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Photochemical alterations of Photosystem II induced by two different photoinhibitory treatments in isolated chloroplasts of pea. A thermoluminescence study

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Suspensions of chloroplasts were submitted to photoinhibitory treatments consisting of an illumination given either in the absence of electron acceptor, or in the presence of electron acceptor. Properties of two thermoluminescence bands: (1) the band at 32–35 ° C due to $S_{2/3}Q_B^-$ recombination and (2) the low-temperature band (near −68 ° C) induced by an illumination at −80 ° C, were studied in the two types of photoinhibited material. In both photoinhibitory treatments the 32 ° C band was reduced in size in photoinhibited chloroplasts in parallel with decrease of rates of O_2 evolution and kept the same shape and position in temperature. These results indicate that the PS II centers remaining active have retained quite normal properties compared to the control. However, in chloroplasts photoinhibited in the absence of acceptor, a high level of luminescence emission was observed after a large number of flashes (more than ten), compared to control chloroplasts. This difference demonstrates an increase in the reduction state of the plastoquinone pool after the photoinhibitory treatment performed when no exogenous acceptor is present. The low-temperature band was charged only by continuous light at −80 ° C (or in the range −100 ° C to −60 ° C) and in the literature it is known as the Z_V band. The band was reduced in size in chloroplasts photoinhibited in the presence of an exogenous acceptor in parallel with the decrease of PS II activity. Conversely, in chloroplasts photoinhibited in absence of acceptor a new low-temperature band appeared which could be differentiated from the usual Z_V band by its insensitivity to ethanol. This band increased with degree of PS II inhibition. This new ethanol-insensitive low-temperature band is proposed to result from recombinations of a charge pair generated in PS II centers photodamaged at the acceptor side.

Introduction

Exposure of photosynthetic organisms to high light intensity in excess of that required for saturation of photosynthesis results in photoinhibition of photosynthesis. The process is characterized by decreased rates of CO_2 fixation at saturating light levels. For higher plants placed in natural conditions it was shown to occur when low temperatures (0–5 ° C) slow the enzymatic processes but absorption of light remains maximum. Insufficient electron transfer away from Photosystem II is supposed to be the major cause of photoinhibition in these conditions (reviews of Powles [1], Krause [2], Anderson and Osmond [3]). In microalgae it was suggested that photoinhibition results in alteration of PS II at the Q_B site because the disappearance of the D1 protein was observed [4,5]. However, studies performed with isolated chloroplasts or PS II membranes have led to the conclusion that components of the primary charge separation are first involved in the degradative process. During illumination lower yields of Q_A^- [6] and of Ph^- [7] were observed. At variance with this latter result, Allakhverdiev et al. [8] have shown that the primary photoreaction between P680 and pheophytin is not impaired, even in highly disorganized PS II. Decrease in rates of O_2 evolution is accompanied by a parallel decrease of the size of the EPR multiline signal, indicating lowering of the capacity for S_2 formation in the samples [9]. Disappearance

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Abbreviations: TL, thermoluminescence; PI, photoinhibition; PS II, Photosystem II; Q_A, the first quinone electron acceptor; Q_B, the second quinone electron acceptor; Chl, chlorophyll; pBQ, *para*-benzoquinone; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid; DCMU, 3-(3-4-dichlorophenyl)-1,1-dimethylurea.

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of D1 occurred more slowly [9–11] and was accompanied by the release of Mn in the medium [9].

I have investigated the properties of chloroplasts submitted to two types of photoinhibitory treatment consisting in a strong illumination performed either in the absence of an efficient electron acceptor or in the presence of a saturating amount of acceptor. The first treatment is the usual one used to observe photoinhibition in suspension of chloroplasts [12] or microalgae [13] and it is supposed that appearance of damage is the consequence of the insufficiently rapid electron draining from PS II. In the presence of electron acceptors a photoinhibitory process is also known to occur [14], but is rarely studied.

The aim of this study was to obtain insight into the electron transfer in PS II reaction center in order to localize the sites of alterations for each photoinhibitory treatment. This was realized by using thermoluminescence (TL). This technique allows the detection and identification of states responsible for recombination reactions involving pairs of charged components (reviewed in Refs. 15–17). The localization of charges on redox compounds depends on the degree of intactness of the electron transfer chain and any breaks in the electron chain near or at the level of PS II should be characterized in this way. In addition to the usual charging of TL bands by actinic flashes at ambient temperature, studies involving charging by illumination at low temperature (near $-80\,^\circ$C) were performed in order to detect very unstable charge pairs with rapid recombination at ambient temperature.

