Light Activation of Mg\(^{2+}\)-dependent Adenosine 5'-Triphosphatase in Isolated Euglena Chloroplasts

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ABSTRACT

Enhancement of Mg\(^{2+}\)-dependent ATPase activity in Euglena gracilis chloroplasts by light in the presence of a sulfhydryl compound has been demonstrated. A number of uncouplers and energy transfer inhibitors were studied for their effects on the light enhancement of ATPase activity simultaneously with their effects on photophosphorylation. Results suggest that the light-enhanced ATPase activity in Euglena chloroplasts is an energy-initiated process and that the energy is supplied through electron flow upon illumination of the chloroplasts. However, by studying the difference in their response toward the various uncouplers and inhibitors, it seems that the two processes (photohydrolysis of ATP and photophosphorylation) share only the latter part of their energy-transferring pathway.

In addition to demonstrating successfully the light enhancement of Mg\(^{2+}\) ATPase in Euglena chloroplasts, we also compared the effect of a number of uncouplers and energy transfer inhibitors on photophosphorylation and photoactivation of Mg\(^{2+}\) ATPase in an effort to determine the relationship between the two reactions.

MATERIALS AND METHODS

The culturing of Euglena gracilis and the isolation of its chloroplasts have been recently described (6). The chloroplasts were washed twice with the grinding medium consisting of 0.4 M mannitol; 25 mM tris-HCl, pH 7.8; and 10 mM NaCl (MTN). Although washing sometimes may decrease the photophosphorylation activity by as much as 30%, it was necessary because the crude chloroplasts preparation usually contained significant amounts of mitochondria which contributed appreciably to ATP hydrolysis in the dark.

The reaction mixtures for assays of photophosphorylation and ATP hydrolysis are described in each figure or table. For photophosphorylation, the samples were illuminated for 2 min in 10- x 60-mm test tubes in a water bath at 23 C with 1.2 \times 10^6 ergs/cm\(^2\)-sec of white light supplied by two 150-w photo flood lights, and passed through 2 inches of water. Phosphate esterification was measured by the method of Lindberg and Ernstcr (14), which was also used for determining the ATP hydrolysis. For photohydrolysis, the samples (1.5 ml) were illuminated for 20 min and the dark controls were placed in the same bath but were wrapped with aluminum foil. The reaction was stopped with 0.1 ml of 50% trichloroacetic acid, the samples were centrifuged in a refrigerated centrifuge, and the supernatant was assayed immediately for inorganic phosphate, to minimize the hydrolysis of ATP by the H\(_2\)SO\(_4\)-molybdate mixture.

Phlorizine was a gift from Dr. William Lynn of Duke University, chlorotri-n-butyltin was purchased from Aldrich Chemical Company, and Valinomycin from Calbiochem. Reduced lipoic acid was prepared according to the method of Gunsalus and Razzell (8). Chlorophyll was determined by the method of Arnon (1).

RESULTS

Optimal Conditions for Light Activation. The optimal pH for light-activated ATPase was between pH 7.5 and 8.0. The requirements for light activation are presented in Table I. MgCl\(_2\) is absolutely required for both light-activated and dark ATPase activity. Omission of pyocyanine (or some other cofactors for electron transport) essentially eliminated photophosphorylation but retained light activation of ATPase partially. The presence of a sulfhydryl compound was necessary, with 40 mM cysteine being the optimal concentration when

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the experiment was carried out aerobically (Fig. 1). However, the optimal concentration varied somewhat with different preparations of chloroplasts. Anaerobically, stimulation by cysteine reached a plateau at 20 mM and the maximal activation which could be attained was generally lower than in air. Other sulfhydryl compounds—glutathione, dithiothreitol, and reduced lipoic acid—did not give any better results in light activation, although in the case of reduced lipoic acid lower concentrations were needed than for cysteine. Furthermore, dithiothreitol and reduced lipoic acid both interfered to a certain extent with the separation and extraction of P, by the procedure of Lindberg and Ernster (14). Ammonia was not found to be absolutely necessary, though it helped in general to produce larger differences between light and dark ATPase activity. The optimal concentration varied from 0.5 to 1.5 mM. Presence of 6 mM inorganic phosphate minimized the effect of resynthesis of 32P into ATP in the light. Higher concentrations of phosphate tended to produce a white precipitate in the reaction mixture.

**Light-triggered or Light-dependent Activation.** The hydrolysis of ATP as a function of time of exposure is presented in Figure 2. Curve I represents ATP hydrolyzed in the dark while Curve II represents this activity when the samples were illuminated. Samples in Curve III were illuminated for 10 min and then placed in the dark. These curves demonstrate that the Mg\(^{2+}\) ATPase in *Euglena* chloroplasts was activated by light but that the activation was light triggered rather than light dependent. Once it was initiated, the ATPase activity continued at the accelerated rate in the dark after the light was turned off (Curve III). The triggering process required a somewhat longer time than that reported for spinach chloroplasts, which needed only 2 min (17). The fact that values in Curve II were consistently slightly lower than those in Curve III is probably caused by a deterioration process of the chloroplasts under extended exposure to light. Preillumination

