The Role of the \( \beta \)DELSEED Motif of F\(_{1}\)-ATPase

PROPAGATION OF THE INHIBITORY EFFECT OF THE \( \epsilon \) SUBUNIT

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In \( F_1\)-ATPase, a rotary motor enzyme, the region of the conserved DELSEED motif in the \( \beta \) subunit moves and contacts the rotor \( \gamma \) subunit when the nucleotide fills the catalytic site, and the acidic nature of the motif was previously assumed to play a critical role in rotation. Our previous work, however, disproved the assumption (Hara, K. Y., Noji, H., Bald, D., Yasuda, R., Kinosita, K., Jr., and Yoshida, M. (2000) \textit{J. Biol. Chem.} 275, 14260–14263), and the role of this motif remained unknown. Here, we found that the \( \epsilon \) subunit, an intrinsic inhibitor, was unable to inhibit the ATPase activity of a mutant thermophilic \( F_1\)-ATPase in which all of the five acidic residues in the DELSEED motif were replaced with alanines, although the \( \epsilon \) subunit in the mutant \( F_1\)-ATPase assumed the inhibitory form. In addition, the replacement of acidic residues in the C-terminal region of the \( \epsilon \) subunit by alanines caused a decrease of the inhibitory effect. Partial replacement of the acidic residues in the DELSEED motif of the \( \beta \) subunit or of the basic residues in the C-terminal \( \alpha\)-helix of the \( \epsilon \) subunit induced a partial effect. We here conclude that the \( \epsilon \) subunit exerts its inhibitory effect through the electrostatic interaction with the DELSEED motif of the \( \beta \) subunit.

\( F_1\), together with the membrane-embedded proton-conducting unit \( F_0\), forms the \( F_0F_1\)-ATP synthase that reversibly couples trans-membrane proton flow to ATP synthesis/hydrolysis (1–6). Isolated \( F_1\) has ATP-hydrolyzing activity, hence is called \( F_1\)-ATPase, and has an \( \alpha\beta\gamma\epsilon\) subunit structure in which \( \alpha \) and \( \beta \) subunits have noncatalytic and catalytic nucleotide-binding sites, respectively. The \( \gamma \) subunit inserts its coiled-coil structure into the central cavity of the \( \alpha\beta\) hexagonal ring (7) and rotates relative to the \( \alpha\beta\gamma \) ring during ATP hydrolysis (8–13). The conformation of the \( \beta \) subunit with a nucleotide-filled catalytic site is significantly different from that of the \( \beta \) subunit with an empty catalytic site; specifically, the C-terminal helical domain is lifted up to or swung down from the nearly immobile N-terminal domain, respectively. The acidic cluster sequence, known as the DELSEED motif, in the C-terminal domain of the \( \beta \) subunit moves in contact with the \( \gamma \) subunit when the catalytic site is filled with nucleotide. This sequence has been well conserved in all \( F_1\)s with minor variations. For example, DELSEED in \( F_1\) from a thermophilic \textit{Bacillus} PSS3 (\( TF_1\)) is assumed to play an essential role in the efficient coupling between catalysis and transport (14). However, a mutant \( \alpha\beta\gamma \) complex of \( TF_1\), in which acidic residues in the \( \beta \)DELSEED sequence were replaced with alanines, showed the rotary motion of the \( \gamma \) subunit with a normal torque value, 40 pNnm (15). Thus, negative charges in this motif do not have a direct role in torque generation, and the role of the \( \beta \)DELSEED motif remains unclear.

