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Protection from Photoinhibition by Low Temperature in *Synechocystis* 6714 and in *Chlamydomonas reinhardtii*: Detection of an Intermediary State

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Received March 21, 1990; Revised Manuscript Received May 17, 1990

ABSTRACT: Photoinhibition was induced in a cyanobacterium strain, *Synechocystis* 6714, and a green alga, *Chlamydomonas reinhardtii*, by exposing them to light intensities from 1000 to 4000 $\mu E/(m^2 \cdot s)$ at various temperatures. The photoinhibition process was followed by measurements of chlorophyll fluorescence and oxygen evolution. During exposure to high light, fluorescent active reaction centers II became low fluorescent inactive centers. This process involved several reversible and irreversible steps. The pathway from the active state to the inactive low fluorescent state was different in *Synechocystis* and *Chlamydomonas*. In the latter there was a reversible intermediary step characterized by an increase of F_0. This state was stable at 5 °C and slowly reversible at room temperature. The high F_0 fluorescence level corresponded to a state of photosystem II centers that were inactive for oxygen evolution. An F_0 decrease occurred in the dark in the absence of protein synthesis and was correlated to a restoration of oxygen evolution. Further experiments suggested that the existence of the intermediate fluorescent state is due to modified closed centers in which the reduced primary acceptor is less accessible to reoxidation. In cyanobacteria this reversible state was not detected. In both organisms, the decrease of F_{max} reflected an irreversible damage of photosystem II centers. These centers need replacement of proteins in order to be active again. The quenching of F_{max} and the irreversible inhibition of oxygen evolution were slowed down in both organisms by decreasing the temperature of the photoinhibitory treatment from 34 to 5 °C. We conclude that low temperature protected the reaction center II from irreversible photodamage.

Photoinhibition is related to the excess of light absorbed by the pigment antennae which cannot be properly dissipated by photosynthesis (Osmond, 1981; Powles, 1984; Kyle, 1987; Cleland, 1988). There is now a large amount of evidence demonstrating that the primary site of lesion is the reaction center of the photosystem II (PSII).[1] Different sites in the reaction center II (RCII) were proposed to be the first target of high light. Studies on isolated chloroplasts, thylakoids, or PSII preparations suggested that the P_{680}–Phe–Q_A portion of the electron transport is the primary site of damage (Cleland et al., 1986; Theg et al., 1986; Arntz & Trebst, 1986; Vass et al., 1988; Styring et al., 1990). As opposed to that, results obtained with intact organisms suggested that the Q_B niche in the D_1 protein is the first site to be damaged (Kyle et al., 1984; Kirilovsky et al., 1988; Ohad et al., 1988). It was observed that the electron transfer through Q_B ($H_2O \rightarrow$ DCBQ or $H_2O \rightarrow$ DCIP) decreased more rapidly than electron transfer which did not involve Q_B ($H_2O \rightarrow$ SiMo) (Kirilovsky et al., 1988, Kyle et al., 1984). Moreover, thermoluminescence measurements showed that modifications of the B band appeared before modifications of the Q band. B and Q signals result from the charge recombination between $S_{2,3}$ and Q_B^- and Q_A^-, respectively (Ohad et al., 1988; Kirilovsky, Ducruet, and Etienne, unpublished data).

The decrease of PSII activity due to photoinhibition can be restored if the cell exposure to high light is not too long. The repair process, which is light dependent, involves de novo synthesis of thylakoid proteins among which the most prominent is D_1 (Ohad et al., 1985; Lönneborg et al., 1988; Kirilovsky et al., 1988).

It is assumed that environmental conditions that reduced the rate of photosynthesis accentuate the effects produced by plants exposed to high light. Exposure of leaves of many plants to high photon flux densities at chilling temperatures produces a damage to the photosynthetic apparatus which is greater than that observed at higher temperatures [reviewed by Oquist et al. (1987)]. The recovery is also temperature dependent, being slower at low temperatures. It was proposed that the inhibition of recovery by low temperatures may also contribute to the particular susceptibility to photoinhibition in plants at chilling

[1] Abbreviations: Chl, chlorophyll; D_1 and D_2, polypeptides of the RCII; DCBQ, dichlorobenzoquinone; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_0, F_v, F_{max}, initial, variable, and maximum fluorescence; P_{680}, a chlorophyll molecule that acts as the primary electron donor in the RCII; Pheo, pheophytin, intermediary electron acceptor; PSII, photosystem II; Q_A and Q_B, primary and secondary quinone electron acceptors, respectively; RCII, reaction center II; Z, electron donor to P_{680}.