Materials and Methods

Plant material

Pisum sativum var. Merveille de Kelvedon was grown from seeds on vermiculite supplemented with nutritive medium in a partially shaded greenhouse. Temperature was 22° C day/18° C night. Illumination was provided by combination of solar illumination and artificial light (Osram lamps HQIL 800 W) giving an average light intensity of about 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ during the 14 h photoperiod.

Isolation of chloroplasts

Intact chloroplasts were isolated from fully expanded leaves of 2-week-old plants using the method of Cerovic and Plesnicar [18]. The degree of chloroplasts intactness was estimated at about 85% using the usual ferricyanide test.

Measurement of electron transport activity

Chloroplasts were osmotically broken by suspension in 5 mM $MgCl_2$ for 30 s then resuspended in the following medium: 350 mM sorbitol, 5 mM $MgCl_2$, 5 mM KCl, 25 mM Hepes (pH 7.5) at chlorophyll concentration of 40 $\mu g \cdot ml^{-1}$. Electron transport was measured polarographically as O_2 evolution (or absorption) rates using a Clarke-type electrode (Hansatech) with transparent water-jacket at a controlled temperature (22° C). Illumination was performed directly in the electrode cuvette (light intensity was 5500 $\mu E \cdot m^{-2} \cdot s^{-1}$). Rates of O_2 evolution were determined after the addition of 1 mM *p*-benzoquinone alone, followed by the addition of 10 mM NH_4Cl after 20 s; stimulation of rates by the addition of uncoupler was 2–2.5. Uncoupled rates of O_2 evolution were in the range 350–450 μmol/mg Chl per h.

Photoinhibitory treatments

These were performed in the cuvette of the O_2 electrode. Samples (1.2 ml, 50 μg Chl) were illuminated at light intensity of 5500 $\mu E \cdot m^{-2} \cdot s^{-1}$ either in the presence or the absence of electron acceptor. In the case of photoinhibition with acceptor, 25 μM methylviologen, 2 μM gramicidin and 250 U catalase were added to chloroplasts. Rates of O_2 absorption were determined (as explained above) in parallel in the absence of catalase, as a control. Photoinhibition in the absence of electron acceptor was performed by adding only 250 U catalase to chloroplasts. The medium was normally oxygenated (250 μM O_2).

At the end of the period of illumination: 1–8 min (times indicated in the legends of the figures), the samples were centrifuged in Eppendorff minivials (1 min, 2000 × *g*) then the pellets were kept in darkness on ice for at least 2 h. Samples submitted to photoinhibition in the presence of methylviologen + gramicidin were washed twice with resuspending medium before storage on ice. Samples of control chloroplasts were directly pelleted in vials without illumination. We checked that 5–8 min of vigorous stirring in the cuvette without illumination had no effect on PS II activity of chloroplasts or on the shape of the TL curves compared to control.

Hill activity was measured in resuspended pellets kept about 4 h on ice (samples used for TL studies were stored 3–5 h on ice) either in the presence of pBQ (PI without acceptor) or with 25 μM methylviologen + 2 μM gramicidin (PI with acceptor). Rates of O_2 evolution/absorption were usually 20 – 30% decreased after 4 h on ice in control chloroplasts, whereas the rates were only 10% lower in photoinhibited chloroplasts.

Thermoluminescence measurements

They were performed using a new set up built in the laboratory as a modification of the apparatus built by Visocekas [19]. The thermoluminescence cuvette, about 0.2 mm wide, was formed by a rubber plate with a 1–2 cm cavity pressed between 1 plexiglas window and an aluminium plate which can be partly dipped in liquid nitrogen. A 'thermocoax' heater on the other side of the plate was used for regulation of temperature, which was measured in the cuvette by a thermocouple. Pellets were resuspended (in very dim light) in 0.2 ml medium (same resuspending medium as above) at 200 μg Chl · ml^{-1}. Other additions when indicated were 10 μM atrazine in ethanol, 2.5% ethanol (alone), or 50 μM methylviologen + 2 μM gramicidin. The suspension was maintained 5 min in darkness at 0 ° C, poured into the cuvette then cooled slowly to reach a precise temperature, at which it was submitted to illumination allowing charging of the TL bands. This was done either (1) by giving one or more saturating flashes at -5 ° C, or (2) with continuous red light (wavelength > 650 nm) at light intensity of 3000 μE · m^{-2} · s^{-1} given at a low temperature: -80 ° C or in the range -100, -60 ° C, in the experiments of Fig. 7. Then the samples were rapidly cooled at -140 ° C by dipping the holder in liquid nitrogen. In these two types of experiment heating was performed from -90 ° C to 80 ° C in 6 min (28.3 C °/min). The luminescence emission was measured at wavelengths above 685 nm by a cooled photomultiplier connected to a photon counting system. Signal recording and regulation of temperature were performed by an HP 85 microcomputer.

