

Photosystem I-Mediated Regulation of Water Splitting in the Red Alga, *Porphyra sanjuanensis*¹

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ABSTRACT

The marine red alga, *Porphyra sanjuanensis* is found mainly in the high intertidal zone and at low tide subject to frequent and extreme water stress, often accompanied by high temperatures and light intensities. Such exposures can lead to severe desiccation which is accompanied by the progressive loss of photosynthetic activity. Even following the loss of more than 90% of the thallus water content the alga recovers rapidly when returned to seawater. This stress-induced, reversible inactivation of photosynthesis is believed to be a protective adaptation which prevents photodamage to the exposed alga. Effects of light, inhibitors of water splitting, and electron donors to PSI on variable fluorescence and water splitting suggest that activity of the oxygen evolving complex is regulated by the PSI-driven reduction of a component of intersystem electron transport.

The high-intertidal red alga, *Porphyra sanjuanensis* is extremely stress tolerant (29). It can withstand exposure to bright sun and high temperatures for many hours each day when the tide is out. In addition, it appears to sustain little damage from the osmotic effects of high salt concentrations during drying or from dilution by rainfall. Certain adaptations are necessary for any plant to resist stress-induced damage (12, 17). Various stresses have been reported to increase susceptibility to photodamage (17, 21, 25, 26). Among these is water stress and plants differ greatly in their ability to withstand water deficits (2). Generally the more resistant plants are those that retain their photosynthetic organs throughout periods of unfavorable exposures (*i.e.* evergreens, some mosses, lichens, and high intertidal marine algae) (3, 18, 26, 29). In some evergreen conifers, protection from photodamage during stress has been reported to involve a mechanism which controls chloroplast photochemical activity (6). We have examined aspects of the photosynthetic activity of *P. sanjuanensis* and conclude that it too has such a mechanism. The site of this control has been localized but its precise mechanism has yet to be elucidated.

In the two high intertidal red algal species *P. sanjuanensis* and *Porphyra perforata*, both F_v ² and O₂ evolution decrease as desiccation proceeds (29). On rehydration both functions rapidly

reappear. This water stress-induced loss of photosynthetic activity probably serves to protect the thylakoids from photodamage (22). Early effects of water stress in higher plants, in addition to stomatal closure, have been reported to be on the dark reactions of CO₂ assimilation (8, 13, 23). When CO₂ assimilation is inhibited, the demand for the photochemical products ATP and NADPH⁺ diminishes and unless photochemistry also declines, production of ¹O₂, O₂⁻, and derived radicals increases (9, 10). These products are damaging to both thylakoid pigments and membrane structure (9). Plants do have mechanisms which quench or dismutate active O₂ species but their capacity may be exceeded during stress, thereby inducing (a) photooxidations (10, 24), (b) photoinhibition (21, 26), and (c) membrane lipid peroxidations (9). In stress-sensitive plants, *i.e.* deciduous or annual plants, mechanisms which protect thylakoids from photodamage may be relatively less effective (1, 25, 29) than in stress-resistant plants. Both of these red algal species appear to regulate photochemistry by controlling OEC activity.

MATERIALS AND METHODS

Algae were collected at Dundarave beach in West Vancouver or from the shore of Stanley Park in Vancouver, B.C. and kept in sea water for up to 3 d before use. In some experiments algal samples were incubated in the dark for at least 1 h in sea water containing 10 μM DCMU, with or without the additions shown in Table I.

For fluorescence measurements light was provided by a 30 W EKZ projector lamp, passed through a Corning 4-96 cutoff filter

Table I. Effects of Electron Donors to PSI or Inhibitors of Water Splitting on Variable PSII Chl *a* Fluorescence and O₂ Evolution in *P. sanjuanensis*

DCMU concentration was 5 μM; DAD, 0.1 mM; Tris, 0.8 M; CCCP, 5 μM; DCPIP and DCPIPH₂, 0.1 mM; antimycin A, 50 μM; TMPD⁺ and TMPD, 0.1 mM; CH₃NH₂, 0.1 mM; NH₂OH, 1.0 mM. Light intensities: F_v , 0.35 W m⁻² (550 nm); O₂ evolution, saturating white light.

