The Carboxyl Terminus of the ϵ Subunit of the Chloroplast ATP Synthase Is Exposed during Illumination†

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ABSTRACT: The ϵ subunit of the chloroplast ATP synthase is an inhibitor of activity of the enzyme. Recombinant forms of the ϵ subunit from spinach chloroplasts lacking the last 10, 32, or 45 amino acids were immobilized onto activated Sepharose. A polyclonal antiserum raised against the ϵ subunit was passed over these immobilized protein columns, and the purified antibodies which were not bound recognized the portions of the ϵ subunit missing from the recombinant form present on the column. The full polyclonal antiserum can strip the ϵ subunit from the ATP synthase in illuminated thylakoid membranes [Richter, M. L., and McCarty, R. E. (1987) *J. Biol. Chem. 262*, 15037−15040]. Exposure of illuminated thylakoid membranes to antibodies recognizing the last 32 amino acids of the ϵ subunit collapses the proton gradient and hinders ATP synthesis with similar efficiency as the full polyclonal preparation. These results indicate that antibodies against the last 32 amino acids of the ϵ subunit are capable of stripping the subunit from the ATP synthase in illuminated membranes. Neither of these effects was seen when the membranes were exposed to the antibodies in the dark. This is direct evidence that the chloroplast ATP synthase undergoes a conformational shift during its activation by the electrochemical proton gradient which specifically alters the conformation of the carboxyl-terminal domain of the ϵ subunit from protected to solvent-exposed. The relation between this shift and activation of the enzyme by the electrochemical proton gradient is discussed.

Chloroplast ATP synthase, CF1CFo,[1] couples the translocation of protons to the synthesis and hydrolysis of ATP. This coupling of ion movement to ATP synthesis was first suggested by Mitchell's chemiosmotic hypothesis (*1*). In chloroplasts, the electrochemical proton gradient formed across the thylakoid membranes during illumination provides the driving force for ATP synthesis. CF1CFo belongs to the large family of F-type ATPases and is similar to ATP synthases found in mitochondria and bacteria. A feature of the chloroplast ATP synthase is that it exists in two distinct states. In the light, when a high electrochemical proton gradient across the thylakoid drives the reaction toward ATP synthesis, the ATP synthase can generate several hundred ATPs per second. In the absence of illumination, the ATP synthase is inactive, showing almost no ATP hydrolysis. This inactive state of the enzyme persists until a proton gradient is generated across the thylakoid membrane, after which ATP synthesis reaches its maximum velocity within milliseconds (*2*).

Conformational changes of CF1CFo in response to the electrochemical proton gradient are likely to be at the heart of the mechanisms that switch the ATP synthase on in the light and off in the dark. A decrease in the affinity for MgADP, changes in the solvent-accessibility of both the γ and ϵ subunits of CF1, and an increased rate of reduction of a regulatory disulfide bond within the γ subunit are manifestations of changes elicited by the electrochemical proton gradient (*3*).

The ϵ subunit is an inhibitor of the activity of CF1CFo or of CF1. Removal of the ϵ subunit causes very high rates of ATP hydrolysis, and its reintroduction strongly inhibits ATPase activity. The formation of an electrochemical proton gradient across thylakoid membranes in the light relieves this inhibition, thereby allowing rapid ATP synthesis. The ϵ subunit is not released during activation in the light (*4*), as its presence is necessary for coupling the proton gradient to ATP synthesis (*5*).

Illumination of thylakoid membranes causes a change in the solvent accessibility of the ϵ subunit of CF1. When thylakoids are illuminated in the presence of pyridoxal 5′-phosphate (PLP), the ϵ subunit is 3 times more reactive with PLP than in nonilluminated samples. A single ϵ subunit lysine residue, Lys-109 (*6*), reacts with PLP in the light. Polyclonal anti-ϵ antibodies reacted with the ϵ subunit of CF1CFo in intact thylakoids in the light (*7*). The antibodies partially stripped the ϵ subunit from the thylakoids, causing a collapse of the proton gradient and a loss of ATP synthesis. These effects were reversed by adding back excess ϵ to the treated thylakoids. Incubation of thylakoids in the dark with the antiserum had no effect on either ATP synthesis or proton conductance (*7*). These results suggest that a part or parts of the ϵ subunit are exposed to the solvent in illuminated thylakoid membranes.