The \( \epsilon \) subunit rotates along with the \( \gamma \) subunit (16–18), and because the \( \epsilon \) subunit has been known as an intrinsic inhibitor of the ATPase activity of \( F_1\) and of the ATP-driven proton translocating activity of \( F_0F_1\)-ATP synthase (19–22), the mechanism of how the \( \epsilon \) subunit inhibits catalysis while it is rotating is intriguing. We found that the \( \epsilon \) subunit of \( TF_1\) can adopt two conformations, an inhibitory state and a noninhibitory state (23). The high resolution structure of the \( \epsilon \) subunit of \textit{Escherichia coli} \( F_1\)-ATPase (\( EF_1\)) (24–26) most likely corresponds to the noninhibitory state. When ATP-Mg is added to \( TF_1\) (\( \alpha\beta\beta\gamma\epsilon\)) or the \( \alpha\beta\gamma\epsilon \) complex, ATP hydrolysis starts slowly, accelerates with time, and then reaches the final rate. This time-dependent activation was not observed for the \( \alpha\beta\gamma \) and \( \alpha\beta\gamma\epsilon \) complexes (23, 27). From the results, we suspected that a slow nucleotide-dependent conformational transition of the \( \epsilon \) subunit in \( TF_1\) during the transition from inhibitory to noninhibitory state occurred. It should be mentioned that \( EF_1\) shows an apparently similar time-dependent activation that is derived from a different mechanism: dissociation of the \( \epsilon \) subunit from \( EF_1\) during catalysis (19).

We had shown that a mutant \( \epsilon \) subunit lacking the C-terminal half had no inhibitory effect on \( TF_1\) (22). In \( EF_1\), a cysteine residue introduced at the C-terminal end of the helix \( \alpha_2^\epsilon \) (residues from Lys\(^{114}\) to Lys\(^{135}\) in \( TF_1\); see Ref. 25) of the \( \epsilon \) subunit was cross-linked with a cysteine introduced in the \( \alpha\)-helix of the \( \beta \) subunit (16–18), and \( EF_1\) showed an apparently similar time-dependent activation that is derived from a different mechanism: dissociation of the \( \epsilon \) subunit from \( EF_1\) during catalysis (19).

A mutant \( \epsilon \) subunit lacking the C-terminal half had no inhibitory effect on \( TF_1\) (22). In \( EF_1\), a cysteine residue introduced at the C-terminal end of the helix \( \alpha_2^\epsilon \) (residues from Lys\(^{114}\) to Lys\(^{135}\) in \( TF_1\); see Ref. 25) of the \( \epsilon \) subunit was cross-linked with a cysteine introduced in the \( \beta \)DELSEED motif (28–30). 4–6 positively charged residues exist in the C-terminal helix \( \alpha_2^\epsilon \) of the \( \epsilon \) subunit of almost all \( F_1\)s, and there has been a change in the helix \( \alpha_2^\epsilon \) helix of the \( \epsilon \) subunit of \( TF_1\). In \( EF_1\), a cysteine residue introduced at the C-terminal end of the helix \( \alpha_2^\epsilon \) (residues from Lys\(^{114}\) to Lys\(^{135}\) in \( TF_1\); see Ref. 25) of the \( \epsilon \) subunit was cross-linked with a cysteine introduced in the \( \beta \)DELSEED motif (28–30).

1 The abbreviations used are: \( TF_1\), \( F_1\)-ATPase from thermophilic \textit{Bacillus} PSS3 (a soluble portion of \( F_0F_1\)-ATP synthase); \( MF_1\), \( F_1\) from bovine heart mitochondrion; \( EF_1\), \( F_1\) from \textit{E. coli}; helix \( \alpha_2^\epsilon \), the C-terminal end of the \( \alpha\)-helices of the \( \epsilon \) subunit (residues from Lys\(^{114}\) to Lys\(^{135}\) in \( TF_1\); see Ref. 25); wt complex, a mutant \( \alpha\beta\gamma\epsilon \) complex; N\(_A\), a mutant complex in which all five acidic residues in the \( \beta \)DELSEED sequence of wt complex were replaced with alanines (\( \beta \)AALSAAA); \( \epsilon\)\textsuperscript{wt}, wild-type \( \epsilon \) subunit of \( TF_1\); \( \epsilon\)\textsuperscript{S107C}, \( \epsilon\)\textsuperscript{K133A}, \( \epsilon\)\textsuperscript{R122A/R126A/K133A}, respectively; PAGE, polyacrylamide gel electrophoresis; TMR, tetramethylrhodamine; \( \gamma \), the part of the \( \gamma \) subunit of \( EF_1\) from Ile\(^{11}\) to Ile\(^{20}\); HPLC, high pressure liquid chromatography; TES, 2-[\( \beta \)-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino]ethanesulfonic acid.

