

# Thermoluminescence and Delayed Luminescence Characterization of Photosystem II<sub>α</sub> and Photosystem II<sub>β</sub> Reaction Centers

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Z. Naturforsch. **43c**, 596–600 (1988); received May 2, 1988

Photosynthesis, Photosystem II, Thermoluminescence, Delayed Luminescence

The thermoluminescence and delayed luminescence characteristics of PS II<sub>α</sub> and PS II<sub>β</sub> centers were investigated in BBY particles and stroma thylakoids, respectively. The BBY particles exhibited a thermoluminescence band at 25 °C (B band) which was associated with the charge recombination of the S<sub>2</sub>Q<sub>B</sub><sup>−</sup> redox couple and underwent period-2 oscillation in a sequence of flashes. In the flash-induced decay of delayed luminescence of BBY particles a component with a half-time of 34 s corresponded to the B thermoluminescence band and was also assigned to S<sub>2</sub>Q<sub>B</sub><sup>−</sup> charge recombination. No corresponding thermoluminescence or delayed luminescence components associated with the secondary acceptor Q<sub>B</sub> could be observed in the glow curve or delayed luminescence decay of stroma thylakoids. These observations indicate that unlike PS II<sub>α</sub> the PS II<sub>β</sub> centers are not associated with the two-electron gate, Q<sub>B</sub>.

## Introduction

An increase in fluorescence intensity during the excitation of DCMU-treated chloroplasts by continuous light reflects the gradual reduction of the primary quinone, Q<sub>A</sub>. The rise of the fluorescence induction curve does not follow a first order kinetics. Analyzing the area growth over the fluorescence induction curve Melis and Homann [1] found two kinetically distinct phases: a rapid sigmoidal phase superimposed with a slower exponential tail. The two phases have been attributed to two different PS II reaction centers, designated PS II<sub>α</sub> and PS II<sub>β</sub> [2]. It has been determined that the two types of PS II reaction center complexes differ in several respects. The antenna size of PS II<sub>α</sub> units is larger and the excitation energy can migrate between the centers [3]. On the other hand the PS II<sub>β</sub> units are separate entities [4]. The two types of PS II reaction centers also differ in their location: PS II<sub>α</sub> is localized in the granal membranes [5] or in BBY-type [6] PS II preparations [7] while PS II<sub>β</sub> is found in the stroma-thylakoid fraction of

chloroplasts [5]. The primary quinone acceptors of PS II<sub>α</sub> and PS II<sub>β</sub> centers are termed Q<sub>α</sub> and Q<sub>β</sub>, respectively. It has been found that Q<sub>β</sub> has a more positive redox potential than Q<sub>α</sub> [8–10]. In addition Q<sub>β</sub> does not reduce plastoquinone via a two-electron gating mechanism [10]. It has been suggested recently that PS II<sub>β</sub> is a precursor form of PS II<sub>α</sub>. It has not the peripheral chlorophyll a/b light-harvesting antenna and a connection to the plastoquinone pool [11].

In recent years thermoluminescence proved to be a useful method in the investigation of PS II photochemistry. A thermoluminescence peak is the result of charge recombination between a positively charged donor and a negatively charged acceptor of PS II. It has been well established that S<sub>2</sub>Q<sub>A</sub><sup>−</sup> and S<sub>2</sub>Q<sub>B</sub><sup>−</sup> recombinations are responsible for the thermoluminescence peaks appearing at around 10 and 30 °C, respectively [12, 13]. Moreover, the peak associated with Q<sub>B</sub><sup>−</sup> exhibits a period two oscillation as a function of exciting flash number [13] reflecting the oscillation of the redox state of the secondary acceptor, Q<sub>B</sub>. The peak position of a thermoluminescence peak strongly depends on the redox potential of the interacting components. Consequently, the thermoluminescence method could provide information on the redox potentials of Q<sub>α</sub> and Q<sub>β</sub> as well as on the relationship between PS II<sub>β</sub> and the plastoquinone pool.

