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Changes in the photosynthetic apparatus of red algae induced by spectral alteration of the light field. II. Further characterization of the light-dependent regulation of the apparent quantum yield of PS I

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Several red algae show a light-dependent regulation of the apparent quantum yield of PS I (Rehm, A.M., Gülzow, M. and Ried, A. (1989) Biochim. Biophys. Acta 973, 131–137). The induction of a reduced quantum yield of PS I by light 1 (L1) (exciting preferably chlorophyll) and its revision either in L2 (exciting the phycobiliproteins) or in darkness show different characteristics in *Porphyra yezoensis*, *Rhodella violacea* and *Porphyridium purpureum*. The process of induction, however, is always faster than its reversion. The ability to regulate the quantum yield of PS I is strongly influenced by temperature, not by NH_4Cl. The 77 K fluorescence spectra of PS I in L1 (452 nm) do not show differences after pretreatment in L1, L2 or darkness which could be correlated with an altered quantum yield of PS I. Also, the wavelength of the actinic L1 (694–720 nm) has no influence on the observable differences in P-700 and cytochrome *f* oxidation after preillumination with L1 or L2. These data are interpreted as evidence against a possible decrease of the apparent quantum yield of PS I as a consequence of a functional decoupling of antenna and reaction center of PS I. The fact that in thylakoids the addition of ascorbate (2–10 mM) largely protects from a reduction of the apparent quantum yield of PS I by L1 preillumination and the clear dependence of the PS I activity upon the redox potential of the medium indicates a control of the quantum yield of PS I by a thylakoidal redox component accessible to external redox components. The redox midpoint potential of this component is +140 mV at pH 7.8. This redox center does not participate in linear electron transport between the photosystems and is located nearby PS I.

Introduction

In a recent paper [1] we have shown that in some red algae a change in the wavelength of the exciting light may produce, in addition to other effects (e.g., a redistribution of excitation energy between PS I and PS II), a strong modulation of the apparent quantum yield of PS I. This regulation cannot be explained by an altered energy distribution between the two photosystems or an altered reduction rate of P-700 (via linear electron transport from PS II to PS I or via a DBMIB-sensitive cyclic pathway around PS I). It was thought to depend on the redox state of a redox component associated with plastoquinone. Meanwhile, as very similar effects were found also in leaves of higher plants [2], the observed modulation appears to be a more generally occurring regulatory phenomenon. Some other reports also describe a regulation of the PS I activity independent of a redistribution of energy between the two photosystems [3–5]. However, there is no obvious indication of a connection between these observations and the phenomenon described by us. A question also remains concerning the significance of PS I heterogenity, which is apparently manifested in measurements of the light-saturation curve of PS I electron transport in *Chlorella* [6] and in isolated spinach thylakoids [7]. However, such heterogenity is not manifested in the kinetics of P-700 photooxidation in spinach thylakoids [8]. Moreover, Allen and Melis found the PS I (P-700) activity of *Scenedesmus obliquus* unchanged under con-

Abbreviations: PQ, Plastoquinone; Chl, Chlorophyll; PS, Photosystem; L1, L2, Light 1, Light 2; PAR, photosynthetic active radiation, measured from 400 to 700 nm; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenolindophenol; Hepes, (4-(2-hydroxyethyl)-1-pipierazineethanesulphonic acid; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylendiamine; CCCP, Carbonyl cyanide-3-chlorphenylhydrazone.

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ditions where a significant loss of the absorption cross-section of PS II after adaptation to state 2 was observed [9].

Any unilateral regulation of one photosystem, of PS I as of PS II [10–16], may have a dual function [14,17,18]: it may provide a protection of the respective photosystem against excessive excitation and secondly facilitate electron flow and in this way improve the overall photosynthetic efficiency by redox poising of components of the electron-transport chain. In this last function, such mechanisms compete with a redistribution of excitation energy. They may be imagined as accessory tools to balance the activities of both photosystems if an even distribution of energy cannot be obtained for energetic (primary overexcitation of PS I), spatial (overexcitation of PS II in higher plants) or other reasons. In some cases they may even replace a redistribution mechanism. All the quoted mechanisms of a fast adaptation to changing light conditions resemble one another phenomenologically in many respects and especially in their wavelength sensitivity. So the question arises, wether all mechanisms are controlled by the same sensor or each has its own detector system. The statement that the diverse regulatory mechanisms act independently does not necessarily exclude a common link in their signal chains. Their relative independence could be caused by additional special effectors. It was the aim of our work to contribute to this problem and to characterize further the regulation of the apparent quantum yield of PS I in red algae. Experiments on isolated thylakoids presented more concrete evidence for a control of the apparent quantum yield of PS I by a redox component which equilibrates with members of the electron-transport chain between the two photosystems.

Materials and Methods

Porphyridium purpureum and *Porphyra yezoensis* were cultured as described elsewhere [1]. *Rhodella violacea* Kornmann (Sammlung von Algenkulturen, Göttingen) was grown in glass tubes in 500 ml of the following medium: 400 ml Jones medium [19], 200 ml seawater, 390 ml distilled water and 10 ml soil extract. Green light was produced by installing a Röhm & Haas Plexiglas filter Nr. 701 in front of 5 Osram fluorescent lamps (L 40W/25-1). Irradiation intensity was $2.5 \cdot 10^{-9}$ mol photons $\cdot cm^{-2} \cdot s^{-1}$ PAR.

