Plant Physiol. (1985) 79, 992–995
0032-0889/85/79/0992/04/$01.00/0

Photoinhibition and Reactivation of Photosynthesis in the Cyanobacterium *Anacystis nidulans*

Received for publication May 15, 1985

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ABSTRACT

The susceptibility of photosynthesis to photoinhibition and its recovery were studied on cultures of the cyanobacterium *Anacystis nidulans*. Oxygen evolution and low temperature fluorescence kinetics were measured. Upon exposure to high light *A. nidulans* showed a rapid decrease in oxygen evolution followed by a quasi steady state rate of photosynthesis. This quasi steady state rate decreased with increasing photon flux density of the photoinhibitory light. Reactivation of photosynthesis in dim light after the photoinhibitory treatment was rapid: 85 to 95% recovery occurred within 2 hours. In the presence of the translation inhibitor, streptomycin (250 micrograms per milliliter), no reactivation occurred. We also found that the damage increased dramatically if the high light treatment was done with streptomycin added. A transcription inhibitor, rifampicin, did not inhibit the reactivation process. Based on these data we conclude that the photoinhibitory damage observed is the net result of a balance between the photoinhibitory process and the operation of the repairing mechanism(s).

It is well documented that photoinhibition of photosynthesis in visible light may occur under conditions when the antenna of photosynthesis absorbs light in excess to what can be deexcited through photosynthesis (17). Excess excitation of the antenna can be obtained simply by increasing the light intensity above the level which saturates the rate of photosynthesis (20, 27). However, it can also occur under moderate light intensities if the rate of photosynthesis is limited by stress factors: freezing temperatures (24), low but nonfreezing temperatures in either chilling sensitive plants (1, 18), or in chilling resistant plant (10), drought (13), and excess salt in the root medium (4) can induce photoinhibition under moderate light intensities.

The general concept is that the primary site of photoinhibition is at, or close to, the reaction center of PSII (17) but PSI may also be affected (21). However, photoinhibition of photosynthesis also may cause partial uncoupling of electron transport as well as an increased rate constant for spillover of excitation energy from PSII to PSI (11). Kyle *et al.* (9) have recently given evidence that one site of inhibition is at the Q_B-protein on the reducing side of PSII, but there is still no consensus on the molecular mechanisms of photoinhibition. Neither is there any understanding of the significance of the different mechanisms (carotenoids [8]; Mehler reaction [5]; photorespiration [19]; spillover of excitation energy from PSII to PSI [16]) proposed to protect an overexcited photosynthetic apparatus from photoinhibition. The very high susceptibility to photoinhibition at low temperatures of the chilling resistant species *Lemna gibba* (10) suggests that low temperatures not only limit the rate of photosynthesis but also make alternative ways of deexciting Chl less efficient. Furthermore, very little attention has been paid to the possibility of repairing mechanisms operating under photoinhibitory conditions. We know that at least moderate photoinhibition may be completely repaired within 6 to 8 h (10, 20) and the results of Kyle *et al.* (9) imply that the repairing system of photodamages in photosynthesis includes synthesis of specific proteins. We hypothesize that a repairing system operating under photoinhibitory conditions may be significant in determining the susceptibility to photoinhibition in photosynthesis; when the damages exceed the capacity of the repairing system, photoinhibition is likely to occur.

In a long-term effort to obtain a better understanding of both the molecular mechanisms of photoinhibition of photosynthesis, and of the significance and the mechanisms of a repairing system for avoiding photoinhibitions of photosynthesis, this initial communication describes the time courses of photoinhibition and reactivation of photosynthesis in the cyanobacterium *Anacystis nidulans*. *A. nidulans* has been chosen for this study because it shows the typical responses in photosynthesis upon photoinhibition and we believe that its procaryotic gene organization makes it a suitable object for using molecular genetic tools to elucidate the mechanisms of both photoinhibition and reactivation of photosynthesis.

