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A New Mechanism for Adaptation to Changes in Light Intensity and Quality in the Red Alga *Porphyra perforata*[1]

III. FLUORESCENCE TRANSIENTS IN THE PRESENCE OF 3-(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA

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ABSTRACT

In the red alga *Porphyra perforata*, the level of chlorophyll fluorescence in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) decreased during illumination of the thallus. The results showed that: (a) this decay was related to the photooxidative activity of photosystem I; (b) Q, the primary electron acceptor of photosystem II, became oxidized during the decay of the fluorescence; (c) reagents which inhibit the back reaction of photosystem II inhibited the decay.

From these results, it is suggested that, when conditions in the chloroplasts of this red alga become too oxidative, excess light energy can be converted to heat as a result of an accelerated back reaction of photosystem II. This may be one of the mechanisms by which this alga can cope with the high salt and high light conditions that can occur in its natural habitat.

In the photosynthesis of red algae, two light-harvesting pigment proteins are involved. Phycobilisomes are mainly connected to PSII and most of Chl *a* belongs to PSI (11). To maintain a suitable ratio of activities of the two photosystems, red algae have evolved a mechanism to control the transfer of light energy from pigment system II to I, called state I-state II transitions (3, 12, 18). The red alga *Porphyra perforata* is an intertidal alga that is periodically exposed to high light and/or high salt conditions. Therefore, it might be expected that this alga has other mechanisms besides the state I-state II transitions to adapt it to live under high light conditions.

Fork and Öquist have reported (5) that the morphology of the pigment systems in *Porphyra* changed after air drying so that light energy absorbed by PSII was preferentially transferred to PSI. Recently, we found another mechanism in *P. perforata* that was termed state II-state III transitions (Satoh and Fork [13, 14]). After a state II to III transition, the light energy reaching the reaction centers of PSII was decreased without any significant change in the PSI activity (Satoh and Fork [13]). All these mechanisms may be useful to avoid the photoinhibition of photosynthesis in a plant such as *Porphyra* which is often exposed to high light intensity. However, in high salt concentrations where the water potential is very low, PSII activity would be expected to be strongly inhibited. Under these conditions, an additional mechanism(s) would be needed to avoid photodamage to this alga.

In this study, we found in *P. perforata* a large light-induced fluorescence decrease in the presence of DCMU. It is proposed that this fluorescence decrease is related to one of the adaptation mechanisms of this alga to conditions of high light and low water potential.

MATERIALS AND METHODS

Porphyra perforata was collected at Half Moon Bay, CA. Thalli were maintained under illumination (8 μmol quanta/m^2·s) in open dishes of sea water at 13°C. Samples were kept in the dark for 1 h or more before use.

Fluorescence spectra at 77K and time courses at room temperature of fluorescence at 685 nm were measured using a fiber optic system to excite and collect the fluorescent light (6). PSI (433 nm) and PSII (550 nm) light, which are mainly absorbed by Chl *a* and phycoerythrin, respectively, were obtained by passing the white light from a 150-w, 21.5-v projector lamp (type DLS) through Balzers interference filters and neutral density filters. For measurements of fluorescence induction in the msec time range, a Nicolet Signal Averager model 1010 was used as a transient time converter.

RESULTS

Figure 1 shows the time courses of Chl fluorescence in *P. perforata* illuminated with PSI (433 nm) and PSII (550 nm) light in the presence of DCMU. Both the PSI and PSII lights were attenuated to excite PSII equally. The initial fluorescence increase resulting from the reduction of Q was so rapid that we could detect only the tail of the increase on the time scale used in Figure 1. Surprisingly, after the initial fluorescence increase, the intensity of the fluorescence decreased during illumination of the thallus in the presence of DCMU. The decay rate of the fluorescence was higher in PSI light than in PSII light (Fig. 1), suggesting that PSI activity was related to the process. In these experiments, we used 20 μM DCMU, and at this concentration, O_2 evolution was completely inhibited. Therefore, this fluorescence decay should not be due to the photooxidation of Q by PSI.