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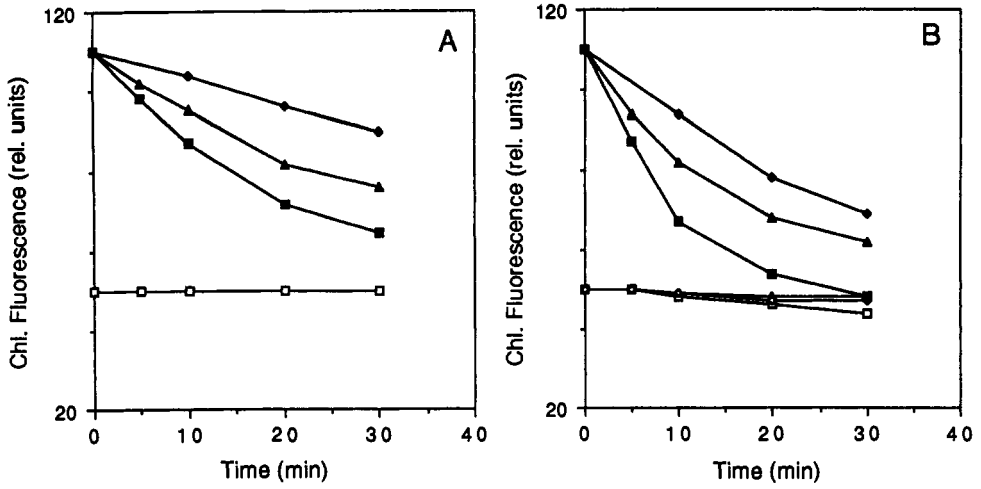

FIGURE 1: Changes in F_{max} and F_0 during photoinhibition in *Synechocystis* 6714. The cells were exposed to the light given by one (◇, ◆), two (△, ▲), or four (□, ■) spot lamps. Each lamp gives an intensity of 1000 $\mu E/(m^2 \cdot s)$. (A) Photoinhibition at 5 °C. (B) Photoinhibition at 25 °C. F_0, open symbols; F_{max}, closed symbols.

temperatures (Oquist et al., 1987; Greer et al., 1986).

In the present work we describe the effect of temperature on photoinhibition in *Synechocystis* 6714 (a cyanobacterium) and *Chlamydomonas reinhardtii* (a green alga). Surprisingly, the low temperature did not increase photoinhibition; on the contrary, it had a protective effect from high light. The damage of the RCII was slowed down by lowering temperature. In *Chlamydomonas*, this irreversible damage was preceded by a fluorescent state inactive for oxygen evolution. A large increase of F_0 was observed at low temperature, which was stable at 5 °C but became reversible at room temperature. The intermediary state reversed in the dark with no protein synthesis. We proposed that the F_0 increase is due to reversible conformational changes in D_1 (and maybe D_2) leading to the isolation of Q_A^-.

Experimental Procedures

Growth Conditions. Synechocystis 6714 cells were grown in the mineral medium described by Herdman et al. (1973) with twice the concentration of nitrate and an illumination of about 70 $\mu E/(m^2 \cdot s)$. The cells were generally grown at 34 °C. They were acclimated to 24 °C during 2 weeks before doing the experiment described in Figure 4A. Other conditions were as previously described (Astier et al., 1986). *C. reinhardtii* was grown at 25 °C in TAP medium (Gorman & Levine, 1965), consisting of 20 mM Tris, 17 mM acetate, and 1 mM phosphate (pH 7).

Photoinhibition Experiments. Cells of *Synechocystis* 6714 or *C. reinhardtii* were harvested by centrifugation and resuspended in fresh growth medium at a final concentration of 30 μg of Chl/mL. The cell suspension (15–30 mL) was incubated in a glass tube (3 cm diameter) refrigerated by cooled water and illuminated by one, two, or four Atralux spots of 150 W [each giving an intensity of about 1000 $\mu E/(m^2 \cdot s)$]. The temperature of the different photoinhibitory treatments was as indicated under Results. The cells were gently stirred by a magnetic bar. Chloramphenicol (200 μg/mL for *Synechocystis* 6714 and 400 μg/mL for *C. reinhardtii*) was present in all the high light incubations, and during recovery in the dark.

Fluorescence Measurements. Fluorescence induction was determined with a fluorimeter described by Vernotte et al. (1982). The fluorescence was excited with a tungsten lamp through 5-59 and 4-96 Corning filters for *Synechocystis* 6714, and though a 4-96 Corning filter for *C. reinhardtii*. The fluorescence was detected in the red region through a 2-64 Corning filter and a Wratten 90 filter. The recording was done through a multichannel analyzer. The cell suspension contained about 1 μg of Chl/mL. The F_{max} level was determined in the presence of 10^{-5} M DCMU.

Oxygen Measurements. (a) Oxygen evolution by whole cells (15 μg of Chl/mL) was measured by polarography using a Clark-type oxygen electrode at 20 °C. (b) The amount of oxygen produced per flash during a sequence of saturating flashes was measured with a rate electrode identical with that described by Joliot and Joliot (1968). The short (5-μs) saturating flashes were produced by a Strobotac (General Radio Co.). The spacing between flashes was 0.6 s. Each experiment was started with dark-adapted cells (500 μg of Chl/mL).

Chlorophyll content was determined in methanol according to Bennett and Bogorad (1973) in *Synechocystis* cells and in acetone (80% in water) according to Arnon (1949) in *Chlamydomonas*.

