Nucleotide-Dependent and Dicyclohexylcarbodiimide-Sensitive Conformational Changes in the ϵ Subunit of *Escherichia coli* ATP Synthase†

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Received July 3, 1991; Revised Manuscript Received August 22, 1991

ABSTRACT: The rate of trypsin cleavage of the ϵ subunit of *Escherichia coli* F_1F_o (ECF_1F_o) is shown to be ligand-dependent as measured by Western analysis using monoclonal antibodies. The cleavage of the ϵ subunit was rapid in the presence of ADP alone, ATP + EDTA, or AMP-PNP + Mg^{2+}, but slow when P_i was added along with ADP + Mg^{2+} or when ATP + Mg^{2+} was added to generate ADP + P_i (+Mg^{2+}) in the catalytic site. Trypsin treatment of ECF_1F_o was also shown to increase enzymic activity on a time scale corresponding to that of the cleavage of the ϵ subunit, indicating that the ϵ subunit inhibits ATPase activity in ECF_1F_o. The ligand-dependent conformational changes in the ϵ subunit were also examined in cross-linking experiments using the water-soluble carbodiimide 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide (EDC). In the presence of ATP + Mg^{2+} or ADP + P_i + Mg^{2+}, the ϵ subunit cross-linked to β in high yield. With ATP + EDTA, the yield of β–ϵ-cross-linked product was much reduced. Prior reaction of ECF_1F_o with dicyclohexylcarbodiimide (DCCD), under conditions in which only the F_o part was modified, blocked the conformational changes induced by ligand binding. When the enzyme complex was reacted with DCCD in ATP + EDTA, the cleavage of the ϵ subunit was rapid and yield of cross-linking of β to ϵ subunit low, whether trypsin cleavage was conducted in ATP + EDTA or ATP + Mg^{2+}. When enzyme was reacted with DCCD in ATP + Mg^{2+}, cleavage of the ϵ subunit was slow and yield of cross-linking of β to ϵ high, under all nucleotide conditions for proteolysis. We suggest that conformational rearrangements of the ϵ subunit are a part of the coupling between events at the catalytic sites and proton translocation in the ATP synthase.

An F_1F_o-type ATP synthase, found in the bacterial plasma membrane, the mitochondrial inner membrane, and the chloroplast thylakoid membrane, catalyzes ATP synthesis in response to a transmembrane proton gradient. This enzyme can also generate a proton gradient by using the energy released from hydrolysis of ATP. The best characterized F_1F_o-type ATPase is the enzyme from *Escherichia coli*, ECF_1F_o[1] (Cross, 1988; Senior, 1988; Futai et al., 1989).

The ECF_1 moiety is extrinsic to the membrane and composed of five distinct subunits in the stoichiometry of $\alpha_3,\beta_3,\gamma,\delta,\epsilon$. Catalytic sites are located on the β subunits, possibly at the interface between α and β subunits (Cross, 1988; Senior, 1988). The γ subunit appears to be involved in regulation of activity (Schumann et al., 1985), while both the δ and ϵ subunits are required for linking F_1 to the membrane-intercalated F_o moiety (Sternweis & Smith, 1977, 1980).

The F_o moiety contains three different subunits in the apparent stoichiometry a1, b2, c10–12 (Foster & Fillingame, 1979a,b). Genetic analyses have led to the suggestion that subunits a and c form a proton wire of ionic residues (Cox et al., 1984). Binding of dicyclohexylcarbodiimide (DCCD) to Asp-61 of subunit c has been shown both to inhibit proton translocation through F_o and to reduce affinity for ATP by producing a conformational change transmitted to the catalytic sites on the F_1 part (Penefsky, 1985). Similar energy-dependent and ligand-induced conformational changes are believed to link proton translocation and catalytic site events during ATP synthesis and ATP hydrolysis (Rosen et al., 1979; Cross et al., 1982), but the subunits involved in transmitting these conformational changes have not been identified.