Figure 2 shows the fluorescence induction on a faster time scale when a strong actinic light was added 20 s and 7 min after preillumination of *Porphyra* with relatively weak 433 nm light. The upper curve shows that, during the 20-s preillumination in the presence of DCMU, Q became reduced almost completely. This is indicated by the fact that the initial fluorescence (F_o) was very high and there was only small variable fluorescence. By contrast, after 7 min of preillumination, Q was largely in its oxidized state

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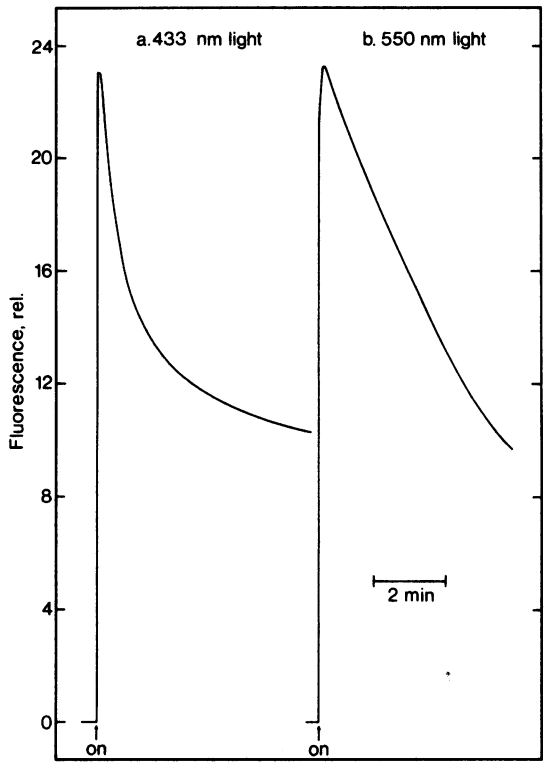

FIG. 1. Time courses of Chl fluorescence in system I (433 nm) and II (550 nm) light in *P. perforata* in the presence of DCMU. Intensities of the PSI and PSII light were 27.6 and 4.4 $\mu w/cm^2$, respectively. The concentration of DCMU was 20 μM.

as indicated by the low F_o and large induction (lower curve). An important point in this result is that the fluorescence intensities finally reached the same level in both cases. These results suggest that the fluorescence quenching observed during illumination of *Porphyra* in the presence of DCMU (Fig. 1) is produced by oxidation of Q by another mechanism different than oxidation via plastoquinone which is completely inhibited by DCMU at the concentration used. Fluorescence spectra at 77K before and after the quenching of the Chl fluorescence were the same suggesting that a change in transfer of light energy (state I–II transition or state II-state III transition [13, 14]) was not the cause of this quenching (data not shown).

Figure 3 shows the effects of NH_2OH, CCCP[3], and antimycin A on the time course of fluorescence quenching. NH_2OH and CCCP inhibited the fluorescence decay strongly (Fig. 3, b and c). Generally speaking, the oxidation of Q might be produced by three mechanisms: (a) oxidation by PSI through the electron transport chain; (b) oxidation by oxidants produced on the water side of PSII (we call this reaction simply the back reaction of PSII; see Refs. 1, 7, 8); and (c) by donation of electrons to other redox substances. Mechanism a is improbable, because we used 20 μM DCMU which completely inhibited oxygen evolution. Furthermore, if Q was oxidized by PSI, CCCP should not inhibit the reaction, because CCCP should accelerate the electron flow between the two photosystems. If Q was oxidized by other substances (mechanism c), addition of NH_2OH should increase the oxidation because NH_2OH at 1 mM acted not as an electron donor but as an inhibitor of PSII (2, 9); therefore, it should decrease the rate of Q reduction. NH_2OH is also known as a potent inhibitor of the back reaction of PSII (1, 7). At high concentrations, CCCP inhibits the oxidizing side of PSII; but, at concentrations around 10 μM, CCCP

[3] Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; DCIP, 2,6-dichlorophenolindophenol; DAD, diaminodurol.

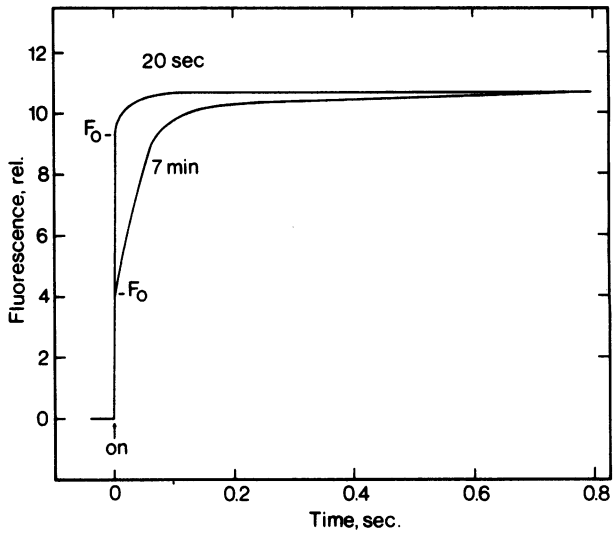

FIG. 2. Induction of Chl fluorescence measured 20 s and 7 min after preilluminating *Porphyra* in the presence of DCMU with 433 nm light. For the measurement, 632.8 nm light from a He-Ne laser (124 $\mu w/cm^2$) was used as the actinic light. Other conditions were the same as in Figure 1.