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[1] Abbreviations: 9-AA, 9-aminoacridine; Borate buffer, 125 mM sodium borate, 75 mM NaCl (pH 8.4); CF1, the soluble, catalytic part of chloroplast ATP synthase; CFo, the intrinsic, proton-conducting part of the chloroplast ATP synthase; TFA, trifluoroacetic acid; Tricine, *N*-tris(hydroxylmethyl)methylglycine.

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In this paper, we show that the C-terminus of the ϵ subunit is exposed to antibodies in the light. The relation of this energy-dependent change to activation of the ATP synthase is discussed.

MATERIALS AND METHODS

Thylakoid membranes were prepared from market spinach (*8*) and could be stored (about 3 mg/mL chlorophyll) at 4 °C in 20 mM Tricine-NaOH (pH 8.0), 400 mM sucrose, and 10 mM NaCl for up to 4 h without significant loss of activity. All ϵ mutant plasmids and inclusion bodies were prepared according to McCarty and Cruz (*9*). ϵC6S gains native activity and folding after rapid dilution of 8 M urea solutions into an ethanol/glycerol mixture (*10*). The mutations $\epsilon\Delta$10C and $\epsilon\Delta$45C were constructed from the ϵC6S plasmid as described elsewhere (*11*). The notation ΔnC indicates that a mutant form of the ϵ subunit has been truncated by *n* amino acids from its C-terminus. The mutation $\epsilon\Delta$32C was constructed from the ϵC6S plasmid using unique site elimination mutagenesis (Clontech, Palo Alto, CA). The forward primer for $\epsilon\Delta$32C was, from 5′ to 3′, CCGCTTTCCTTTAGTTAGCTTC. The reverse primer altered a unique *Xho*I cut site to *Pvu*II using the primer GACTGCTTTACCGCAGCTGCCTCGCGCG.

Illumination of Thylakoids. Thylakoids equivalent to 435 μg of chlorophyll were diluted to 1 mL in 50 mM Tricine-NaOH (pH 8.0), 50 mM NaCl, 5 mM $MgCl_2$, and 10 μM pyocyanine, a mediator of cyclic electron flow. In clear plastic tubes, 57 μL of diluted thylakoids was added to 30 μL of borate buffer containing either antibodies or bovine serum albumin at approximately 30 mg of protein/mL. Thylakoids were illuminated with saturating white light in a shaking water bath for 10 min at 10 °C. Immediately following illumination, thylakoids were placed on ice in the dark for 15 min before use. Dark control samples were treated identically to other samples, but these tubes were tightly wrapped with aluminum foil.

Measurement of ATP Synthesis. ATP synthesis activity was measured from thylakoids equivalent to 20 μg of chlorophyll diluted to 2 mL of 50 mM Tricine-NaOH (pH 8.0), 50 mM NaCl, 5 mM $MgCl_2$, 10 μM pyocyanine, 1 mM ADP, 2 mM Na_2HPO_4, and 10 μM diadenosine pentaphosphate. A 500 μL aliquot of this mixture was removed and placed in s boiling water bath for 45 s and then placed on ice as preillumination control. Remaining thylakoids were illuminated with saturating white light in a shaking water bath for 90 s at 10 °C. Immediately after illumination, samples were placed in boiling water bath for 45 s and placed on ice. Denatured thylakoid membranes were removed by centrifugation prior to determination of the amount of ATP formed using the luciferin/luciferase assay as described by the supplier (Roche).

Quenching of 9-Aminoacridine Fluorescence. Thylakoids equivalent to 20 μg of chlorophyll were diluted to 2 mL in 50 mM Tricine−NaOH (pH 8.0), 50 mM NaCl, 5 mM $MgCl_2$, 10 μM pyocyanine, and 2 μM 9-aminoacridine (9-AA). Thylakoids were then placed in a stirred cuvette in a Shimadzu RF-5000 spectrofluorometer. 9-AA fluorescence was excited at 399 nm. At the indicated times, the cuvette was exposed to red actinic light, and the resulting drop in fluorescence was monitored at 430 nm, with appropriate filters used to prevent the actinic light from reaching the emission photodetector.