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is a possibility that the negative charges in the bDELSEED motif interact with positive charges in the C-terminal helix \( \alpha_2 \) of the \( \epsilon \) subunit. Therefore, this electrostatic interaction may be critical for inhibition of ATPase activity by the \( \epsilon \) subunit. To examine this possibility, we compared the ATPase activities of the mutant \( \alpha_3\beta_3\gamma \) and the \( \alpha_3\beta_3\gamma\epsilon \) complexes of TF1, in which acidic residues in the bDELSEED sequence and basic residues in the \( \epsilon \) subunit were replaced with alamines. The results indicate that the interaction between the negative charges in the bDELSEED motif and the positive charges in the C-terminal end of helix \( \alpha_2 \) of the \( \epsilon \) subunit is essential for the \( \epsilon \) subunit to play a role as an inhibitor for F1-ATPase.

**EXPERIMENTAL PROCEDURES**

**Construction of Expression Plasmid for \( \alpha_3\beta_3\gamma \) Complex and \( \epsilon \) Subunit of TF1**—The \( \alpha_3\beta_3\gamma \) complex of TF1, containing the mutations (\( \alpha1C193S, 10 \) His at N termini of \( \beta, \gamma \), S107C, wt) was considered to be a wild-type complex in this work. The expression plasmid for the wt complex was pKABG1 (31), and the mutations in the bDELSEED sequence were introduced into the plasmid as described previously (15). The expression plasmid for the wild-type \( \epsilon \) subunit (\( \epsilon^{wt} \)) was pTE2 (27), and that for the mutant \( \epsilon \) subunit (S45C/N125C, \( \epsilon^{NCX} \)) of TF1, was prepared as described previously (23). Alanine mutations were introduced into the plasmids (K133A, E140A, K121A, R122A/R126A, \( \epsilon^{PRA} \)) using a polymerase chain reaction method with \( \alpha1C193S \), NdeI and HindIII sites were introduced into the 5' and 3' termini of the mutant \( \epsilon \) subunit gene, respectively. The polymerase chain reaction products were digested by NdeI and HindIII, and the fragments obtained were cloned into pTE21c vector (Novagen Inc.) for efficient expression.

**Proteins**—The mutant \( \alpha_3\beta_3\gamma \) complexes of TF1 were purified as described (31). The wild-type and mutant \( \epsilon \) subunits of TF1, were prepared as described previously (23, 33). The \( \alpha_3\beta_3\gamma \) complexes were reconstituted from \( \alpha_3\beta_3\gamma \) and the \( \epsilon \) subunit by incubation for 30 min at room temperature. To remove the unbound \( \epsilon \) subunit, the mixture was concentrated several times with a centrifuge concentrator (Microcon 100; Millipore Amicon), and their purity was analyzed by 6% (w/v) PAGE without SDS (native-PAGE) in the presence of 20 \( \mu \)M ATP and 2 mM MgCl\(_2\).

**Measurement of ATPase Activity**—ATPase activity was measured spectrophotometrically with an ATP regenerating system at 25 °C (34). The assay mixture contained 50 mM Tris-HCl (pH 8.0), 100 mM KCl, 2.5 mM ATP, 0.1 mM NADH, 50 mM pyruvate, 2 mM MgCl\(_2\), 0.2 mM NADH, 50 mM pyruvate kinase, 50 \( \mu \)g/ml lactate dehydrogenase, and 20 \( \mu \)g/ml TMR maleimide into the 6% (w/v) native-PAGE for efficient expression. The assay mixture contained 50 mM Tris-HCl (pH 8.0), 100 mM KCl, 2.5 mM ATP and 2 mM MgCl\(_2\). Measurement of ATPase Activity—The mutant \( \alpha_3\beta_3\gamma \) complex was incubated with 20 \( \mu \)M ATP, 2 mM MgCl\(_2\), and 100 mM KCl in 50 mM Tris-HCl (pH 8.0) for 30 min at 25 °C. 100 \( \mu \)l of the complex was subjected to gel filtration HPLC (G-3000SWXL (Tosoh, Japan)) equilibrated with 20 mM Tris-HCl, 100 mM KCl, and 50 mM Tris-HCl (pH 7.0). The flow rate was 0.5 ml/min. A peak fraction was concentrated with Microcon 100, and their subunit composition was analyzed by 6% (w/v) native-PAGE and 14% (w/v) SDS-PAGE.

