The Structure of the Chloroplast F$_1$-ATPase at 3.2 Å Resolution\(^*\)

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The structure of the F$_1$-ATPase from spinach chloroplasts was determined to 3.2 Å resolution by molecular replacement based on the homologous structure of the bovine mitochondrial enzyme. The crystallized complex contains four different subunits in a stoichiometry of $\alpha_3\beta_3\gamma\delta$. Subunit $\delta$ was removed before crystallization to improve the diffraction of the crystals. The overall structure of the noncatalytic $\alpha$-subunits and the catalytic $\beta$-subunits is highly similar to those of the mitochondrial and thermophilic subunits. However, in the crystal structure of the chloroplast enzyme, all $\alpha$- and $\beta$-subunits adopt a closed conformation and appear to contain no bound adenine nucleotides. The superimposed crystallographic symmetry in the space group $R32$ impaired an exact tracing of the $\gamma$ and $\epsilon$-subunits in the complex. However, clear electron density was present at the core of the $\alpha_3\beta_3$-subcomplex, which probably represents the C-terminal domain of the $\gamma$-subunit. The structure of the spinach chloroplast F$_1$ has a potential binding site for the phytotoxin, tentoxin, at the $\alpha$-$\beta$-interface near $\beta$Asp$^{83}$ and an insertion from $\beta$Gly$^{56}$-$\text{Asn}^{60}$ in the N-terminal $\beta$-barrel domain probably increases the thermal stability of the complex. The structure probably represents an inactive latent state of the ATPase, which is unique to chloroplast and cyanobacterial enzymes.

The membrane-bound proton translocating ATP synthase of chloroplasts, CF$_1$, catalyzes ATP synthesis and ATP hydrolysis coupled to proton translocation across the thylakoid membrane (for a recent review see Ref. 1). The enzyme is comprised of nine different polypeptides organized in two separate sectors. The membrane-integrated $\tilde{F}_0$ complex, composed of subunits I, II, III, and IV, mediates proton transport and provides specific sites for the attachment of the catalytic $F_1$ complex. The membrane extrinsic $F_1$ domain is an assembly of five different polypeptides in a stoichiometry of $\alpha_3\beta_3\gamma\delta\epsilon$. In contrast to the homologous bacterial and mitochondrial enzymes, isolated CF$_1$ is a latent ATPase that requires activation.

The overall shape, dimension, and mass distribution of the chloroplast ATPase were revealed by electron microscopy and image analysis (2, 3). The catalytic $F_1$ complex resembles a pseudo-hexagonal, symmetrical ring of alternating $\alpha$ and $\beta$ subunits. The central cavity of the $\alpha_3\beta_3$ core complex is partially filled with subunit $\gamma$. Previous studies on subunit deficient chloroplast F$_1$ demonstrated that the $\epsilon$-subunit is necessary to preserve the arrangement of the central $\gamma$-subunit. Removal of subunit $\delta$ showed no effect on the integrity of the central mass in the $\alpha_3\beta_3$ hexagon (4). Interaction of the catalytic $F_1$ domain and the membrane embedded $F_0$ complex, however, was affected in CF$_1$-deficient in subunit $\delta$ (5).

The location and the orientation of the catalytic and the regulatory sites in the chloroplast $F_1$ domain were identified in photo-labeling and fluorescence resonance energy transfer studies (6), which demonstrated that nucleotide binding is associated with the $\alpha$ and $\beta$ subunits. Binding sites are located on each of the six $\alpha\beta$-interfaces.