Construction of Immobilized ϵ Subunit Columns. Inclusion bodies containing the ϵ subunit were incubated for 15 min with 30 units of DNase I (Boehringer Mannheim) in 20 mM Tris-HCl (pH 8.0), 2 mM $MgCl_2$ at 37 °C, and the DNase was removed by centrifugation at 16000*g* for 5 min. Inclusion bodies (approximately 2−4 mg) containing the ϵ subunit were solubilized in 8 M urea, 20 mM Na_2HPO_4 (pH 7.0). The pellets were incubated in the urea−phosphate solution for 2 h at room temperature then centrifuged at 16000*g* for 5 min to remove insoluble material. The protein concentrations of unfolded ϵ subunit solutions were estimated by the Bradford method (*12*) as corrected by Richter et al. (*13*) and were adjusted to 2 mg/mL in urea−phosphate.

Four immobilized ϵ subunit columns were prepared as described below. The four ϵ subunit preparations used were C6S, Δ10C, Δ32C, and Δ45C. Approximately 5 mL of cyanogen bromide-activated Sepharose (Sigma) was gravity-packed into a column with an inner diameter of 1.5 cm. A solution of 5 mg of ϵ subunit in 2.5 mL of urea−phosphate was added directly to the column. The column was then sealed and allowed to sit at room temperature for 2 h. The column was then washed extensively with urea−phosphate followed by 10 column volumes of 50 mM Tris-HCl (pH 8.0). The column was sealed and allowed to sit at room temperature for 3 h. The column was then washed with eight successive cycles of 50 mM Tris-HCl (pH 8.0), 1 M NaCl followed by 50 mM glycine (pH 3.5), 1 M NaCl. The column was then equilibrated with borate buffer at 4 °C.

Isolation of Antibodies for the C-Terminus of the ϵ Subunit. Rabbit antiserum against purified ϵ subunit was obtained previously (*7*) and was stored at −80 °C. Two milliliters of the antiserum was added directly to the immobilized ϵ subunit column. The column was then sealed and allowed to sit overnight at 4 °C. Fresh borate buffer was then added, and the first 10 mL of effluent from each column was collected. Solid ammonium sulfate was added to 50% of saturation, and the mixture was kept at 4 °C for 1 h followed by centrifugation for 30 min at 4 °C and 3000*g*. The pellet was dissolved in 3 mL of borate buffer and then passed through a 10 mL column of Sephadex G-50 equilibrated with borate buffer. The protein contents of 1 mL fractions were determined by the method of Bradford (*12*), and peak fractions were combined, diluted with additional borate buffer, and concentrated using centrifugal concentration (Millipore) to 2 mL. Preparations from all columns gave approximately equivalent protein content (30 mg/mL by the Bradford method) which is appropriate given that each type of antibodies only accounts for a small amount of the total protein content. The sample was then further purified by column centrifugation through Sephadex G-50 equilibrated with borate buffer. Samples were free of residual ammonium ion as tested by Nessler's reagent.

Specificity of isolated antibodies was determined by spot blot of approximately 10 pmol of unfolded ϵ subunit in urea−phosphate onto PVDF. Proteins were fixed to the PVDF using a 1% glutaraldehyde solution in 50 mM $NaHPO_4$ (pH 7.5) (*14*). PVDF was probed using the isolated antibodies at 1:10 000 dilutions. Secondary antibody was goat anti-rabbit IgG−peroxidase conjugate (Sigma), and the blots were developed using an ECL detection kit (Amersham Pharmacia Biotech).

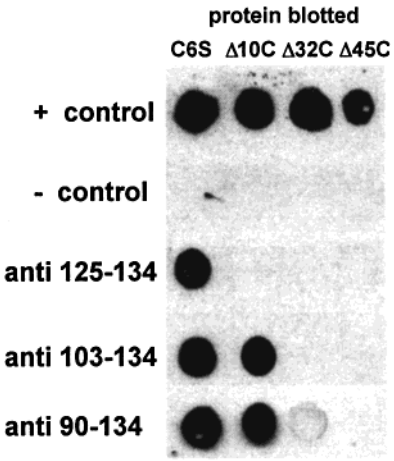

FIGURE 1: Specificity of purified antibodies after immobilized ϵ chromatography. ϵ subunit was solubilized in 50% acetonitrile/0.1% TFA and dot-blotted onto PVDF (5 pmol per dot) and the PVDF exposed to purified antibodies at 1:10 000 dilution. + control, antiserum passed through a column containing immobilized bovine serum albumin; − control, antiserum passed through an immobilized ϵC6S column; anti 125−134, antiserum passed through an immobilized $\epsilon\Delta$10C column; anti 103−134, antiserum passed through an immobilized $\epsilon\Delta$32C column; anti 90−134, antiserum passed through an immobilized $\epsilon\Delta$45C column.

