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A NEW MECHANISM FOR ADAPTATION TO CHANGES IN LIGHT INTENSITY AND QUALITY IN THE RED ALGA, *PORPHYRA PERFORATA*

I. RELATION TO STATE 1–STATE 2 TRANSITIONS *

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Time courses of chlorophyll fluorescence and fluorescence spectra at 77 K after various light treatments were measured in the red alga, *Porphyra perforata*. Photosystem (PS) I or II light (light 1 or 2) induced differences in the fluorescence spectra at 77 K. Light 2 decreased the two PS II fluorescence bands (F-685 and F-695) in parallel, while light 1 preferentially increased F-695. Light 1 and 2 also produced different effects on the activities of PS I and II. Preillumination with light 1 increased PS II activity and decreased PS I activity. However, preillumination with light 2 decreased PS II activity with no effect on PS I activity. These results show that there are at least two mechanisms that can alter the transfer of light energy in *P. perforata*. The dark state in this alga was found to be State 2 and light 1 induced a State 2–State 1 transition which retarded the transfer of light energy from PS II to PS I. Light 2 induced another change (which we have called a State 2–State 3 transition) that was accompanied by a change only in PS II activity.

Introduction

In red algae, pigment systems I and II that belong to PS I and PS II, respectively, are quite different with regard to their pigment composition and colors of light that they absorb. Light absorbed by the phycobilin proteins is delivered mainly to PS II while most light absorbed by chlorophyll *a* is delivered to PS I [1,2]. Moreover, these algae often live where the quality and intensity of the light that penetrates the ocean water can vary. These algae seem to have evolved several adaptation mechanisms that allow them to cope with the changes in light intensity and quality. One such mechanism has been termed the State 1-State–2 transition [3–5]. State 1 has been proposed to be the state obtained after long illumination in light 1 (light that is mainly absorbed by pigment system I) and in this state, transfer of light energy from pigment system II to I is reduced, resulting in higher chlorophyll fluorescence at room temperature. State 2 has been defined as the state obtained after long illumination in light 2 (absorbed mainly by pigment system II) and in this state energy transfer from PS II to PS I is enhanced, resulting in low fluorescence at room temperature [3–5]. Therefore, this state transition is a useful mechanism for algae to balance PS I and PS II activities under conditions of changing light quality.

Much research has been done on the State 1–State 2 transitions in red algae [2,4,6]. Unfortunately, there are still many contradictions in the

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Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; PS, photosystem.

literature concerning this phenomenon. Murata [4] found a fluorescence decrease upon illumination of dark-adapted *Porphyridium* cells, suggesting that the dark state was the high-fluorescence state (State 1). Ley and Butler [2] reported the the dark state was a mixed condition of States 1 and 2 but closer to State 1 in *Porphyridium*. By contrast, Ried and Reinhardt [6] concluded that the dark state was State 2 in many species of red algae.

Many phenomena are known to affect the intensity of PS II fluorescence besides the State 1–State 2 transitions [7–10]. Decreases in PS II fluorescence result from formation of high-energy states in the thylakoid membranes [8], depletion of Mg^{2+} from the reaction mixture [9], or photoinhibition of the chloroplasts [10]. Phosphorylation of thylakoid membrane proteins such as the light-harvesting chlorophyll *a/b* proteins [11,12] also produces a decrease in PS II fluorescence. Therefore, in a study of adaptation mechanisms of plants, it is important (1) to determine the number and characteristics of the mechanisms which a plant has evolved to adapt to different conditions – one of which is the State 1–State 2 transitions, (2) to determine the efficiency of each mechanism, and (3) to determine how each phenomenon can be observed separately.

In this paper, we found that there are at least two mechanisms that affect energy transfer in *Porphyra*. One is the State 1–State 2 transition which controls the distribution of light energy between the two photosystems and the other is a new State 2–3 transition which results in a decrease in light energy reaching PS II and has no effect on PS I activity.

