Photoinhibition of Electron Transport Activity of Photosystem II in Isolated Thylakoids Studied by Thermoluminescence and Delayed Luminescence

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The effect of photoinhibition on the primary (Q_A) and secondary (Q_B) quinone acceptors of photosystem II was investigated in isolated spinach thylakoids by the methods of thermoluminescence and delayed luminescence. The amplitudes of the Q (at about 2 °C) and B (at about 30 °C) thermoluminescence bands which are associated with the recombination of the $S_2Q_A^-$ and $S_2Q_B^-$ charge pairs, respectively, exhibited parallel decay courses during photoinhibitory treatment. Similarly, the amplitudes of the flash-induced delayed luminescence components ascribed to the recombination of $S_2Q_A^-$ and $S_2Q_B^-$ charge pairs and having half life-times of about 3 s and 30 s, respectively, declined in parallel with the amplitudes of the corresponding Q and B thermoluminescence bands. The course of inhibition of thermoluminescence and delayed luminescence intensity was parallel with that of the rate of oxygen evolution. The peak positions of the B and Q thermoluminescence bands as well as the half life-times of the corresponding delayed luminescence components were not affected by photoinhibition. These results indicate that in isolated thylakoids neither the amount nor the stability of the reduced Q_B acceptor is preferentially decreased by photoinhibition. We conclude that either the primary target of photodamage is located before the Q_B binding site in the reaction center of photosystem II or Q_A and Q_B undergo simultaneous damage.

Introduction

Photosynthetic organisms exposed to higher light intensity than that required to saturate photosynthesis gradually loose their photosynthetic capacity. The phenomenon is called photoinhibition [1, 2]. There is a general agreement that photoinhibition is related to a damage in photosystem II (PS II) [1–12]. However, the opinions differ concerning the exact site of photodamage in the electron transport chain. Works using algal cells as experimental object suggest that the inhibitory damage occurs at the level of the Q_B binding protein [3–6]. On the other hand the majority of experiments performed with isolated chloroplasts, thylakoids or PS II membrane preparations advocate a primary site of photoinhibition in the P_{680}-Pheo-Q_A section of the electron transport chain in the reaction center complex of PS II [7–12]. Exceptions of this generalization are the reports of Ohad *et al.* [13, 14].

Recently thermoluminescence (TL) and delayed luminescence (DL) proved to be useful methods in the investigation of PS II photochemistry. In the glow curve of thylakoids, peaks appearing at around 2 and 30 °C are ascribed to $S_2Q_A^-$ and $S_2Q_B^-$ charge recombinations, respectively [15, 16]. Similarly, the decay of delayed luminescence components with half life-times of about 3 and 30 s are also attributed to the $S_2Q_A^-$ and $S_2Q_B^-$ recombinations, respectively [17, 18]. Thus it can be expected that the effect of photoinhibition on the Q_A and Q_B acceptors can be easily followed by the application of thermoluminescence and delayed luminescence. Recent thermoluminescence investigation of photoinhibition in *Chlamydomonas reinhardii* cells led to the conclusion that in the first stage of photoinhibition the Q_B binding site is modified while the Q_A acceptor is only slightly influenced [19]. Considering that in isolated thylakoids the process of photoinhibition may differ from that occurring in intact cells we carried out ther-

Abbreviations: PS II, photosystem II; Q_A, primary quinone acceptor of PS II; Q_B, secondary quinone acceptor of PS II; Chl, chlorophyll; P_{680}, reaction center chlorophyll of PS II; Pheo, pheophytin; HEPES, N-2-hydroxy-ethyl-piperazine-N′-ethane sulfonic acid; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; DMQ, 2-5-dimethyl-*p*-benzoquinone.

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moluminescence and delayed luminescence measurements of photoinhibited spinach thylakoids.

The results provide evidence that *in vitro* inhibition of the light-induced reduction of Q_A and Q_B acceptors follows exactly the same time course during photoinhibition. This observation suggests that in contrast to algal cells, the Q_B protein in isolated thylakoids is not the specific primary site of photoinhibition. The electron transport chain is modified at a site between P_{680} and Q_A or Q_A and Q_B are simultaneously damaged under the photoinhibitory treatment.

