Catalytic Properties and Sensitivity to Tentoxin of *Chlamydomonas reinhardtii* ATP Synthases Changed in Codon 83 of *atpB* by Site-directed Mutagenesis

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The participation of the amino acid β83 in determining the sensitivity of chloroplast ATP synthases to tentoxin was reported previously. We have changed codon 83 of the *Chlamydomonas reinhardtii* *atpB* gene by site-directed mutagenesis to further examine the role of this amino acid in the response of the ATP synthase to tentoxin and in the mechanism of ATP synthase and hydrolysis. Amino acid β83 was changed from Glu to Asp (βE83D) and to Lys (βE83K), and the highly conserved tetrapeptide βT82-E83-G84-L85 (ΔTEGL) was deleted. Mutant strains were produced by particle gun transformation of *atpB* deletion mutants *cw15ΔatpB* and *FUD50* with the mutated *atpB* genes. The transformants containing the βE83D and βE83K mutant genes grew well photoautotrophically. The ΔTEGL transformant did not grow photoautotrophically, and no CF₁ subunits were detected by immunostaining of Western blots using CF₁ specific antibodies. The rates of ATP synthesis at clamped ΔpH with thylakoids isolated from *cw15* and the two mutants, βE83D and βE83K, were similar. However, only the phosphorylation activity of the mutant βE83D was inhibited by tentoxin with 50% inhibition attained at 4 μM. These results confirm that amino acid β83 is critical in determining the response of ATP synthase to tentoxin. The rates of the latent Mg-ATPase activity of the CF₁s isolated from *cw15*, βE83D, and βE83K were similar and could be enhanced by heat, alcohols, and octylglucoside. As in the case of the membrane-bound enzyme, only CF₁ from the βE83D mutant was sensitive to tentoxin. A lower alcohol concentration was required for optimal stimulation of the ATPase of the βE83K-CF₁ than that of CF₁ from the other two strains. Moreover, the optimal activity of the βE83K-CF₁ was also lower. These results suggest that introduction of an amino acid with a positively charged side chain in position 83 in the “crown” domain affects the active conformation of the CF₁-ATPase.

The eukaryotic unicellular green algae *Chlamydomonas reinhardtii* constitutes a powerful experimental model system for the study of the photosynthetic machinery. It is accessible to genetic analysis and grows photoautotrophically on minimal medium or heterotrophically with acetate as the sole carbon source. These properties have been used to isolate numerous photosynthetic mutants which have helped to examine the function of the photosynthetic apparatus (1).

The chloroplast of *C. reinhardtii* contains approximately 80 copies of its 196-kb circular genome (2). Due to recent progress in the molecular genetics of *C. reinhardtii*, chloroplast proteins can be altered by site-directed mutagenesis of the corresponding genes followed by transformation into the chloroplast (3–7). Chloroplast transformation was first demonstrated in 1988 by Boynton and co-workers (3), by complementation of an *atpB* deletion mutant with the cloned wild type gene. The transforming DNA integrates into the recipient chloroplast DNA by homologous recombination. Goldschmidt-Clermont (5) has constructed a chimeric selectable marker using the chloroplast *apbA* promoter and the bacterial gene aminoglycoside adenine-transferase (*aadA*), which confers resistance to spectinomycin and streptomycin in *Escherichia coli*.

Tentoxin, a cyclic tetrapeptide (cyclo-1-leucyl-N-methyl-(z)-dehydrophenylalanyl-glycyl-N-methyl-alanyl) produced by the fungus *Alternaria tenuis*, is a potent inhibitor of the chloroplast ATP synthase of certain sensitive plant species (8, 9), which acts as an competitive inhibitor of the CF₁-ATPase with respect to nucleotide substrates. It was proposed that 1 mol of tentoxin/mol of CF₁ bound at a site near the interface between the N-terminal domains of subunits α and β, interferes with the cooperative interaction between nucleotide binding sites inhibiting the enzyme activity (10). Binding of tentoxin to a second, low affinity site stimulates the ATPase activity of CF₁ (11–13). Avni et al. (7) have shown that amino acid β83 is involved in conferring tentoxin sensitivity. *Nicotiana* species having a glutamic acid residue at position 83 are resistant, whereas species with an aspartic acid residue in this position are sensitive to tentoxin. The peptide sequence around position 83 in *C. reinhardtii* β subunit was replaced by the corresponding sequence from a tentoxin-sensitive tobacco β subunit. This exchange of five amino acids including Glu⁸³ to Asp⁸³ converted the normally tentoxin-resistant to a tentoxin-sensitive *C. reinhardtii* ATP synthase.

