Biochimica et Biophysica Acta, 1142 (1993) 115-122
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BBABIO 43795

Photoinhibition of photosynthesis in vivo: Involvement of multiple sites in a photodamage process under CO_2- and O_2-free conditions

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(Received 2 November 1992)

Key words: Bicarbonate; D1 protein; Electron transport; Chlorophyll fluorescence; Photosystem I; Reaction center II; (*L. gibba*)

Intact *Lemna gibba* plants were illuminated by photoinhibitory light in air, in air minus O_2, in air minus CO_2, and in pure N_2. In pure N_2, the degree of photoinhibition increased 3-5-times compared with that in air. This high degree of photoinhibition is described as photodamage. Photodamage was found to constitute a syndrome, that is, it is due to inactivation of multiple sites. These sites include RC II component(s) from P680 to Q_A; the Q_B-site; and a component of PS I. In photodamage, the donor side of PS II and PS II excitation energy transfer remain unimpaired, but the size of the PS I antenna seems to decrease. Photodamage is distinguishable from photoinactivation. Photoinactivation occurred in air and could be attributed to inhibition of electron transport from Q_A^- to Q_B. During photoinactivation the D1 protein of RC II became degraded faster than the detectable inhibition of Q_B reduction. The photoinhibition-induced rise in F_0 occurred only during the process of photodamage but not during that of photoinactivation, and was a secondary event which arose as a consequence of photodamage. Atmospheric O_2 alleviated photodamage but increased photoinactivation. The light-induced D1 degradation and inhibition of Q_A to Q_B electron transfer were enhanced in vivo not only by O_2 but also by depletion of CO_2.

Introduction

Photoinhibition is a process by which photosynthetic rate is decreased as a result of exposure of oxygen-evolving organisms to light intensities greater than those saturating photosynthesis. Photoinhibition consists of photoinactivation of reaction centre II and breakdown of D1 protein [1]. Based on experiments in vivo with green algae or cyanobacteria, and in vitro with thylakoid membranes or PS II particles, it is proposed that, during photoinactivation, the acceptor side of RC II is altered reversibly and electron flow from Q_A^- to Q_B is slowed down [1-5], followed by double reduction of Q_A^-, which is irreversible [5]. The inactivation of the acceptor side may induce changes in the donor side of PS II [1,2]. Inactivation of the donor side results in formation of cation radicals at RC II which may further damage the RC II-D1 protein and accelerate photoinhibition [6-8].

Some reports have shown that PS I photochemical activity is also inactivated by photoinhibitory treatment in vitro in the absence of an added electron acceptor [9]. Photoinhibition of PS I requires either the presence of oxygen [9,10] or extremely reducing conditions [11,12].

The photoinhibition described above occurs in air. Photoinhibition can be strongly affected by composition of the gas phase. Photoinhibition is alleviated by the presence of CO_2 in vivo [13,14] or by addition of bicarbonate in vitro [15]. The role of oxygen is more complex. In presence of O_2 the organism can dissipate a part of the excess energy through pseudocyclic electron flow [14,16] or through photorespiration [9,13]. Anaerobic conditions can greatly enhance susceptibility to photoinhibition [17,18]. On the other hand, the presence of O_2 may be required for photoinhibition, since oxygen free-radicals formed by photosynthetic reduction of oxygen [19] may be responsible for breakdown of the RC II-D1 protein [20,21]. Some authors have suggested that photoinhibition has different

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Abbreviations: Asc, ascorbate; DI, Q_B-binding reaction centre protein of Photosystem II; DCIP, 2,6-dichlorophenol indophenol; DPC, diphenylcarbazide; MV, methylviologen; PFD, photon flux density; PQ, plastoquinone; PS I, Photosystem I; PS II, Photosystem II; Q_A^-, the quinone which is the primary electron acceptor of PS II; Q_B, the quinone which is the secondary electron acceptor of PS II; RC I, II, Photochemical reaction centre of Photosystem I, II.

mechanisms under anaerobic and aerobic conditions [17,22]. The anaerobic conditions gave an increase in F_0 and aerobic condition gave an decrease in F_v in isolated chloroplasts [17,22]. However, the different sites of in activation in each case are still unclear.

In the present work we find involvement of multiple sites during a photodamage process in vivo. The photodamage occurs under conditions of depletion of O_2 and CO_2 which are similar to the anaerobic conditions reported earlier [15,17,18]. The results serve to distinguish photodamage from the photoinactivation which occurs in air. Further features of photodamage are described.

