Biochimica et Biophysica Acta 933 (1988) 288–298
Elsevier

BBA 42756

Changes in the properties of reaction center II during the initial stages of photoinhibition as revealed by thermoluminescence measurements

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(Received 30 October 1987)

Key words: Thermoluminescence; Photoinhibition; Reaction center II; Charge recombination; Herbicide binding protein; (*C. reinhardtii*)

Analysis of the early events occurring during photoinhibition of *Chlamydomonas reinhardtii* cells by the thermoluminescence technique shows a shift of the B-emission band at 30 °C ascribed to charge recombination of $S_2Q_B^-$ to lower temperatures (15–17 °C). The appearance of this modified emission band is gradual, affects the whole population of reaction centers and occurs already at relatively low light intensities and short periods of exposure (20–60 min, 300–1000 $W \cdot m^{-2}$). Under these conditions a reduction of only 30–40% occurs in the intensity of the emission band ascribed to charge recombination of $S_2Q_A^-$. The loss of the $S_2Q_B^-$ response at 30 °C is interpreted as a destabilization of this state and seems to correlate with an increase in the value of the intrinsic fluorescence F_0 while the reduction in the $S_2Q_A^-$ signal parallels the reduction of the maximal variable fluorescence in the presence of DCMU. Measurements of oxygen flash yield and oscillation under these conditions show that the S-states cycle is not impaired. Following more extensive photoinhibition the B-type signal was completely lost while the $S_2Q_A^-$ band emission persisted and remained at approx. 20% of its initial value. The light intensity, required for the complete shift of the B-emission band at 30 °C to 15 °–17 °C, seems to be sufficient to accelerate the rate of D1 protein synthesis which continues for a while even if the cells are reexposed to low light intensity (recovery). These results indicate that during the initial stage of photoinhibition changes are induced in the reaction center which lead to some alteration of the D1 protein, resulting in a destabilisation of the $S_2Q_B^-$ charge recombination. These events may be connected with the light-dependent turnover of the D1 protein.

Introduction

It is well established that prolonged exposure (several hours) of plants, algae, and cyanophytes to light intensity exceeding that required for saturation of photosynthesis causes a loss of photosynthetic activity (photoinhibition) [1–4]. While most investigators of this phenomenon agree that the target of this process is Photosystem II [1–6], the mechanism whereby loss of activity is achieved is still subject to controversy. Based on the well-documented observation that the thylakoid polypeptide of 32.5 kDa, considered to function as an apoprotein for the secondary quinone acceptor Q_B and bind to PS II herbicides [7], is rapidly synthesized and degraded in a light-dependent process [8], Kyle et al. [5] proposed that photoinhibition

Abbreviations: PS II, Photosystem II; Chl, chlorophyll; TL, thermoluminescence; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

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may be due to the accelerated degradation of the Q_B protein, which may exceed the rate of its de novo synthesis. Since at the time of this proposal, Q_B, the herbicide binding protein (presently known as D1), was not considered as being a part of the Photosystem II reaction center proper and thylakoids from photoinhibited chloroplast could still reduce silocomolybdate [5], it was further proposed that the reaction center II, which was considered to consist of the CP44 and CP47 polypeptides [9,10], is not inactivated by photoinhibition [5,11]. This view was supported also by the observation that recovery from photoinhibition requires de novo synthesis of chloroplast proteins among which the most prominent was D1 [11]. The above-mentioned proposal was questioned by many authors on the basis of in vitro experiments demonstrating loss of PS II activity as being associated with loss of P-680 or Q_A absorption [13–16]. Following recent advance in our knowledge of bacterial reaction centers structure [17] and the inferred role of the D1 and D2 polypeptides in the function of the photochemical reaction center II and binding of the Q_B and Q_A quinone acceptor, respectively [17,18], the difference of opinions regarding photoinhibition become semantic and one could agree that the target of photoinhibition is indeed reaction center II. Nonetheless, the primary events leading to this process remain to be elucidated.

In the present work advantage was taken of the potential of the thermoluminescence technique for the study of the early events in photoinhibition in vivo. As reported before [19,20], this technique enables the detection and quantitation of the $S_2Q_A^-$ and $S_2Q_B^-$ states and their stability under various conditions.

The results presented here demonstrate that during the initial period of photoinhibition of *Chlamydomonas* cells two major phenomena occur, a reduction in the intensity of the emission band ascribed to $S_2Q_A^-$ charge recombination and a reduction and shift in the emission temperature of the B-band ascribed to $S_2Q_B^-$ charge recombination [21] from 30 °C to 12–17 °C, indicating destabilisation of this state. The time-course of these changes during photoinhibition and their recovery, the light dependence and the correlation with an increased rate of the D1 polypeptide synthesis suggest that the observed phenomena represent the early stages of Photosystem II loss of activity in photoinhibition.

Materials and Methods

Cultivation of cells, photoinhibition and recovery conditions

Chlamydomonas reinhardtii y-1 cells were grown in batch cultures in a mineral medium containing acetate as a carbon source as described before [22]. Cells at the end of the logarithmic phase of growth ($(1.1–2) \cdot 10^6$ cells/ml) were harvested by centrifugation ($1000 \times g$ for 3 min) washed and resuspended in fresh growth medium at a final concentration of 10^7 cells/ml (30–40 μg Chl/ml, non-dividing conditions [22]). The cell suspension (25–50 ml) was incubated in a glass tube 3 cm diameter, immersed in a glass water jacket kept at 25 °C and illuminated with a tungsten halogen lamp adjusted to give 100 $W \cdot m^{-2}$ to 2000 $W \cdot m^{-2}$ by varying the distance and voltage. Illumination below 100 $W \cdot m^{-2}$ was provided by fluorescent tubes (cool white). The cells were stirred gently by a magnetic bar. The thickness of the water layer in the water jacket was approx. 1 cm. Light intensity was measured inside the tube containing the cells using a YSI - Kettering Radiometer light meter. For recovery from photoinhibition the cells were incubated on a rotary shaker (60 cycles/min) at 25 °C and illuminated by white fluorescent lamps (approx. 30 $W \cdot m^{-2}$) or covered by aluminum foil (recovery in the dark).

