Inhibition of Photophosphorylation in Spinach Chloroplasts by Inorganic Sulfate*

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SUMMARY

Illumination of chloroplasts in the presence of sulfate, ADP, and Mg²⁺ causes 35 to 50% inhibition of a subsequent phosphorylation reaction; ADP cannot be replaced by other nucleotides. Compounds that normally inhibit photophosphorylation prevent the appearance of the sulfate-induced inhibition. No evidence was found for phosphorylation site specificity of the sulfate inhibition.

In chloroplasts inhibited by preliminary illumination with sulfate, the light-induced pH rise and the P/Δε ratio in a phosphorylating Hill reaction are inhibited as much as photophosphorylation is. The pH rise can be restored by dicyclohexylcarbodiimide.

Both the trypsin- and dithiothreitol-activated Ca²⁺-dependent ATPase activities associated with the chloroplast coupling factor (CF₁) are decreased in sulfate-inhibited chloroplasts. Removal of CF₁ from the membranes followed by recombination with CF₁ from noninhibited chloroplasts restores the pH rise completely and phosphorylation to a large extent. From these and other experimental results it is concluded that sulfate, in the presence of ADP and Mg²⁺, interacts with CF₁ on chloroplast membranes under conditions of coupled electron flow so as to cause a modification of CF₁ structure.

Despite extensive studies of chloroplasts and mitochondria, details of the mechanism of ATP formation are at present unknown. It is clearly established, however, that in chloroplasts the terminal phosphorylation reaction is catalyzed by chloroplast coupling factor 1 (CF₁) (1-4), a high molecular weight protein that in many of its properties resembles coupling Factor 1 of mitochondria (5). Treatment of chloroplasts with the antibody against CF₁ (3) or removal of CF₁ in dilute EDTA solution (1) was shown to inhibit phosphorylation severely. Activity could be restored to CF₁-depleted chloroplast membranes by recombination with CF₁ in the presence of Mg²⁺ (1). In addition, a latent ATPase activity which is unmasked by treatment either with heat, sulfhydryl reagents, or trypsin was shown to be associated with CF₁ (2-4).

An approach to elucidating the molecular mechanism of ATP formation in chloroplasts has been made with analogues of the phosphate anion. Thus arsenate seems to replace phosphate in photophosphorylation, serving as a competitive inhibitor to phosphate (6). Both on the basis of its chemical similarity and its known chemistry, it is presumed that chloroplasts form an unstable adenosine 5'-diphosphoselenate, which is rapidly hydrolyzed and therefore has not been detected. Sulfate was recently shown to inhibit photophosphorylation (7) and is competitive with phosphate both in this function (8) and in inhibition of carbon dioxide fixation (9). The formation of adenosine 5'-diphosphosulfate was suggested (8) but not shown. We now report, however, that sulfate has a very different effect on chloroplasts than arsenate does. In the presence of ADP and Mg²⁺ and under conditions of coupled electron flow, sulfate causes loss of enzymatic activity of CF₁, which is so far irreversible.

METHODS

Chloroplast Preparation—Greenhouse-grown spinach leaves were homogenized in a Waring Blender for 10 sec with a medium containing 0.8 M sucrose-20 mM Tricine-NaOH (pH 7.8)-10 mM NaCl 5 mM ascorbate. After filtering through Miracloth (Chicopee Mills, Inc., New York), the homogenate was centrifuged at 1500 x g for 30 min and chloroplasts were isolated from the supernatant fluid by centrifugation at 1500 x g for 7 min. The chloroplasts were washed once in the grinding medium and resuspended in sucrose-Tricine-NaOH-NaCl. Chlorophyll was determined by the method of Arnon (10).

Sulfate Inhibition—In a final volume of 2.0 ml, chloroplasts (200 μg of chlorophyll) were illuminated at 23° in a reaction mixture containing 50 mM Tricine-NaOH (pH 8.5), 5 mM MgCl₂, 2.5 mM ADP, 10 mM K₂SO₄, and 50 μM pyocyanine. A light intensity of 3 x 10⁵ ergs per cm² per sec was provided by tungsten photoflood lamps, with the beam filtered through 7 cm of water. K₂SO₄ was omitted from the controls. After 60 sec the chloroplasts were diluted in 20 ml of sucrose-Tricine-NaOH-NaCl-ascorbate, collected by centrifugation, and resuspended in 1 ml of sucrose-Tricine-NaOH-NaCl.