Results

Photoinhibition of Cyanobacteria. (A) Protection by Low Temperature. During photoinhibition in living cells, protein synthesis which occurs continuously counteracts the damaging effect of high illumination (Ohad et al., 1985; Green et al., 1986; Lönneborg et al., 1988). To avoid the effect of recovery processes, all experiments were performed in the presence of chloramphenicol.

Cells of *Synechocystis* 6714 were exposed at a constant temperature to high light given by one, two, or four lamps. Each lamp corresponded to an intensity of about 1000 $\mu E/(m^2 \cdot s)$. Photoinhibition kinetics were followed by measurements of oxygen evolution an chlorophyll fluorescence. The maximum fluorescence F_{max} decreased during photoinhibition while F_0 remained constant. Figure 1 shows the changes of F_{max} in photoinhibited cells at 5 and 25 °C under three different light intensities. The extent of F_{max} decrease was strongly affected by light and temperature. At a given temperature, F_{max} decrease was slowed down by lowering the light intensity. At a given light intensity, F_{max} decrease was slowed down by lowering the temperature. PSII activity estimated from the oxygen yield under continuous illumination decreased also more slowly at 5 °C than at 25 °C independently of the light intensity used during the photoinhibitory treatment (Figure 2).

The major antenna system for PSII in cyanobacteria is the phycobilisome, a large extrinsic pigment–protein complex bound to the PSII centers. A temperature-dependent reversible

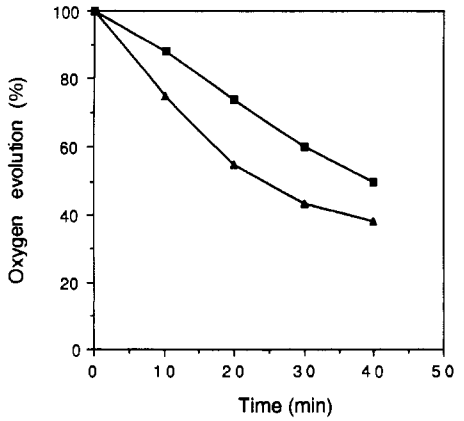

FIGURE 2: Comparison of the decrease of oxygen evolution during photoinhibition at 5 °C (■) and at 25 °C (▲) of *Synechocystis* 6714 cells. One hundred percent of oxygen evolution = 150 μmol of O_2/(mg of Chl·h). The cells were exposed to the light provided by two lamps.

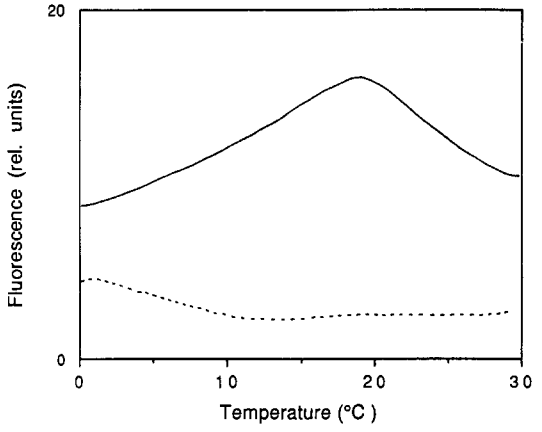

FIGURE 3: Temperature dependence of Chl *a* fluorescence in *Synechocystis* 6714 grown at 34 °C (—) and at 24 °C (---). Excitation was done by a tungsten lamp through 5-59 and 4-96 Corning filters. The Chl *a* fluorescence in the presence of 10^{-5} M DCMU was detected in the red region.

dissociation of phycobilisomes from the thylakoids was observed in several strains of cyanobacteria below 5 °C (Schreiber et al., 1979; Manodori & Melis, 1985). In *Synechocystis* 6714 the variable fluorescence ($F_v = F_{max} - F_0$) induced by excitation of the phycobilisomes and the half-time of fluorescence rise was the same at 5 °C and at 25 °C (data not shown). Therefore, the energy transfer from the phycobilisomes to the PSII was not modified in the range of temperatures used in photoinhibition experiments.

Lowering the temperature has a protective effect for cyanobacteria cells during photoinhibition.

(B) Relationship between the Protective Effect by Low Temperatures and the Physical State of Membrane Lipids. The temperature of phase transition between the liquid crystalline and the solid states depends on the fatty acid composition; the higher is the degree of unsaturation of fatty acids, the lower is the phase transition temperature (Ladbrooke & Chapman, 1969). In the thylakoids of higher plants and most algae the content of linolenic acid (an unsaturated fatty acid) is very high (Benson, 1964), and in consequence the phase transition appears at very low temperature. In cyanobacteria the content of saturated fatty acids is high (Hirayama, 1967) and the phase transition occurs at room temperature. Moreover, the fatty acid content changes depending on the growth temperature (Holton et al., 1964). Murata et al. (1975) have reported that the transition of the physical phase of lipids appeared at 13 or 24 °C in the cyanobacterium *Synechococcus* R2 grown at 28 and 38 °C, respectively.