Results and Discussion

Broken chloroplasts were submitted to a high light intensity (5500 μE · m^{-2} · s^{-1}) either in the presence or the absence of an efficient exogenous electron acceptor.

In the absence of exogenous electron acceptor, impairment of the photosynthetic process proceeds rapidly: 50% inhibition of O_2 evolution occurs at approx. 4–5 min (Fig. 1). Oxygen exerts a protective effect on PS II activity, since in a totally O_2-deprived medium, using glucose + glucose oxidase [12,20], PS II activity was totally suppressed in 90 s (not shown). Similar results were obtained with PS II particles [8]. O_2 probably acts as an electron acceptor giving H_2O_2 at the level of PS I, which is destroyed by the addition of catalase to the medium [21].

A still faster impairment of the PS II activity was observed when photoinhibition was performed in the presence of electron acceptor: half-times for inactivation were about 2 min (Fig. 1). In the latter conditions, where a high level of oxygen evolution occurs, we did not try to carry out experiments in O_2-deprived medium using glucose + glucose oxidase.

For thermoluminescence studies, pellets of photoinhibited and control chloroplasts were kept in the dark at 0 ° C for at least 2 h before charging of the TL bands.

Charging of TL bands with actinic flashes

After one actinic flash fired near -5 ° C a single TL band emission peaking at 32 ° C was observed: Fig. 2A and B (control and chloroplasts photoinhibited without acceptor). It corresponds to the well-known TL band due to recombination of the radical pair, $S_2Q_B^-$ [22,23]. A shoulder on the left side (at lower temperatures) of the band is always present near 12 ° C but its size varies from one batch of the chloroplasts to another. The TL band has almost the same shape and position in photoinhibited and in control samples but its amplitude is decreased in the former. The shoulder at 12 ° C is more apparent in photoinhibited chloroplasts, although unchanged in size. The decrease in amplitude of the TL band is parallel to the decrease of PS II electron transfer activity (Fig. 1). While these studies were in progress similar results were published by Vass et al. [24]. The results presented here confirm the earlier report [24]. It is of note that quite different data have also been

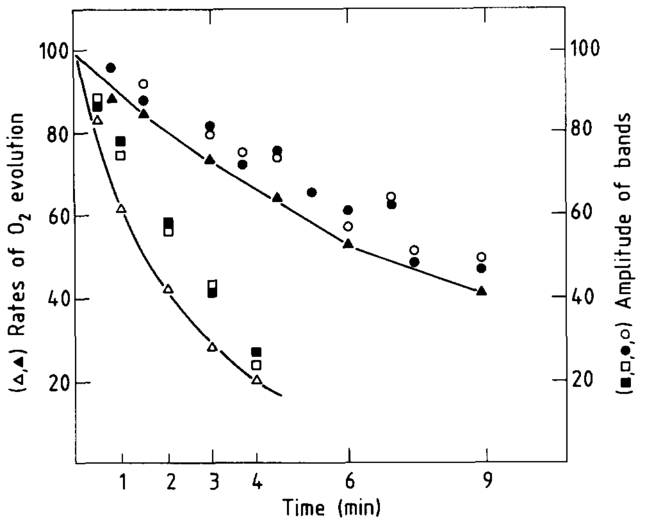

Fig. 1. Relationship between the amplitude of the 32 ° C (●,■) and 9 ° C (○,□) band emission of luminescence and the rate of electron transfer (as oxygen evolution rates): ▲,△, as a function of the duration of the photoinhibitory treatment. Photoinhibition in the absence of electron acceptor (●,○,▲) or in the presence of electron acceptor (■,□,△) (see Methods). Uncoupled rates of O_2 evolution in the control were 400–450 μmol/mg Chl per h (100%). Each data point is average of 3–5 individual measurements on different batches of chloroplasts.