Thermoluminescence, fluorescence kinetics and oxygen flash yield measurements

For thermoluminescence measurements aliquots (1.5–3 ml) were taken from the cell suspension and the cells were sedimented by centrifugation in a microfuge (approx. $5000 \times g$ for 30 s) at room temperature. The pellet was resuspended in a final volume of 80 μl growth medium containing 30% glycerol (35–60 μg Chl) and evenly spread on a square filter paper (4 cm^2) mounted on the sample holder as described before [19]. The cells were dark adapted for 1.5 min, then cooled at 0–2 °C and a saturating light flash was given followed by immediate immersion in liquid N_2. Thermoluminescence emission was measured and re-

corded as described previously [19]. When used, DCMU or Ioxynil ($5 \cdot 10^{-6}$ M) were added from methanol solutions to the cell suspension prior to the adsorption on the sample holder paper. All operations were carried out in dim green safe light. For variable fluorescence measurements samples (3–5 μg Chl/ml) were taken and fluorescence transients in the absence or presence of DCMU were recorded and analysed using the apparatus described before [24]. Oxygen flash yield was measured using a Joliot-type electrode as previously reported [25] and oxygen evolution by whole cells at saturating light was measured by polarography using a Clark-type oxygen electrode.

Radioactive labeling of thylakoid protein by $^{35}SO_4^{2-}$

Cell suspensions were incubated as above in growth medium containing only 0.1 μmol/ml sulfate. $^{35}SO_4^{2-}$ (30 μCi/μmol, 1 μmol/ml) was added as indicated in the legends to figures and incubation continued for 10 min. The cells were then cooled in ice, washed free of radioactive sulfate using normal growth medium and thylakoids were isolated as previously described [26]. The thylakoid polypeptide pattern was resolved by SDS-polyacrylamide gel electrophoresis [5] followed by autoradiography and densitometry scanning using a Biomedical Instrument scanner equipped with a laser beam.

Results

Changes in the thermoluminescence response following incubation of Chlamydomonas cells at increasing light intensities

The glow curves of *Chlamydomonas* cells grown under standard light intensity (35–50 $W \cdot m^{-2}$) are shown in Fig. 1. A thermoluminescence emission peak at about 28–30 °C could be detected following excitation of dark adapted cells by one saturating flash at 0–2 °C before freezing them in liquid N_2. Addition of $5 \cdot 10^{-6}$ M DCMU to the cells suspension prior to the excitation flash caused the disappearance of this TL peak and appearance of a higher intensity emission at about 10–15 °C, whereas addition of the Photosystem II inhibitor Ioxynil, a phenol-type herbicide which competes with Atrazine for binding to Photosystem II [27], resulted in a further shift toward a lower temperature of the emission band (0–2 °C) and a considerable lowering of the emission intensity. The intensity of the Ioxynil-induced band was affected by the temperature of the sample during the excitation flash. Maximum intensity was obtained if the cells were cooled to approx. −5 °C to −10 °C before the flash. Such cooling (+2° to −5 °C) had no significant effect on the temperature or intensity of the emission bands obtained in absence of Photosystem II inhibitors or in the presence of DCMU. The emission bands of *Chlamydomonas* cells obtained under the above experimental conditions can be ascribed to the charge recombination of $S_2Q_B^-$ obtained in the absence of PS II inhibitors, or the B band, and $S_2Q_A^-$ obtained in the presence of inhibitors, the D (Q or DCMU) -band [19].

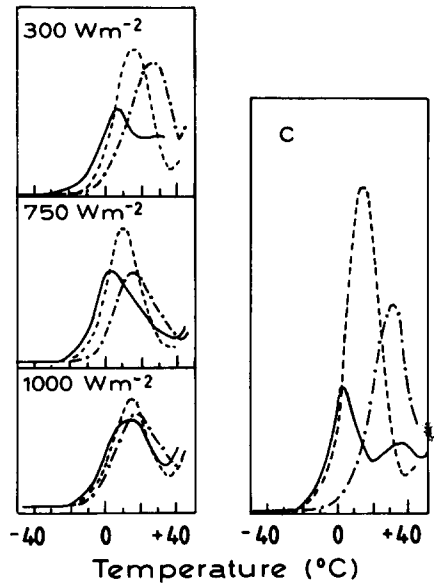

Fig. 1. Thermoluminescence response of control and photoinhibited cells. *Chlamydomonas* cells (45 μg Chl/ml) were incubated at light intensities as indicated for 25 min and the TL response was measured in absence (·–·–·) or presence of DCMU (- - - - -) or Ioxynil (———) Trace C: control cells. The values of F_v/F_o were 3.1 (control) and 2.1, 1.2 and 0.8 for the samples exposed to 300, 750 and 1000 $W \cdot m^{-2}$, respectively. The excitation flash was given at 0–2 °C for the control and DCMU and −10 °C for the Ioxynil-treated samples, respectively; the chlorophyll concentration was 45 μg per sample for the control and 35 μg for all other samples.