We have recently measured the kinetics of cleavage of the subunits of ECF_1 as a function of nucleotides, Mg^{2+}, and/or P_i present (Mendel-Hartvig & Capaldi, 1991). These studies, in conjunction with cross-linking (Mendel-Hartvig & Capaldi, 1991), indicate that the structure of the ϵ subunit is sensitive to the presence of nucleotide and P_i in the enzyme.

If changes in the ϵ subunit are important in linking ATP hydrolysis with proton pumping through F_o, they should occur in ECF_1F_o. To test this, we have conducted trypsin digestion and cross-linking experiments on purified ATP synthase, including experiments in the presence and absence of DCCD to block proton translocation. Results are interpreted in terms of the role of the ϵ subunit in the functioning of the ATP synthase.

Materials and Methods

ECF_1F_o was isolated from *E. coli* strain AN1460 according to Foster and Fillingame (1979a,b). The enzyme was isolated at concentrations of 0.5–1 mg/mL protein from a 10–40% sucrose gradient containing 0.25 mg/mL egg phosphatidylcholine (Sigma), 0.01% sodium cholate, and 0.25% sodium deoxycholate in 50 mM Tris-HCl, pH 7.5, 5 mM $MgSO_4$, 1 mM DTE, and 6 mM PAB. Sucrose gradient fractions were immediately pooled and dialyzed against 1000 volumes of 50 mM Tris-HCl, pH 7.5, 5 mM $MgSO_4$, 1 mM DTT, and 10%

† This research was supported by National Institutes of Health Grant HL24536.

[1] Abbreviations: DCCD, dicyclohexylcarbodiimide; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; ECF_1F_o, *Escherichia coli* ATP synthase; DTE, dithioerythritol; PAB, *p*-aminobenzamidine; MOPS, 3-(*N*-morpholino)propanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; LDAO, *N*,*N*-dimethyldodecylamine *N*-oxide; ECF_1, water-soluble, membrane-extrinsic ATPase part of the *E. coli* ATP synthase; PMSF, phenylmethanesulfonyl fluoride.

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glycerol for 12 h at 4 °C and then against 1000 volumes of 50 mM Tris-HCl (or 50 mM MOPS, where indicated), pH 8.0, 0.5 mM $MgSO_4$, 1 mM DTT, and 10% glycerol, overnight. Vesicles were collected by centrifugation at 100000*g* for 1 h at 4 °C after dilution in 1 volume of double-distilled water. Finally, the ECF_1F_o vesicles were resuspended in a minimum amount of 50 mM Tris-HCl (or 50 mM MOPS), pH 8.0, 0.5 mM $MgSO_4$, 1 mM DTT, and 20% glycerol, and samples were stored in liquid N_2.

Trypsin Cleavage of ECF_1F_o. Trypsin cleavage of ECF_1F_o (2 mg/mL) was conducted in a buffer of 50 mM Tris-HCl, pH 8.0, 1 mM DTT, and 20% glycerol with the following additions made to give aliquots containing 5 mM ADP and 5 mM $MgCl_2$: 5 mM ATP and 5 mM $MgCl_2$; 5 mM ADP and 1 mM EDTA; 5 mM ATP and 1 mM EDTA; 5 mM ADP, 5 mM $MgCl_2$, and 5 mM NaH_2PO_4; 5 mM AMP-PNP and 5 mM $MgCl_2$, respectively. Samples were allowed to incubate for 30 min at room temperature before addition of trypsin (1:50 w/w protease to protein). At various times, aliquots were withdrawn, inhibited by PMSF (1 mM) and a 10-fold excess of soybean trypsin inhibitor, and immediately assayed for ATPase activity, activation by 0.5% LDAO (Lotscher et al., 1984), and inhibition by 50 μM DCCD (Satre et al., 1980).

