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ΔpH-dependent fluorescence quenching and its photoprotective role in the unicellular red alga *Rhodella violacea*

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Abstract

Plants have developed various photoprotective mechanisms to resist irradiation stress. One of the photoprotective mechanisms described in the literature for LHC2-containing organisms involves a down-regulation of photosystem (PS) 2 occurring simultaneously with the build-up of a proton gradient across the thylakoid membrane (ΔpH). It is often correlated with deepoxidation of xanthophylls located in LHC2. In *Rhodophyta* instead of LHC2, the peripheral antenna of PS2 consists of a large extramembrane complex, the phycobilisome (PBS), which transfers its excitation to the core antennae of PS2 composed of the CP43 and CP47 protein-chlorophyll complexes and there is no xanthophyll cycle. In the red alga *Rhodella violacea* a ΔpH-dependent chlorophyll (Chl) *a* fluorescence quenching can be formed. We characterised this quenching, studied the effects of various irradiances and inhibitors. Under photoinhibitory conditions, the ΔpH-dependent Chl fluorescence quenching exerts a photoprotective role and delays the kinetics of photoinhibition. It is the first time that such a photoprotective mechanism is described in PBS-containing organisms.

Additional key words: chlorophyll; irradiance; light-harvesting chlorophyll-protein complex; photoinhibition; photosystems 1 and 2; phycobilisome.

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Abbreviations: Chl, chlorophyll; LHC2, light-harvesting complex of photosystem 2; DCCD, *N,N'*-dicyclohexyl carbodiimide; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LED, light emitting diode; PBS, phycobilisome; PS, photosystem; Q_A, primary electron acceptor; Q_B, secondary electron acceptor.

Introduction

Photosynthetic organisms convert radiant energy into chemical energy. The energy absorption occurs at the antenna of two membrane-pigment complexes, PS1 and PS2. The absorbed energy is transferred to the reaction centres which operate in series to oxidise water and reduce CO_2. The electron transfer is coupled to proton translocation across the thylakoid membrane, generating a proton motive force used by the ATPase for the synthesis of ATP molecules. The ΔpH is formed at different points of the electron transfer chain. The production of one molecule of oxygen is accompanied by a release of four protons into the lumen. Plastoquinone molecules reduced by PS2 are protonated by protons coming from the stroma and when reoxidized by PS1 they release the protons into the lumen (Crofts and Wraight 1983, Ort and Yocum 1996). In the stroma, protons are bound by carbon dioxide reduction. Photochemistry is the main pathway of deactivation of the excitons. Fluorescence and thermal deactivation are the alternative ways to deactivate the excitons. At room temperature, fluorescence comes mainly from the Chl *a* antenna of PS2. Its yield is modulated by the oxidoreduction state of the primary acceptor of PS2, the plastoquinone Q_A (Duysens and Sweers 1963, van Gorkom 1974). Photochemical conversion is maximal when all PS2 centres are open (Q_A oxidised) and this situation corresponds to the minimal fluorescence of PS2, the F_0 level. When the centres become closed (Q_A reduced), fluorescence rises to a maximal fluorescence F_m. The fluorescence yield of PS2 is also modulated by non-photochemical quenching mechanisms ascribed to three main processes: state transitions, ΔpH-dependent Chl fluorescence quenching, photoinhibition (Sauer and Debreczeny 1996). These processes can be recognised because they develop under different irradiances, have different kinetics of relaxation in the dark, and are inhibited by specific chemicals (Demmig *et al.* 1987, Quick and Stitt 1989, Lee *et al.* 1990, Horton *et al.* 1996).

