State Transitions or ΔpH-Dependent Quenching of Photosystem II Fluorescence in Red Algae

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ABSTRACT: Fluorescence changes attributed to state transitions have been shown to exist in phycobilisome-containing organisms. Contradictory conclusions have been derived from studies about the mechanism of state transitions carried out either in cyanobacteria or in red algae. In this paper, fluorescence changes induced by light 1 and light 2 are reinvestigated in a unicellular red alga, *Rhodella violacea*, by performing 77 K fluorescence spectra and fluorescence yield measurements at room temperature in the presence of uncouplers and inhibitors of the electron transfer. We show that transfer of light 1-adapted cells to light 2 (green light) induces a large quenching of photosystem II which is suppressed by subsequent incubation in light 1 (far-red or blue light). The level of the photosystem I-related fluorescence does not change during these transfers. We demonstrate that the large quenching of photosystem II induced by low intensities of green light is completely suppressed by addition of NH_4Cl, an uncoupler that inhibits ATP synthesis by canceling the ΔpH across the membrane. DCCD, which is an inhibitor of the ATPase that swells the ΔpH, maintains the quenched state even under light 1 illumination. The opposite effects of DCMU and DBMIB on state transitions are demonstrated to be due to a suppression (by DCMU) or maintenance (by DBMIB) of the ΔpH and not to a change in the redox state of the plastoquinone. We conclude that, in *R. violacea*, the fluorescence change commonly associated with state 2 transition is in fact a ΔpH-dependent quenching. This type of quenching has always been associated with near-saturating light intensities. Here, we show that very low intensities of a light that activates only the photosystem II induce a ΔpH across the membrane that is not dissipated since the ATPase is not activated. The ΔpH is dissipated only under conditions in which the photosystem I turns, confirming that the thioredoxin must be reduced to activate the ATPase. We suggest that the fluorescence changes, induced by various light conditions, in cyanobacteria and red algae could be associated with different phenomena.

Higher plants, algae, and cyanobacteria convert light energy into chemical energy. Light-driven reactions take place in the thylakoids at the level of two membrane pigment−protein complexes, named photosystem I (PS I)[1] and photosystem II (PS II). Quinone reduction and oxygen evolution occur in PS II, while plastocyanin oxidation and NADPH reduction occur in PS I. ATP (chemical energy) and NADPH (reducing power) are used in the bioconversion of CO_2 into sugars. ATP is synthesized during the electron transfer from PS II to PS I and during the cyclic electron transfer around the PS I, while the reduction of NADP is related only to linear electron transfer. Photosynthetic organisms developed different mechanisms to respond to variations of the incident light. It is often assumed that such responses serve to optimize the utilization of the captured energy, to balance the production of ATP and NADPH, and/or to avoid photodamage. In green algae and leaves, when the PS II is preferentially illuminated, or when the balance between the incoming irradiation and the dissipative capacity of carbon assimilation is broken (under high white light illumination), a large quenching of the PS II fluorescence level is induced. This quenching is a consequence of a redistribution of the absorbed energy (state transitions) [see Williams and Allen (1987)] and/or of an increase of radiationless energy dissipation which depends on a ΔpH across the membrane (q_e) [see Krause and Weis (1991)]. Under weak illumination, state transitions are the only form of quenching observed, while under high illumination, the ΔpH-dependent quenching is predominant (Walters & Horton, 1991). Very high light intensities induce a quenching of PS II fluorescence associated with photoinhibition [see Prasil et al. (1992)].

Bonaventura and Myers (1969) and Murata (1969) first showed that exposure of algae to light predominantly absorbed by PS II causes a relative decrease of the PS II fluorescence. When PS I is preferentially illuminated, this relative decrease is prevented. The state produced by light 1 is called state 1 and that produced by light 2 state 2. The molecular bases of state transitions in green algae and higher plants have been widely studied over the years [reviewed in Williams and Allen (1987), Biggins and Bruce (1989), and Allen (1992)]. The hypothesis of a preferential migration of LHCII from PS II to PS I (from grana to stroma lamellae) causing an increase in the part of the incident light absorbed by PS I in state 2 is now the most widely accepted hypothesis (Horton et al., 1981; Horton, 1983; Barber, 1983; Haworth

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[1] Abbreviations: Chl, chlorophyll; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCCD, *N,N*′-dicyclohexylcarbodiimide; DCMU, 3-(3,4-dichlorophenyl)-1,1′-dimethylurea; F_o and F_{max}, initial and maximum fluorescence level, respectively; LHCII, light-harvesting chlorophyll *a*/*b* protein; PQ, plastoquinone; PS I, photosystem I; PS II, photosystem II; Q_A and Q_B, primary and secondary quinone electron acceptors in PS II, respectively; RC II, reaction center II.

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et al., 1982a,b; Kyle et al., 1982, 1983, 1984; Holzwarth, 1986). Preferential illumination of PS II leads to the reduction of the plastoquinone (PQ) pool. A membrane-bound kinase is, in turn, activated, and several polypeptides of the LHCII are phosphorylated. The phosphorylated LHCII moves away from the PS II and migrates to the PS I. The membrane-bound kinase is deactivated in the dark or when the PS I is predominantly illuminated, conditions in which the PQ pool is more oxidized. In these conditions, the LHCII is dephosphorylated by a light-independent phosphatase protein (Bennett et al., 1980; Horton & Black, 1980, 1981; Allen et al., 1981; Horton et al., 1981; Steinback et al., 1982; Telfer et al., 1983; Canaani et al., 1984).

It was first proposed that the principal role of state transitions was the redistribution of the absorbed energy in order to maximize the overall efficiency of photosynthesis under any given light condition (Bonaventura & Myers, 1969; Murata, 1969). More recently, Bulté et al. (1990) suggested that a main function of state transitions could be the balance between the production of ATP and NADPH. They showed that the inhibition of ATP production in intact cells of *Chlamydomonas reinhardti* leads to a transition to state 2 while an increase of the ATP content causes reversion to state 1. They proposed that ATP depression causes starch degradation and in turn NADPH formation that reduce the PQ pool via a NADPH/PQ oxidoreductase. In this condition, the LHCII kinase is activated and the state 2 transition is produced. Thus, oxidative conditions and a high concentration of ATP favor state 1, whereas reducing conditions and ATP depression favor state 2. This hypothesis is supported by the works of Vallon et al. (1991) and Delosme (1991) which related the fast or slow activity of the cytochrome b_6/f complex to its location near PS I in the presence of a high ATP concentration or near PS II when the ATP concentration is low.

In red algae and cyanobacteria, fluorescence yield changes linked to the wavelength of illumination have also been observed. A quenching of PS II fluorescence was observed in phycobilisome-containing cells illuminated with light 2, conditions in which the PQ pool is reduced, or in cyanobacteria cells incubated in the dark under anaerobic conditions in which the content of ATP is low and the PQ pool is also reduced (Murata, 1969; Mohanty & Govindjee, 1973; Ried & Reinhardt, 1977, 1980; Ley & Butler, 1980; Williams et al., 1981; Fork & Satoh, 1983; Biggings et al., 1984; Bruce et al., 1985; Mullineaux et al., 1986; Olive et al., 1986; Dominy & Williams, 1987). The PS II light-harvesting antenna of these organisms is very different from LHCII since it consists of a large extramembrane phycobilisome [see for review Gantt (1980) and Glazer (1984)]. Hence, the mechanism of changes in energy distribution due to state transitions is likely to be different in plants and phycobilisome-containing organisms.

