Photoinactivation of the F₁-ATPase from Spinach Chloroplasts by Dequalinium Is Accompanied by Derivatization of Methionine β183*

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In contrast to the F₁-ATPases from bovine mitochondria and the thermophilic Bacillus PS3, which are reversibly inhibited by dequalinium in the absence of irradiation, the Mg²⁺-ATPase activity of heat- or dithiothreitol-activated chloroplast F₁ (CF₁) from spinach chloroplasts is slightly stimulated by dequalinium. Conversely, dequalinium is a partial inhibitor (maximal inhibition is 85–90%) of the Ca²⁺-ATPase activated by heat, dithiothreitol, or octylglucoside. The Mg²⁺- and Ca²⁺-ATPase activities of CF₁ respond differently in the presence of lauryl dimethylamine oxide (LDAO) in the assay medium. Whereas the Mg²⁺-ATPase activity of heat- or dithiothreitol-activated CF₁ is stimulated up to 14-fold by increasing concentrations of LDAO, the Ca²⁺-ATPase is inhibited in a biphasic manner by increasing concentrations of LDAO. In the presence of LDAO, dequalinium does not stimulate the heat-activated Mg²⁺-ATPase over that promoted by LDAO alone. That dequalinium slightly stimulates Mg²⁺-ATPase activity although it inhibits Ca²⁺-ATPase activity can be reconciled by assuming that dequalinium binds to two sites in CF₁, a stimulatory site that also binds LDAO and an inhibitory site. By acting as a partial inhibitor of the Mg²⁺-ATPase activity that it activates, the combined effect of dequalinium is modest stimulation. Irradiation of heat- or dithiothreitol-activated CF₁ or the α₃β₃γ subcomplex of CF₁ in the presence of 12 μM dequalinium led to rapid photoinactivation. ATP and ADP, separately or in combination with Mg²⁺, protect against photoinactivation. After photoactivating the α₃β₃γ subcomplex of CF₁ with [¹⁴C]dequalinium, tryptic and peptic digests of the isolated, derivatized β subunit were fractionated by high performance liquid chromatography. Sequencing of the isolated, radioactive tryptic and peptic peptides revealed that Met⁸⁰³, which is at or near the catalytic site, is derivatized in a single β subunit when CF₁ is photoactivated with [¹⁴C]dequalinium.

The ATP synthase complex of chloroplast thylakoid membranes catalyzes ATP synthesis coupled to a proton electrochemical gradient across the thylakoid membrane generated by light. The membrane-bound enzyme comprises an integral membrane protein complex, CF₀, and a peripheral membrane protein complex, CF₁. CF₀ mediates transmembrane proton conduction, whereas CF₁ contains the catalytic sites for ATP synthesis.

The CF₁ portion of ATP synthase can be easily removed from the thylakoid membrane. Isolated CF₁ is composed of five different subunits, designated α, β, γ, δ, and ε in order of decreasing molecular weight with a stoichiometry of α₂β₃γδε, and has a molecular mass of 400 kDa (1). CF₁ contains six binding sites for adenine nucleotides (2), three of which participate directly in catalysis, whereas the other three, which are called noncatalytic sites, do not have a well defined functional role. Isolated CF₁ is a latent ATPase, which can be activated with dithiothreitol (DTT) (3), trypsin (4), heat (4, 5), organic solvents (6), or octylglucoside (7). DTT treatment reduces the disulfide bond within the γ subunit (8), while heat activation relieves inhibition caused by the ε subunit (9, 10). Trypsin digestion partially cleaves the γ subunit, which significantly decreases the affinity of CF₁ for the ε subunit (11, 12). The effects of octylglucoside and organic solvents on CF₁ are more complicated. Octylglucoside may remove the inhibitory ε subunit, decrease inhibition by free Mg²⁺, and also increase the affinity for substrate (7, 13). One effect of organic solvent is to overcome inhibition by the ε subunit. A second activating effect may be a decrease of the affinity for inhibitory MgADP (14).