MATERIALS AND METHODS

Culture Conditions. *Anacystis nidulans* 625 (*Synechococcus* 6301) was grown in batch cultures in an inorganic medium (22) at 38°C. PAR was 50 μmol m^{-2} s^{-1}, as measured with a Li-Cor quantum radiometer (Lambda Instruments Lincoln, NE) and the light sources were incandescent lamps (Philips PAR 38 150 inches/220 V). The cells were grown in 0.5 L of the medium in rectangular flasks (1.5 L), gently shaken, with light coming in from above, using equipment described before (15). Air was flushed over the culture, which gave cells adapted to low inorganic carbon.

Photoinhibitory Treatment and Reactivation. The cells were harvested by centrifugation at 2000*g* for 5 min and washed once in medium at 38°C, after which the algal pellet was resuspended in 10 ml of the medium, buffered with 1 mM Tris (pH 7.2), made up by CO_2-free water. From this stock an algal suspension of 1 ml was transferred to each of eight test tubes containing 9 ml of the buffered medium with a concentration of inorganic carbon of about 100 μM. The tubes were placed in a glass water bath at 38°C. The algal concentration was 1.5×10^9 cells/ml and the Chl *a* concentration, calculated by the method of Stevens and Myers (23) was approximately 2.5 μg/ml. The test tubes were closed with rubber stoppers and the photoinhibitory treatment

started. The light source was a halogen lamp (Osram, power star, HQI-IS 400 W, Berlin) and the incident PAR was varied by placing the lamp at different distances from the algal samples. Control tubes were incubated at 38°C in dim light equal to about 5 μmol m^{-2} s^{-1}. At time intervals from zero to 135 min algal cells were withdrawn from the photoinhibitory treatment and tested for photoinhibition of photosynthesis by measuring photosynthetic O_2 evolution and Chl *a* fluorescence kinetics.

Reactivation of photosynthesis after photoinhibition was done at 38°C in dim light of a PAR of 5 μmol m^{-2} s^{-1}. When used, the concentrations of the translation inhibitors streptomycin and kanamycin, and the transcription inhibitor rifampicin, were added to final concentrations of 250 μg/ml.

Photosynthesis Measurements. Photosynthesis was measured using a Clark-type O_2 electrode (Hansatech, Ltd., Norfolk, U.K.) at a nonsaturating PAR of 55 μmol m^{-2} s^{-1} and a temperature of 38°C. After different times of photoinhibition and reactivation, 1 ml of the algal sample was transferred to the electrode and bubbled with O_2 free gas (1% CO_2 in argon) for 30 s to lower the O_2 concentration and to avoid O_2 saturation during the measurements. Prior to the measurements, 20 μl of a 1 M HCO_3^- solution was also added to certify saturating carboxylating conditions. Light source was an Atlas projector lamp (A1, 215 24 V/150 W). The signal from the electrode was registered on a chartpen recorder and when stable rates were obtained photosynthesis was calculated as μmol O_2 evolved·mg Chl^{-1}·h^{-1}. The electrode was calibrated to the absolute mode by using dithionite and air-saturated water at 38°C.

Fluorescence Measurements. Fluorescence kinetics were measured at 77 K using the trifurcated fiberglass centered fluorescence spectrometer earlier described (11). The emission peaks at 698 and 725 nm were ascribed to PSII and PSI, respectively (3). The instrumental settings are given in figure legends.

RESULTS AND DISCUSSION

Photoinhibitory treatments of *A. nidulans* inhibited the photosynthetic O_2 evolution rapidly followed by a quasi steady state level (Fig. 1). The initial rapid phase of photoinhibition was much more pronounced at a PAR of 1000 μmol m^{-2} s^{-1} than at 500 and 250 μmol m^{-2} s^{-1}. The quasi steady state level of photosynthetic O_2 evolution was reached within about 30 min of photoinhibitory treatment. This level decreased roughly in proportion to increased PAR but even at a PAR of 1000 μmol m^{-2} s^{-1} (20 times higher than the PAR used for growing the algae) the activity was 20% of that of the control.