Additive	Effect on F_v + DCMU	Effect on O ₂ Evolution - DCMU
None	220 min decay	
DAD ^a	5 min decay	Rapid inhibition
Tris, CCCP	45 min decay	Slow inhibition
DCPIPH ₂ ^a	90 min decay	None
DCPIP	No effect	None
Antimycin A	No effect	None
TMPD ⁺	No decay, F_v increase	None
TMPD ^a	45 min	None
CH ₃ NH ₂	No decay, F_v increase	None
NH ₂ OH	Partial decay or partial recovery	Inhibition

* Ascorbate (5 mM) added to maintain the reduced forms.

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² Abbreviations: F_v , variable Chl *a* fluorescence emission; APC, allophycocyanin; DAD, diaminodurene; DCPIP, dichlorophenolindophenol; CCCP, carbonyl cyanide-*m*-chlorophenylhydrazine; OEC, the oxygen evolving complex; P₆₈₀, the PSII reaction center; PC, phycocyanin; PE, phycoerythrin; PQ, plastoquinone; Q_A, the primary electron acceptor of PSII; TMPD, *N,N,N',N'*-tetramethylphenylenediamine.

and controlled by a Compur electronic shutter with an electronic timer (SFU, ES-217). A bifurcated fiber optics conducted the excitation (8.7 W m^{-2}) to the sample and the fluorescence to an EMI 468R photomultiplier protected from excitation light by a red Corning 2-64 cutoff filter or Balzers narrow band interference filters. Fluorescence emission was recorded on a Tektronix 5031N storage oscilloscope.

Other fluorescence measurements were performed using a dual wavelength fluorescence spectrophotometer (Perkin-Elmer MPF-44B). Samples, $1 \times 3 \text{ cm}$, of the single cell layered thalli were immersed in sea water in a cuvette between two nylon screen grids at 45° to the excitation beam. Excitation was 0.35 W m^{-2} at either 433 or 550 nm with the excitation slit set at 5 nm and the emission slit set at 1 nm. Emission spectra were scanned at 60 nm min^{-1} and recorded on an x-y recorder. The kinetics of single waveband emission was recorded on a strip chart recorder at constant excitation and emission wavelengths. Because of the thinness of the algal thallus, self absorption is not considered to have affected the results appreciably. Low temperature emission spectra were recorded as described above, except that the thallus was mounted in a transparent Dewar flask containing liquid N_2 . O_2 evolution was recorded on a strip chart recorder using a Clarke-type electrode.

The blue, oxidized form of TMPD, TMPD^+ was prepared by dissolving $\text{TMPD} \cdot \text{HCl}$ in 1% v/v methanol in sea water and aging this solution overnight in the presence of air. Concentrations of inhibitors of O_2 evolution or electron donors to PSI were shown in Table I. All solutions were maintained at pH 7.8.

RESULTS

The fluorescence emission spectrum of untreated *Porphyra sanjuanensis* excited with 550 nm light (0.35 W m^{-2}) is similar to that of Figure 1A. The fluorescence peak at 580 nm, the shoulder at 640 nm, the peak at 661 nm, and the shoulder at 685 nm arise from PE, PC, APC, and PSII Chl *a*, respectively (20). In continuous illumination, within 5 min after adding DCMU, there was an approximate 5-fold increase in the magnitude of the 685 nm peak (Fig. 1B). After 1 h dark adaptation, the single waveband emission time courses shown in Figure 2 were obtained with the DCMU treated samples using 550 nm