Exposure of the cells for a given time to light intensities above that saturating photosynthesis (photoinhibition) resulted in a progressive lower-

ing of the TL bands intensity, a shift of the B-band emission toward lower temperatures and a shift of the D-band induced by Ioxynil toward slightly higher temperature (Fig. 1). The loss of the emission peak at 30 °C (B-band) coincided with the appearance of a new emission band at 12–17 °C which might represent a modified B-band response ('B', Figs. 1 and 2). The quantitative changes in the intensity of the B- and D-bands and appearance of the 'B' band in cells exposed to increasing light intensities at 25 °C are shown in Fig. 2. A progressive loss of the emission intensity at 30 °C (B-band) concomitant with the appearance of the emission peak at 12–17 °C ('B' band) were observed. While the intensity of the emission at 30 °C was reduced below 10% of its initial value in cells exposed to 1200 $W \cdot m^{-2}$ and could be barely detected in cells exposed to 2000 $W \cdot m^{-2}$, the new emission peak at 15–17 °C ('B') appeared already in cells exposed to 150 $W \cdot m^{-2}$ and reached a relatively constant value in cells illuminated by approx. 500–1000 $W \cdot m^{-2}$. As opposed to the TL bands emitted in the absence of

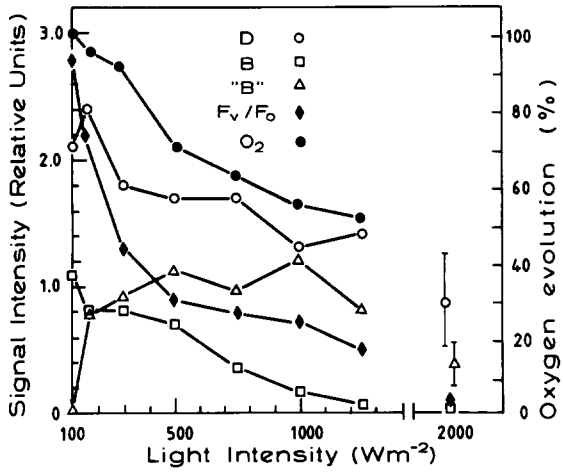

Fig. 2. Changes in the thermoluminescence, variable fluorescence and oxygen-evolution activities as a function of the light intensity during photoinhibition. *Chlamydomonas* cells (35 μg Chl/ml) were photoinhibited for 35 min at light intensities as indicated and the TL response was measured after one excitation flash at 0–2 °C. D, the DCMU-treated samples (D-band); B, 'B', the emission in absence of inhibitor at 30 °C and 15–17 °C, respectively; the data obtained at 2000 $W \cdot m^{-2}$ are averaged from three different experiments; F_v/F_o, the variable fluorescence measured in the presence of DCMU; ●———●, oxygen evolution measured as described in Materials and Methods.

Photosystem II inhibitors, the band elicited by DCMU was reduced only by about 30% even in cells exposed to 1250 $W \cdot m^{-2}$ and varied in different experiments between 25% and 50% of its initial value in cells exposed to 2000 $W \cdot m^{-2}$.

As an indication of the degree of photoinhibition, the variable fluorescence F_v normalized to F_o and oxygen evolution were also measured. The fast decrease of the F_v/F_o parameter to about 30% of its initial value and the subsequent relative stability of the F_v/F_o value in cells exposed up to 1250 $W \cdot m^2$ paralleled the changes in the D-band signal. The appearance of the modified 'B'-type signal preceded the loss of oxygen-evolving activity which decreased to about 56% of its initial value in cells exposed to 1200 $W \cdot m^{-2}$ in which the B-band signal was almost completely shifted to the 'B'-type band (Fig. 2).

Correlation between recovery from photoinhibition and recovery of the TL signals

The changes observed in the TL response of *Chlamydomonas* cells exposed to increasing light intensities could be due to changes induced in the thylakoid environment by high photosynthetic activity (pH [20,21], ATP concentration, ion composition and concentration) or may represent alteration of the reaction center II properties correlated with the photoinhibition process. It has been reported before that photoinhibited cells can recover Photosystem II activity. The recovery process is light dependent, requires de novo synthesis of chloroplast translated proteins and is completed within 3–4 h [11].

To test whether a correlation exists between recovery from photoinhibition and that of TL response, cells were exposed to photoinhibition conditions for 60 min and then allowed to recover their activity in the light or dark for 3 h. Measurements of the TL response during photoinhibition and recovery showed a 55% reduction in the emission of the DCMU band and almost complete loss of the B band emission with concomitant appearance of the lower intensity emission of the 'B' band after 60 min of photoinhibition (Fig. 3A). Both the B- and D-thermoluminescence bands regained their initial values after 3 h of recovery in the light. A significantly lower recovery was obtained in the dark-incubated cells. The increase in