When plants or cyanobacteria are transferred to higher irradiances than the irradiance to which they are acclimated, there is a sudden increase in the photon pressure on the pigment antennae and, within seconds, photosynthesis reaches over-saturation. Plants have developed various photoprotective mechanisms to resist radiant stress (Demers *et al.* 1991, Demmig-Adams and Adams 1996). When photoprotection is only partly effective (in the case of strong and/or prolonged radiant stress), photoinhibition may occur (Arsalane *et al.* 1994). The first result of photoinhibition is a loss of PS2 activity which can be reversible without protein synthesis and is accompanied by a loss of the variable fluorescence. This inhibition of electron transfer is followed by a damage to the proteins and then by lipid peroxidation (Baker and Horton 1987, Kirilovsky *et al.* 1988, Prášil *et al.* 1992, Vass *et al.* 1992, Krasnovsky 1994). In higher plants, green and brown algae, the light-harvesting complex of PS2 (LHC2) consists of an intramembrane protein-Chl-carotenoid complex (Jansson 1994, Grossman *et al.* 1995, Green and Durnford 1996). One of the photoprotective mechanisms described in the literature for LHC2-containing organisms involves a down-regulation of PS2 occurring simultaneously with the build-up of a proton gradient across the thylakoid membrane (ΔpH) (Horton *et al.* 1991, Horton and Ruban 1992, Ruban *et al.* 1992). The ΔpH induces a fluorescence quenching often correlated with deepoxidation of xanthophylls located in LHC2 (Bilger and Björkman 1990, Olaizola *et al.* 1994).

In *Rhodophyta* (also called *Rhodophyceae* or red algae), instead of LHC2 the peripheral antenna of PS2 consists of a large extramembrane complex, the phycobilisome (PBS), which transfers its excitation to the core antennae of PS2 composed of the CP43 and CP47 protein-Chl complexes while PS1 has an intramembrane Chl *a* antenna complex (LHC1) (Gantt 1980, 1981, 1986, 1996, Glazer 1984). Studies on the unicellular red alga *Rhodella violacea* were initiated in this laboratory some years ago. In *R. violacea* the PBS has a simple hemidiscoidal structure analogous to that found in cyanobacteria. When *R. violacea* is grown under low irradiance, the PBS rods contain one proximal hexamer of C-phycocyanin (C-PC) and two distal hexamers of B-phycoerythrin (B-PE) associated with specific linker polypeptides; the PBS core is composed of allophycocyanin (APC) hexamers (Koller *et al.* 1977, Mörschel *et al.* 1977, Klotz and Glazer 1985, Bernard *et al.* 1996).

In *R. violacea* whenever PS2 is either preferentially excited by a weak green radiation or strongly excited by "white light", a large ΔpH-dependent fluorescence quenching is found (Delphin *et al.* 1996). This quenching can also be produced by white saturating "multi-turnover" pulses (Delphin *et al.* 1998). In this last case, even though the build-up of the ΔpH occurs during pulses, full development of the fluorescence quenching requires several tens of seconds. Its dissipation is very slow in the dark, where it takes more than 15 min. In contrast, under far-red irradiation, in the presence of an uncoupler (in the dark or under irradiation) or in the presence of DCMU which blocks oxygen evolution and photoreduction of plastoquinone, the fluorescence quenching relaxes in few seconds (Delphin *et al.* 1998).

The goal of the present study was first to show that complementary information can be obtained with the three different fluorimeters utilized for the experiments. We demonstrated that the ΔpH-dependent fluorescence quenching can be formed under different irradiances and that it is very persistent. We then demonstrated that the ΔpH dependent fluorescence quenching corresponds to a decrease in the effective antenna size of PS2. Finally we showed that, under photoinhibitory conditions, the ΔpH-dependent Chl fluorescence quenching exerts a photoprotective role and delays the kinetics of photoinhibition.

Materials and methods

The unicellular red alga *R. violacea* (strain 115-79 from the Göttingen University) was grown photoautotrophically in sterile artificial seawater (Jones *et al.* 1963) with the addition of vitamin B_{12} at 25 mg m^{-3}. Cultures were incubated at 20 °C in glass culture flasks continuously flushed with sterile air, irradiance by cool fluorescent tubes was 40 μmol m^{-2} s^{-1} in a 16/8-h light/dark cycle. To standardise the culture conditions and to minimise self-shading, cells were regularly diluted every 3 d to 700 cells per mm^3 with fresh medium.

For the photoinhibition experiments, the cells were concentrated at 10 g(Chl *a*) m^{-3} in their growth medium and irradiated in the photoinhibition set-up described elsewhere (Kirilovsky *et al.* 1990). The cell suspension (15 to 30 cm^3) was placed in a glass vessel (3 cm diameter), thermostated at 20 °C. It was gently stirred and irradiated by two *Atralux* spots of 150 W. Samples were taken and dark-adapted for 20 min before the measurements in order to allow full dissipation of the ΔpH.