Materials and Methods

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

Fluorescence spectra at 77 K 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 [13]. Green light (light 2) was obtained by passing the white light from a 150 W, 21.5 V projector lamp (type DLS) through Corning glass filters, 4-96 and 3-96. For light 1, the output of the projector lamp was passed through a Schott cutoff filter RG-10 ($\lambda >$ 690 nm). For measurements of fluorescence induction in the millisecond time range, a Nicolet Signal Averager Model 1010 was used as a transient time converter.

Light-induced absorbance changes of the cytochrome *c*-553 in *Porphyra* were measured with a single-beam spectrophotometer. The actinic light (632.8 nm) which was mainly absorbed by phycocyanin was of a low intensity so that the rate of cytochrome *c* oxidation was light limited.

Results

Fig. 1 shows the time courses of chlorophyll fluorescence on different time scales when dark-adapted *Porphyra* thalli were illuminated with light 2. Chlorophyll fluorescence increased rapidly upon illumination. This increase was followed by a slow decay that produced a peak P. The fluorescence transients produced by the dark-to-light transition are known as the Kautsky effect and usually show three maxima termed P, M_1 and M_2 [7,14] within the time range shown in Fig. 1.

Fig. 2A shows the fluorescence spectra at 77 K before and after illumination of dark-adapted *Porphyra* cells with light 2 for 2 min. PS II illumination induced a parallel decrease in both PS

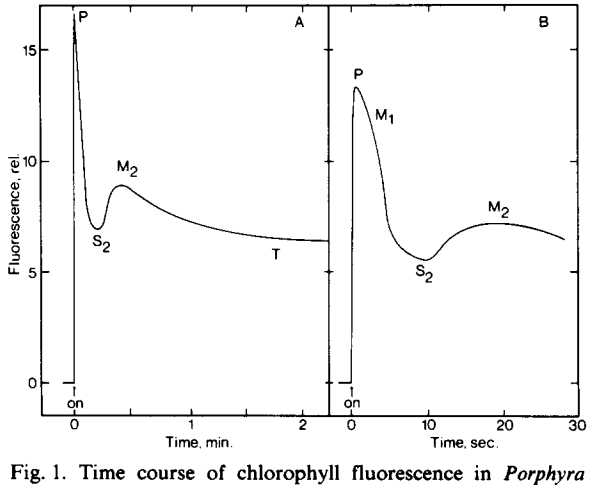

Fig. 1. Time course of chlorophyll fluorescence in *Porphyra perforata* on two time scales. Dark-adapted thalli were illuminated with light 2 (green light, 235 μW/cm^2).