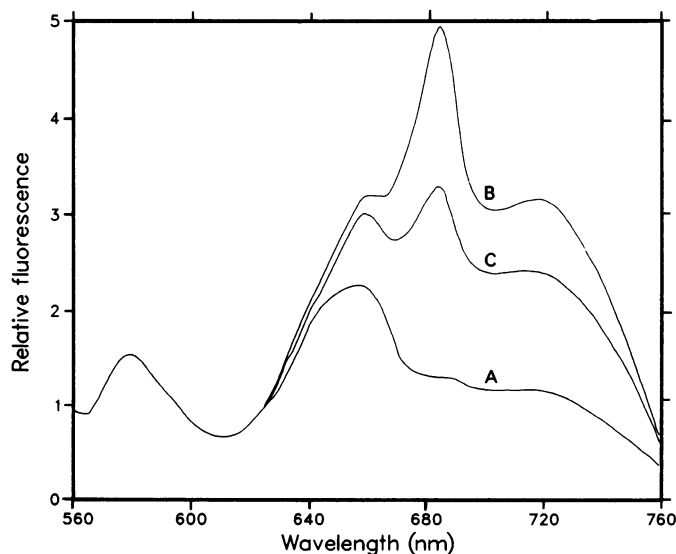


FIG. 1. Fluorescence emission spectrum of *P. sanjuanensis* with 550 nm, 0.35 W m^{-2} excitation. A, Before the addition of DCMU or on completion of the F_V decay after DCMU was added; B, 5 min after the addition of DCMU; C, with the addition of NH_2OH either before or after the F_V decay was completed when DCMU was added.

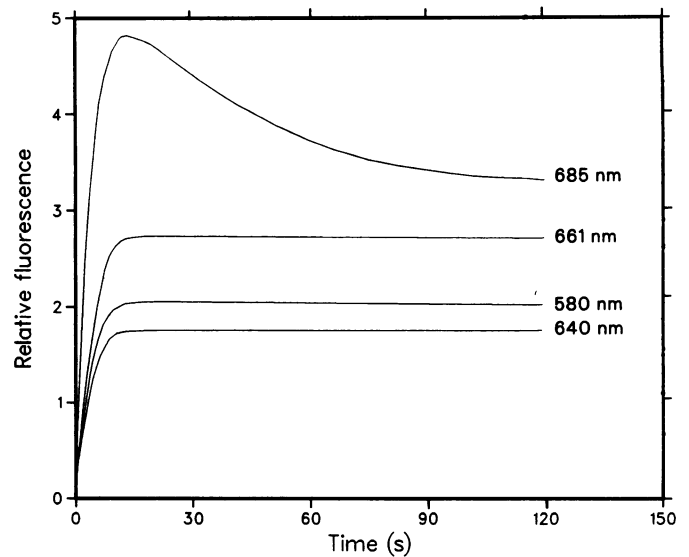


FIG. 2. Time courses of single waveband fluorescence emission in dark-adapted DCMU-treated *P. sanjuanensis*. Excitation was at 550 nm, 8.7 W m^{-2} .

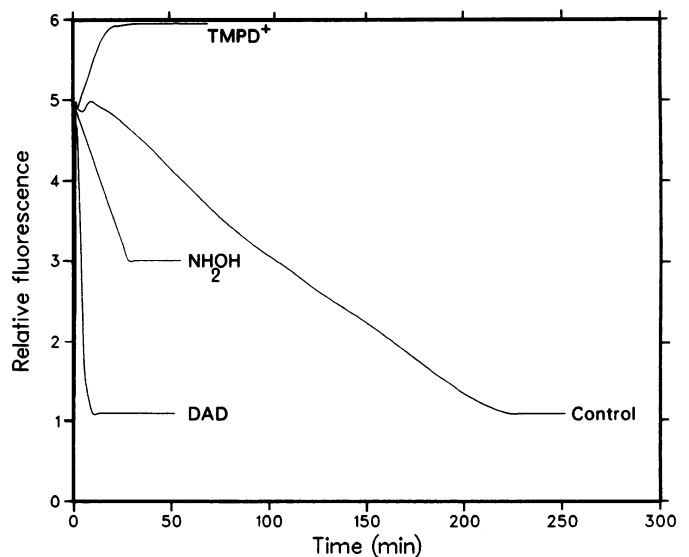


FIG. 3. Effects of additives on F_V in DCMU-treated *P. sanjuanensis*. Samples were incubated 1 h in DCMU and illuminated at $t = 0$; other additions made at $t = 5$ min. Excitation as in Figure 1.

excitation (8.7 W m^{-2}). Only the 685 nm emission decayed while the phycobilin emissions remained constant. This indicates that there was no change in energy transfer via phycobilisomes and that the 685 nm quenching is independent of phycobilin excitation.