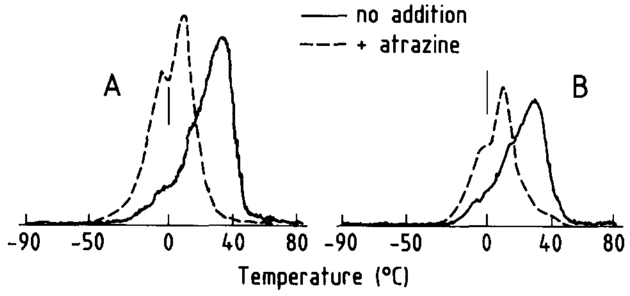

Fig. 2. Thermoluminescence glow curves in control chloroplasts (A) and chloroplasts photoinhibited in absence of acceptors (B). Inhibition of PS II activity, 40%. Charging of bands by 1 saturating flash given at -5 ° C. No addition, ———; + 10 μM atrazine, - - - - - -. Bars indicate 0 ° C.

reported [13]; these contradictions will be dealt with in the Discussion.

After photoinhibition performed in the presence of acceptor the results were similar (Fig. 1).

The curve for the decrease in the TL band is slightly higher than that of PS II activity for the same photoinhibitory treatment (Fig. 1). This is probably due to a systemic change in the amplitude of the $S_2Q_B^-$ TL band in centers dark-adapted after strong illumination compared to the non-illuminated control.

In the presence of atrazine, the $S_2Q_B^-$ band disappeared and was replaced by a band attributed to $S_2Q_A^-$ [22,25] peaking at 9 ° C (Fig. 2A and B). The $S_2Q_A^-$ band has the same shape in control and in chloroplasts photoinhibited (with or without acceptor). It is decreased in photoinhibited chloroplasts compared to the control with a time disappearance as seen for the $S_2Q_B^-$ band (Fig. 1). Again, this is in agreement with Vass et al. [24].

The proximity of the positions of the low-temperature shoulder in Fig. 2A,B and the $S_2Q_A^-$ band near 10 ° C leads up to suggest that the shoulder could be due to $S_2Q_A^-$ recombination in PS II centers lacking functional Q_B [26]. These PS II centers seem to be very resistant to photoinhibition in agreement with earlier suggestions [6,27].

When a series of flashes were used to charge the TL bands, the band peaking at 32–35 ° C oscillates with a periodicity of 4 depending of the number of flashes: Fig. 3. Maxima were observed after 1, 5 and 9 (or 10) flashes in control and 1, 5 and 10 flashes in photoinhibited chloroplasts (without acceptor). After two or more flashes the TL band is attributed to recombination reactions involving a mixture of $S_2Q_B^-$ and $S_3Q_B^-$ states [23,23]. The behavior of control and photoinhibited chloroplasts is very similar. Moreover, when comparing the shape of the TL bands during the sequence, especially after 9–10 flashes, an increase of the band at 10–12 ° C was clearly observed: it is particularly striking at 40 flashes (Fig. 4, compare 9 flashes and 40 flashes) giving a defined peak, whereas another shoulder near − 10 ° C is apparent. With the addition of an efficient electron acceptor (methylviologen) + an uncoupler (gramicidin) to the resuspending medium of dark-adapted chloroplasts, the TL bands remain of low size after 9–10 flashes with a low 10–12 ° C band. These results reinforce our interpretation concerning the origin of the band at 10–12 ° C as being due to $S_2Q_A^-$ recombinations. In addition, the band charged by three flashes is shown since it exhibits a 10–12 ° C band more clearly individualized. The small size of this band indicates that the recombination reaction responsible for this luminescence involves only a low fraction of the total PS II centers.

