respectively. The photographs of the fluorescence image and Coomassie Brilliant Blue R-250-stained gel were scanned by a flat head scanner, GT-7600UF (EPSON, Japan), and the intensity of the bands was quantified by the image processing and analyzing program Scion Image (Scion Co.).

Measurement of ATPase Activity—ATPase activity was measured spectrophotometrically with an ATP-regenerating system at 25 °C (42). The reaction mixture consisted of 0.2 ml of 50 mM TES-NaOH (pH 8.0), 100 mM MgCl$_2$, 0.25 mM phosphoonylpyruvate, 2 mM MgCl$_2$, 0.2 mM NADH, 50 $\mu$m pyruvate kinase, 50 $\mu$m lactate dehydrogenase, and 50 $\mu$m ATP-Mg. The reaction was initiated by the addition of 2 $\mu$m of subunit complexes of TF$_1$ into 1 ml of assay mixture, and the changes in the absorbance at 340 nm were monitored in a spectrophotometer UV-2200 (Shimadzu, Kyoto, Japan).

Effect of Adenine Nucleotides on the Formation of the Disulfide Bridge—Formation of the intramolecular cross-link in the $\epsilon$ subunit in $\alpha_{3}\beta_{3}\gamma\epsilon$ complex was examined in the presence and absence of 2 mM ATP-Mg, AMP-PNP-Mg, or ADP-Mg. The isolated $\alpha_{3}\beta_{3}\gamma^{NC-RED}$ complex (1 mg/ml) was incubated with 4 mM magnesium nucleotides for 10 min at room temperature in 50 mM TES-NaOH (pH 7.0), and 100 mM NaCl. For a control, 4 mM MgCl$_2$ was added instead of magnesium nucleotide. The cross-linking was induced by the addition of equal volume of CuCl$_2$ solution (in 50 mM TES-NaOH (pH 7.0) and 100 mM NaCl) resulting in a final CuCl$_2$ concentration of 0, 10, 20, and 50 $\mu$m, respectively. After an incubation for 1 h at room temperature, the reaction was quenched by addition of 10 mM EDTA, and the solution was incubated for further 10 min. 0.1% (w/v) SDS and 25 $\mu$m TMR maleimide were then added to label cysteines that did not form the

**Fig. 1. Structural model for the mutant $\epsilon^{NC}$ subunit.** The structure of the $\epsilon$ subunit of E. coli $F_{1}$-ATPase is shown. The corresponding residues to the mutated residues in $\epsilon^{NC}$ are shown in black, and the amino acids in single letter code with the residue number of TF$_1$ $\epsilon$ subunit are indicated. The corresponding residues of E. coli $F_{1}$ $\epsilon$ subunit are shown in parentheses. N and C termini are marked with "N" and "C," respectively. Coordinate and sequence alignment were taken from Ref. 31. The figure was generated by the software Ras-Mol (46).
Complexes maintain the correct subunit stoichiometry including the possible conformational change in the maleimide. Judging from the differences in the mobility on the PAGE analysis of the isolated $\alpha_2\beta_2\gamma$ complexes containing reduced or oxidized $\epsilon^{NCX}$ mutant after treatment with 10 mM DTT. Lane 1, $\alpha_2\beta_2\gamma^{NCX-RED}$; lane 2, $\alpha_2\beta_2\gamma^{NCX-OX}$; lane 3, $\alpha_2\beta_2\gamma^{NCX-OX}$; and lane 4, $\alpha_2\beta_2\gamma^{WT}$. The gel was stained with Coomassie Brilliant Blue R-250. B, the same gel shown in A was illuminated by an UV transilluminator before staining, and only the region around $\epsilon$ subunit is shown. C, non-denaturing PAGE analysis of the isolated $\alpha_2\beta_2\gamma$ complexes containing reduced or oxidized $\epsilon^{NCX}$ mutant after treatment with 10 mM DTT. Lane 1, $\alpha_2\beta_2\gamma^{NCX-RED}$; lane 2, $\alpha_2\beta_2\gamma^{NCX-OX}$; lane 3, $\alpha_2\beta_2\gamma^{NCX-OX}$; and lane 4, $\alpha_2\beta_2\gamma^{WT}$.

disulfide bridge, and the solution was incubated for 10 min. Then 10 mM $N$-ethylmaleimide was added. Incorporation of TMR maleimide into the $\epsilon$ subunit was analyzed with 15% SDS-PAGE without reducing reagent as described above.

