The C-Terminal Domain of the ϵ Subunit of the Chloroplast ATP Synthase Is Not Required for ATP Synthesis†

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Received August 7, 2002; Revised Manuscript Received October 3, 2002

ABSTRACT: The ϵ subunit of the ATP synthases from chloroplasts and *Escherichia coli* regulates the activity of the enzyme and is required for ATP synthesis. The ϵ subunit is not required for the binding of the catalytic portion of the chloroplast ATP synthase (CF1) to the membrane-embedded part (CFo). Thylakoid membranes reconstituted with CF1 lacking its ϵ subunit (CF1-ϵ) have high ATPase activity and no ATP synthesis activity, at least in part because the membranes are very leaky to protons. Either native or recombinant ϵ subunit inhibits ATPase activity and restores low proton permeability and ATP synthesis. In this paper we show that recombinant ϵ subunit from which 45 amino acids were deleted from the C-terminus is as active as full-length ϵ subunit in restoring ATP synthesis to membranes containing CF1-ϵ. However, the truncated form of the ϵ subunit was significantly less effective as an inhibitor of the ATPase activity of CF1-ϵ, both in solution and bound to thylakoid membranes. Thus, the C-terminus of the ϵ subunit is more involved in regulation of activity, by inhibiting ATP hydrolysis, than in ATP synthesis.

ATP synthases are membrane enzymes that couple the exergonic flow of protons down the electrochemical potential gradient created by electron and proton transport to the endergonic synthesis of ATP from ADP and P_i. Closely related ATP synthases are present in the inner mitochondrial membrane, the thylakoid membrane of chloroplasts, and the plasma membrane of some bacteria, including that of *Escherichia coli*. For reviews, consult refs *1*−*3*.

ATP synthases consist of two separable oligomeric proteins: F1[1] and Fo. F1 contains the catalytic sites of the enzyme and is water-soluble once it is detached from the membrane. Fo is embedded in the membrane and translocates protons across the membrane. CF1, for chloroplast F1, is made up of five different polypeptides labeled α−ϵ in order of decreasing molecular mass. There are three copies of the α and β subunits, but only one each of the γ, δ, and ϵ polypeptides. CFo is comprised of four polypeptides in a somewhat uncertain stoichiometry. Multiple copies of subunit III (or c), the smallest and most hydrophobic protein in the ATP synthase, are present.

The ϵ subunit of the CF1CFo plays a dual role. It is a key player in the regulation of activity and is required for ATP synthesis. CF1 lacking its ϵ subunit (CF1-ϵ) has high ATPase activity when free in solution or bound to CFo (*4*). Native (*4*) or recombinant (*5*) ϵ subunit inhibits this ATPase activity to the very low level that is characteristic of isolated, intact CF1 and of thylakoid membranes. Membranes that contain bound CF1-ϵ cannot synthesize ATP in the light, partly at least because the membranes are so leaky to protons that electron transport cannot generate the ΔpH necessary to activate CF1CFo and to drive ATP synthesis. Native (*4*) or recombinant (*5*) ϵ subunits restore ΔpH formation and ATP synthesis to these membranes.

The C-terminal domain of the ϵ subunit of the *E. coli* ATP synthase is not required for the growth of the bacterium on succinate (*6*, *7*), indicating that this domain is dispensable for oxidative phosphorylation. Moreover, the C-terminal domain is not present in the ϵ subunits of some species (*8*). It is, however, unclear whether the truncated ϵ subunits of *E. coli* F1 are inhibitors of the ATPase activity of the enzyme bound to the membrane. The ϵ subunit is required for the binding of ECF1 to ECFo (*9*). Interactions of ECF1 with ECFo are likely to modulate the activity of the enzyme. Thus, it is difficult to distinguish between the effect of ECFo interactions and that of the ϵ subunit.

The ϵ subunit of the chloroplast ATP synthase is not required for the specific binding of CF1 to CFo (*10*). Thus, we can reconstitute thylakoid membranes devoid of CF1 with CF1-ϵ and simply add the ϵ subunit to the reconstituted membranes (*4*). In this paper we report that the deletion of 45 amino acids (of a total of 134) from the C-terminus of the ϵ subunit diminishes the extent of inhibition of ATPase activity by this subunit. However, the initial rates of light-

† This work was supported by NSF Grants MCB9723945 and MCB0110232. V.T.'s visit was supported by Cooperation in Applied Science and Technology (CAST).