In order to modify their lipid content *Synechocystis* 6714 cells were grown at two different temperatures, 24 and 34 °C. The temperature of phase transition of the thylakoids was measured by using chlorophyll *a* as a native fluorescence probe. A maximum of Chl *a* fluorescence appeared at the temperature of phase transition in curves of fluorescence versus temperature (Murata & Fork, 1975). Phase transition occurred at about 19 °C in cells grown at 34 °C and at about 0–1 °C in cells grown at 24 °C (Figure 3).

We compared the effect of temperature on photoinhibition in both types of cells. *Synechocystis* 6714 cells grown at 34 or 24 °C were exposed to high light at temperatures varying from 5 to 34 °C. Figure 4A shows that a continuous increasing protection from photoinhibition was obtained by progressive lowering of temperature in cells grown at 24 °C. In cells grown at 34 °C, we observed a break of the increasing protection between 20 and 15 °C (Figure 4B). The rate of F_{max} decrease was similar between 15 and 5 °C. The break was more evident in the Arrhenius plot of photoinhibition (Figure 4C).

These results suggested that the state of the membrane lipids has a role in the mechanism of photoinhibition and in the protective effect of low temperature.

Photoinhibition in C. reinhardtii. (A) Temperature Dependence of F_{max} and F_0 Level during Photoinhibition. Chlamydomonas cells were exposed to high light (provided by one and four lamps) at 5 and 23 °C. Measurements of

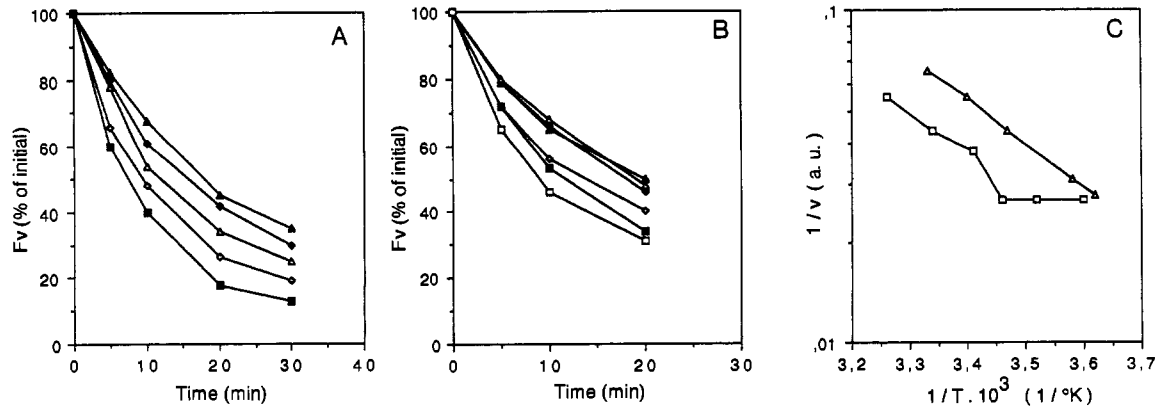

FIGURE 4: Effect of temperature on the decrease of variable fluorescence during photoinhibition in *Synechocystis* 6714. (A) Cells grown at 24 °C were exposed to light given by two lamps [1000 μE/(m^2·s) each] at different temperatures: 3 °C (▲), 6.5 °C (◆), 13 °C (△), 21 °C (◇), and 28 °C (■). (B) Cells grown at 34 °C were photoinhibited at 5 °C (▲), 11 °C (◆), 16 °C (△), 20 °C (◇), 26 °C (■), and 34 °C (□). (C) Arrhenius plots of photoinhibition of cells grown at 24 °C (△) and at 34 °C (□).