acted as an uncoupler of photophosphorylation. We also tested the effects of CCCP on the back reaction of PSII in *Porphyra* and found that, like NH_2OH, low concentrations of CCCP inhibited the back reaction as reported previously by Homann (7) and Ikegami and Katoh (8). The results that NH_2OH and CCCP strongly inhibited the fluorescence quenching suggest that the oxidation of Q occurred through the back reaction of PSII (mechanism b). By contrast, antimycin A which inhibits cyclic electron flow around PSI (17) accelerated the quenching (Fig. 3d). When cyclic electron flow is inhibited by antimycin A, there may be an accumulation either of oxidants or reductants produced by PSI.

The data shown in Figures 1 and 3 suggest that either an oxidant or reductant formed by PSI may accelerate the back reaction of PSII. To check whether reductants are related to this process, we observed the effects of artificial electron donors to PSI on the fluorescence quenching. Figure 4 shows that electron donors such as reduced DCIP (Fig. 4b) or DAD (Fig. 4c) decreased the rate of fluorescence quenching. If the reductants are related to the quenching, electron donors to PSI should not decrease the rate but rather should increase it. These results suggest, therefore, that an oxidant may give rise to the acceleration of the back reaction of PSII.

Figure 5 shows fluorescence transients in the presence of DCMU before and after preillumination of *Porphyra* with PSI light. In this experiment, weak excitation light was used compared to that used in Figure 2. For the illuminated sample, a 2-min dark treatment following the 6 min preillumination was used to allow Q to become reoxidized in the dark. The data in Figure 5 show that F_o was not changed by the preillumination. However, in contrast to the data shown in Figure 2, it can be seen that the maximum fluorescence level was not noticeably decreased. Since there was no change in the levels of F_o, these data suggest that the fluorescence quenching was not caused by a decrease of light energy reaching PSII which may result from a state II to III transition (Satoh and Fork [13]). Rather, the decreased F_m level indicates that the quantum yield of the PSII reaction was decreased, which is compatible with the idea that the back reaction of PSII was accelerated by the light treatment.

DISCUSSION

The data presented in this paper clearly demonstrate that, under certain conditions, the chloroplasts of the red alga *P. perforata*

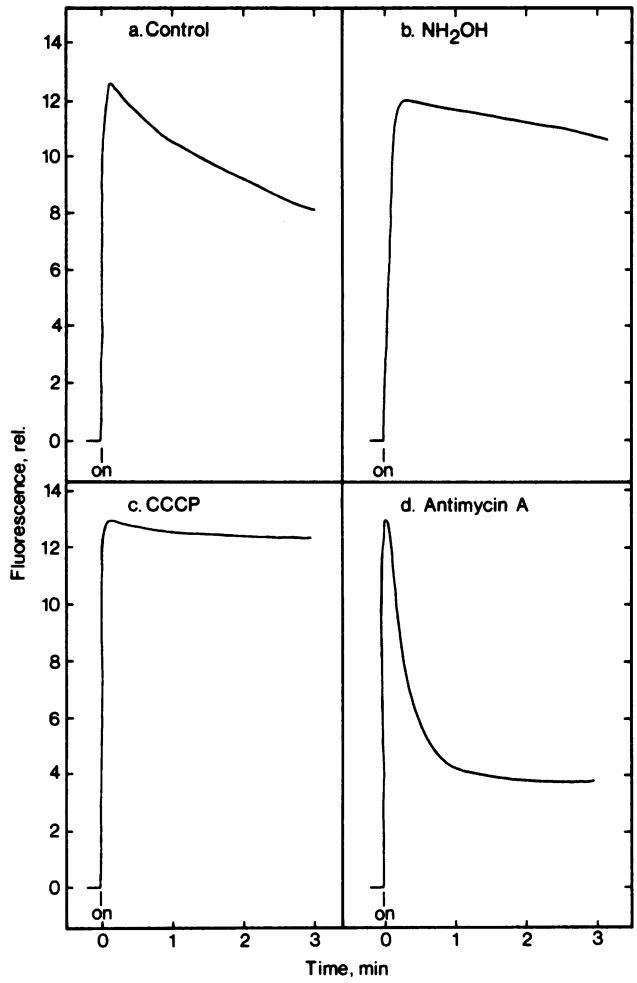

FIG. 3. Effects of NH_2OH, CCCP, and antimycin A on the fluorescence quenching in *Porphyra* in the presence of DCMU. Concentrations of DCMU, NH_2OH, CCCP, and antimycin A were 20 μM, 1 mM, 5 μM, and 50 μM, respectively. PSI light (433 nm) was used as the excitation light. Other conditions were the same as in Figure 1.

