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Changes in the photosynthetic apparatus of red algae induced by spectral alteration of the light field. I. A decrease in the apparent quantum yield of PS I caused by preillumination with light 1

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Preillumination with light 1 strongly diminishes the apparent quantum yield of Photosystem I in *Porphyridium purpureum*, *Porphyra yezoensis* and some other red algae. At limiting light 1 intensities, the photooxidation rate of cytochrome *f* and of P-700 is reduced by 40–60% after preillumination with light 1 compared with light 2 preillumination. Addition of DCMU or DBMIB preceding the preillumination period completely abolishes the effect, whilst addition immediately after the preillumination does not affect it. We conclude that this effect (1) is not related to a redistribution of energy, (2) is not caused by an acceleration of the simultaneous reduction of cytochrome *f* and P-700 via plastoquinone (by cyclic or noncyclic electron transport) and (3) depends on the redox state of an electron carrier nearly connected with the plastoquinone pool. In many respects, as in fluorescence or oxygen-exchange kinetics, this effect mimics the phenomena which are expected to result from a true redistribution of excitation energy between the two photosystems.

Introduction

During our earlier work on the distribution of excitation energy between PS I and PS II in red algae [1–5], we became more and more aware of the fact that a change in the wavelength of the exciting light may induce a plurality of regulatory events. These different mechanisms of adaptation to changing light conditions are difficult to distinguish because of the similarity of the conditions that evoke them and the resemblance of the observable phenomena.

Over the past few years, several working groups involved in the problem of regulation of energy distribution have reported on observations which show that thylakoid phosphorylation or changes in the wavelength may induce changes in the photosynthetic apparatus obviously independent of a redistribution of energy between the photosystems [6–13] (see also Refs. 14–20). Most of these observations were made on chloroplasts or thylakoids of higher plants and concern variations within the domain of PS II. Satoh and Fork [6–8] described an effect of this kind ('state 2–state 3 transition') with a red alga, *Porphyra perforata*. However, there are suggestions of special effects on PS I, too. Forti and Grubas [12] reported a stimulatory effect of thylakoid phosphorylation on PS-I-dependent photophosphorylation, found up to saturating light intensities, which is obviously not connected to a redistribution of energy between PS I and PS II. On the other hand, an unexpected reduction of the relative activity of PS I has been observed after preillumination with light 2 in *Cryptomonas ovata* by Snyder and Biggins [13]. In this connection also, the 'low intensity state' observed in intact leaves by Canaani and Malkin [14] should be considered. It is interpreted to result from a loss of excitonic interaction between antennae and reaction centers of PS I.

In the present paper we report on a modulation of the apparent quantum yield of PS I measured in pure light 1 as a new effect of preillumination with light 1 or light 2, which is the predominant effect in some species of red algae.

Abbreviations: Cyt., Cytochrome; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; L_1, L_2, light 1, light 2; PAR, photosynthetic active radiation, measured from 400 to 700 nm; PS, Photosystem; Q_A, primary quinone acceptor of PS II; PES, Provasoli-enriched seawater.

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Materials and Methods

P. purpureum (Bory) Drew et Ross (formerly *P. cruentum*) strains 1380-d and 1380-f (Sammlung von Algenkulturen, Göttingen) were grown at 15–18 °C and under a light-dark cycle of 16–8 h in glass tubes of 6 cm diameter using the medium of Jones et al. [21]. The cultures were aerated with air or with air enriched in CO_2 (1.5%). They were illuminated either with white light (Osram fluorescent lamps Type L 40W/25-1), $1.5 \cdot 10^{-8}$ mol photons $\cdot$ cm$^{-2} \cdot$ s^{-1} PAR or with red light, $2.49 \cdot 10^{-9}$ mol photons $\cdot$ cm$^{-2} \cdot$ s^{-1} PAR, by installing a Röhm & Haas Plexiglas filter Nr. 502 (> 620 nm) in front of the light source. The cells were harvested in the late logarithmic phase. *P. yezoensis*, obtained from Dr. Kornmann, Helgoland, was grown at 15 °C in white light, $2.45 \cdot 10^{-10}$ mol photons $\cdot$ cm^{-2}s^{-1} PAR, in Provasoli-enriched seawater (PES) medium [22] plus 1% soil extract. 6–8-week-old well-grown and homogeneously pigmented thalli were used for the measurements.