Materials and Methods

Thylakoids were isolated from market spinach as described in [20] and suspended in a medium composed of 50 mM HEPES (pH 7.5)/5 mM $MgCl_2$/10 mM NaCl/0.4 M sorbitol to give 1–2 mg Chl/ml and stored on ice until use. For photoinhibitory treatment samples were diluted with the suspension medium to 100 μg Chl/ml and illuminated in a flat Petri dish for a period of time ranging from 5 to 60 min. White light with an incident intensity of 500 W/m^2 was provided by a 650 W Narva halogen lamp. Throughout the photoinhibitory treatment samples were continuously stirred and a constant temperature of about 4 °C was maintained. Before measurements samples were diluted further with the suspension medium as indicated later.

Thermoluminescence was measured as previously described [16]. Single-flash excitation was given either at 5 °C or at −20 °C (for DCMU-treated samples). After quick cooling glow curves were recorded from −40 °C to 80 °C at a heating rate of 20 °C/min.

For delayed luminescence measurements samples were diluted to 30 μg Chl/ml. The single-flash induced delayed light emission was detected at 20–25 °C as in [18]. The amplified photomultiplier signal was stored in a multichannel analyzer (model ICA 70, Central Res. Int. Phys., Budapest, Hungary) connected to a small computer (Commodore-64). Delayed luminescence curves were resolved into exponential components with an IBM AT personal computer using a least-squares curve-fitting program.

The rate of photosynthetic oxygen evolution was measured at saturating light intensities using a Clark-type electrode at 25 °C. The assay medium contained 0.1 M sorbitol/10 mM K_2HPO_4/20 mM NaCl/14 mM $MgCl_2$/2 mM EDTA/50 mM HEPES (pH 7.5)/1.5 mM DMQ and thylakoids resulting 50 μg Chl in a final volume of 3 ml [21].

Fluorescence induction was measured at 10 μg Chl/ml as previously described [22].

Results

Untreated isolated spinach thylakoids excited by a saturating flash at 5 °C exhibited a TL band at about 28 °C (Fig. 1, left side). This band can be accounted for by $S_2Q_B^-$ charge recombination [15, 16]. In the presence of DCMU, the B band was replaced by a band at about 2 °C (Fig. 1, right side). This band arises from $S_2Q_A^-$ recombination and designated the Q or D band [15, 16]. When thylakoids were exposed to higher light intensity (500 W/m^2) than that required to saturate photosynthesis for various periods

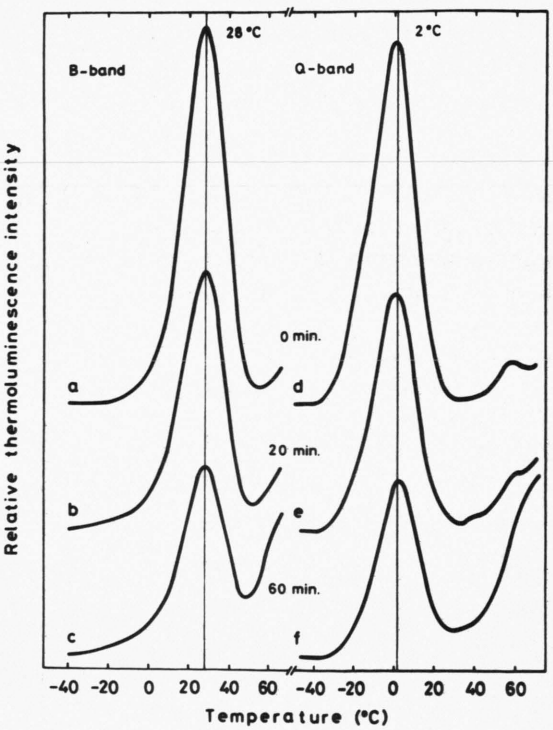

Fig. 1. Changes in thermoluminescence characteristics of isolated thylakoids during photoinhibition. Samples were illuminated with 500 W/m^2 white light for 0, 20 and 60 min. The B and Q thermoluminescence bands were excited by a single flash in the absence (curves a, b, c) and presence (curves d, e, f) of 1 μM DCMU, respectively. Before thermoluminescence measurements thylakoids were dark-adapted for 5 min at 25 °C. All samples contained 30% glycerol.