For the fluorescence induction kinetics at 20°C three different set-ups were used: (*1*) a pulse-amplitude modulated (*PAM*) fluorimeter (model *101*, *Walz*, Effelrich, Germany). It combines excitation with radiation pulses of adjustable duration, quality and intensity with the detection by a non-actinic, modulated red beam provided by light-emitting diodes (LEDs, *Stanley HPG 5566X* - Schreiber *et al.* 1986). The detecting beam is at 690 nm very weak (0.026 μmol m^{-2} s^{-1}) modulated in pulses at a frequency of 1.6 KHz of 1 μs duration each. The fluorescence was detected beyond 700 nm by a photodiode. Its signal was fed to a synchronous amplifier. A 10-s strong "white light" (1400 μmol m^{-2} s^{-1}) was obtained from a *KL-1500* lamp (*Schott*, Mainz, Germany). "White light" pulses of 800 ms were produced by an electronic shutter (*Uniblitz*, Vincent, USA, opening time 2 ms) put in front of another *KL-1500* quarz iodine lamp and controlled by the accessory module *PAM-103*. Two irradiances were used for the pulses: 3 000 and 50 μmol m^{-2} s^{-1}. To measure the maximal fluorescence level attained during the 800 ms pulses, values were averaged in the time range 400-800 ms (acquisition time 33 μs) after shutter opening. Weak far-red radiation (100 μmol m^{-2} s^{-1}) obtained from a LED *102-FR* (*Walz*) and filtered through a *Schott RG9* filter (735±20 nm) was used to fully oxidize the plastoquinone pool in dark-adapted cells. Values acquisition, shutter control, and pulse averaging were driven by homemade software through a 12-bit analogic digital converter as described by Arsalane *et al.* (1993). The *PAM* fluorimeter was used to evaluate the apparent antenna size of PS2 before or after the formation of the ΔpH dependant fluorescence quenching.

(*2*) A double-modulation fluorimeter (*P.S.I. Instruments*, Brno, Czech Republic) (Trtilek *et al.* 1997). The instrument contains two heads equipped with red diodes emitting at 626 nm (*Hewlett-Packard*) or green diodes emitting at 550 nm (ultrabright, *Marl*) which provide actinic flashes of 20 μs duration, blue diodes emitting at 415 nm are used as detecting flashes of 2.5 μs duration. The actinic flashes are fired every 2 ms and the detecting flash during which the fluorescence intensity is measured is fired 150 μs after each actinic flash. The initial fluorescence level F_0 is measured during the first four detecting flashes fired in the absence of actinic flashes. The fluorescence is detected beyond 700 nm by a photodiode through a *Corion LG* 697 nm filter. This instrument is used in the 100 μs to 10 s time range with cells diluted to 2 g(Chl *a*) m^{-3} concentration. The software defines and archives the experimental protocol, downloads the experimental protocol to the control unit, and is used to initialise the experiment, to retrieve values from the control unit, and to visualise and export them. This set-up was used to compare fluorescence induction under two irradiances: the red irradiation which excites both photosystems and the green irradiation which excites preferentially PS2.

(*3*) A laboratory-built continuous fluorimeter that uses green LEDs with an interference filter (*Corion*, Franklin, MA, USA, 550±20 nm) to provide continuous irradiation of adjustable intensity and duration which serves both as actinic and as detecting beam. The fluorescence is detected at 680 nm by a photomultiplier (*Hamamatsu Photonics H 5700-50*, Japan) protected by an interference filter (680±10 nm, *Corion*). Values are collected in a computer with a *PCL 818* (*Advantech*) data acquisition card, with a 33 μs time resolution. The system uses a software developed in our laboratory. During one experiment, values are collected with different time resolutions and averaged to obtain the maximum information with a minimal file size. The results are displayed on logarithmic time scale to display together all the kinetics. The software controls both the LEDs and the acquisition of values. This set-up was useful to monitor the fluorescence induction for minutes and to study the effects of inhibitors (DCMU, nigericin, NH_4Cl) added during the irradiation.