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[1] Abbreviations: F1, the catalytic portion of ATP synthases; Fo, the proton conducting part of ATP synthases; CF1, chloroplast F1; CFo, chloroplast Fo; ECF1, F1 from *Escherichia coli*; CF1-ϵ, CF1 lacking its ϵ subunit; C6S, recombinant, full-length ϵ subunit from CF1; Δ45c, recombinant ϵ subunit from which 45 amino acids were deleted from the C-terminus; ACMA, 9-amino-6-chloro-2-methoxyacridine; Tricine, *N*-tris(hydroxymethyl)methylglycine; PMS, *N*-methylphenazonium methosulfate; STN, 0.4M sucrose, 0.02 M Tricine−NaOH (pH 8.0), and 0.01 M NaCl; decyl glucoside, *n*-decyl β-D-glucopyranoside.

10.1021/bi026594v CCC: $22.00
Published on Web 11/27/2002

dependent ATP synthesis by thylakoid membranes reconstituted with the truncated ϵ subunit (Δ45c) were similar to those reconstituted with full-length ϵ. Thus, the C-terminal domain of the ϵ subunit is not required for photophosphorylation but is likely to be involved in regulation of activity.

MATERIALS AND METHODS

CF1 (*11*) and CF1-ϵ (*4*) were prepared from market spinach and were stored at 4 °C as an $(NH_4)_2SO_4$ precipitate. Before use, aliquots of the suspensions were centrifuged at about 16000*g* for 5 min and the pellets dissolved in 20 mM Tris-HCl (pH 8.0) with either 1 mM EDTA or 0.1 mM $CuCl_2$. Residual $(NH_4)_2SO_4$ was removed by centrifugal filtration through Sephadex G-50 equilibrated with the Tris−EDTA buffer. When the CF1 and CF1-ϵ were to be used for membrane reconstitution experiments, they were treated as described above except that the buffer used was 5 mM Tricine−NaOH (pH 8.0) and 1 mM EDTA.

Recombinant ϵ subunits [C6S (full length) and Δ45c] were expressed as inclusion bodies in *E. coli* (*5*). The inclusion bodies were dissolved in buffered 8 M urea and rapidly diluted in a solution containing ethanol and glycerol. This procedure generates full-length ϵ subunit that is nearly as effective as the native, wild-type ϵ subunit as an inhibitor of the Ca^{2+}-ATPase activity of CF1-ϵ (*5*). The recombinant ϵ subunits used contained Ser at position 6 in place of Cys (C6S). Cys6 is the only Cys residue in the ϵ subunit, and its replacement with Ser, which has no effect on activity, obviates dimer formation by oxidation.

Dr. Mark L. Richter of the University of Kansas kindly provided us a clone of the gene for the δ subunit of spinach CF1. The δ subunit was expressed in *E. coli* and isolated as described for the recombinant ϵ subunits. Folding of the δ subunit was achieved by dissolving the protein pellets in 0.2% SDS, followed by removal of the detergent by passage of the solution through a 3 mL column of AG11 A8 resin (Bio-Rad). Native δ subunit treated with SDS regains activity after removal of the SDS by this protocol (*12*).

CF1 was completely stripped from spinach thylakoid membranes by treatment with 2 M NaBr (*13*). Stripped membranes were reconstituted with CF1-ϵ, and the excess CF1-ϵ was removed by washing. Five microliters of recombinant ϵ subunits in dilution buffer was added for every 95 μL of reconstituted membranes, equivalent to 5 μg of chlorophyll in a buffered sucrose solution (STN). Various molar ratios of ϵ to CF1 were generated for ATPase inhibition, and a ratio of 40:1 ϵ subunits to CF1-ϵ was used for ATP synthesis and ACMA fluorescence quenching. The amount of CF1-ϵ bound was estimated to be 1 nmol (mg of chlorophyll)$^{-1}$. The procedure used to remove the ϵ subunit from CF1 also removes some of the δ subunits which are required for ATP synthesis. Thus, recombinant δ subunit was routinely added at a molar ratio of 3:1 δ subunits to CF1 during reconstitution.