Oxygen evolution, cytochrome *f* and P-700 turnover were estimated as described previously [1]. P-430 turnover was measured as difference of absorption between 430 nm and 445 nm. Care was taken to prevent absorption changes due to P-700 during these experiments.

Fluorescence spectra were obtained using a system with a four-branched fiber optic. Two branches channelled the actinic light (L1 = 452 nm, L2 = 550 nm) while the emitted light was collected through the remaining arms. One arm was connected to a spectrograph combined with a diode array and an O-SMA detector controller (Spectroscopy Instruments). The fluorescence spectra were stored in a computer. The obtained spectra were corrected for the wavelength sensitivity of the sensor system and for scattered light. Two methods were developed for the preparation of the sample. Cells of *Rhodella* or *Porphyridium* were either sedimented on a cellulose nitrate filter (Satorius, pore size 0.65 μm) or 150 μl of the cell suspensions were placed between two dialysis membranes fixed on two metal rings, resulting in a thickness of the sample of 1 mm. Effects of reabsorption were negligible under our experimental conditions. The samples were preilluminated or dark-adapted in a home-built apparatus and were kept in temperature-controlled medium during illumination by use of a fiber optic. This system allows one to take the sample out of the medium and drop it into liquid nitrogen without any change or interruption of the preillumination conditions within less than 5 s.

We used three methods to normalize the fluorescence spectra: (1) normalization at the emission maxima of phycocyanin and allophycocyanin at 640–655 nm under L2 (550 nm) excitation (APC correction); (2) normalization by reference to the chlorophyll content of the sample; chlorophyll extraction and estimation [20] was done right after the fluorescence measurement (Chl correction); (3) normalization by use of fluorescein as an internal standard. Fluorescein fluorescence was detected in L1 (452 nm) by a photomultiplier which was protected by a Schott OG 515 and a IL 534 interference filter. Concentration of fluorescein was $1 \cdot 10^{-5}$ M (Fluorescein correction).

Prolonged illumination of the fluorescein-containing suspension led to a loss of fluorescein fluorescence. Further difficulties in using fluorescein as an internal standard arose from the strong dependence of the fluorescence yield on ionic strength and pH of the medium.

The isolation procedure of intact thylakoid vesicles (PBS vesicles) described by Dilworth and Gantt [21] was modified in two aspects. (1) Since the ionic strength and composition of the isolation buffer may strongly influence not only the activity but also the regulatory competence of the thylakoids, several buffers were tested. The following two buffers gave the best results and were henceforth regularly used in the experiments: (I) citrate 300 mM, K_2HPO_4 500 mM, $MgCl_2$ 1 mM, $MnCl_2$ 1 mM, KOH (pH 7.5–8.0); (II) Hepes 30 mM, glucose 600 mM, KCl 100 mM, $MgCl_2$ 1 mM, $MnCl_2$ 1 mM, KOH (pH 7.5–8.0). The two buffers did not induce any significant differences in the ability of the thylakoids to regulate the quantum yield of PS I. However, at 4° C the thylakoids were stable for several hours in buffer I, whereas a decoupling of phycoerythrin from

the phycobilisome occurred in buffer II during this time. (2) *Porphyridium* cells were not washed in deionized water but in the isolation buffer prior to cell breakage in the sonifier.

$DCPIPH_2$ oxidation of the vesicles by PS I was monitored at 597 nm in an Aminco DW-2 spectrophotometer at 10 °C. Excitation light was passed through a Schott RG 665 filter. The sample contained 12–15 mg Chl/l and the following additions: DCMU, $1{,}5 \cdot 10^{-5}$ M; methylviologen, $5 \cdot 10^{-4}$ M; DCPIP, $2.5 \cdot 10^{-5}$–$2 \cdot 10^{-4}$ M; TMPD, $8 \cdot 10^{-4}$ M; ascorbate, $(1–3) \cdot 10^{-4}$ M. The redox potentials were calculated from the ratio $DCPIP_{reduced}/DCPIP_{oxidized}$ underlying a midpoint potential of DCPIP at pH 7.0 $E_{m7.0} = +217$ mV.

Results

The ability to reduce the apparent quantum yield of PS I in L1 as described for *Porphyra yezoensis* and *Porphyridium purpureum* (1) was found as a general phenomenon in many red algae (*Rhodella violacea, Porphyridium aerugineum, Porphyra umbilicalis, Palmaria palmata, Chondrus crispus* and *Cryptopleura ramosa*), whereas the extent, the induction pattern and some other characteristics of this reaction differed widely. Variability was also observed within one species under different culture conditions. In the case of *Porphyra yezoensis* a stronger decrease of the apparent quantum yield of PS I could be observed in a combination of L1 and L2 compared to a pure L1 illumination. This phenomenon was not detectable in *Porphyridium purpureum* or *Rhodella violacea. Rhodella violacea* grown in green light did not show any decrease in the apparent quantum yield of PS I after illumination with weak L1 (699 nm, $1.55 \cdot 10^{-10}$ mol photons $\cdot$ cm$^{-2} \cdot$ s^{-1}), whereas cells grown in white light reduced the apparent quantum yield of PS I up to 40% under these conditions, behaving comparable to *Porphyridium purpureum* grown in white light [1].

