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CHARGE ACCUMULATION AND RECOMBINATION IN PHOTOSYSTEM II STUDIED BY THERMOLUMINESCENCE

I. PARTICIPATION OF THE PRIMARY ACCEPTOR Q AND SECONDARY ACCEPTOR B IN THE GENERATION OF THERMOLUMINESCENCE OF CHLOROPLASTS

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In the glow curves of chloroplasts excited by a series of flashes at +1°C the intensity of the main thermoluminescence band appearing at +30°C (B band; B, secondary acceptor of Photosystem II) exhibits a period-4 oscillation with maxima on the 2nd and 6th flashes indicating the participation of the S_3 state of the water-splitting system in the radiative charge recombination reaction. After long-term dark adaptation of chloroplasts (6 h), when the major part of the secondary acceptor pool (B pool) is oxidized, a period-2 contribution with maxima occurring at uneven flash numbers appears in the oscillation pattern. The B band can even be excited at −160°C as well as by a single flash in which case the water-splitting system undergoes only one transition ($S_1 \rightarrow S_2$). The experimental observations and computer simulation of the oscillatory patterns suggest that the B band originates from charge recombination of the S_2B^- and S_3B^- redox states. The half-time of charge recombination responsible for the B band is 48 s. When a major part of the plastoquinone pool is reduced due to prolonged excitation of the chloroplasts by continuous light, a second band (Q band; Q, primary acceptor of Photosystem II) appears in the glow curve at +10°C which overlaps with the B band. In chloroplasts excited by flashes prior to DCMU addition only the Q band can be observed showing maxima in the oscillation pattern at flash numbers 2, 6 and 10. The Q band can also be induced by flashes after DCMU addition which allows only one transition of the water-splitting system ($S_1 \rightarrow S_2$). In the presence of DCMU, electrons accumulate on the primary acceptor Q, thus the Q band can be ascribed to the charge recombination of either the S_2Q^- or S_3Q^- states depending on whether the water-splitting system is in the S_2 or the S_3 state. The half-time of the back reaction of Q^- with the donor side of PS II (S_2 or S_3 states) is 3 s. It was also observed that in a sequence of flashes the peak positions of the Q and B bands do not depend on the advancement of the water-splitting system from the S_2 state to the S_3 state. This result implies that the midpoint potential of the water-splitting system remains unmodified during the $S_2 \rightarrow S_3$ transition.

Introduction

The photosynthetic energy-conversion process involves a light-induced separation of positive and negative charges in the reaction-center complex.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; PQ, plastoquinone; PS, Photosystem.

The initial charge-separation is stabilized by a series of electron-transport steps at the two sides of the reaction center. Consequently, positive charges are permanently stored in the water-splitting system.

On the other hand, on the acceptor side of PS II the semireduced form B^- of the secondary acceptor B represents a long-lived trapping state for the negative charge [1,2]. Part of the energy stored during the charge separation process is lost by a thermally induced back-reaction of the accumulated charges leading to radiative charge recombination. The charge recombination gives rise to either delayed luminescence or thermoluminescence depending on the measuring conditions. The equivalence of the two phenomena has recently been demonstrated by Desai et al. [3].

The majority of the available information about the charge recombination process was obtained from delayed luminescence investigation [4–6]. Recently, however, thermoluminescence has emerged as a promising complementary method for studying the backreactions of PS II.

It has been found that the S_4, S_3 and S_2 states of the water-splitting system are involved as oxidized substrates for thermoluminescence [7–10]. Participation of the acceptor side of PS II in the generation of thermoluminescence has not been thoroughly investigated. Inoue and Shibata [8] have assumed that the thermoluminescence characteristics of chloroplasts are determined mainly by the redox state of the donor side of PS II [7,11]. However, recent reports attribute a significant role to the acceptor side of PS II as well in determining the peak positions of the thermoluminescence bands. It has been found that in the presence of various PS II inhibitors the primary acceptor Q contributes to the thermoluminescence [9,12,13]. Furthermore, on the basis of flash experiments it has been concluded that the single-reduced secondary acceptor B^- also participates in the generation of thermoluminescence [9,10].

In order to obtain further information concerning the involvement of the primary and secondary acceptors in the charge recombination process, we extended these studies, and carried out a detailed investigation into the oscillations of the thermoluminescence intensity in chloroplasts, which had been dark adapted for various time periods, and subjected to a series of flashes both in the presence and absence of DCMU.

Materials and Methods

Intact chloroplasts were isolated from maize as described earlier [14]. The suspension of chloroplasts contained 0.4 M D-sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 2 mM EDTA and 50 mM Hepes (pH 7.5) with 100 μg Chl/ml. 0.4 ml aliquots of samples were preilluminated by white light (10 W/m^2) for 30 s at 30°C and dark-adapted for 5 min before thermoluminescence measurements. Thermoluminescence was excited by continuous white light or by xenon flashes (General Radio, Stroboslave, 3 μs, 0.5 J). After excitation of the samples thermoluminescence measurements were performed at a heating rate of 20°C/min by using an apparatus similar to that described in Ref. 15. Pulsed polarographic measurements of oxygen yields in a sequence of flashes were carried out as described in Ref. 16. The rate of oxygen evolution was measured using a Clark-type electrode as reported earlier [17]. Computer analysis of glow curves was carried out as described in Ref. 18.