The study of fluorescence changes related to different types of illumination (light 1 versus light 2) in phycobilisome-containing organisms was carried out in red algae and in cyanobacteria. It was assumed that the same mechanism of state transitions exists for both organisms. However, contradictory conclusions were derived from these studies. Allen's group (Allen et al., 1985; Mullineaux et al., 1986; Sanders & Allen, 1988; Harrison et al., 1991), Dominy and Williams (1987), and Williams and Dominy (1990), who studied state transitions in different strains of cyanobacteria, proposed that state transitions were controlled by the redox state of the plastoquinone as in green algae and higher plants. Allen et al. (1985) presented evidence for a light 2-dependent phosphorylation of some polypeptides of the thylakoids and the phycobilisomes. They proposed that the phycobilisomes became dissociated from PS II when these polypeptides were phosphorylated in state 2. However, subsequent works of other laboratories did not support this hypothesis. Biggins's group (Biggins et al., 1984; Biggins & Bruce, 1985, 1989), who used the red alga *Porphyridium cruentum* in their studies, suggested that the redox state of plastoquinone and the phosphorylation reactions were not involved in state transitions in phycobilisome-containing organisms. They (Biggins et al., 1984) did not detect any change in protein phosphorylation during the conversion of *P. cruentum* cells between state 1, state 2, and darkness. They proposed that transition to state 1 was induced by a local electrochemical gradient around PS I generated by cyclic electron transfer that results in a small change in the thylakoid conformation, increasing the spillover between the PS II and PS I. By using the phosphoprotein phosphatase inhibitor NaF and the protein kinase inhibitor staurosporine, we have recently demonstrated that state transitions in the red alga *Rhodella violacea* were independent of protein phosphorylation (Delphin et al., 1995).

Another difference between cyanobacteria and red algae is their dark-adapted state. It has been largely demonstrated that green algae and cyanobacteria cells are in state 2 in the dark (Williams & Salamon, 1976; Williams et al., 1980; Fork & Satoh, 1983; Mullineaux & Allen, 1990). It was proposed that the plastoquinone is reduced in the dark by oxidation of chlororespiratory (green algae) (Bennoun, 1982; Wollman & Delepelaire, 1984) or respiratory (cyanobacteria) substrates [see review in Schmetterer (1994)]. In red algae, studies about state transitions in *Porphyra* (Satoh & Fork, 1983) and *Porphyridium* (Murata, 1970; Ley & Butler, 1980; Biggins & Bruce, 1985) showed that the dark level of the PS II fluorescence was intermediate between state 1 and state 2. The dark state seemed to be closer to state 1 (Murata, 1970; Ley & Butler, 1980). This result suggested that the plastoquinone pool is oxidized in the dark or that the fluorescence level did not depend on the redox state of the plastoquinone in red algae. The fact that the two different hypotheses about the induction of state transitions were the result of works in cyanobacteria or in red algae and the differences in the dark-adapted state of these cells suggest that fluorescence changes observed under light 1 or light 2 could have different origins in these two organisms.

In this controversial context, we have undertaken a series of experiments to re-examine the state transition mechanisms in red algae. Founding our conclusion upon 77 K fluorescence emission spectra and room-temperature fluorescence yield measurements in a unicellular red alga, *R. violacea*, we show that low intensities of green light induce a quenching of PS II fluorescence which is completely suppressed by addition of the uncoupler NH_4Cl. The PS II quenching is also relaxed by far-red illumination or DCMU addition, while it is maintained by DBMIB or DCCD. We also show that the PS I-related fluorescence is not modified during this process. We conclude that, in red algae, the so-called state 2 is a ΔpH- dependent PS II quenched state. Moreover, in green plants, the ΔpH-dependent quenching was always associated with near-saturating light intensities. Here, we demonstrate that low intensities of light 2 could

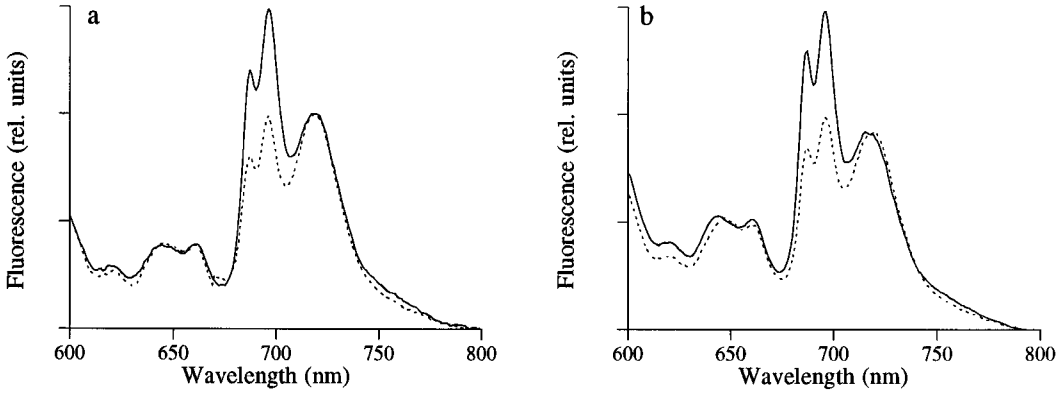

FIGURE 1: Fluorescence emission spectra (77 K) of cells under different light illuminations. (a) Cells were illuminated for 4 min with far-red light (solid line) or green light (25 μE m^{-2} s^{-1}) (dashed line). (b) Cells were illuminated for 4 min with 10 μE m^{-2} s^{-1} white illumination (solid line) and with 500 μE m^{-2} s^{-1} white light. The 77 K fluorescence emission spectra of dark-adapted cells were identical to those of cells illuminated with far-red light. The excitation wavelength was 560 nm, with a 10 nm slit width.

generate a large ΔpH responsible for the PS II quenched state.

MATERIALS AND METHODS

Culture of Algae. The unicellular marine red alga *R. violacea* (strain 115-79, from Göttingen University) was grown photoautrophically in sterile sea water enriched with $NaNO_3$ (0.2 g L^{-1}), Na_2HPO_4 (0.03 g L^{-1}), vitamin B_{12} (2.5 $\times$ 10^{-2} μg mL^{-1}), $FeCl_3 \cdot 6H_2O$ (0.6 mg L^{-1}), and NaEDTA (12.5 μM). The cultures were grown at 20 °C with a 16 h light/8 h dark photoperiod. They were continuously bubbled with sterile air. Light was provided by cool-white Philips fluorescent tubes at an intensity of 60 μE m^{-2} s^{-1}. To standardize the culture conditions, cells were regularly diluted every 2 days. Exponential phase cells, at a concentration of about 10^6 cells/mL, were collected from 2-day-old cultures. The harvested cells were kept in the dark or at low intensities of white light.

Fluorescence Measurements. The 77 K temperature fluorescence emission spectra were recorded on a Hitachi F-3010 Fluorescence Spectrophotometer. The excitation monochrometer was set to 560 $\pm$ 5 nm. Emission was scanned from 600 to 800 nm (slit width of 3 nm). Cell suspension (3 mL) was preilluminated in a refrigerated cuvette (1 cm diameter). Then, 150 μL of the suspension was quickly filtered and the filter immediately plunged into liquid nitrogen. Different light regimes were provided by a 150 W quartz-iodine lamp. The white light intensities ranged from 1 to 1000 μE m^{-2} s^{-1}, while the green light intensities (540 $\pm$ 10 nm) ranged from 0.25 to 25 μE m^{-2} s^{-1}. The decreasing intensities for both lights were obtained with neutral density filters added in front of the lamp. For the deconvolution of fluorescence spectra, data were first expressed as a function of wavenumbers (cm^{-1} $\times$ 10^{-3}). Fitting was carried out with the general curve fit of Kaleidragraph (Abelbeck software) programmed for eight Gaussian components whose maxima were deduced from the fourth derivative of the spectra. Data were finally expressed as a function of wavelength.