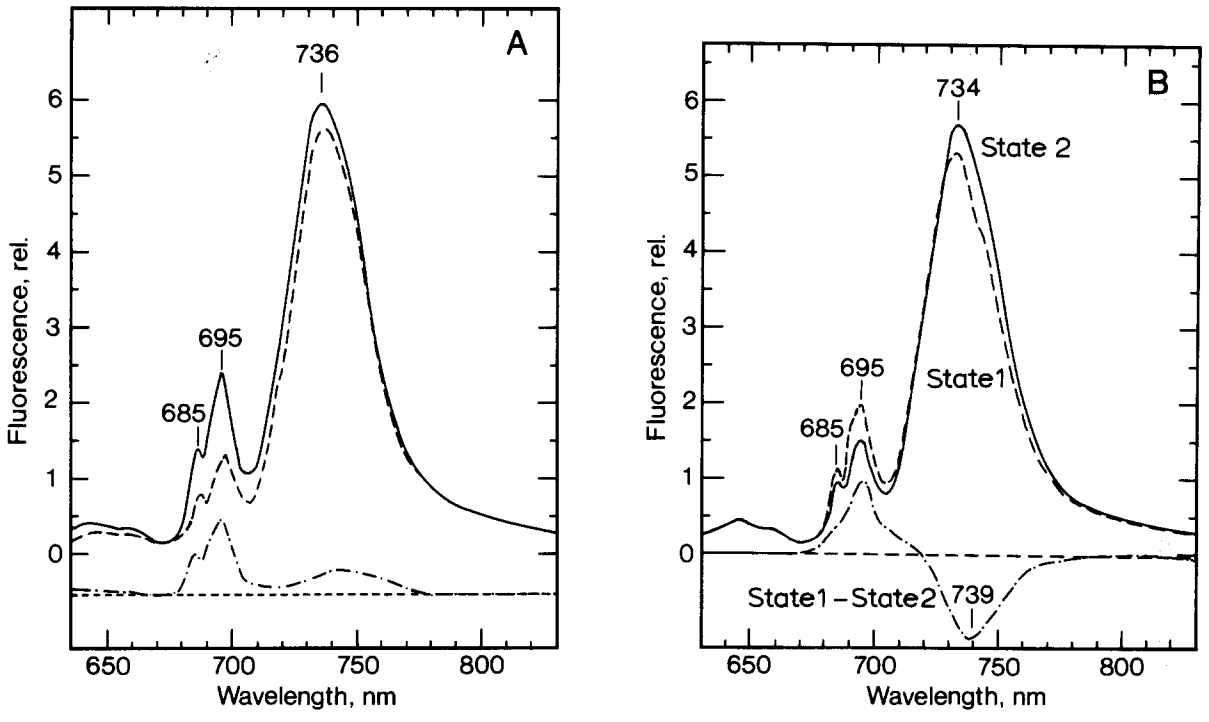

Fig. 2. Fluorescence spectra at 77 K of *Porphyra* before and after illumination of the thalli with light 2 for 2 min (A) or with light 1 for 30 min (B). The dark-adapted thallus (———) or preilluminated thallus (— — —) was cooled to 77 K and the fluorescence spectra measured. The excitation light for measurements of fluorescence at 77 K was the same light 2 used for Fig. 1, but the intensity was lowered to 58 $\mu W/cm^2$. (·–·–·) Difference spectrum between the dark-adapted and preilluminated cells.

II fluorescence bands (F-685 and F-695) and a slight decrease in PS I fluorescence. Sometimes a decrease in phycobilin fluorescence was observed. In contrast to the blue-green alga, *Synechococcus lividus* [15] and the green alga, *Scenedesmus obliquus* [16], these results may indicate that light 2 produces some changes in pigment arrangement within the thylakoid membranes of *Porphyra* as discussed below. In *Synechococcus* [15] and *Scenedesmus* [16] we could not find any large change in the fluorescence spectra at 77 K after illumination with light 2. By contrast, the difference spectrum obtained after light 1 illumination differed from that obtained after light 2 illumination.

Fig. 2B shows fluorescence spectra at 77 K before and after illumination of the dark-adapted *Porphyra* thallus with light 1 for 30 min (State 2–State 1 transition; see below). Illumination with light 1 induced a fluorescence increase at 695 nm and a decrease in PS I fluorescence. It can be noted that F-695 increased significantly more than did F-685. These results show that light 1 produced changes in arrangements of pigment proteins within the thylakoid membranes, and that the mechanism involved seems to be different from that induced by light 2. Therefore, it is unreasonable to conclude that the dark state is a mixed condition of States 1 and 2 and that light 1 or 2 converts the state to pure State 1 or 2.

In order to check whether the changes we observed in the fluorescence spectra were related to redox changes of Q, we also measured fluorescence spectra under conditions were Q is oxidized or reduced (Fig. 3). Fluorescence at the F_0 level was measured using a very low intensity of exciting light in order to keep Q mostly in the oxidized state. Fluorescence at the F_m level was measured under the same conditions after Q was totally reduced with strong light. The fluorescence spectra induced by redox changes of Q differed from all of the fluorescence spectra mentioned above, except