In all cases, the reversion of the reduction of the apparent quantum yield of PS I in L2 needed substantially more time than the induction by L1. In *Porphyridium purpureum*, the half-time for the induction of a minimum quantum yield of PS I is $t_{1/2} = 30$ s whereas $t_{1/2}$ for the reversion in L2 or in darkness is 90 s and 200–220 s, respectively (Fig. 1). For *Porphyra yezoensis* the reported differences in the half-times were still greater: for the induction by L1 $t_{1/2} = 15$ s, by L1 + L2 $t_{1/2} = 39$ s and for the reversion in L2 $t_{1/2} =$ 220–250 s [1]. *Rhodella* grown in white light reached the half-maximum decrease of the apparent quantum yield of PS I after 5–7 s in L1 (699 nm, $5.72 \cdot 10^{-10}$ mol photons $\cdot$ cm$^{-2} \cdot$ s^{-1}). The half-time for the reversion in L2 (550 nm, $9 \cdot 10^{-10}$ mol photons $\cdot$ cm$^{-2} \cdot$ s^{-1}) was about 25 s. The reversion kinetics in darkness showed a fast and slow phase (data not shown).

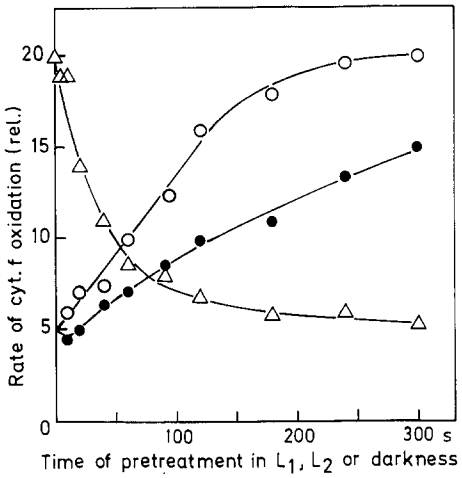


Fig. 1. Rate of oxidation of cytochrome *f* in L1 (699 nm, $3.5 \cdot 10^{-10}$ mol photons $\cdot$ cm$^{-2} \cdot$ s^{-1}) as a function of the time of preillumination with L1 (699 nm, $9.0 \cdot 10^{-10}$ mol photons $\cdot$ cm$^{-2} \cdot$ s^{-1}, △) or L2 (550 nm, $4.1 \cdot 10^{-10}$ mol photons $\cdot$ cm$^{-2} \cdot$ s^{-1}, ○) or of incubation in darkness (●). Each individual preillumination period of variable length was preceded by standard pretreatment of 10 min with L1 (699 nm, $9.0 \cdot 10^{-10}$ mol photons $\cdot$ cm$^{-2} \cdot$ s^{-1}, ○, ●) or L2 (550 nm, $4.1 \cdot 10^{-10}$ mol photons $\cdot$ cm$^{-2} \cdot$ s^{-1}, △). Organism: *P. purpureum*.

The ability of *Porphyridium purpureum* to regulate the quantum yield of PS I was strongly influenced by temperature, with a distinct maximum at 18 °C, the growth temperature (Fig. 2). Higher temperatures (above 20 °C) reduced the ability for regulation of the PS I quantum yield mainly by reducing the quantum yield of PS I in L2. At about 30 °C the PS I quantum yield became independent of light quality and showed a value close to the minimum quantum yield of 18 °C. In *Porphyra yezoensis* the apparent quantum yield of PS I after L1 and L2 preillumination showed a similar temperature dependence, but at temperatures above 25 °C the oxidation rate of cytochrome *f* and P-700 became further reduced by an additional irreversible process. At the temperature optimum the ratio between the maximum and minimum apparent quantum yield of PS I is 2.7 in *Porphyridium purpureum* (Fig. 2) and up to 3.7 in *Porphyra yezoensis*.

The fact that the regulation of the apparent quantum yield of PS I can be observed over a wide range of L1 and L2 intensities [1] can be interpreted as an evidence for a mechanism not directly influenced by the proton gradient across the thylakoid membrane. The influence of NH_4Cl on oxygen evolution, the reduction rate of cytochrome *f* and on the quantum yield of cytochrome *f* oxidation under different light regimes is summarized in Table I for *Porphyra yezoensis*. The decrease in steady-state oxygen evolution and the increase in the cytochrome *f* reduction rate by about 15% indicate that NH_4Cl (5 mM) is a weak uncoupler of photophosphorylation. A stronger effect was not observed by higher concentrations of NH_4Cl. In the presence of NH_4Cl,