Immunodetection of HPLC Fragments. ϵC6S was prepared from inclusion bodies as described above to a final concentration of 10 mg/mL in 8 M urea, 50 mM ammonium bicarbonante. Two hundred micrograms of ϵC6S was diluted to 1 mg/mL into 50 mM ammonium bicarbonate, followed by the addition of 4 μg of Glu-C endoproteinase (Sigma). The solution was kept at 37 °C overnight. Twenty-five microliters of the solution was then injected into an HPLC (Waters) and separated on a C-18 column (Metachem) using a 2−60% acetonitrile (0.1% TFA) gradient, flowing at 0.8 mL/min over a course of 60 min, and taking 1 min fractions. The fractions were then dried in a Speedvac (Heto) and resuspended in 30 μL of 50% acetonitrile followed by the addition of 3 μL of 1 mg/mL fish gelatin (BioFX). Three microliters of each fraction was then blotted directly onto dry PVDF and then fixed with glutaraldehyde (*14*). The PVDF membrane was then exposed to the positive control antibody preparation at a dilution of 1:10 000 followed by secondary detection with goat anti-rabbit conjugated with horseradish peroxidase. Detection was done using an ECL kit (Amersham Pharmacia Biotech). Intensity of detection on X-ray film following ECL was calculated using NIH Image software.

RESULTS

Isolation of Antibodies against the C-Terminus of the ϵ Subunit. Truncated forms of the ϵ subunit immobilized onto a column matrix should retain all antibodies from the polyclonal antiserum that recognize epitopes on the region of the ϵ subunit present on the column. Antibodies against epitopes missing from the truncated forms of the ϵ subunit should not bind to the column and should be present in the column effluent.

Figure 1 shows the ability of purified antibodies, after passage through immobilized ϵ columns, to recognize several truncated forms of the ϵ subunit. Purified antibodies that had been passed through a column that contained bound bovine serum albumin (positive control) should contain antibodies that recognize epitopes over the entire ϵ peptide. Purified antibodies passed through a column bound with full-length ϵ subunit (negative control) should contain no antibodies to the ϵ subunit. Purified antibodies passed through a column bound with ϵ subunits deficient in the last 10 (anti 125−134), 32 (anti 103−134), or 45 (anti 90−134) amino acids should retain only the antibodies against epitopes present within these residues. When these preparations are used to immunodetect the ϵ subunit, any polyclonal mixture containing antibodies against any epitope of the ϵ subunit would be able to immunodetect full-length ϵ (ϵC6S). As shown in Figure 1, all polyclonal preparations except the negative control detected ϵC6S. Both the ϵ present on the immobilized column and on the blotting membrane (PVDF) may be assumed to be denatured protein. Thus, antibodies against epitopes found only on native, folded protein cannot be excluded. However, the passage of the anti-ϵ serum through a column to which full-length ϵ subunit is attached cleared the serum of antibodies against the denatured ϵ subunit.

Figure 1 also shows that the different preparations of the same antiserum now have altered specificity. Sample anti 125−134, in contrast to the positive control, is capable of detecting only full-length ϵ subunit (ϵC6S) but not any of its truncated forms ($\epsilon\Delta$10C, $\epsilon\Delta$32C, or $\epsilon\Delta$45C). The immobilized ϵ columns were effective in removing antibodies that recognize any epitope present on the first 124 amino acids of the ϵ subunit, but have not removed antibodies against epitopes found between residues 125 and 134. Since these residues are found only on ϵC6S, anti 125−134 will only bind to full-length ϵ and will not recognize any of the other truncated forms. Sample anti 103−134 can detect both ϵC6S and $\epsilon\Delta$10C but is unable to detect $\epsilon\Delta$32C or $\epsilon\Delta$45C, showing it has been cleared of antibodies against epitopes on all but the last 32 amino acids of the full-length ϵ subunit. Sample anti 90−134 can detect ϵC6S and $\epsilon\Delta$10C, but not $\epsilon\Delta$32C even when amounts of $\epsilon\Delta$32C were present at 10 times the amount used in Figure 1. This suggests that there is no epitope between residues 90 and 103.