**Conformational Transition of the \( \epsilon \) Subunit in \( \alpha_3\beta_3\gamma \) Complex**—Formation of the intramolecular cross-link in the mutant \( \epsilon \) subunit in \( \alpha_3\beta_3\gamma \) complex was examined in the presence or the absence of 20 \( \mu \)M ATP-Mg. The isolated \( \alpha_3\beta_3\gamma\epsilon^{NCX} \) complex (1 mg/ml) was incubated with 20 \( \mu \)M ATP, 2 mM MgCl\(_2\), and 100 mM KCl in 50 mM Tris-HCl (pH 8.0) for 30 min at 25 °C. 100 \( \mu \)l of the complex was subjected to gel filtration HPLC (G-3000SWXL (Tosoh, Japan)) equilibrated with 20 mM ATP-Mg in the period 5–6 min after the addition of ATP. The ATPase activity of the N5A mutant complex, in which all of the five acidic residues in the bDELSEED sequence of the wt complex were replaced with alamines, was measured and compared with the activity of the wt complex. The reaction was initiated by the addition of the \( \alpha_3\beta_3\gamma \) and \( \alpha_3\beta_3\gamma\epsilon^{NCX} \) complexes were compared.

**Analysis of Stability of Mutant \( \alpha_3\beta_3\gamma\epsilon \) Complex in the Presence of ATP-Mg**—The mutant \( \alpha_3\beta_3\gamma\epsilon \) complex (1 mg/ml) was incubated with 20 \( \mu \)M ATP, 2 mM MgCl\(_2\), and 100 mM KCl in 50 mM Tris-HCl (pH 8.0) for 30 min at 25 °C. 100 \( \mu \)l of the complex was subjected to gel filtration HPLC (G-3000SWXL (Tosoh, Japan)) equilibrated with 20 \( \mu \)M ATP, 100 mM MgCl\(_2\), and 100 mM Tris-HCl (pH 7.0). The flow rate was 0.5 ml/min. A peak fraction was concentrated with Microcon 100, and their subunit composition was analyzed by 6% (w/v) native-PAGE and 14% (w/v) SDS-PAGE.

**RESULTS**

**Effect of the \( \epsilon \) Subunit on ATPase Activity of N5A Mutant Complex**—The ATPase activity of the N5A mutant complex, in which all of the five acidic residues in the bDELSEED sequence of the wt complex were replaced with alamines, was measured and compared with the activity of the wt complex. The reaction was initiated by the addition of the \( \alpha_3\beta_3\gamma \) and \( \alpha_3\beta_3\gamma\epsilon^{NCX} \) complexes, designated as the \( -\epsilon \) complex and the \( +\epsilon \) complex, respectively, to the reaction mixture. As reported previously (27), ATP hydrolysis at low ATP concentration by the wt(\(+\epsilon\)) complex was very slow in comparison with the wt(\(-\epsilon\)) complex, and the inhibitory role of the \( \epsilon \) subunit is thus evident (Fig. 1A). It should be noted that the inhibition by the \( \epsilon \) subunit was relieved slowly and that the ATPase activity was recovered gradually by the reaction period. At 2 \( \mu \)M ATP, this recovery was much faster, and the inhibitory effect of the \( \epsilon \) subunit was only observed at the very initial phase of the reaction (Fig. 1B). As stated previously, this recovery is due to the nucleotide-dependent conformational transition of the \( \epsilon \) subunit (23). On the contrary, the N5A(\(+\epsilon\)) complex hydrolyzed ATP at the same rate as the N5A(\(-\epsilon\)) complex at both 20 \( \mu \)M and 2 \( \mu \)M ATP (Fig. 1, C and D). Apparently, the N5A complex lost sensitivity to the inhibition by \( \epsilon \) subunit.