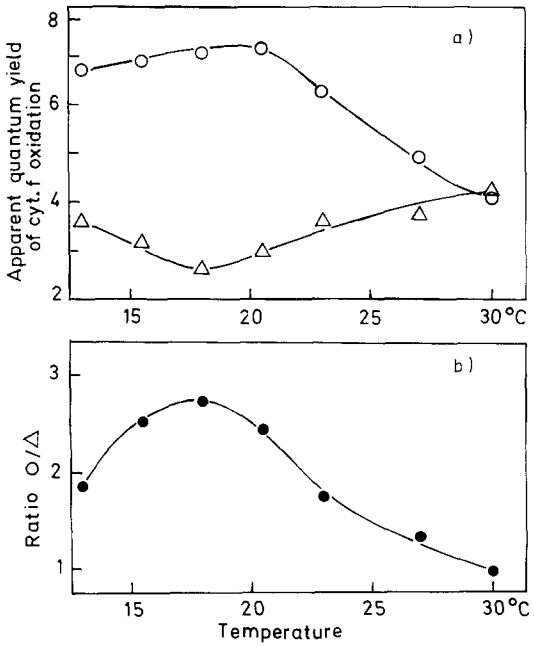


Fig. 2. (a) Apparent quantum yield of cytochrome *f* oxidation of L1 (694 nm) after preillumination with L1 (694 nm, $4.15 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}) (△) or L2 (550 nm, $2.2 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}) (○) as a function of the temperature during pretreatment. Each individual point represents the slope of the plot: rate of cytochrome *f* oxidation versus L1 intensity (see Fig. 4) for the given temperature. (b) Ratios of the apparent quantum yields of cytochrome *f* oxidation after preillumination with L2 or L1 versus temperature. Organism: *P. purpureum*.

the quantum yield of cytochrome *f* oxidation is slightly enhanced after dark pretreatment but reduced after preillumination. However, the differences of the apparent quantum yield of cytochrome *f* oxidation after preillumination with L2 or L1 + L2 remained almost unchanged. The influence of NH_4Cl on the apparent quantum yield of cytochrome *f* oxidation in L1 was small. In the presence of CCCP ($(1-5) \cdot 10^{-6}$ M) cytochrome *f* and P-700 could hardly be oxidized even in L1 (694 nm), so a clear estimation of the apparent quantum yield of PS I was not possible.

TABLE I

Apparent quantum yield (defined by the slope of the plot: rate of cytochrome *f* oxidation vs. L1 intensity) of cytochrome *f* oxidation in L1 (694 nm), oxygen evolution and reduction rate of cytochrome *f* after different pretreatment of a *P. yezoensis* thallus in the presence or absence of 5 mM NH_4Cl.

Pretreatment	Quantum yield of cytochrome *f* oxidation in L_1 (%)		Effect of NH_4Cl (%)	
	control	$+NH_4Cl$	reduction-rate of cyt. f	oxygen evolution
Dark	105	125	+16–17	–
L_2	100	70	+11–14	
L_1	42	38	+18	−15%
$L_1 + L_2$	28	17	+12	

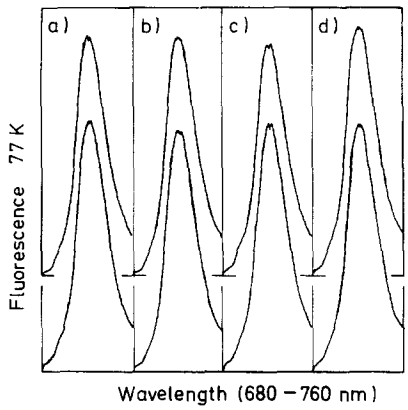


Fig. 3. 77 K fluorescence spectra of *R. violacea* cells excited with L1 (452 nm). The spectra were normalized by reference to the chlorophyll content of the samples (Chl correction, upper row), or to the fluorescence signal at 640–655 nm in L2 (APC correction, lower row). All spectra are plotted from 680 to 760 nm. The cells were pretreated before freezing at 77 K as follows: (a) 50 min darkness; (b) 10 min L2 (550 nm, $3.75 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1}); (c, d) 10 min L2 (550 nm, same intensity as (b), followed by 10 min L1 (699 nm, $4.6 \cdot 10^{-11}$ mol photons·cm^{-2}·s^{-1} in (c) or $4.55 \cdot 10^{-10}$ mol photons·cm^{-2}·s^{-1} in (d). The shape of the fluorescence spectra is unaltered, the observable differences in the heights of the maxima at 720–721 nm being within the error of 5–10%.

P-430 turnover in pure L1 measured as difference of absorption at 430–445 nm showed the same differences after L1 or L2 preillumination as reported for P-700 and cytochrome *f* (not shown). Any contamination of this signal by P-700 was prevented by the use of L1 too low to oxidize P-700.

In search for other symptoms of a possible decoupling of part of the PS I antenna, we recorded fluorescence emission spectra of *Rhodella violacea* at 77 K after different pretreatments (Fig. 3). In red algea the blue light (452 nm) used for excitation is almost exclusively absorbed by chlorophylls and carotenoids associated with PS I and therefore predominantly excites PS I. All spectra exhibited an emission maximum at 721–722 nm with two small shoulders at 685 nm and 695 nm. The emission band at 685 nm is complex and can be attributed partly to APC-B, to the 95 kDa linker polypeptide and to a chlorophyll-protein complex of PS II. The origin of the emission maximum at 695 nm is not yet clear, but it is believed to belong to a fluorescence emitter of PS II. Under excitation with blue light the two emission bands at 685 nm and 695 nm contribute to the emission maximum of PS I at 721–722 nm with less than 10%. Large changes in the fluorescence yields of the emission bands at 685 nm and 695 nm correspond to only minor changes (within the limits of error) of the emission at 721–722 nm.