Photophosphorylation by these previously illuminated chloroplasts was carried out at the same temperature and light intensity in a reaction mixture which contained, in 2.0 ml, 50 mM Tricine-NaOH (pH 8.5), 5 mM MgCl₂, 2.5 mM ADP, 5 mM K₂HPO₄ containing 2 x 10⁶ cpm of ³²P, 50 μM pyocyanine, and

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The abbreviations used are: CF₁, chloroplasts coupling Factor 1 (the latent, Ca²⁺-dependent ATPase); Tricine, tris(hydroxy-methyl)methylglycine.

Grant GM-14479.
TABLE I
Inhibition of photophosphorylation caused by sulfate

Chloroplasts (100 μg of chlorophyll per ml) were treated either in the light or in the dark for 60 sec with 5 mM K₂HPO₄, 5 mM K₂HAsO₄, or 10 mM K₂SO₄, in the presence of ADP and Mg²⁺. The chloroplasts were then washed in sucrose-Tricine-NaOH-NaCl-ascorbate, and phosphorylation was measured in a pyocyanine-dependent reaction. Details of these procedures are given in the text.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Illumination</th>
<th>Subsequent phosphorylation (μmoles ATP/mg chlorophyll/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₂SO₄ + ADP + Mg²⁺</td>
<td>-</td>
<td>171</td>
</tr>
<tr>
<td>K₂HPO₄ + ADP + Mg²⁺</td>
<td>+</td>
<td>83</td>
</tr>
<tr>
<td>K₂HAsO₄ + ADP + Mg²⁺</td>
<td>+</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>170</td>
</tr>
</tbody>
</table>

100 μg of chlorophyll. After 2 min in the light the reaction was stopped by adding trichloracetic acid to a final concentration of 3%. ATP was measured in the supernatant fluid according to Avron (11); radioactivity was determined with a Nuclear-Chicago gas flow counter. Light intensities were measured with a YSI-Kettering radiometer, model G5.

Light-induced pH Rise—Chloroplasts were resuspended in 50 mM NaCl, 2.5 mM MgCl₂, and 25 μM pyocyanine to a final concentration of 50 μg of chlorophyll per ml and equilibrated to 15°C. The light-induced pH rise was measured as previously described (12).

Analytical Methods—Ferricyanide reduction was calculated from the decline in extinction at 420 nm in the denatured reaction mixture supernatant solution (13). Protein (14) and P₁ (15) were estimated colorimetrically.

Reagents—Phenazine methosulfate, trypsin, dithiothreitol, and phlorizin were purchased from Nutritional Biochemicals, methylviologen from British Drug Houses, and N,N'-dicyclohexylcarbodiimide from Sigma. GDP, CDP, and UDP were from P-L Laboratories and adenosine 5'-methylene diphosphonate was a product of Miles Laboratories. Tricine (16), pyocyanine (17), and diaminodurene (2,3,5,6-tetramethylphenylenediamine) (18) were prepared by methods already described. 32P₁ was purchased from New England Nuclear.

RESULTS

Illumination of chloroplasts in the presence of sulfate, ADP, and Mg²⁺ results in marked inhibition of a subsequent phosphorylation reaction, in spite of a washing step between the two illuminations (Table I). No significant inhibition was observed when the experiment was repeated with phosphate or arsenate in place of sulfate.

Fig. 1 shows the effect of sulfate concentration on the formation of inhibition. Phosphorylation was about 50% inhibited by prior illumination in the presence of ADP, Mg²⁺, and 10 mM K₂SO₄; higher concentrations of sulfate were not further effective. Impairment of the phosphorylation mechanism occurs rapidly under these conditions, with a half-time on the order of 5 to 10 sec (Fig. 2).

Requirements for Formation of Sulfate Inhibition—As shown in Table II, inhibition by sulfate was dependent on both ADP (Experiment 1) and Mg²⁺ (Experiment 2) and on the electron carrier pyocyanine (Experiment 3). Either phosphate or arsenate, when present together with sulfate, completely protected against inhibition (Experiment 1). ATP not only failed to replace ADP, but partially protected against the inhibition when present with ADP (Experiment 4). Inasmuch as these observations suggest an interaction between sulfate and the phos-
Sulfate Inhibition of Photophosphorylation

The basic reaction mixture for the formation of sulfate inhibition contained K$_2$SO$_4$, ADP, and Mg$^{2+}$, as described under "Methods." K$_2$SO$_4$ was omitted from controls. ADP, Mg$^{2+}$, and other compounds were included or omitted where indicated. After illumination, the chloroplasts were washed in sucrose-Tricine-NaOH-NaCl-ascorbate and phosphorylation was measured in a pyocyanine-dependent reaction.