The F₁-ATPases are inhibited by a variety of amphipathic cations that include substituted phenothiazines (15, 16), substituted xanthenes (17–20), substituted acridines (17, 19, 21), and mono- and bisalkylquinoliniums (17, 19, 22–24). The binding sites for quinacrine mustard and dequalinium have been partly defined by labeling studies on TF₁ and MF₁. Inactivation of MF₁ with quinacrine mustard is due, at least in part, to modification of one or more of the carboxylic acid side chains in the DELSEED segment of β subunit (25), which, according to the x-ray structure, participates in a catch contact with Lys⁴⁰³ and Lys⁴⁰⁶ on the γ subunit (26). Dequalinium inhibits MF₁ noncompetitively in the absence of light (17, 22). Irradiation of MF₁ in the presence of dequalinium at 350 nm rapidly inactivates the enzyme. Photoinactivation of MF₁ with [¹⁴C]dequalinium results in the labeling of Phe⁴⁰³ and Phe⁴⁰⁶ in mutually exclusive reactions and a side chain within residues 440–459 of the β subunit (23). To a certain extent, photo-cross-links the α and β subunits of MF₁, indicating that the reagent spans the distance between Phe⁴⁰³ or Phe⁴⁰⁶ and a side chain within residues 440–459 of the β subunit.

Dequalinium interacts differently with TF₁. Although it inhibits TF₁ and MF₁ noncompetitively, a long lag is introduced.

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¹ The abbreviations used are: CF₀, the membrane sector of the chloroplast ATP synthase; CF₁, MF₁, and TF₁, the F₁-ATPase from chloroplasts, mitochondria, and the thermophilic Bacillus PS3, respectively; CF₁(–δ), CF₃, deficient in the δ subunit; DTT, dithiothreitol; LDAO, lauryldimethylamine oxide; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; PAGE, polyacrylamide gel electrophoresis; AMP-PNP, adenosine 5’-(β,γ-iminotriphosphate).
when TF₂ is assayed in the presence of dequalinium, a phenomenon not observed with MF₁. Irradiation of TF₂ in the presence of dequalinium inactivates the enzyme, which is accompanied by derivatization of Phe⁴²⁰ (equivalent to Phe⁷⁶⁴⁴ of MF₁) in the nucleotide binding domain of a single β subunit (24).

Groth and Junge (27) reported that dequalinium blocks proton release from the CF₁CF₃-ATP synthase under conditions of proton slip, a conducting state observed when ADP and Pᵢ are not present to consume the proton electrochemical potential generated when thylakoids are illuminated. They assumed that the DELESEED segment of the β subunit, which is derivatized when MF₁ is inactivated with quinacrine mustard, is part of the binding site for dequalinium on CF₁ (27). Given that MF₁ and TF₂ respond differently to dequalinium and that different residues in the two enzymes are derivatized when they are photoinactivated with [¹⁴C]dequalinium, it was of interest to determine if the interaction of dequalinium with CF₁ resembles more its interaction with MF₁ or TF₂.

EXPERIMENTAL PROCEDURES

Materials—Reagents for gel electrophoresis were purchased from Bio-Rad. Enzymes and biochemicals used in assays and dequalinium dichloride were purchased from Sigma. LDAO (30% aqueous solution) was purchased from Calbiochem. Pepsin was purchased from Sigma. Sequence grade modified trypsin was purchased from Promega. HPLC solvents were purchased from Fisher. Centricon 30 was obtained from Amicon. [¹⁴C]Dequalinium with a specific radioactivity of 2.9 cpm/pmol was synthesized as described previously (23).

CF₁ was prepared by modification of previously described methods (28, 29). After CF₁ was purified by anion exchange on DEAE-cellulose (Whatman) and DEAE-Sephadex (Pharmacia Biotech Inc.), considerable Rubisco remained, which was removed as follows. A solution of CF₁ (150 mg) in buffer A (50 mM Tris-SO₄, pH 8.0, 1 mM EDTA, and 10 mM DTT) containing 0.5 M (NH₄)₂SO₄ was applied to a 2.5 × 8 cm column of Butyl-Toyopearl-650S (Toso-Haas), which was equilibrated with the same buffer. After application, the column was washed with two volumes of the same buffer. Rubisco was eluted by washing with 100 ml of 0.2 M (NH₄)₂SO₄ in buffer A. The column was then washed with 100 ml of 0.15 M (NH₄)₂SO₄ in buffer A, which eluted a small amount of CF₁, deficient in the δ subunit, CF₁ (−δ), a trace amount of residual Rubisco, and contaminating proteins that migrate behind the α subunit on SDS-PAGE. Finally, pure, intact CF₁, as assessed by SDS-PAGE was eluted with buffer A. The specific activity of CF₁ was 26 μmol of ATP hydrolyzed min⁻¹ mg⁻¹ when assayed at 30 °C in the presence of 30 μM octylglucose (7).