At present, most detailed structural information about the $F_1$ complex comes from the high resolution structure of the bovine mitochondrial ATPase (7, 8). Additional structural information was obtained from the rat liver $\alpha_3\beta_3\gamma$ complex (9), the $\alpha_3\beta_3$ core complex from the thermophilic bacterium $PS3$ (10), and the $\alpha_3\beta_3\gamma\delta$ complex from Escherichia coli (11). For the chloroplast enzyme, however, we still rely on the limited structural information from electron microscopy, cross-linking, and fluorescence resonance energy transfer studies because no high resolution structure was available until now. Even though the chloroplast enzyme shares many structural and functional characteristics with the mitochondrial and bacterial enzymes, CF$_1$ is unique in several aspects of enzyme activation and sensitivity toward specific energy transfer inhibitors. Control of the catalytic activity in membrane-bound CF$_1$ is achieved by the transmembrane proton gradient, the inhibitory tight binding of ADP to a catalytic site, and the redox modulation of $\gamma$Cys$^{196}$ and $\gamma$Cys$^{205}$. In addition to these mechanisms, isolated CF$_1$ is activated by heat, proteolytic cleavage in the vicinity of the regulatory disulfide in the $\gamma$-subunit, alcohol, and mild detergents (summarized in Ref. 1). Removal of the $\delta$-subunit shows no effect on the catalytic activity. In contrast CF$_1$-$\epsilon$ is always activated (12).

The $\alpha$ and $\beta$ subunits of CF$_1$ share 58.5 and 67.5% sequence identity, respectively, with their mitochondrial counterparts, suggesting that they have similar overall structures. In contrast, the smaller subunits are not well conserved. Particular interest is focussed on subunit $\gamma$, which contains a regulatory insert of about 20 amino acids ($\gamma$183–206) including two redox-active cysteine residues in the chloroplast enzymes of higher plants and green algae. The important role of the $\gamma$-subunit in the catalytic cycle was demonstrated in sophisticated experiments which showed that subunit $\gamma$ rotates in the $\alpha_3\beta_3$ core and

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The atomic coordinates and structure factors (code 1FX0) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/)

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\(\dagger\)The abbreviations used are: CF$_1$, chloroplast F$_1$; AMPPPN, adenosine 5’-($\beta$,$\gamma$)-imino-triphosphate; Tricine, N,N,N,N,N-hydroxymethyl-2-ethylglycine; rms, root mean square.
is probably related to sequential conformational changes of the αβ-pairs (13–15). Three different conformations of the β-subunit, which contains most of the catalytic binding site, were found in the high resolution structure of the bovine MF$_1$ complex (7). Depending on the nucleotide bound in the catalytic site the subunits were named γ$_{DP}$, β$_{TP}$, and β$_{G}$. The conformation of the three noncatalytic α-subunits was very similar in the bovine MF$_1$ structure because all α-subunits were filled with the nonhydrolyzable nucleotide analogue AMP-PNP (7). Deletion of the 20 C-terminal residues of the γ-subunit resulted in an active chloroplast enzyme and questioned the significance of the γ-rotation for the catalytic process in the chloroplast ATPase (16). Hence, rotation of subunit γ was recently also visualized for isolated CF$_1$ (17).

Another striking difference that is expected to be reflected in the structure of the chloroplast ATPase is the specific interaction of the extrinsic CF$_1$ complex of certain plant species with the fungal phytotoxin, tenuxtoxin. Binding of a single molecule of tenuxtoxin that has no effect on mitochondrial or bacterial ATPases is sufficient to inhibit CF$_1$, whereas binding of a second and third molecule results in its reactivation (18, 19).