Absorbance spectra were obtained with an Aminco DW2 spectrophotometer, using a slit width of 3 nm. Photooxidation and reduction of cytochrome *f* was monitored in the double-beam mode of the Aminco DW2 as absorbance difference between 402 and 421 nm [24,25]. The slit width was 5 nm and the intensity of the measuring beams was $3.4 \cdot 10^{-12}$ mol photons $\cdot$ cm$^{-2} \cdot$ s^{-1}. It was ascertained by light – dark difference spectra and by analysis of the kinetics of the observed absorbance changes that, under our conditions, there was only a negligible overlap by absorbance changes of other constituents of the thylakoid membrane. Evaluation of the absorbance difference between 421 nm and the isosbestic point at 411 nm gave exactly the same result. The sample was kept in a 3 ml cuvette and was illuminated by two Volpi Intralux 25OH lamps through a bifurcated fiber optic. Light 1 (699 nm or 694 nm) was obtained by Schott & Gen. IL interference filters (half-bandwidth 11 or 12 nm) and light 2 by a Schott & Gen. PAL 550 interference filter (half-bandwidth 20 nm). The photomultiplier was protected from the excitation and from fluorescence light by two Schott glass filters, BG 12 and BG 28. During the preillumination period, the *Porphyridium* suspension was stirred to prevent sedimentation of cells and any gas deficiency. In the experiments with *Porphyra*, the PES medium was exchanged every 30 min. It was carefully checked that the physiological condition of the thallus was unchanged after renewal of the medium.

Light-1-induced P-700$^+$ formation was monitored at 699 nm in the single-beam mode of the Aminco DW2 with a slit-width of 7.5 nm. A Schott IL 699 nm interference filter was fixed in front of the photomultiplier. Fluorescence emission of the sample had no observable effect on the measurement. Excitation light 1 was obtained with a blue Schott glass filter BG 12 or with a Schott interference filter IL 443, and light 2 with a Schott PAL 550 interference filter.

During light 1 preillumination, P-700 and cytochrome *f* are partly oxidized and the plastoquinone pool should be strongly oxidized. A short period of light 2 (3–5 s) followed by a dark interval of 5 s was found to be sufficient to obtain maximum physiological reduction of P-700 and cytochrome *f*. In all cases, the light 2 intensity used for this reduction was less than half saturating photosynthetic oxygen evolution. The relative rate of photooxidation of cytochrome *f* and P-700 was defined by the slope of the tangent that goes through the starting point of illumination with light 1 (see Fig. 1). This slope is strongly dependent on the lag phase that precedes the oxidation. This delay should depend, besides, on the excitation balance between PS I and PS II, and on other factors, e.g., the proton gradient and the redox state of the plastoquinone pool. It was proved that these additional factors were largely stabilized by our illumination schedule, independent of preillumination with light 1 and light 2. Evaluation of the steepest curve slope after the lag phase of oxidation gave in principle identical but somewhat more scattered results (cf. Fig. 2).

Fluorescence emission was measured using laboratory-constructed apparatus. *Porphyridium* suspensions were stirred in a cuvette, whereas *Porphyra* thalli were fixed on a Clark-type electrode by means of a dialysis membrane. A layer of 0.2 ml PES medium was put on top of the fixed thalli. The surface was carefully ventilated with a mild stream of air. During the measurements the temperature was kept constant at 15 °C in all cases. Actinic light was provided by two projectors. Light 1 (452 nm) was filtered through a Schott interference filter IL 452 combined with an Oriel Blue dichroic filter and a set of cut-off and heat reflecting filters; light 2 (550 nm) was passed through two Schott interference filters IL 550 and PAL 550 and a heat-reflecting filter. Excitation light was passed through a four-branched fiber optic. Two branches were used for the excitation light, while the emitted light was collected through the remaining arms. To select for the Photosystem II fluorescence signal, the following filters were fixed in front of the photomultiplier (Hamamatsu R 928): Schott IL 684, Schott RG 645, KG 3 and Calflex 113 (Balzer).