Only Antibodies against the C-Terminus of ϵ Are Required for Uncoupling. The ability of thylakoid membranes to establish a proton activity gradient (ΔpH) in the light can be monitored by the quenching of the fluorescence of 9-AA upon illumination. Exposure of thylakoids to the anti-ϵ serum in the light was shown (*7*) to decrease the extent of 9-AA fluorescence quenching. This decrease in ΔpH was attributed to the partial stripping of the ϵ subunit from the membrane, and the addition of excess ϵ reverses this collapse. The ϵ subunit is required to block proton conductance through the CFo.

Thylakoid membranes pre-illuminated without antibodies present (using bovine serum albumin in borate buffer) are capable of light-dependent quenching of 9-AA fluorescence (Figure 2, a). Thylakoids pre-illuminated in the presence of the negative control serum that is devoid of anti-ϵ antibodies (Figure 2, b) also show the formation of a proton gradient. Thylakoids exposed to antibodies, the positive control, show a partial collapse of the ΔpH if the exposure was done in the light (Figure 2, c) but not in the dark (Figure 2, d). Since both the positive and the negative controls are from the same antiserum, the altered reactivity suggests that the full-length ϵ subunit present in the column through which the negative

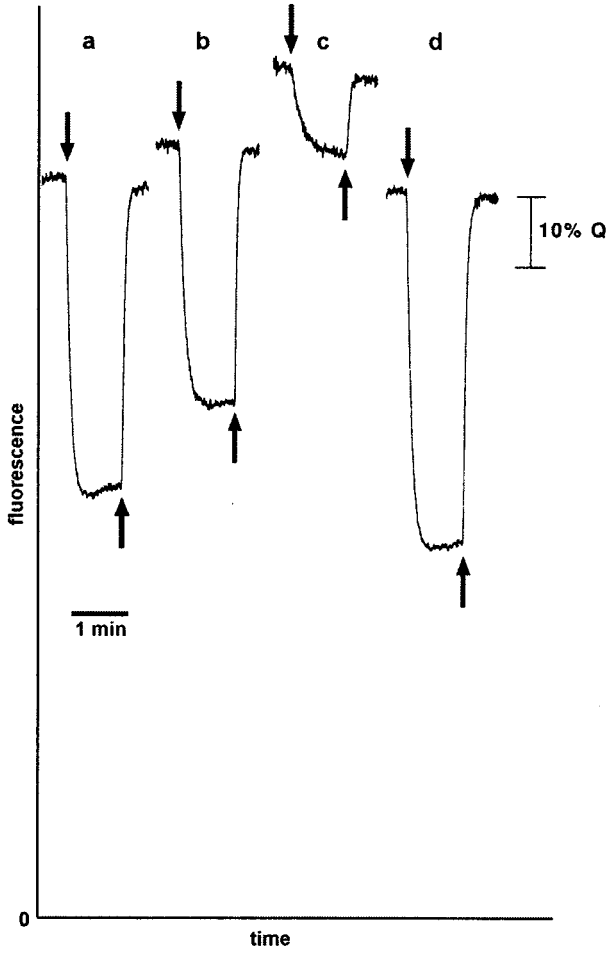

FIGURE 2: Ability of immobilized ϵ chromatography to remove antibodies responsible for proton gradient collapse. The fluorescence of 9-aminoacridine in the light was monitored as an indication of the formation of ΔpH. Red actinic light was turned on at the time indicated by the downward arrow and turned off as indicated by the upward arrow. Trace a: control thylakoids that have been exposed to an equivalent volume of 30 mg/mL bovine serum albumin instead of purified antibodies. Trace b: thylakoids exposed for 10 min with saturating white light to antibodies which had passed through a column containing immobilized ϵC6S prior to assay (negative control). Trace c: thylakoids exposed for 10 min with saturating white light to antibodies which had passed through a column containing immobilized BSA prior to assay (positive control). Trace d: thylakoids exposed to antibodies that had passed through a column containing bound ϵC6S without prior illumination.

control passed has removed the antibodies needed to collapse the proton gradient of thylakoid membranes.