When CF₁, prepared as described above, was repleted to the Butyl-Toyopearl-650S column in buffer A containing 0.5 M (NH₄)₂SO₄, and then applied to a Sephacryl S-300 linear gradient of 0–0.2 M (NH₄)₂SO₄ in a total volume of 600 ml, CF₁ (−δ), as assessed by SDS-PAGE, eluted as a single peak in fractions 65–90 of the 6.5 ml fractions collected. The column was then washed with 50 ml of buffer A, which removed a single peak containing mostly δ subunit and trace amounts of the α, β, γ, and ε subunits as assessed by SDS-PAGE. This peak probably represents free δ subunit containing a trace of intact CF₁.

CF₁ (−δ) prepared in this manner was stored at 4 °C after precipitation with 50% saturated (NH₄)₂SO₄. The α₂β₂γ subcomplex, CF₁ depleted of the δ and ε subunits, was prepared from CF₁ (−δ) by the method described for preparing CF₁ (−ε) (10).

Methods—Peptide separations by HPLC were conducted as described previously (23, 24). Protein concentrations were determined by the method of Bradford (30) with Coomassie Blue Plus from Pierce, but using a correction factor of 1.5 based on an absorbance coefficient of 0.6 at 600 nm (19). Protein precipitated during dialysis and was recovered by centrifugation. Analysis of the supernatant and precipitate revealed that the supernatant contained mostly δ subunit with small amounts of the α and γ subunits, whereas the precipitate contained mostly α and γ subunits and a trace of the β subunit. The supernatant was applied to a hydroxypatite column (Bio-Rad HTP, 1.5 × 5 cm) equilibrated with 25 mM Tricine-NaOH, pH 8.0, 0.2 mM EDTA, 0.1 mM ATP, and 0.1 mM DTT at 4 °C. The column was washed with two volumes of equilibration buffer. The β subunit was eluted with the same buffer and contained 30 mM sodium phosphate, pH 8.0 (38). The solution containing β subunit was dried under vacuum and dissolved in 6 μM guanidine HCl. About 1.5 mg of pure β subunit was obtained from 6 mg of α₂β₂γ subcomplex. Based on the amount of [¹⁴C]incorporated per mol of β subunit, 27% of the isolated β subunit was labeled by [¹⁴C]dequalinium.

Prior to pepsin digestion, the denatured β subunit in guanidine HCl was dialyzed against 2 liters of 1% (w/v) formic acid overnight at 4 °C. An aqueous solution of pepsin was added to a final pepsin:protein ratio of 1:50. Dialysis was performed at 30 °C for 4 h with continuous stirring and was interrupted by freezing at −20 °C. Prior to trypsin digestion, the denatured β subunit in guanidine HCl was dialyzed against 2 liters of distilled water overnight at 4 °C. The β subunit precipitated during dialysis and was collected by centrifugation. The protein was suspended in 500 μl of 0.1 M Tris-HCl, pH 8.0. Trypsin was added to a final protease:protein ratio of 1:50. Dialysis was carried out at 37 °C for 4 h with continuous stirring and was interrupted by freezing at −20 °C.

RESULTS

The Effects of LDAO on the Mg²⁺- and Ca²⁺-ATPase Activities of CF₁—The neutral detergent LDAO stimulates the ATPase activity of F₁-ATPase preparations from Escherichia coli with and without the ε subunit (39), and it also stimulates TF₂-ATPase preparations with and without the δ and ε subunits (40). Since the ATPase activity of latent CF₁ is activated by octylglucose (7) or methanol (14), the effects of LDAO on the Mg²⁺-ATPase activity of CF₁ and the α₂β₂γ subcomplex of CF₁ were examined. Fig. 1A illustrates that LDAO stimulates the Mg²⁺-ATPase activity of heat- or DTT-activated CF₁, latent CF₁ and the latent or DTT-activated α₂β₂γ subcomplex of CF₁. Each was stimulated maximally at LDAO concentrations between 0.2 and 0.4%. In this range of LDAO concentration, heat-activated CF₁, latent CF₁, and the latent α₂β₂γ subcomplex were stimulated about 12-, 14-, and 6-fold, respectively. DTT-activated CF₁ and the DTT-activated α₂β₂γ subcomplex were stimulated 14- and 6-fold, respectively. The finding that LDAO stimulates the latent α₂β₂γ subcomplex only half as much as observed for latent CF₁ and heat-activated CF₁ indicates that the LDAO-induced stimulation is caused by displacement of the ε subunit from an inhibitory position. The same argument applies to the difference in LDAO-induced stimulation observed for DTT-activated CF₁ and the DTT-activated α₂β₂γ subcomplex. A similar difference is observed when stimulation of E. coli F₁ by LDAO is compared with that of E. coli F₁ deleted of the ε subunit (39).