Tryptic fragments were identified by $NaDodSO_4$–polyacrylamide gel electrophoresis followed by immunoblotting with antibodies against each of the ECF_1 subunits as described earlier (Mendel-Hartvig & Capaldi, 1991).

EDC Cross-Linking of ECF_1F_o. ECF_1F_o vesicles (2 mg/mL) equilibrated in 50 mM MOPS, pH 8.0, 0.5 mM DTT, and 20% glycerol, in the presence or absence of various ligands (described above), were incubated with 5 mM EDC for 3 h at room temperature as described earlier (Mendel-Hartvig & Capaldi, 1991). The cross-linking was inhibited by the addition of 4 M Tris to a final concentration of 100 mM prior to $NaDodSO_4$–polyacrylamide gel electrophoresis.

Other Methods. Protein concentrations were determined according to Markwell et al. (1978). Samples for $NaDodSO_4$–polyacrylamide gel electrophoresis were dissolved in 2% $NaDodSO_4$, 5% glycerol, and 0.12 M Tris-HCl (pH 6.8); 1.5-mm-thick slab gels were run as described by Laemmli (1970) using a 3% acrylamide gel as stacker and a 10–18% gradient of acrylamide in the separating gel. Staining with Coomassie brilliant blue and destaining were carried out according to Downer et al. (1976). Immunoblotting was conducted according to Towbin et al. (1979) using a buffer containing 10 mM CAPS, pH 11.0, and 10% methanol. Alkaline phosphatase conjugated goat anti-mouse antibodies were used for color development (Hawkes et al., 1982).

ATPase activity was assayed using the ATP regenerating system described previously (Lotscher et al., 1984). To measure ligand-induced differences in the DCCD binding affinity of the F_o moiety, ECF_1F_o aliquots (1 mg/mL) were incubated in 50 mM Tris-HCl, pH 8.0, 0.5 mM DTT, and 20% glycerol in the presence and absence of various ligands. At 5-min intervals, 50 μM DCCD (final concentration) was added to each sample. The ATPase activity of each sample was measured at 10-min intervals.

Results

Characteristics of the Reconstituted Vesicles of ECF_1F_o. ECF_1F_o was reconstituted into vesicles with phosphatidylcholine during the final steps of purification, and stored in 50 mM Tris-HCl, pH 8.0, 1 mM DTT, and 20% glycerol, with 0.5 mM $MgSO_4$ added to prevent stripping of the F_1 part from F_o. Samples were incubated for 30 min at room temperature with various nucleotides, EDTA, or Mg^{2+} before the proteolysis reaction was begun.

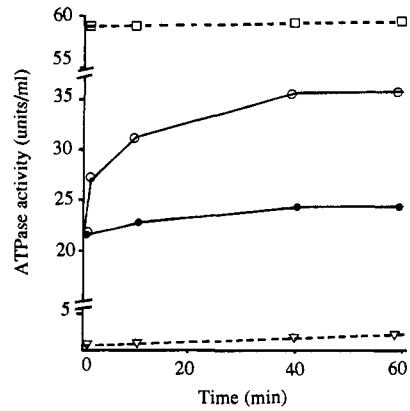

FIGURE 1: Effect of trypsin cleavage on the ATPase activity of ECF_1F_o. ECF_1F_o was incubated with trypsin (1:50 w/w) in the presence of 5 mM ATP and 2 mM EDTA (○) or 5 mM ATP and 5 mM Mg^{2+} (●). At specified times, proteolysis was inhibited by addition of trypsin inhibitor, and samples were assayed for ATPase activity. 0.5% LDAO activation (□), and 50 μM DCCD inhibition of activity (▽).

Preparations had ATPase activities ranging from 19 to 22 μmol of ATP hydrolyzed min^{-1} (mg of enzyme)$^{-1}$ and were inhibited 90–95% by incubation with 50 μM DCCD for 1 h at room temperature. Addition of 0.5% LDAO activated the reconstituted enzyme 3.5–4-fold (to 70–80 μmol of ATP hydrolyzed min^{-1} mg^{-1}). A similar activity was obtained by LDAO treatment of the DCCD-inhibited enzyme, indicating that the hydrophobic carbodiimide had reacted in F_o (in subunit c) rather than in the F_1 part [see Lotscher et al. (1984) for a discussion of the effects of LDAO].