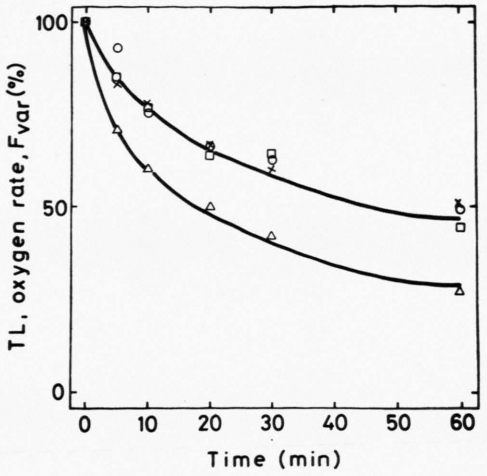

Fig. 2. Time course of photoinactivation of PS II activity in thylakoids exposed to 500 W/m^2 at 4 °C. ○, intensity of the B TL band; □, intensity of the Q TL band measured in the presence of 1 μM DCMU; x, rate of oxygen evolution measured in the presence of 1.5 mM DMQ as electron acceptor; △, relative variable fluorescence ($(F_{max}-F_o)/F_o$). The initial rate of oxygen evolution was 110 μM O_2/mg Chl/h.

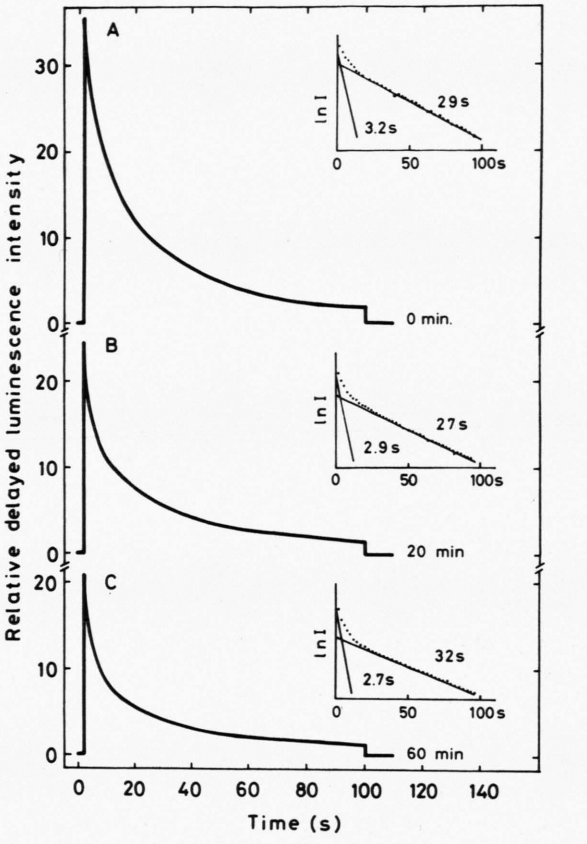

Fig. 3. Effect of photoinhibition on delayed luminescence characteristics. Single-flash induced delayed light emission was measured in thylakoids photoinhibited for various periods of time: A, 0 min; B, 20 min; C, 60 min. Experimental conditions for photoinhibition were the same as indicated in Materials and Methods and in Fig. 1. The insets show the resolved exponential components with half life-times.

of time the amplitude of the B and Q bands gradually decreased (Fig. 1). The decrease of the emission intensities of the B and Q bands followed exactly the same time course during photoinhibition (Fig. 2). Although the intensity of the B and Q bands diminished considerably during photoinhibition the peak positions and consequently the half life-times of the two bands remained constant (Fig. 1). The light-saturated electron transport rate of PS II measured from H_2O to dimethylbenzoquinone decreased by the same extent as the amplitude of the B and Q bands (Fig. 2). However, the loss of the variable fluorescence ($(F_{max}-F_o)/F_o$) which is a generally used test of photoinhibitory damage to photosynthetic organelles, ran ahead of the inhibition of the electron transport rate (Fig. 2). This observation is consistent with previous reports [6, 9, 12].