Results

P. purpureum and *P. yezoensis* showed, upon a transition from light 1 to light 2, the well-known fluorescence and oxygen-exchange kinetics which are normally considered as an expression of the so-called state 1–state 2 phenomenon [26,27], which is believed to be caused by a redistribution of excitation energy between PS I

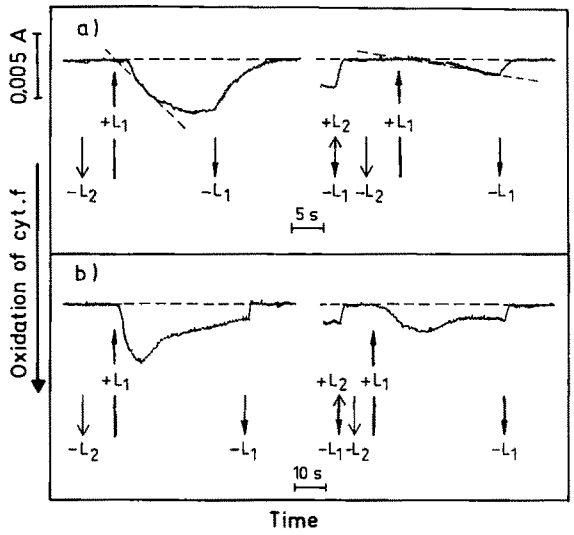

Fig. 1. Time-course of photooxidation of cytochrome *f* in *P. purpureum* after light 2 (left) or light 1 preillumination (right). Photooxidation rate (a) and maximum attained oxidation state (b) of cytochrome *f* are strongly reduced by light 1 preillumination. Illumination schedule: Left: 5 min light 2 preillumination (550 nm, $5.2 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}), 5 s dark, light 1 (699 nm, $7.0 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1} in (a), $1.0 \cdot 10^{-9}$ mol photons·cm^{-2}·s^{-1} in (b). Right: 5 min light 1 preillumination (699 nm, $1.0 \cdot 10^{-9}$ mol photons·cm^{-2}·s^{-1}). Other conditions as above, except that the dark phase is preceded by 5 s light 2 (550 nm, $2.2 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}). ↑, light on; ↓, light off.

and PS II. As could be expected according to this interpretation, the photooxidation of cytochrome *f* in light 1 with a lower light 2 background was much faster after preillumination with light 2 than with light 1. Surprisingly, however, we observed the same preillumination effect also in pure light 1 (699 nm) (Fig. 1a). Also, the transient maximum degree of photooxidation attained after light 2 was much higher than after light 1 preillumination (Fig. 1b).

By varying the ratio of the intensities of light 1 and light 2 during preillumination, it was assured that the observed difference did not result simply from an intensity effect. In *Porphyridium* the diminution of the rate of cytochrome *f* photooxidation was saturated at fairly low light intensities (Fig. 2). Half-saturation was obtained with $3\text{–}5 \cdot 10^{-11}$ mol photons · cm^{-1} · s^{-1} of incident light of 694 nm or with $9 \cdot 10^{-11}$ mol photons · cm^{-2} · s^{-1} of light of 699 nm, corresponding to the ratio of absorption at these two wavelengths. The direction of the preillumination effect did not change when the ratio of the absorbed light 1 and light 2 used for preillumination had been reversed. Light 1 (694 or 699 nm) was varied from $2.5 \cdot 10^{-12}$ to $1.0 \cdot 10^{-9}$ mol photons · cm^{-2} · s^{-1}, light 2 (550 nm) from $7.0 \cdot 10^{-12}$ to $1.5 \cdot 10^{-9}$ mol photons · cm^{-2} · s^{-1}.

Action spectra for O_2 evolution of *Porphyridium* measured with light 1 or light 2 background (not shown) indicated that in accordance with the data of Ley and Butler [28] (and also in our *Porphyridium* strain), at 699 nm less than 5% of the total absorbed light is absorbed by PS II. Thus, the observed effect cannot be explained by regulation of the energy transfer from PS II to PS I.