R. violacea was chosen for the experiment shown in Fig. 3, because cells grown in green light did not show any decrease in the apparent quantum yield of PS I after preillumination with weak L1 (up to $1.5 \cdot 10^{-10}$ mol photons · cm^{-2} · s^{-1}) but a strong reduction of the

quantum yield of PS I after pretreatment with L1 intensities above $(2.5-3.0)\cdot 10^{-10}$ mol photons $\cdot$ cm$^{-2}\cdot$ s^{-1}. Cells were incubated in darkness for 10 min or preilluminated with either L2 (550 nm, $3.75\cdot 10^{-10}$ mol photons $\cdot$ cm$^{-2}\cdot$ s^{-1}) alone or with two intensities of L1 (699 nm, $4.6\cdot 10^{-11}$ and $4.55\cdot 10^{-10}$ mol photons $\cdot$ cm$^{-2}\cdot$ s^{-1}) before they were dropped into liquid nitrogen. The L1 intensity of $4.55\cdot 10^{-10}$ mol photons $\cdot$ cm$^{-2}\cdot$ s^{-1} was sufficient to produce a decrease in the apparent quantum yield of PS I of 50%. The fluorescence spectra were corrected either by the Chl correction method (Fig. 3, upper row) or by the APC correction method (Fig. 3, lower row, see Materials and Methods). The different pretreatments did not change the shape of the fluorescence spectra. The maximum differences of the four samples in the heights of the emission peak at 721–722 nm was 4% after APC correction and 7% after Chl correction. Similar results were obtained with *P. purpureum* with maximum differences in the PS I emission peak at 717 nm of 5–10% (data not shown). The use of fluorescein as an internal standard to correct the fluorescence spectra yielded more scattered results. Nevertheless, no correlation was observed between a reduced quantum yield of PS I induced by L1 preillumination and a change of the fluorescence emission of PS I at 77 K in *P. purpureum* and *R. violacea*.

In contrast to whole cells, thylakoid membranes are more suitable for clarifying the sensor system and the mechanism of the regulation of PS I quantum yield, due to the accessibility of the isolated thylakoids to electron donors and acceptors. We therefore prepared PBS vesicles which were able to evolve oxygen in L2 and which showed the same phycoerythrin/Chl and P-700/Chl ratios as intact cells. About 30–50% of the water-splitting complex was inactivated by the isolation procedure. The cytochrome *f* content estimated via oxidation in L1 was reduced to 60–75% in the vesicles compared to whole cells. Fig. 4 demonstrated that the PBS vesicles are able to regulate the quantum yield of PS I in the same manner as intact cells. In this experiment, L1 preillumination reduced the apparent quantum yield of PS I to 46.5% compared to dark pretreatment. In contrast to intact cells, preillumination with L2 also causes a reduction, although only to 75%. This difference may be easily explained by the fact that in PBS vesicles the water-splitting system was partly impaired. Therefore the L2 preillumination will have resulted in a partial oxidation of the electron-transport chain as is obtained in intact cells by irradiation with L1 of low intensity. In L2 and darkness P-700 was fully reduced while during L1 preillumination about 18% of P-700 were in the oxidized state. The reduction of P-700 after its oxidation by actinic L1 was very slow compared to *Porphyridium* cells. The half-time of the reduction of P-700 was $t_{1/2} = 13$ s; this is more than 10-times longer as in cells. So the reduction rate of P-700 cannot

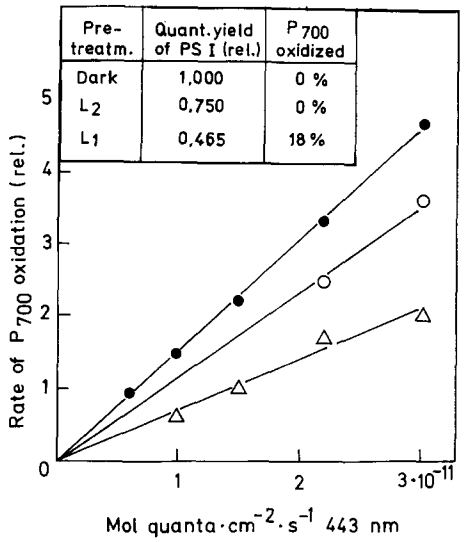


Fig. 4. Rate of P-700 oxidation of PBS vesicles (*P. purpureum*) as a function of absorbed quanta of L1 (699 nm). The thylakoids were preincubated in darkness (●) or preilluminated with L1 (440 nm, △) or L2 (550 nm, ○). The inset shows the apparent quantum yield of P-700 oxidation (defined by the slopes) and the portion of P-700 oxidized during pretreatment. The sample (31 mg Chl *a*/l) contained $2\cdot 10^{-4}$ M methylviologen.

be responsible for the observed differences in the oxidation by L1. We therefore conclude that the PBS vesicles exhibit a real regulation of the quantum yield of PS I which shows the same characteristics as in intact cells. Illumination with L2 of high intensity resulted in a partly oxidized P-700 during preillumination and, like preillumination with L1, in a strongly reduced quantum yield of PS I.