In contrast to the Mg²⁺-ATPase activity, which is stimulated by LDAO, the Ca²⁺-ATPase shows a biphasic response to increasing concentrations of LDAO illustrated in Fig. 1B. The
Ca\textsuperscript{2+}-ATPase activity of heat-activated, DTT-activated CF\textsubscript{1} and the latent \(\alpha_3\beta_3\gamma\) subcomplex are progressively inhibited by increasing concentrations of LDAO up to about 0.025%. Thereafter, the activity progressively activates, reaching a plateau where little further activation is observed with increasing LDAO concentration. A possible explanation for this apparent anomaly is as follows. The sharp dip in Fig. 1\textbf{B} occurring at about 0.025% LDAO may represent the critical micelle concentration of LDAO under the ionic conditions of the experiment. In water, the critical micelle concentration of LDAO is 0.05% (41). At LDAO concentrations over 0.025%, increasing concentrations of micelles are present, which might stimulate ATPase activity.

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Dequalinium Stimulates the Mg\textsuperscript{2+}-ATPase Activity of CF\textsubscript{1} Whereas It Inhibits Ca\textsuperscript{2+}-ATPase Activity—When isolated from spinach chloroplasts, CF\textsubscript{1} is a latent ATPase that can be activated by a variety of methods. After heat activation or DTT activation, CF\textsubscript{1} has low Mg\textsuperscript{2+}-ATPase activity of 0.54 \(\mu\)mol of Pi mg\textsuperscript{-1} min\textsuperscript{-1} or 0.59 \(\mu\)mol of Pi mg\textsuperscript{-1} min\textsuperscript{-1}, respectively, illustrated in Fig. 2\textbf{A}. Since dequalinium inhibits the ATPase activities of MF\textsubscript{1} and TF\textsubscript{1} (23, 24), it was surprising to find that...
it stimulates the Mg\textsuperscript{2+}-ATPase activity of heat-activated CF\textsubscript{1} and DTT-activated CF\textsubscript{1}, whereas it has no effect on the Mg\textsuperscript{2+}-ATPase activity of latent CF\textsubscript{1}. Heat-activated CF\textsubscript{1} and DTT-activated CF\textsubscript{1} behaved differently in the presence of dequalinium. The heat-activated Mg\textsuperscript{2+}-ATPase was maximally stimulated by about 50% with 10 \( \mu \)M dequalinium. At higher concentrations, stimulation declined. In contrast, the Mg\textsuperscript{2+}-ATPase activity of DTT-activated CF\textsubscript{1} was stimulated maximally by about 80% with 30 \( \mu \)M dequalinium. However, in this case, stimulation did not decline with increasing dequalinium in the concentration range examined. In the presence of 0.2% LDAO, dequalinium does not stimulate the Mg\textsuperscript{2+}-ATPase activity of heat-activated CF\textsubscript{1} over that stimulated by LDAO alone. In fact, concentrations of dequalinium greater than 15–20 \( \mu \)M inhibit Mg\textsuperscript{2+}-ATPase activity in the presence of 0.2% LDAO.

Whereas dequalinium inhibits the Mg\textsuperscript{2+}-ATPase activity of the \( \alpha\beta\gamma\delta \) subcomplex of CF\textsubscript{1} before it is activated with DTT, the subcomplex is stimulated over 2-fold in the presence of 30 \( \mu \)M dequalinium after it is activated with DTT. In the presence of 0.2% LDAO, dequalinium only slightly stimulated the Mg\textsuperscript{2+}-ATPase activity of the DTT-activated \( \alpha\beta\gamma\delta \) subcomplex over that observed in the presence of LDAO alone.