The ATPase and ATP synthesis activities of the reconstituted membranes as well as their ability to generate ΔpH were determined. The Ca^{2+}-ATPase activity of CF1-ϵ in solution or membrane bound was assayed at 37 °C in a reaction mixture that contained 50 mM Tris-HCl (pH 8.0), 5 mM ATP, and 5 mM $CaCl_2$. P_i was determined colorimetrically. When reconstituted membranes were used, it was necessary to remove the Mg^{2+} added during the incubation of the membranes with CF1-ϵ. Mg^{2+} is a potent inhibitor of Ca^{2+}-ATPase activity (*14*, *15*). The membranes were pelleted by centrifugation (10 min at 16000*g*), resuspended in STN plus 10 mM EDTA at a concentration of 0.5 mg of chlorophyll mL^{-1}, and incubated overnight at 4 °C. Mg^{2+}-dependent ATPase activity was determined at 37 °C in a reaction mixture (1 mL) that contained 50 mM Tris-HCl (pH 8.0), 2.5 mM (with decyl glucoside) or 5 mM (with Na_2SO_3) $MgCl_2$, and 5 mM ATP. Either 50 mM Na_2SO_3 or 10 mM decyl glucoside was present in the assay medium to prevent inhibition by free Mg^{2+}. When decyl glucoside was used, 0.1 mL of 100 mM octyl glucoside was added before P_i determination to remove the turbidity caused by the decyl glucoside.

ATP synthesis assay mixtures (1 mL) contained 50 mM Tricine−NaOH (pH 8.0), 50 mM NaCl, 5 mM $MgCl_2$, 1 mM ADP, 2 mM potassium phosphate buffer (pH 8.0), 0.05 mM PMS, 0.01 mM diadenosine pentaphosphate, a potent inhibitor of adenylate kinase, and reconstituted NaBr-treated thylakoids equivalent to 10 μg of chlorophyll. Samples were either illuminated (2 kW m^{-2}) or kept in the dark for 15−90 s at either room temperature or 30 °C. Trichloroacetic acid was added to a final concentration of 0.5% and ATP in the samples determined by the luciferin/luciferase method. Contaminating ATP in ADP was removed by treating an ADP solution with hexokinase and glucose, heating the sample to denature the hexokinase, and precipitating the ADP with ethanol (*18*).

The light-dependent quenching of the fluorescence of ACMA, which was taken as an indication of the magnitude of ΔpH (*19*), was assayed at room temperature in a mixture (1 mL) that contained 50 mM Tricine−NaOH (pH 8.0), 50 mM NaCl, 5 mM $MgCl_2$, 5 μM PMS, 2 mM ascorbate, and reconstituted membranes equivalent to 10 μg of chlorophyll. ACMA fluorescence was excited at 410 nm and measured at 475 nm. The reaction mixtures were stirred. Actinic light (660 nm) was provided by a quartz halide lamp, and blue filters were used to prevent the actinic light from entering the emission slit.

The concentration of the ϵ subunit was determined by the Bradford assay (*20*) with the correction determined by Richter et al. (*21*). CF1, CF1-ϵ, and δ subunit concentrations were determined by the Lowry (*22*) or Bradford assay.

RESULTS

Thylakoid membranes that contain bound CF1-ϵ have high ATPase activity and cannot make ATP or generate a significant ΔpH in the light (*4*). Native or recombinant ϵ subunits strongly inhibit ATPase activity and restore ATP synthesis and ΔpH formation (*4*, *5*). The ability of the truncated form of the ϵ subunit, Δ45c, to inhibit ATPase activity and restore ATP synthesis and ΔpH formation was compared to that of full-length ϵ subunit.

Recombinant full-length ϵ almost completely inhibited the Ca^{2+}-ATPase activity of CF1-ϵ in solution (Figure 1A) or bound to thylakoid membranes (Figure 1B). In contrast, the maximum extent of inhibition of Ca^{2+}-ATPase activity by Δ45c in the experiment shown was 10% for the soluble enzyme (Figure 1A) and about 50% for the membrane-bound enzyme (Figure 1B). The extent of inhibition of the Ca^{2+}-