**The \( \epsilon \) Subunit Does Not Dissociate from the N5A(\(+\epsilon\)) Complex during ATP Hydrolysis**—The results shown in Fig. 1 could be explained even if it were by the dissociation of the \( \epsilon \) subunit from the N5A complex rapidly during ATP hydrolysis. To confirm this, we incubated the N5A(\(+\epsilon\)) complex with 20 \( \mu \)M ATP-Mg under the same conditions used for the measurement of the ATPase activities. After a 30-min incubation, the complex was isolated by gel filtration HPLC in the presence of 20 \( \mu \)M ATP-Mg (Fig. 2). The homogeneity of the N5A(\(+\epsilon\)) complex was further demonstrated by native-PAGE.
because the complex electrophoresed as a single band (Fig. 2, inset b). The electrophoretic mobility of the N5A(+e) complex was slightly slower than that of the N5A(-e) complex. SDS-PAGE analysis of the isolated complex showed that the N5A(+e) complex contained all of the α, β, γ, and ε subunits with the correct stoichiometry (Fig. 2, inset a). These results strongly suggest that the N5A complex lost its sensitivity to inhibition by the ε subunit without dissociation of this subunit.

**Conformational Transition of εNCX in the N2A(+e) Complex**—To confirm the nucleotide-dependent conformational change of the ε subunit in the N2A(+e) complex, we used a mutant ε subunit, εNCX, in which two cysteine residues were introduced at the interface of the two domains (S48C/N125C) (23). εNCX inhibited the wt complex but not the N2A complex as wild-type ε (Fig. 1, A and C). When the N-terminal β strand domain and C-terminal α-helical domain packed closely in εNCX (“closed” form) as determined by NMR and x-ray crystallography (24–26), an interdomain disulfide is readily formed by the assistance of the low concentrations of CuCl2. However, if these two domains assume the “open” form, a disulfide bond is no longer formed, and the free sulfide residues are susceptible to labeling by fluorescent maleimide. This efficiency of labeling by TMR maleimide is therefore a good indicator of the existence of the open form of εNCX in which a disulfide bond is very rarely formed. Electrophoretic mobility of εNCX containing a disulfide is slightly faster than that of εNCX with TMR-labeled sulfides, and this distinction was also adopted as a means to assess the open and closed forms. In the presence of 2 mM ATP and 50 mM CuCl2, εNCX both in the wt’ and the N2A complex was largely unlabeled by TMR maleimide, and the protein bands corresponding to εNCX, with a disulfide were dominant (Fig. 3). On the other hand, disulfide formation in εNCX in the N2A complex did not occur in the absence of 20 mM ATP, similar to wt’ complex. These results imply that the ε subunit in the complex can undergo a nucleotide-dependent conformational transition to the noninhibitory state, although 20 mM ATP-Mg was insufficient to this transition.

**Effect of Partial Elimination of Negative Charges in the βDELSDEED Motif**—To clarify the important residues in the βDELSDEED motif for inhibition by the ε subunit, we generated seven mutant αβγε complexes in which the acidic residues in the βDELSDEED sequence were substituted by alanines. Substitution of the last three acidic residues (DELSAAA) efficiently suppressed the propagation of the inhibitory effect of the ε subunit (Table I). The replacement of the first two acidic residues (AALSDDD) also resulted in suppression of the ε-induced inhibition, although the mutation showed a partial effect (Table I). A single replacement at Asp⁹⁰⁹, Glu⁹¹⁹, or Asp⁹²⁶ also has some suppression on the ε-induced inhibition. Taken together, nearly all of the acidic residues in the βDELSDEED sequence cumulatively contribute to the inhibitory effect of the ε subunit, and the last three are the most critical.