Results and Discussion

Fig. 1 shows the glow curves of chloroplasts excited at different temperatures by continuous light or by flash illumination. Adopting the terminology used by Inoue and Shibata [8,19] the main thermoluminescence band appearing at about +30°C in the glow curves is denoted as B band in the present paper. It was found that the B band can be excited at very low temperatures (T_{ex} = −60°C and −160°C) not only by continuous light (solid line) but by a single flash as well (dotted curve).

At temperatures lower than −35°C the higher S states cannot be formed and the S_0 and S_1 states are converted only into the S_2 state during light excitation [8].

Consequently, it follows that at low temperature excitation the positive charges responsible for the B band can be ascribed to the S_2 state of the water-splitting system. At the acceptor side of PS II electron transfer is also slowed down by lowering the temperature and as a result electrons accu-

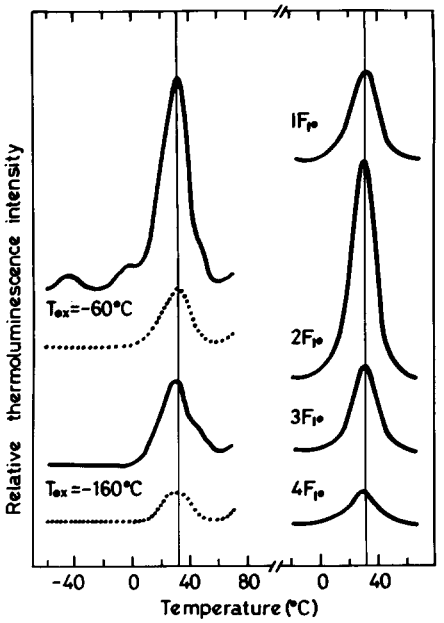

Fig. 1. Glow curves of isolated maize chloroplasts excited under different illumination conditions. Glow curves on the left side were excited by continuous light of 10 W/m^2 for 2 min at −60°C (upper curve) and at −160°C (bottom curve) as indicated by T_{ex} on the curves. Dotted glow curves were excited by a single flash. Glow curves on the right side were excited by various numbers of flashes at +1°C as indicated by F on each curve and cooled quickly down to −40°C before measurement of thermoluminescence.

mulate on Q [20]. By increasing the temperature of the sample during the course of thermoluminescence measurement the electrons gradually proceed from Q to B.

The semireduced form of the secondary acceptor, B^- represents a long-lived state which can backreact with the S_2 state of the donor side in a charge recombination reaction [21]. Thus, we can assume that the B band, charged by low temperature excitation, originates from the recombination of the S_2B^- redox state.

When thermoluminescence was excited by flashes at +1°C, the glow curve exhibited a main band again at about +30°C which underwent a quadruple oscillation in intensity as a function of the flash number (Fig. 1, right side).

Since the oscillation pattern exhibited a maximum after the second flash, we have previously suggested that the positive charges responsible for the B band are supplied by the S_3 state of the water-splitting system [13]. However, the appearance of a considerable thermoluminescence band immediately after the first flash could not be explained.

Our low temperature measurements provide evidence that the S_2 state can account for the appearance of the B band. In the light of this observation the high amplitude of the B band after the first flash in a flash series may also be explained by the contribution of S_2 state. Taking into account the results obtained by low temperature excitation and by flash excitation of thermoluminescence, we conclude that the B band can arise from either the S_2 or S_3 states depending on the redox state of the water-splitting system.

The most striking observation was that the peak position of the B band did not depend on the excitation flash number within experimental error (±2°C) (Fig. 1). The peak position of a thermoluminescence band is mainly determined by the redox distance between the interacting donor and acceptor molecules participating in thermoluminescence [18]. Since the S states have different midpoint potentials [22], one would expect a shift in the thermoluminescence band position with the advancement of the water-splitting system from the S_2 state to the S_3 state. After two successive flashes, which produce a high concentration of the S_3 state, the peak position of the B band should appear at lower temperature than after the first flash when the majority of the centers are in the S_2 state. However, in contrast to this expectation the peak position of the B band did not depend on whether the water-splitting system was in S_2 or S_3 state (Fig. 1).

To determine the source of electrons participating in the generation of the B band, chloroplasts, dark-adapted for various time periods in order to allow the relaxation of the acceptor side of PS II, were subjected to a sequence of exciting flashes and thermoluminescence was measured.