Trypsin Treatment of ECF_1F_o Increases ATPase Activity by Cleavage of the ε Subunit. Figure 1 shows a time course of the effect of trypsin treatment on the ATPase activity of ECF_1F_o in the presence of ATP + EDTA. There was a rapid activation of the enzyme from an activity of around 20 μmol of ATP hydrolyzed min^{-1} mg^{-1} to 35–40 μmol of ATP hydrolyzed min^{-1} (mg of enzyme)$^{-1}$.

The time course of proteolysis was also examined by $NaDodSO_4$–polyacrylamide gel electrophoresis. The activation of ECF_1F_o seen with ATP + EDTA was paralleled by cleavage of the ε subunit, as seen both from Coomassie brilliant blue stained gels (Figure 2A) and from Western blotting experiments using monoclonal antibodies to selected individual subunits (Figure 2B). Only cleavage of the ε subunit followed the same time course as activation; cleavage of the γ subunit was subsequent to this activation, as shown in Figure 2B. There was cleavage of the β subunit which could be followed on 10% polyacrylamide gels (not shown), but this was much slower than the observed increase in ATPase activity. No cleavage of the α, δ, a, b, or c subunits was observed during the time course of the experiment. Thus, activation of ECF_1F_o upon trypsin cleavage can be attributed to cleavage of the ε subunit, and this subunit, therefore, acts to inhibit ATPase activity in the intact ATP synthase, as it does in isolated ECF_1.

The sizes of the cleavage products of the ε subunit in ECF_1F_o were the same as those observed upon trypsin cleavage of ECF_1 alone (Mendel-Hartvig & Capaldi, 1991), indicating that this subunit is modified from the C terminus, which must be exposed in the intact ATP synthase.

Enzyme which had been activated by trypsin cleavage was still inhibited by DCCD modification of the c subunit of F_o (Figure 1). Therefore, the removal of the C terminus of the ε subunit did not alter the interaction between F_1 and F_o parts in any major way.