<table>
<thead>
<tr>
<th>Reaction Mixture</th>
<th>Rate of ATP Hydrolysis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Light (μmoles ATP mgchl h)</td>
</tr>
<tr>
<td>Control</td>
<td>92.1</td>
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<tr>
<td>−MgCl₂</td>
<td>10.0</td>
</tr>
<tr>
<td>−MgCl₂ + CaCl₂</td>
<td>8.3</td>
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<tr>
<td>−Pyocyanine</td>
<td>55.6</td>
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<tr>
<td>−Cysteine</td>
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<tr>
<td>−NH₄Cl</td>
<td>55.1</td>
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<tr>
<td>−Sodium phosphate</td>
<td>43.8</td>
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</tbody>
</table>

**Fig. 1.** Effect of cysteine concentration on photophosphorylation and Mg\(^{2+}\) ATPase. The reaction mixture for light activation of Mg\(^{2+}\) ATPase was as described in Table I, with cysteine concentration varied as indicated. The reaction mixture for photophosphorylation in a final volume of 1.5 ml contained 20 μmoles of tris-HCl buffer, pH 7.8, 35 μmoles NaCl, 4 μmoles ADP, 4 μmoles phosphate buffer containing 500,000 cpm 32P, 50 nmoles pyocyanine, 5 μmoles MgCl₂, and chloroplasts containing 20 μg chlorophyll: 2 min of illumination.

**Fig. 2.** Time-course of ATP hydrolysis in light and in the dark by chloroplasts of *Euglena gracilis*. I: Samples in the dark; II: samples in the light; III: samples in the light for 10 min and then kept in the dark. Reaction mixture for ATP hydrolysis was as described in Table I.
of the chloroplasts in the absence of ATP or cysteine for 5 or 10 min produced no activation.

The Effect of Uncouplers and Energy Transfer Inhibitors on Photophosphorylation and Light Activation of Mg²⁺ ATPase. The mechanism of light-triggered or light-dependent ATPase activity is not well understood. Generally it is believed that the light-dependent process requires energy and the energy is supplied by light-potentiated electron transport through coupling, which results in a high energy intermediate. It has been further suggested that the energy transfer of these two processes (photophosphorylation and photohydrolysis of ATP) is closely related, sharing at least part of the energy conservation pathway (17). If this is the case, then uncouplers and energy transfer inhibitors known to affect ATP formation could also affect light-activated ATPase.

Ammonia, Butyramine, and Quainacrine. All three uncouplers of photophosphorylation (4, 7, 13) inhibited ATP synthesis in a similar manner, but only quainacrine inhibited ATP hydrolysis as well (Table II). CCCP, an uncoupler of both oxidative and photosynthetic phosphorylation (9), had no significant effect on light-activated ATPase. This lack of effect on ATP hydrolysis was probably due to the presence of cysteine, which also reversed the inhibition by CCCP of ATP synthesis. While 10 μM CCCP inhibited photophosphorylation by 50% in the absence of cysteine, it had no significant effect on either reaction in the presence of cysteine. Similar effects of cysteine have been reported for spinach chloroplasts (18). The inhibition of ATP synthesis by ammonia and butyramine was not reversed by cysteine.

Valinomycin, which affects cation permeability of membranes (3), inhibited photophosphorylation and ATP photohydrolysis in an identical manner (Table II). Chlorotri-n-butyltin and phlorizin, both inhibitors of energy transfer and acting at the terminal steps in the ATP-synthesizing pathway (10, 11), showed different kinetics for the inhibition of ATP synthesis and ATP hydrolysis (Table II). While phlorizin was more potent as an inhibitor of ATP synthesis than of ATP hydrolysis, chlorotri-n-butyltin was more effective against ATP hydrolysis as compared to ATP synthesis.

DISCUSSION AND CONCLUSION

As a result of their study on the activation of Mg²⁺-dependent ATPase in spinach chloroplasts, McCarty and Racker (15) concluded that the activation was caused by the modification of a single enzyme by the sulhydryl reagents, and this activation can be further accelerated by light. A similar explanation can perhaps be applied here, except that in Euglena chloroplasts the modification of the enzyme by sulhydryl reagents cannot take place prior to illumination, although the sulhydryl reagents can maintain the enzyme in the light-activated form.

It has been suggested by Petrack et al. (17) that the two processes (ATP synthesis and ATP hydrolysis) share at least in part their energy-transferring pathway. Our results show that when an inhibitor acts at a site close to the site of ATP formation, such as phlorizin (10) or chlorotri-n-butyltin (11), both reactions are affected. If the site of action of the inhibitor is closer to the site of energy coupling, it may affect both reactions (quinacrine, Valinomycin), or it may affect ATP synthesis drastically while having no effect on ATP photohydrolysis (ammonia, butyramine). It thus appears that in Euglena chloroplasts only the latter part of the energy conservation pathway is shared by both reactions.

While our previous work with chlorotri-n-butyltin (11, 12) indicated that all coupling sites were kinetically equivalent as far as ATP synthesis was concerned, this seems not to be the case for ATP hydrolysis. The trialkyltin inhibitors bind irreversibly to the ATP-synthesizing sites, and probably those sites most accessible to diffusion are inactivated first. Those sites would be the most accessible ones to ATP as well. Phlorizin, being bound reversibly, would establish an equilibrium with all sites being equivalent. It will thus affect the ATPase activity less than ATP synthesis (Table II), since the initial rate of the latter is higher and rate limiting in the overall process of photophosphorylation (12) under saturating light.

As has been suggested before (5), the Mg²⁺ ATPase, which is not light dependent, shows no relationship to either ATP synthesis or its photohydrolysis. None of the inhibitors or uncouplers added changed the rate of the dark reaction significantly.

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LITERATURE CITED