The effect of L1 illumination on the quantum yield of PS I was strongly diminished after addition of ascorbate (2–10 mM) to the thylakoids (see Table II). PS I quantum yield was reduced to 30% after L1 preillumination in the absence of ascorbate. In the presence of ascorbate, however, the quantum yield of PS I was reduced only to 82% after a L1 preillumination compared to a dark pretreatment. In both cases about 70% of P-700 were oxidized during L1 preillumination.

TABLE II

Apparent quantum yield of P-700 oxidation in L1 (699 nm) of PBS vesicles from *P. purpureum* after different pretreatment in the presence or absence of 8 mM ascorbate.

Pretreatment	Quantum yield of P700-oxidation +DCMU ($7\cdot 10^{-6}$ M) +MV ($2\cdot 10^{-4}$ M)	
	− ascorbate	+ ascorbate ($8\cdot 10^{-3}$ M)
Dark	1.00	1.05
L_1, 440 nm $5.7\cdot 10^{-11}$ mol photons $\cdot$ cm$^{-2}\cdot$ s^{-1}	0.30	0.84

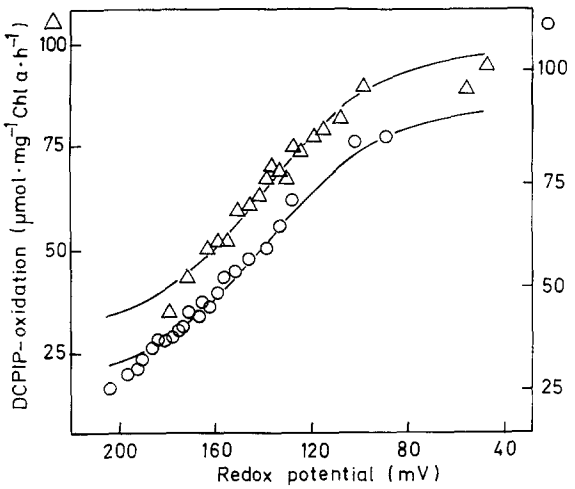


Fig. 5. $DCPIPH_2$ oxidation of two preparations of PBS vesicles (*P. purpureum*) in L 1 (660 nm) as a function of the redox potential at pH 7.8. The redox potential was calculated from the ratio of $DCPIP_{reduced}/DCPIP_{oxidized}$. The two samples contained: 15 mg Chl/l; $1.5 \cdot 10^{-5}$ M DCMU; $5 \cdot 10^{-4}$ M methylviologen; $(2.5-20) \cdot 10^{-5}$ M DCPIP; $8 \cdot 10^{-4}$ M TMPD and $(1.5-4) \cdot 10^{-4}$ M ascorbate. The titration was performed from low to high redox potentials. The solid curves are standard Nernst-equations for a redox component with a midpoint potential of +140 mV and a one-electron reaction. For further details see text.

Ascorbate enhanced the reduction rate of P-700, but still the reduction was more than 5-times slower than in *Porphyridium* cells.

To find out whether a redox component located in the membrane is responsible for the regulation of the quantum yield of PS I as suggested previously [1], we studied the dependence of PS I activity on the redox potential of the medium. Thylakoids were kept in the dark for 5–10 min and care was taken that the redox potential of the medium was stable prior to the measurement (see Materials and Methods). The plots of two experiments shown in Fig. 5 were fitted by a Nernst curve of a titration with a one-electron step ($n = 1$). The calculated midpoint potential in both experiments was +140 mV, but the maximum and minimum activities of PS I differed slightly. The ratio of the calculated maximum and minimum PS I activities was 3.0 in one experiment (upper curve) and 3.3 in the other (lower curve). The values are in good agreement with the differences in the quantum yield of PS I in thylakoids after dark pretreatment and L1 preillumination (see Table II) and the ratio of the maximum and minimum quantum yields of PS I in intact cells after L1 and L2 preillumination (see Fig. 2b). The titration was performed from low to high redox potentials. After the termination of the titration, the redox-potential was lowered again by addition of small amounts of ascorbate that completely reduced DCPIP and restored PS I activity within 15 min to 75% and 90% of the maximum values in these two experiments. So we conclude that tolerable although not completely negligible processes of inactivation took place during the measurements. The region within which this system allows us to attribute the observed PS I activity to the redox potential of the medium is very small. It is limited by the oxidation of TMPD (the ultimate electron donor of PS I) at potentials higher than +180 to +200 mV and by the reduction of DCPIP by ascorbate at potentials lower than +40 to +60 mV. This reduction of DCPIP by ascorbate can be observed after switching off the actinic L1 and is absent at redox potentials higher than +60 mV. Although the region of the redox titration of the activity of PS I is very small, the good fit of the measured values by a Nernst curve justifies the conclusion that a redox component of the thylakoid membrane with a midpoint potential $E_m = +140$ mV at pH 7.8 controls the apparent quantum yield of PS I.

Discussion

The ability for a light-dependent regulation of the apparent quantum yield of PS I does not seem to be restricted to red algae and cyanobacteria. Aware of our work, Weinzettel et al. [2] found a reduction of the apparent quantum yield of cytochrome *f* oxidation after L1 preillumination of intact leaves of *Sinapis alba*.