We investigated in more detail the role of the acidic amino

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1 The abbreviations used are: kb, kilobase; PCR, polymerase chain reaction; Chl, chlorophyll; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)methyl]glycine; AMP-PNP, adenosine 5’-β,γ-imino-triphosphate.
acid residue at position 83 of the b subunit of the ATP synthase by site-directed mutagenesis. The atpB deletion mutant cw15ΔatpB was prepared from the cell wall deficient strain cw15 by replacing the atpB open reading frame by the aadA cassette (14). The use of the cell wall deficient strain cw15 allowed us to isolate well preserved thylakoids and to characterize electron transport and photophosphorylation activities of these membranes (15, 16).

EXPERIMENTAL PROCEDURES

C. reinhardtii cw15 was cultivated in high salt medium (1). The cell wall deficient atpB deletion mutant cw15ΔatpB (14) was cultivated in high salt medium containing 0.4% acetate and 150 μg/ml spectinomycin (high salt medium containing acetate/spectinomycin) at low light intensity (∼50 lux). The atpB deletion mutant FUD50 (17) was cultivated in Sager-Granick medium (1) containing 0.2% acetate.

All cloning procedures were done using commercially available restriction endonucleases and DNA-modifying enzymes according to the suppliers instructions. DNA fragments were isolated from agarose gels using the Glass Select DNA Isolation Kit (5 Prime) or by freezing the sliced agarose fragments in the presence of 80% phenol, followed by chloroform extraction, and ethanol precipitation of the DNA from the supernatant. Transformation into E. coli JM109 or DH5α was done according to the instructions supplied in the pAlter site-directed mutagenesis kit (Promega Biotech). Plasmid DNA was isolated by alkaline miniprep as described in the T7 DNA sequencing protocol (Promega Biotech) or by the Qiagen plasmid kit (Qiagen GmbH, Hilden, Germany). DNA sequencing was done using Sequenase version 2.0 (USB) and denatured plasmid DNA as template. Site-directed mutagenesis was done using the pALTER site-directed mutagenesis kit (Promega Biotech) according to the suppliers instructions, except that T7 DNA polymerase was used for second strand synthesis instead of T4 DNA polymerase. The mutagenesis procedure was performed using single-stranded DNA from plasmid pALTER containing a 2.8-kb HindIII-EcoRI fragment with the complete atpB gene (18) and the mutagenic primers shown in Fig. 1. Introduction of the mutation into the atpB gene was confirmed by PCR, restriction analysis, and DNA sequencing using atpB-specific primers. The 0.9-kb EcoRV-PstI fragments containing the mutant sites were cloned back into suitable transformation vectors containing extended 5′- and 3′-flanking regions and anti-biotics resistance cassettes as markers for successful transformation.

Chloroplast transformation was done by vortexing in the presence of glass beads (6) or with the PDS1000/He apparatus of Bio-Rad. Cells were spread in 0.2% soft agar onto high salt medium-agar plates and bombarded on acetate containing medium, incubated overnight at low temperature, and then spread onto acetate containing plates containing 10 to 300 mU of aosp or lysc, respectively, in the orientation shown, with the 2.5 to the right. The bases changed to give a aosp or lysc, respectively, are indicated in bold letters, as well as the single base change introduced to yield a new AseI restriction site. The 12-base pair deletion created in the ΔTEGL primer is indicated by a dashed line.

5 ml of Fractogel TSK DEAE-650 (M) in a chromatography column, rinsed with loading buffer, and eluted with a 10-300 mU (NH4)2SO4 gradient in loading buffer. The purest fractions, corresponding to a protein peak eluting at about 130 mU (NH4)2SO4, were collected, supplemented with 1 mU ATP, precipitated by addition of (NH4)2SO4 to 50% saturation, and stored at 4°C. For enzymatic assays, the CF1 pellet was collected by 15 min centrifugation at 12,000 × g, dissolved in 20 mM Tricine/NaOH, pH 8.0, to a concentration of 0.5 to 1 mg/ml. Salts were removed by column centrifugation through Sephadex G-25 equilibrated with 20 mM Tricine/NaOH, pH 8.0.