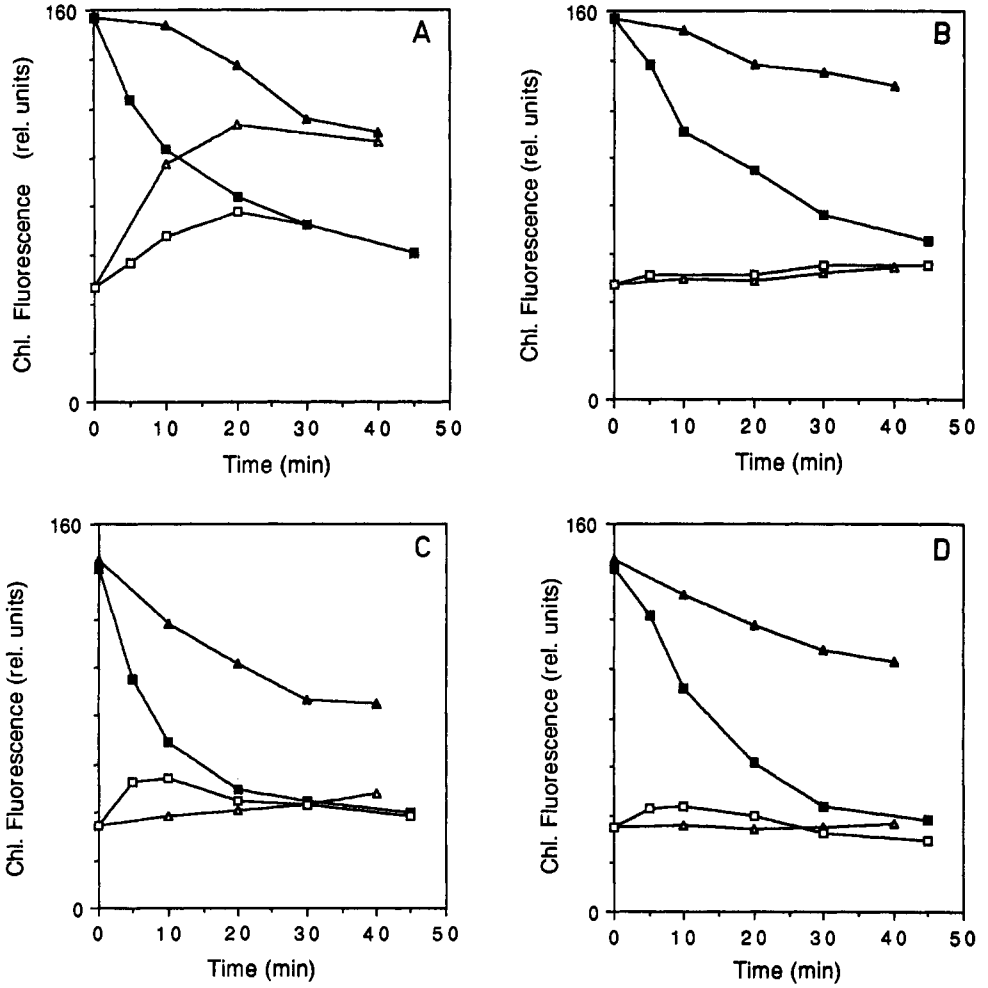

FIGURE 5: Changes in F_{max} and F_0 during photoinhibition at 5 °C (A and B) and at 23 °C (C and D) in *C. reinhardtii*. The fluorescence measurements were done immediately after the photoinhibition treatment (A and C) or after 1 hour of dark incubation at room temperature (B and D). The cells were exposed to the light given by one (△, ▲) or four lamps (□, ■). F_0, open symbols; F_{max}, closed symbols.

fluorescence were done immediately after cell exposure to light. Photoinhibitory treatment produced changes in both F_0 and F_{max}.

Temperature and light dependence of F_{max} behavior was similar in *Chlamydomonas* and *Synechocystis* cells. We observed that also in *Chlamydomonas* cells F_{max} decreased slower at lower light intensities and at lower temperatures (Figure 5A,C).

F_0 behavior was completely different in *Chlamydomonas*. A large increase of F_0 level was observed during the photoinhibitory treatment at 5 °C (Figure 5A). F_0 reached the value of F_{max}, and then they decreased together. The variable fluorescence was totally suppressed after 20–30 min of cell exposure to high light (Figure 5A). At room temperature the increase of F_0 was smaller and never reached the F_{max} level (Figure 5C). Two temperature-dependent processes occurred during photoinhibition in *Chlamydomonas*: one responsible for F_{max} decrease and the other responsible for F_0 increase. Further experiments were done in order to characterized the F_0 increase.

(B) Intermediary State. The large increase in F_0 corresponded to inactive centers. Figure 6 shows the relationship between F_v/F_{max} and oxygen evolution.

The high initial fluorescent state was stable at 5 °C (data not shown) but not at room temperature. Figure 5B,D shows the level of F_{max} and F_0 when the fluorescence measurements were carried out after 1 hour of dark incubation at room temperature. The high level of F_0 was no longer observed. The decay of F_0 was concomitant with an increase in PSII activity

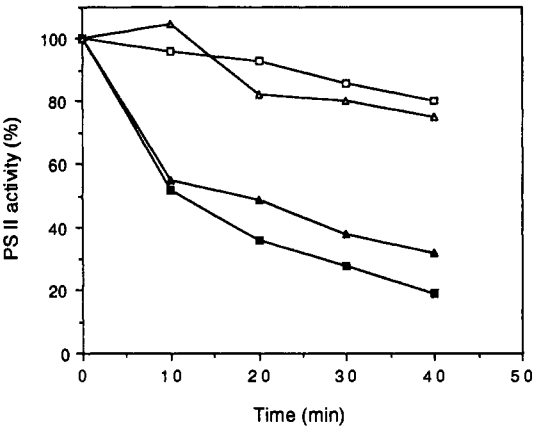

FIGURE 6: Inhibition of PSII activity during photoinhibition in *C. reinhardtii*. Oxygen evolution (△, ▲) and the ratio F_v/F_{max} (□, ■) were calculated from measurements carried out immediately after cell exposure to high light (closed symbols) and after dark incubation at room temperature (open symbols). The cells were exposed to the light provided by one lamp at 5 °C. One hundred percent of oxygen evolution = 210 μmol of O_2/(mg of Chl·h).