In DCMU-treated samples, not dark-adapted, and continuously illuminated at 550 nm (0.35 W m^{-2}), the 685 nm fluorescence decayed (Fig. 3) to nearly the level of the untreated controls in about 220 min. Absorbance changes cannot account for this decay since there was no difference in the thallus absorption spectrum before and after DCMU treatment (data not shown). The decay was not accompanied by a recovery of O_2 evolution indicating that electron transport remained blocked. Satoh and Fork (22) reported a similar decay in PSII fluorescence in the high intertidal *P. perforata* treated with DCMU and antimycin A. Low temperature emission spectra (not shown) were not influenced by the stage of F_V decay at the time of freezing,

indicating that the decay depends on processes inoperative at 77°K. This result suggests that the decay does not involve a state transition or PSII → PSI energy transfer.

The dependence of the decay on light intensity (Fig. 4A) suggests that it is light driven. Independence of the end point on intensity appears to indicate that the decay represents the effect of light absorption on a pool of finite size. Recovery of F_V was rapid in the dark (Fig. 4B) and the total amount of emission recovered (yield *versus* time) depended on the length of the dark interval (Fig. 4C). The near maximum F_V amplitude was regained with a brief interruption of excitation light and the rate of the subsequent decline to the original decay curve depended on the dark time.

Satoh and Fork (22) reasoned that the loss of PSII fluorescence was probably due to a back reaction which quenched the Chl *a* emission. We carried out further experiments to investigate possible mechanisms and control of such a back reaction. The 685 nm fluorescence was monitored using continuous 550 nm excitation (0.35 W m^{-2}). Upon illumination of DCMU-treated samples, the emission was recorded until it reached its maximum in about 5 min (Fig. 3). At this maximum, various chemicals known to influence electron transport or other photosynthetic activities were added (Table I). The addition of 0.1 mM DAD, with 5 mM ascorbate, accelerated the decay so that it was complete in 5 min (Fig. 3). TMPD accelerated the decay to a lesser extent (about 45 min to completion). In contrast, addition of 0.1 mM TMPD⁺ eliminated the decay and increased the emission (Fig. 3).

The effects of these and various other additions on both F_V and DCMU-free O_2 evolution are summarized in Table I. NH_2OH , which inhibited O_2 evolution, accelerated the decay but only to the extent that about half of the maximum emission was lost (to about 3 units in Fig. 3). The decay was slightly more rapid with 10 mM NH_2OH (data not shown), but the end result was not different. In DCMU treated samples in which the decay had gone to completion, about half (3 units in Fig. 3) of the fluorescence rapidly recovered upon the addition of NH_2OH . In addition to its effect on F_V (see above) DAD (\pm ascorbate) almost completely inhibited O_2 evolution. Antimycin A, DCIP, DCIPH₂, CH_3NH_2 , TMPD, and TMPD⁺ had no significant effect on O_2 evolution. With NH_4Cl , O_2 evolution was inhibited less than 5% after 30 min. Tris, CCCP, and NH_2OH inhibited O_2 evolution but required about 25 min to exert their maximum effects.

Lack of an observed effect of antimycin A in these experiments is most likely explained by our procedures. When this inhibitor of cyclic photophosphorylation was added at $t = 5$ min (Table I; Fig. 3) no acceleration of the decay was observed. On the other hand, there was pronounced acceleration in samples incubated with antimycin A for 12 h in darkness (data not shown), an effect also reported by Satoh and Fork (22).