Materials and Methods

Plant material

Axenic cultures of *Lemna gibba* L. G_3 were precultured as previously described [23]. The plants were then grown for 6–9 days at a PFD of 250 μmol m^{-2} s^{-1} at 22°C. Plants at the 4-frond stage were selected for the experiments.

Photoinhibitory treatment and photosynthesis measurement

Plants were photoinhibited using tungsten-halogen lamps, at PFDs specified in results. The gas phases were controlled by flushing the reaction chamber with: air; 99.99% N_2; N_2 plus 360 ppm CO_2 (equivalent to air minus O_2); air minus CO_2. CO_2 was removed from air by pumping it through a column filled with Carbosorb (BDH Chemicals, UK).

Net photosynthetic rate was measured as CO_2 uptake using an infra-red gas analyser (Uras 2G) in a closed gas exchange system as previously described [24,25].

Thylakoid preparation

Immediately after the photoinhibitory treatment, plants were homogenised with cold homogenisation medium consisting of sorbitol (0.33 M), NaCl (10 mM), $MgCl_2$ (5 mM), EDTA (2 mM) and Tricine (50 mM) adjusted to pH 7.6 with KOH. The homogenate was filtered through four layers of fine nylon mesh directly into a centrifuge tube. 1/4 volume of the homogenisation medium containing sucrose (0.33 M) instead of sorbitol was smoothly injected into bottom of the tube to form two clear phases of liquid. The mixture was centrifuged at $1000 \times g$ for 3 min. The procedure described above is based on that of Mills and Joy [26]. The pellet of chloroplasts was resuspended with the sorbitol-containing medium for further experiments. For photosynthetic electron transport measurements, the thylakoid suspension was used at once; for the 77 K fluorescence detection, the samples of suspension were frozen in liquid N_2 before the measurements; for the gel electrophoresis, the suspension was centrifuged at $10\,000 \times g$ for 10 min and the pellet of thylakoid membrane was resuspended with the Tricine buffer and preserved at −80°C.

Chlorophyll concentration was determined according to Arnon [27].

Measurements of electron transport

To measure PS II-dependent electron transport, thylakoid suspension was added to Tricine buffer containing 10 μM DCIP, to give a final chlorophyll concentration of 4 μg ml^{-1}. The kinetics of photochemical reduction of DCIP were measured spectrophotometrically using either H_2O or DPC (10 μM) as electron donors [28]. The actinic light was obtained by use of a 665 nm short-pass filter to favour excitation of PS II [28]. For the PS I electron transport measurement, O_2 uptake was detected using Clark-type polarographic electrode as previously described [29]. Ascorbate (5 mM) with DCIP (0.1 mM) was used as an electron donor to methyl viologen (50 μM) in the presence of DCMU (10 μM) and NaCN (10 mM).

Measurements of chlorophyll fluorescence

For room temperature fluorescence measurements, a PAM system (Waltz, Effeltrich, Germany) with standard accessories (PAM 101, 102 and 103) and computer analysis was used. The fluorescence decay after a single turnover flash (given by the xenon flash lamp, XST-103, Waltz) was measured according to Schreiber [30]. The flash intensity is intended to be high enough for one charge separation at every reaction centre and short enough to prevent double hits. During the whole period of experiments, the *Lemna* plants floated on growth medium in a home-made aluminium cuvette in which temperature and gas compositions were controlled. Lights for adaptation and photoinhibition were supplied with Olympus ILK-4 and Schott KL1500 lamps respectively. The plants were dark adapted for 10 min before the flash.

77 K fluorescence emission was measured using a Perkin-Elmer LS-5 luminescence spectrometer as described previously [31]. Thylakoid suspension (see above) with a final chlorophyll concentration 7.0 μg ml^{-1} was frozen in liquid N_2 for at least 10 min before the measurement. Excitation was at 435 nm. The fluorescence emission maxima at 692 nm (PS II) and 737 nm (PS I) were normalised to the 550 nm fluorescence maximum of fluorescein (sodium salt) [32], present at a final concentration of 1.0 μM.

Pulse-chase and analysis of RCII-D1 protein

During the pulse period, 20 plants were incubated for 20 min in 4 ml cultivating medium containing 143 μCi [^{35}S]methionine (> 1000 Ci $mmol^{-1}$, Amersham) under a PFD of 250 μmol m^{-2} s^{-1} at 20°C. At the end

of the pulse, 10 plants were removed for thylakoid preparation. The other plants were rinsed and incubated for 60 min in cultivating medium containing 1 mM non-radioactive methionine under various atmospheric and light conditions.

Thylakoid membrane and the D1 protein were separated by LDS-electrophoresis and autoradiography as previously reported [25]. D1 was quantified densitometrically.