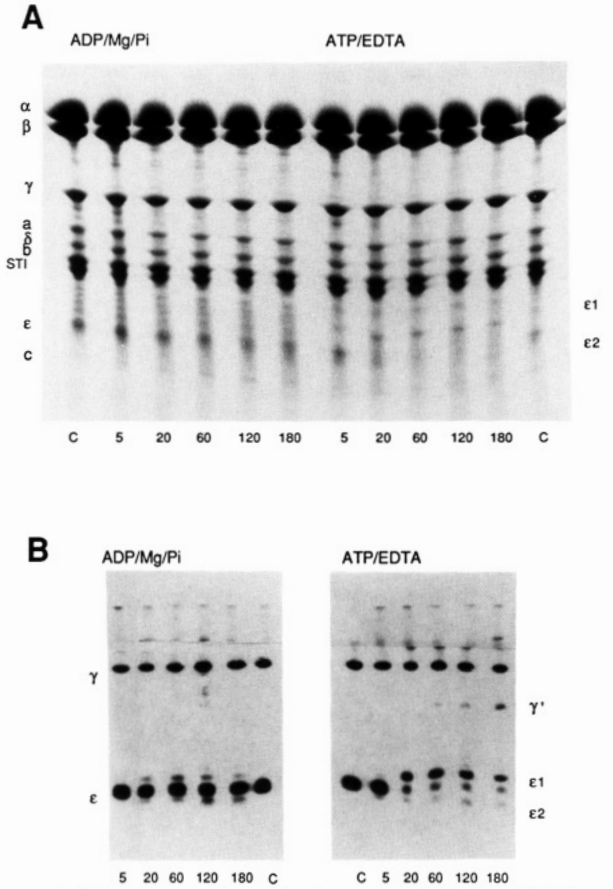

FIGURE 2: Time course of trypsin cleavage of ECF_1F_o. ECF_1F_o was incubated with trypsin (1:50 w/w) in the presence of Mg^{2+} + ATP (Mg^2 + ADP + P_i after catalysis) (lanes 1–6) or ATP + EDTA (lanes 7–12). At the time indicated (5, 20, 60, 120, and 180 min), proteolysis was inhibited by addition of trypsin inhibitor. (A) Samples were electrophoresed on a 10–18% $NaDodSO_4$–polyacrylamide gel. (B) Immunoblot of the same samples with monoclonal antibodies against the γ and ε subunits. Lane C in each gel is a control experiment in which trypsin inhibitor was added to ECF_1F_o samples prior to addition of trypsin.

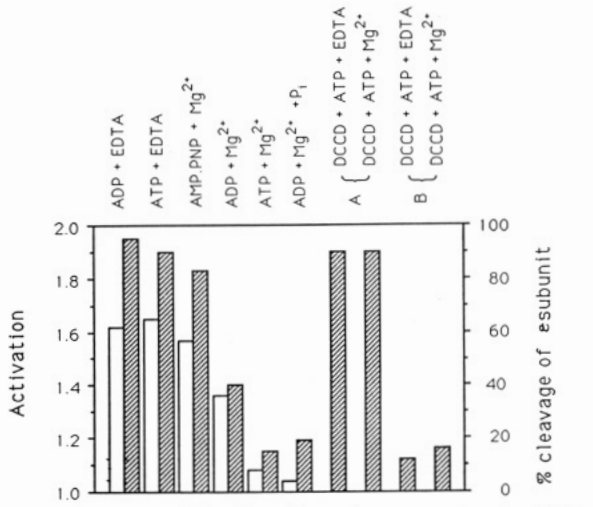

FIGURE 3: Summary of the effect of trypsin treatment on the ATPase activity and ε-subunit cleavage in ECF_1F_o under different nucleotide conditions and after DCCD reaction of the F_o part. ATP, ADP, AMP-PNP, $NaHPO_4$, and $MgCl_2$ were added to 5 mM, respectively, EDTA to 1 mM. Enzyme was reacted with 50 μM DCCD before protease digestion: (A) in the presence of 5 mM ATP and 1 mM EDTA and (B) in the presence of 5 mM ATP and 5 mM $MgCl_2$. Open bars are the effect on ATPase activity calculated as activation after 60-min proteolysis. Hatched bars are the percent cleavage of the ε subunit from $NaDodSO_4$–polyacrylamide gel electrophoresis, measured for samples incubated with trypsin for 60 min.

Activation of ATPase Activity and Cleavage of the ε Subunit Are Nucleotide-Dependent. The effect of trypsin treatment on ECF_1F_o was followed under a variety of nucleotide conditions, and the results are summarized in Figure 3. The rapid activation along with concomitantly rapid cleavage of the ε subunit seen in ATP + EDTA was also observed in enzyme suspended in ADP + EDTA, and in AMP-PNP + Mg^{2+}, a nonhydrolyzable analogue of ATP.

When ECF_1F_o was suspended in ATP + Mg^{2+}, there was very little increase in ATPase activity (Figure 1) and only very slow cleavage of the ε subunit within the 60 min of the proteolysis experiment (Figure 3). Similarly, low activation and slow cleavage of the ε subunit were obtained in the presence of ADP + Mg^{2+} + P_i. The rates of both activation of the enzyme and cleavage of the ε subunit for ECF_1F_o suspended in ADP + Mg^{2+} were intermediate between the rates in ATP + EDTA or ATP + Mg^{2+} (Figure 3) but variable. The above effects of nucleotide on the state of the ε subunit are the same as seen with isolated ECF_1 under different occupancies of nucleotide in catalytic sites (Mendel-Hartvig & Capaldi, 1991).