Mg-ATPase activity of these preparations was measured by photometric determination of released inorganic phosphate (P) as described (22). 5 μg of enzyme were incubated for the indicated times in 20 mM Tricine/NaOH, pH 8.0, with 4 mM MgCl2 and 6 mM ATP present, and the additions indicated in the legends of figures and tables. Reaction mixes lacking either enzyme or ATP were preincubated at the indicated temperature, and the reactions were started by adding enzyme or substrate. The reaction was stopped by addition of 3% trichloroacetic acid, and inorganic phosphate released was determined photometrically.

Active thylakoid membranes were prepared as described (15, 16). Cells were grown at 20°C in high salt medium containing 0.2% acetate at a light intensity of 900 lux in a 14-h-light to 10-h-dark cycle, in 400-ml cultures bubbled with sterile air. The cultures were grown to a cell density of 6 × 108 cells/ml and then harvested by centrifugation (10 min, 1000 × g), washed with medium A (0.3 M sucrose, 10 mM Tricine, pH 8.0, 50 mM NaCl, and 5 mM MgCl2), and resuspended in the same medium at 4°C. All further steps were carried out at this temperature. The cells were twice passed through a Yeda Press at 5 × 105 N/m2, 5.5 × 105 N/m2, respectively, and harvested by a 1-min centrifugation step at 3000 × g. The resulting pellet was resuspended and washed twice in 2 mM Tricine, pH 8.0, 50 mM NaCl, 1 mM MgCl2 and resuspended in a small volume of solution A.

ATP formation was measured at clamped proton gradient by employing the ΔP clamp device (23). The proton gradient was controlled by monitoring the fluorescence quench of 9-aminoacridine and adjusting the light intensity correspondingly. The reaction medium contained in a final volume of 2.5 ml: 25 mM Tricine, pH 8.0, 50 mM KCl, 5 mM MgCl2, 50 μM phenazine methosulfate, 10 mM dithiothreitol, 50 mM valinomycin, 10 mM glucose, 12 units/ml hexokinase, 5 μM 9-aminoacridine, and thylakoid vesicles to give a final concentration of 25 μg Chl/ml. After preillumination at 20°C for 2 min, the ΔP was adjusted to 60% fluorescence quench and after 1 min 1 mM ADP and 5 mM [32P]Pi were added. Rates of photophosphorylation were calculated from the organic [32P]-labeled phosphate determined in the samples taken after 10, 20, 30, and 40 s according to the method of Sugino and Miyoshi (24).

RESULTS

The oligonucleotide primers presented in Fig. 1 were used to introduce the desired changes (bold letters) into the atpB gene by site-directed mutagenesis as follows: (a) change of codon 83 from glutamic acid to aspartic acid (E83D), (b) change of codon 83 from glutamic acid to lysine (E83K), and (c) deletion of the tetrapeptide Thr82-Glu83-Glu84-Leu85 (ΔTEGL). In the βE83D and βE83K oligonucleotides a second, silent point mutation, creating a new AseI restriction site (underlined), was intro-
The DNA fragments containing the mutant sites were isolated from total DNA by PCR, and in the case of the βE83D and βE83K transformants the mutations were confirmed by restriction analysis (Fig. 3). Subsequently, in all three types of transformants, the mutations were verified by sequence analysis of the corresponding PCR products. The presence of the CF₀CF₁ complex was examined by SDS-polyacrylamide gel electrophoresis of the thylakoid proteins followed by immunostaining of Western blots with a CF₁-specific antiserum. The thylakoids from the spectinomycin- and kanamycin-resistant transformants containing the ΔTEGL mutation were completely devoid of the ATP synthase complex (not shown). Thus, this highly conserved peptide segment seems to be important for the assembly or stability of the ATP synthase complex.

Thylakoids prepared from cw15 and the transformants cw15D221 and cw15K221 containing the mutations βE83D and βE83K, respectively, were assayed for their photophosphorylation capacity. All three thylakoid preparations yielded similar rates at a clamped pH (60% fluorescence quench). Photophosphorylation activity of cw15 and βE83K was not inhibited by tentoxin, whereas that of βE83D was sensitive to tentoxin with 50% inhibition attained at 4 μM (Fig. 4A). The degree of inhibition was enhanced by preincubation of thylakoids with tentoxin and maximal inhibition was attained after 1 h with 5 μM tentoxin (Fig. 4C). Fig. 4B shows the response to tentoxin of the ethanol-enhanced Mg-ATPase activity of CF₁, isolated from the wild type and the two transformants. The ATPase activity of cw15 and βE83K CF₁ was not inhibited by tentoxin, whereas that of βE83D was inhibited with 50% inhibition attained at a concentration of 0.5 μM tentoxin. Preincubation with tentoxin was also required for maximal inhibition (Fig. 4C). Hence, the isolated CF₁-ATPase is about 10-fold more sensitive to tentoxin than photophosphorylation.