Room-temperature fluorescence was measured at 18.5 °C with a modulated fluorometer (PAM chlorophyll fluorometer; Walz, Effelrich, Germany) adapted to a DW1 Hansatech oxygen electrode as previously described (Arsalane et al., 1994). Saturating multiple turnover white light pulses (3200 μE m^{-2} s^{-1}) were produced by an electronic shutter (Uniblitz, Vincent, USA, opening time of 2 ms) put in front of a KL-1500 quartz-iodine lamp (Schott, Mainz, Germany) and controlled by the accessory module PAM-103. To prevent variations due to fluorescence induction kinetics, data were averaged in the time range 400−800 ms (acquisition time of 33 μs) after shutter opening. Different background continuous illuminations were obtained from another KL-1500 lamp that was either used as white light or filtered at 540 $\pm$ 10 nm for green light (Oriel 70617 AM-4064) or at 435 $\pm$ 15 nm for blue-violet light (Seavem). Far-red light (100 μE m^{-2} s^{-1}) was obtained from a light emission diode (102-FR, Walz) filtered through a Schott RG 9 filter (735 $\pm$ 20 nm). Data acquisition, shutter control, and pulse averaging were driven by homemade software through a 12-bit analogic digital converter as previously described (Arsalane et al., 1993).

RESULTS

The Wavelength of Illumination Influences the 77 K Fluorescence Emission Spectra in R. violacea

In red algae, the emission spectra obtained by illuminating the cells with blue, red, or far-red lights which are preferentially absorbed by the antenna of the PS I are different from that obtained by green light illumination, absorbed by phycoerythrin, one of the components of the phycobilisome (the antenna of the PS II). Figure 1a shows the 77 K fluorescence emission spectra of dark-adapted *R. violacea* cells exposed for 3 min to far-red light in order to preferentially excite PS I (light 1) or to green light to specially activate PS II (light 2). Fluorescence emission at 685 and 695 nm originates from PS II. The first peak mainly derives from the phycobilisome terminal emitter and the second from the Chl *a* core antenna of PS II. The third main peak, observed at 718 nm, is related to the Chl *a* antenna of PS I. Phycocyanin (at 643 nm) and allophycocyanin (at 661 nm) also contribute to the fluorescence spectrum. The ratio between PS II and PS I fluorescence significantly differed in dark-adapted cells exposed to green light or to far-red light. The values of the ratio F695/F718, which were used as an indicator of fluorescence changes, varied from 1.5−1.7, in the dark or under light 1 (Figure 1a, solid line), to 1−1.2, under light 2 (Figure 1a, dashed line). These fluorescence patterns were associated with state 1 and state 2, respectively.

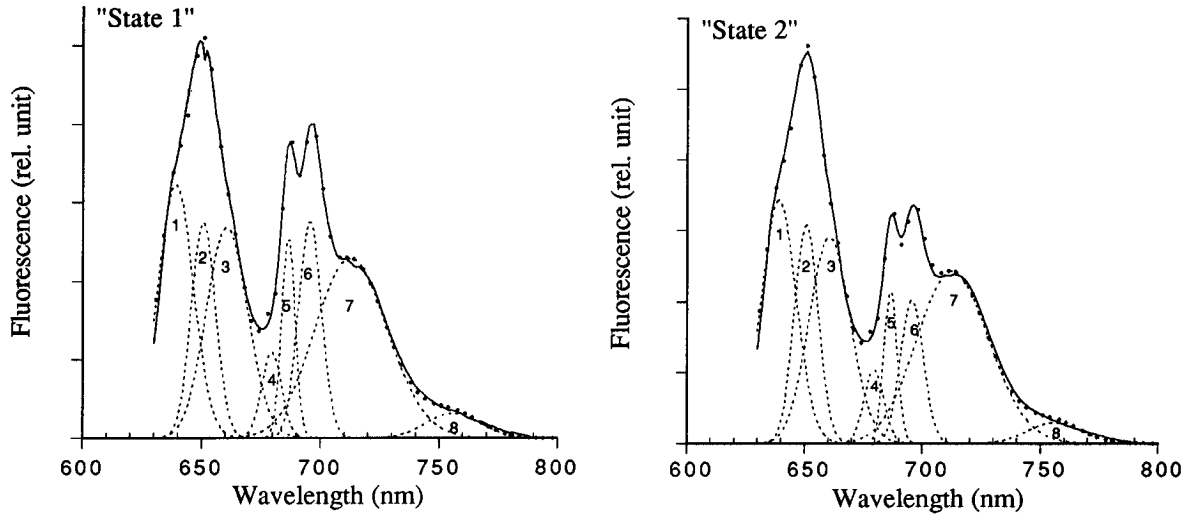

FIGURE 2: Deconvolution of state 1 and state 2 77 K fluorescence spectra in eight Gaussian components. The main peak, around 650 nm, related to *Spirulina* phycocyanin, is used as the probe: solid line, experimental data; dashed lines, deconvoluted Gaussian components; and bold points, sum of the Gaussian components ($R = 0.999\ 33$ for state 1 and $R = 0.9807$ for state 2). Mean wavelength and the standard deviation of each Gaussian: 1, 639 nm (1.8); 2, 650.7 nm (1.2); 3, 660.5 nm (2); 4, 679.5 nm (0.9); 5, 686.5 nm (0.6); 6, 695.5 nm (1); 7, 712.5 nm (3); 8, 756 nm (2).

Table 1: Comparison of the Elementary Gaussian Components G6 (PS II Fluorescence) and G7 (PS I Fluorescence) to G2 (Probe Fluorescence), in State 1 and State 2, as Shown in Figure 2[a]

	state 1		state 2	
ratio of areas	G2/G6	G2/G7	G2/G6	G2/G7
M	**1.19** $\pm$ 0.07	**0.48** $\pm$ 0.03	**1.79** $\pm$ 0.11	**0.50** $\pm$ 0.03

[a] Bold numbers denote the average value of each ratio, calculated from five experiments.

In order to determine the origin of the changes in the F695/F718 ratio, an external fluorescence probe, *Spirulina maxima* phycocyanin, was added to cell suspensions. Low-temperature emission spectra obtained in green and far-red light were deconvoluted into eight elementary Gaussian components as shown in Figure 2. The areas of the Gaussian components corresponding to F695 and F718 (curves 6 and 7 in Figure 2) were calculated in cells under light 1 and light 2 illumination. They were then compared to the area of the Gaussian component characteristic of the added probe, F650 (curve 2 in Figure 2). The calculated ratios F650/F695 and F650/F718 (Table 1) showed that PS I fluorescence was similar in far-red light and green light, while PS II fluorescence was higher in cells illuminated with far-red light than in cells illuminated with green light. In conclusion, the changes in the F695/F718 ratio were due to a variation of the PS II fluorescence with no concomitant change of the PS I fluorescence. These results suggested that a redistribution of the absorbed energy between PS II and PS I was not induced when one of the photosystems was preferentially illuminated. We can say that, more than state 2 (which involves changes in the cross section of the antennae of both PS II and PS I or in the spillover), light 2 induced a quenching of PS II fluorescence in *R. violacea* cells. Nevertheless, along with other studies, we will also call the PS II quenched state apparent "state 2" since it was induced by light 2 and the high PS II fluorescence state apparent "state 1" since it was induced by light 1.

These types of spectra were observed under other conditions in *R. violacea* cells. When dark- or far-red-adapted cells were illuminated with near-saturating white light intensities, a decrease in the F695/F718 ratio was observed, whereas when the cells were illuminated with low intensities of white light, they remained in a high PS II fluorescence state (Figure 1b).