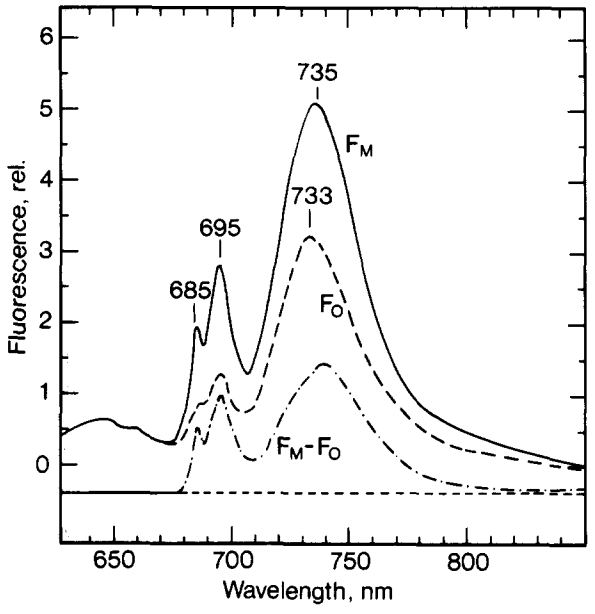

Fig. 3. Fluorescence spectra at 77 K of *Porphyra* at the F_0 and F_m level. The intensity of the exciting light described in Fig. 2 was lowered to 2.9 $\mu W/cm^2$. The fluorescence spectrum of the F_0 level was measured soon after the onset of the exciting beam and that of F_m level was obtained with the same thallus after illumination of the thallus with 40-times stronger light for 3 min at 77 K. The dark-adapted thallus was used.

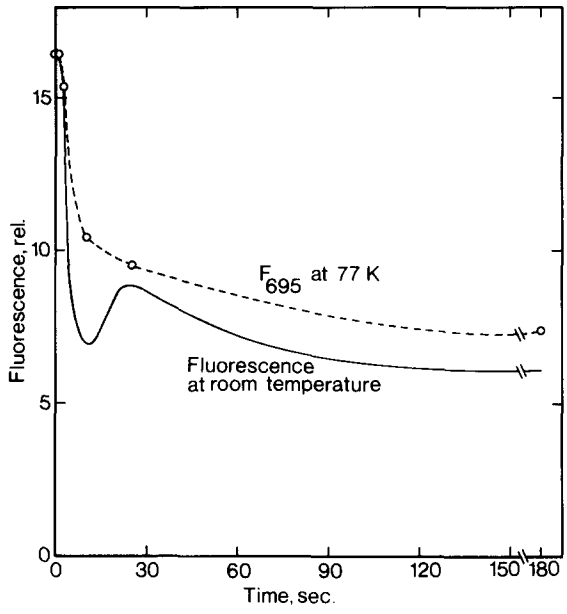

Fig. 4. The fluorescence time course and fluorescence intensity at 695 nm at 77 K after illumination of *Porphyra* with light 2 for various periods. Fluorescence intensities at 77 K were measured as in Fig. 2.

that the two PS II fluorescence bands (F-685 and F-695) changed in parallel as was the case with light 2 illumination.

Fig. 4 shows the time course of chlorophyll fluorescence at room temperature and the fluorescence intensities at 695 nm at 77 K. To obtain these values, cells were illuminated with light 2 for various periods at room temperature and then dropped into liquid nitrogen. The fluorescence spectra were measured after a sufficient time of illumination of the cells at 77 K so that both Q and P-680 were in the fully reduced state. 1 s illumination of the cells did not affect the 695 nm fluorescence band at 77 K. The decrease of F-695 started after 3 s of illumination of the cells (that corresponded to M_1, see Fig. 1B) and the intensity dropped sharply to a lower level after 10 s of illumination. The shapes of the decay curve of F-695 and the fluorescence time course at room temperature did not correspond completely. However, the decay curve of F-695 seems to be related at least to the M_1S_2 part of the fluorescence time course. These results show that the fluorescence transients at room temperature are produced by at least two different mechanisms; one, by changing the redox level of Q and another, perhaps, by changing the pigment arrangement in the thylakoid membranes. Murata [4] observed a similar fluorescence decay in dark-adapted *Porphyridium* cells and did not observe a fluorescence increase in light 1, suggesting that the dark state was State 1. The reason that Murata failed to observe an effect of light 1 on dark-adapted cells may be due to the very short illumination time employed (90 s) [4]. Light 1 is about 10-times slower in changing the fluorescence emission spectrum compared to light 2.