**TABLE II**

Requirements for formation of sulfate inhibition in chloroplasts

The basic reaction mixture for the formation of sulfate inhibition contained K$_2$SO$_4$, ADP, and Mg$^{2+}$, as described under "Methods." K$_2$SO$_4$ was omitted from controls. ADP, Mg$^{2+}$, and other compounds were included or omitted where indicated. After illumination, the chloroplasts were washed in sucrose-Tricine-NaOH-NaCl-ascorbate and phosphorylation was measured in a pyocyanine-dependent reaction.

<table>
<thead>
<tr>
<th>Addition or omission</th>
<th>Control</th>
<th>Sulfate-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>294</td>
<td>191</td>
</tr>
<tr>
<td>Minus ADP</td>
<td>277</td>
<td>278</td>
</tr>
<tr>
<td>Plus 5 mm K$_2$HPO$_4$</td>
<td>280</td>
<td>279</td>
</tr>
<tr>
<td>Plus 5 mm K$_2$HAsO$_4$</td>
<td>239</td>
<td>248</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>191</td>
<td>115</td>
</tr>
<tr>
<td>Minus Mg$^{2+}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>625$^*$</td>
<td>320</td>
</tr>
<tr>
<td>Minus pyocyanine</td>
<td></td>
<td>684</td>
</tr>
<tr>
<td>Experiment 4</td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td>166</td>
</tr>
<tr>
<td>Minus ADP</td>
<td></td>
<td>323</td>
</tr>
<tr>
<td>Plus 0.25 mm ATP</td>
<td>241</td>
<td>116</td>
</tr>
<tr>
<td>Plus 2.5 mm ATP</td>
<td></td>
<td>232</td>
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<td></td>
</tr>
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<td>116</td>
</tr>
<tr>
<td>Plus 1 mm NH$_4$Cl</td>
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<td>Experiment 6</td>
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<td></td>
</tr>
<tr>
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<td>120</td>
</tr>
<tr>
<td>Plus 1 mm phlorizin</td>
<td></td>
<td>252</td>
</tr>
</tbody>
</table>

* Summer spinach.

In light of the following experiments (Experiments 5 and 6), ADP was not replaced by AMP, ATP, adenosine 5'-methylene diphosphonate, or other nucleoside diphosphates.$^3$ That GDP failed to replace ADP was surprising, since both are phosphorylated in chloroplasts with comparable efficiency (19, 20).

**Sulfate Inhibition of Photophosphorylation Supported by Different Electron Flow Patterns—Inhibition of chloroplasts by sulfate results in a 35 to 50% decline in pyocyanine-dependent phosphorylation (Tables I and II). Since cyclic phosphorylation might involve two sites of ATP formation (21-22), the possibility was considered that sulfate might affect only one of these. Results shown in Table III make this unlikely. Phosphorylation was inhibited to an almost identical extent, whether supported by noneucaryotic electron flow through System II (ferri cyanide as electron acceptor), a true cycle involving only System I (phenazine methosulfate in the presence of 3-(3,4-dichlorophenacyl)-1,1-dimethylurea), or noneucaryotic electron flow through System I only (ascorbate-diaminodiurene as donor, methylviologen-oxygen as acceptor, in the presence of 3-(3,4-dichlorophenacyl)-1,1-dimethylurea) (24). Moreover, 35 to 50% inhibition of pyocyanine-dependent phosphorylation is also found with ferri cyanide in place of pyocyanine during the initial exposure to sulfate and light.$^3$

**Electron Transport in Sulfate-Inhibited Chloroplasts—Electron transport from water to ferri cyanide was not impaired by sulfate inhibition. Rates of 643 and 661 mmoles of ferri cyanide reduced per mg of chlorophyll per hour in control and sulfate inhibited membranes, respectively, were associated with the phosphoryl-
TABLE IV

Effect of sulfate inhibition on trypsin- or dithiothreitol-activated Ca2+-ATPase Activity