Figure 3 shows the results of the 9-AA fluorescence quenching by thylakoids that had been exposed to the different antibody preparations in the light and the dark prior to assay. The extent of 9-AA fluorescence quenching in thylakoid membranes was only slightly affected by illumination. The slight drop in illuminated samples is probably due to photodamage during the 10 min exposure of the thylakoids to saturating white light. Thylakoids treated with negative control antiserum preparation gave very similar results to those treated with bovine serum albumin.

The antibodies purified after passage through the immobilized Δ10C column (anti 125−134) should recognize only the last 10 amino acids of the polypeptide. When thylakoid membranes were exposed to these antibodies in the light, but not the dark, an inhibition of the extent of 9-AA

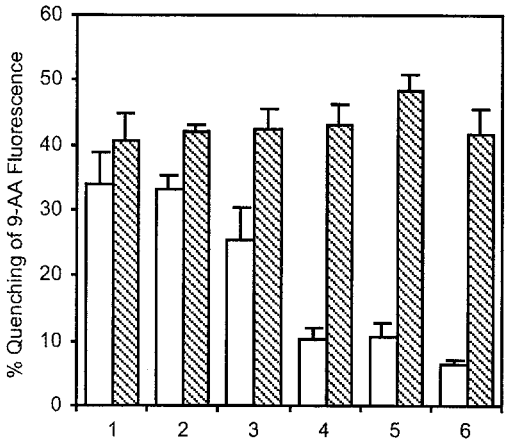

FIGURE 3: Effect of purified antibodies on 9-AA fluorescence quenching. Quenching of 9-AA fluorescence monitored over 60 s of illumination with actinic light. Percent quenching is the minimal fluorescence during illumination compared to the maximal fluorescence after illumination. Open bars indicate thylakoids exposed to purified antibodies while illuminated; hatched bars indicated thylakoids exposed to purified antibodies in the dark. Thylakoids were incubated with bovine serum albumin (1), with negative control antiserum (2), with anti 125−134 serum (3), with anti 103−134 serum (4), with anti 90−134 serum (5), and with positive control antiserum (6).

fluorescence quenching was seen. This effect, however, is much smaller than the effect seen in the positive control preparation. The antibodies present in anti 125−134 must recognize some epitope of the ϵ subunit which is only exposed on illuminated membranes, but this epitope alone does not appear to be sufficient for the removal of the ϵ subunit seen when all antibodies against the ϵ subunit are present.

Both anti 103−134 and anti 90−134 decrease the extent of 9-AA fluorescence quenching to a similar extent as that in the positive control. Both of these preparations also contain the antibodies present in anti 125−134, suggesting that more than one antibody/antigen interaction may be required to remove the ϵ subunit effectively from the thylakoid membrane. These antibodies affect 9-AA fluorescence quenching only when the thylakoids are first illuminated in the presence of the antibody. In the absence of illumination, no epitope between residue 90 and the end of the polypeptide chain was sufficiently exposed to the solvent to be recognized by the antibodies.

Figure 4 shows rates of ATP synthesis of thylakoids exposed to the different antibody preparations in the light or dark. The effects of the antibodies on ATP synthesis are similar to those on 9-AA fluorescence quenching. The treatment of thylakoids with anti 125−134 in the light decreased ATP synthesis rates somewhat, but not to the extent seen with anti-ϵ. Both anti 103−134 and anti 90−134 inhibit ATP synthesis to a greater extent than anti 125−134. The positive control gave the strongest inhibition of ATP synthesis, suggesting that there may be additional epitopes beyond residues 90−134 involved in the inhibition. However, antibodies against epitopes present in residues 90−134 are about 70% as effective as anti-ϵ in the light-dependent inhibition of ATP synthesis.