Differences in nucleotide binding of the chloroplast ATPase were reported depending on whether isolated or membrane-bound CF$_1$ were studied (20–22). In addition magnesium was shown to play a crucial role in nucleotide binding (23, 24). Depending on their binding characteristics sites were designated I–5 (21) or A–C (25, 26). Isolated CF$_1$ contains one or two endogenous bound nucleotides probably located on the noncatalytic α-subunit (26–28). The presence of adenine nucleotides at the noncatalytic sites, however, is not a prerequisite for catalytic turnover but promotes the release of inhibitory tightly bound ADP from a catalytic site (29). Tight binding of adenine nucleotides to noncatalytic sites is stimulated by the presence of magnesium, which also stabilizes the inhibitory enzyme-bound ADP located on a catalytic site (25). Fluorescence resonance energy transfer studies demonstrated that the asymmetry of the nucleotide binding sites of CF$_1$ is not a permanent feature and that site switching in the catalytic cycle is controlled by magnesium (30, 31). The molecular structures of the rat liver (9) and the thermophilic ATPase (10) suggest that the asymmetry of the catalytic sites in the F$_1$ complex resolved in the bovine F$_1$ (7) is controlled by magnesium and/or subunit γ. Both structures show 3-fold symmetry and were obtained in the absence of magnesium and for the thermophilic structure also in the absence of the γ-subunit.

In this paper we present the first high resolution structure of a chloroplast α$_1$β$_2$γ complex. The structure was obtained in the absence of magnesium and shows 3-fold symmetry. Thus, subunits γ and ε, which are present in single copies in the complex, are not clearly resolved. Despite the different nucleotide content the chloroplast α and β subunits adopt a conformation that resembles the conformations of the bovine MF$_1$ complex containing bound nucleotides. This difference might be related to the unique latent ATPase activity of the chloroplast enzyme.

**EXPERIMENTAL PROCEDURES**

**Crystallization and Data Collection—**Purification and crystallization of the membrane extrinsic F$_1$ domain of the chloroplast ATP synthase have been described previously (28). Briefly, CF$_1$ was released from thylakoid membranes of spinach plants in a medium containing 300 mM sucrose, 2 mM Tricine, pH 7.5, 2 mM dithiothreitol, 0.875 mM EDTA, and 0.002% (v/v) phenylmethylsulfonyl fluoride. Membrane fragments were removed by centrifugation, and the supernatant was applied to anion exchange chromatography on POROS HQ (Applied Biosystems). The protein was eluted from the anion exchange column by applying a linear gradient of 225–1500 mM NaCl in 25 mM Bis-trispropane/Tris borate, pH 7.5. In contrast to the former protocol subunit ε was removed from the catalytic F$_1$ domain by incubation with 20% (v/v) ethanol/14% (v/v) glycerol in 25 mM Bis-trispropane/Tris borate, pH 7.5, when CF$_1$ was bound to the anion exchange column. Rhomboidal crystals, space group P3$_2$, were grown in 25 mM HEPES, pH 7.5, 1 mM dithiothreitol, 0.002% (v/v) phenylmethylsulfonyl fluoride, 0.01% (v/v) NaN$_3$, using 15 mM ammonium sulfate as a precipitant at 20 °C by the micro batch technique. Adenine nucleotides, ADP (0.02 mM), and AMP-PNP (1 mM) were added to the crystallization buffer to stabilize the purified protein. A first diffraction set was collected at 3.5 Å resolution from flash-frozen crystals on beam line X11 (EMBL/DESY Hamburg). Subsequently a second data set at 3.2 Å resolution was measured on beam line BW7B at EMBL/DESY. Diffraction data were processed with the programs DENZO (32) and MOSFLM (33), revealing unit cell parameters of a = 147.70 Å, b = 147.70 Å, and c = 185.05 Å, α = 90°, β = 90°, and γ = 120°. Further crystallographic parameters are given in Table I.