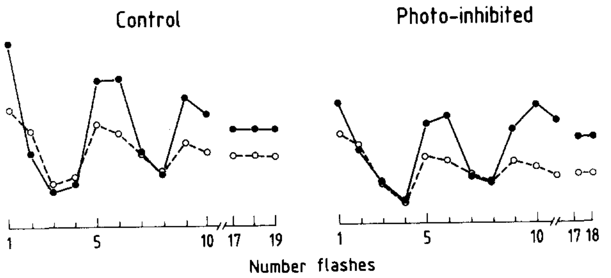

Fig. 3. Variation of amplitude of 32–35 ° C bands in function of the number of flashes used to charge the bands. Control and photoinhibited chloroplasts (photoinhibition in the absence of electron acceptor, inhibition of O_2 evolution rates, 40%). No additions, ●———●; addition of 50 μM methylviologen + 2 μM gramicidin (added to resuspending medium before TL measurements), ○------○. The dark-adapted chloroplasts received one saturating flash at 20 ° C then were allowed to relax 5 min in darkness at 20 ° C in order to accumulate about 100% state S_1 and 87% $Q_B$ox in samples [23]; then 1 − n flashes were fired at − 5 ° C.

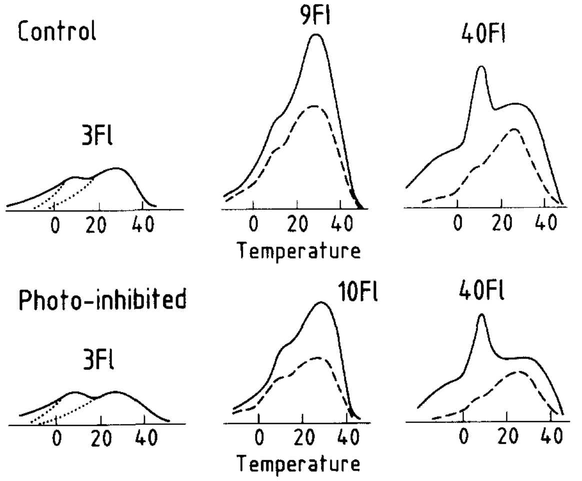

Fig. 4. Variation of shape of 32–35 ° C bands (with apparent 12 ° C band) in relation with the number of flashes used to charge the bands. Same experiment as in Fig. 3. No addition, ———; addition of methylviologen, gramicidin (see Fig. 3), ------. Experiment with three flashes, no addition of methylviologen + gramicidine; dotted lines indicate tentative of deconvolution of the curve to evidence $S_2Q_B^-$ (32 ° C) and $S_2Q_A^-$ (12 ° C) recombinations.

Damping of the oscillations is seen during the flash sequence in the control and in photoinhibited chloroplasts (without acceptor) which is complete after 15 flashes (Fig. 3). A high level of luminescence is observed for samples excited by more than nine flashes. This is more marked in chloroplasts photoinhibited without acceptor, whereas chloroplasts photoinhibited with acceptor behave as the control (not shown). Addition of methylviologen plus an uncoupler (gramicidin) to the resuspending medium of dark-adapted chloroplasts results in a less marked damping with a large decrease of the luminescence level after 15–20 flashes, especially in photoinhibited chloroplasts. Following Rutherford and Inoue [28], who observed similar phenomena, the increase in luminescence level is thus ascribed to charge accumulation in the plastoquinone pool able to feed Q_B with negative charges and displacing the equilibrium to

Q_A^-. It seems that in chloroplasts photoinhibited without acceptor the PQ pool remains more reduced than in the control due to an absence of efficient electron draining away from PS II. This is a consequence of the lack of electron acceptor acting at the level of PS I. This conclusion is confirmed by EPR measurements in this material which showed reduced Rieske iron-sulfur protein even after more than 3 h dark-adaptation, while in control material the Rieske iron-sulfur center was largely oxidized (Rutherford, A.W. and Farineau, J., unpublished data).

In conclusion, since TL bands exhibit nearly similar characteristics in control and photoinhibited chloroplasts (photoinhibition with or without acceptor) it seems that the PS II centers remaining active for O_2 evolution are not apparently damaged: there is no special modified $S_2/_3Q_B^-$ peak formed in the conditions of these experiments. It has been reported that, a short time after a photoinhibitory treatment (without acceptor) applied to *Chlamydomonas* cells, the TL peak induced by one flash is seen at a lower temperature than normal $S_{2/3}Q_B^-$: at 12–17°C (after a moderate photoinhibitory treatment), and near 10°C after a severe photoinhibition [13]. This can be interpreted as due partly to an increase in the reduction state of the PQ pool (as was shown above for chloroplasts) leading to some $S_{2/3}Q_A^-$ recombinations and possibly $S_{2/3}QH_2$ [23,28]. The band shift reportedly disappears with dark-adaptation (in the case of a moderate treatment) and the recovery of the B band is probably attributable to a slow reoxidation of PQH_2. However, we can not rule out the possibility that some of the differences in the phenomena reported here and in Ref. 24 with chloroplasts compared to the work in *Chlamydomonas* cells [13] may be due to reactions occurring in vivo which are absent in vitro.