Other Procedures—Protein concentration was determined by the method of Bradford (43) using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

Formation of the Intramolecular Disulfide Bridge in $\epsilon^{NCX}$ Does Not Prevent the Assembly of the $\alpha_2\beta_2\gamma$ Complex—Formation of the intramolecular disulfide bridge in $\epsilon^{NCX}$ must restrict the possible conformational change in the $\epsilon$ subunit, and this restriction may affect the efficient assembly of the $\alpha_2\beta_2\gamma$ complex. Therefore, we first investigated the effect of the formation of a disulfide bridge in $\epsilon^{NCX}$ on the reconstitution of the complex. $\epsilon^{NCX}$ was first oxidized by the incubation with CuCl$_2$ or reduced with DTT and then mixed with the recombinant $\alpha_2\beta_2\gamma$ complex. The reconstituted complex was isolated by ultrafiltration after gel filtration HPLC in the presence of 1 mM ATP-Mg and confirmed the presence of the $\epsilon$ subunit in the complex using native PAGE as shown in Fig. 2C. More than 90% of the $\epsilon$ subunit remained bound to the complex after the chromatography (data not shown).

Relation between the Conformation of $\epsilon$ Subunit and a State of the $\alpha_2\beta_2\gamma$ Complex—The efficiency of cross-link formation in the $\epsilon$ subunit in the complex was investigated under various conditions. In the presence of 2 mM ATP-Mg the $\alpha_2\beta_2\gamma$ complex is converted from the suppressed state to the activated state (28), and the cross-link was efficiently formed in the presence of 20 mM CuCl$_2$ (Fig. 4A). This efficiency was slightly less than that of the isolated $\epsilon$ subunit where 10 mM CuCl$_2$ was sufficient to complete the cross-link (Fig. 2B). No cross-link was formed with these concentrations of CuCl$_2$ when ATP was omitted. Therefore, the conformation of $\epsilon^{NCX-OX}$ should be very similar to that in the activated state complex. This structure must be similar to the reported three-dimensional structure of $\epsilon$ subunit of E. coli F$_1$ (30–32) because the distance between two $\alpha$-carbons of the residues corresponds to Ser$^{148}$ and Asn$^{125}$ of TF$_1$ $\epsilon$ subunit in the crystal structure of E. coli F$_1$. $\epsilon$ subunit is 5.1 Å, sufficiently short to form the disulfide bridge when they are substituted with cysteines. This result agrees well with the previous study on the E. coli F$_1$, where a similar mutant $\epsilon$ subunit could easily take the cross-linked conformation in the complex (33). On the contrary, the conformation of the $\epsilon$ subunit must be somehow different in the $\alpha_2\beta_2\gamma$ complex in the suppressed state.

Which Is The Trigger of Interconversion, Nucleotide Binding
or Hydrolysis—As described above, we noticed that the efficiency of the cross-link formation in eNCX could be a useful indicator for the conformational change of the e subunit in the complex. The gradual change in the ATPase activity of αβγε complex during the ATPase measurement implies that ATP-Mg itself could trigger the interconversion of the complex from the suppressed state to the activated state (28). We investigated the effects of other nucleotides on the formation of the cross-link to determine whether the conformational change in the e subunit, which corresponds to the change in the ATPase activity, is induced by ATP binding or by ATP hydrolysis. As shown in Fig. 4A, AMP-PNP could induce the cross-link although the efficiency appeared to be slightly lower than ATP. ADP was less effective but could induce the cross-link formation. These results suggest that the binding of ATP, not the hydrolysis, can induce the conformational change in the e subunit.

Nucleotide Binding to the Catalytic Site Induces the Conformational Change in the e Subunit—We investigated the cross-link formation in eNCX by using the non-catalytic site-deficient mutant (ΔNC) complex to clarify if the nucleotide binding to the catalytic sites induces the conformational change in the e subunit. The ΔNC complex shows only the initial burst of ATP hydrolysis activity and then immediately drops into the inactive state (ADP-inhibited form) (38). This rapid inactivation is due to the deficiency of the nucleotide binding capability at the non-catalytic site which is responsible for the release of inhibitory ADP-Mg from the catalytic site(s). The formation of the intramolecular cross-link in the e subunit in this ΔNC complex was clearly nucleotide-dependent (Fig. 4B). The effects of the nucleotides were more remarkable than those for the wild type complex, and ADP was as effective as ATP in this mutant complex. Therefore the nucleotide binding to the catalytic site(s) must be the trigger for the conformational change in the e subunit.