Induction of the Quenched PS II Fluorescence State by Green and White Light

Effect of Different Light Intensities on the Formation of the PS II Quenching by Green and White Light. We compared the efficiency of different intensities of green and white light to generate the quenching of the PS II fluorescence. The time course of the transition to the PS II quenched state was determined for two light intensities: 2.5 and 25 μE m^{-2} s^{-1} for green light and 10 and 500 μE m^{-2} s^{-1} for white light. Cells preilluminated for 5 min with far-red light were transferred to green or white light, and 77 K fluorescence spectra were recorded after various periods of illumination. The ratio F695/F718, calculated from the 77 K fluorescence emission spectra, was used to measure the extent of the transition to the PS II quenched state. Figure 3 shows the kinetics of the decrease of the ratio F695/F718. Both intensities of green light illumination produced the same decline of PS II fluorescence, but the kinetics were quite different depending on the light intensity (Figure 3a). When cells were exposed to the highest intensity, the emission ratio decreased rapidly (30−60 s) to a very low level (dashed line). By contrast, under the lower green light irradiation, the decay was slower (solid line). Only after 5 min of light incubation was the maximal quenched level reached. Intensities as low as 0.25 μE m^{-2} s^{-1} were also effective in inducing the large PS II fluorescence quenching in 5 min (data not shown). These results indicated that very low light intensities of green light are sufficient to induce maximum quenching.

The kinetics of PS II fluorescence quenching, under 10 and 500 μE m^{-2} s^{-1} of white light, are shown in Figure 3b. The lowest intensity was unable to generate fluorescence changes, whatever the incubation time. Under the strongest intensity, *Rhodella* cells reached the quenched PS II fluorescence state within 4 min. Only intensities higher than 200 μE m^{-2} s^{-1} were able to decrease the F695/F718 ratio from 1.65 to 1.1 in 4 min (see Figure 3d). It is worth noting

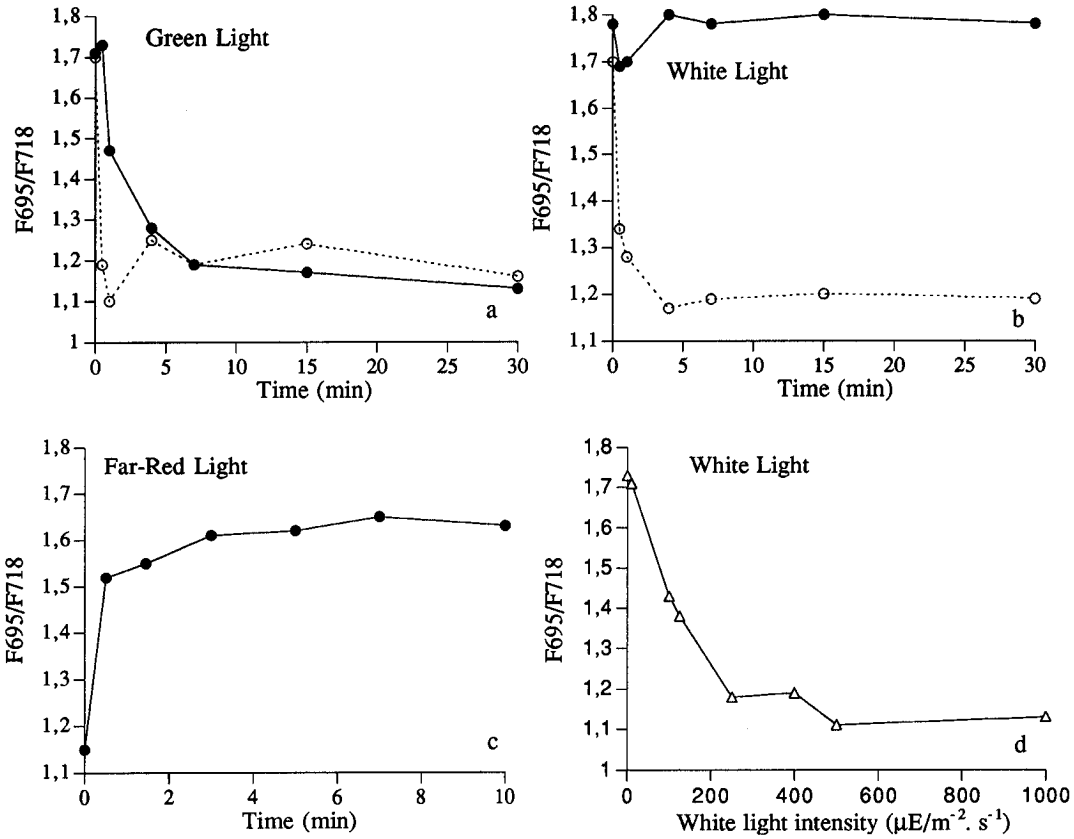

FIGURE 3: Kinetics of state transitions expressed as changes in the ratio F695/F715. The ratio F95/F718 was measured from 77 K emission spectra. Transition to state 2 was induced (a) by two green light intensities differing by a factor of 10, 2.5 μE m^{-2} s^{-1} (solid line) and 25 μE m^{-2} s^{-1} (dashed line), and (b) by two white light intensities, 10 μE m^{-2} s^{-1} (solid line) and 500 μE m^{-2} s^{-1}. (c) Kinetics of state 1 transition under far-red light illumination, in green light-adapted cells. (d) Cells preadapted to far-red light were incubated for 4 min under white light intensities ranging from 10 to 1000 μE m^{-2} s^{-1}.

that the lowest green light intensity (0.25 μE m^{-2} s^{-1}) we used was able to induce a significant decrease of the ratio F695/F718, while much higher white light intensities were needed to obtain the same effect.

The kinetics of recovery from the quenched PS II fluorescence state was followed during illumination by far-red light in cells preadapted to green light (at 25 μE m^{-2} s^{-1}) for 5 min. The transition was biphasic with a fast phase in the first 30 s in which the highest F695/F718 ratio was almost reached and a slower phase in the following 5 min of illumination (Figure 3c). The quenched state was also suppressed in the dark. The kinetics of recovery in the dark depended on the time of preadaptation to green light, and they were slower than under far-red illumination (data not shown).

Fluorescence Yield Measurements at Room Temperature. At room temperature, most of the fluorescence is emitted from PS II. Its yield depends on the oxidoreduction state of Q_A [reviewed by Briantais et al. (1986)]. When Q_A is oxidized, a minimal level of fluorescence is observed (F_o), since the excitons are efficiently trapped by the open centers. When Q_A is fully reduced, the fluorescence level reaches a maximum (F_{max}), since the centers are unable to trap excitons. Quenching of this fluorescence may be "photochemical", involving the use of the absorbed energy to charge separation, or may be due to a "nonphotochemical" energy dissipation. In a PAM fluorometer, the yield of chlorophyll fluorescence can be continuously monitored using a modulated low-intensity nonactinic light. The F_{max} levels were attained by illuminating the cells with saturating multiple turnover (800 ms, 3200 μE m^{-2} s^{-1}) pulses. Application of these brief pulses of intense light which transiently closes all PS II centers and removes photochemical quenching serves to distinguish photochemical from nonphotochemical quenching at any time. The time course of the fluorescence level in dark-adapted cells illuminated successively by far-red light, by blue (3 μE m^{-2} s^{-1}) or white (3 μE m^{-2} s^{-1}) light, and finally by green light (4 μE m^{-2} s^{-1}) is shown in Figure 4. As expected, the F_o and F_{max} levels of dark-adapted cells were not modified by the far-red illumination (data not shown) which confirmed that the dark state was similar to state 1. When far-red illumination was followed by blue (Figure 4b) or white light (Figure 4c), the cells remained in this apparent state 1. In the onset of green illumination, a transient increase of the fluorescence level was observed, indicating that some PS II centers became closed. The shape of the fluorescence induction depended on the previous illumination. A saturating pulse which was given after 4 min of light incubation showed that a large quenching of F_{max} was always developed during the green illumination. The maximal level of F_{max} was recovered when the cells were shifted to far-red light (Figure 4). Figure 5 shows the values of F'_{max} and F'_o under green light and far-red illumination. The superscript prime indicates the value of the respective variable in a light-adapted state. As already said, F'_{max} and F'_o under far-red illumination were identical to F_{max} and F_o, respectively, measured in dark-adapted cells. Nonphotochemical quenching, under green light incubation, was quantified by the Stern−Volmer-type fluorescence coefficients, SV_o and SV_{max} (Demming-Adams, 1990; Gilmore & Yamamoto, 1991). SV_o ($F_o/F'_o - 1$) is linearly dependent