For *P. yezoensis* it was shown that a strong correlation exists between the quantum yield of PS I and the extent of the fluorescence-rise ($\Delta F/F_1$) after switching from L1 to L2 [1]. We did not find such a strong correlation in other red algae. Therefore, this result must not be generalized. A transient fluorescence rise after a L1–L2 switch could equally result from a redistribution of excitation energy as from a slowly developing process of nonradiative energy dissipation in PS II or from a reversible reduction of the apparent quantum yield of PS I in L1, or from any combination of these mechanisms. The observation that the induction of a reduced quantum yield of PS I by L1 preillumination was invariably faster than the reversion in L2 (see Fig. 1) segregates this effect from the state 1–state 2 transition in some marine Florideae, where identical kinetics in both directions have been found [22]. The phenomenon described in this paper differs also in some other aspects from the previously reported effects with Florideae [23,24]. This indicates that really two or even more different fast regulation processes can contribute to the adaptation of the photosynthetic apparatus of red algae to different light qualities. There are several indications that the phenomenon described by Ried and Reinhardt [22–25] may reflect a real regulation of the energy distribution between PS I and PS II with no or only minor contamination by other effects. Some other observations on *P. cruentum* [26], *P. yezoensis* [27] and possibly also some observations on blue-green algae [28,29], organisms which we have found to show a prominent regulation of the apparent quantum yield of

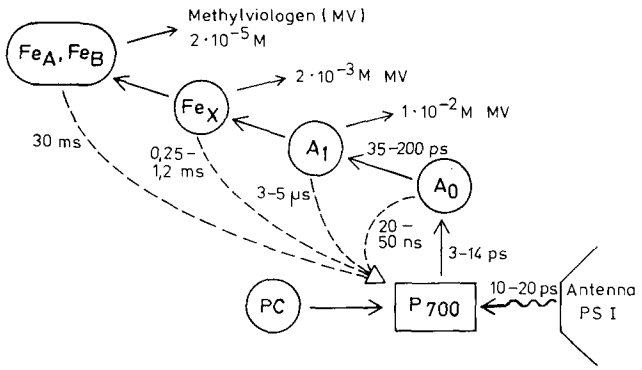
PS I [1] may more likely refer to a combination of at least two effects: a regulation of the energy distribution between the two photosystems and of the apparent quantum yield of PS I.

The effect of temperature on the regulation of the quantum yield of PS I cannot be easily understood. Besides reports on other effects observed after increasing the temperature [30–34], Weis reported an increase in spill-over (PS II–PS I) with rising temperature [35] and concluded that the observed mechanism would help to reestablish a good redox poise under changing environmental conditions. Any temperature-dependent change in the activity of the Calvin cycle will react – by changing the NADPH/$NADP^+$ and ATP/ADP ratios – on photosynthetic electron and proton transport. In this way also the regulation of the apparent quantum yield of PS I could be affected. Increasing the temperature also induces a higher membrane fluidity in pea thylakoids [36] and enhances the rate of cytochrome *f* reduction in red algae [37] and cyanobacteria [38]. An increased rate of plastoquinone oxidation at higher temperature decreases the portion of reduced plastoquinone which could interact with the '+140 mV' redox center. On the other hand, increasing the temperature can lead to an altered interaction of lipids and polypeptides, which can result in a reduced quantum yield of PS I.

The main intention of this work was to contribute to two central questions. (1) What is the nature of the changes in PS I responsible for the observed reduction of the apparent quantum yield induced by L1 preillumination? (2) What is the nature and location of the sensor system and how does it affect PS I? Concerning the first problem, some possible mechanisms have been excluded on the basis of already published data [1]: the reduction of the apparent quantum yield of PS I is not based (1) on any kind of redistribution of excitation energy, or (2) on a retardation of electron donation by photosynthetic control or by a modulation of the electron-transfer rate via plastocyanin, nor is it (3) only simulated by an accelerated electron donation by PS II or by a cyclic electron flow around PS I involving plastoquinone. Two main possibilities remain: the apparent quantum yield of PS I is reduced in L1 either by a decrease in the excitonic interaction between a chlorophyll antenna and P-700, or by a change within the PS I core. This change could result in an enhanced charge recombination between an early electron acceptor of PS I and $P\text{-}700^+$ or in the establishment of an energy trap dissipating energy in a nonradiative way.