Since these observations were made with intact cells at fairly low light intensities, the effect of illumination with light 1 could consist in a retardation of cytochrome *f* photooxidation or in an acceleration of the simultaneous reduction as well. Actually, the reduction of cytochrome *f* after switching off the actinic light 1 was enhanced after preillumination with light 1. Calculations made on the basis of data like those shown in Fig. 4 and data obtained by variation of the intensity of the measuring beam suggested, however, that this effect depends on the diminution of the oxidizing action of the measuring beam which acts as light 1. The measuring beam used in all experiments considered here, had, however, such a low intensity ($3.4 \cdot 10^{-12}$ mol photons · cm^{-2} · s^{-1}) that it did not change the state of maximum reduction of cytochrome *f* obtained in the dark or in low light 2. As will be shown below (cf. Fig. 5), the intensity of the measuring beam was therefore also insufficient to induce a diminution of the apparent quantum yield of PS I to any appreciable extent (cf. Figs. 2 and 4). Nevertheless, the measuring beam was always switched off during the preillumination.

The conception that the photooxidation rate itself is affected by preillumination was confirmed by experiments made in the presence of DCMU and of DBMIB (Fig. 3). The inhibitors (at concentrations chosen to give nearly maximum inhibition of O_2 evolution: 100% with DCMU and 60–70% with DBMIB) were added in the dark 5 s after the preillumination. It was assured in parallel experiments that the time of 25 s between addition of the inhibitor and the measurement of cytochrome *f* photooxidation would be sufficient to obtain the full inhibitory effect on oxygen evolution. In experiments where DBMIB was to be added, the intensity of

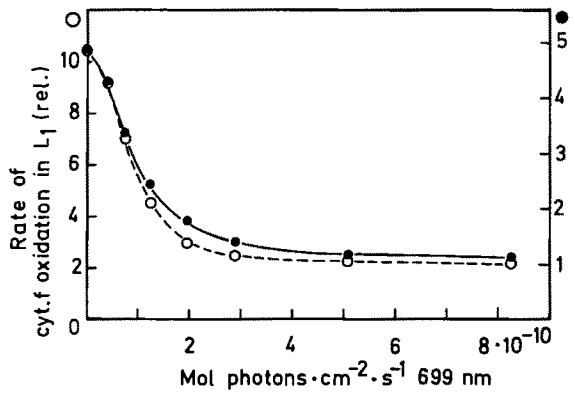

Fig. 2. Rate of cytochrome *f* photooxidation as function of the intensity of light 1 during preillumination. Illumination schedule as in Fig. 1. The value plotted at light 1 intensity 0 is obtained from algae preilluminated with light 2 ($2.3 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}). ○, values calculated from the steepest curve slope; ●, values calculated from the slope of the tangent (cf. text). Organism: *P. purpureum*.

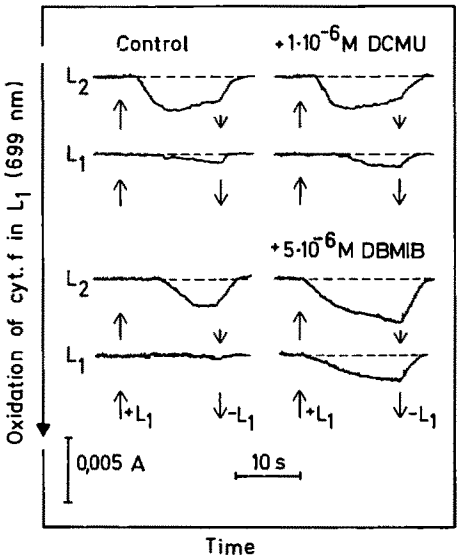

Fig. 3. Effect of DCMU ($1 \cdot 10^{-6}$ M) and DBMIB ($5 \cdot 10^{-6}$ M) on the time-course of cytochrome *f* photooxidation when added after a preillumination with light 1 or light 2 as marked by L_1 or L_2 at the left. Preillumination with light 1 or light 2 as in Fig. 1 was followed by 30 s dark. The inhibitors were added 5 s after the beginning of the dark period. Cytochrome *f* photooxidation was measured in light 1 (699 nm) of $3.1 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1} (when DCMU was added) or $2.0 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1} (when DBMIB was added). Organism: *P. purpureum*.

actinic light 1 was reduced by 50% in order to obtain almost the same photooxidation rate after addition of DBMIB as with or without DCMU. The interruption of electron flow from PS II by DCMU had no effect on the observed phenomenon. As an expected consequence of the inhibition of plastoquinone oxidation, DBMIB strongly accelerated cytochrome *f* photooxidation but did not eliminate the difference between the samples preilluminated with light 1 or light 2. This inefficiency of both inhibitors with respect to the preillumination effect shows that neither reduction of cytochrome *f* by PS II nor by any other process involving plastoquinone can be responsible for the observed effect.