Epitopes of Antibodies Required for Uncoupling Partially Identified by HPLC. Recombinant ϵ subunit (ϵC6S) was subjected to proteolysis, and the resulting fragments were

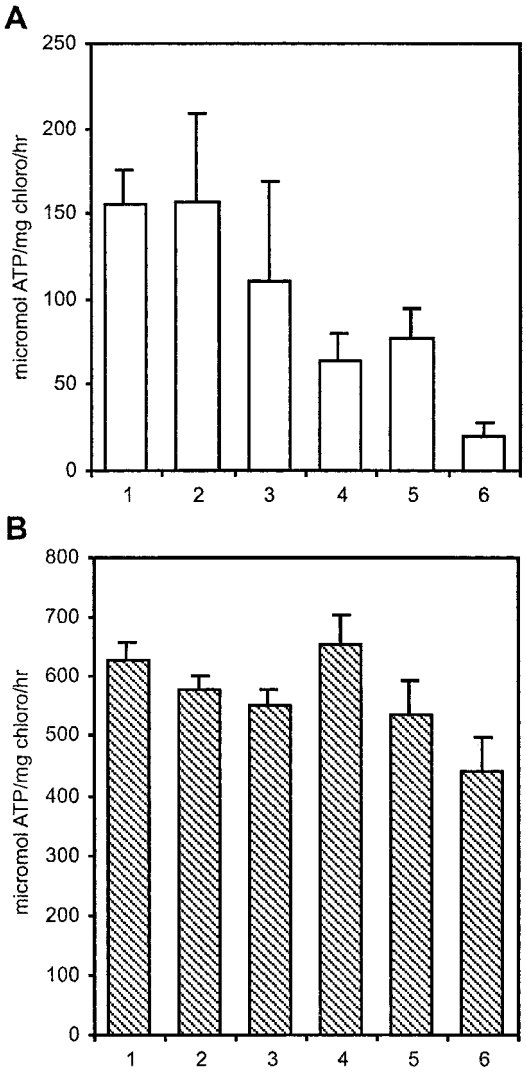

FIGURE 4: Effect of purified antibodies on ATP synthesis. (A) Rates of ATP synthesis of thylakoids after exposure to purified antibodies during illumination. (B) Rates of ATP synthesis of thylakoids after exposure to purified antibodies without illumination. The difference between synthesis rates with and without illumination results from photodamage during the illumination prior to the synthesis assay. Thylakoids were treated with either bovine serum albumin or antisera as described in the legend to Figure 3.

separated using reversed-phase liquid chromatography. The fragments were attached to a PVDF membrane and immunodetected with the positive control antibody preparation. Several fragments were recognized by the antibodies (Figure 5). Polypeptides that eluted at 14, 23−24, 30−31, and 48−52 min were detected by the antibody. Samples of these fractions were analyzed by laser-diffusion or electrospray mass spectroscopy. Fraction 14 contained a 679.3 Da peptide that corresponds to the Glu C ϵ subunit peptide 128−134 (predicted mass 678.3 Da). The fraction that eluted at 23 min contained a 1524.9 Da peptide that is almost identical to the predicted mass (1525.9 Da) of the fragment containing residues 115 through 127. The fraction that eluted at 30 min contained a 2654 Da peptide that is almost identical (2655.3 Da) to the fragment containing residues 74 through 97. A 5525.0 Da polypeptide was present in fraction 51. This polypeptide is ϵ subunit fragment 22−73 (predicted mass 5526.5 Da).