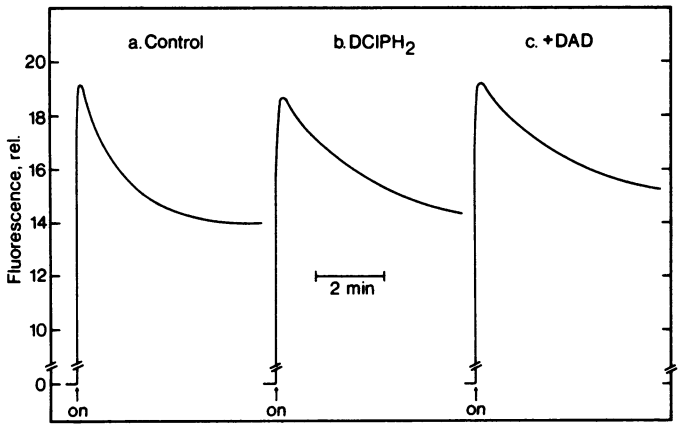

FIG. 4. Effects of electron donors to PSI on the fluorescence quenching in *Porphyra* in the presence of DCMU. Where indicated, 0.1 mM DCIP + 5 mM ascorbate or 0.1 mM DAD + 5 mM ascorbate were added. Other conditions were the same as in the Figure 3a.

consume light energy by accelerating the back reaction of PSII. Under high salt and high light (conditions to which this alga is periodically exposed), high potential oxidants produced by PSII can damage the chloroplasts. Illumination of the cells in the

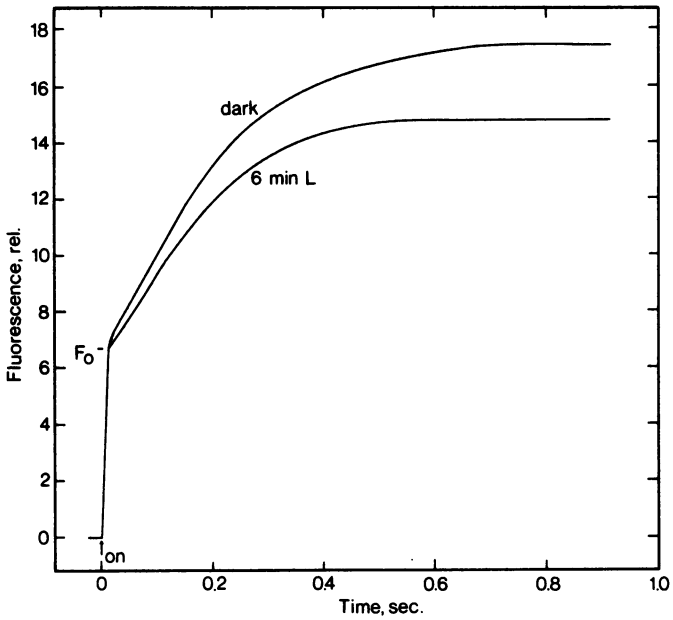

FIG. 5. Induction of Chl fluorescence before and after 6 min preillumination of *Porphyra* in the presence of DCMU. After the preillumination with 433 nm light, the thallus was kept in the dark for 2 min and the induction was measured. Green excitation light for measurement of the induction was obtained by passing white light from the lamp through Corning glass filters 4-96 and 3-96 and a Calflex C heat-reflecting filter (Balzers), and had an intensity of 5.9 μw/cm². Other conditions were the same as in Figure 2.

presence of DCMU also produces oxidants, but in this case the redox potentials of the oxidants may be much lower than those of the oxidants produced when the oxidizing side of PSII is inhibited.

In this study, we observed an acceleration of the back reaction of PSII which could serve to inhibit the formation of oxidants with high redox potentials in desiccated plants where oxidation of water was inhibited. Therefore, this mechanism as well as the other mechanisms reported previously (5, 13, 14) seem to be acting to prevent photoinhibition under natural conditions. It is possible that this acceleration of the back reaction is common to many plants although its effectiveness may differ between species. It may be that the redox state of a particular substance controls the back reactions of PSII. To control the rate of the back reaction, this substance must be located close to the reaction center of PSII, and also must be oxidizable by PSI as well as by PSII. The identity of this substance has not yet been determined. However, Cyt b_{559} may meet the conditions described for the substance above, since it is a) located very close to the reaction center of PSII, b) can be photooxidized by PSII even at 77K (4, 10, 15), and c) is also photooxidized by PSI at room temperature (4, 16). It is also possible that a mobile redox substance such as the superoxide anion may give rise to the effects observed here.

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