(Figure 6). We measured the amount of oxygen produced per flash in cells photoinhibited at 5 °C, in two conditions: at 5 °C, to avoid the reversal of the intermediary state, and at 20 °C, after F_0 decay. Oxygen evolution was drastically reduced in photoinhibited cells at 5 °C (Figure 7). Concomitant with the F_0 decay (at room temperature) a large increase of the oxygen yield per flash was observed (Figure 7).

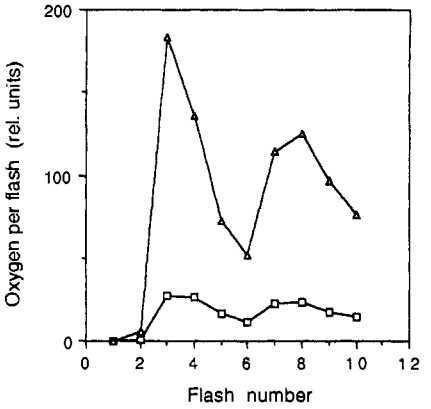

FIGURE 7: Oxygen yield per flash during a series of short saturating flashes in photoinhibited cells (20 min at 5 °C). Sequence 1 (□): Photoinhibited cells were dark adapted at 5 °C and the sequence measured at this temperature. Sequence 2 (△): After photoinhibition, the sample was incubated in the dark at 20 °C for 15 min and the sequence measured at 20 °C.

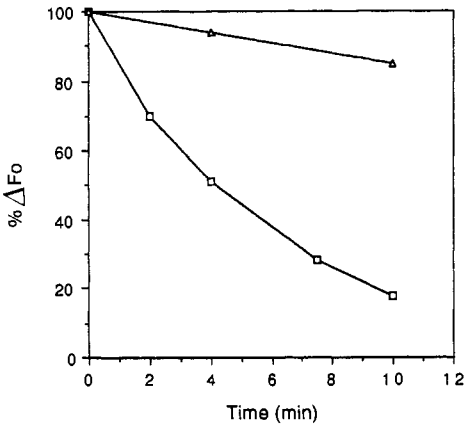

FIGURE 8: Kinetics of F_0 decay in *C. reinhardtii*. The cells were exposed to a light intensity of 1000 $\mu E/(m^2 \cdot s)$ at 5 °C for 20 min. They were then transferred to the dark without any addition (□), or in the presence of NH_2OH and DCMU (△). ΔF_0 initial = F_0 after 20 min of photoinhibition − F_0 of the control. Chloramphenicol (400 $\mu g/mL$) was present all the time.

F_0 decay and PSII activity recovery occurred in the dark in the presence of chloramphenicol. This process needed several minutes (Figure 8). The decay kinetics of F_0 at room temperature was slowed down in the presence of DCMU or NH_2OH, which are known to block respectively the reoxidation of Q_A^- by Q_B or by back reaction with the positive charges on the donor side. After 10 min of room temperature incubation, F_0 decreased 85% without any addition and only 65 or 52% in the presence of NH_2OH or DCMU, respectively. In the presence of both inhibitors the decay was almost blocked (Figure 8). These results suggested that the high fluorescent state was due to an accumulation of Q_A^- in a special environment preventing its reoxidation by forward or back reactions at a normal rate. It is important to point out that F_0 decay was very slow compared to the normal rates of Q_A^- reoxidation ($t_{1/2}$ forward reaction, 200 μs; and $t_{1/2}$ back reaction, 3 s).

(*C*) *Change in the Equilibrium* $Q_A^-Q_B \leftrightarrow Q_AQ_B^-$. After 10 or 20 min of photoinhibition at 5 °C, due to the increase of F_0 (Figure 5A), there remained a small amount of variable fluorescence. We measured the fluorescence decay after a saturating flash in order to monitor Q_A^- reoxidation kinetics. The decay during the first milliseconds after the flash with F_0 as the asymptotic level can be fitted by a sum of three exponentials. Table I shows that the amplitude of the fast component was decreased in photoinhibited cells and its half-time was increased. These results suggest a modification in the rate of reoxidation of Q_A^- by Q_B. In samples photoinhibited at 5 °C we also observed a damping in the oscillations of the intensity of luminescence measured 150 μs after a series of short saturating flashes (data not shown). This was also an indication of a modified state of the oxygen evolving centers.

Table I: Decomposition in Three Exponentials of the Fluorescence Decay

	fluorescence decay, phase amplitude (%)			$t_{1/2}$
	fast	medium	slow	fast phase (μs)
control	70	21	9	140
10 min PI[a] (5 °C)	66	20	14	170
20 min PI (5 °C)	65	20	15	210

[a] PI, photoinhibition.

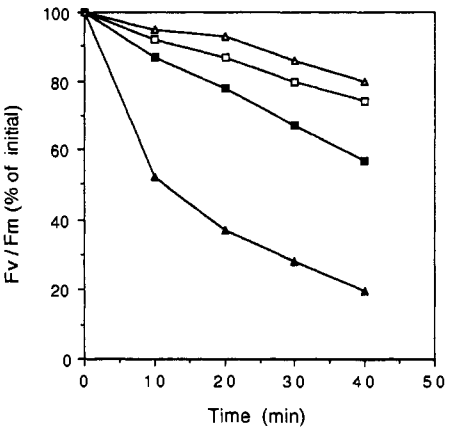

FIGURE 9: Effect of temperature on PSII activity. The ratio F_v/F_{max} was calculated from measurements done immediately after photoinhibitory treatment (closed symbols) or after F_0 decay (open symbols). The cells were exposed to a light intensity of 1000 $\mu E/(m^2 \cdot s)$ at 5 °C (△, ▲) or at 23 °C (□, ■).