DCCD Modification of F_o Prevents Conformational Switching. The possibility that trypsin cleavage of the F_1 part of ECF_1F_o was sensitive to DCCD modification of the F_o part was examined in a series of experiments. The pattern of trypsin cleavage proved to be determined by which nucleotides were present during the DCCD modification, rather than the conditions under which proteolysis occurred. Thus, when ECF_1F_o was reacted with DCCD in the presence of ATP + EDTA to give 90% inhibition of ATPase activity, subsequent trypsin treatment gave rapid cleavage of the ε subunit, whether proteolysis was conducted in ATP + EDTA or in ATP + Mg^{2+} (Figure 3). Similarly, the cleavage of the ε subunit was slow in ECF_1F_o modified by DCCD (90% inhibition) in ATP + Mg^{2+}, whether proteolysis was conducted in the same nucleotide conditions, or if ATP + Mg^{2+} had been exchanged for ATP + EDTA by dialysis prior to the addition of trypsin.

These results indicate that DCCD modification of subunit c prevents the nucleotide-dependent switching of the conformation of ECF_1 that is being monitored by the sensitivity of the ε subunit to trypsin treatment.

EDC Cross-Linking of a β Subunit to the ε Subunit in ECF_1F_o Is a Nucleotide-Dependent and DCCD-Sensitive Reaction. We have previously found that the water-soluble carbodiimide EDC reacts with ECF_1 to form a cross-link between a β and the ε subunit, the yield of which was sensitive to the presence of different ligands in the catalytic site (Mendel-Hartvig & Capaldi, 1991). Figure 4 shows a representative experiment in which ECF_1F_o was reacted with EDC in the presence of Mg^{2+} + ATP and ATP + EDTA, respectively. The Coomassie brilliant blue stained gel and corresponding Western blots for the two cross-linking conditions (not presented) showed clear differences in the yield of β–ε-cross-linked product. In the presence of Mg^{2+} + ATP (hydrolyzed to ADP + Mg^{2+} + P_i in the catalytic sites), most of the ε subunit was cross-linked to a β subunit. In the presence of EDTA + ATP, there was internal cross-linking of the ε subunit, with some of this product being further cross-linked to the β subunit, but in low yield. DCCD modification of F_o was found to lock the ε subunit into the arrangement present in the buffer conditions in which the carbodiimide reaction was carried out. Thus, when ECF_1F_o was reacted with DCCD in ATP + EDTA, there was very poor cross-linking of ε to β, whether the EDC reaction was conducted in ATP + EDTA

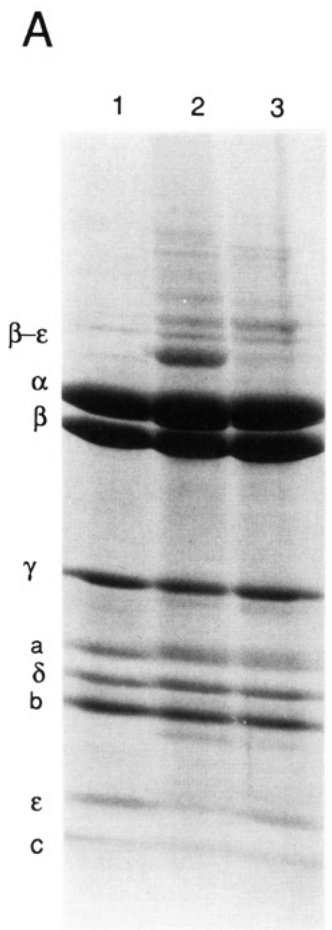

FIGURE 4: Formation of cross-links by EDC. ECF_1F_o (1.5 mg/mL) was incubated with 1 mg/mL EDC in the absence (lane 1) and presence of 5 mM ATP + 5 mM $MgSO_4$ (lane 2) or 5 mM ATP + 2 mM EDTA (lane 3), as described under Materials and Methods. After incubation for 3 h at room temp, the cross-linking reaction was stopped by addition of 100 mM Tris (final concentration). The samples were subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue.

or ATP + Mg^{2+}. Also, if ECF_1F_o was modified with DCCD in ATP + Mg^{2+}, the yield of β–ϵ-cross-linked product was high when EDC was added in the presence of either ATP + Mg^{2+} or ATP + EDTA (result not shown).