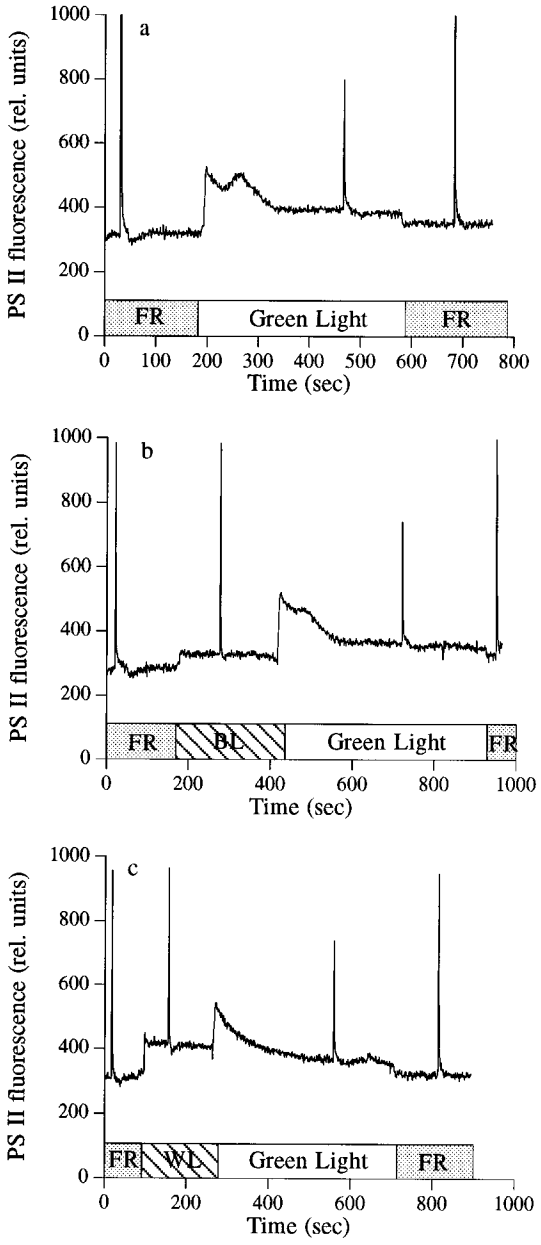

FIGURE 4: Measurements of fluorescence yield by a PAM fluorometer at room temperature during different types of illumination. Dark-adapted cells were illuminated successively with (a) far-red (FR), green (4 μE m^{-2} s^{-1}), and far-red light; (b) far-red, blue-violet (BL) (3 μE m^{-2} s^{-1}), green (4 μE m^{-2} s^{-1}), and far-red light; and (c) far-red, white (WL) (3 μE m^{-2} s^{-1}), green (4 μE m^{-2} s^{-1}), and far-red light. The weak measuring modulated light was maintained during all the experiments. Saturating pulses (3200 μE m^{-2} s^{-1}, 800 ms duration) were applied to assess F_{max} and F'_{max}.

on changes in the rate constant of nonradiative de-excitation in the antenna, and SV_{max} ($F_{max}/F'_{max} - 1$) is affected by changes in the antenna and in the reaction center (Olaizola & Yamamoto, 1994). Calculated from Figure 5, SV_o is equal to 0.1 and SV_{max} is equal to 0.6. SV_o was never higher than 0.1, whereas SV_{max} was sometimes higher. The larger increase on SV_{max} compared to that of SV_o suggested that the fluorescence quenching was mostly produced by changes in the reaction center.

Regulation of the Induction of the Quenched PS II Fluorescence State

Effect of Uncouplers of Photophosphorylation and Inhibitors of ATP Synthesis. We studied the effect of different

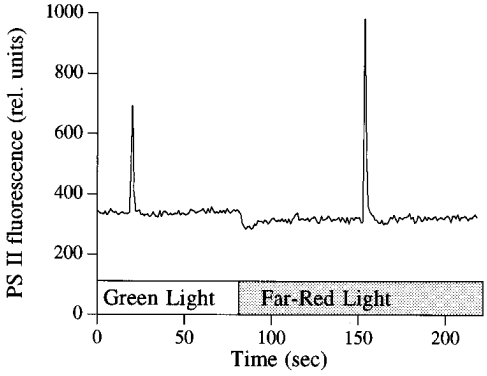

FIGURE 5: Measurement of F'_o and F'_{max} under green (12 μE m^{-2} s^{-1}) and far-red illumination. F'_o and F'_{max} under far-red light were identical to those of the dark state that we considered as F_o and F_{max}. To measure F'_o under green illumination, far-red light was turned on, upon turning of the green light. Quenching of F_o and F_{max} was calculated using the Stern−Volmer coefficients: SV_o ($F_o/F'_o - 1$) and SV_{max} ($F_{max}/F'_{max} - 1$).

types of chemical treatments on the fluorescence state of cells to establish a possible link between the development of the quenching and other events occurring in the thylakoids during the different light regimes. In order to check whether the adaptations we observed were related to the formation of a ΔpH or ATP synthesis, two treatments that modify these variables were tested. The uncoupler of photophosphorylation, NH_4Cl, which cancels the ΔpH but retains the electrical field across the membrane, was added to samples exposed to either green or white light. Figure 6 shows the decrease of the F_{max} level after 4−5 min of incubation in different intensities of green light (0.3, 4, and 16 μE m^{-2} s^{-1}) (left) or of white light (50, 200, and 800 μE m^{-2} s^{-1}) (right). The higher the light intensity, the larger the decrease in F_{max}. As already shown, green light was more effective than white light in inducing the quenched state; 50 μE m^{-2} s^{-1} of white light was insufficient to induce any quenching, while 0.3 μE m^{-2} s^{-1} of green light induced some. The addition of NH_4Cl to the cells illuminated by green light was immediately followed by an increase of the steady-state level of fluorescence. This increase was proportional to the light intensity used. After 1 min of NH_4Cl incubation, a saturating pulse showed that F_{max} had reached its highest level even in green light. In white light, F_{max} was only partially reversed. Further incubation with far-red light completely reversed the fluorescence quenching in the cells incubated under 200 μE m^{-2} s^{-1}. Far-red light was unable to supress the fluorescence quenching induced by 800 μE m^{-2} s^{-1}, indicating that a photoinhibitory effect might occur in this case.