In untreated centers there is a subpopulation of centers which is blocked in forward electron transfer after formation of $S_2Q_A^-$ [26]. This can be seen as a low-temperature shoulder after one flash and a separate band after three flashes (i.e., when the majority of the centers are in state S_0). These centers are less sensitive to photoinhibition.

Charging of TL bands with continuous light at low temperature

Figs. 5A,B show TL profiles observed when dark-adapted samples were submitted to moderate illumination: 5 s red light, 3000 $\mu E \cdot m^{-2} \cdot s^{-1}$ at −80°C.

With continuous illumination essentially two TL bands were observed (Fig. 5A,B): a peak at 32°C and a band peaking at −68°C. An additional, very broad band is observed at temperature higher than 50°C [29]: this is poorly developed in control samples but is of greater amplitude in photoinhibited chloroplasts.

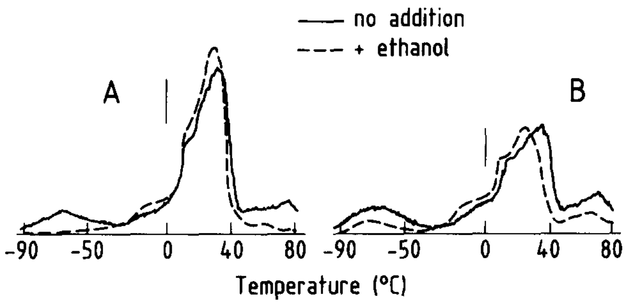

Fig. 5. Thermoluminescence glow curves in control chloroplasts (A) and chloroplasts photoinhibited in the absence of acceptors (B). Same experiment as for Fig. 2 except that charging of bands was by 5 s continuous light (3000 $\mu E \cdot m^{-2} \cdot s^{-1}$) at −80°C. No addition, ———; addition of 2.5% ethanol, ------.

The band with peak at 32°C is identified as arising from $S_2Q_B^-$ recombination, since illumination of PS II centers at low temperatures, in the range −100°C to −60°C, allows charging of S states to S_2 and the reduction of Q_A. The charge on Q_A migrates to Q_B when heated above −60°C. In addition, the shoulder corresponding to $S_2Q_A^-$ recombinations near 12°C is seen. However, $S_2Q_B^-$ band charged by continuous light has a 20–30% lower amplitude compared to when it is charged by one flash (Table I).

The band at −68°C is small. Its size is increased in chloroplasts photoinhibited without acceptor (10–30%) as compared to the control (Fig. 5A,B). The size of the −68°C band depends on the magnitude of the PS II inhibition (Fig. 6A). In chloroplasts photoinhibited with acceptor, the band at −68°C is decreased in parallel with lowering of PS II activity (Fig. 6B). In the control, the band at −68°C is known as the Z_V band [30,31].

It is reported that the appearance of this band is inhibited by ethanol in the control (Fig. 5A) and in samples photoinhibited in the presence of electron acceptor (Fig. 6B). The suppression of the band by ethanol in the control is accompanied by a corresponding 10% increase in the usual $S_2Q_B^-$ recombination, i.e., the TL band at 32°C (Table I). Ethanol addition results in the only partial disappearance of the −68°C band in

TABLE I

Change in the amplitude of the 32°C band and of the low-temperature (−68°C) band in control chloroplasts charged either at ambient temperature (one flash) or at low temperature (−80°C)

Sizes of each band (32°C and −68°C) was taken equal to 100% in chloroplasts charged by 5 s continuous light at −80°C.