**Structure Determination and Refinement—**The structure was solved by molecular replacement using the program AMoRe (34). The atomic coordinates of a poly-alanine model derived from the refined structure of bovine mitochondrial F$_1$ (7) were used as a search model in these calculations. All conformations of αβ-dimers found in the mitochondrial F$_1$ structure (7) were tested. Data from 15–5 Å resolution were included in the molecular replacement calculations. A clear solution with a correlation coefficient of 0.71 was obtained with the α$_1$β$_2$γ$_{DP}$-dimer and the closely related α$_{DP}$β$_{TP}$γ$_{DP}$-conformer. In these dimers the nucleotide binding sites on the α-subunits are filled with the nonhydrolyzable nucleotide analogue AMP-PNP, whereas the β-subunits contain either ADP or ATP. Calculations using the β$_G$ conformation of the β-subunit where no nucleotides are bound in the catalytic site resulted in a correlation coefficient of 0.63. These results suggested that a αβ-dimer in the chloroplast α$_1$β$_2$γ complex resembles the dimer formed by nucleotide-filled α and β-subunits in the mitochondrial enzyme. The model was mutated to the chloroplast sequence in O (35). Initial rigid body refinement was carried out by the program CNS (36) for all reflections from 15 to 5 Å. According to the structure of the mitochondrial F$_1$ complex, three domains in the α and β subunits were defined in the rigid body minimization corresponding to residues α$_{19–95}$, α$_{96–379}$, and α$_{380–510}$, and β$_{99–82}$, β$_{83–363}$, and β$_{364–474}$, respectively. Further crystallographic refinement was accomplished by Cartesian coordinate molecular dynamics and restrained B-factor minimization in iterative cycles. The model was revised at each cycle of the refinement by inspection of the $F_F$ and $2F_F$ maps and manual rebuilding in O (35). A bulk solvent correction was applied in the final refinement cycle. The model converged to a final crystallographic R-factor of 31.9% (R$_{free}$ = 35.0%) for reflections from 6–3.2 Å. Model geometry was verified with PROCHECK (37). Figures were produced using the programs Bobscript (38), Molscript (39), and Swiss-PdbViewer (40). The structure has been submitted to the Protein Data Bank (PDB accession code 1FXO).

**SDS-Polyacrylamide Gel Electrophoresis—**Proteins were analyzed by SDS-polyacrylamide gel electrophoresis on 12% polyacrylamide gels according to Laemmli (41). After electrophoresis, subunit composition of the purified complex was detected by silver staining (42).

**RESULTS AND DISCUSSION**

**Overall Structure of the Chloroplast aβ-Dimer—**Previously we have described purification and crystallization of the chloroplast F$_1$ domain (28). However, these crystals diffracted only up to 6–8 Å resolution. Analysis of the crystals by SDS-poly-
acrylamide gel electrophoresis revealed that subunit δ was degraded, whereas all other subunits remained intact (data not shown). Thus, in subsequent preparations subunit δ was removed from the CF₁ complex and the remaining α₃β₂γε complex was used in crystallization experiments (Fig. 1). No trace of subunit δ could be detected in this complex either by silver staining or by immunoblotting. The α₃β₂γε complex crystallized in the space group R32 having one single αβ-pair and 0.33 γ-subunit and 0.33 ε-subunit in the asymmetric unit of the crystal. In contrast to the structure of the rat liver enzyme, unambiguous determination of the γ structure was not possible because of the 3-fold crystallographic symmetry in the complex. The single α and β subunits in the asymmetric unit represent a superposition of the three copies present in the chloroplast α₃β₂γε complex. With the exception of the C termini the electron density is well defined, suggesting that all α and β subunits are in similar conformations even though, because of local disorder in parts of the C-terminal domain, alternative conformations in the individual subunits cannot be completely excluded. A representative plot of part of the 3.2 Å resolution electron density map with the refined model coordinates superimposed is shown in Fig. 2.

The final model of the chloroplast αβ-dimer includes 942 residues (α25–501 and β19–485). The final parameter of refinement and model stereochemistry are summarized in Table I. Residues αLeu345, αGly355, αLeu357, αGlu369, αPhe385, αSer401, αThr407, αAsn409, Arg413, αTyr464, αThr461-Phe482, αGlu489, βLeu159, βAla331, βGly365, βGly381, βAla406, βPhe407, βGly409, βGlu416, βAsp417, βGly446, βLeu452, and βAsp467-Ser468 were not clearly visible in the electron density map; several other surface exposed residues in the C-terminal domain display high temperature factors for their side chain atoms. All residues except βArg325 have main chain dihedral angles that fall within the allowed regions of the Ramachandran diagram as defined by the program PROCHECK (37).