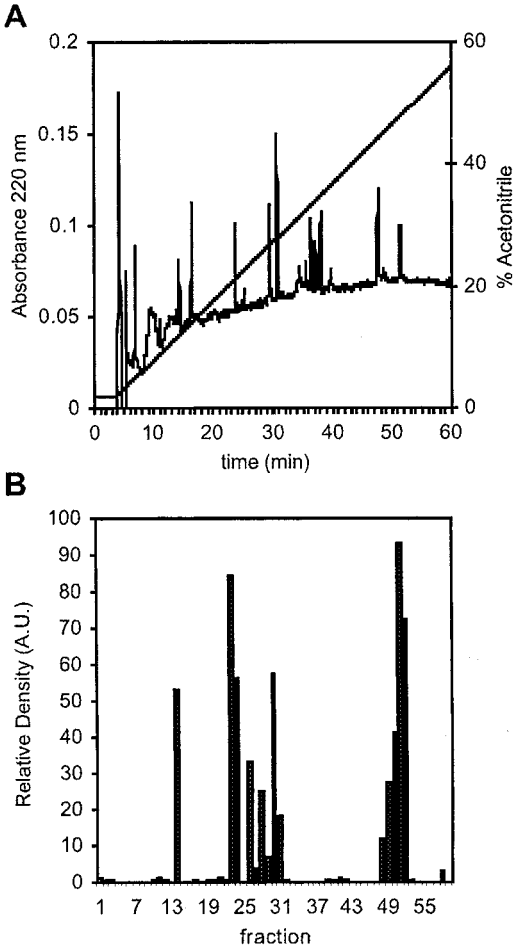

FIGURE 5: Immunodetection of HPLC fragments by purified anti-ϵ antibodies. Recombinant ϵ subunit (ϵC6S) was digested with endoproteinase Glu-C, and the peptides were separated by reversed-phase (C-18) liquid chromatography. (A) Elution profile determined at 220 nm. The right axis shows the acetonitrile gradient. (B) Immunodetection of peptides in the fractions (one per minute) using anti-ϵ serum. Four fractions, 14, 24, 30, and 51 min, each contained at least one polypeptide that was readily detected.

DISCUSSION

Our results show that antibodies against the C-terminus of the ϵ subunit can strip the ϵ subunit from CF1CFo in thylakoid membranes only when the membranes are illuminated. Since no effect is seen when the thylakoid membranes are exposed to the antibodies without illumination, the ϵ subunit must be involved in a conformational shift that exposes portions of its C-terminus that are solvent-protected in the dark. These results are in agreement with the finding that a single lysine on the C-terminus of ϵ (Lys-109) increases its reactivity with pyridoxal phosphate when thylakoids are illuminated (*6*).

In the similar ϵ subunit of the ATP synthase from *Escherichia coli*, evidence for conformational shifts in the region surrounding the ϵ subunit has been reported. The C-terminal region of the ϵ subunit cross-links to different subunits of the ATP synthase depending on the nucleotides bound to the enzyme (*15*). The *E. coli* ϵ subunit is thought to undergo a nucleotide-dependent shift as part of the catalytic process (*16*). Although cross-linking of the N- and C-terminal domains of the ϵ subunit does not inhibit ATP synthesis by the *E. coli* ATP synthase, it increases the rate

of ATP hydrolysis (*17*). Cysteine residues in the C-terminal region and N-terminal domain were introduced into the ϵ subunit of F1 from the thermophilic bacterium, *Bacillus* PS3. The mutant ϵ subunit was reconstituted with $\alpha_3\beta_3\gamma$. Disulfide bond formation occurred only when MgATP was present, indicating that nucleotide binding induces a change in the structure of the ϵ subunit (*18*).

In our experiments, nucleotides were not added to the medium. Thus, the exposure of the C-terminal region of the chloroplast ϵ subunit is driven by the formation of the electrochemical proton gradient, not by the binding of nucleotides to the catalytic sites. In no way, however, does this fact preclude the possibility that nucleotide binding will also induce conformational shifts in the ϵ subunit of CF1CFo. The influence of nucleotides on the interaction of the ϵ subunit with the remainder of the chloroplast ATP synthase remains to be investigated.

The conformational shift seen in the ϵ subunit of chloroplast ATP synthase is a response to the formation of the electrochemical proton gradient. The movement of the C-terminus of the ϵ subunit may relieve the inhibition of the activity of the ATP synthase imposed by the ϵ subunit. Since the ϵ subunit is involved in both the coupling and the inhibition of ATP synthase, it seems that at least the inhibitory function may be predominantly controlled by the C-terminus. This possibility was previously suggested from the facts that N-terminally truncated forms of the ϵ subunit partially inhibit ATP hydrolysis by CF1CFo deficient in the ϵ subunit, but did not block proton conductance through CFo (*10*). Current work in our laboratory has also shown that the inhibition of ATPase activity by the ϵ subunit is much more sensitive to truncation from the C-terminus than restoration of ATP synthesis (K. F. Nowak, unpublished data). Future work will try to understand how this separation of functions is achieved.

The ϵ subunit of *E. coli* (*19*, *20*) and the analogous δ subunit of yeast (*21*) and bovine (*22*) mitochondrial F1 contain a C-terminal α-helical coiled-coil domain and an N-terminal β sandwich. Interestingly the coiled-coil domain of the δ subunit of yeast F1 was in a different conformation from that of the bovine enzyme.

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