DISCUSSION

The results of our experiments indicate that in *Porphyra sanjuanensis*, both photochemistry and electron transport are influenced, or perhaps regulated, by a light-driven mechanism affecting the activity of PSII. In illuminated DCMU-treated samples, F_V decayed to a minimum value. The decay rate depended on intensity of the excitation light. Recovery to the maximum F_V amplitude occurred rapidly in the dark but the total amount of fluorescence emission depended on the length of the dark interval. Recovery of F_V in the dark indicates that while these treatments influenced the activity of PSII partial reactions, they had no irreversible effects. It is generally accepted that at any given instant the amplitude of variable Chl *a* fluorescence is an indicator of the redox state of the primary PSII electron acceptor, Q_A (19). Karukstis and Sauer (14) reported

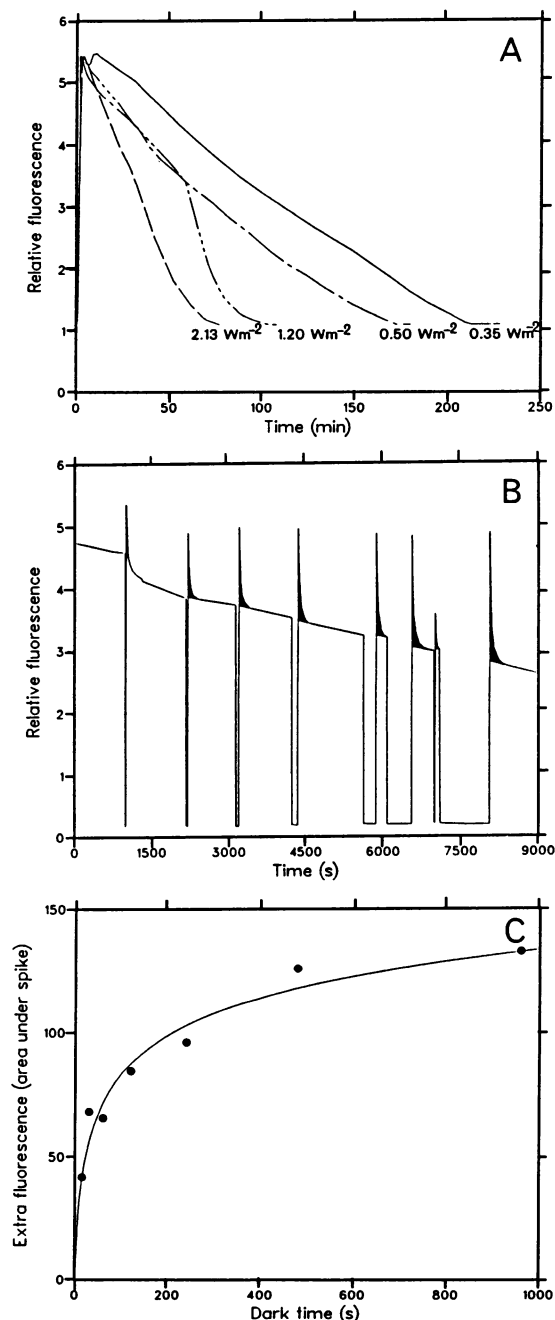


FIG. 4. A, The effect of light intensity on the F_V decay in DCMU-treated *P. sanjuanensis*. B, Recovery of F_V when the light-induced decay was interrupted by dark intervals of 15, 30, 60, 120, 240, 480, 15, and 960 s. Note that on reillumination F_V rapidly decayed to the initial decay rate and that the area under the dark-induced spikes increased with the length of the dark interval. The small spike after the second 15 s dark interval indicates that the magnitude of the dark recovery depends on the dark time and unrelated to the sequence of light interruptions. C, Graph of the area under the dark F_V recovery spikes in B as a function of the duration of the dark interval. Each point represents the mean of four determinations. Equation for the drawn curve is $1/\text{area} = 0.22657 (1/\text{time}_{\text{C(dark)}}) - 0.03888$, suggesting a first order process. Excitation was at 550 nm; for curves B and C intensity was 0.35 W m^{-2} .