Results

After *Lemna* plants were illuminated by high light for 90 min, the degree of photoinhibition measured by CO_2 uptake was much higher in the gas phases lacking CO_2 than in those containing CO_2 (Fig. 1). In pure N_2 the degree of photoinhibition was the highest (80–90%, Fig. 1) and the photoinhibition induced in this condition recovered very slowly compared with that induced by other stress conditions [17,25]. We describe the photoinhibition in N_2 as photodamage. In air the degree of photoinhibition was much lower than that in N_2 (Fig. 1) and is described as photoinactivation. At PFD of 2800 μmol m^{-2} s^{-1}, O_2 alleviated photodamage but increased photoinactivation (Fig. 1).

Table I shows electron transport partial reactions in photoinhibited plants. In pure N_2, the electron transport from H_2O to DCIP measured under actinic light favouring PS II was inhibited by 43% which was higher than the degree of inhibition in any other gas phase. This indicates that the photodamage process is located at PS II. A similar degree of inhibition of DCIP reduction was obtained using DPC as an electron donor, showing that the water-splitting complex in PS II remained unimpaired. The question remains: how can photodamage inhibit CO_2 uptake to a much greater extent (Fig. 1) than PS II electron transport (Table I)? One possibility is that additional sites besides that in PS II may be inhibited. To check this, PS I electron transport from reduced DCIP to methyl viologen was measured before and after 90 min photoinhibitory treatment. In N_2, the rate of PS I light-saturated electron transport decreased by 8% and the rate of PS I light-limited electron transport decreased by 27%, while in the other atmospheric conditions PS I was not significantly influenced during photoinhibition (Table I). This result indicates that a site in PS I between P700 and the Fe-S centres is inactivated during the photodamage process. This is in agreement with previous reports using isolated chloroplasts, which showed that under extremely reducing conditions a site between A_0 and Fe-S X of PS I was photoinhibited [12].

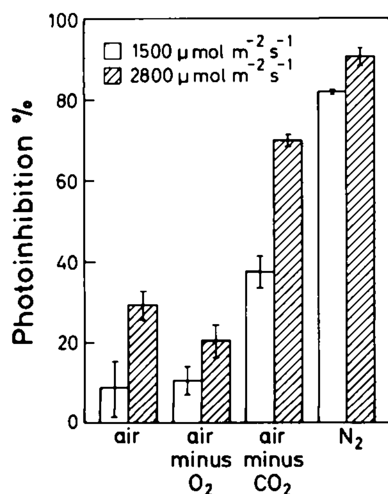

Fig. 1. Loss of CO_2 up take after photoinhibitory treatment. Plants were illuminated for 90 min under the PFD and atmospheric conditions as indicated. Vertical bars represent S.D.

TABLE I

Localization of photoinactivation and photodamage in the photosynthetic electron transport chain

Thylakoids were prepared from plants illuminated for 90 min under PFD of 2800 μmol m^{-2} s^{-1} in the gas phases indicated. The 'Control' was at PFD 250 μmol m^{-2} s^{-1} in air. DCIP reduction was measured under actinic light preferentially absorbed by PS II. The rates of electron transport are expressed as a percentage of the control rate. The control rate was 268 ± 40 μmol DCIP (mg chlorophyll)$^{-1}$ h^{-1} for the PS II-dependent reactions $H_2O \rightarrow$ DCIP and DPC $\rightarrow$ DCIP, 70 ± 12 μmol O_2 (mg chlorophyll)$^{-1}$ h^{-1} for the PS II- and PS I-dependent reaction $H_2O \rightarrow$ MV, and 167 ± 15 μmol O_2 (mg chlorophyll)$^{-1}$ h^{-1} for the PS-I-dependent reaction Asc $\rightarrow$ MV. The low rate of PS II + PS I reaction is possibly due to coupling of ATP synthesis, since uncouplers were absent during the measurements. HL, measured under saturating light; LL, measured under unsaturating light (PFD of 300 μmol m^{-2} s^{-1}).