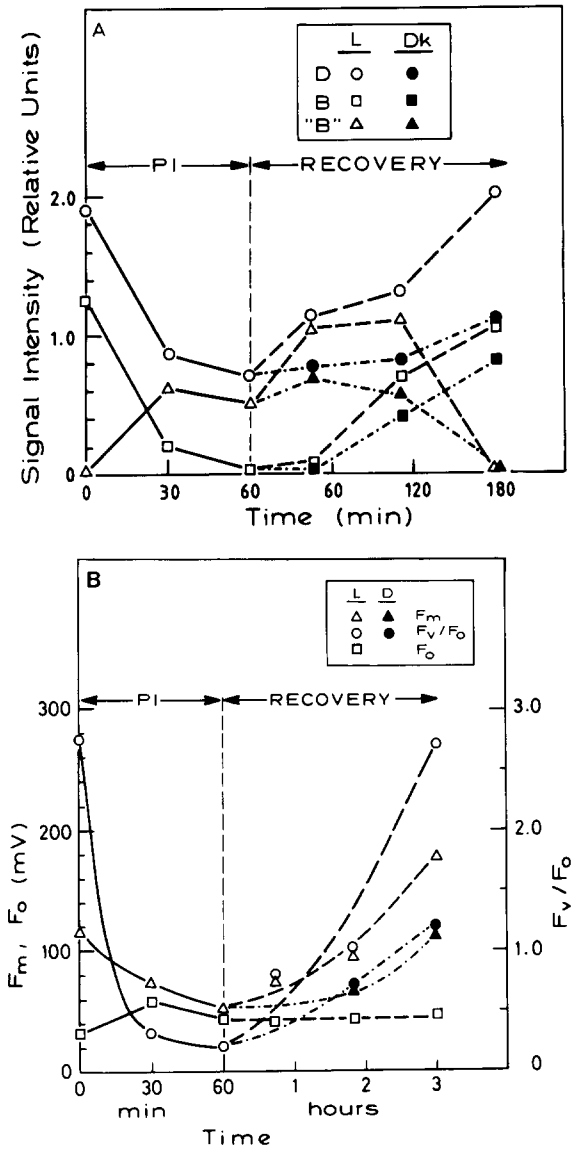

Fig. 3. Changes in TL (A) and fluorescence kinetics (B) response of cells during the photoinhibition and recovery process. *Chlamydomonas* cells (35 μg Chl/ml) were photoinhibited at 2000 $W \cdot m^{-2}$ and allowed to recover at 20 $W \cdot m^{-2}$ white light. The thermoluminescence response was obtained after one flash excitation at 0–2 °C. Open figures: light-incubated samples (L); closed figures: dark-incubated samples (Dk); solid lines; photoinhibition (PI); broken lines: recovery; D, the signal in the presence of $5 \cdot 10^{-6}$ DCMU (D-band) emitted at 10 °C; B, 'B', signals in absence of DCMU emitted at 30 and 15 °C, respectively.

the emission of the D-band started with the onset of the recovery process in the light and did not change in the dark-treated cells.

As opposed to that, the recovery of the B-band intensity showed a lag of about 1 h in both light- and dark-incubated cells. The increase in the emission intensity at 30 °C was accompanied by a comparable loss of emission at 15–17 °C (Fig. 3A). In the cells exposed to light during the recovery process the intensity of the 'B'-emission band almost doubled during the first hour, while no such change was observed in the intensity of this band in dark-incubated cells (Fig. 3A). This phenomenon could be related to the process of repair of the altered reaction center which implies the degradation of the altered reaction centers D1 protein and exchange with newly synthesized D1, a process known to be light dependent [5,8]. Measurements of variable fluorescence parameters (Fig. 3B) showed an initial rise in the value of the intrinsic fluorescence F_o during photoinhibition and stabilization at a level 30–40% higher than the initial value. The maximal fluorescence level in presence of DCMU (F_m) was reduced only by about 50% during the photoinhibition, corresponding to the loss of oxygen evolution (compare with Fig. 2) and the reduction in the $S_2Q_A^-$ signal. As also observed for this signal, the rise in the F_m value during the recovery period was enhanced by light. The changes in the value of F_v/F_o ($(F_m - F_o)/F_o$) reflected the changes in the F_o and F_m parameters. These results demonstrate that the changes in the intensity of the D-band and the temperature shift in the emission of the B-band induced by photoinhibition correspond well with those of the variable fluorescence and can be reversed during the recovery process. The time-course and light effect indicate that the changes in the TL response of whole cells induced by photoinhibition and recovery might be correlated with the mechanism of loss and recovery of Photosystem II activity proper.

The origin of the 'B' emission band at 15–17 °C

So far, the TL signal at 15–17 °C emitted by cells which have been exposed to photoinhibition conditions was considered to be a modified form of the B-emission band and thus to originate from $S_2Q_B^-$ charge recombination. If this were the case one would expect this signal to be subject to oscillations induced by consecutive saturating light flashes given to dark-adapted cells cooled at

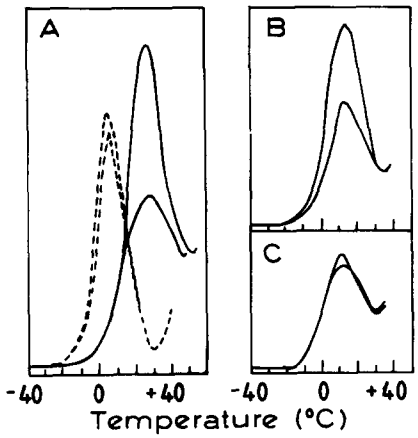

Fig. 4. Oscillation of TL response induced by two consecutive flashes of control and photoinhibited cells. Cells (35 μg Chl/ml) were exposed for various times to various light intensities. Samples were taken and the thermoluminescence response in absence (continuous lines) or presence of $(5 \cdot 10^{-6}$ M DCMU, broken lines) was measured after one flash (lower trace) or two flashes (upper trace) given at 0–2° C; panel A, control cells; panels B and C, cells photoinhibited at 1250 $W \cdot m^{-2}$ for 20 min and 1750 $W \cdot m^{-2}$ for 45 min, respectively.