Charging of bands		Amplitude	
	Band:	−68°C	32°C
(a) 1 Fl (−5°C)		0	120
(b) 5 s CL (−80°C)		100	100
5 s CL + 2.5% ethanol		0	108

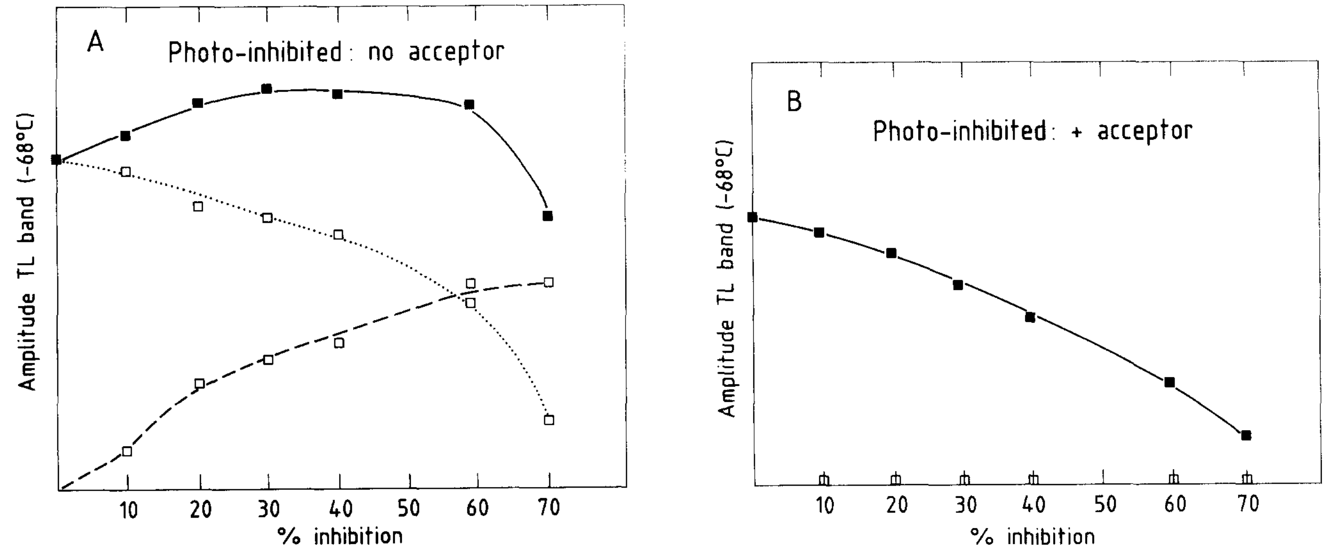

Fig. 6. Dependence of amplitude of low temperature (−68° C) band on degree of inhibition of O_2 evolution rates. Bands were charged by 5 s moderate continuous light at −80° C. Photoinhibition performed either in the absence of electron acceptor (A) or in the presence of acceptors (25 μM methylviologen +2 μM gramicidin (B). No addition to resuspending medium: ■———■; addition of 2.5% ethanol: □-----□. Curve indicated as (□·····□) is difference between curves without and with addition of ethanol to medium.

chloroplasts photoinhibited without acceptor (Figs. 6A, 7). The part resistant to ethanol is different from the usual Z_V band in that the emission is very low when charged at −60° C and much higher when charged at −100° C (Fig. 7). The fraction of the band resistant to ethanol (measured after an illumination at −80° C, Fig. 6A) increases in size in parallel with the inhibition of PS II activity. These results indicate that the TL band at low temperature in this photoinhibited material is made up of two bands, one of which is the usual Z_V band and decreases during photoinhibition (curve 'Difference' in Fig. 6A) while the other is a new state generated in photodamaged reactions centers.

In control chloroplasts, the size of the low-temperature band oscillates with a periodicity of 4, as a function of the number of preilluminating flashes (given at −5° C) before the illumination performed at −80° C (Fig. 8). Maxima are after 0 and 4 flashes and minima

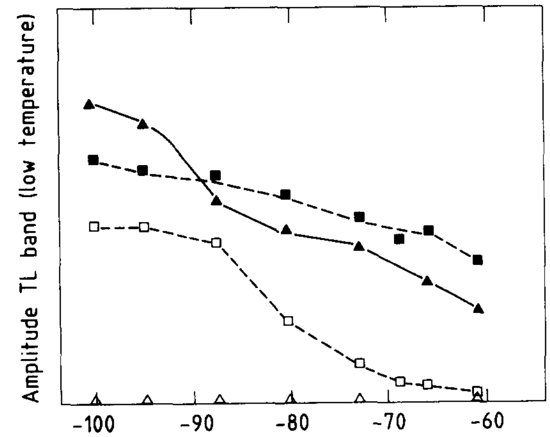

Fig. 7. Dependence of the amplitude of the low-temperature band on temperature where samples were illuminated to charge the bands. Charge by 5 s continuous light at temperatures indicated in abscissae. Control chloroplasts, ▲,△; chloroplasts photoinhibited in absence of electron acceptors, 40% inhibition of O_2 evolution rates, ■,□. No addition, ▲,■; addition of 2.5% ethanol, △,□.