	$H_2O \rightarrow$ DCIP	DPC $\rightarrow$ DCIP	$H_2O \rightarrow$ MV	Asc $\rightarrow$ MV	
	HL	HL	HL	HL	LL
Control	100	100	100	100	100
Photoinhibitory treatment					
air	86.7	86.0	80.2	101	100
air minus O_2	88.5	90.6	80.6	97.6	99.0
air minus CO_2	67.9	70.2	65.9	92.2	86.7
N_2	56.8	57.2	25.6	91.4	73.0

To probe additional effects on PS I and PS II, we used 77 K fluorescence emission spectroscopy. We obtained 77 K fluorescence emission spectra of thylakoids isolated from *Lemna* plants before and after photoinhibition under the four atmospheric conditions described (Table II). Two emission maxima, at 692 and 737 nm, were observed in all cases. The fluorescence emission at 692 nm, which may reflect the state of the PS II core and recombination luminescence of the RC II [33], showed a pattern of changes (Table II) consistent with those in electron transport (Table I). Fluores-

cence emission of PS II was lowered by the CO_2-free gas, and was lowered by 50% after photoinhibition in N_2 (Table II). Fluorescence emission at 737 nm, which may be ascribed to LHC I and the PS I core [34,35], decreased by 42% after photoinhibitory treatment in pure N_2 (Table II). The drastically decreased 77 K fluorescence emission of PS I again suggests that PS I may be inactivated during the photodamage process. The size of the PS I antenna may have decreased. This is supported by the decreased 77 K fluorescence emission at 737 nm (Table II) as well as by the inhibited PS I light-limited electron transport (Table I). 77 K fluorescence of PS II as well as PS I was relatively unaffected by photoinactivation, especially in the absence of O_2 (Table II).

Most previous investigations on the mechanism of photoinhibition have been carried out in air and the site of inactivation was located at PS II. Table I showed that in N_2, PS II-dependent electron transport was inhibited 2.5-times more than that in air. This indicates that the photodamage brought about by CO_2- and O_2-free conditions results either from more photoinactivated sites or from acceleration of the damage at a single site within PS II. To estimate the site of block-