The overall structure of the q and β subunits (Fig. 3, A and B) is highly similar to those of the mitochondrial (7, 8) and the thermophilic subunits (10). As in the MF₁ and TF₁, structures (7–10), both subunits have almost identical folds and are arranged alternately about a central axis (Fig. 3C). Three different domains can be distinguished in each subunit: a N-terminal six-stranded β-barrel (α25–96 and β19–93), a central domain of β-strands with associated α-helices that contains the nucleotide binding site (α97–371 and β94–381), and a C-terminal α-helical bundle (α372–501 and β382–485). In comparison with the mitochondrial enzyme, the chloroplast β-subunit contains two insertions in the N-terminal β-barrel domain (βLys329 and βGlu338–Asn400), a single residue inserted in the central domain of the β-subunit (βGlu224), and a deletion of 8 residues between helix B and strand 6 in the central nucleotide binding domain of the α-subunit (αLys187–Asp194). The insertions βLys329 and βGlu338 and the deletion in subunit α show only minor effects on the structure. However, the insertion βGly338–Asn400, which is located in an extended loop between strands 3 and 4, seems to affect the structure of the chloroplast F₁ complex. As shown in Fig. 3C the additional residues wrap up part of the β-barrel domain of the adjacent α-subunit and appear to provide additional contacts, thereby increasing the stability of the CF₁ crown region (Fig. 3C).

The conformation of the chloroplast α-subunit is similar to the three conformations found in the bovine mitochondrial structure. The Ca atoms superimpose with rms deviations of 1.31, 1.31, and 0.95 Å with the mitochondrial α₃TP, α₃FP, and α₃C conformation. In contrast the chloroplast β-subunit superimposes well only with the β₃TP or β₃FP conformation of the mitochondrial enzyme. The Ca atoms show rms deviations of 1.24 and 1.19 Å, respectively. Comparison with the nucleotide-free β₃C conformation of the mitochondrial structure reveals a rms deviation of 3.89 Å. Thus the chloroplast α and β-subunit seem to reflect the closed nucleotide-filled conformation of the mitochondrial enzyme rather than the open nucleotide-free conformation. However, the high temperature factors of the C-terminal domains of the α- and β-subunits raise the possibility that more than one single, discrete conformation exists in the crystal structure. Different conformations might exist in particular of the catalytic β-subunit. To discriminate these different conformations and to reduce the effects of model bias, residues βGlu338-Phe410 which are involved in the conformational changes associated with nucleotide binding and catalytic turnover, were omitted in the structure refinement. Fig. 4 shows the calculated electron density map and the protein backbone corresponding to the β₃FP and β₃C conformation of the bovine mitochondrial ATPase (7). Although the corresponding calculated electron density is somewhat weaker than when all res-
idues in the C-terminal region were included in the refinement, the density clearly indicate a closed conformation of the β-subunit. Calculations of a composite omit, cross-validated, σ-A weighted map, where small regions of the model (10%) are systematically excluded in the refinement, support this conclusion (Fig. 4B).

As an additional test, whether the catalytic β-subunits in the crystal is present as a mixture of closed and open conformations, crystallographic refinement was carried out with a model containing the βE and βDP conformation at various occupancies. In comparison with a model having the βE-subunit exclusively in the closed conformation (1.00 βDP), refinement of a model with 0.66 βDP conformation and 0.33 βE conformation increased the free R-factor by 3.5%. These calculations further support the suggestion that the closed conformation is the predominant form of the catalytic β-subunit in the crystal, although local disorder and structural distinct conformations cannot be completely excluded at the present resolution.