We found no evidence for a decoupling of a chlorophyll antenna of PS I from the core complex as described by Gross and Grenier in Mg^{2+}-deficient chloroplasts [4]. The strong fluorescence emission at 77 K of intact cells excited by L1 of 452 nm can be ascribed to the PS I core [39] and results from a reduced energy transfer between the fluorescing chlorophyll species and P-700 [39–41]. The unchanged fluorescence properties of PS I at 77 K give no hint of an altered coupling of antennae and reaction centers (see Fig. 4), especially if we take into account that under the low excitation intensities used in our experiments virtually no excitation annihilation processes occur in the antenna complex [42]. However, we cannot entirely exclude a loss of excitation transfer from antenna to P-700 to participate in the observed reduction of the quantum yield of PS I. It is not yet clear if possible differences in the rates of energy transfer from antenna to P-700 can be neutralized by cooling to 77 K. If a decoupling of antenna and reaction center would be responsible for the regulation of the apparent quantum yield of PS I we should find also remarkable differences in the quantum yield of cytochrome *f* oxidation after L1 preillumination when the oxidation rate of cytochrome *f* is measured in actinic L1 of different wavelength. As already mentioned, we found no differences with L1 of 694 nm and 699 nm [1]. Even in L1 of 720 nm obtained with an Oriel interference filter (half-bandwidth = 10.8 nm) we detected a strong reduction of the apparent quantum yield of cytochrome *f* oxidation after L1 preillumination, and the calculated ratios of maximum and minimum quantum yield of PS I were the same (within the error of 7%) in L1 of 694 nm, 699 nm and 720 nm (data not shown). However, also this result is no unequivocal indication for an unaltered excitonic interaction between antennae and reaction centers of PS I if red algae have a chlorophyll antenna like blue-green algae [43] absorbing in the far red (C-710) and therefore having absorption characteristics similar to P-700 itself.

However, on the basis of the present results it seems more likely that the quantum yield of PS I is modulated by a change in the PS I core itself. The scheme of the electron transfer processes in PS I which combines data from the literature [39,42,44–53] may facilitate the formulation of conditions for a charge recombination process in PS I which could be responsible for the observed decrease of the apparent quantum yield of PS I.



Such reactions must compete very effectively with the forward reaction as with reduced plastocyanin for electron donation to P-700$^+$. Recombination of Fe_A, Fe_B (reduced) or Fe_X (reduced) with P-700$^+$ is much slower than the reduction of P-700$^+$ by plastocyanin (see Scheme I). To be competitive, this recombination reaction must be at least 100-fold enhanced in L1. Recombination of A_1^- or A_0^- with P-700$^+$ would be fast enough to compete efficiently for the reduction of P-700$^+$. If this type of recombination were to be involved in the decrease of the apparent quantum yield of PS I, we would have to assume a strong retardation of electron transport from $A_0 - A_1$ to the iron-sulfur centers as a primary effect of preillumination with L1.

The absorption changes at 430 nm under conditions of weak actinic L1 where no absorption changes of P-700 can be observed are correlated with redox reactions of the iron-sulfur centers in PS I [44,48,49,54–56]. The signal at 430 nm cannot be attributed to one center only (Fe_X, Fe_A, Fe_B) but is a combination of signals of all three redox centers. In all cases of possible recombination reactions mentioned above one should expect that P-700 and P430 behave similarly at an L1-L2 change as we have observed. Any recombination process also is expected to produce triplet states at an enhanced rate, whose detection – planned for the near future – could enable to distinguish this mechanism from the other ones.

The quantum yield of PS I seems to be controlled by a redox component with a mid-point potential of +140 mV at pH 7.8 (Fig. 5). The experiments on PBS vesicles suggest that this redox center is associated with the thylakoid membrane and can equilibrate with plastoquinone and with added ascorbate. In the presence of ascorbate (8 mM) we measured a nearly maximum apparent quantum yield of PS I even under conditions (in the presence of DCMU and methylviologen) where cytochrome *f* was oxidized to 70% and the PQ pool was largely oxidized (Table II). We therefore conclude that a redox center which is accessible to PQ and ascorbate but which obviously does not participate in the linear electron transport between the two photosystems may act as a sensor. Its equilibration with the PQ pool can be suppressed by artificial electron donors. If this redox center were to be located close to the CPI complex, its oxidation or reduction would be able to induce changes in the interaction of the redox components in PS I, especially between P-700, A_0, A_1 or the iron-sulfur centers.

The measured value of +140 mV for the midpoint potential, which is higher than the midpoint potential of plastoquinone, is consistent with the fact that after dark pretreatment where more than 40% of plastoquinone is oxidized [57], the apparent quantum yield of PS I is high [1]. It was not expected that the '+140 mV' redox component seems to be a one electron carrier but interacts with plastoquinone. The nature of the redox component as a one-electron carrier could arise from an insufficient equilibration of the redox center with the redox potential of the medium measured via DCPIP absorption, all the more since the titration was made without special redox mediators. The fast restoration of the initial PS I activity (up to 90%) by lowering the redox potential seems to rule out this possibility. Yet we have no clear idea in which way plastoquinone reduces the '+140 mV' redox center. The observation that the equilibration of the redox component with the PQ pool is not markedly influenced by lowering of the ΔpH across the thylakoid membrane (see results with NH_4Cl) suggest that this process is not accompanied by a proton translocation across the thylakoid membrane.

Our results indicate that in red algae at least three regulation processes poise the electron transport between the two photosystems: (1) energy redistribution between PS I and PS II [22–25]; (2) modulation of the apparent quantum yield of PS I (Ref. 1, this report); and (3) modulation of the apparent quantum yield of PS II (Ref. 10, unpublished data). All of them seem to be under control of a redox component which can interact with members of the electron transport chain between the two photosystems. It cannot be decided, however, if each of these processes has its own individual sensor, although for a fast, sensitive and differentiated answer to altered environmental conditions this would seem more favorable.

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