that some of the PSII fluorescence may be due to charge recombination. The large increase in Chl *a* fluorescence observed following the addition of DCMU, despite the small PSII Chl *a* complement (11) suggests that in *P. sanjuanensis* the bulk of the emission arises from charge recombination. The data obtained with electron donors to PSI in the presence of DCMU supports this idea since it might be expected that when intermediate electron carriers are reduced F_v would be high, but the opposite was found. This result also shows that no electron transfer occurred between PQH₂ and P₆₈₀ when DCMU was present. Our findings, then, can be accounted for in terms of effects on processes leading to the reduction of Q_A (increase in F_v) or its oxidation (decrease in F_v). Oxidation of Q_A could result from electron transfer toward PSI either through transfer to P₇₀₀ or the reduction of an acceptor pool. The presence of DCMU and the nonrecovery of O₂ evolution indicate that this explanation is unlikely. Alternatively, DCMU could induce a transient increase in energy transfer from PSII to PSI chlorophylls but evidence for this is lacking (Fig. 1). The time-dependent fluorescence decay would then seem to be due to either the activation of a quenching back reaction (22) involving a nonemitting charge recombination between Q_A and a component on the oxidizing side of PSII (7) or a decrease in electron transfer to P₆₈₀. A decrease in electron transport is the interpretation favored by us and this could result from the light-dependent inactivation of OEC. Partial restoration of emission by NH₂OH is consistent with OEC inactivation as this substance both inhibits water splitting and can donate electrons to PSII (5).

The rapid and opposite effects of TMPD⁺ and electron donors to PSI suggest a key to a mechanism by which water splitting may be regulated. DCPIPH₂ and TMPD which are known to reduce electron donors to PSI (16, 27, 30), did not affect water splitting in *P. sanjuanensis*, but accelerated the light-driven F_v decay. On the other hand, TMPD⁺ which has been reported to oxidize PQH₂ (4, 28), prevented the decay and even increased the amplitude of F_v , yet had no effect on water splitting. DAD, which also reduces PSI intermediates (27), strongly accelerated the decay and inhibited O₂ evolution. Possible explanations for these effects are either that a quenching back reaction is enhanced when (a) component(s) of the intersystem electron transport chain is (are) reduced or (b) that this blocks water splitting by OEC. Satoh and Fork (22) suggested that the redox state of a mobile substance near PSII controlled a quenching back reaction but the effect of NH₂OH appears to indicate that water splitting itself is controlled. The known inhibitors of water splitting, CCCP, and Tris (15, 31) both accelerated the decay and inhibited O₂ evolution. It is clear that chemical treatments which either reduce intersystem electron carriers or inhibit water splitting both cause a drop in F_v . On the other hand, treatments which reoxidize the intersystem carriers prevent the light-driven decay of F_v , indicating that the OEC remains active under these conditions.

Light dependency of the decay suggests that the reduction of a rather large acceptor pool is involved. Time-dependent recovery likewise suggests that whatever the regulating substance may be, it becomes reoxidized in the dark. Although it is probably necessary to add the dark reoxidation (Fig. 4B) to the decay rate, that the pool size appears large may indicate that PQH₂ is the regulator. Satoh and Fork (22) reported PSI excitation to be more efficient in inducing the fluorescence decay in *P. perforata* than PSI excitation (we have obtained this result with *P. sanjuanensis*), which clearly demonstrates that the control is mediated by PSI. These results are consistent with the idea that when the reoxidation of Q_A is blocked, cyclic electron flow from PSI can reduce a component of the intersystem transport chain, possibly PQ, which in turn regulates the activity of OEC.

Our findings can be interpreted to account for the loss of

variable fluorescence when O₂ evolution ceases due to desiccation in *P. sanjuanensis* and *P. perforata* (29). It is under the conditions of water stress that the risk of photodamage is greatest. Therefore, it is consistent with the ecology of *P. sanjuanensis* that the protective mechanism operates maximally when dark biochemical reactions of CO₂ assimilation are stress inhibited. Regulation might occur even in the absence of water stress if under high light intensity water splitting activity exceeded the electron accepting capacity of PSI.

CONCLUSIONS

1. In *P. sanjuanensis* the bulk of the variable Chl *a* fluorescence appears to arise from the recombination of Q_A⁻ and P₆₈₀.
2. Our data suggest that the activity of OEC is regulated by the action of PSI in reducing a component, possibly PQ, of the intersystem electron transport chain.
3. The function of this control is probably to prevent photodamage to the alga at times when electrons derived from water splitting exceed the capacity of PSI to utilize them, *i.e.* under stress or high light intensity.

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