These results were confirmed by 77 K fluorescence spectra (Table 2). After exposure (5 min) to low green light (2.5 μE m^{-2} s^{-1}) or to white light (500 μE m^{-2} s^{-1}) of far-red preadapted cells, the ratio F695/F718 was low. At that stage, NH_4Cl was added and allowed to act for 3 min. Following the addition of NH_4Cl, the decrease of PS II fluorescence, induced during green irradiation, was reversed (Table 2). Compared to the initial state, the F695 level was slightly lower in the presence of the uncoupler. A following far-red illumination (3 min) almost completely reversed the quenching of PS II fluorescence (Table 2). Illumination of *Rhodella* cells with a green light intensity of 25 μE m^{-2} s^{-1} gave similar results (data not shown). When NH_4Cl was added at the beginning of the experiment, during preincubation in

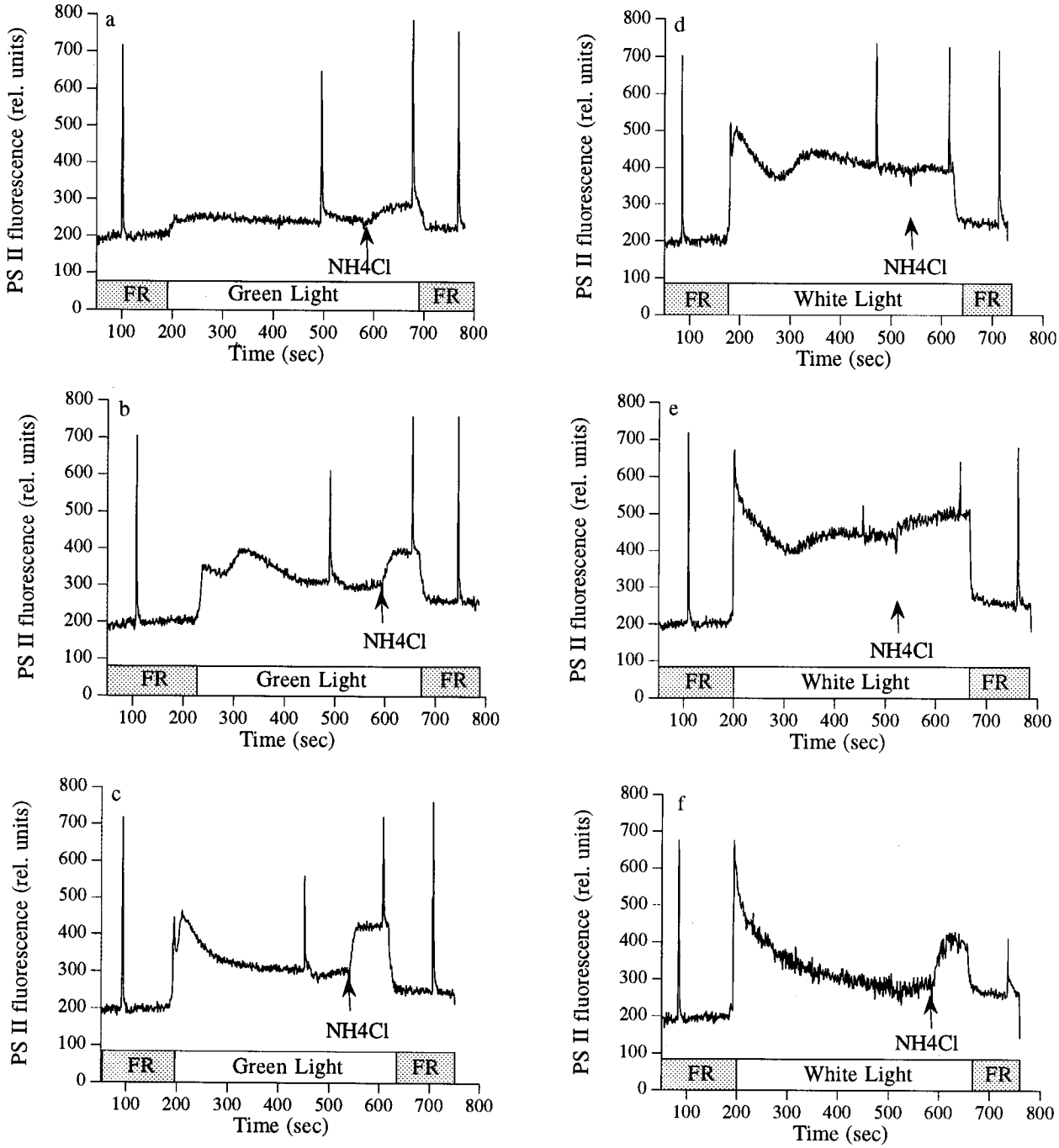

FIGURE 6: Effect of NH_4Cl on the PS II fluorescence yield in cells illuminated with green light, (a) 0.3 μE m^{-2} s^{-1}, (b) 3 μE m^{-2} s^{-1}, and (c) 12 μE m^{-2} s^{-1}, or with white light, (d) 50 μE m^{-2} s^{-1}, (e) 200 μE m^{-2} s^{-1}, and (f) 800 μE m^{-2} s^{-1}. NH_4Cl was added after 5 min of green or white illumination. A saturating pulse was applied to assess F'_{max} during the different regimes of light.

far-red light, green light incubation was not able to transfer the cells to the quenched PS II state (Table 2).

Table 2 also shows that the presence of NH_4Cl was not able to completely eliminate the PS II fluorescence quenching induced during the saturating white light illumination. When cells exposed to white light in the presence of NH_4Cl were transferred back to far-red light, the reversion was not complete, thus indicating that a photoinhibitory effect also occurred (Table 2). When the experiment was realized with 180 μE m^{-2} s^{-1}, the far-red illumination totally reversed the quenched state (data not shown).

These results indicate that the quenching of PS II fluorescence, induced by continuous green or white light, was completely or partially abolished in the presence of NH_4Cl which suppresses the ΔpH and inhibits the synthesis of ATP. In comparison, DCCD, an inhibitor of photophosphorylation that directly prevents ATP synthesis but swells ΔpH, was tested (Figure 7). Dark-adapted cells were incubated for 10 min with DCCD (50 μM). Then, the cells were successively illuminated with far-red and green light. In the presence of DCCD, the PS II quenched state was induced during the green light illumination. The quenched state was maintained when the cells were illuminated again with far-red light. The addition of NH_4Cl immediately suppressed the PS II quenching (Figure 7).

Effect of Electron Transfer Inhibitors. The effects of DCMU and DBMIB on the induction of the quenched state were also tested. DCMU blocks the transfer of electrons

Table 2: Effect of NH_4Cl on the Quenched State Induced by Green or White Light[a]

	Fr_i	*L*	*N*	Fr_f
green light a	1.76	1.28	1.65	1.7
b			1.8	1.8
white light a	1.78	1.17	1.3	1.4
b			1.4	1.47

[a] 77 K emission spectra were recorded in the following conditions: preadaptation to far-red light for 4 min (Fr_i); (a) adaptation to green light at 2.5 μE m^{-2} s^{-1} for 7 min (*L*) or to white light at 500 μE m^{-2} s^{-1} for 4 min (*L*); following addition of 1 mM NH_4Cl and an incubation during 3 min (*N*); and final far-red incubation for 3 min (Fr_f). (b) Alternativaly, NH_4Cl was added at the beginning of the light incubation (*N*). In the table are shown the means of three experiments. In each experiment, five spectra of each condition were recorded. Error is ±0.04.

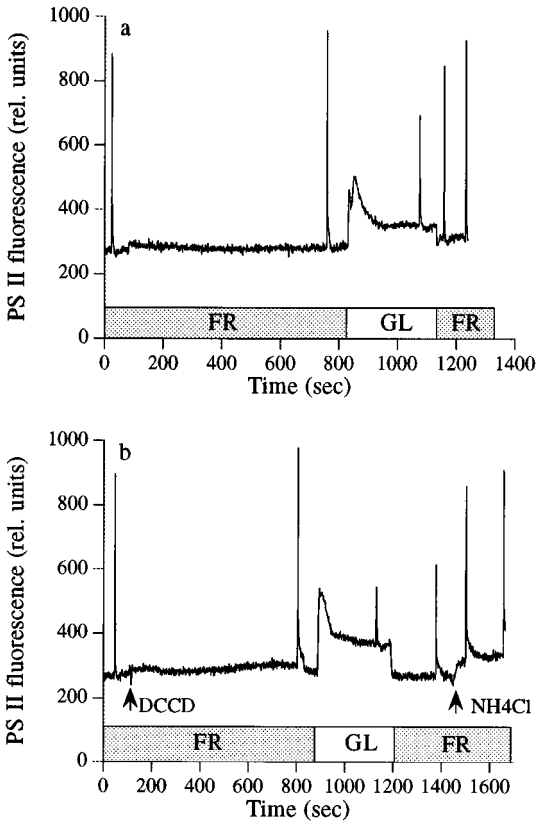

FIGURE 7: Effect of DCCD. Dark-adapted cells were illuminated successively with far-red, green (12 μE m^{-2} s^{-1}), and far-red light in the absence (a) or in the presence (b) of DCCD. Saturating pulses were applied to obtain F'_{max}.