We examined whether the mutations in codon 83 had additional effects on the catalytic properties of the mutant ATPases. The general characteristics of all three CF₁ preparations were similar to those reported before for C. reinhardtii (21) although the rates with the latent enzyme were lower. As shown in Table II, the low basal rates of the Mg-ATPase activities of all three CF₁ preparations were enhanced by elevating the assay temperature, by octylglucoside, by ethanol, and by methanol. In the presence of 20% ethanol the apparent K₅₀ for ATP was significantly lower (Fig. 5, B and C). Maximal stimulation of the Mg-ATPase activity by octylglucoside was attained at a concentration of about 1% (Fig. 5A). While the basal, heat- and detergent-stimulated ATPase activities were similar for the wild type and mutant enzymes, the ATPase activity of the βE83K CF₁ elicited by alcohols was significantly lower (Fig. 5, B and C). Maximal stimulation of the Mg-ATPase activities of the cw15 and βE83D enzymes was obtained at an ethanol concentration of 23% (ν/ν) and a methanol concentration of 30% (ν/ν), whereas the maximal ATPase activity of the βE83K enzyme was attained at 20% ethanol and 25% methanol (Fig. 5, B and C). The degree of stimulation of the βE83K enzyme by alcohols was less than half that of the cw15 and the βE83D enzymes (Fig. 5, B and C).

The lower activity and the shift in the concentration of alcohols needed for maximal stimulation of ATPase with the βE83K enzyme could be caused by rapid time-dependent inactivation of this enzyme, in particular at higher organic solvent concentrations. We tested this possibility by measuring the time course of the Mg-ATPase activity of all the three preparations at 17 and 25% ethanol. The specific activities of the βE83K enzyme at both ethanol concentrations were equal and about 30% lower than its maximal activity at 20% ethanol. At 17% ethanol the ATPase activities of all three enzymes were similar (Fig. 6A), but at 25% ethanol the ATPase activities of
Detailed construction of the transformation vectors and the kanamycin cassette will be described elsewhere. Strains cw-D221 and cw-K221 were used for determination of photophosphorylation activity and for routine preparations of CF₁. CF₁ preparations from strains D101 and K101 displayed the same catalytic properties as those prepared from strains cw-D221 and cw-K221.

At both ethanol concentrations the rates of all three CF₁ were decreased (Fig. 4). Effective inhibition by tentoxin required preincubation of the enzyme with tentoxin as reported previously (Fig. 4C) (8, 25). Photophosphorylation of isolated thylakoids of the βE83D mutant was also inhibited by tentoxin with 50% inhibition obtained at 4 mM. The fact that the enzymes with the bulkier amino acid side chains (Glu and Lys) in position 83 were sensitive to tentoxin whereas that of the mutant with Asp in position 83 was resistant may indicate that in the resistant species the access of tentoxin to its binding site is sterically hindered.

**DISCUSSION**

The sensitivity of certain CF₀CF₁ ATP synthases to tentoxin has been attributed to the presence of aspartic acid in position 83 of the β subunit. This was demonstrated by Avni et al. (7) by analysis of resistant and sensitive tobacco strains and subsequent mutagenesis studies with the C. reinhardtii atpB gene. The C. reinhardtii atpB sequence from codons 74–91 (VRAVMNPNTEGLMRGMEV) was replaced by the corresponding tobacco sequences of the resistant (VRAVSATDELTRGMEV) or sensitive lines (VRAVSMATDELTRGMEV) by changing four or five amino acids, respectively (underlined). The resulting mutant enzyme containing Glu in position 83 was not resistant to tentoxin whereas the enzyme with Asp in position 83 was inhibited by tentoxin.