Significance of the Conformational Flexibility of e Subunit for Switching—The results shown in the present study indicate that a certain conformation of the e subunit is important for the inhibitory effect, and the conformation of the e subunit in the complex is variable. Moreover, our study suggests that the e subunit at work as an inhibitor may take a different conformation from the reported one (30–32), which is the conformation facilitating formation of the intramolecular cross-link spontaneously but incompetent to suppress the ATPase activity (see Fig. 3).

It is well known that the C-terminal helical domain of the e subunit is dispensable for ATP synthesis but necessary for the inhibition of ATP hydrolysis (29, 44, 45). A movement of this C-terminal helical domain close to the bottom part of αβγ hexagon was suggested from cross-linking studies (21, 32, 35, 36). Furthermore, Schulenberg and Capaldi (33) have also reported the effect of the intramolecular cross-link formation in e subunit of E. coli F0F1-ATP synthase which was similar to our mutant e subunit. In their case, the cross-link formation between two domains of e subunit resulted in the increase of the ATPase activity. However the H+ pumping activity and the ATP synthesis activity were not affected, and they concluded that the conformational change in the e subunit is not necessary for the enzyme function. They also suggested that the enzyme activity of F0F1 from E. coli is modulated by the e subunit because the ATPase activity increased by the extent of the intramolecular cross-link. This result may be explained if the switching of ATPase and H+ pumping in F0F1 was directly managed by the e subunit (28, 29).

In conclusion, our present study indicates that the movement of the C-terminal α-helical domain relative to the N-terminal β sandwich domain in the e subunit is required for the switching from the suppressed state to the activated state. Furthermore, the conversion to the activated state of the complex is triggered by the ATP binding to the catalytic site(s). We cannot conclude yet that the role of the e subunit as a regulator in the complex is universal among F0F1 from various sources, but we could provide important information on the role of two domains in the e subunit of TF1 from this study. The function of the e subunit can be divided into several aspects. The C-terminal helical domain is unnecessary for the functional coupling (29, 44, 45) but necessary for the inhibitory effect. This helical domain should reside in a proper position, which is different from the reported three-dimensional structure (30–32), in the complex for the inhibition. Information on the structure of the e subunit, which is active as an inhibitor, is important to figure out the molecular mechanism of the regulation of F0F1 by the e subunit.

Acknowledgment—We thank Dirk Bald for critical reading of the manuscript.

REFERENCES

1. Senior, A. E. (1988) Physiol. Rev. 68, 177–231
2. Futai, M., Nouni, T., and Maeda, M. (1989) Annu. Rev. Biochem. 58, 111–136
3. Boyer, P. D. (1993) Biochim. Biophys. Acta 1140, 215–230
4. Boyer, P. D. (1997) Annu. Rev. Biochem. 66, 717–749
5. Abrahams, J. P., Leslie, A., Lutter, R., and Walker, J. E. (1994) Nature 370, 621–628
6. Yokoyama, K., Hisahori, T., and Yoshida, M. (1989) J. Biol. Chem. 264, 21837–21841
7. Matsui, T., and Yoshida, M. (1995) Biochim. Biophys. Acta 1231, 139–146
8. Kihara, C., Matsui, T., Hisahori, T., and Yoshida, M. (1996) J. Biol. Chem. 271, 2433–2438
9. Duncan, T. M., Bulygin, V. V., Zhou, Y., Hutcheon, M. L., and Cross, R. L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10964–10968
10. Schmitz, H., Engelbrechti, S., and Jener, W. (1996) Nature 381, 623–625
11. Noji, Y., Yasuda, R., Yoshida, M., and Kinohisa, K. Jr., and Yoshida, M. (1997) Nature 386, 299–302
12. Yasuda, R., Noji, H., Kinohisa, K., Jr., and Yoshida, M. (1998) Cell 93, 1117–1124
13. Aggerel, R., Ogilvie, I., and Capaldi, R. A. (1997) J. Biol. Chem. 272, 19621–19624
14. Kato-Yamada, Y., Noji, H., Yasuda, R., Kinohisa, K. Jr., and Yoshida, M. (1998) J. Biol. Chem. 273, 19375–19377
15. Bulygin, V. V., Duncan, T. M., and Cross, R. L. (1998) J. Biol. Chem. 273, 31769–31769
16. Hisahori, T., Kondoh, A., and Yoshida, M. (1999) FEBS Lett. 463, 35–38
17. Junge, W., Lill, H., and Engelbrecht, S. (1997) Trends Biochim. Sci. 11, 429–433
18. Sambongi, Y., Iko, Y., Tanabe, M., Omote, H., Iwamoto-Kihara, A., Ueda, I., Yanagida, T., Wada, Y., and Futai, M. (1995) Science 266, 1722–1724
19. Jones, P. C., Hermolin, J., Jiang, W., and Fillingame, R. H. (2000) J. Biol. Chem. 275, 31340–31346
20. Tsunoda, S. P., Aggerel, R., Noji, H., Kinohisa, K., Jr., Yoshida, M., and Capaldi, R. A. (2000) FEBS Lett. 470, 244–248
21. Aggerel, R., and Capaldi, R. A. (1996) J. Biol. Chem. 271, 13888–13891
Domain Movement of γ Subunit for the Activation of F1-ATPase