between Q_A^- and Q_B in the PS II; in its presence, the plastoquinone pool is oxidized. DBMIB is an inhibitor of the electron transfer between the plastoquinone pool and the cytochrome b_6/f complex; the plastoquinone pool is reduced in its presence. *Rhodella* cells were placed for 5 min in green light, and then, DCMU (20 μM) was added. Figure 8a shows the 77 K spectra recorded just before the addition of DCMU and 5 min after. Figure 8b shows the 77 K spectra of cells that were incubated under green illumination in the absence or presence of DBMIB (10 μM) and then transferred to far-red illumination for 5 min. These results might suggest that, when the plastoquinone is oxidized (in the presence of DCMU), the PS II fluorescence is at its highest level, while when it is reduced (in the presence of DBMIB), the PS II fluorescence is quenched.

We further studied the effect of DBMIB using PAM measurements. The fluorescence level, in *Rhodella* cells successively illuminated by far-red light, green light, and far-red light, is shown in Figure 9. DBMIB was added after 4 min of green illumination. This addition induced the closure of all the PS II centers as a consequence of the reduction of the plastoquinone pool; however, it did not modify the level of F_{max} (Figure 9b,c). The addition of NH_4Cl during the green illumination immediately induced the increase of the F_{max} level (Figure 9b). Figure 9a shows that, when the cells were transferred from green light to far-red light, they attained the apparent state 1 in 1 min. When cells were transferred to far-red in the presence of DBMIB, they remained in the quenched state. Addition of NH_4Cl suppressed the quenched state. We conclude that, in the presence of DBMIB, the ΔpH formed during the green illumination was maintained during the far-red incubation. We propose that it is the presence of a ΔpH that kept the cells in the quenched state.

DISCUSSION

In the present article, we show that, in the red alga *R. violacea*, the PS II fluorescence level is maximal when PS I is preferentially illuminated by far-red or blue light, but also in the dark or under low white light. In cells adapted to any of those conditions, illumination by green light (low, medium, or high) induces a large PS II quenching. In reference to other studies, we called state 1 the state induced by light 1 and state 2 (or PS II quenched state) the state induced by light 2. The apparent state transitions are very fast (they occur in seconds) in *R. violacea* cells as in other red algae [for example Ried and Reinhardt (1977) and Biggins and Bruce (1985)]. The corresponding transition rates in cyanobacteria strains appear to be slower (they occur in some minutes), but they are faster than those of green algae and higher plants [Fork and Satoh (1983) see review by Williams and Allen (1987)].

We have recently shown that in *R. violacea* state transitions are independent of protein phosphorylation (Delphin et al., 1995). The protein kinase inhibitor staurosporine and the phosphoprotein protein inhibitor NaF had no effect in state 2 and state 1 transitions, while in *C. reinhardti*, they suppressed state transitions (Delphin et al., 1995). In the present article, we show that the level of PS I-related fluorescence does not change while a large PS II fluorescence quenching is developed when *Rhodella* cells are shifted from light 1 to light 2. The large quenching of F_{max} and the almost invariance of F_o suggest that the fluorescence quenching was in the reaction center and not in the antenna of PS II. These results indicate that the fluorescence changes induced by light 1 or light 2 in *R. violacea* cells are not related to changes in the association of phycobilisomes to PS II or in the size of the antennae of PS I and PS II.

Our results clearly demonstrate that in *R. violacea* the PS II quenched state (or apparent state 2) is induced by a ΔpH across the membrane and not by the reduction of the plastoquinone. Satoh and Fork (1983) suggested that a PS II quenched state (that they named state 3) induced in dark-adapted cells of the red alga *Porphyra perforata* by illumination with light 2 was related to an electrochemical gradient across the membrane. This state 3 was not mentioned again in the literature. In addition to the confirmation that indeed

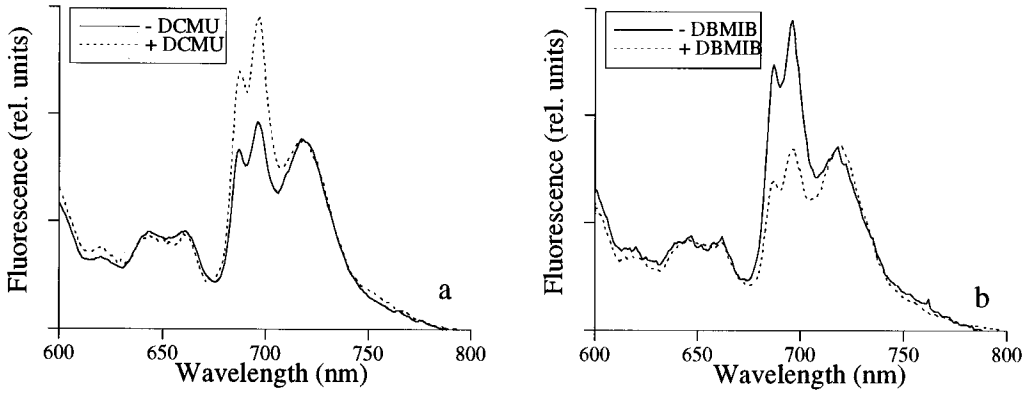

FIGURE 8: Effect of DCMU and DBMIB on state transitions in *R. violacea* cells. (a) The 77 K emission fluorescence spectra were recorded before the addition of 20 μM DCMU (solid line) to green-adapted cells and 5 min after (dashed line). (b) Green light-adapted cells were transferred to far-red light and incubated for 5 min in the absence (solid line) or in the presence of 10 μM DBMIB (dashed line). The excitation wavelength was 560 nm, with a 10 nm slit width.

light 2 induces a ΔpH-dependent PS II fluorescence quenching in red algae, our contribution was to demonstrate that the transition to the apparent state 1 was obtained in conditions that suppressed the ΔpH across the membrane even when the plastoquinone remained reduced. These results also indicated that a local electrochemical gradient near the PS I was not necessary for the transition to state 1 as proposed by Biggins's group (Biggins et al., 1984).

The uncoupler NH_4Cl, by relaxing the ΔpH across the membrane, inhibits the synthesis of ATP. When NH_4Cl was added to green light-adapted cells in which the plastoquinone was reduced in the presence of a ΔpH, a transition to state 1 was induced although the plastoquinone remained reduced. When NH_4Cl was added before the cell transfer to green illumination, its presence inhibited the formation of the PS II quenched state. DCCD, which inhibits the ATPase activity without suppressing the ΔpH, did not suppress the quenching even under far-red illumination. Therefore, it is the ΔpH and not the ATPase activity which regulates the nonphotochemical quenching in *Rhodella* cells. The results obtained with DCMU and DBMIB are generally interpreted in terms of the oxidoreduction state of the plastoquinone pool. In this work, we demonstrate that DBMIB not only inhibits the reoxidation of the plastoquinone pool but also inhibits the dissipation of the ΔpH under far-red illumination. DCMU, in addition to inducing the oxidation of the plastoquinone pool under illumination, can act as an uncoupler and suppress the ΔpH. Therefore, the effects of DCMU and DBMIB can be interpreted as antagonist effects on the ΔpH across the membrane. These results are different from those found with other organisms. In green algae and leaves, the fluorescence changes induced by low light 2 intensities are not ΔpH-dependent (Walters & Horton, 1991). In cyanobacteria, NH_4Cl has no effect on the PS II fluorescence level (D. Kirilovsky and J.-C. Duval, unpublished results).