Results

Formation of ΔpH-dependent fluorescence quenching during continuous green irradiation: As for all other oxygen evolving organisms, if dark-adapted *R. violacea* cells are irradiated, the intensity of Chl fluorescence emission shows a characteristic variation in time called fluorescence induction. At any phase of the fluorescence induction curve, the Chl *a* fluorescence yield is controlled by more than one physiological process due to simultaneous variations in photochemical and non-photochemical quenching (Satoh and Katoh 1981). The shape of fluorescence induction is strongly influenced by the intensity of excitation. Under strong irradiance several maxima and minima can be detected, under weaker irradiance the fluorescence induction is more monotonous.

In the past, when irradiation was provided by a lamp controlled by a shutter, the intermediary steps were classically described as OIDPS (Briantais *et al.* 1986). More recently with the development of the LEDs, the shutter could be avoided and the time resolution improved. This lead to the discovery of a new intermediary maximum J described by Strasser *et al.* (1995).

The first important modulator of the fluorescence yield at room temperature is photochemistry and hence the oxido-reduction state of the primary acceptor of PS2, the plastoquinone Q_A. Q_A is reduced by photons and reoxidized by the plastoquinone pool through the two-electron gate Q_B. During the photoreduction of the platoquinone pool, Q_A is in changing equilibrium with the pool and several transients can be observed: the first maximum J (if detected) is followed by a second maximum I (easier to detect in conventional fluorimeters) then by a dip D and a slow rise up to P. This constitutes the first fast part of the fluorescence induction corresponding to the attainment of the maximal concentration of reduced plastoquinone pool under a given irradiation. Under strong irradiance, the plastoquinone pool is reoxidized by PS1 at a slower rate than its fast reduction by PS2. When the radiant energy becomes limiting, a transitory reduction of the plastoquinone pool does not necessarily occur.

After the maximum level P, a slow decrease to S is observed. It takes minutes and can correspond to a partial reoxidation of the plastoquinone pool and hence of Q_A and to a non-photochemical quenching of the fluorescence generally attributed to the build-up of a ΔpH across the thylakoid membrane.

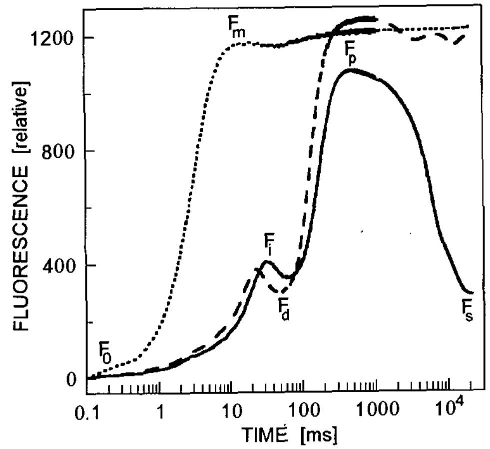

Fig. 1. Fluorescence inductions of dark-adapted *Rhodella violacea* cells under green irradiation, obtained with the continuous fluorimeter. The sample contained 5 g(chlorophyll) m^{-3}. The continuous irradiation was provided by green LEDs. *Solid line*: control cells; *dotted line*: preincubation of dark-adapted cells with 10 μM DCMU; *broken line*: preincubation of dark-adapted cells with 100 μM nigericin. The results are plotted on a logarithmic time-scale. The F_0 fluorescence level was taken as the origin of variable fluorescence expressed in arbitrary units.

When dark-adapted *R. violacea* cells were irradiated by a continuous green radiation which excites mainly PS2, a classical OIDPS fluorescence induction was observed (Fig. 1, *solid line*). Under the continuous green irradiance of 40 μmol m^{-2} s^{-1}, we did not detect the F_j level, an initial rise to F_i was followed by a dip to F_d then by a slower rise to F_p, which lasted around 200 ms. DCMU prevents the electron transport in PS2 from the primary bound plastoquinone Q_A to the secondary mobile plastoquinone Q_B (Bennoun 1970, Velthuys 1981, Wraight 1981) and the build-up of a ΔpH. In the presence of DCMU, the fluorescence induction consisted of a simple photochemical rise to the maximal fluorescence F_m (Fig. 1, *dotted line*), this rise was completed when each PS2 centre had a reduced Q_A. Under the green irradiation of 40 μmol m^{-2} s^{-1}, the F_0 to F_m rise took around ten milliseconds. In the control cells, the first rise from F_0 to F_i took twenty milliseconds. The irradiance used in this experiment was not strong enough to allow the detection of the F_j level described by Strasser *et al.* (1995) the detection of which needs high irradiances allowing a faster photoreduction of Q_A than its reoxidation by bound Q_B. However, we will show that during photoinhibition the F_j level becomes visible when the electron transfer between Q_A and Q_B is hindered (Kirilovsky *et al.* 1988) (see below and Fig. 4). In the control cells, the second slower part of the induction, which lasted for around 10 s, was a decrease from F_p to F_s. If the cells were preincubated in the dark with nigericin (a proton-transporting ionophore which disrupts membrane potential across the thylakoid membrane), the slow fluorescence decrease from F_p to F_s was suppressed (Fig. 1, *broken line*). This indicates that in *R. violacea* under green irradiation, the F_p to F_s decrease is mainly due to the development of a ΔpH dependent fluorescence quenching and cannot be attributed to a partial reoxidation of the plastoquinone pool by PS1. We attribute the faster kinetics in the presence of nigericin and the higher F_p to the decoupling of the electron transport and to the suppression of the ΔpH dependent fluorescence quenching.