Delayed luminescence originates from the same charge recombination mechanism as thermoluminescence. Accordingly, delayed luminescence investigation of photoinhibited spinach thylakoids correlated well with the thermoluminescence results. In the decay of delayed luminescence three components could be distinguished in the seconds to minutes time scale (Fig. 3 and 4). The slowest component with a half life-time of 28–30 s (Q_B component), corresponds to the B thermoluminescence band and originates from $S_2Q_B^-$ recombination [17, 18]. As expected, this component was abolished in the presence of DCMU (Fig. 4). The second component, decaying with a half life-time of approximately 3 s, was intensified in DCMU-treated thylakoids. This component is attributed, like the Q thermoluminescence band, to $S_2Q_A^-$ charge recombination [17, 18] and designated in this paper as the Q_A component. In the presence of DCMU, a delayed luminescence component with a half life-time of about 500 ms was also observed (Fig. 4). The origin of this component is not clarified yet but it probably arises from the recombination of Q_A^-, or an other acceptor located before the DCMU action site, with an unspecified donor of PS II. Fig. 5

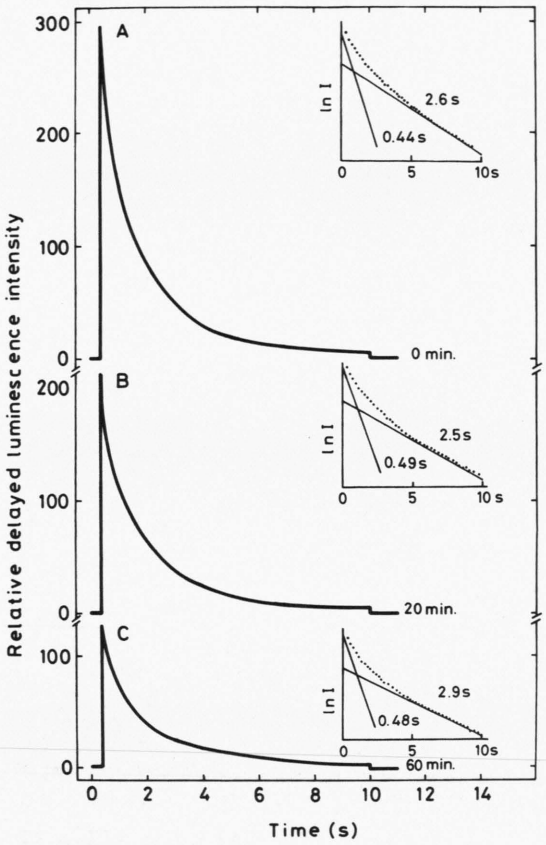

Fig. 4. Effect of photoinhibition on delayed luminescence characteristics measured in the presence of DCMU. 1 μM DCMU was added to samples previously photoinhibited for various periods of time as indicated in Fig. 3.

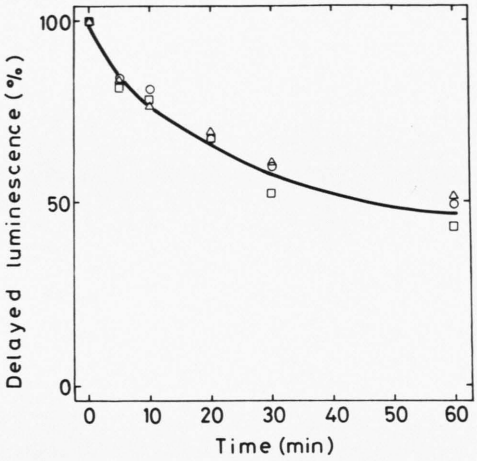

Fig. 5. Time course of photoinhibition of delayed luminescence components in thylakoids. Amplitudes of exponential components obtained from the resolution of decay curves are plotted as a function of photoinhibition time. ○, Q_B component ($t_{1/2} \simeq 30$ s); □, △, Q_A components ($t_{1/2} \simeq 3$ s) measured with or without 1 μM DCMU, respectively.

shows the decrease of the amplitudes of delayed luminescence components as a function of photoinhibitory time. The decay course of the Q_B and Q_A component was parallel with each other and also with those of the B and Q thermoluminescence bands (compare with Fig. 2). It is important to note that the Q_A component obtained from delayed luminescence curves measured either with or without DCMU decayed identically (Fig. 5). Corresponding to the constant peak positions of the Q and B thermoluminescence bands, the half life-times of the Q_A and Q_B delayed luminescence components did not change with the duration of the photoinhibitory treatment (compare Fig. 3 and 4).