**Effect of Partial Elimination of Positive Charges in the C-terminal α-Helix of the ε Subunit**—Next we investigated whether the positively charged residues in the C-terminal α-helix of the ε subunit contribute to the inhibitory role of the ε subunit. For this purpose, we designed three mutant ε subunits of TF3, in which some positively charged residues in the C-terminal α-helix were replaced with Ala, and they were designated ε⁺¹α, ε⁺³α, and ε⁺⁴α according to the number of the substituted Ala from the positively charged residue(s). The ATPase activities of the wt’ complex with these mutant ε subunits were measured in the presence of 20 mM ATP (Fig. 4). The wt’ (+E⁺⁴α) complex hydrolyzed ATP at the almost same rate as the wt’ (−ε) complex. The wt’ (+E⁺⁴α) complex was isolated by gel filtration HPLC in the presence of 20 mM ATP-Mg (Fig. 5), suggesting that the complex obtained with this mutant ε subunit was stable. The wt’ (+E⁺¹α) and wt’ (+E⁺³α) complexes showed partial effects on the inhibitory role of the ε subunit. These results suggested that the ε subunit exerts the inhibitory role through the positive charges in the C-terminal α-helix of the ε subunit.

**Table I**

| Mutation | ATPase activity | % |
|----------|----------------|---|
|        | +ε             | −ε             | +ε/−ε  |
| DELSEED (wt’) | 2.8 ± 0.6 | 25 ± 3 | 11 |
| AALSAAA (N2A) | 3.1 ± 0.2 | 3.4 ± 0.3 | 85 |
| DELSEED | 1.6 ± 0.2 | 1.9 ± 0.3 | 85 |
| AALSAAA | 1.6 ± 0.2 | 2.8 ± 0.4 | 68 |
| DELSEED | 6.5 ± 0.7 | 20 ± 2 | 35 |
| DALSDD | 0.32 ± 0.03 | 0.79 ± 0.1 | 41 |
| DELSEED | 3.1 ± 0.3 | 16 ± 2 | 19 |
| DELSEDEA | 2.0 ± 0.3 | 13 ± 0.9 | 15 |
| DELSEDEA | 0.90 ± 0.06 | 1.6 ± 0.05 | 55 |

**Fig. 3. Effect of nucleotides on the formation of cross-links within ε subunit.** The αβγε complex was incubated with ATP at 0 or 20 μM or 2 mM and with 2 mM MgCl2 in the absence (−) or the presence (+) of 50 μM CuCl2, respectively. After incubation for 1 h at 25 °C, non-cross-linked sulphydril groups were labeled with TMR maleimide, and the samples were subjected to 15% SDS-PAGE. The gel was visualized by UV illumination (upper panel) and stained with Coomassie Brilliant Blue (lower panel). Only the region around the ε subunit is shown. The conditions for the incubation were indicated.
Inhibitory effect of the inhibitory conformation (Fig. 3). Therefore, we here propose that the

Procedures."

Experimental conditions were described under “Experimental Procedures.”

Inset b

An absorbance unit 4.0

supported—

Because the resolution of the yeast mitochondrial

transition of the

are two possible explanations for this observation. Either the

subunit to an inhibitory state, or the

subunit cannot interact with the

DELSEED motif under certain conditions (28–30). A mutant

subunit whose C-terminal domain was truncated could not inhibit the ATPase activity of the

F1-F0-ATP synthase (22). A nucleotide-dependent conformational change in the

subunit of EF1 was reported by Wilkens and Capaldi (26). We also have reported the nucleotide-dependent

formation of an interdomain cross-link in the

subunit of TF1 (23). Hence there are several results that strongly suggest the direct interaction of the

DELSEED motif with the C-terminal domain of the

subunit and a large conformational transition of the

subunit. Finally, these possibilities were supported recently by the newly reported three-dimensional structures of the MF1 (37) and the γ-ε complex from EF1 (38) as described below.

Interaction between the βDELSEED Motif and the C-terminal α-Helix of the ε Subunit Involves Electrostatic Interactions—Elimination of negative charges in the βDELSEED motif cumulatively desensitized F1 from ε-induced inhibition (Table I), indicating that the βDELSEED motif interacts with the ε subunit not by specific interactions between certain residues but through a cluster of negative charges. In addition, elimination of positive charges in the C-terminal α-helix of the ε subunit also gave similar results (Fig. 4). The cumulative contribution of the negative charges in the βDELSEED motif and the positive charges in the C-terminal region of the ε subunit strongly suggest that the electrostatic interaction between these two regions dominates the inhibition of the F1-

ATPase activity.