Comparison of the Crown Region—The overall structure of the N-terminal six-stranded β-barrel domain is highly similar in the mitochondrial (7), thermophilic (10), and chloroplast enzymes (Fig. 5). However, the chloroplast and the thermophilic β-subunit contain an insert of five and seven residues, respectively. In the chloroplast αβγε complex, these residues form an extended loop located between strands 3 and 4 (Fig. 5B), whereas in the thermophilic enzyme a more rigid structure of an extended β-strand is formed by the additional residues (Fig. 5C). Both structures should promote additional contacts in the N-terminal crown-region of the F1 complex, which might increase the thermal stability of the enzyme. In the thermophilic enzyme a hydrogen bond between βAsn40 and αArg90 and van der Waals interaction of residues βAsn38–αSer21 and βGlu41–αMet48 are proposed to cause this increased stability (10). The high resolution structure of the chloroplast enzyme suggests that a salt bridge βAsp83–αArg297 enhances the stability of the enzyme. Both residues are within 3.4–3.5 Å, whereas the corresponding residues in the thermophilic (βAsp68–αArg296) and the mitochondrial (βGlu67–αArg304) enzyme are 7.7–9.9 and 5.7–11.6 Å apart. The proposed increased stability of the chloroplast F1 complex caused by the additional contacts in the β-barrel domain is reflected by the fact that the isolated enzyme tolerates incubation at 60 °C for heat activation of the latent ATPase activity (43).

Structure of the γ-Subunit—In contrast to the rat liver mitochondrial F1 complex that also crystallized in the space group R32 (9), unambiguous tracing of the γ-subunit in the chloro-
plast αβ3γ complex was not possible because of the superimposed 3-fold crystallographic symmetry. The calculated electron density maps of the chloroplast ATPase, however, showed significant, additional electron density in the central cavity of the chloroplast αβ3 core complex, which probably corresponds to the 3-fold superimposed C terminus of the chloroplast γ-subunit (Fig. 6). Nevertheless, a clear interpretation of this density was not possible at the present resolution of the diffraction data. Inspection of the additional density in the center of the αβ3 domain suggests that the extreme C-terminal end of the chloroplast γ-subunit is less ordered than the corresponding residues in the mitochondrial enzyme. Whether this disorder is due to the superimposed 3-fold crystallographic symmetry or is an intrinsic characteristic of the chloroplast γ-subunits remains to be solved.

A less ordered, unfolded structure rather than a more buried well ordered α-helical conformation might account for the observed fast modification of γCys232 located at the extreme C-terminal end of the γ-subunit and might also explain why this portion of the γ-subunit is not crucial for rotational catalysis in the chloroplast enzyme as suggested by Sokolov et al. (16). These conclusions drawn from our present data, however, are preliminary and have to be considered with caution. Data at higher resolution and the addition of cross-linking agents might allow a clear interpretation of the complete density attributed to the γ-subunit in the near future.