Fig. 2B shows that dequalinium inhibits the Ca\textsuperscript{2+}-ATPase activity of CF\textsubscript{1} activated by treatment with DTT, heat, or octylglucoside. Unlike the Mg\textsuperscript{2+}-ATPase activity, the Ca\textsuperscript{2+}-ATPase activity is inhibited at all concentrations of dequalinium examined. A \( K_b \) value of 4.0 \( \mu \)M was determined from a Dixon plot for inhibition of heat-activated Ca\textsuperscript{2+}-ATPase activity by dequalinium.

**Photoinactivation of CF\textsubscript{1} with Dequalinium and the Effects of Ligands on Photoinactivation**—When heat-activated CF\textsubscript{1} was irradiated at 350 nm in the presence of dequalinium, and then assayed for Mg\textsuperscript{2+}-ATPase activity in the presence of octylglucoside (7), first order decay of enzyme activity was observed. Although in the presence of LDAO, low concentrations of dequalinium no longer stimulate the Mg\textsuperscript{2+}-ATPase of heat-activated CF\textsubscript{1}, it has no effect on the rate of photoinactivation of heat-activated CF\textsubscript{1} by dequalinium (data not shown). Pseudo-first order rate constants, summarized in Table I, were determined for photoinactivation of heat-activated CF\textsubscript{1} by 12 \( \mu \)M dequalinium in the presence or absence of ligands. Inorganic phosphate and pyrophosphate had almost no effect on the rate of photoinactivation, whereas ADP, ATP, and Mg\textsuperscript{2+} provided protection against photoinactivation. The combination of ADP plus Mg\textsuperscript{2+} and ATP plus Mg\textsuperscript{2+} were the most effective, each decreasing the rate of inactivation nearly 10-fold. Ca\textsuperscript{2+} alone or in combination with ADP or ATP also provides protection against photoinactivation, but is not as effective as Mg\textsuperscript{2+} alone or in combination with ADP or ATP. Since ATP in the presence of Mg\textsuperscript{2+} or Ca\textsuperscript{2+} is hydrolyzed by heat-activated CF\textsubscript{1}, the latter combinations contained a mixture of MgADP and MgATP or CaADP and CaATP.

**Photoinactivation of Heat-activated CF\textsubscript{1} with \( ^{14}\text{C}\)Dequalinium Is Caused by Derivatization of Met\textsuperscript{183}**—Fig. 3 shows the pattern of radioactive peptides obtained when a sample of heat-activated CF\textsubscript{1} that had been photoinactivated by 80% with \( ^{14}\text{C}\)dequalinium was submitted to SDS-PAGE. About 1.4 mol of reagent/mol of CF\textsubscript{1} was incorporated. The percentages of the \( ^{14}\text{C} \) in the labeled CF\textsubscript{1} applied to the gel that were recovered in the \( \alpha, \beta, \gamma, \delta, \) and \( \epsilon \) subunits are shown in Fig. 3. The 20% \( ^{14}\text{C} \) found in slices 2 and 3 apparently represents derivatized CF\textsubscript{1} that resisted depolymerization by SDS. A comparable band was not observed in the lane containing unmodified CF\textsubscript{1} submitted to SDS-PAGE on the same gel. It is clear that the \( \beta \) subunit was predominantly labeled with \( ^{14}\text{C}\)dequalinium. With the exception of the \( \alpha \) subunit, which was slightly labeled compared with \( \beta \), very little radioactivity was covalently bound to the other subunits.

A tryptic digest of the isolated \( \beta \) subunit prepared from the \( \alpha\beta\gamma\delta \) subcomplex derivatized with \( ^{14}\text{C}\)dequalinium as described under “Experimental Procedures” was submitted to HPLC on a C\textsubscript{4} column equilibrated with 12 mM HCl. After the column was washed with 12 mM HCl for 10 min, it was developed with a linear gradient of 0–15% acetonitrile in 12 mM HCl for 10 min and then with a linear gradient from 15 to 53% acetonitrile in 12 mM. A single radioactive peak eluted from the