In order to decide whether a reduction of the photochemical efficiency of PS I or only a retardation of electron transport through plastocyanine is responsible for the light-1-dependent deceleration of cytochrome *f* photooxidation, P-700 photooxidation was measured under similar conditions (Fig. 4a,b). In this case, broad-band blue light with a maximum at 400 nm was used as actinic light 1. This light should be absorbed by PS II to a higher degree than 699 nm light, yet to less than approx. 20%. Similar experiments made with narrow-band light of 443 nm, which is absorbed more exclusively by PS I, gave comparable results. The P-700 photooxidation was slowed down by light 1 preillumination to a similar degree as cytochrome *f* photooxidation and this effect was also unimpaired by DCMU or by DBMIB. The slope of the straight line given by plotting the relative photooxidation rate versus the absorbed quantum fluence rate of actinic light 1 was reduced after light 1 preillumination to 46% in the case of P-700 and to 51% in the case of cytochrome *f* (Fig. 4a,b). Similar results were obtained with *P. yezoensis*: after light 1 preillumination, the photooxidation rate of P-700 was approx. 45%, and that of cytochrome *f* approx. 50% of the photooxidation rate after light 2 preillumination.

The preillumination effect on cytochrome *f* photooxidation as on P-700 photooxidation was severely impaired by DCMU and by DBMIB, however, when the inhibitors were added before the preillumination period. In the presence of DCMU; even low intensities of light 2 acted as light 1 and any specific wavelength dependence disappeared. Likewise, in the presence of DBMIB, the wavelength-dependent preillumination effect vanished completely at higher preillumination intensi-

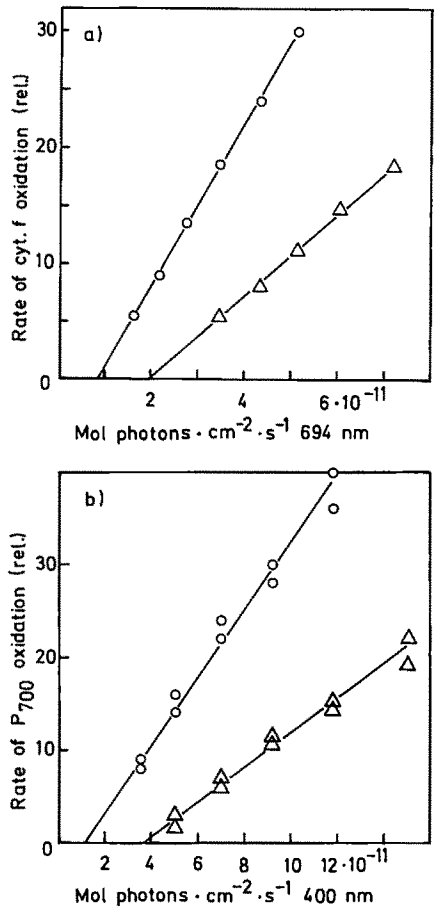

Fig. 4. Relative rate of cytochrome *f* photooxidation (a) and of P-700 photooxidation (b) measured after light 1 (△) or light 2 (○) preillumination as a function of absorbed photon fluence rate. *P. purpureum* has been preilluminated for 5 min with light 2 (550 nm, $3.0 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}) or light 1 (694 nm, $5.1 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1} in cytochrome *f* measurements (a) or broad-band blue light (max. at 400 nm) of $5.0 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1} in P-700 measurements (b)). Actinic light for the photooxidation of cytochrome *f* and P-700 was the same light 1 as during preillumination, but at varying intensities.