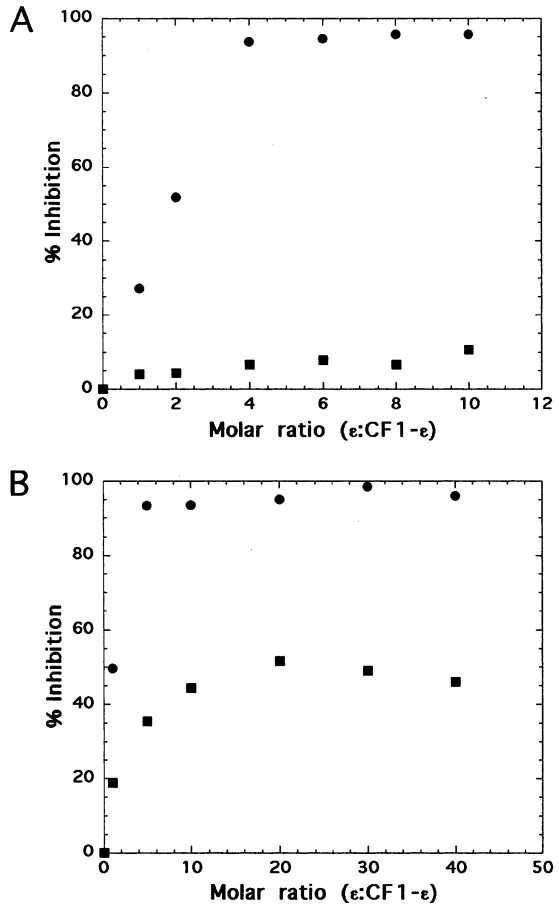

FIGURE 1: Inhibition of the Ca^{2+}-ATPase activity of CF1-ϵ in solution or bound to thylakoid membranes. In panel A, 5 μg of CF1-ϵ (12.5 pmol) in solution was incubated for 5 min with the indicated amount of either full-length ϵ or Δ45c and Ca^{2+}-ATPase activity determined. The control activities were 15.1 μmol of P_i min^{-1} (mg of CF1-ϵ)$^{-1}$ for full-length ϵ and 13.6 μmol of P_i min^{-1} (mg of CF1-ϵ)$^{-1}$ for Δ45c. In panel B, thylakoid membranes were reconstituted with CF1-ϵ, and unbound CF1-ϵ was removed. Reconstituted membranes containing the amounts of ϵ indicated were collected by centrifugation at 16000*g*, resuspended in STN plus 10 mM EDTA to a final chlorophyll concentration of 0.5 mg mL^{-1}, and incubated overnight at 4 °C. Aliquots of 10 μL were assayed for Ca^{2+}-ATPase activity. It was assumed that 1 nmol of CF1-ϵ was bound to the membranes per milligram of chlorophyll. The control rates were 318 μmol h^{-1} (mg of chlorophyll)$^{-1}$ for full-length ϵ and 231 μmol h^{-1} (mg of chlorophyll)$^{-1}$ for Δ45c. Key: circles, full-length ϵ; squares, Δ45c.

ATPase activity of soluble CF1 by Δ45c was variable but always considerably less than that of full-length ϵ. In 16 experiments, the mean inhibition of Ca^{2+}-ATPase activity by full-length ϵ at a molar ratio of ϵ to CF1-ϵ of 10:1 was 94 $\pm$ 4%, whereas that by Δ45c also at a molar ratio of 10:1 was 16 $\pm$ 12%. The reason for this variability is unknown. The extent of inhibition of the Ca^{2+}-ATPase activity of bound CF1-ϵ by Δ45c was less variable.

Although full-length ϵ strongly inhibited Mg^{2+}-ATPase activity of CF1-ϵ in thylakoids assayed in the presence of 50 mM sulfite to overcome inhibition by Mg^{2+} (*16*), Δ45c had no effect, even at a molar ratio of 40:1 (Δ45c:CF1-ϵ) (data not shown). Either sulfite promotes the dissociation of Δ45c or the Δ45c binds but does not inhibit. In addition to oxyanions, alcohols (*23*) and some detergents (*17*) prevent inhibition of ATPase activity by Mg^{2+}. Although short-chain alcohols (*4*) and alkyl glucosides (*17*) cause release of the ϵ subunit, CF1 treated with *n*-decyl β-D-glucopyranoside (decyl glucoside) retains most of its ϵ subunit (*17*). Decyl glucoside is, however, as effective as octyl glucoside in overcoming inhibition by Mg^{2+}. Mg^{2+}-ATPase activity of CF1-ϵ, incubated prior to assay with full-length ϵ, Δ45c, or combinations of full-length ϵ and Δ45c, was assayed in the presence of 10 mM decyl glucoside. Full-length ϵ strongly inhibited ATPase activity, but Δ45c was without effect even at a molar ratio of Δ45c to CF1-ϵ of 20:1 (Figure 2). However, Δ45c partially prevented inhibition by full-length ϵ when the subunits were added as a mixture to CF1-ϵ. Thus, Δ45c binds to CF1-ϵ but does not inhibit. It is likely that $\epsilon\Delta$45c has a lower binding affinity for CF1-ϵ, but the magnitude of this difference cannot be determined from the data shown.