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**Table I**

*Effect of ligands on the photoinactivation of CF\textsubscript{1} by dequalinium*

| Ligand | \( k_{\text{inact}} \) \( \text{min}^{-1} \) | Ligand | \( k_{\text{inact}} \) \( \text{min}^{-1} \) |
|--------|----------------|--------|----------------|
| None   | \( 3.45 \times 10^{-2} \) | ADP + P\textsubscript{i} | \( 2.56 \times 10^{-2} \) |
| P\textsubscript{i} | \( 3.45 \times 10^{-2} \) | ADP + Mg\textsuperscript{2+} | \( 4.66 \times 10^{-3} \) |
| PP\textsubscript{i} | \( 3.83 \times 10^{-2} \) | ATP + Mg\textsuperscript{2+} | \( 6.33 \times 10^{-3} \) |
| ADP   | \( 2.46 \times 10^{-2} \) | ATP + Mg\textsuperscript{2+} | \( 4.89 \times 10^{-3} \) |
| ATP   | \( 1.29 \times 10^{-2} \) | P\textsubscript{i} + Mg\textsuperscript{2+} | \( 1.13 \times 10^{-2} \) |
| Mg\textsuperscript{2+} | \( 1.05 \times 10^{-2} \) | P\textsubscript{i} + Mg\textsuperscript{2+} | \( 8.02 \times 10^{-3} \) |
| Ca\textsuperscript{2+} | \( 2.35 \times 10^{-2} \) | ADP + Ca\textsuperscript{2+} | \( 2.13 \times 10^{-2} \) |
| ATP + Ca\textsuperscript{2+} | \( 2.13 \times 10^{-2} \) | | |
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**FIG. 4. Summary of the purification of the major radioactive peptides in a peptic digest of β subunit from CF<sub>1</sub>, inactivated with [14C]dequalinium.**

The β subunit was photoinactivated and digested as described under “Experimental Procedures.” The peptic digest was first submitted to HPLC on a C<sub>4</sub> column (as described under “Experimental Procedures”) and eluted with a gradient (10–42%) of acetonitrile in 12 mM HCl in 160 min. Peak P1, which contained the majority of 14C, eluted from the C<sub>4</sub> column concentrated under vacuum and submitted to HPLC on a phenyl column, which was equilibrated with 12 mM HCl and eluted with a gradient (15–35%) of acetonitrile in 12 mM HCl in 200 min. The material in peaks P1-A and P1-B were submitted to automatic sequence analysis. The percentages in parentheses represent yields of radioactivity obtained for each column step.

The peptic digest of the labeled tryptic peptide was obtained in only 5% yield, a peptic digest of the labeled β subunit was fractionated by HPLC using the scheme illustrated in Fig. 4. Two radioactive fractions, P1-A and P1-B, were isolated, which accounted for 9.1% and 32.1%, respectively, of the total 14C in the peptic digest submitted to the fractionation procedure. Automatic Edman degradation of the materials in P1-A and P1-B revealed the same tripeptide with the sequence: I-X-E. There are five segments in the β subunit of CF<sub>1</sub> containing an I-X-E sequence. Only one of the tripeptide segments contains Met in the position designated X. This is Met<sup>183</sup>. The finding that the sequence of the labeled peptic peptides corresponds to the sequence of the labeled tryptic peptide strongly implicates Met<sup>183</sup> as the site of derivatization when CF<sub>1</sub> is photoinactivated with [14C]dequalinium.

To ensure that photoinactivation of the different forms of CF<sub>1</sub> examined in this study proceeds with modification of Met<sup>183</sup>, 1-mg samples of latent CF<sub>1</sub>, heat activated CF<sub>1</sub>, in the presence or absence of 0.1% LDAO, DTT-activated CF<sub>1</sub>, and the latent α<sub>β</sub>γ subcomplex of CF<sub>1</sub> were inactivated with 12 μM [14C]dequalinium using the spectrophotometric assay in the presence of octylglucoside to monitor inactivation. After photoinactivation, each sample was denatured and digested with pepsin. The peptic digests were submitted to HPLC under the conditions described above for the large scale preparation of peptic peptides derived from the isolated β subunit derivatized with [14C]dequalinium. The profiles of radioactivity in the eluents of the peptic digests were essentially identical to the profiles obtained in the large scale fractionation of peptic peptides derived from the isolated β subunit derivatized with [14C]dequalinium. From these analyses, we conclude that Met<sup>183</sup> is the major site of derivatization when each form of CF<sub>1</sub> was photoinactivated with [14C]dequalinium. Given that 27% of the β subunit was derivatized on 80% photoinactivation of the α<sub>β</sub>γ subcomplex of CF<sub>1</sub> by [14C]dequalinium, we also conclude that modification of Met<sup>183</sup> in a single β subunit is sufficient for complete photoinactivation.