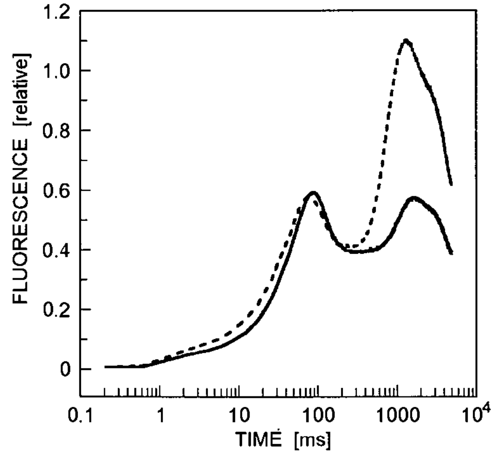

Fig. 2. Comparison of fluorescence inductions under green and red irradiations, obtained with the double modulated fluorimeter. The dark-adapted *Rhodella violacea* cells containing 2 g(chlorophyll) m^{-3} were irradiated by green flashes spaced at 2 ms (*broken line*) or red flashes spaced at 2 ms (*solid line*). The results are plotted on a logarithmic time-scale. The F_0 fluorescence level was taken as the origin of variable fluorescence expressed in arbitrary units.

Ability of green, red, and "white" irradiation to induce the fluorescence quenching: To compare fluorescence induction under red or green radiation, the experiment was done in the double modulated fluorimeter, by using one set of red LEDs and one set of green LEDs. The actinic flashes were fired every 2 ms. The fluorescence yield was measured by weak detecting blue flashes fired 150 μs after each actinic flash. The fluorescence induction observed can be regarded as the effect of a quasi-continuous irradiation. The F_0 level was first measured by four detecting flashes fired in the absence of actinic flashes. Then the actinic flashes were turned on and the classical OIDPS fluorescence induction (previously described) was observed under both red and green irradiation. The red and green irradiances were not determined accurately but if one considers the time needed to reach F_i (70 ms under the green irradiation, 80 ms under the red irradiation), the green flashes were slightly more actinic than the red flashes (Fig. 2). However both irradiances were weaker than in the continuous fluorimeter where F_i under continuous green irradiation was reached in 20 ms. The F_0 to F_m rise detected in the presence of DCMU took 30 ms under green irradiation and 40 ms under red irradiation (values not shown). The F_p level attained under red irradiation was smaller than that under green irradiation. This difference can be attributed to the preferential excitation of PS2 by the green radiation whereas under the red radiation both photosystems are excited and the plastoquinone pool becomes less reduced during the first phase (fast rise: F_0 to F_p) of the fluorescence induction. The slow F_p to F_s decrease, seen under the two irradiations, indicates that in both cases a ΔpH dependent fluorescence quenching was developed (Fig. 2). The fluorescence quenching can also be produced during a "white light" irradiation as described in Delphin *et al.* (1998). We will use the "white" irradiation of the *PAM* fluorimeter in the next paragraph. One characteristic of *R. violacea* cells grown in the conditions described above is that they developed, more rapidly than other strains, a ΔpH dependent fluorescence quenching which is slow to dissipate in the dark. This ΔpH persistence forbids the use of the same sample for successive fluorescence induction if a long dark time is not allowed for the dissipation of the ΔpH.