In order to determine the relationship between state transitions (State 1–State 2 or State 2–State 1) and changes induced by light 1 or 2, we measured activities of PS I and PS II. Fig. 5 shows the time courses of photooxidation of cytochrome *c*-553 before and after illumination of *Porphyra* with light 1 for 30 min (State 2 and 1, respectively; see below). After the illumination, DCMU and DBMIB were added to inhibit rereduction of the

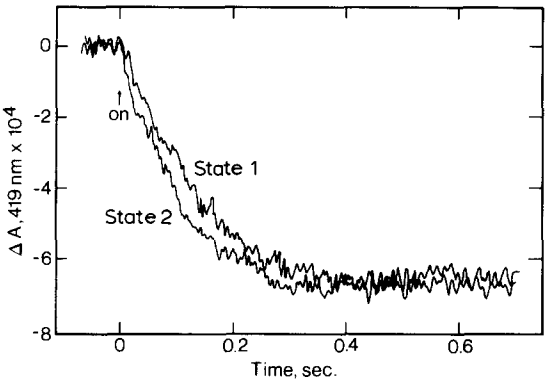

Fig. 5. Time courses of photooxidation of cytochrome *c*-553 (measured at 419 nm) in States 1 and 2 in *Porphyra*. State 1 was obtained by illuminating the thallus with light 1 for 30 min. State 2 was obtained by dark adaptation of the the thallus for more than 1 h (see Discussion). The actinic light was 632.8 nm light from an He-Ne laser (128 $\mu W/cm^2$). 10 μM DCMU, 10 μM DBMIB and 5 mM ascorbate were added soon after the pretreatment and after 1 min of incubation, the absorbance changes were measured.

cytochrome by PS II or by cyclic electron flow around PS I. The data in Fig. 5 clearly show that the rate of photooxidation of the cytochrome was decreased after light 1 illumination of the cells.

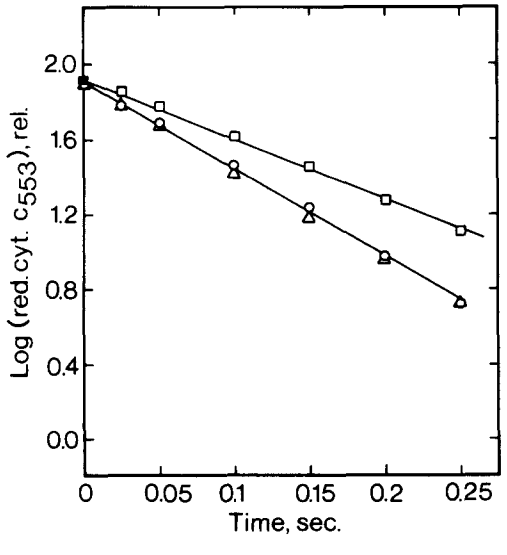

Fig. 6. Photooxidation of cytochrome *c*-553 (measured at 419 nm) in states 1, 2 and 3 in *Porphyra*. State 3 (△) was obtained by illuminating the dark-adapted thallus (State 2 (○) with light 2 for 3 min. state 1 (□) was obtained by illuminating the thallus with light 1 for 30 min (see Discussion). Other conditions were the same as in Fig. 5.

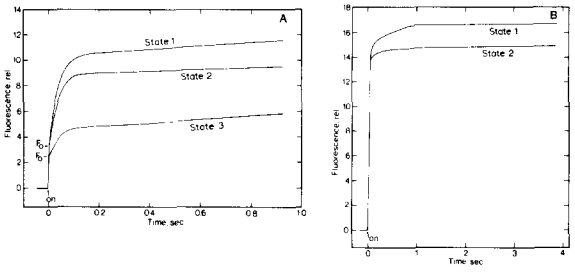

Fig. 7. Time courses of fluorescence induction in *Porphyra* in the presence of DCMU in States 1, 2 and 3. 10 μM DCMU was added soon after the pretreatment and after 1 min of incubation, the time courses were measured. State 3 was obtained by illumination of the thallus with light 2 for 2 min. Other conditions were the same as in Fig. 5.

Since we used very low actinic light, the decreased rate of photooxidation of the cytochrome after light 1 suggests that the quantum yield of PS I was decreased. Fig. 6 compares the kinetics of the photooxidation of cytochrome *c*-553 on a semilogarithmic plot before and after illumination of dark adapted *Porphyra* with light I for 30 minutes or light 2 for 3 min (States 2, 1 and 3, respectively; see below). In contrast to the effect of light 1, light 2 did not change the rate of photooxidation of the cytochrome.