In the present work thermoluminescence and delayed luminescence investigations substantiate the fluorescence results of Thielen and van Gorkom [10]

**Abbreviations:** DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; HEPES, N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid; PS, Photosystem; Q<sub>A</sub>, primary quinone electron acceptor of photosystem II; Q<sub>B</sub>, secondary electron acceptor of photosystem II.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen  
0341-0382/88/0700-0596 \$ 01.30/0



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according to which the PS II<sub>β</sub> centers are not associated with the two-electron acceptor, Q<sub>B</sub>.

## Materials and Methods

Chloroplasts were isolated from spinach as previously described [14]. Photosystem II sub-chloroplast particles were obtained by a modification of the procedure of Berthold *et al.* [6]. Chloroplasts were resuspended in 50 mM Hepes (pH 7.5)/15 mM NaCl/5 mM MgCl<sub>2</sub> and incubated with Triton X-100 (Triton/Chl = 25:1 w/w) at 0 °C for 30 min. Triton incubation of the thylakoids was carried out at a chlorophyll concentration of 2 mg/ml. The membranes of the grana partition region were precipitated by centrifugation at 40000 × g for 30 min and kept at -70 °C until use. In preparation for thermoluminescence and delayed luminescence measurements the BBY particles were resuspended in a medium containing 50 mM Hepes (pH 7.5)/0.4 M sucrose/15 mM NaCl/5 mM MgCl<sub>2</sub>. Stroma thylakoid membranes were isolated according to the procedure of [15] except that French press treatment replaced Yeda press treatment before differential centrifugation [16]. The 100000 × g fraction consisted of stroma thylakoids and had a Chl *a*/Chl *b* ratio of 6.3. Chlorophyll concentrations were determined in 80% acetone using the procedure of Arnon [17]. Ferricyanide treatment of BBY particles was carried out in the presence of 50 µg K<sub>3</sub>Fe(CN)<sub>6</sub>/ml at 0 °C for 60 min at a chlorophyll concentration of 150 µg/ml. After incubation the BBY particles were washed twice in the measuring buffer.

Thermoluminescence was measured using an apparatus previously described [18]. The samples were excited by xenon flashes (General Radio, Stroboslave, 3 µs, 0.5 J) at -15 °C. After excitation the samples were quickly cooled to -50 °C. Thermoluminescence measurements were performed at a heating rate of 20 °C/min.

Delayed luminescence was excited in a 1 cm cell. The emitted light was detected by a photomultiplier (EMI 9558b) situated at right angle to the exciting light source. The emitted delayed luminescence was observed by opening of an Uniblitz shutter 80 ms after the flash. The photomultiplier signal was amplified by a homemade differential amplifier, stored in a multichannel analyzer and plotted on a X-Y recorder. Delayed luminescence decays were resolved into exponentials from semilogarithmic plots. The PS

II<sub>α</sub> and PS II<sub>β</sub> contents of the BBY and stroma thylakoid membranes were determined from the semi-logarithmic plot of the area growth over the fluorescence induction curves.

## Results and Discussions

To compare the thermoluminescence and delayed luminescence characteristics of PS II<sub>α</sub> and PS II<sub>β</sub> centers BBY particles and stroma thylakoids were isolated. It has been shown that BBY particles are highly enriched in PS II<sub>α</sub> reaction centers [7]. On the other hand stroma thylakoids isolated from the nonappressed regions of spinach chloroplast membrane contain mainly PS II<sub>β</sub> centers [5]. The PS II<sub>α</sub> and PS II<sub>β</sub> ratio was determined in the BBY and stroma thylakoid preparations from the semilog plot of the growth of the area over the induction curve. It was found that in the BBY particles PS II<sub>α</sub> accounts for about 85% of the total PS II reaction centers while PS II<sub>β</sub> makes up the remainder. In the stroma thylakoid fraction 85% of the reaction centers is of the PS II<sub>β</sub> type. These data are in good agreement with the PS II<sub>α</sub> and PS II<sub>β</sub> content of similar thylakoid preparations [5, 7]. The glow curve of BBY particles exhibits a peak at around 25 °C (Fig. 1). It has been