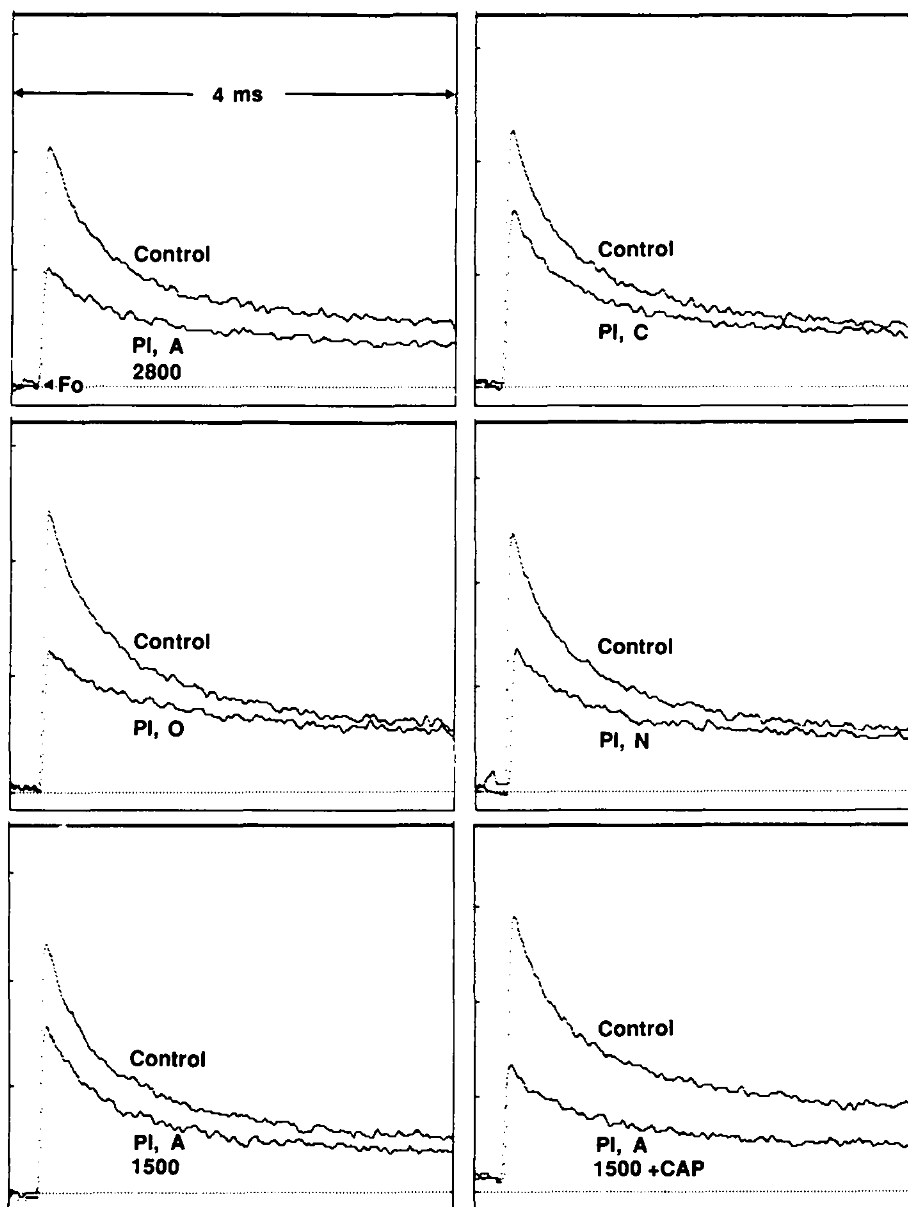

Fig. 2. The fast phase of fluorescence decay was slowed down by presence of O_2, absence of CO_2, and addition of chloramphenicol during photoinhibition. Intact plants were given a single turnover flash before (Control) and after (PI) photoinhibition of 90 min in air (A), air minus O_2 (C), air minus CO_2 (O) and pure N_2 (N) under a PFD of 2800 μmol m^{-2} s^{-1} or 1500 μmol m^{-2} s^{-1} in air and in the presence or absence of chloramphenicol (CAP) at a final concentration of 500 μg ml^{-1}.

TABLE II

77 K fluorescence emission during photoinhibition

Lemna plants were photoinhibited at PFD of 2800 μmol m^{-2} s^{-1} for 90 min in the atmospheres indicated. Fluorescence spectra were obtained for thylakoid extracts at equal chlorophyll concentration (7 μg ml^{-1}), and were normalised to fluorescence at 550 nm from 1 μM fluorescein. The normalized values for total fluorescence emission are given in arbitrary units ±S.D. The 'Control' condition was the same as that in Table I.

	PSII (F_{692})	PSI (F_{737})	F_{692}/F_{737}
Control	0.47±0.03	0.66±0.06	0.727±0.030
Photoinhibition			
air	0.38±0.04	0.58±0.07	0.665±0.010
air minus O_2	0.43±0.01	0.62±0.03	0.691±0.012
air minus CO_2	0.30±0.02	0.49±0.00	0.605±0.029
N_2	0.24±0.04	0.38±0.09	0.629±0.030

age within RC II during photoinhibition, intact plants were given a single turnover flash after Q_A and the PQ pool were oxidised in the dark. The kinetics of fluorescence decay following the flash (Fig. 2) represent Q_A reoxidation [30,36]. The fluorescence decay during 40 ms showed three exponential components (data not shown). The first component reflects the forward electron flow from Q_A^- to Q_B [36,37] and the $t_{1/2}$ of this phase is shown in Table III. The fast phase was slowed down by increasing PFDs (Fig. 2 and Table III). After 90 min illumination at PFD of 2800 μmol m^{-2} s^{-1}, the $t_{1/2}$ of the fast phase increased in all gas phases. The increase of $t_{1/2}$ of the fast phase was 20% greater in the presence of O_2 than in absence of O_2, and 19% greater in the absence of CO_2 than in presence of CO_2 (Fig. 2 and Table III). Photoinhibition in pure N_2 resulted in a similar degree of inhibition of Q_A^- to Q_B electron transport as that which occurred in air (Table III). In contrast, under PFD of 2800 μmol m^{-2} s^{-1}, the inhibition of CO_2 uptake was almost 3-times higher in N_2 than in air (Fig. 1), and inhibition of PS II-dependent electron transport 2.5-times higher in N_2 than in air (Table I). The higher degree of photoinhibition of CO_2 uptake might be attributed to PS I inhibition. The higher inhibition of PS II electron transport in N_2 could be due to inhibition of RC II components from P680 to Q_A, since water oxidation itself seems to have been unimpaired (Table I) and since the inhibition of electron flow from Q_A to Q_B was inhibited to a similar degree in N_2 as in air (Table III).

After photoinhibitory treatment, intrinsic fluorescence, F_0, increased in N_2 and, to a lesser extent, in air minus CO_2. F_0 did not increase in the other gas phases (Table III). The increased F_0 can be due to RC II inactivation [38] by trapping of Q_A^- [3,4] or by Q_B^- destabilization [1,2]. F_0 did not rise in air in vivo, although the Q_A reoxidation was inhibited (Fig. 2 and Table III). In addition, F_0 does not rise in vitro when an electron acceptor such as ferricyanide is present [1]. Therefore the rise of F_0 in N_2 indicates that Q_A^- oxidation is blocked due to the over-reduction of the PQ pool. The fluorescence kinetic studies (Fig. 3) show that, during photoinhibition in N_2, F_0 started to rise after 20 min and came to a maximal level at 75 min, but in air F_0 did not rise at any time (Fig. 3A). In N_2 the net photosynthetic rate was photoinhibited by over 50% even after an illumination for 10 min (Fig. 3B), while F_0 did not change (Fig. 3A). This suggests that the rise in F_0 is a secondary event arising as a consequence of photodamage. Moreover, the rise in F_0 during photodamage may indicate that PS II charge separation is inactivated [38]. This is supported by the