**DISCUSSION**

It is clear from the results presented that photoinactivation of the various forms of CF<sub>1</sub> with dequalinium is caused by derivatization of Met<sup>183</sup>. This residue corresponds to Met<sup>167</sup> of MF<sub>1</sub> (42). Fig. 5 shows that Met<sup>167</sup> of MF<sub>1</sub> is among a cluster of apolar amino acid residues that line a cavity surrounding the side chain of Glu<sup>199</sup>, a conserved residue near the catalytic nucleotide binding site. It is possible that the positive charge on one of the quinaldinium moieties of dequalinium interacts with the side chain of the equivalent of Glu<sup>199</sup> in CF<sub>1</sub>. Comparison of the unliganded catalytic site, β<sub>PC</sub>, illustrated in Fig. 5A, with the catalytic site of MF<sub>1</sub> liganded with AMP-PNP clearly shows that the side chain of Met<sup>167</sup> shifts away from the entrance of this cavity when the catalytic site is liganded. In β<sub>PC</sub> (data not shown), the side chain of Met<sup>167</sup> is also shifted away from the entrance to the cavity. This is consistent with the observation that ADP and ATP, especially when complexed with Mg<sup>2+</sup>, protect CF<sub>1</sub> against photoinactivation by dequalinium. It is interesting that photoinactivation of TF<sub>1</sub> with dequalinium, which has a glutamine residue in the position corresponding to Met<sup>167</sup> of MF<sub>1</sub> (43), leads to derivatization of Phe<sup>420</sup>, which corresponds to Phe<sup>424</sup> of MF<sub>1</sub>. Fig. 5 shows that Phe<sup>424</sup> is near the entrance of the hydrophobic cavity. The change in orientation of helix B with respect to helix C is extraordinarily large in going from the unliganded catalytic site shown in Fig. 5A to the liganded catalytic site shown in Fig. 5B. Derivatization of either Met<sup>183</sup> in CF<sub>1</sub> or Phe<sup>424</sup> in TF<sub>1</sub> probably blocks this conformational change.

The finding that dequalinium binds to or near a catalytic site of CF<sub>1</sub> is difficult to reconcile with the results of Groth and Junge (27), who reported that ADP blocks proton slip mediated by CF<sub>0</sub>F<sub>1</sub> at the electrogenic step, whereas dequalinium blocks the proton release step. To explain the differential effects of dequalinium and ADP on proton slip, Groth and Junge (27) assumed that dequalinium does not bind to a catalytic site.

The anomalous finding that, in the absence of light, dequalinium stimulates Mg<sup>2+</sup>-ATPase activity, whereas it inhibits Ca<sup>2+</sup>-ATPase activity of CF<sub>1</sub> activated by dithothreitol or heat, can be explained by assuming that, in addition to binding to an inhibitory site or at near a catalytic site, dequalinium binds to a stimulatory site that also binds LDAO. The finding that dequalinium stimulates the Mg<sup>2+</sup>-ATPase activity of heat-activated CF<sub>1</sub> at lower concentrations of LDAO, whereas it inhibits the ATPase activity at higher concentrations of LDAO, is consistent with this assumption. LDAO stimulates the α<sub>β</sub>γ subcomplex of TF<sub>1</sub> by promoting dissociation of inhibitory MgADP from a catalytic site during turnover (40). Since isolated CF<sub>1</sub> contains ADP tightly bound to a single catalytic site, which is converted to inhibitory MgADP in the presence of medium Mg<sup>2+</sup> (44, 45), that part of activation of CF<sub>1</sub> by LDAO that is independent of the α subunit is likely to occur by the same mechanism. In experiments not shown, it was found that addition of low concentrations of dequalinium to heat- or DTT-activated CF<sub>1</sub> in the presence of 0.2% LDAO does not stimulate Mg<sup>2+</sup>-ATPase activity above the level observed in the presence of LDAO alone. This suggests that dequalinium also stimulates the Mg<sup>2+</sup>-ATPase activity of CF<sub>1</sub> in the same manner as LDAO. However, in the presence of LDAO, higher concentrations of dequalinium still slightly inhibit the Mg<sup>2+</sup>-ATPase of heat-activated CF<sub>1</sub>. This also suggests that CF<sub>1</sub> contains a low affinity inhibitory site for dequalinium that does not bind LDAO. In contrast, the Ca<sup>2+</sup>-ATPase of CF<sub>1</sub> is not inhibited by CaADP bound to a single catalytic site (46). Fig. 2B shows that
Dequalinium Derivatizes Met\textsuperscript{B183} When It Photoinactivates CF\textsubscript{1} (26).