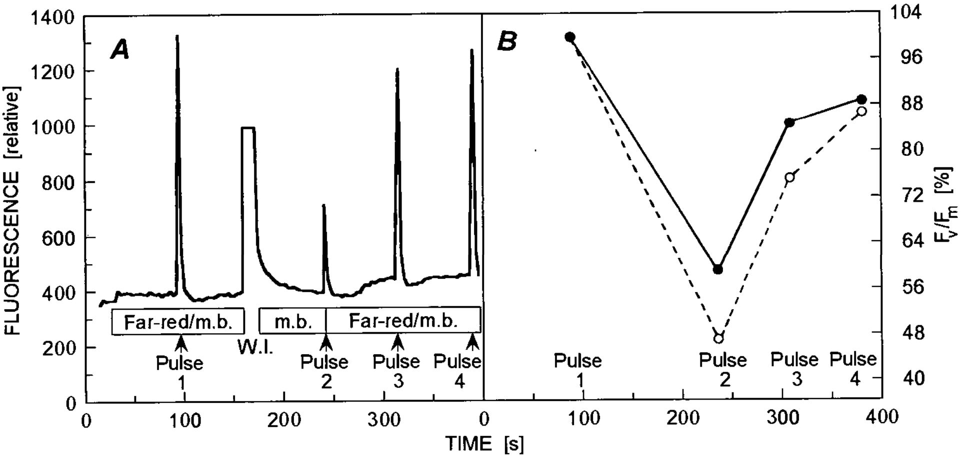

Fig. 3. Evaluation of the influence of the fluorescence quenching on the antenna size, obtained with the *PAM* fluorimeter with samples containing 10 g(chlorophyll) m^{-3}. The experimental procedure is depicted in *A*. Far-red/m.b. = far-red irradiation in addition to the weak modulated beam (m.b.); m.b. = modulated beam only; W.I. = "white" irradiation (1400 µmol m^{-2} s^{-1}). The % of initial F_v/F_m ratio is shown in *B*: it is computed for each pulse of a series of four white pulses (the four pulses are indicated along the time axis). The *solid line* corresponds to the results obtained with the 800 ms white pulse of 3000 µmol m^{-2} s^{-1}, the *dotted line* corresponds to those with the 800 ms pulse of 500 µmol m^{-2} s^{-1}.

Comparison of quantum efficiency in dark-adapted and preirradiated samples: Using the *PAM* fluorimeter, the quantum efficiency of a strong "white" pulse (800 ms, 3000 µmol m^{-2} s^{-1}) and a comparatively weaker white pulse (800 ms, 50 µmol m^{-2} s^{-1}) were compared before and after a 10 s "white" irradiation (1400 µmol m^{-2} s^{-1}) during which the ΔpH-dependent fluorescence quenching was formed. The following experimental procedure was followed: the dark-adapted cells were preirradiated for one minute by far-red to fully oxidize the plastoquinone pool, then a first pulse was fired, followed by the 10 s "white" irradiation. A second pulse was fired after one minute of darkness, then after one minute of far-red irradiation (to induce maximal fluorescence yield), a third pulse was fired. The last measurement was repeated a second time (fourth pulse) (Fig. 3*A*). The pulses were utilized to reduce Q_A. During the strong flash (3000 μmol m^{-2} s^{-1}) of 800 ms, Q_A was fully reduced at the end of the induction curve (not shown here, but represented in Fig. 2 of Delphin *et al.* 1998). During the weaker flash (50 μmol m^{-2} s^{-1}), Q_A was only partly reduced during the fluorescence induction (see Fig. 2 of Delphin *et al.* 1998). The F_0 and F_m levels reached during each of the pulses were measured, the ratio $(F_m - F_0)/F_m = F_v/F_m$ was computed and taken as a measure of the relative quantum efficiency of the strong and weak flashes (Krause and Weis 1991). The results expressed as % of initial F_v/F_m show (Fig. 3*B*) that the 10 s "white" irradiation during which the fluorescence yield was strongly reduced by the ΔpH fluorescence quenching also fully reduced the plastoquinone pool. This can be deduced from Fig. 1 showing that if ΔpH formation was prevented by the presence of an uncoupler, the F_s level was very close to F_p without appreciable reoxidation of the plastoquinone pool during the first 10 s of irradiation. Therefore the level attained during the weak flash which is a result of a dynamic equilibrium between Q_A reduction and Q_A^- oxidation is certainly not in favour of a larger Q_A oxidation after the 10 s "white" irradiation. The ΔpH-induced fluorescence quenching diminished the F_v/F_m during the non-saturating pulse more strongly than during the saturating pulse. So, after the development of the ΔpH-induced fluorescence quenching, the weak flash (which was already less efficient than the strong flash on dark-adapted cells) has become even less efficient than the strong pulse, or, in other words, the effective antenna size of PS2 has become smaller. This effect was reversed by a two minute far-red irradiation which was previously shown to reverse the fluorescence quenching (see Fig. 6*B* in Delphin *et al.* 1998).