In this work we have exchanged a single codon of the C. reinhardtii atpB gene, Glu83 by Asp, to determine whether in fact this change alone determines tentoxin sensitivity. The Mg-ATPase activity of the isolated CF₁ containing the Asp residue in position 83 (βE83D) was indeed inhibited by tentoxin at concentrations similar to those required to inhibit enzymes from sensitive plants and from the sensitive cyanobacterium Anacystis nidulans (Fig. 4B) (7, 8, 25). Effective inhibition by tentoxin required preincubation of the enzyme with tentoxin as reported previously (Fig. 4C) (8, 25). Photophosphorylation of isolated thylakoids of the βE83D mutant was also inhibited by tentoxin with 50% inhibition obtained at 4 μM (Fig. 4A). The difference of 1 order of magnitude between the sensitivity to tentoxin of photophosphorylation and CF₁-ATPase activity (Fig. 4) might be explained by different accessibility of tentoxin to its binding site. It is known that the conformation of isolated CF₁ differs from the conformation of the CF₁ sector in the membrane-bound CF₀CF₁ complex (26, 27). On the other hand, photophosphorylation of the mutant enzyme produced by Avni et al. (7) containing a tobacco/C. reinhardtii hybrid β subunit was rather sensitive to tentoxin even after a relatively short preincubation time. The apparent higher affinity of this mutant enzyme to tentoxin could be the result of the additional changes made in the sequence of the tobacco/C. reinhardtii hybrid gene (7).

The fact that the enzymes with the bulkier amino acid side chains (Glu and Lys) in position 83, irrespective of their charge, are resistant to tentoxin whereas the enzyme with Asp in position 83 is sensitive indicates that in the resistant species the access of tentoxin to its binding site is sterically hindered.
amino acid corresponding to position 83 of the \( \beta \) subunit of \( C. \) reinhardtii is located in the lower region of the crown structure at the interface where the alternating \( \alpha \) and \( \beta \) subunits are in close contact. Tentoxin contains several hydrophobic residues that might be important for the interaction with a hydrophobic pocket partly screened by amino acid \( \beta 83 \). The spatial structure

**Fig. 4.** A, inhibition of photophosphorylation activity of thylakoids from the different strains by tentoxin. Thylakoids were incubated in a reaction mix without ADP with the indicated concentrations of tentoxin for 45 min at room temperature. Photophosphorylation activity was measured as described under "Experimental Procedures," at clamped \( \Delta pH \) (60% fluorescence quench). 100% control activities for cw15 (○) and \( \beta E 83 K \) (■) thylakoids were 62 \( \mu \)mol of ATP formed per mg Chl/h and 82 \( \mu \)mol of ATP formed per mg Chl/h for the \( \beta E 83 D \) (●) mutant. B, inhibition of the ethanol-stimulated Mg-ATPase activity by tentoxin of cw15 (○), \( \beta E 83 K \) (■), and \( \beta E 83 D \) (●) CF1. 5 \( \mu \)g of enzyme were preincubated in a reaction mix without ATP and ethanol for 45 min at room temperature with the indicated concentrations of tentoxin. ATPase activity was assayed after addition of 20% ethanol and ATP as described under "Experimental Procedures." 100% of control activities of CF1s were as follows: cw15, 32; \( \beta E 83 K \), 36 and \( \beta E 83 D \), 48 \( \mu \)mol of ATP hydrolyzed per mg protein/min. C, preincubation with tentoxin required for inhibition of photophosphorylation with \( \beta E 83 D \) thylakoids (■) and of the ethanol-enhanced Mg-ATPase activity of isolated \( \beta E 83 D \)-CF1 (●). Thylakoids were preincubated in a reaction mix without ADP for the indicated times with 5 \( \mu \)g tentoxin. CF1 was preincubated in a reaction mix, without ATP and ethanol, with 2 \( \mu \)g tentoxin. 100% of control activities were 170 (without preincubation) and 80 (after 60-min preincubation) \( \mu \)mol of ATP formed per mg Chl/h and 51 \( \mu \)mol of ATP hydrolyzed per mg of protein/min.

**TABLE II**

Mg-ATPase activity of CF1s from cw15 and \( \beta E 83 D \) and \( \beta E 83 K \) transformants

Activities were determined after incubation of reaction mixes containing the indicated concentrations of effectors for 2 min at 37 °C as described under "Experimental Procedures." Specific activity is given in \( \mu \)mol of ATP hydrolyzed per mg of protein/min.

| CF1          | 37 °C        | 65 °C        | 37 °C        | 20% ethanol | 25% methanol | 1.2% octylglucoside |
|--------------|--------------|--------------|--------------|--------------|--------------|-------------------|
|               | No addition  |              |              |              |              |                   |
| cw15         | 1°           | 21           | 36           | 52           | 46           |                   |
| \( \beta E 83 D \) | 1.3°         | 21           | 39           | 63           | 47           |                   |
| \( \beta E 83 K \) | 1.1°         | 22           | 32           | 47           | 52           |                   |

* Assays without effector at 37 °C were run for 10 min.