Discussion

According to our present knowledge, the PS II reaction center complex consists of a heterodimer composed of D_1 and D_2 subunit polypeptides and cytochrome *b*-559. D_1 provides binding site for Q_B while Q_A is very probably located on D_2 [23–26]. Investigations performed with algal cells led to the conclusion that the primary effect of photoinhibitory attack is a damage of the Q_B binding site (on the D1 protein) [3–6]. At variance with this conclusion experiments carried out with isolated chloroplasts, thylakoids or PS II membranes suggested that the primary photodamage impairs electron transfer at the level of Pheo or Q_A (on the D2 protein) [7–12]. Taking the advantage of TL in studying the effect of photoinhibition on the Q_A and Q_B acceptors Ohad *et al.* [19] recently carried out a detailed photoinhibitory study of *Chlamydomonas reinhardii* cells. They found that in the first stage of photoinhibition the Q_B binding site was modified even by moderate light irradiances. Exposure of the cells to a light intensity of 500 W/m^2 resulted in a shift of the B thermoluminescence band from 30 °C to 15–17 °C (B^1 band). The appearance of this modified B band has been attributed to a change in the conformation of the D_1 protein which caused a destabilization of the $S_2Q_B^-$ state. This destabilization of the $S_2Q_B^-$ charge recombination was accompanied by a reduction in the half life-time of the B^1 TL band to approximately half of that of the original B signal. The amplitude of the TL band ascribed to the Q_A acceptor was de-

creased only by 30–40% under similar photoinhibitory treatment. These results can hardly be reconciled with the observations obtained in the present work in isolated thylakoids. In spinach thylakoids the peak position of the B band did not change during photoinhibition (Fig. 1). Its amplitude gradually decreased but its half life-time remained constant. In contrast to the result observed on *Chlamydomonas* cells, the diuron type inhibitor, DCMU could bind to the thylakoid membranes until the B band was completely abolished (data not shown). The binding of the herbicide resulted in the disappearance of the B band with a concomitant appearance of the Q band at lower temperature. The most surprising observation was that the decrease in the amplitude of the Q and B bands followed exactly the same time course during photoinhibition, indicating that the reduction of the Q_B and Q_A acceptors is simultaneously retarded by photoinhibitory treatment.

The parallel time courses of inhibition of the amplitudes of DL components substantiated the TL observation that the reduction of Q_B and Q_A acceptors is simultaneously diminished during photoinhibition. The constant values of the half life-times of delayed luminescence components also contradict any preferential photo-induced change in the redox state of the Q_B acceptor in the first stage of photodamage.

The assumption that inhibition occurred at the water-splitting site of PS II would explain a parallel decline in the reduction of Q_A and Q_B acceptors. However, the well documented observation that artificial electron donors can not restore the loss of variable fluorescence during photoinhibition [1–3, 10] excludes this possibility.

On the basis of our TL and DL measurements, according to which the amounts of reduced Q_A and Q_B acceptors follow parallel decay courses, we conclude that in isolated thylakoids the Q_B binding site is not the primary target of photoinhibition. We propose two alternatives to reconcile the data presented here and reported previously in the literature.

I. Photoinhibition impairs electron transfer at a site in the Z-P_{680}-Pheo-Q_A section of the electron transport chain. Our methods do not make possible a more precise localization of the site of action. However, based on data in recent literature [9, 12] a damage at Q_A or between Pheo and Q_A would be the most probable.

II. It was suggested that the primary cause of photoinhibition is the light-induced accumulation of the reactive quinone species Q_B^{2-} which damages the Q_B binding site [3]. The accumulation of the reduced form of the primary quinone acceptor (Q_A^-) might be responsible for a similar damage to the Q_A binding site. In isolated thylakoids and PS II preparations which do not have CO_2 fixation capability high light intensity reduces fully all of the acceptor pools. Due to the equal amount of permanently reduced Q_B and Q_A both the Q_B and Q_A binding sites are damaged to the same extent. In intact algal cells electrons are continuously drained off the plastoquinone pool towards PS I resulting in a partially reduced electron transport chain *i.e.* there is a smaller population of reduced Q_A than that of Q_B. This can explain the slower photodamage of the Q_A binding site compared to that of Q_B observed in algal cells [6, 19].

We prefer the second alternative because it can explain the primary photodamage to the Q_B binding site in algae cells as well as the parallel impairment of Q_A and Q_B reduction in isolated thylakoids.

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