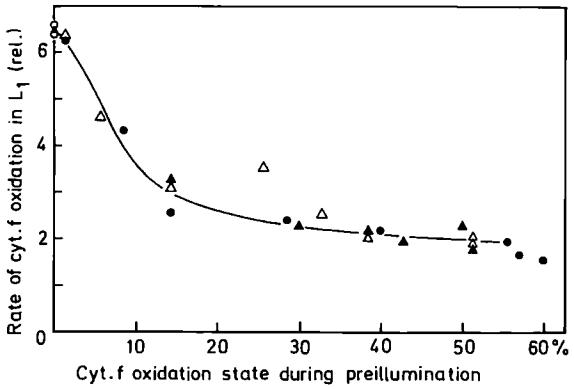

Fig. 5. Rate of cytochrome *f* photooxidation measured after light 1 (694 nm) (△,▲) or light 2 (550 nm) (○,●) preillumination in presence (▲,●) or absence (△,○) of 10^{-6} M DCMU as a function of cytochrome *f* oxidation state (% oxidized from the total photooxidable) attained during the preillumination period. Actinic light was 694 nm light ($5.8 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}). Light 1 intensity during preillumination was varied between $1.3 \cdot 10^{-11}$ and $9.7 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}, light 2 intensity was varied between $2.3 \cdot 10^{-11}$ and $1.7 \cdot 10^{-9}$ mol photons·cm^{-2}·s^{-1}. Organism: *P. purpureum*.

ties, but in this case the resulting photooxidation rate was about twice as high as in the unpoisoned algae, even when preilluminated with light 2. It was identical with the photooxidation rate obtained when DBMIB was added after the light 2 preillumination period. This result may be interpreted as evidence for a control of the apparent quantum yield of PS I by the redox state of a component of (or associated to) the electron-transport chain between PS II and PS I located between the inhibition sites of DCMU and DBMIB. This view is illustrated by the representation of the data in Figs. 5 and 6. For all control experiments without inhibitor these plots show a clear but nonlinear dependence of the photooxidation rate of cytochrome *f* on the oxidation state attained at steady-state during the preillumination period. The same correlation was found when DCMU was present (Fig. 5). There was, however, no such dependence after addition of DBMIB (Fig. 6).

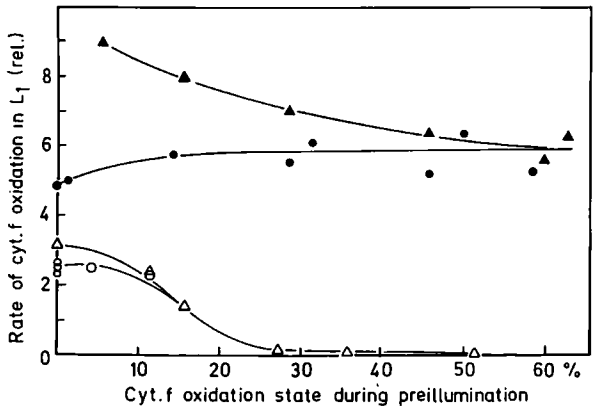

Fig. 6. Conditions as in Fig. 5, but preillumination and measurement in presence (▲,●) or absence (△,○) of $5 \cdot 10^{-6}$ M DBMIB. Actinic light (694 nm) had an intensity of $2.0 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}.