Catalytic and Noncatalytic Nucleotide-binding Sites—The high resolution structures of the mitochondrial and thermophilic ATPase revealed that the nucleotide binding sites are located at the interfaces between the α- and β-subunits (7–10). Noncatalytic sites are predominately formed by the α-subunits, and catalytic sites are mainly located on the β-subunits. Folds and structural motifs in the different ATPases are highly similar. Clear differences, however, were reported in the occupancy of the nucleotide binding sites, which might reflect different physiological states or an artificial state of the enzyme caused by the crystallization conditions. Inspection of the noncatalytic nucleotide binding sites in our crystal structure of the chloroplast αβ3γ complex revealed that no nucleotides were bound to the α-subunit, even though the conformation of the α-subunit resembles the closed, AMPPNP-filled conformation of the bovine mitochondrial enzyme. The same closed conformation of the α-subunits lacking bound nucleotides was also found in the thermophilic αβ3 core complex (10). This complex, however, was assembled from individual subunits overexpressed in E. coli (44), and the significance of the nucleotide occupancy in this complex might be questioned. The chloroplast αβ3γ complex crystallized in this study, however, was isolated from its natural source and retained catalytic activity (12, 28). Thus, the closed conformation might also exist in the absence of nucleotides in the native F1 complex. The situation is similar for the catalytic binding sites on the chloroplast β-subunits. Again, no bound nucleotides were detected in the crystal structure. However, some additional density was detected in the P-loop region (αGly170-Thr177 and βGly172-Thr179) of the chloroplast catalytic and noncatalytic binding sites that might represent bound phosphate or sulfate. The refinement showed no significant changes in model geometry or free R-factor when sulfate was included in the catalytic and noncatalytic site. Thus, at the present resolution we cannot discriminate whether the P-loop contributes to the additional density or whether it indicates a phosphate ion bound to the P-loop. Because of the 3-fold crystallographic symmetry, we also have to be cautious about the occupancy of the nucleotide binding sites in the chloroplast αβ3γ complex in general because substoichiometric binding of adenine nucleotides to 1 or 2 sites in the crystal cannot be completely excluded on the basis of the present data. Substoichiometric binding of 1 ADP was determined in the purified chloroplast ATPase by luciferin/luciferase (28). Although crystals were grown in the presence of 1 mM AMPPNP and 0.02 mM ADP, rebinding of adenine nucleotides to the catalytic and noncatalytic sites is limited because magnesium, which was shown to stimulate the tight binding of nucleotides (25), was excluded in the crystallization trials. Thus, the substoichiometric nucleotide content found in the purified CF1 complex might also reflect the situation in the crystallized enzyme. To resolve any substoichiometric binding in the CF1 structure, ADP at an occupancy of 0.33, and 0.66 was included in the crystallographic refinement. The adenine nucleotide was located in the β-subunit according to the structure of the bovine mitochondrial F1 complex (7) with B-factors set to the P-loop region of the chloroplast structure. The different calculations showed no significant change in the free R-factor. In addition, no clear negative peak was visible at the position of the nucleotide in the electron density difference maps. Thus, on the basis of the present data, we cannot com-
pletely rule out the possibility that at least a single site in the chloroplast F₁ complex is occupied, even though the electron density map in the nucleotide binding domain shows no clear density corresponding to a bound adenine nucleotide.

Mechanistic Implications of the Chloroplast Structure—In contrast to the bacterial and mitochondrial enzymes CF₁, either in isolated or membrane-bound form, is a latent ATPase. Nucleotides cannot exchange effectively with medium nucleotides until the enzyme is activated by reduction of the γ-disulfide or displacement of the ε-subunit (12). Activation always precedes catalysis and controls the catalytic cycle of the enzyme to prevent the futile hydrolysis of ATP in the dark under physiological conditions.

The structure of the chloroplast α₃β₃ε complex described in this work might reflect the inactive, latent state of the enzyme that is not found in the bacterial or mitochondrial enzymes because those F₁ complexes exhibit high rates of ATP hydrolysis without previous activation. Catalytic and noncatalytic binding sites in the structure are in a closed conformation and contain no or substoichiometric amounts of adenine nucleotides. Therefore, any catalytic turnover, which might result in conformational changes in the γ-subunit or in the catalytic

Fig. 7. Suggested potential Tentoxin binding site of the chloroplast ATPase (A) and corresponding domains in the mitochondrial (B) and thermophilic F₁ complex (C). Surface representation of residues within 20.0 Å of the essential βAsp⁶⁵ in CF₁ and the corresponding residues βAsp⁶⁷ in MF₁ and βAsp⁶⁸ in TF₁. Structures are shown in stereo view with the crystallographic axis in the vertical position as they would appear from the outside of the α₃β₃ε complex.
β-subunit, is blocked efficiently in the inactive oxidized complex. Transformation of this inactive, transient state of the chloroplast F1 complex into an active state might be related to conformational changes in the γ-subunit caused by reduction of the disulfide or by altered interaction with the ε-subunit. These conformational changes involving the transformation of the nucleotide binding sites might be related to a displacement of the central axis of subunit γ as discussed for the symmetrical rat liver F1 complex (9) to convert the symmetrical inactive form into the asymmetrical form described for the bovine mitochondrial ATPase (7, 8) that reflects the binding change mechanism (45).