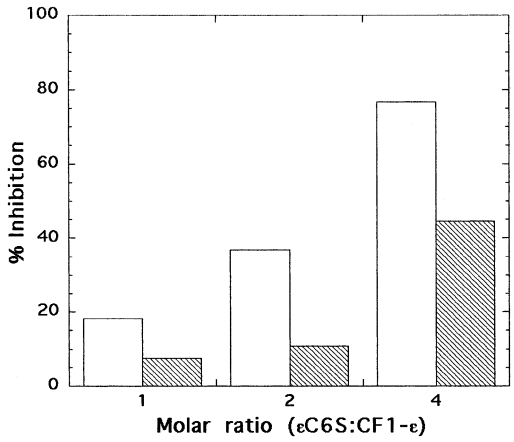

FIGURE 2: Inhibition of Mg^{2+}-ATPase activity by full-length ϵ and competition with Δ45c. CF1-ϵ was incubated with the indicated amounts of full-length ϵ in the presence and absence of a 20:1 molar ratio of Δ45c to CF1. Mg^{2+}-ATPase activity was determined in the presence of 10 mM decyl glucoside. Key: open bars, ϵ alone; hatched bars, ϵ plus Δ45c. The control ATPase activity was 31 μmol of P_i released min^{-1} (mg of CF1-ϵ)$^{-1}$.

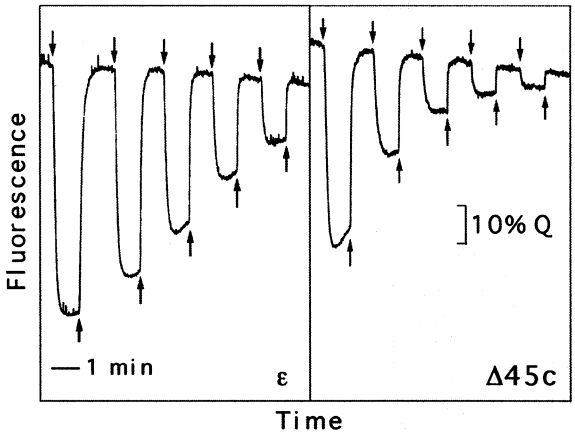

FIGURE 3: ΔpH formation as detected by light-dependent quenching of ACMA fluorescence. Thylakoid membranes containing bound CF1-ϵ were incubated with either full-length ϵ or Δ45c at a 40:1 molar ratio of ϵ subunit to CF1-ϵ, and ACMA fluorescence was determined. The down arrows indicate light on; the up arrows, light off. Negligible quenching was seen in the absence of added ϵ subunit.

Thylakoid membranes reconstituted with CF1-ϵ show no light-dependent quenching of ACMA fluorescence. Substantial quenching was seen after incubation of the membranes with full-length ϵ or Δ45c (Figure 3). The extent of ACMA fluorescence quenching decreased with repeated illuminations of 1 min each. This effect was especially dramatic in

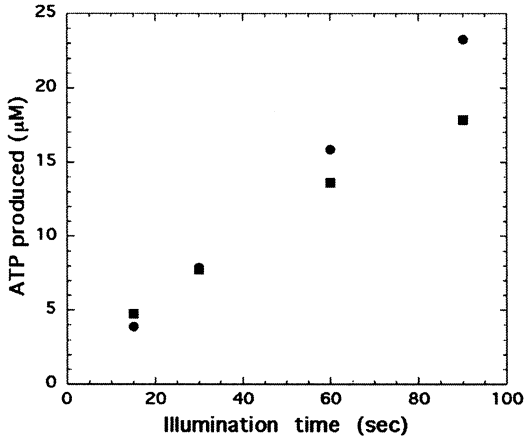

FIGURE 4: Time course of ATP synthesis in the light by reconstituted thylakoid membranes. Thylakoid membranes containing CF1-ϵ were incubated with either full-length ϵ or Δ45c at a molar ratio of 40:1 ϵ subunits to CF1-ϵ, and ATP synthesis was determined. No ATP formation was detected in membranes that were not reconstituted with ϵ. Key: circles, ϵ; squares, Δ45c.