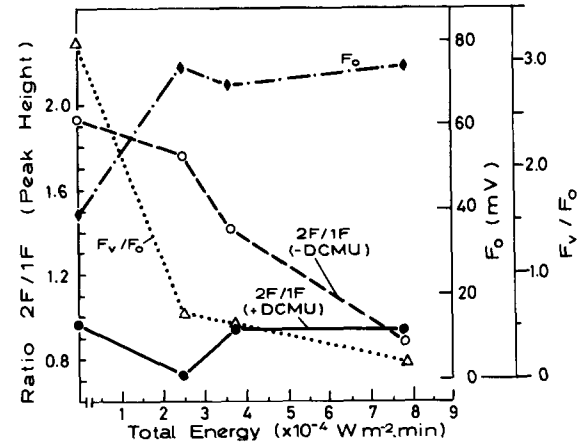

Fig. 5. Loss of oscillations of the 'B'-band emission as a function of the total energy given to a cell suspension during photoinhibition. Same experimental conditions as in Fig. 4. The data are expressed as the ratio of the emission intensity after the second flash to that after the first flash (2F/F) in the absence (B, 'B' bands) or in the presence (D band) of DCMU $(5 \cdot 10^{-6}$ M); F_o and F_v/F_o data are also given as a measure of the degree of photoinhibition.

0°–2° C prior to freezing in liquid N_2 [21].

The B-band emission of control cells increased in intensity following excitation of the sample, cooled at 0°–2° C, by a second flash (Fig. 4) and decreased again after three flashes (data not shown). As expected [21], no change was induced by this protocol in the intensity of the D-emission band elicited in the presence of DCMU (Fig. 4A). The 'B'-emission band at about 15°–17° C exhibited by cells after partial photoinhibition showed a similar oscillation to that of the B-band. However, no oscillations could be detected in the emission of the 'B'-band in cells which have been exposed to severe photoinhibitory conditions and in which the TL emissions in absence of DCMU was shifted to 10–12° C, and thus coincided with that of the emission in presence of DCMU (Fig. 4C). The progressive reduction in the oscillation of the 'B'-emission band as a function of the total energy $(W \cdot m^{-2} \cdot min^{-1})$ impingent on the cell suspension is shown in Fig. 5. The results are expressed as the change in the ratio of the emission after two flashes relative to that elicited by one flash. The oscillation of 'B'-emission band under these experimental conditions decreased with the degree of photoinhibition and was almost

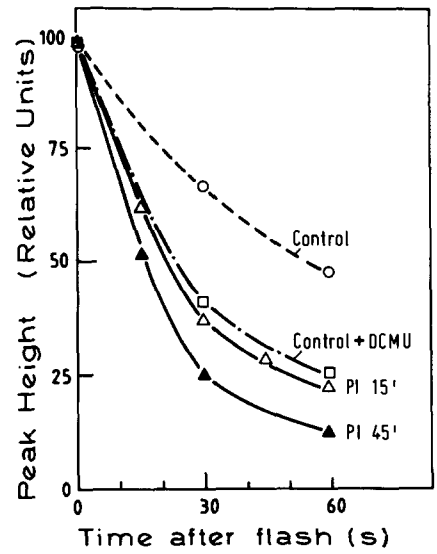

Fig. 6. Stability of $S_2Q_B^-$ as a function of time after flash in control and photoinhibited cells. Photoinhibition was carried out at 1000 $W \cdot m^{-2}$ for 15 min and 45 min (PI 15', PI 45') respectively. The samples were dark adapted, flashed once, and further incubated at 20° C for times (s) as indicated before freezing in liquid N_2 and measure of the TL response in absence of DCMU (B- and 'B'-band in control and photoinhibited samples respectively). Control + DCMU, D-band measured in the presence of $5 \cdot 10^{-6}$ M DCMU, flashed and incubated at 0–2° C before freezing in liquid N_2.

completely abolished when the cells have lost approx. 80% of their Photosystem II activity as indicated by loss of variable fluorescence ($F_v/F_o \approx 0.1$). As expected, the intensity of the D-emission band obtained in the presence of DCMU did not oscillate when the same protocol was used and the ratio of the emission after two flashes to that obtained after one flash remained practically constant throughout the photoinhibition process (Fig. 5).

These results suggest that the 'B' signal arises from the $S_2Q_B^-$ charge recombination. The shift toward a lower temperature of the 'B'-band could be due to a change in the properties of the D1 protein resulting in the destabilisation of the $S_2Q_B^-$ state. The half-life of the 'B' emission band at 20 °C was only about half of that of the B signal and decreased with increase in the photoinhibition (Fig. 6).

Changes in the water-oxidising complex during photoinhibition

The apparent destabilisation of the $S_2Q_B^-$ state in cells exposed to increasing light intensities described above could be due to change in the water-oxidation complex [28]. To test this possibility the quadruple oscillation of the oxygen yield following a train of saturating flashes was measured in control and partially photoinhibited cells in which the B-signal was shifted to the lower emission temperature ('B'), but still showed oscillations as in Fig. 5. The results (Fig. 7) showed that the partially photoinhibited cells exhibited a normal oscillation pattern but with a reduced amplitude. However, the oxygen yield per flash normalized to the third flash was identical in the photoinhibited cells to that of the control cells (Fig. 8), indicating that while the B signal was