TABLE III

Effect of photoinactivation and photodamage on Q_A reoxidation and RC II activity

Fluorescence decay was recorded under the conditions described in Fig. 2. Y_{max} was the maximal fluorescence following one flash. F_0 and Y_{max} values after photoinhibitory treatment are expressed as percentages of the values before photoinhibitory treatment. The 100% values for F_0 and for Y_{max} were the averages from the controls of each experiment.

Photoinhibitory condition	$t_{1/2}$ (μs) of fast phase			F_0 ±S.D. (%)	Y_{max} (%)
	control	photo-inhibited	increase in $t_{1/2}$ (%)	photo-inhibited	photo-inhibited
1500 μmol m^{-2} s^{-1}					
air	486	547	13± 5	96±2	76.5
air minus O_2	493	496	1± 2	93±7	74.9
air minus CO_2	459	700	53± 8	102±2	77.4
N_2	508	545	7±15	98±6	78.0
air + CAP	509	587	15	102	61.0
2800 μmol m^{-2} s^{-1}					
air	478	646	35± 7	102±5	64.9
air minus O_2	529	563	6±11	102±4	76.2
air minus CO_2	512	937	83±14	107±2	63.3
N_2	460	616	34± 2	112±2	74.4

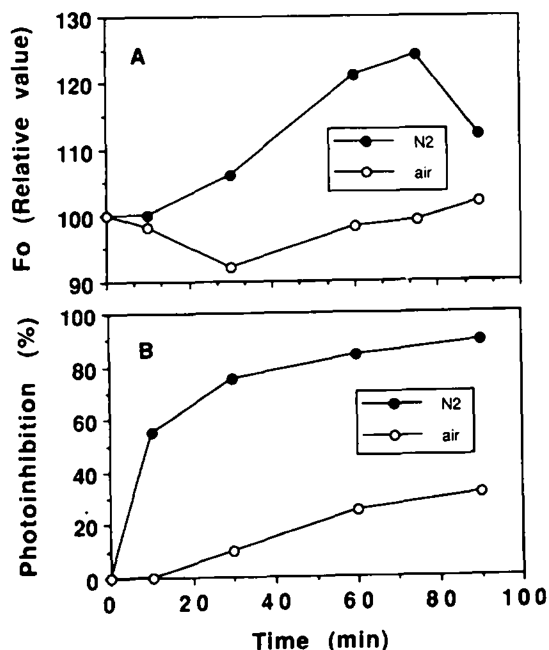

Fig. 3. Photodamage occurred earlier than the rise in initial fluorescence (F_0). Plants were photoinhibited under a PFD of 2800 μmol m^{-2} s^{-1} in air or pure N_2. F_0 (A) and net photosynthetic rate (B) were measured at the times indicated and are expressed as percentages of photoinhibition (B).

decrease in 77 K fluorescence emission at 692 nm (Table II), which is thought to be fluorescence from the photoactive pheophytin *a* that functions as an electron acceptor in RC II [33].

The lowered photosynthetic rate (Fig. 1) and PS II-dependent electron transport (Table I) in N_2 could partially result from impaired energy transfer from the

TABLE IV

Effect of increased actinic light intensity on rate of PS II electron transport after photoinhibition

The plants were photoinhibited in the same conditions as in Table I. PS II electron transport was measured in the actinic light as indicated.

PFD used for measurement	PSII activity ($H_2O \rightarrow$ DCIP, μmol (mg Chl)$^{-1}$ h^{-1}				
	control	photoinhibition			
		air	air minus O_2	air minus CO_2	N_2
560 μmol m^{-2} s^{-1}	240	237	213	135	140
2200 μmol m^{-2} s^{-1}	268	237	232	182	152

antenna to the reaction centre of PS II. This interpretation would be consistent with the observed rise of F_0 (Table III and Fig. 3) if the latter is caused by partial detachment of antenna pigments from the RCs [39]. In this case, the decreased photosynthetic rate in N_2 (Fig. 1 and Table I) could be reversed by increasing the actinic light intensity. To test this, we measured PS II electron transport under the actinic light intensities below and above saturation. Table IV shows that after the plants were illuminated by high light in N_2, the degree of photoinhibition of electron transport could not be improved even if the measuring light intensity was increased by 5 times. This indicates that energy transfer from the antenna to the reaction centre of PS II is unlikely to have impaired in these experiments.