Potential Tentoxin-binding Site at the αβ-Interface—The isolated and membrane-bound chloroplast ATPase of certain sensitive species of plants is inhibited by the cyclic tetrapeptide, tentoxin, which is produced by several phytopathogenic fungi (46). The precise binding site and the mechanism of the phytotoxin are not identified yet. Labeling studies suggest a high affinity inhibitory binding site, probably located on the αβ-interface and one or two low affinity binding sites that cause reactivation of the enzyme (18–19). Mutational studies suggested that residue Asp263 in the N-terminal domain of the β-subunit controls the sensitivity to the inhibitor (47, 48). However, tentoxin-resistant enzymes of the thermophilic bacterium PS2 and E. coli also contain aspartate in the corresponding position of their β-subunit. Thus, the binding motif seems to be more complex and can probably not be reduced to single residues but rather to a certain structural conformation formed at the catalytic αβ-interface. Residues α95–96, α95–96, α131–133, α297, β26–29, β51–54, β78, β80–85, and β242, which are located within 10.0 Å of Asp263 in the structure of the chloroplast αβ2γ complex, might be involved in the binding of the phytotoxin. Comparison with the corresponding domain in the mitochondrial and thermophilic enzymes (Fig. 7) suggests that the potential salt bridge βAsp81–αArg297 controls the overall conformation of the binding pocket and adjusts the αβ-interface to the inhibitor. Neither the mitochondrial nor the thermophilic ATPase show contacts between the corresponding residues in their structures. The F1 subunits of the different species, however, suggest that the compensatory of the positively charged αArg297 in the chloroplast enzyme by the salt bridge might be essential to promote the binding of the toxin as in the thermophilic and the mitochondrial F1 complex, positively charged residues αArg128, αLys132, and αArg132 are exposed at the surface of the potential binding cleft (Fig. 7, B and C). Additional negative surface charge at the binding site is promoted in the chloroplast F1 complex by βAsp53 and βThr54, which are located at the top of the cleft. Adjacent to these residues a hydrophobic surface is formed by residues αLeu129, αSer132, and αPro133, which might provide essential contacts for the inhibitor. Further analysis of the CF1 structure suggests that residues αMet274 and αLeu277 located at the inner face of the cleft might also contribute to this hydrophobic cluster. In the mitochondrial structure access to the potential binding site located around βGlu67 is blocked by αArg128 and βGlu42. In the thermophilic structure the corresponding BAsp68 is accessible and located almost in the center of the binding cleft. However, the surface charge distribution at the entrance site of the binding pocket clearly differs from those in the CF1 structure. In the thermophilic enzyme, charged residues αGlu130 and αArg132 are located at the position corresponding to the hydrophobic cluster in the chloroplast structure, and a hydrophobic surface instead of a negatively charged entrance site is found at the top of the binding cleft.

On the basis of the present structural information obtained with the chloroplast F1 complex, a sequence alignment of sensitive and resistant species suggests that residues αAla96 and αPro133 are involved in the binding and might confer the sensitivity of the F1 complex to the phytotoxin. In resistant species alanine is substituted by hydrophobic residues with bulky side chains, and proline is replaced by basic or hydrophobic residues with extended side chains. The mechanism by which tentoxin inhibits and activates the chloroplast ATPase remains still open and additional structural information and studies of protein dynamics seem necessary. Recent progress in co-crystallization of CF1 and tentoxin2 promises more detailed structural information on the inhibitory complex and might also identify the reason for the reactivation of the chloroplast ATPase caused by tentoxin.

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