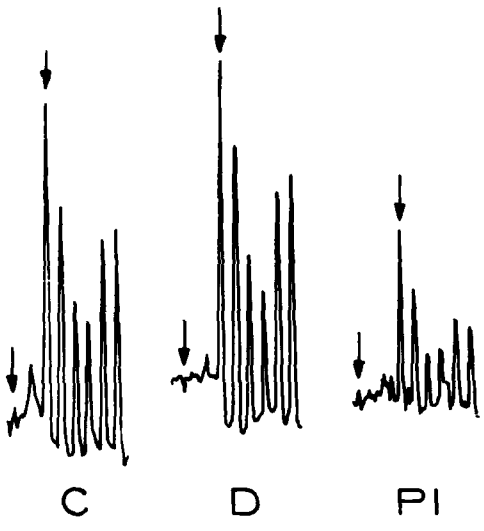

Fig. 7. Oxygen yield as a function of flash number in control and photoinhibited cells. Cells (35 μg Chl/ml) were photoinhibited for 20 min at 400 $W \cdot m^{-2}$ and the oxygen yield measured in control cells before (trace C) or after dark adaptation (trace D); photoinhibited cells (trace PI); the B and D (DCMU) emission bands heights in the control were 27 and 37 units emitted at 28 and 7 °C, respectively, and 17 and 22 units for both 'B'- and D-bands of the photoinhibited samples.

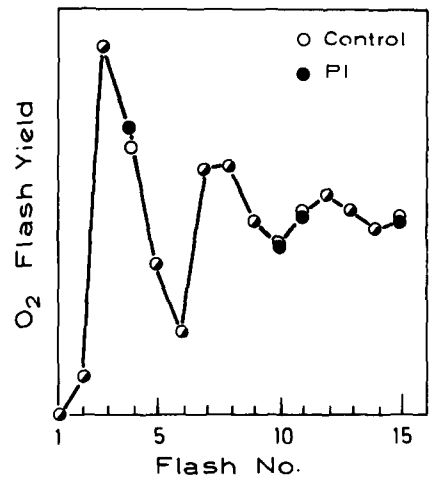

Fig. 8. The flash oxygen yield of control and photoinhibited cells normalized to the third flash. Same experimental conditions as in Fig. 7.

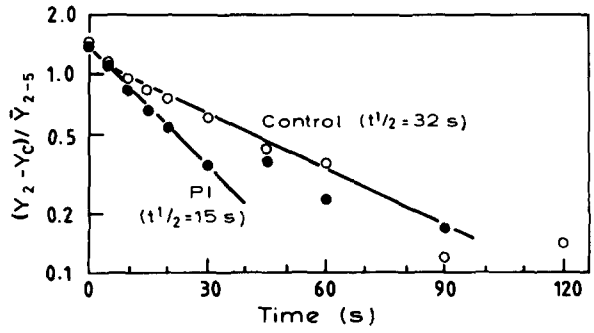

Fig. 9. Decay of flash oxygen yield as a function of time interval between flashes in control and photoinhibited cells. The relative amount of S_2 was calculated from the oxygen yield in the second flash (Y_2) corrected for the Y_2 value obtained without preflashes (Y_c) divided by the average oxygen evolution in the second to fifth flash (Y_{2-5}) [29]. Same experimental conditions as Figs. 7 and 8.

already altered, the S states cycle was still operative. However, measurements of the decay time of the S states (Fig. 9) showed a reduction in the half-life time from 32 s in the control cells to 15 s in the photoinhibited cells comparable to $t_{1/2}$ obtained for the $S_2Q_B^-$ in photoinhibited cells calculated from Fig. 6.

Correlation between the loss of the B-band emission and increase in the turnover of the D1 protein

It has been demonstrated before that the Q_B-binding D1 protein turns over in the light considerably faster than all other thylakoid protein [8]. Kyle et al. [5] proposed that the light-dependent degradation of D1 could be induced by an alteration of the D1 protein due to formation of radicals at the Q_B site and might be the major reason for the phenomenon of photoinhibition. This proposal was substantiated by the observation that recovery from photoinhibition correlated with de novo synthesis of D1 [11]. The question thus arises whether cells in which the B signal has been altered to the 'B' form will respond by an accelerated synthesis of the D1 polypeptide. To answer these questions *Chlamydomonas* cells were exposed for 20 min to increasing light intensities and pulse-labelled with $^{35}SO_4^{2-}$ during the last 10 min of the incubation. Electrophoretic separation of the thylakoid polypeptides and autoradiography showed a progressive increase in the radioactive