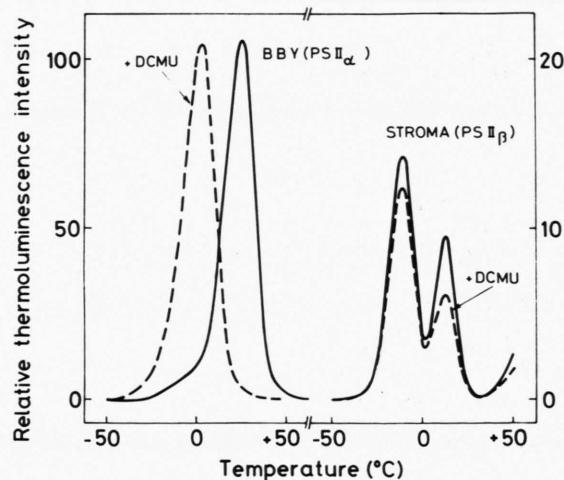


Fig. 1. Thermoluminescence glow curves of untreated (—) and 10 µM DCMU-treated (---) BBY particles and stroma thylakoids. The measuring buffer contained 50 mM Hepes (pH 7.5)/0.4 M sucrose/15 mM NaCl/5 mM MgCl<sub>2</sub>. The PS II<sub>α</sub>:PS II<sub>β</sub> ratios were 85:15 and 15:85 in BBY and stroma samples, respectively. Thermoluminescence was excited by a xenon flash at -15 °C. After excitation the samples were quickly cooled to -50 °C and measured at a heating rate of 20 °C/min.

demonstrated that the so-called B band appearing at 30 °C in the glow curve of whole chloroplasts originates from charge recombination of the  $S_2Q_B^-$  redox couple [12, 13]. On the basis of the similar peak position it can be assumed that the thermoluminescence peak of BBY particles is also associated with  $S_2Q_B^-$  recombination. This assumption is further supported by the effect of DCMU on the 25 °C thermoluminescence peak. Following DCMU treatment the B band, as in whole chloroplasts, is replaced by a peak at about 5 °C. In the literature this peak is designated as Q or D band and it can be accounted for by  $S_2Q_A^-$  charge recombination [12, 13]. In the BBY particles the amplitude of the B band oscillates with a periodicity of four as a function of exciting flash number [19]. The period four oscillation demonstrates that the BBY particles possess an active water-splitting system which can undergo the various S state transitions [19]. This was also confirmed by polarographic measurements. The electron transport rate measured from water to pBQ was approximately 800  $\mu\text{M O}_2/\text{mg Chl/h}$  in the BBY sample. It has been published that the oscillatory pattern of thermoluminescence in whole chloroplasts greatly depend on the oxidation state of the secondary acceptor pool [13, 20]. When the  $Q_B$  pool is oxidized the period-4 oscillation of the thermoluminescence intensity is converted into a period-2 oscillation. The period-2 oscillation can also be induced in BBY particles treated with ferricyanide according to the procedure of

Robinson and Crofts [21]. This treatment almost completely oxidizes the  $Q_B$  pool and the period-2 oscillation of the B band reflects the amount of  $Q_B^-$  as a function of flash number. After an uneven number of flashes a considerable amount of  $Q_B$  is in the single reduced state and can undergo charge recombination. On the other hand after an even number of flashes the doubly reduced  $Q_B^{2-}$  is rapidly reoxidized to  $Q_B$  by the plastoquinone pool and does not participate in a charge recombination reaction. The damping of the period-2 oscillation indicates a limited size of available plastoquinone pool (Fig. 2). The fact that the BBY particles exhibit the B thermoluminescence band which oscillates with a periodicity of two proves that in the PS II $_\alpha$  centers the primary quinone,  $Q_A$  is connected to the plastoquinone pool *via* the two electron gate secondary quinone molecule,  $Q_B$ . This result is in agreement with earlier observations [10, 22].