Photoinactivation without photodamage occurred under high PFD in atmospheres containing CO_2. The

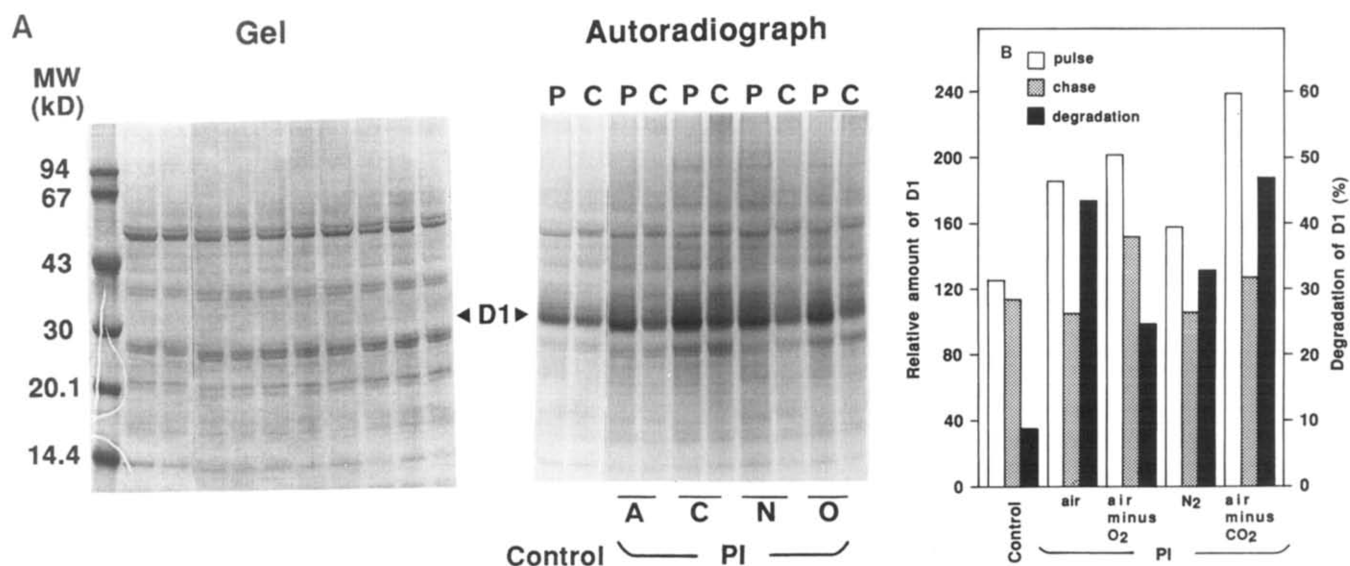

Fig. 4. D1 degradation in vivo was accelerated by presence of O_2 and absence of CO_2. Plants were pulse-labeled with [^{35}S]methionine for 20 min (P) and chased for 60 min (C) under PFD of 1500 μmol m^{-2} s^{-1} in air (A), air minus O_2 (C), air minus CO_2 (O) and pure N_2 (N). D1 is quantified by means of a densitometric scan from Panel A, and the result is shown in Panel B. 'Relative amount of D1' refers to area of the peaks of D1 obtained from the scan.

rate of CO_2 fixation (Fig. 1) and PS II activity (Tables I and II) decreased by a lesser degree during photoinactivation than during photodamage. Fluorescence relaxation following a single turnover flash (Fig. 2) showed that the rate of electron flow from Q_A^- to Q_B was inhibited by 26% as represented by the $t_{1/2}$ after the plants were illuminated for 90 min under PFD of 2800 μmol m^{-2} s^{-1} in air (Table III). This inhibition was largely lowered in air minus O_2 (Fig. 2 and Table III). These results indicated that the photoinactivation is O_2 dependent and largely due to the inactivation of Q_B-site of the D1 protein. Pulse-chase experiments showed that during the photoinactivation period of only 60 min, the RC II D1 protein degraded in air by more than 40% (Fig. 4). Thus D1 degradation was more rapid than inhibition of electron transfer to Q_B (Table III). D1 degradation was accelerated by presence of O_2 and absence of CO_2 (Fig. 4). D1 degradation without loss of electron transfer to Q_B could result from efficient D1 synthesis and processing [20,40], since loss of Q_B reduction was increased by chloramphenicol which is an inhibitor of protein synthesis (Fig. 2 and Table III).