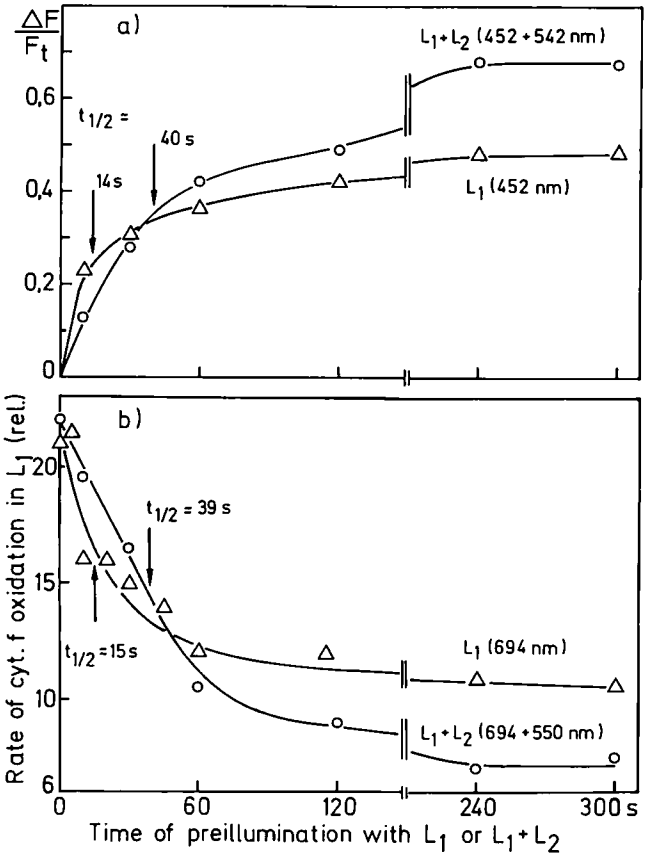

Fig. 7. Fluorescent transient ($\Delta F/F_T$) after a light 1–light 2 change (a) and relative rate of cytochrome *f* photooxidation (b) in *P. yezoensis* as a function of the time of preillumination with light 1 (△) or light 1 + light 2 (○). The illumination schedule for fluorescence measurements (a) was: light 2 (550 nm, $2.4 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}) until steady-state was reached, *x* s light 1 (452 nm, $5.7 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}) or *x* s light 1 + light 2, light 2. The protocol for cytochrome *f* measurements was: light 2 (550 nm, $2.6 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}) until steady-state, *x* s light 1 (694 nm, $5.8 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}) or light 1 + light 2, 3 s light 2, 2 s dark, light 1 (actinic light 694 nm, $3.7 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}).

To obtain comparable photooxidation rates in the presence of both inhibitors the intensity of the actinic light 1 was reduced to $1.5 \cdot 10^{-11}$ mol absorbed photons·cm^{-2}·s^{-1} in the experiment shown in Fig. 6. Under this condition, light 1 preillumination led to complete suppression of cytochrome *f* photooxidation in untreated cells, as can be derived from Fig. 4. As a consequence, the effect appears enhanced compared with Fig. 5.

A reduction of the apparent quantum yield of PS I to about 50% by preillumination with light 1, which is only slowly reversed in light 2, should lead to a strong reduction of Q_A and of the whole electron-transport chain immediately after a light 1–light 2 transition. This eventually could be the main, or even exclusive, reason for the fluorescence transient and the depression of oxygen evolution observed in these two algae after a light 1–light 2 transition. The results drawn in Fig. 7a,b support this view. The dependence of the fluorescence

transient (as $\Delta F/F_T$) and of the photooxidation rate of cytochrome *f* on the length of preillumination with light 1 was estimated in two parallel experiments with identically grown thalli of *P. yezoensis*. Different wavelengths (452 and 694 nm) had to be chosen as light 1 in both experiments. The intensities of both lights were adjusted to give nearly equal overexcitation of PS I compared with PS II. All other conditions were kept identical. There was no obvious difference in the dependence of both phenomena on the duration of the light 1 preillumination period. Additional illumination with low-intensity light 2 during the light 1 preillumination had a double effect on both parameters: the maximum rate of cytochrome *f* photooxidation and the maximum fluorescence transient were markedly enlarged, while the preillumination time necessary to produce the half-maximum effect was more than doubled. This general congruence of fluorescence transients and cytochrome *f* photooxidation rate in all three points may be taken as strong indication of a causal connection between them.

Discussion

The data presented here demonstrate a strong decrease of the apparent quantum yield of PS I in *P. purpureum* and *P. yezoensis* by illumination with light 1 of low or medium intensity. The possibility can be safely excluded that this effect could be based on a regulation of an exciton transfer from PS II to PS I. Furthermore, the fact that P-700 photooxidation is affected in exactly the same way as cytochrome *f* photooxidation excludes the involvement of a modulation of the electron-transfer rate via plastocyanine, as could be suggested by the work of Gross et al. [29–31] on redox state- and pH-dependent conformation changes of plastocyanine. The data shown in this paper do not allow us to decide between the remaining three main lines of interpretation of the effect. It could depend on a partial decoupling of energy transfer between antennae and reaction centers of PS I, on a change in the reaction centers of PS I themselves, which diminishes the photochemical efficiency or, lastly, on a partial switch to a fast light-1-induced cyclic electron transport around PS I. As follows from the experiments with DBMIB, this cyclic pathway should not involve plastoquinone.