In the stroma lamellae only PS II $_\beta$  and PS I centers are located. In the glow curve of stroma thylakoid fragments the B band does not appear. However, the Q band can be observed at about 10 °C and a low temperature band of unknown origin at -10 °C. This suggests that in the PS II $_\beta$  centers the electrons cannot proceed from  $Q_A$  to  $Q_B$  and accumulate on the primary quinone molecules. Consequently on DCMU addition the peak position of the Q band did not change and its amplitude was independent of the excitation flash number (Fig. 2). It can be concluded that the secondary quinone acceptor pool is absent from the stroma membrane region and the PS II $_\beta$  centers are not associated with the two-electron gate,  $Q_B$ . The decrease in the amplitude of the Q band after DCMU addition can be attributed to a secondary effect of DCMU on the reaction centers. The peak arising at -10 °C is probably the  $A_T$  band described earlier [23]. This band was not influenced by DCMU (see Fig. 1). The amplitude of the Q band was considerably smaller in the PS II $_\beta$  than in the PS II $_\alpha$  centers. The explanation of this phenomenon is based on the distribution of antenna chlorophyll in the two different particles. While in the BBY particles all of the chlorophyll  $a$  belong to the PS II $_\alpha$  centers, in the stroma thylakoids about 70% of the total chlorophyll of stroma thylakoids is associated with the PS I complex [24]. The peak temperatures of the Q band in the BBY and stroma particles were about 5 and 10 °C, respectively. The peak position of a thermoluminescence band is determined by the re-

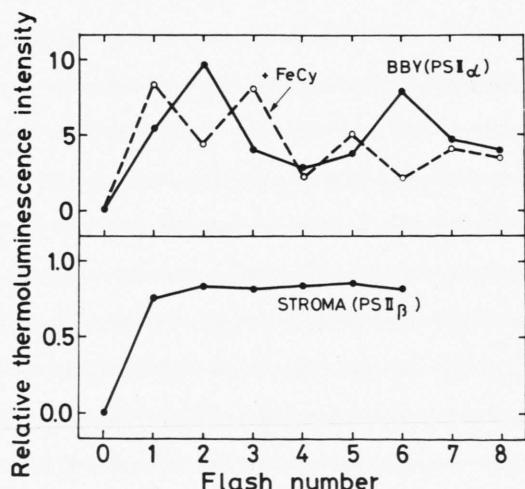


Fig. 2. Oscillation of thermoluminescence intensity at 30 °C in BBY (85% PS II $_\alpha$ ) and at 5 °C in stroma thylakoids (85% PS II $_\beta$ ). Flashes were given at 1 s intervals. Other measuring conditions are indicated in Fig. 1.

dox distance between the interacting donor and acceptor components. The similar peak position of the Q band in the glow curves of BBY and stroma particles suggests that the midpoint redox potentials of the  $Q_\alpha$  and  $Q_\beta$  primary acceptors are approximately the same. This conclusion is at variance with previous results [8, 9] according to which the primary electron acceptor of the PS II $_\beta$  centers,  $Q_\beta$  has a midpoint redox potential considerably higher than that of the PS II $_\alpha$  centers. Two alternatives can be considered to explain this discrepancy. The Q band observed in the glow curve of stroma thylakoids is not associated with  $Q_\beta$  but originates from  $Q_\alpha$  acceptors present as a small percentage in the stroma thylakoids. However, in this case we ought to conclude that in stroma thylakoids the  $Q_\alpha$  centers are not associated with the two-electron gate,  $Q_B$  since the B band can not be observed in the glow curve and the thermoluminescence intensity does not oscillate as a function of flash number (Fig. 2). It can be assumed that during the isolation of stroma thylakoids the PS II $_\alpha$  centers present in a very low amount in the stroma thylakoids lose their  $Q_B$  acceptors and the peak at 10 °C originates from  $Q_\alpha$  originates from  $Q_\alpha$  acceptors. Independently of this possibility a thermoluminescence peak corresponding to the  $Q_\beta$  acceptors should appear in the glow curve. However, no thermoluminescence band was observed in the temperature region extending from -50 to 50 °C which would correspond to  $Q_\beta$  (Fig. 1). This suggests that  $Q_\beta$  does not participate in charge recombination reaction. The second alternative which is not in accordance with previous observations [8, 9] is that the redox potentials of  $Q_\alpha$  and  $Q_\beta$  are approximately the same and  $Q_\beta$  is responsible for the peak at 10 °C.