Chloroplasts were illuminated in the presence of K2SO4, ADP, and Mg2+, washed in sucrose-Tricine-NaOH-NaCl-ascorbate, and resuspended in sucrose-Tricine-NaOH-NaCl as described under "Methods." Controls were illuminated in the absence of K2SO4. Trypsin-activated Ca2+-ATPase activity was assayed according to McCarty (personal communication). In a final volume of 0.9 ml, chloroplasts containing 50 μg of chlorophyll were incubated with 20 mM Tris-HCl (pH 8.0), 2 mM EDTA, 2 mM ATP, and 400 μM of freshly dissolved trypsin. After 10 min at 20°, 0.1 ml of trypsin inhibitor (10 mg per ml) was added. Aliquots (0.1 ml) were incubated for 20 min at 37° with 0.9 ml of a solution containing 20 mM Tris-HCl (pH 8.0), 5 mM CaCl2, and 4 mM ATP. Reactions were stopped with trichloracetic acid. Dithiothreitol activation was carried out at 20° for 45 min in 80 mM Tris-HCl (pH 7.0, to a final concentration of 330 μg of chlorophyll per ml. After 5 min at room temperature, the suspension was centrifuged at 27,000 × g for 10 min and the CF1-depleted membranes were removed by centrifugation. The latter exhibited only about half of the light-induced pH rise of controls. Sulfate inhibition in chloroplasts was formed by illumination in the presence of K2SO4, ADP, and Mg2+ as described under "Methods." K2SO4 was omitted from controls. The methods of preparing CF1-deficient membranes and for reconstituting the resolved membranes with CF1 are given in Table V. Reconstituted membranes were washed twice in 10 mM NaCl before light-induced pH shifts were measured. C, CF1-deficient control chloroplasts; S, CF1-deficient sulfate-inhibited chloroplasts; S(CF1), CF1 from control chloroplasts; S(CF1), CF1 from sulfate-inhibited chloroplasts.

Effect of Sulfate Inhibition on Trypsin- or Dithiothreitol-activated Ca2+-dependent ATPase Activity

Results presented thus far suggest that sulfate may inhibit photophosphorylation by interacting with CF1. It might be expected therefore that the ATPase activities associated with CF1 would show similar inhibitions. Chloroplasts, after sulfate exposure, were incubated with trypsin which releases CF1 from the membrane and activates its Ca2+-dependent ATPase. Other chloroplasts were incubated with EDTA to release CF1, and then with dithiothreitol to activate the ATPase. In some experiments the activation was performed before and in other experiments after removal of the green membranes by centrifugation. The latter protocol eliminated any possibility that the inhibited membranes were affecting either activation or assay of the ATPase. Activities, on either a chlorophyll or protein basis, were decreased by values varying between 17 and 45% in CF1 released from sulfate-inhibited chloroplasts (Table IV).
Sulfate Inhibition of Photophosphorylation

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Figure 5. Kinetic analysis of the effects of sulfate inhibition on photophosphorylation. Control and sulfate-inhibited chloroplasts were prepared as described under "Methods." Phosphorylation was measured in a reaction mixture which contained, in a total volume of 2.0 ml, 50 mM Tricine-NaOH (pH 8.5), 5 mM MgCl₂, and 50 μM pyocyanine together with the following compounds: A, 2.5 mM ADP, variable Pi, and 100 μg of chlorophyll; B, variable ADP, 2 mM Pi, and 10 μg of chlorophyll; C, 2.5 mM ADP, 5 mM Pi, and 100 μg of chlorophyll. Each reaction mixture contained activities that might be restored by removing the damaged CF₁ and replacing it with untreated enzyme. To examine this possibility, sulfate-inhibited chloroplasts were extracted with 0.75 M EDTA, centrifuged, and then recombined with Mg²⁺ ions and either with their own or with fresh CF₁ derived from noninhibited controls. Similar experiments were carried out with CF₁-depleted control membranes, which were then recombined with CF₁ from either control or sulfate-treated chloroplasts. With either type of chloroplast, phosphorylation was restored more completely by CF₁ derived from control chloroplasts than from sulfate-treated chloroplasts (Table V). These results were obtained with saturating amounts of CF₁, although similar results were found at limiting CF₁ concentrations. Even with control CF₁ added back, the sulfate-treated chloroplasts did not phosphorylate as well as control chloroplasts with control CF₁ added. Superficially this might appear to show that some of the damage to chloroplasts is associated with the residual green membranes, rather than being limited to their CF₁ molecules. However, a single EDTA extraction is known to remove only 50 to 70% of the CF₁ from chloroplasts, as judged by residual trypsin-activated Ca²⁺-ATPase activity (25). Thus the sulfate-treated membranes still contain a significant fraction of damaged CF₁ molecules even after EDTA extraction and restoration by control CF₁.