The clear dependency of this diminution of the apparent quantum yield of PS I on the degree of oxidation of cytochrome *f* during the preillumination period together with the action of inhibitors provide strong evidence that the effect is controlled by the redox state of plastoquinone, or a tightly connected redox component, just as the phosphorylation of the light-harvesting complex, LHC II, in green plants. A more thorough and detailed discussion of the underlaying mechanism and of the function and ecological significance of this effect will be given in a forthcoming paper on the basis of further results obtained by other experimental approaches.

The reduction of the apparent quantum yield of PS I by illumination with light 1 seems to be a widely distributed phenomenon. We found it (to a largely varying extent) also in several marine red algae as *Porphyra umbilicalis, Palmaria palmata* and *Chondrus crispus* (and also in a cyanobacterium). In these algae other effects induced by a change in the wavelength of light are more or less superimposed on this effect, sometimes masking it. Also, in *P. purpureum*, it is not possible to explain all effects observed at a change between light 1 and light 2 by a reduction of PS I efficiency. For instance, the fluorescence decay after switching from light 1 to light 2 does not correspond with the kinetics of the reversion of the decrease in the cytochrome *f* photooxidation rate.

This light-1-induced reduction of PS I efficiency cannot generally be taken as an expression of reduced vitality of the organism, as a regulation mechanism of second choice, operating only when a more efficient regulation of energy distribution is impaired as a consequence of detrimental conditions during the pretreatment of the alga. We found it regularly in *Porphyridium* grown under optimal culture conditions at every age of the culture and at different developmental stage of the cells (growth was to about 80% synchronous). We have detected this effect also in some freshly collected marine algae. However, in most cases, the effect was strongly pronounced after longer culture of the algae at the laboratory. It seems possible that the effect is also involved in some published observations on energy distribution in red algae. This does not seem to be the case, however, in the experiments reported by Biggins [33], although these are, like ours, made with *P. purpureum*. It has to be considered that these data were obtained under experimental conditions quite different from ours (e.g., at much higher light intensities) on glutaraldehyde-fixed material grown under divergent culture conditions. Moreover, differences between the strains used in these experiments may be responsible for the discrepancies.

Special attention should be paid to the similarity of the effect described here and of its consequence for fluorescence, oxygen exchange, etc., with the symptoms attributed to a real redistribution of excitation energy. Both phenomena seem to be controlled by the redox state of the same, or by nearly connected, components of the electron-transport chain and both will result in similar oxygen and flourescence kinetics, in an enlarged inequality of PS I and PS II activity in light 2 after preillumination with light 1 (state 1) and consequently in higher enhancement values, E_2 (cf. Ref. 32). If the reduction of the apparent quantum yield of PS I in light 1 is combined with any kind of a decrease of PS II

activity in light 2 (or as a consequence of phosphorylation of thylakoids) as described from several organisms [6–11,15,16], this combination is hard to distinguish from a genuine redistribution of excitons, even when fluorescence emission spectra are considered.

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Addendum

Just after our submission of this manuscript for publication a critical, thorough investigation of state transitions in *Scenedesmus* was published [34], which should be mentioned here. Allen and Melis did not find, after transfer of this alga to state 2, any increase of the optical cross-section of PS I corresponding to the observed 20% decrease in energy gain of PS II.

We agree with the authors that, until now, there is little convincing evidence of an energy transfer from PS II to PS I and there seems to be no single criterion which could demonstrate such a mechanism absolute unambiguously. In spite of the difficulties quoted, the uncertainties and negative results, we think, however, that there are several lines of circumstantial evidence, at least for some organisms, in favor of a regulation of energy distribution between PS II and PS I that is not connected with a substantial dissipation of energy.

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