A Model for the Conformational Transition of the ε Subunit

F1-F0, structure was limited to 3.9 Å (36), the details of the ε subunit (called δ in mitochondrial F1) could not be clearly defined. Nevertheless, the electron density of the δ subunit (bacterial ε subunit) can readily accommodate the structure of the isolated ε subunit from EF1 (24, 26). The δ subunit (bacterial ε subunit), however, lies on the c subunit ring, far from the αβ3 subunit, where a part of the ε subunit cannot interact with the DELSEED motif of the β subunit. When the ε subunit acts as an inhibitor, a large conformational transition must occur in the ε subunit to achieve interaction with the catalytic portion. Cross-linking experiments showed that the C-terminal domain of the ε subunit is close to the βDELSEED motif under certain conditions (28–30). A mutant ε subunit whose C-terminal domain was truncated could not inhibit the ATPase activity of the F1-F0-ATP synthase (22). A nucleotide-dependent conformational change in the ε subunit of EF1 was reported by Wilkens and Capaldi (26). We also have reported the nucleotide-dependent formation of an interdomain cross-link in the ε subunit of TF1 (23). Hence there are several results that strongly suggest the direct interaction of the βDELSEED motif with the C-terminal domain of the ε subunit and a large conformational transition of the ε subunit. Finally, these possibilities were supported recently by the newly reported three-dimensional structures of the MF1 (37) and the γ-ε complex from EF1 (38) as described below.
and Activation of αβγε Complex—Just when we concluded the present study, the crystal structure of the γ’ (residues γ11-γ258)-ε complex in EF$_1$ (38) and that of the αβγε complex in MF$_1$ (37) were reported. These two structures revealed that the ε subunit in EF$_1$ and the corresponding subunit in MF$_1$, δ, adopted very different conformations. The two helices of EF$_1$-ε extended and entwined the coiled-coil structure of the γ subunit, and the C-terminal end reached out toward the αβγ subunit. In contrast, the two α-helices of MF$_1$-δ abutted the β-sandwich structure and the C termini located on the surface of ε subunits.

The interaction between the βDELSEED motif and the C-terminal α-helix of the ε subunit must be possible if the conformation of the ε subunit in the complex is similar to that reported in the γ’-ε complex of EF$_1$ (38). In contrast, the cross-linking in ε$^{NCX}$ must occur when the structure of the ε subunit in the complex is in the same conformation as that of the δ subunit in MF$_1$ (37). If this is the case, our results should be well explained by the large conformational transition of the ε subunit. The conformation of the ε subunit in the γ’-ε complex of EF$_1$ (38) and the δ subunit in MF$_1$ (37) correspond to the inhibitory state and noninhibitory state, respectively.

Interestingly, the ε subunit of MF$_1$ lay between the γ subunit and the δ subunit like a wedge (37). This subunit seems to mimic the structure of the C-terminal α-helical domain of the bacterial ε subunit in the complex. The C-terminal part of the MF$_1$-ε subunit contains four positively charged residues at the counterpart of the C-terminal α-helix of the MF$_1$-δ subunit, which is shorter and contains only two positively charged residues. It is therefore an intriguing assumption that the C-terminal region of the ε subunit of MF$_1$ interacts with βDELSEED motif and inhibits the activity in MF$_1$-ATPase under certain conditions, although the role of this subunit is not known very well.

Here we propose a model for the conformational transition of the bacterial ε subunit to explain the activation of αβγε complex (Fig. 6). When the ε subunit is in the inhibitory form, the C-terminal α-helix of the ε subunit interacts with βDELSEED motif through their respective charges. The apparent bridge formed between the ε subunit and a β subunit makes the αβγε complex inactive. When the conformational change is induced in the β subunit by ATP binding, the electrostatic interaction is broken, and the ε subunit resumes the inhibitory form. Then the αβγε complex becomes active. From the present results and our previous reports, it is evident that ATP-Mg stimulates the conformational transition of the ε subunit from the inhibitory state to the noninhibitory state. The information on the trigger for this conformational transition of the ε subunit is requisite for further understanding of the internal regulation of the F$_1$-ATPase.

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