During photoinhibition of *Synechocystis* 6714 no modification of the equilibrium Q_A/Q_B was detected (Kirilovsky, Ducruet, and Etienne, unpublished date).

(*D*) *Temperature Dependence of PSII Activity.* As already noticed in Figure 6, F_v/F_{max} was a good indicator of PSII activity. Figure 9 shows the decrease of F_v/F_{max} during photoinhibition at 23 and 5 °C calculated from measurements performed just after photoinhibitory treatment or after F_0 decay. The inhibition of PSII activity was faster at 5 °C than at 23 °C (Figure 9). Almost all the inhibition of PSII activity is due to the intermediary state at 5 °C but to the irreversible damage at 23 °C. The comparison of the irreversible damage (after F_0 decay) at both temperatures showed that it was slightly faster at 23 °C than at 5 °C.

Therefore, the PSII activity decreased rapidly at low temperatures, but most of the inhibition was reversible. The irreversible damage of the RCII appeared more rapidly at higher temperatures like in cyanobacteria.

Discussion

In this work we described the effect of temperature on photoinhibition in two unicellular organisms: *Synechocystis* 6714 (a cyanobacterium) and *C. reinhardtii* (a green alga). We demonstrated that low temperature protected from photoinhibition in both organisms and the existence of an intermediary reversible state in *Chlamydomonas*.

Effect of Temperature on the Irreversible Damage of the RCII. We consider the decrease of F_{max} as an indicator of

PSII centers having an irreversible damage. Recovery of F_{max} requires light and protein synthesis (Ohad et al., 1985; Kirilovsky et al., 1988).

At any given temperature F_{max} decreased more rapidly when the light intensity was increased. Therefore the frequency of the photons falling on photosystem II obviously plays a role.

As far as electron flow through photosystem II is concerned, lowering the temperature decreases both the rate of the S transitions on the donor side and of the reoxidations of Q_A^-, Q_B^-, and Q_B^{2-} on the acceptor side. Therefore, the evacuation of positive charges and electrons is slowed down while the primary charge separation is unaffected. Under the same strong illumination more intermediary oxidized donors and reduced acceptors will be accumulated at 5 °C than at 25 °C. Thus one would expect that temperature decrease at a given light level or a light increase at a given temperature are qualitatively equivalent in causing photoinhibition. This is not the case since in *Chlamydomonas* and in *Synechocystis* the attainment of the irreversible damage of the RCII was slowed down by lowering temperature. It seems unlikely that the accumulation of $Z^+ P^+ I^-$ or Q_A^- can be the only cause of damage to photosystem II. Configurational changes of the centers and proteolytic activities might also be important factors involved in the mechanism of photoinhibition.

Relationship between Physical State of Membrane Lipids and Photoinhibition. The physical state of the membrane lipids influenced also the kinetics of photoinhibition. In cyanobacteria, the temperature of phase transition is above 0 °C. Moreover, it varies with the growth temperature. We have demonstrated that the increasing protection by lowering temperature presented a break at the phase transition point. The decrease of F_{max} and of PSII activity became independent of temperature when the membrane lipids were in the mixed solid–liquid crystalline state. These results suggest that conformational changes of proteins, highly dependent on temperature and fluidity of membrane lipids, are involved in the mechanism of photoinhibition.

Occurrence of an Intermediary State of Photoinhibition in Chlamydomonas. The pathway from an active state with a large variable fluorescence and oxygen activity to an inactive low fluorescent state was different in *Synechocystis* 6714 and *C. reinhardtii*. When fluorescence measurements were done just after photoinhibitory treatment, we observed a large increase of F_0 only in *Chlamydomonas*. In cyanobacteria F_0 remained constant or decreased slightly. The large increase of F_0 was responsible for a very rapid decrease of the ratio F_v/F_{max} in *Chlamydomonas*. The high fluorescence level of F_0 corresponded to a state of PSII inactive for oxygen evolution. This state was stable at 4 °C but reversible at room temperature, in the dark, without protein synthesis. F_0 decay and oxygen evolution recovery occurred concomitantly. The increase of F_0 is an indicator of a reversible modification of the RCII, which does not need D_1 replacement.

The existence of this intermediary state and its stability at 4 °C gives an explanation for the previous observation of Kyle (1985) that the photosystem II activity is more rapidly inhibited at lower temperatures. Our contribution was to show that a large part of this inactivation is reversible and is not linked to any degradation of PSII.