Effects on the kinetics of photoinhibition of nigericin (uncoupler) and DCCD (ATPase inhibitor): When cells grown at 40 μmol m^{-2} s^{-1} are transferred to an irradiance of 2000 μmol m^{-2} s^{-1} for periods longer than a few seconds or minutes (which were the durations used for all the previous experiments), they progressively loose their photosynthetic activity because photoinhibition occurs. This can be monitored by the fluorescence variations shown in Figs. 4 and 5. The samples were taken from the photoinhibition vessel and fluorescence induction was monitored in the continuous fluorimeter after dark-adaptation for 15 min to allow the dissipation of the transthylakoid proton gradient. *Solid line* in Fig. 4 corresponds to the fluorescence induction of control cells, *broken line* corresponds to the fluorescence induction of cells exposed for 40 min to 2000 μmol m^{-2} s^{-1} of "white light". The F_j level which was undetectable in *solid line* appears clearly in *broken line* showing that the first result of photoinhibition is to inhibit the electron transfer between Q_A and Q_B as already published for cyanobacteria (Kirilovsky *et al.* 1988, 1990). The fluorescence induction of the cells after 40 min exposure to 2000 μmol m^{-2} s^{-1} also exhibits decreased F_p and F_m levels. The decrease in the F_p level can be the result of two phenomena. The amount of fluorescent and active PS2 is lower and the competition between reduction of the plastoquinone pool by PS2 and its oxidation by PS1 is modified. DCMU (10 μM) was added in the cuvette at the end of the fluorescence induction. This addition produced a fast rise to the F_m level. The decrease in the F_m level only corresponds to the loss of variable fluorescence in photoinhibited PS2 centers. This experimental procedure allowed to detect in a single run F_0, F_p, and F_m and to determine the part of the fluorescence quenching produced by photoinhibition. The differences F_p *minus* F_0 and F_m (measured with 10 μM DCMU present) *minus* F_0 were plotted as a function of the duration of the strong irradiation [min]. The experiments were done first with the control cells, then in the presence of nigericin which prevents the formation of the ΔpH (Delphin *et al.* 1998, Yahyaoui *et al.* 1998), or in the presence of DCCD which inhibits the ATPase (Shoshan and Selman 1980). In the presence of nigericin (or in the presence of NH_4Cl), the destruction of variable fluorescence was faster than in the control (Fig. 5). On the other hand, the kinetics of photoinhibition were not or little influenced by the presence of DCCD (values not shown).

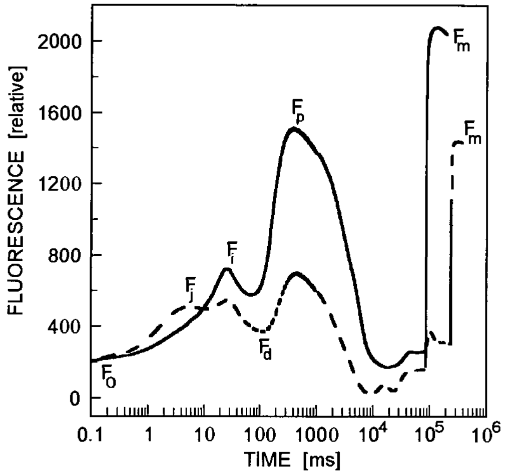

Fig. 4. Comparison of the fluorescence inductions of control cells and photoinhibited cells under green irradiation, obtained with the continuous fluorimeter at 5 g(chlorophyll) m^{-3}. Two fluorescence inductions are shown: *solid line* is the fluorescence induction after dark incubation, *dotted line* is the fluorescence induction after 40 min of photoinhibitory treatment by an irradiance of 2000 μmol m^{-2} s^{-1} at 20 °C at 10 g(chlorophyll) m^{-3}. The results are plotted on a logarithmic time-scale. DCMU (10 μM) was added before the end of irradiation to allow the determination of F_m. The F_0 fluorescence level was taken as the origin of variable fluorescence expressed in arbitrary units.