22. Laget, P. P., and Smith, J. B. (1979) Arch. Biochem. Biophys. 197, 83–89
23. Sternweis, P. C., and Smith, J. B. (1980) Biochemistry 19, 526–531
24. Pullman, M. E., and Monroy, G. C. (1963) J. Biol. Chem. 238, 3762–3769
25. Hashimoto, T., Yoshida, Y., and Tagawa, K. (1990) J. Bioenerg. Biomembr. 22, 27–38
26. Hashimoto, T., Shiroiwa, M., Ichikawa, N., Yoshida, Y., and Tagawa, K. (1996) Seikagaku 68, 775
27. Ort, D. R., and Oxborough, K. (1992) Annu. Rev. Plant Physiol. Plant Mol. Biol. 43, 269–291
28. Kato, Y., Matsui, T., Tanaka, N., Muneyuki, E., Hisabori, T., and Yoshida, M. (1997) J. Biol. Chem. 272, 24906–24912
29. Kato-Yamada, Y., Bald, D., Koike, M., Motohashi, K., Hisabori, T., and Yoshida, M. (1999) J. Biol. Chem. 274, 33991–33994
30. Wilkens, S., Dahlquist, F. W., McIntosh, L. P., Donaldson, L. W., and Capaldi, R. A. (1995) Nat. Struct. Biol. 2, 961–967
31. Uhlin, U., Cox, G. B., and Gus, J. M. (1997) Structure 5, 1219–1230
32. Wilkens, S., and Capaldi, R. A. (1998) J. Biol. Chem. 273, 29645–29651
33. Schulenberg, B., and Capaldi, R. A. (1999) J. Biol. Chem. 274, 28351–28355
34. Stock, D., Leslie, A. G. W., and Walker, J. E. (1999) Science 286, 1700–1705
35. Dallmann, H. G., Flynn, T. G., and Dunn, S. D. (1992) J. Biol. Chem. 267, 18953–18960
36. Tang, C., and Capaldi, R. A. (1996) J. Biol. Chem. 271, 3018–3024
37. Capaldi, R. A., and Schulenberg, B. (2000) Biochim. Biophys. Acta 1458, 263–269
38. Matsui, T., Muneyuki, E., Honda, M., Allison, W. S., Dou, C., and Yoshida, M. (1997) J. Biol. Chem. 272, 8215–8221
39. Kunkel, T. A., Behenek, K., and McClary, J. (1991) Methods Enzymol. 204, 125–139
40. Hisabori, T., Kato, Y., Motohashi, K., Kroth-Pancic, P., Strotmann, H., and Amano, T. (1997) Eur. J. Biochem. 247, 1158–1165
41. Laemmli, U. K. (1970) Nature 227, 680–685
42. Stiggal, D. L., Galante, Y. M., and Hatefi, Y. (1979) Methods Enzymol. 55, 308–315
43. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
44. Kuki, M., Noumi, T., Maeda, M., Amemura, A., and Futai, M. (1988) J. Biol. Chem. 263, 17437–17442
45. Hisabori, T., Motohashi, K., Koike, M., Kroth, P., Strotmann, H., and Amano, T. (1998) in Photosynthesis: Mechanisms and Effects (Garab, G., ed) Vol. 3, pp. 1711–1714, Kluwer Academic Publishers Group, Dordrecht, Netherlands
46. Sayle, R. A., and Milner-White, E. J. (1995) Trends Biochem. Sci. 20, 374