This temperature-dependent increase of F_0 was also observed by Greer et al. (1988) during exposure to high light of leaves of kiwifruit. They related the F_0 increase to a substantial reduction in the rate constant for photochemistry as a result of damage at, or near, the PSII reaction centers; this damage became extreme at low temperatures. It remains to be determined whether the state characterized by the high level of F_0 can be reversed without replacement of damaged proteins in higher plants as is the case in *Chlamydomonas*. Since almost all the publications about the effect of temperature in higher plants pointed out a greater photoinhibitory damage at low temperature, it seems possible that the intermediate state is not reversible or is reversed very slowly in higher plants.

A possible explanation for the fluorescent state is the existence of modified closed centers in which the reduced primary acceptor Q_A^- is less accessible to reoxidation as shown by the slow kinetics of F_0 decay. The fact that the presence of hydroxylamine and DCMU blocked the decay of F_0 supported this explanation. This high level of F_0 was also observed during photoinhibition of PSII particles from spinach in anaerobic or reducing conditions. The presence of ferricyanide during anaerobic photoinhibition prevented F_0 increase (Setlik et al., 1990). Conformation changes of D_1 (or D_2) leading to an increase of the activation energy of recombination and electron transport reactions may explain the increase of F_0.

Another indication of changes in D_1 conformation comes from the measurements of the decay of fluorescence after one flash and luminescence oscillations. Both measurements showed a modification in the Q_A/Q_B equilibrium in centers still active for oxygen evolution. Ohad et al. (1988, 1990) also pointed out a change of the Q_B binding site as the primary modification during photoinhibition.

We propose that a modification in the Q_B site is followed by larger conformational changes of the D_1 protein leading to a reversible isolation of Q_A^- from Q_B and Z, both embedded in D_1.

Conclusion

In cyanobacteria, the degradation of D_1 is preceded by an irreversible modification of the Q_B site which leads to the inhibition of oxygen evolution. In *Chlamydomonas*, reversible conformational changes cause a modification in the equilibrium Q_A/Q_B followed by a reversible accumulation of Q_A^- before irreversible damages take place.

The Q_A^- stable state occurring in *Chlamydomonas* may be due to a shift in the redox potential of the Q_A/Q_B^- couple induced by the modifications of the proteinous environment.

It is obvious that experiments are still needed to fully understand the complexity of photoinhibition, but we have clearly demonstrated that the irreversible damage of the RCII is slowed down by lowering temperature in both *Synechocystis* and *Chlamydomonas*.

Acknowledgments

We thank Dr. J. Garnier for providing *C. reinhardtii* cells.

Registry No. Chl *a*, 479-61-8; O_2, 7782-44-7.

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Structure, Stability, and Receptor Interaction of Cholera Toxin As Studied by Fourier-Transform Infrared Spectroscopy†

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Received September 26, 1989; Revised Manuscript Received May 11, 1990

ABSTRACT: The structure and thermal stability of isolated B and A subunits of cholera toxin, as well as the interaction of the B subunit with a ganglioside G_{M1} receptor, were studied by Fourier-transform infrared spectroscopy. The B subunit of the toxin is highly folded; its secondary structure consists predominantly of β-sheets. The temperature dependence of the infrared spectrum indicates that the B subunit undergoes thermal unfolding in the temperature range between approximately 66 and 78 °C. Binding to the ganglioside G_{M1} receptor or to its oligosaccharide moiety results in only marginal, if any, change in the secondary structure of the B subunit; however, the receptor-associated subunit does show a markedly increased thermal stability. The secondary structure of the enzymatically active A subunit is less ordered and much less stable than that of the B subunit. The relatively loose folding of the A subunit is likely to be of importance for the effective membrane translocation of this subunit.

The clinical manifestations of cholera are attributable to cholera toxin, an enterotoxic protein produced by *Vibrio cholerae* [for recent reviews of cholera toxin chemistry and biological activity, see Lai (1980), Fishman (1982), Finkelstein (1988), and Moss and Vaughan (1988)]. The toxin molecule is composed of two structurally and functionally distinct subunits, A and B (Lannroth & Holmgren, 1973; Cuatrecasas et al., 1973). The A promoter consists of two polypeptide chains, A_1 (M_r 23 000) and A_2 (M_r 5500), that are linked by a single disulfide bridge (Gill, 1976). The B subunit is believed to contain five identical polypeptide chains (M_r 11 600) arranged in a noncovalently associated pentameric ring (Gill, 1976; Lai et al, 1977; Dwyer & Bloomfield, 1982; Ludwig et al., 1986; Ribi et al., 1988). The functions served by these two subunits are complementary (Lai, 1980; Fishman, 1982; Finkelstein, 1988; Moss & Vaughan, 1988). Upon penetration through the cell membrane, the toxic A protomer enzymatically activates intracellular adenylate cyclase, leading to the increased levels of cAMP. The primary role of the B subunit is to initiate the toxin–target cell interaction by binding to specific receptor sites on the membrane surface.

The receptor for cholera toxin has been identified as monosialoganglioside G_{M1} (van Heyningen et al., 1971; Cuatre-

† Issued as National Research Council of Canada Publication No. 30582.

0006-2960/90/0429-8106$02.50/0 Published 1990 by the American Chemical Society