membranes reconstituted with Δ45c. This decrease may reflect loss of the ϵ subunit in the light, photoinhibition of photosystem I, or damage to the membranes caused by prolonged exposure of the membranes to ethanol. Even during the first period of illumination, the extent of ACMA fluorescence quenching was lower in membranes reconstituted with Δ45c than with full-length ϵ. For example, for the data shown in Figure 3, the quenching expressed as $\Delta F/F$, where ΔF is the change in fluorescence from the dark to light transition and F is the total ACMA fluorescence, was 0.76 for membranes reconstituted with full-length ϵ and 0.55 for those reconstituted with Δ45c. This result may indicate that the ΔpH is lower in the membranes reconstituted with Δ45c.

The ability of NaBr-treated thylakoids reconstituted with CF1-ϵ to synthesize ATP in the light was restored by incubation with full-length ϵ or Δ45c (Figure 4). In four experiments, the rate of ATP synthesis during a 15 s illumination of membranes incubated with Δ45c was 103 $\pm$ 15% (range 85−122%) of that in membranes reconstituted with full-length ϵ. Even though the maximum rates varied from experiment to experiment, the initial rate of phosphorylation within a given experiment by membranes incubated with Δ45c was always close to that of membranes reconstituted with full-length ϵ subunit. There can be no doubt that the last 45 amino acids of the ϵ subunit of CF1 are not required for photophosphorylation. In some experiments the rate of ATP synthesis by membranes incubated with Δ45c fell off more rapidly with time of illumination than in the experiment shown in Figure 4. In all experiments, however, the time course was more linear in membranes reconstituted with full-length ϵ than with Δ45c. For example, the rate of ATP synthesis by membranes containing Δ45c for the period of 60−90 s of illumination was 61% (average of four experiments) of those containing full-length ϵ. This observation is consistent with the possibility that some of the Δ45c dissociates during illumination.

The rates of ATP synthesis by the reconstituted membranes are lower than those in freshly prepared thylakoids under the same conditions. Considering that the reconstituted membranes had been treated with 2 M NaBr, incubated with CF1-ϵ, centrifuged several times, and incubated with the ϵ preparations, this fact is not too surprising. The rates of ATP synthesis by the reconstituted membranes are, however, significant. For example, a rate of 90 μmol of ATP formed h^{-1} (mg of chlorophyll)$^{-1}$ corresponds to a turnover rate for CF1 of 25 s^{-1}.

DISCUSSION

The results presented in this paper show that the C-terminal domain of the ϵ subunit of CF1 is not required for ATP synthesis. However, a role for this domain in regulation of activity is indicated by the decrease in the extent of inhibition of ATPase activity by Δ45c. Complementation studies in *E. coli* (*6*, *7*) and analysis of deduced sequences of ϵ subunits (*8*) from a variety of sources indicated that the C-terminal domain is dispensable for oxidative phosphorylation. Xiong et al. (*7*) also showed that recombinant ECF1 ϵ subunit from which 43 amino acids had been deleted from the C-terminus and 2 serines added was a weaker inhibitor of the ATPase activity of ECF1 in solution. Membranes that contained this truncated form of the ϵ subunit were nearly as active in ATP-dependent proton translocation as those containing wild-type ϵ subunit. The ATPase activity of the membranes containing the truncated ϵ subunit was slightly higher than that of wild-type membranes.

In our experiments, direct reconstitution of thylakoid membranes containing CF1-ϵ with recombinant full-length or truncated ϵ subunits was used. This approach was possible since CF1, unlike ECF1, binds tightly and specifically to Fo in the absence of the ϵ subunit (*10*). Thus, it is possible to show unambiguously that the ϵ subunit of CF1 inhibits the ATPase activity of membrane-bound CF1. The ATPase activity of thylakoid membranes containing a full complement of ϵ subunit is very low, whereas membranes containing CF1-ϵ have high ATPase activity that is nearly completely inhibited by full-length ϵ subunit. Δ45c inhibits the Ca^{2+}-ATPase activity of membrane-bound CF1-ϵ to a maximum of about 50%. The Mg^{2+}-ATPase activity of CF1-ϵ, assayed in the presence of either sulfite or decyl glucoside, was inhibited by full-length ϵ but not by Δ45c. The observation that Δ45c prevents the inhibition of decyl glucoside-stimulated Mg^{2+}-ATPase activity by full-length ϵ shows that Δ45c binds to CF1-ϵ. The rate of light-activated Mg^{2+}-ATP hydrolysis was significantly higher in membranes reconstituted with Δ45c than with full-length ϵ (K. F. Nowak, unpublished observations).