Discussion

Under green irradiation exciting mainly PS2, dark-adapted *R. violacea* cells show a classical "OIDPS" fluorescence induction. The first fast part of the induction F_0 to F_p corresponds to the reduction of the plastoquinone pool with transitory maximum F_i and minimum F_d, the nature of which was not studied in detail in the present work except for the fact that the time needed to reach F_i was longer than the time needed to reach F_m in the presence of DCMU (as already mentioned by Strasser *et al.* 1995).

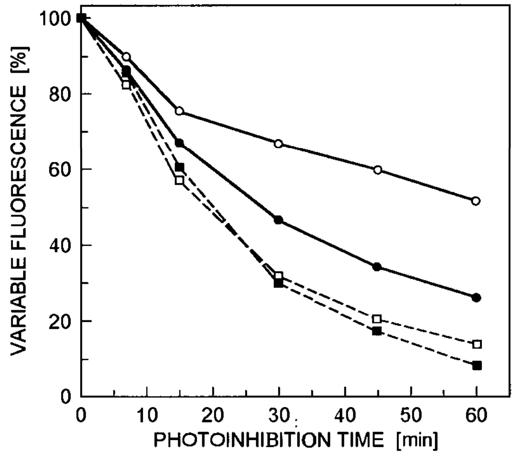

Fig. 5. Photoinhibition kinetics: The cells at 10 g(chlorophyll *a*) m^{-3} were exposed to a strong irradiance of 2000 μmol m^{-2} s^{-1} at 20 °C. Samples were taken and dark-adapted for 15 min to allow the relaxation of the ΔpH fluorescence quenching. The fluorescence inductions were measured in the continuous fluorimeter under green irradiation at 5 g(chlorophyll *a*) m^{-3}. The differences F_p - F_0 and F_m - F_0 were then plotted *versus* the duration in minutes of the strong irradiation. The *solid lines* (*with circles*) corresponds to the control cells, the *dotted lines* (with *squares*) corresponds to the cells preincubated 10 min in the dark with 100 μM nigericin. The *full symbols* correspond to F_p - F_0, the *empty symbols* to F_m - F_0.

The photochemical rise to F_j could not be detected in control cells under the irradiance which we used. The second slower phase, a decrease from F_p to F_s corresponds to a ΔpH-dependent, non-photochemical quenching. It is suppressed if cells are preincubated with nigericin (a proton-transporting ionophore which disrupts membrane potential), or NH_4Cl, the uncoupler used in previous publications (Delphin *et al.* 1996, 1998). In *R. violacea*, the ΔpH quenching can be formed to various extent by green, red, or "white" irradiation. We showed by comparing the efficiency of "white" pulses of different irradiances that when the non-photochemical fluorescence quenching was present, the quantum efficiency was smaller and the apparent antennae size reduced. We then utilized an irradiance strong enough to produce significant inhibition of PS2 activity within one hour of exposure of living cells at 20 °C (their growth temperature). The uncoupler has two effects, the main effect being the prevention of a ΔpH built-up during irradiation, and the second, indirect consequence being that the uncoupled electron flow is somewhat faster than the coupled electron flow. One could compare the effect of uncoupling to a slight increase in irradiance. We have shown (Kirilovsky *et al.* 1990), that indeed the photoinhibition kinetics depend on irradiance, however, the effect observed here is too large to be simply attributed to an increased rate of electron transfer (Fig. 1 shows that the same time is needed to reach F_p in the presence or absence of nigericin). We therefore believe that when the ΔpH was absent then the photoinhibiton kinetics became faster. The main conclusion of this paper is therefore that in *R. violacea* there exists a photoprotective mechanism correlated with a persistent ΔpH-dependent fluorescence quenching. It is the first time that such a photoprotective mechanism is described in PBS-containing organisms.

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