The delayed luminescence observations are in agreement with the thermoluminescence results. Fig. 3 shows that the flash-induced delayed luminescence of BBY particles can be resolved into three exponential components with half-lives of 0.6 s, 3.2 s and 34 s. The half-times of the two slower components are in good agreement with those of delayed luminescence components observed in whole chloroplasts and attributed to  $S_2Q_A^-$  and  $S_2Q_B^-$  recombinations [25]. In ferricyanide-treated BBY particles the component with half-time of 34 s oscillated with a periodicity of two as a function of flash number (not shown). Consequently, this component is associated with the secondary acceptor,  $Q_B$ . The delayed luminescence results demonstrate that in the PS II $_\alpha$

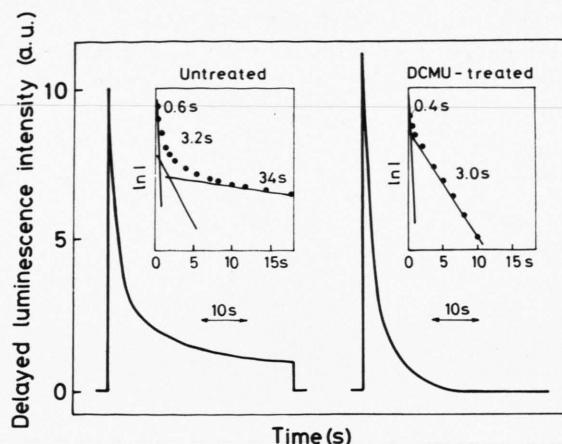


Fig. 3. Delayed luminescence of untreated and 10  $\mu$ M DCMU treated BBY particles (85% PS II $_\alpha$ ). Insets: curve resolutions into exponentials with half-decay times.

centers the electrons are transferred from the primary acceptor to the plastoquinone pool *via* the two-electron gate,  $Q_B$ . The addition of DCMU before flash excitation completely eliminated the slowest component with a concomitant intensification of the two fast ones (Fig. 3). The second component with a half-time of 3 s is related to the primary acceptor,  $Q_A$  [25]. The origin of the fastest component is unknown. In the stroma lamellae only the first two delayed luminescence components could be observed indicating that the PS II $_\beta$  centers are not associated with the secondary quinone acceptor,  $Q_B$  (Fig. 4).

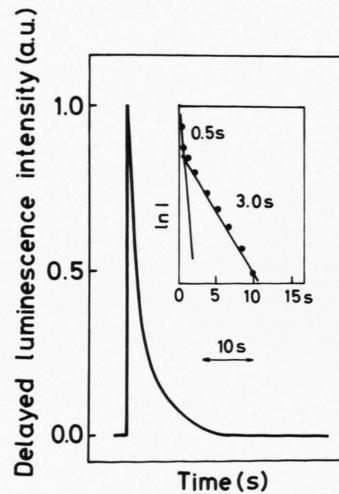


Fig. 4. Delayed luminescence of stroma thylakoid membranes (85% PS II $_\beta$ ). Inset: curve resolution into exponentials.

On the basis of the thermoluminescence and delayed luminescence observations we can say that in the PS II<sub>a</sub> centers the primary quinone acceptor, Q<sub>a</sub> is oxidized *via* the two-electron gate secondary acceptor, Q<sub>B</sub>. However, the PS II<sub>b</sub> centers lack the secondary acceptor, Q<sub>B</sub> and the two-electron gating mechanism.

### Acknowledgements

We thank Dr. I. Vass for the valuable discussions. This work was supported by Research Funds of the Hungarian Academy of Sciences AKA (219/86) and OKKFT Tt(310/86).

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