Restoration of Light-induced pH Rise in Sulfate-inhibited Chloroplasts—The restoration of phosphorylation by replacement of CF₁ (Table V) suggested that the light-induced pH rise might also be restored in this way. Fig. 4 shows that this function could be completely restored to the level of the appropriate controls if the sulfate-treated chloroplasts were stripped of CF₁ by EDTA and then recombined with CF₁ from control chloroplasts. Moreover, CF₁ from sulfate-inhibited chloroplasts could not restore the pH rise to control chloroplast membranes as well as normal CF₁ could. In agreement with previous observations (26), EDTA-treated chloroplasts without added CF₁ showed only a very small light-induced pH shift.

Kinetic Analysis of Effects of Sulfate Inhibition on Photophosphorylation—Phosphorylation rates are 35 to 50% lower as a result of preliminary sulfate treatment at all concentrations of phosphate or ADP and at all light intensities (Fig. 5). Lineweaver-Burk analysis revealed that, although \( V_{max} \) values were decreased in inhibited membranes, \( K_m \) values for phosphate and ADP were unchanged (0.74 mM and 21 μM, respectively) as was the half-saturating light intensity (8.3 × 10⁶ ergs per cm² per sec). These results suggest that 35 to 50% of the chloroplast CF₁ molecules are probably completely inactive in the inhibited membranes. They seem to us less suggestive of a partial inactivation of every CF₁ molecule, although this possibility has not been ruled out.

Attempts to Show Sulfate Binding—The possibility that sulfate might be bound to inhibited CF₁ molecules, thus leading to the irreversible inhibition, was examined with \(^{35}\)SO₄. After establishing the inhibition in the presence of radioactive sulfate, chloroplasts were washed three times in either grinding buffer (sucrose-Tricine-NaOH-NaCl-ascorbate) or in 10 mM nonradioactive K₂SO₄. Although phosphorylation remained 50% inhibited compared to washed controls, the chloroplasts contained no 10⁴ cpm of \(^{35}\)P. After 10 sec (B) or 2 min (A and C) reactions were terminated with trichloroacetic acid. Lineweaver-Burk analysis of these results is shown. chlor., chlorophyll.
radioactivity above that of minus ADP controls. The specific activity of the original sulfate solution (10^6 cpm per amole) would have allowed detection of 1 atom of sulfur for every 5000 chlorophyll molecules. In addition, CF1 was extracted by means of EDTA from chloroplasts inhibited by radioactive sulfate, chromatographed on Sephadex G-75, and found to contain no radioactivity.

The possibility was also considered that sulfate might catalyze the binding of ADP to chloroplasts during sulfate inhibition. This was ruled out in experiments similar to those described above, but with 14C-labeled ADP.

**DISCUSSION**

The inhibition of photophosphorylation by sulfate ions, previously shown (8) to be competitive with respect to phosphate, was found in the present study to include a large apparently irreversible component. This fact has permitted us to examine the requirements for the formation of the inhibition separately from its expression in the various chloroplast reactions, carried on in a second light reaction. We are able to conclude that the inhibition is primarily, if not entirely, due to an alteration in CF1, and that formation of the inhibited state requires those conditions of coupled electron transport in which CF1 is essentially prepared to catalyze anhydro bond formation.

Asada, Deura, and Kasai (7) concluded that inhibition by sulfate was reversible on washing. In their experiments, however, washing of chloroplasts was attempted only after illumination with a mixture of phosphate and sulfate, in a phosphorylation reaction mixture. We have found that less phosphate is required to prevent the irreversible inhibition from occurring than is needed to compete with sulfate directly in a phosphorylation reaction. Thus in the earlier experiments the sulfate to phosphate ratio was not large enough to bring on the irreversible inhibition described here, even though it was sufficient to cause some direct inhibition of photophosphorylation.