Studies of the effects of the time of photoinhibitory treatment at a PAR of 500 μmol m^{-2} s^{-1} on the 77 K fluorescence kinetics of F698 (PSII) showed that the F_v/F_o ratio decreased parallel with the inhibition of photosynthetic O_2 evolution (Fig. 2). In accordance with earlier reports (2, 11), we conclude that this finding is consistent with the general view that excess light

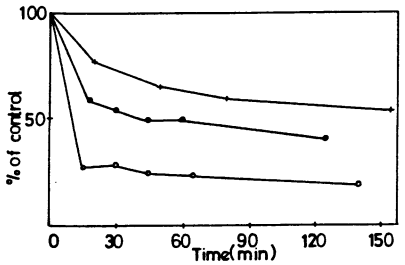

FIG. 1. Photosynthetic O_2 evolution (% of control) *versus* time of photoinhibitory treatment. Control rate was 123 μmol·mg^{-1} Chl·h^{-1}. The PAR of the photoinhibitory light was: (+), 250 μmol·m^{-2}·s^{-1}; (●), 500 μmol·m^{-2}·s^{-1}; (○), 1000 μmol·m^{-2}·s^{-1}.

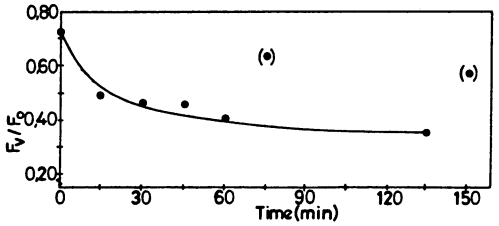

FIG. 2. The ratio F_v/F_o of 698 at 77 K as a function of time of photoinhibitory treatment. PAR of the photoinhibitory light was 500 μmol·m^{-2}·s^{-1}; (●) represents controls treated in dim light, 5 μmol·m^{-2}·s^{-1}. Excitation at 578 nm (half band width 10 nm).

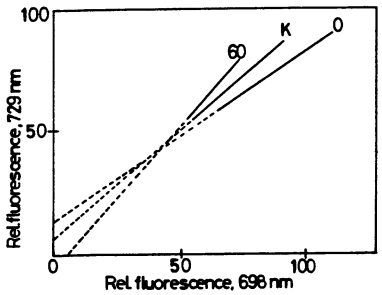

FIG. 3. A plot of F_{729} *versus* F_{698} at 77 K of *A. nidulans* before and after 60 min of photoinhibition in a PAR of 500 μmol·m^{-2}·s^{-1}. The results from a control (K) treated in dim light (5 μmol·m^{-2}·s^{-1}) for 60 min are also presented (- - -) extrapolations. Excitation was at 578 nm (half band width 10 nm).

inhibits the photochemical efficiency of PSII.

When a dark adapted algal sample is frozen at 77 K in darkness and then exposed to a weak actinic light the fluorescence intensity increases from a low initial value, F_o, to a high value, F_m, as the reaction centers of PSII become closed (7). By simultaneous recordings of the fluorescence kinetics of F698 (PSII) and F729 (PSI) during PSII trap closure at 77 K we obtained *x-y* plots like those presented in Figure 3. It has been shown that the variable portion of the PSI fluorescence originates from an increased spillover of energy from PSII to PSI when the PSII traps become closed (7). The same authors also showed that F729/F698 = $k_{T(II\rightarrow I)}\cdot\psi_{FI}/k_{FII}$, where $k_{T(II\rightarrow I)}$ is the rate constant for spillover of excitation energy from PSII, to PSI, k_{FII} is the rate constant for fluorescence in PSII and ψ_{FI} is the probability of fluorescence in PSI. Furthermore, the intercept obtained when the straight line is extrapolated to the *y*-axis is proportional to α, the fraction of excitation energy initially directed to PSI (3).