Discussion

Based on studies in vivo, our results indicate that multiple sites are involved in a photodamage process that is induced by light in CO_2- and O_2-free atmospheres. The inactivated sites include the RC II components from P680 to Q_A, the Q_B site, and a component of PS I. The donor side of PS II and excitation energy transfer within PS II seem to be unimpaired. We suggest that the photodamage results from the over-reducing condition and depletion of bicarbonate. Bicarbonate is essential for keeping the D1-D2 reaction centre functional. In the D1 protein, one of the HCO_3^--binding sites possibly locates at the pairs of Arg-257 and His-252 [41]. In our experiments, when pure N_2 was introduced into the chamber containing the plants, internal CO_2 and O_2 could be extremely low. This may have caused partial removal of bicarbonate from its D1 binding site, since in *Lemna* the thylakoid has a relatively low pH under physiological condition, and this favours the dissociation of HCO_3^- to CO_2 [41]. High light may promote the removal of bicarbonate, since high light drives CO_2 assimilation. The removal of bicarbonate results in inactivation of quinone reactions [42]. Moreover, it may also lead to inactivation of another component, as indicated in the present work, located between P680 and Q_A^-, due to disruption of the PS II complex after bicarbonate removal.

We suggest that photodamage is a process distinct from photoinactivation. Photoinactivation occurs in air and its site of action is generally agreed to lie within PS II. At the early stage of photoinactivation in vivo, high light drives PS II charge separation at high rates and results in over-reduction of the PQ pool due to rate limitation of NADPH oxidation and CO_2 assimilation. When the PS II is gradually inactivated, the PQ pool becomes gradually oxidised due to a rate limitation of PS II electron flow [1]. A high turnover and high ratio of PQ/PQH_2 favour the breakdown of D1 [7,43]. In contrast, photodamage is favoured by reducing conditions and by absence of bicarbonate from the reaction centres, when the PQ pool is kept in a fully reduced state. F_0 rises during photodamage but not during photoinactivation (Table III and Fig. 3). During photoinactivation, reducing conditions may be transient, and thus insufficient to cause a rise in F_0. Photoinactivation is strongly temperature-dependent and is enhanced at low temperatures [9,25,44]. In contrast, photodamage is relatively temperature-independent [9,25] but the following recovery strongly depends on the temperature used during photodamage [25]. High temperature during photodamage inhibits subsequent recovery [25]. Moreover, oxygen alleviates photodamage but aggravates photoinactivation (Fig. 1, 2 and Tables I, III). From this point of view the contradictory effects of O_2 on photoinhibition may be explained.

Under favourable conditions, photodamage does not occur [13] because a series of protective mechanisms may function [1]. Presence of atmospheric CO_2 enables carbon assimilation to use the excess energy produced under high light. In the presence of O_2, the Mehler reaction [14], photorespiration [13], thylakoid energization [39,45] and operation of the zeaxanthin cycle [46] may enable the organism to dissipate the excess absorbed light energy. In these cases an apparent non-photochemical fluorescence quenching may be observed [45]. Moreover, during photoinhibition in air PS I cyclic electron transport may be gradually activated, resulting in greater thylakoid energisation [47,48]. This may prevent further photodamage. In pure N_2 these protective mechanisms are minimised and the excess, undissipated energy may inactivate or damage multiple sites within the photosystems.

It has been reported that light-dependent D1 degradation requires the presence of oxygen at the acceptor site [1,21]. A fast turnover and a high ratio of $PQ1PQH_2$ in the PQ pool promotes the breakdown of D1 during photoinhibition [7,43]. In CO_2- and O_2-free conditions, the rate of O_2 evolution and the O_2 concentration are extremely low, and the PQ pool is over-reduced in high light. However, D1 is then degraded rapidly (Fig. 4) and Q_B reduction is inactivated. We suggest that the rapid degradation of D1 is a result of removal of bicarbonate from the HCO_3^--site of D1. One important function for this site is protonation of Q_B^{2-} or Q_B^- [41]. The accelerated D1 degradation and Q_B-site inactivation (Table III and Fig. 4) may result from the pres-

ence of long-lived Q_B^{2-} or Q_B^- which cannot be protonated.

The mechanisms of inactivation of PS I (especially light-limited, Tables I and II) during photodamage require further investigation.

Acknowledgements

This work was supported by grants from the Norwegian Research Council for Science and the Humanities (NAVF) and the Norwegian Agricultural Research Council (NLVF). We are grateful to I. Ohad and O. Prasil for the valuable discussions. We thank N.F. Tsinoremas and Ø. Norby for help with 77 K fluorescence and PS I electron transport respectively.

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