These experiments suggest a role of the C-terminal domain of the ϵ subunit in regulation of activity. Why Δ45c is a partially effective inhibitor of Ca^{2+}-ATPase activity but fails to inhibit Mg^{2+}-ATPase activity is unknown. However, Mg^{2+} is the physiologically significant divalent cation, and Mg^{2+}-ATP could modulate the effects of the ϵ subunit. The binding of Mg^{2+}-ATP and Mg^{2+}-ADP affects the structure of ECF1 as revealed by specific cross-link formation (*24*).

As an ATPase inhibitor, the ϵ subunit must also be an inhibitor of ATP synthesis. The ATPase activity attributable to CF1 in thylakoid membranes in the dark is very low. The steady-state rate of ATP synthesis in the light is at least 3 orders of magnitude higher than that of ATP hydrolysis in the dark. Clearly, CF1 in thylakoids undergoes activation in the light and deactivation in the dark. Dissociation of Mg^{2+}-

ADP from CF1 (*25*, *26*) and reversal of inhibition by the ϵ subunit are two energy-dependent events in activation.

As indicated by the reaction of Lys109 with pyridoxal phosphate (*27*) and interaction with antibodies (*28*), the C-terminal domain of ϵ is exposed during energization of thylakoid membranes by illumination. This movement of the C-terminus may be one of the changes elicited by ΔpH formation that nullify ϵ inhibition. The observation that Δ45c is a weaker inhibitor than full-length ϵ is in accord with this notion. Although cross-linking of the C-terminal helix−turn−helix domain to the β-sandwich domain of the ϵ subunit from ECF1 did not affect ATP-dependent proton transport, ATP hydrolysis by the membranes was activated (*29*). These observations are consistent with a role of the C-terminus of the ϵ subunit in regulation of activity.

The extent of the quenching of ACMA fluorescence in thylakoid membranes reconstituted with Δ45c was significantly lower than that in the same membranes reconstituted with full-length ϵ, indicating that ΔpH is lower in membranes containing truncated ϵ subunit. Yet, the initial rates of ATP synthesis by the two types of membranes are the same. In view of the sharp dependence of the rate of photophosphorylation on the magnitude of ΔpH [a decrease in ΔpH of 0.3 unit was associated with a 10-fold decrease in the rate of ATP synthesis (*30*)], these results are surprising. Reduction of the disulfide bond in the γ subunit of CF1 weakens the interaction of the ϵ subunit and shifts the curve that relates the rate of ATP synthesis to ΔpH to lower ΔpH values (*31*). Deletion of the C-terminal domain would be expected to weaken the interaction of the ϵ subunit with other subunits of the synthase and could reduce the energetic cost of overcoming ϵ inhibition. However, the ACMA fluorescence quenching and photophosphorylation assays were necessarily carried out under different illumination conditions, thereby complicating the interpretation of these results.

The movement of the ϵ subunit during ΔpH formation may not be restricted to the C-terminus. The falloff of ACMA fluorescence quenching with repeated light−dark cycles in ϵ-reconstituted membranes suggests that changes take place in the light that cause dissociation of ϵ. The effect of these changes or movements of ϵ may be amplified by truncation of the C-terminus. Loss of this domain may destabilize the interactions to such an extent that the ϵ dissociates even more readily, as seen in the ACMA quenching data shown here. The observation that the rate of ATP synthesis in membranes containing Δ45c is linear for only 15−30 s is consistent with dissociation of the Δ45c under turnover conditions.

We can conclude that energy-dependent movements of the ϵ subunit allow the abrogation of ATPase inhibition, the maintenance of the proton gradient, and the reestablishment of ATPase inhibition in the dark. The regulation of the chloroplast ATP synthase during light to dark and dark to light transitions and by the redox state of the γ subunit distinguishes it from the ATP synthases in mitochondria and *E. coli*. Further experimentation will be needed before the energy-dependent movements of the ϵ subunit of chloroplasts may be interpreted in light of the high-resolution structures of $\gamma-\epsilon$ from ECF1 (*32*) and of bovine mitochondrial F1 (*33*).

ACKNOWLEDGMENT

We thank Dr. Mark L. Richter of the University of Kansas for providing the clone of the δ subunit of CF1.

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BI026594V