Discussion

The studies described here show that ligand-dependent changes in the ϵ subunit, observed previously in ECF_1 (Mendel-Hartvig & Capaldi, 1991), also occur in the intact ATP synthase. Moreover, these structural changes are sensitive to DCCD modification of the F_o part of ECF_1F_o, as would be expected if they are important in conformational coupling between catalytic sites and the proton channel. When ADP + Mg^{2+} + P_i are present in catalytic sites of ECF_1F_o, the ϵ subunit is in a conformation relatively insensitive to trypsin cleavage, but this polypeptide is readily cross-linked to a β subunit by the water-soluble carbodiimide EDC. In the presence of ADP + EDTA or ATP + EDTA, or when ADP + Mg^{2+} are added in the absence of P_i, the ϵ subunit of ECF_1F_o is digested rapidly by trypsin but much less efficiently cross-linked to the β subunit.

Our previous studies with isolated ECF_1 established that the conformational changes in the ϵ subunit are related to P_i binding, such that there is a reciprocal relationship between ϵ structure and P_i binding. Binding of P_i affects the ϵ-subunit conformation, and at the same time, binding of the ϵ subunit appears to control P_i binding in the catalytic sites. For example, Dunn et al. (1987) have established that the binding of the ϵ subunit to ECF_1 decreases the off rate of P_i in unisite catalysis. Also, Wood et al. (1987) have found that the ϵ subunit promotes ^{18}O exchange between P_i and ATP bound in the high-affinity catalytic site, indicating a longer residency of P_i when the ϵ subunit is present.

An important finding of the present work is that the structure of the ϵ subunit is influenced by the binding of DCCD to the c subunit of the F_o part of the complex. DCCD blocks proton translocation through F_o and thereby inhibits both ATP synthesis and ATP hydrolysis by the intact ATP synthase. Previous studies have shown that modification of F_o by DCCD both alters nucleotide binding in the catalytic sites of the F_1 part (Penefsky, 1985) and blocks the nucleotide-dependent fluorescence changes of aurovertin bound to the β subunit (Matsuno-Yagi et al., 1985). These observations were taken to indicate that DCCD induces a structural change in F_o that is transmitted to the catalytic sites, as much as 100 Å from the proton channel.

Our results show that DCCD modification of subunit c, in inhibiting ATPase activity, also blocks the structural changes in the ϵ subunit that are generated by changes in nucleotides present. Structural changes in the ϵ subunit of CF_1 have also been shown to accompany proton-driven ATP synthesis (Richter & McCarty, 1987). Taken together, all of the data indicate that conformational rearrangements of the ϵ subunit are a part of the coupling between proton translocation through F_o and catalytic site events in F_1.

It is well documented that the ϵ subunit is an inhibitor of ATPase activity in isolated ECF_1. Results presented here show for the first time that the ϵ subunit affects the rate of ATP hydrolysis in ECF_1F_o. Protease digestion of the ϵ subunit increases the rate of ATP hydrolysis, although not as dramatically as in ECF_1. The rate-limiting step in ATP hydrolysis is product (ADP + P_i) release. If the release of P_i depends on switching of the ϵ subunit between two conformations or switching between two binding sites, this switching could be rate-limiting. The inhibitory effect of the ϵ subunit in ATP hydrolysis would thereby be a secondary consequence of the primary function of this subunit in coupling catalytic site events with proton pumping within the ATP synthase.