The protection by phosphate or arsenate (Table II) indicates a common binding site with sulfate, perhaps the one involved in photophosphorylation. This finding is in agreement with the observation of competition between phosphate and sulfate when both are present during a single photophosphorylation reaction, or during photosynthetic carbon dioxide fixation (8, 9). The requirements for light and an added electron carrier (pyocyanine or ferriyanide) show that electron transport is needed, and preventing the inhibition by ammonium chloride indicates a requirement for maintenance of the high energy conditions of the membranes. The further requirement for ADP and Mg2+ ions, combined with prevention of sulfate inhibition by phlorizin, a known energy transfer (27) and ATPase (28) inhibitor, indicates that almost all of the steps of phosphorylation except for the final attachment of phosphate to ADP are required for the inhibited state to occur.

The high degree of specificity for ADP in the present experiments is striking in that it was not replaced by AMP, ATP, adenosine 5'-methylene diphosphonate, or other nucleotides. Even GDP, which is reported to be phosphorylated directly by chloroplasts rather than through a nucleotide kinase (19, 20), was without effect. This specificity is reminiscent of but more extreme than that for the inhibition of chloroplast ATPase, in which ADP is considerably more effective than GDP (2, 20).

Previous illumination with sulfate inhibits later photophosphorylation supported by any sort of electron flow pattern (Table III), uncouples phosphorylation from associated ferriyanide reduction as shown by a lower P/E ratio, and causes a partial inhibition of the light-induced pH rise (Fig. 3). That the pH rise as well as phosphorylation was restored by removing existing CF1 from these chloroplasts and replacing it with the enzyme from control chloroplasts indicates that all of the effects are caused by damage to CF1. This conclusion is strengthened by the failure of CF1 from inhibited chloroplasts to reconstitute adequately the pH rise or phosphorylation in EDTA-extracted control chloroplasts (Fig. 4, Table V). Also consistent is the restoration of the pH rise to sulfate-treated chloroplasts by NADN1,N4-dicyclohexylcarbodimide (Fig. 3), a reagent previously shown to restore the pH rise in chloroplasts depleted in CF1 (25) or with CF1 damaged by exposure to acid and polyaniions (12). As before, restoration of the pH rise by control CF1 is not considered as evidence for a direct participation of the enzyme in the mechanism for ion fluxes. A change in the conformation of CF1 on the sulfate-inhibited membranes might allow passive proton leakage, and thus cause a dissipation of the pH gradient or membrane potential (or both) necessary for ATP formation (29).

Conclusive evidence as to the alteration of CF1 during sulfate inhibition is found in the decrease of Ca2+-dependent ATPase activity detectable in trypsin-treated chloroplasts (Table IV) or in EDTA extracts of chloroplasts. This decrease was observed even when both activation and assay of the ATPase were carried out apart from the chloroplast membranes.

The nature of the damage to CF1 is not yet known. No signs were found of binding of sulfate-35S or ADP-14C either to inhibited chloroplasts or to CF1 extracted from them. (These data, together with the irreversible nature of the change, seem to rule out the previous suggestion (8) of formation of ADP-sulfate as being responsible for inhibition of photophosphorylation.) Further, no change was seen in the elution pattern of CF1 from Bio-Gel P-300, or the banding observed after electrophoresis of treated CF1 on polyacrylamide gel, thus making it unlikely that inhibition resulted from dissociation into subunits.

There is a possibility that light might have two roles in the present inhibition—first to bring on the high energy state and then to cause photodynamic or photooxidative damage to CF1 brought into a sensitive configuration due to the presence of sulfate, ADP, Mg2+, and the high energy state of the membranes. Irreversible inactivation of electron transport (30, 37) and of phosphorylation (30, 31) in chloroplasts caused by prolonged illumination at high light intensity was described previously. The present results differ in that 30 sec of moderate light intensity are sufficient when all reagents are present, and in that damage is apparently confined to CF1. Several amino acids in proteins are rapidly photooxidized by illumination in the presence of oxidation-reduction dyes in solution, however (38), and it may be that a particular amino acid might be highly sensitized to photooxidation because of the specific conditions used in the present study. The only evidence opposing this concept as yet is the observation that sulfate inhibition is not prevented by performing the illumination in the absence of O2. Photo-induced inhibition of electron transport close to Photosystem II (27) and the high intensity long term photooxidation of phosphorylation (30, 31) also occur anaerobically.

Further experiments are currently directed toward the identification of the site(s) on CF1 affected by sulfate treatment. If
successful they may provide further insight into the reactivity of CF1 with respect to the binding of phosphate or sulfate, and the way in which its conformation or reactivity is affected by conditions which permit anhydro bond formation.

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REFERENCES