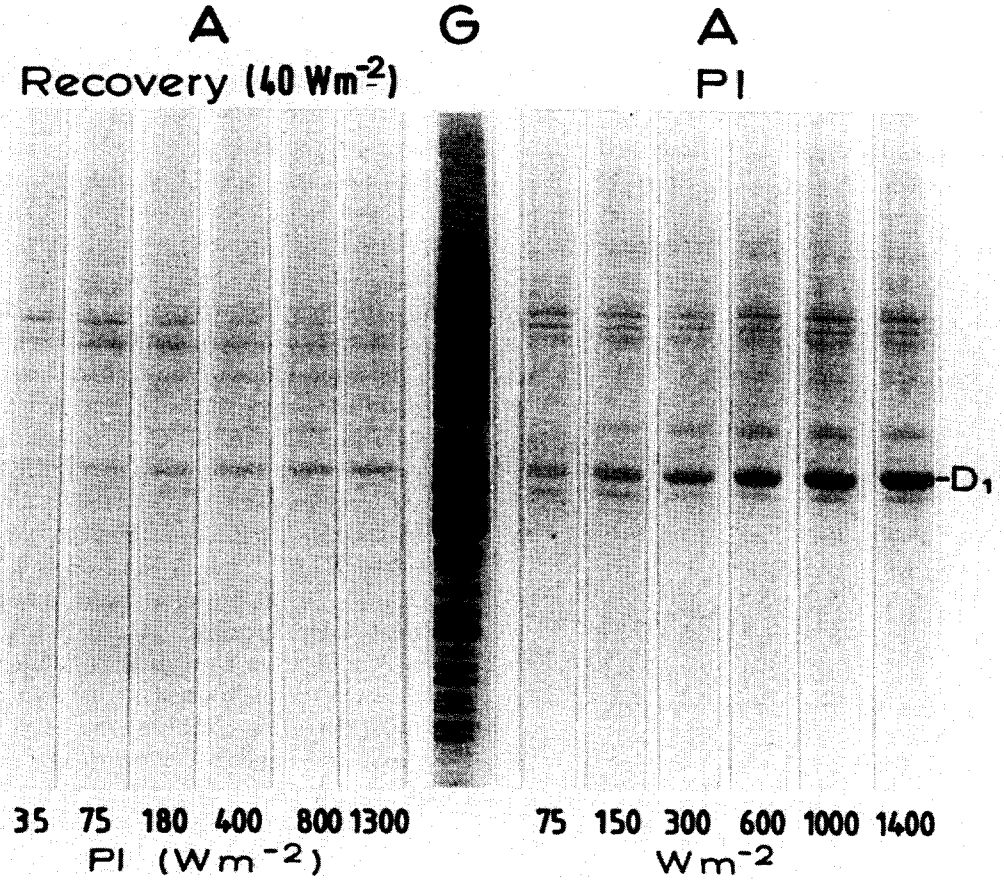

Fig. 10. Incorporation of $^{35}SO_4^{2-}$ into thylakoid proteins as a function of the light intensity during or prior to the labeling period. Cells were incubated at light intensities as indicated at the lower part of the figure for 20 min and then either labelled at the same light intensity (photoinhibition conditions (PI panel) or transferred to 40 $W \cdot m^{-2}$ light for 10 min and labeled for an additional 10 min (recovery panel). G, stained gel; A, autoradiogram of LDS-PAGE resolved polypeptides. The D1 polypeptide is identified from the high relative labeling and by immunoblotting (data not shown).

labeling of the D1 polypeptide with increasing light intensity (Fig. 10).

A significant rise in the radioactivity of D1 could be detected already in cells exposed to 300 $W \cdot m^{-2}$ (Fig. 10) which causes the shift in the temperature emission to 15–17 °C ('B' signal) (Fig. 2) without abolishing the ability of the 'B' emission to oscillate as a function of the number of exciting flashes (Fig. 5, 0.6 $W \cdot m^{-2} \cdot min$). In a second experiment, cells have been exposed to similar light intensities for 20 min, then transferred to low light (40 $W \cdot m^{-2}$, recovery conditions) for 20 min and pulse-labeled with $^{35}SO_4^{2-}$ during the last 10 min of the incubation. The results show that albeit exposed to the same light intensity during the radioactive labeling, the incorporation of ^{35}S into the D1 protein was higher in the cells exposed to higher light intensity during the first incubation (Fig. 10). The increase in the specific labeling of D1 under these experimental conditions was quantitated by scanning densitometry and clearly show the above-described effect

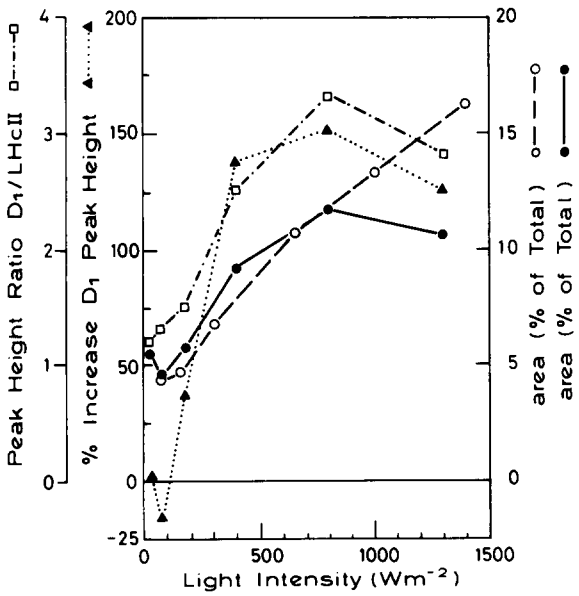

Fig. 11. Densitometer scanning of the radioactivity of the D1 polypeptide from the experiment of Fig. 10. o-----o, area (% of total) of the D1 protein labeled during photoinhibition; ●———●, D1 area (% of total) of the D1 polypeptide labeled during recovery at 40 $W \cdot m^{-2}$; □— — —□, peak height ratio of D1 to LHCII) and ▲———▲, % increase in the D1 peak height of thylakoids labeled during the recovery at the 40 $W \cdot m^{-2}$ light intensity.

(Fig. 11). These results indicate that the light intensities causing the loss of the B-signal and appearance of the 'B'-emission band are 'recognised' by the cells as a photoinhibitory condition and induce a 'recovery' response even though the total loss of photosynthetic activity in cells exposed to 300–600 $W \cdot m^{-2}$ (3–4-times the photosynthesis saturating light) under the protocol used in this work is quite limited (compare with Fig. 2, $F_v/F_o \approx 1.3–0.8$, oxygen evolution, 70–80% of initial values).