Acknowledgments

The excellent technical assistance of Kathy Chicas-Cruz is gratefully acknowledged.

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Evidence from Total Internal Reflection Fluorescence Microscopy for Calcium-Independent Binding of Prothrombin to Negatively Charged Planar Phospholipid Membranes†

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Received February 6, 1991; Revised Manuscript Received August 19, 1991

ABSTRACT: Measurements to test for a proposed Ca^{2+}-independent interaction of prothrombin with membranes containing acidic phospholipids are described. Fluorescein-labeled bovine prothrombin and its amino- and carboxy-terminal peptides, prothrombin fragment 1 and prethrombin 1, were added at various concentrations in the presence or absence of Ca^{2+} to the aqueous space bathing substrate-supported planar membranes composed of 1-palmitoyl-2-oleoyl-3-*sn*-phosphatidylcholine (POPC), POPC/bovine brain phosphatidylserine (bovPS) (70:30 mol/mol), or POPC/1,2-dioleoyl-3-*sn*-phosphatidylglycerol (DOPG) (70:30 mol/mol). Total internal reflection fluorescence microscopy (TIRFM) at the membrane–solution interface showed a significant enhancement by acidic lipids of prothrombin and prothrombin fragment 1 binding in the presence of 5 mM Ca^{2+}, with apparent dissociation constants of 0.4 and 1 μM, respectively. TIRFM measurements indicated that bovPS and DOPG also significantly enhanced the binding of fluorescein-labeled prothrombin to the planar membranes in the absence of Ca^{2+}, with apparent dissociation constants (13–30 μM) at least an order of magnitude larger than the Ca^{2+}-dependent constant for prothrombin binding. Association of prethrombin 1 but not prothrombin fragment 1 with membranes in the absence of Ca^{2+} was enhanced by the presence of bovPS in the membranes, which suggests that the Ca^{2+}-independent binding site(s) is (are) in the prethrombin 1 but not the fragment 1 portion of prothrombin.

In the coagulation cascade, prothrombin is the substrate of the prothrombinase complex which consists of the enzyme factor X_a, its cofactor, factor V_a, and a platelet membrane or a negatively charged phospholipid vesicle. Factors X_a and V_a assemble on these phospholipid surfaces and accelerate the conversion of prothrombin to thrombin. The thrombin proteolysis products of prothrombin are prethrombin 1 (the C-terminal two-thirds of the prothrombin molecule) and fragment 1 (the N-terminal third). The fragment 1 portion of prothrombin contains doubly negatively charged γ-carboxyglutamic acid residues. Binding of prothrombin in the presence of Ca^{2+} to negatively charged membranes is well documented and is reported to require the occupation of Ca^{2+}-binding sites in the fragment 1 portion and to involve the γ-carboxyglutamic acid residues (Suttie & Jackson, 1977; Jackson & Nemerson, 1980; Nemerson & Furie, 1980; Nelsestuen, 1984).

We have previously obtained evidence which suggests that bovine prothrombin interacts with phosphatidylserine (PS)[1]

†Supported by USPHS (SCOR) Grant HL-26309 to B.R.L. and NSF-PYI Grant DCB 85529856 to N.L.T.
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[1] Abbreviations: PS, bovPS, phosphatidylserine, bovine brain PS; POPC, 1-palmitoyl-2-oleoyl-3-*sn*-phosphatidylcholine; DMPC, 1,2-dimyristoyl-3-*sn*-phosphatidylcholine; DOPG, 1,2-dioleoyl-3-*sn*-phosphatidylglycerol; NBD-PE, 1-acyl-2-[12-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]dodecanoyl]phosphatidylethanolamine; FITC, fluorescein-5-isothiocyanate; TES, *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid; Na_2EDTA, disodium ethylenediaminetetraacetate; TIRFM, total internal reflection fluorescence microscopy; K_d, equilibrium dissociation constant.

0006-2960/91/0430-10991$02.50/0