Figure 3 shows that after 60 min of photoinhibitory treatment at a PAR of 500 μmol m^{-2} s^{-1} energy was no longer initially directed to PSI (the actinic light of 578 nm was preferentially absorbed by the phycobilisome). However, the slope of the straight line increased 23% (mean of seven determinants) as compared with the control kept in dim light and 68% as compared with the slope at the onset of the photoinhibitory treatment. As the photoinhibitory treatment did not significantly affect ψ_{FI} (data not shown), as assayed by monitoring F729 upon excitation of only PSI Chl at 703 nm (12), we believe that the increased slope reflects a proportional increase of the rate constant of spillover from PSII to PSI, provided that k_{FII} is not affected. When the slope of the straight line was plotted as a function of the time of photoinhibitory treatment at 500 μmol m^{-2} s^{-1} it was found that the slope increased approximately antiparallel (Fig. 4) with the inhibition of the photochemical efficiency of PSII (Fig. 2). These findings confirm the earlier observations that photoinhibition of photosynthesis is accompanied by an increased rate constant for spillover of excitation energy from PSII to PSI, (1, 12) and a decrease in α (12, 25).

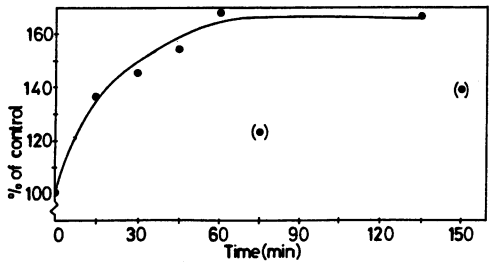

FIG. 4. The slope of F_{729}/F_{698} at 77 K, plotted as a function of time in photoinhibitory light (500 μmol·m^{-2}·s^{-1}. (●), controls held in dim light.

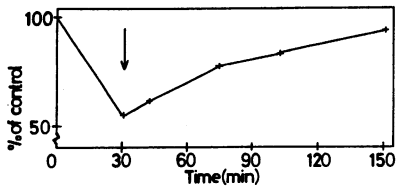

FIG. 5. Photosynthetic O;2 evolution *versus* time, during photoinhibition and reactivation. Cells were photoinhibited for 30 min at a PAR of 500 μmol·m^{-2}·s^{-1}. The arrow indicates transfer to reactivating conditions (5 μmol·m^{-2}·s^{-1}). Results are expressed as a % of controls.

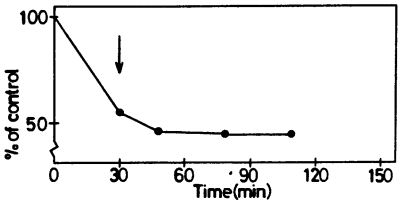

FIG. 6. Photosynthetic O_2 evolution *versus* time, during photoinhibition and reactivation for cells photoinhibited at 500 μmol·m^{-2}·s^{-1}. The arrow indicates transfer to 5 μmol·m^{-2}·s^{-1}. At the time of transfer, streptomycin was added to 250 μg/ml. Results are expressed as a % of controls.

These results show (Figs. 1–4) that the cyanobacterium *A. nidulans* respond to photoinhibitory treatments with changes in the function of photosynthesis that are typical for higher plants (12, 17) and algae (20, 26). The finding, that the quasi steady state level is at least roughly proportional to the PAR used during the photoinhibitory treatment, supports the suggestion that the balance between the inhibiting and reactivating processes is crucial for the magnitude of the net inhibitory effect.

When transferred to a dim PAR of 5 μmol m^{-2} s^{-1}, after a photoinhibitory treatment for 30 min at a PAR of 500 μmol m^{-2} s^{-1} (about 50% inhibition), *A. nidulans* reactivated within 1 h its photosynthetic O_2 evolution to 80 to 95% of the control activity (Fig. 5). The reactivation of photosynthetic O_2 evolution was paralleled by a reactivation of the photochemical efficiency of PSII as assayed by fluorescence kinetics of F698 at 77 K (data not shown). This is in agreement with the results obtained with moderately photoinhibited (about 50%) *Amphidinium carterae* (20), *Lemna gibba* (11) and *Chlamydomonas reinhardii* (14). *A. nidulans*, however, differed by showing an approximately five times faster time course of reactivation and a lack of lag phase after the onset of reactivation.