**Fig. 5.** Enhancement of the Mg-ATPase activity of cw15 (○), \( \beta E 83 D \) (●), and \( \beta E 83 K \) (■) CF1s by octylglucoside (A), ethanol (B), and methanol (C). Activities were determined after incubation of reaction mixes containing the indicated concentrations of effectors for 2 min at 37 °C as described under "Experimental Procedures." ATPase activities are in \( \mu \)mol of ATP hydrolyzed per mg protein/min.
of tentoxin is wedge shaped and could fit into the αβ interface domain. The more extended Glu and Lys residues in position β3 may sterically hinder the access of tentoxin to this binding domain inducing resistance to tentoxin. Tentoxin was shown to inhibit the AMP-PNP-induced release of tightly bound ADP from CF₁ (10), suggesting that tentoxin may bind at the interface between an α and β subunit and thereby preclude conformational changes required for catalytic turnover. Tentoxin acting as a wedge can prevent the transfer of information between different nucleotide binding sites on the ATP synthase and inhibit the enzymatic activity.

The transformants where the highly conserved tetrapeptide β82-E83-G84-L85 was deleted could not grow photoautotrophically probably because the CF₁ CF₂ complex was not assembled. As revealed by the crystal structure of mitochondrial F₁ (28), the four amino acids deleted in the β subunit form a loop connecting two β sheets of the β subunit in the stabilizing crown structure, and their deletion would prevent proper folding of the mutant β subunit. Changing only one residue, a Glu to a Lys, in the βE83K mutant yielded an active ATP synthase which was not sensitive to tentoxin and exhibited similar rates of photophosphorylation to those of the wild type and the βE83D mutant.

The Mg-ATPase activities of the cw15, βE83D, and βE83K CF₁’s were quite similar at elevated assay temperature (Table II) or in the presence of octylglucoside (Fig. 5A), but significant differences were found with the alcohol-enhanced Mg-ATPase activity (Fig. 5, B and C). The higher methanol versus ethanol concentrations required to attain maximal rates of ATP hydrolysis indicate that the dielectric properties of the assay medium plays an important role in the activation process by alcohols. Denaturation by alcohols was ruled out (Fig. 6, A and B) as a possible reason for the lower activity of the βE83K enzyme as compared with the cw15 and βE83D enzymes. We propose that the presence of a Lys residue in position 83, which according to the three-dimensional structure of F₁ is in close vicinity to residue Argβ82, alters the active conformation of the CF₁-ATPase induced with alcohols. On the other hand, the octylglucoside-induced activation of the CF₁-ATPase does not seem to be affected in the same manner by the introduction of Lys in position 83.

It is very interesting that both the sensitivity of CF₁ to tentoxin and the ATPase activity enhanced by alcohols are affected by mutations at codon 83. It is likely that the primary effect of organic solvents is to alter the conformation of the enzyme, lowering the dielectric constant of the medium (29, 30). The release of tightly bound, inhibitory ADP, which is a rate-limiting step in catalysis, is stimulated by alcohols (31) and by octylglucoside (32). In the presence of ethanol the Mg-ATPase activity of CF₁ is no longer inhibited by ADP (29). Ethanol also removes the ε subunit from the CF₂ complex and stimulates the ATPase in this way (33). It will be interesting to determine which of these effects is affected by the mutations in codon 83. Our results demonstrate that the stimulatory effects of octylglucoside and ethanone are based on different mechanisms. The ATPase activity of the βE83K enzyme is stimulated to the same extent as that of the cw15 and βE83D enzymes by octylglucoside but affected differently by methanol and ethanol.

Tentoxin inhibits multisite catalysis by binding at one of three αβ interfaces of the enzyme (probably near amino acid β83), interferes with the interactions between catalytic sites, and inhibits the release of ADP from the catalytic site (10). The results presented here demonstrate that the domain around amino acid β83 in the crown domain participates in activation and inhibition of the catalytic activity of CF₁, probably via long range conformational effects that affect the affinity of the tight sites for ADP. Other mutants in the crown domain (αE83K (34) and βC63W (35)) were shown to affect the coupling efficiency of ATP synthase, probably by interfering with conformational signaling. These results imply that the crown domain is involved in determining the conformational states of the catalytic sites by transmitting conformational signals essential for catalysis.

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