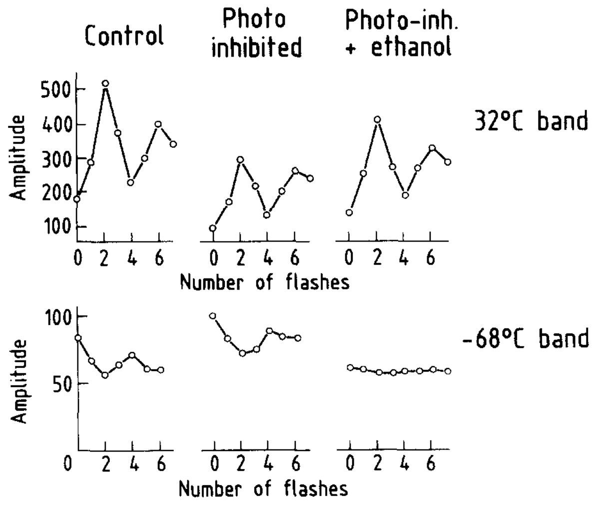

Fig. 8. Dependence of amplitudes of low-temperature (−68° C) and 32° C bands on number of preilluminating flashes given at −5° C to dark-adapted chloroplasts. Control and photoinhibited chloroplasts (photoinhibition in the absence of electron acceptors, 40% inhibition of PS II activity); photoinhibited + ethanol: addition of 2.5% ethanol to resuspending medium for TL measurements. Bands charged by 5 s continuous illumination at −80° C. Amplitudes in relative units; amplitudes of 32° C band and of −68° C band in photoinhibited chloroplasts (without preilluminating flashes) were taken equal to 100%.

after 2 or 6 flashes as reported [31]. Oscillations of the amplitude of the $S_2/_3Q_B^-$ (32° C) band show exactly the opposite pattern (Fig. 8). The oscillation patterns of the $-68°$ C band are very similar in control and photoinhibited chloroplasts (with or without acceptor) except that in the latter they are less marked.

After the addition of ethanol to photoinhibited chloroplasts (without acceptor) the remaining band (at $-68°$ C) exhibits practically no oscillations as a function of the number of preilluminating flashes, whereas the 32° C band is increased in size and oscillates normally compared to untreated chloroplasts (Fig. 8).

In conclusion, the low-temperature band observed in chloroplasts photoinhibited without acceptor is complex and originates from both normal and photodamaged PS II centers. The addition of ethanol eliminates the usual TL band at $-68°$ C and reveals a TL band arising from damaged PS II centers with special properties. The latter band is not seen when photoinhibition is performed in the presence or an efficient electron acceptor. This result shows that the electron transfer properties of chloroplasts photoinhibited in the presence or the absence of electron acceptors are quite different. It indicates that the electron transfer lesion in the two states is also different. This is to be expected, since photoinhibition in the presence of an acceptor is likely to result in the acceptor side outrunning the donor side and thus to the occurrence of donor side lesions. Conversely, in the absence of an acceptor, the electron-acceptor side of PS II becomes overreduced, which should result in lesions at this level. A major problem in interpreting these results at a mechanistic level is that the components involved in the formation of the low temperature bands are unknown. Some of the properties of the usual Z_V band have been correlated with those of an EPR signal at $g = 4$ which was attributed to a fraction of the centers in the S_2 state [32]. Ethanol perturbs the equilibrium between this state and the usual S_2 state, stable at high temperatures, which is manifest as the multiline signal [32,33]. The increase in the normal $S_2Q_B^-$ band observed here when ethanol is added to eliminate the Z_V band fits with the assignment of the Z_V band to S_2 $(g = 4)$ Q_A^- recombination. Other explanations, however, cannot be ruled out. The new band is observed in material photoinhibited without an electron acceptor – a lesion at the electron acceptor side is expected in such material. Thus, possible candidates as charge pairs include S_2 with modified Q_A^- or with Ph^- [8]. Experiments using other spectroscopic techniques are required to resolve these questions.

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