To obtain more information on the nature of the reactivation process we added the translation inhibitor streptomycin to an algal suspension at the onset of reactivation in dim light following a 30-min photoinhibitory treatment at a PAR of 500 μmol m^{-2} s^{-1} (Fig. 6). Streptomycin totally inhibited reactivation as did another translation inhibitor, kanamycin (data not shown). The

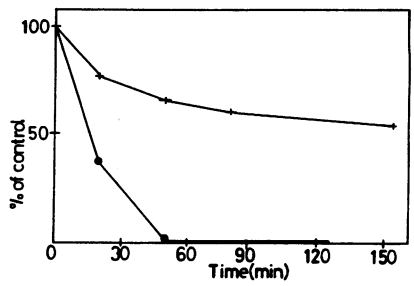

FIG. 7. Photosynthetic O_2 evolution in a control and in a sample containing 250 μg/ml of streptomycin as a function of time in photoinhibitory light (250 μmol·m^{-2}·s^{-1}). (+), Control; (O), streptomycin.

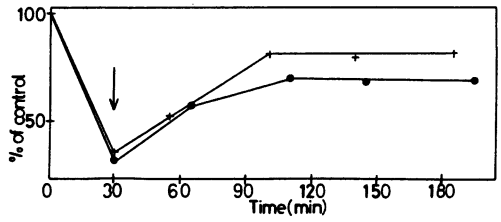

FIG. 8. Photosynthetic O_2 evolution during photoinhibition and reactivation at 500 μmol·m^{-2}·s^{-1}. At the time of transfer, rifampicin was added to 250 μg/ml. (+), Control; (O), streptomycin. Results are expressed as a % of controls.

antibiotics were also added to a control sample kept in dim light. No decrease in the activity of photosynthetic O_2 evolution caused by the antibiotics was seen during the experimental time period used. These studies with translation inhibitors clearly show that protein synthesis is required for reactivation of photosynthesis following a photoinhibitory damage of photosynthesis. Kyle *et al.* (9) and Ohad *et al.* (14) arrived at the same conclusion in their studies of photoinhibition and reactivation of photosynthesis in *C. reinhardii*. A somewhat similar effect under photooxidative conditions was demonstrated in Eloff *et al.* (6).

We also added the translation inhibitor streptomycin to an algal sample prior to photoinhibitory treatment at a PAR of 250 μmol m^{-2} s^{-1} (Fig. 7). Streptomycin more than doubled the rate of photoinhibition during the initial phase and completely eliminated the following quasi steady state phase of photosynthetic O_2 evolution: complete inhibition was reached after 50 min. This finding is consistent with the view that a repairing mechanism is operating during the photoinhibitory treatment and that the quasi steady state level of inhibition obtained at a certain PAR (Fig. 1) reflects the net photoinhibitory effect as a function of the balance between the processes of inhibition and repair.

The protein synthesis required for reactivation of photosynthesis after photoinhibition was insensitive to rifampicin, a transcription inhibitor (Fig. 8). This is an unexpected result and it implies that the protein(s) are translated by stable mRNA(s) or that rifampicin does not inhibit the trancription of the operon(s) involved. The insensitivity of the repairing mechanism to rifampicín is not because of rifampicin not being taken up since we know that rifampicin of the concentration used inhibits cell growth and RNA synthesis (Roger Kalla, personal communication).

The results of this work clearly establish that the responses of the photosynthesis function of the cyanobacterium *A. nidulans* to excessive light are similar to those earlier reported for higher plants and algae. The proportionality between the quasi steady state level of photosynthetic O_2 evolution and the PAR used for inhibiting photosynthesis, as well as the doubling of photoinhibition susceptibility and elimination of the quasi steady state phase upon inhibition of protein synthesis at the translation

level, support the hypotheses that the net photosynthesis inhibition under excessive light, is the result of a balance between the processes of photoinhibition and reactivation. If a system for repairing damages in photosynthesis caused by excessive visible light, are operational under a wide range of PARs it is possible that such a system requires a specific regulation as indicated by the insensitivity to rifampicin. The lack of a lag phase after the onset of reactivation furthermore shows that the repairing mechanism is active or activated during the photoinhibitory treatment. Further work will be directed towards studies of the protein(s) involved in photoinhibitory damages of photosynthesis and of the nature and significance of the repairing mechanism(s).

Acknowledgment—We are grateful to Mrs. G. Malmberg for skillful technical assistance.

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