Fig. 7 shows fluorescence time courses in the presence of DCMU on two different time scales before and after illumination of dark-adapted cells with light 1 for 30 min or with light 2 for 2 min (States 2, 1 and 3, respectively; see below). Light 1 induced a large increase in variable fluorescence without a large change in F_0 fluorescence. The fluorescence time curve seemed to be composed of at least two components, a fast and a slow one. The origin of these two components is not clear, but these components may correspond to PS II_α and PS II_β found in chloroplasts isolated from higher plants [17]. As is shown in Fig. 7A and B, the extents of both of the two components were increased by light 1 and the rate of the fast component was slightly higher. In contrast to light 1 treatment, illumination with light 2 induced a marked decrease in F_0 fluorescence, a decrease in the fast component, and an increase in the slow component of the fluorescence time course. Since this state appears to be different from State 2, we have defined it as State 3 as is discussed below.

Discussion

All the data presented here show that the mechanisms involved in the light 2 effects are different from those of light 1. The effects of light 2 were not just the opposite to the effects of light 1. Light 1 induced an increase in one of the PS II fluorescence bands (F-695) and a decrease in a PS I fluorescence band (Fig. 2), as well as a decrease in PS I activity (Fig. 5) and an increase in the variable fluorescence (Fig. 7). The increase in F_v can be considered to be an increase in PS II activity. All these effects are characteristics of the State 2–State 1 transition observed in the blue-green alga, *S. lividus* [15] and in the green alga, *S. obliquus* [16]. Therefore, the changes induced by light 1 seem to represent a State 2–State 1 transition that induces changes in transfer of light energy from PS II to PS I [3–5].

By contrast, the changes induced by light 2 were different from the State 1–State 2 transitions. Both PS II fluorescence bands (F-685 and F-695) were decreased in parallel, PS I fluorescence did not increase (Fig. 2), PS I activity remained the same (Fig. 6) and F_0 was quenched markedly (Fig. 7). These data suggest that light 2 treatment did not induce changes in transfer of light energy from pigment system II to I, but the arrangement of the pigments in PS II seems to have been modified so that less light energy arrived at the reaction center of PS II (Fig. 7). It is also possible that light 2 gives rise to (or perhaps increases the amount of) a quencher close to the reaction center of PS II. However, in this case the mode of action of this quencher must be different from that of the quencher Q [18], since the quenching by Q produced difference spectra (Fig. 3) that are not the same as those shown in Fig. 2A. This light 2-induced state is, therefore, quite different from States 1 and 2. We propose to identify this condition as State 3. The State 2–State 3 transition is induced by PS II light and seems to be related to formation of the high-energy state in the thylakoid membranes (in preparation). Light energy distribution between the two photosystems is not altered by the State 2–State 3 transition, but energy transfer from the light-harvesting pigments to the reaction centers of PS II is decreased or, perhaps the light energy arriving at the reaction centers is quenched more after the transition. This mechanism may be related to the adaptation of this alga to high light conditions, since *Porphyra* lives in the high intertidal zone where it can be exposed to extreme desiccation often in combination with high light intensities and high salt concentrations. A mechanism to eliminate excess light energy may be important in order to prevent photoinhibition resulting from formation of strong reductants or oxidants by PS I and PS II under conditions where normal electron flow is retarded.

Another noteworthy result is that the dark state appears to be State 2 in *Porphyra*. Since there are at least two light-induced transitions, State 2–State 1 and State 2–State 3, some of the confusion and contradictions mentioned in the Introduction may now be resolved.

The fluorescence transients produced by a dark-to-light transition (the Kautsky effect) were found to be independent of the State 1–State 2 transitions. Thus. light 2 induced a large fluorescence transient as shown in Fig. 1 even though the dark state was State 2. In contrast to the blue-green alga, *Synechococcus* [15], and the green alga, *Scenedesmus* [16], the Kautsky transient in *Porphyra* seems to be overlapped by another transition, the State 2–State 3 transition, which seems to have a close relation to the M_1S_2 decay (Fig. 3).

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