Discussion

Thermoluminescence signals from intact cells have been analyzed before [30]. The emission temperatures and intensity on a chlorophyll basis in *Chlamydomonas* cells were fairly reproducible for a given batch of cells within the duration of an experiment. However, some variation in the emission temperature was observed. The emission temperature of the B-band ascribed to charge recombination of $S_2Q_B^-$ [19–21] varied between 25° and 30 °C and only occasionally lower temperatures were observed. The emission of the D (DCMU) -band ascribed to $S_2Q_A^-$ charge recombination [19,21,30] varied between 5 and 12 °C. Thus it was not difficult to discern between these two bands and the appearance of the 'B'-band considered as a modified B-type band which shifted progressively from 22° to 12 °C as a function of the photoinhibition conditions. On a few occasions in which samples were analyzed after short periods of exposure to light intensities in the range of 400–800 $W \cdot m^{-2}$ the B-band showed the usual peak at 28–30 °C, but of a lower intensity while a new shoulder appeared at 20–22 °C (data not shown), indicating an altered population of reaction centers being formed at the expense of the normal population exhibiting the usual B-thermoluminescence band. With increasing exposure to high light intensities the whole population of reaction centers was changed to the 'B'-type. This result could be due to a change in the soluble phase environment within the chloroplast. At high light intensities one would expect a high rate of ATP synthesis related to a high and constant pH. Alkalinization of the medium was shown to affect the thermoluminescence B-band which is reduced

in its intensity but continues to oscillate with the number of excitation flashes [21]. However, the emission of the B-band at alkaline pH does not change to a lower temperature as observed here for the 'B'-band. At low pH, a shift of the thermoluminescence B-band to 27°C was reproted [20], while the 'B'-emission shift as reported here is to significantly lower temperatures. Furthermore, if the temperature shift were due to a change in the pH, one would expect it to dissipate in the dark when cells are incubated at 25°C, fairly rapidly [31]. However, this was not the case, and recovery of the B-signal once shifted to 15–17°C required 1.5–3 h of incubation in the light. Thus one can conclude that the alteration of the B-emission band to the 'B'-type represents a true change in the properties of the reaction center II which can be interpreted as a destabilisation of the $S_2Q_B^-$ state as also indicated by the reduction of its $t_{1/2}$ by 50%.

The destabilization could be due to a change in the D1 protein conformation compatible with a change in the energy of activation of an approx. 4–6 kJ/mol corresponding to the shift in the emission temperature of 12–15°C. This change does not prevent the ability of D1 to bind herbicides as evidenced by the appearance of the D band at even lower temperatures upon addition of DCMU or Ioxynil. Cells exhibiting the 'B' signal with an emission at 15–17°C continued to evolve oxygen and displayed normal S-cycle oscillations. The reduction in the $t_{1/2}$ of the S-states could be attributed to the destabilisation of the $S_2Q_B^-$ state. One should note that under photoinhibitory conditions which caused a shift of the whole population of reaction centers to the 'B' emission of 15–17°C, the loss of active reaction centers as measured by fluorescence kinetics in continuous light is only partial ($(F_m - F_o)/F_o$ or F_v/F_o, approx. 0.6). Part of this loss is only apparent, since the F_o component increases significantly during the initial photoinhibition period, while the maximal fluorescence F_m (in the presence of DCMU) is not significantly reduced at this stage.

As the photoinhibition time and/or light intensity increases the damage to reaction center II increases as well, and the emission band in the absence of DCMU ('B'?) is further shifted to 10–12°C. At this stage, addition of DCMU does not cause an increase in the emission intensity nor a shift to lower temperature significantly as expected for a D-band emission. Furthermore, addition of Ioxynil does not shift the emission temperature to 0–2°C any longer. Both these facts indicate that at this stage the PS II herbicide binding effect is lost. The TL signal does not show oscillations with the flash number and one could interpret these results as indicating that the $S_2Q_B^-$ state cannot be detected any longer. At this stage the intensity of the remaining thermoluminescence signal which can be considered as a 'D'-type emission ($S_2Q_A^-$) is also significantly reduced and corresponds to the lowering of the F_v/F_o value which is now mostly due to loss of maximal fluorescence in presence of DCMU (F_m) and is accompanied by significant loss of O_2 evolution (Fig. 2) or DCIP reduction [5]. The results indicate that at this stage part of the reaction-center population has been inactivated completely.

Thus the results presented above can be interpreted as a series of gradual changes induced in the reaction center II by photoinhibitory conditions: (1) a change in the whole population of reaction center II affecting the (D1)-$S_2Q_B^-$ stability and accompanied by a corresponding rise in the F_o value while only a slight loss of the $S_2Q_A^-$ signal occurs; followed by (2) a complete loss of the (D1)-$S_2Q_B^-$ signal, and loss of the $S_2Q_A^-$ which can be related to a total loss of reaction center II activity.

The initial changes in the reaction center II expressed as destabilisation of the $S_2Q_B^-$ state can be interpreted as being the result of a change in the D1 polypeptide conformation. This could be the trigger for, or the result of, initiation of the D1 degradation process which is known to be light dependent. The correlation between the initial changes in reaction center II thermoluminescence properties and the increase in the rate of D1 synthesis and initiation of the 'recovery' response support the conclusion that the two phenomena are related and represent the earlier changes so far observed in the process of photoinhibition.

Acknowledgements

This work was supported by a Grant on Solar Energy Conversion by Means of Photosynthesis

awarded to the Institute of Physical and Chemical Research (RIKEN) by the Science and Technology Agency of Japan, and partially by a research grant awarded to I.O. by the Israel-American Binational Research Foundation, BIRD, the FMC Agricultural Group, Princeton, NY, U.S.A., and Luxembourg Chemicals, Tel Aviv. I.O. was supported at the RIKEN Institute by a grant based on the Versailles Summit Corporation Program on Photosynthesis and Photoconversion.

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