Mills and Mitchell (1984) and Junesch and Graber (1984) have demonstrated that proton flux through the ATP synthase is a function of the fraction of active units and that this fraction depends on both the ΔpH and the redox state of the thioredoxin. The rate of ATP synthesis increased in the presence of thiols (reduced thioredoxin). Here, we show that very low intensities of green light, which activates only the PS II, are sufficient to create a large ΔpH capable of inducing PS II fluorescence quenching. In this condition, the thioredoxin is mostly oxidized and the ATP synthesis seems to be negligible. The ΔpH is not decreased. Subsequent far-red illumination or low intensities of blue or white light that activate the PS I suppress the PS II quenched state by inducing ATP synthesis and concomitant ΔpH decrease.

White light illumination can also induce a ΔpH-dependent PS II fluorescence quenching in *R. violacea* cells. However, while 0.3 μE m^{-2} s^{-1} of green light was able to generate a large fluorescence quenching, white light intensities lower than 100 μE m^{-2} s^{-1} did not induce any quenching. This result can be explained by the fact that, under nonsaturating white light, both photosystems turn and the ΔpH is consumed in the synthesis of ATP. At near-saturating and saturating intensities, the induced ΔpH is larger than that which the cell is capable of using and the PS II quenching is induced. By increasing the light intensity, we formed an additional quenching that could not be suppressed by addition of NH_4Cl. In most cases, subsequent far-red illumination was sufficient to completely reverse this additional quenching. Since this ΔpH-independent quenching appeared at saturating light intensities, it is very unlikely that it was related to state transitions. Since it recovered in seconds under far-red illumination or after addition of DCMU, it could not be associated with damage of the RC II (photoinhibition). Additional PS II quenching associated with photoinhibitory effects was observed when illumination was done at intensities higher than 300 μE m^{-2} s^{-1}; far-red illumination had no effect on this type of quenching.

In green algae and leaves, under weak illumination, the only quenching observed is related to the redistribution of the absorbed energy between PS II and PS I (state transitions) (Walters & Horton, 1991). Under near-saturating illumination, the ΔpH-dependent quenching is predominant (Krause & Weis, 1991; Walters & Horton, 1991). Most of this quenching results from an increase of radiationless energy dissipation in the LHCII. A quenching of both F_o and F_{max} is observed in this case, indicating a decrease of the photon flux to the centers. The formation of this quenching is accompanied by the accumulation of deepoxidated xanthophyll (Demming et al., 1987; Bilger et al., 1989; Gilmore & Yamamoto, 1991) and conformational changes (most probably, aggregation) of the LHCII (Horton et al., 1991). It is assumed that this quenching is a mechanism of photoprotection. It was also proposed that part of the quenching of F_{max} is due to energy dissipation in the reaction center either by nonphotochemical radiationless decay (Briantais et al.,

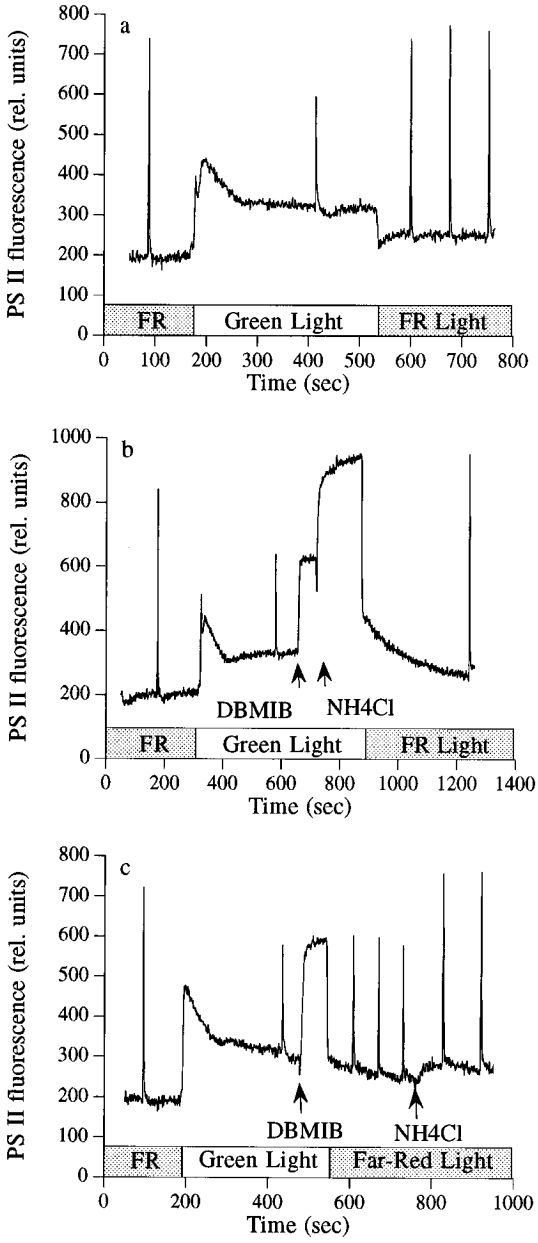

FIGURE 9: Measurements of fluorescence yield in cells illuminated successively with far-red, green (12 μE m^{-2} s^{-1}), and far-red light (a) without additions. (b) DBMIB was added after 5 min of green illumination, and NH_4Cl was added 1 min after. (c) DBMIB was added after 5 min of green illumination as in panel b; 2 min later, the cells were transferred to far-red light and NH_4Cl was added after 4 min of far-red incubation. Saturating pulses were applied to measure F'_{max}.

1979; Weis & Berry, 1987) or by photochemical charge separation followed by charge recombination facilitated by limitation of electron donation from the oxygen-evolving site (Krieger et al., 1992; Kreiger & Weis, 1993). The role in photoprotection of this mechanism is being discussed. In red algae, low intensities of light 2 and near-saturating intensities of white light induce only one type of PS II fluorescence quenching triggered by the formation of a transthylakoid ΔpH. The unvariance of the F_o levels in state 1 and state 2 suggests that the PS II fluorescence occurs in the reaction center itself and not in the antenna. Preliminary determinations of saturation curves of oxygen evolution (data not shown) also suggest that photon flux to the centers is similar in the absence or in the presence of the quenching. We propose that the quenching of F_{max} induced in red algae is the consequence of a mechanism that occurs in closed centers to avoid recombination reactions which might generate triplet chlorophyll and as a consequence damaging oxygen radicals. The trigger is an increase in the transthylakoid ΔpH, and the quenching is maintained until it is consumed. It seems that *Rhodella* cells perceive illumination by light 2 as a stress condition since green light activates only the PS II inducing the transient closure of PS II centers and a large ΔpH across the membrane. The ΔpH is maintained since the ATPase is not activated. Further studies are needed to determine if this quenching of PS II fluorescence is really related to a protection mechanism from high light in red algae and to elucidate the mechanism of this ΔpH-dependent quenching in an organism which does not have LHCII or the carotenoid cycle.

No clear distinction has been made in the literature for the nature of the so-called state transitions in cyanobacteria and red algae. We have clearly demonstrated that the fluorescence changes until now associated with state transitions in red algae are, in fact, related to the energization state of the photosynthetic membranes. The mechanism of state transition in cyanobacteria remains enigmatic. Nevertheless, in a preliminary re-examination of the case of cyanobacteria, we have indications that the transmembrane ΔpH is not directly involved in the fluorescence diminution under light 2 illumination. We can therefore suggest that the fluorescence changes associated with state transitions in cyanobacteria have a different origin than do those in red algae. We believe that there are always modifications induced by the preferential excitation of one photosystem or the other but that each type of photosynthetic organism developed a different strategy.

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