![Diagram of hydrophobic residues in the catalytic sites of the \( \beta_E \) and \( \beta_{TP} \) subunits of MF\textsubscript{1}](image)

Dequalinium is a partial inhibitor of the Ca\textsuperscript{2+}-ATPase activity of CF\textsubscript{1}. Dequalinium is also a partial inhibitor of MF\textsubscript{1} and TF\textsubscript{1} (22, 24). Maximal inhibition of each enzyme by dequalinium is not greater than 85–90%. Therefore, dequalinium may act both as an activator of the Mg\textsuperscript{2+}-ATPase activity of CF\textsubscript{1}, by promoting dissociation of inhibitory MgADP from a catalytic site, and as a partial inhibitor of the Mg\textsuperscript{2+}-ATPase activity that it activates. The combined effect of dequalinium is the relatively modest stimulation of Mg\textsuperscript{2+}-ATPase activity observed, compared with that promoted by LDAO.

In contrast to its effect on Mg\textsuperscript{2+}-ATPase activity, dequalinium only inhibits the Ca\textsuperscript{2+}-ATPase activity of heat-, DTF-, and octylglucoside-activated CF\textsubscript{1}. Unlike the Mg\textsuperscript{2+}-ATPase activity, the Ca\textsuperscript{2+}-ATPase activities of heat-activated CF\textsubscript{1}, DTF-activated CF\textsubscript{1}, and the latent \( \alpha_3 \beta_3 \gamma \) subcomplex are inhibited by low concentrations of LDAO. These observations support an earlier report (46) that the Ca\textsuperscript{2+}-ATPase activity of CF\textsubscript{1} is not subject to inhibition caused by binding of CaADP in a single catalytic site. Consistent with this argument, Leckband and Hammes (47) reported that the initial rate of Ca\textsuperscript{2+} hydrolysis by heat-activated CF\textsubscript{1} is triggered by free Ca\textsuperscript{2+}. In contrast, free Mg\textsuperscript{2+} inhibits the Mg\textsuperscript{2+}-ATPase activity of CF\textsubscript{1} by interacting with ADP bound to a catalytic site to form the inhibited CF\textsubscript{1}-ADP \( \text{Mg} \) complex.

The finding that derivatization occurs at or near a catalytic site when CF\textsubscript{1} or TF\textsubscript{1} are photoinactivated with dequalinium, whereas a different site is derivatized when MF\textsubscript{1} is inactivated with the reagent, might be related to the insensitivity of spinach CF\textsubscript{1} and TF\textsubscript{1} and sensitivity of MF\textsubscript{1} to inhibition by aurovertin (48). In the case of MF\textsubscript{1}, photoinactivation with dequalinium is accompanied by derivatization of Phe\textsuperscript{403} or Phe\textsuperscript{406}, in mutually exclusive reactions, and a side chain within residues 440–459 of the \( \beta \) subunit. To a limited extent, \( \alpha \)-\( \beta \) crosslinking accompanies photoinactivation (23). The crystal structure of MF\textsubscript{1} liganded with 2 aurovertin molecules has been solved (49). One aurovertin molecule is bound to \( \beta_{TP} \), and the other is bound to \( \beta_E \). In \( \beta_{TP} \), the pyrone ring of bound inhibitor interacts with the side chain of Tyr\textsuperscript{199} and the methoxy oxygen attached to the pyrone is in van der Waals contact with a carboxylate oxygen of Glu\textsuperscript{199} of \( \alpha_{TP} \). At an \( \alpha_{TP}/\beta_{TP} \) interface, Phe\textsuperscript{403} is 16.4 Å from Tyr\textsuperscript{458}, which is within the spanning distance of the two 4-amino-2-methylquinolinium moieties of dequalinium. Whether or not Tyr\textsuperscript{458} is derivatized when MF\textsubscript{1} is photoinactivated with dequalinium has not been established. However, an investigation is in progress to identify the residue derivatized within residues 440–459 of the \( \beta \) subunit when MF\textsubscript{1} is inactivated with \( [\text{\textsuperscript{14}C}] \) dequalinium.

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