In chloroplasts preilluminated for 30 s by continuous light and kept in dark for 5 min the amplitude of the B band exhibited a period-4 oscillation against the exciting flash number with maxima appearing after the 2nd and 6th flashes (Fig. 2a, solid line). After a 2-h dark-adaptation the oscillation pattern was changed into another period-4 oscillation showing maxima on the 1st

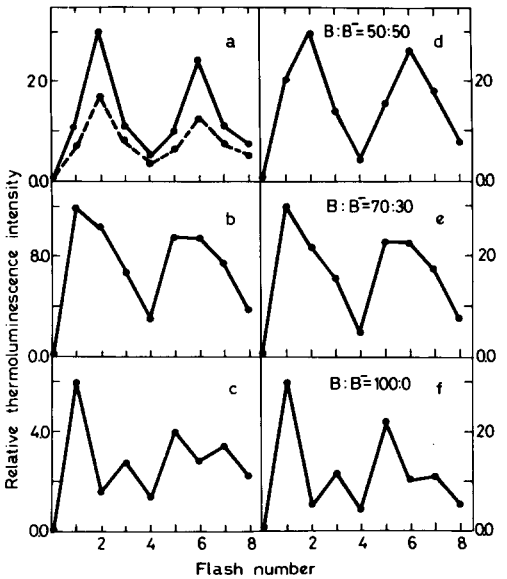

Fig. 2. Oscillation of the intensity of the main thermoluminescence band at +30°C (B band) after a variable number of flashes. (a) (———) Chloroplasts were preilluminated at +30°C for 30 s by continuous light of 10 W/m^2 followed by 5 min dark adaptation. After flash excitation at +1°C the sample was quickly cooled down to −40°C and thermoluminescence was measured. (- - - - - -) is the same as (———) except that chloroplasts were stored for 6 h in the dark at +6°C before preillumination; (b) chloroplasts were stored for 2 h in the dark at +6°C before flash excitation; (c) as (b) except that chloroplasts were stored for 6 h; (d), (e) and (f) computer-simulated oscillations assuming that after each flash the intensity of the B band is determined by the sum of the centers present in S_2B^- and S_3B^- states. The miss parameter is 8%. The redox state of the B pool before the first flash and consequently the initial distribution of the four possible redox states of the reaction centers depends on the dark adaptation time of chloroplasts; (d) the B pool is 50% oxidized and $S_0B : S_0B^- : S_1B : S_1B^- = 15 : 15 : 35 : 35$; (e) the B pool is 70% oxidized and $S_0B : S_0B^- : S_1B : S_1B^- = 21 : 9 : 49 : 21$; (f) the B pool is 100% oxidized and $S_0B : S_0B^- : S_1B : S_1B^- = 30 : 0 : 70 : 0$.

and 5th flashes (Fig. 2b). Following a prolonged dark adaptation of chloroplasts (6 h) the period-4 oscillation was superimposed with another oscillation of period-2 (Fig. 2c).

It has been established that in a sequence of flashes the redox state of the two electron acceptor, B oscillates with a periodicity of two between the single-reduced state and the fully oxidized state [1].

Obviously, it can be assumed that the amplitude oscillations of the B band occurring with a period of two, can be accounted for by the participation of the single-reduced secondary acceptor, B^- in the generation of thermoluminescence.

When the B pool is half-reduced, i.e., B/B^- is approx. 1, excitation of thermoluminescence either by an uneven or an even number of flashes leaves the B pool in a half-reduced state and the thermoluminescence intensity is determined by the four successive charge accumulating states of the water-splitting system (Fig. 2a, solid line). After a prolonged dark adaptation of chloroplasts the B pool is almost completely oxidized [1,2,26] and consecutive flashes shift its redox state back and forth between the single-reduced state and fully oxidized state. Consequently, a period-2 contribution appears gradually in the oscillation pattern of the thermoluminescence amplitude (Fig. 2b and c). Illumination of long-term dark adapted chloroplasts by continuous light restores the initial ratio for which B/B^- is approx. 1 and the oscillation pattern is again determined by the charge accumulating states in the water-splitting system (Fig. 2a, dashed line).

In summary, the changes in the oscillation patterns can be well interpreted by assuming that the pool of the single-reduced secondary acceptor, B^-, is the source of electrons responsible for the B band. The same conclusion was reached by Rutherford et al. [10] although they did not report the observation of a period-2 oscillation.

Computer simulation of the oscillatory pattern of thermoluminescence at various B/B^- ratios supports the explanation given above. Two assumptions are made in the model calculation. (i) Both the S_2B^- and S_3B^- states are involved in the generation of the B band. The validity of this assumption is experimentally verified in the previous section of the paper. (ii) After 5 min dark-adaptation of chloroplasts the distribution of the four possible redox states is taken to be $S_0B : S_0B^- : S_1B : S_1B^- = 15 : 15 : 35 : 35$ which gradually proceeds, with the same oxidation rate of B^- in the S_0B^- and S_1B^- centers, towards the distribution $S_0B : S_0B^- : S_1B : S_1B^- = 30 : 0 : 70 : 0$ during prolonged dark adaptation of the chloroplasts.

Period-4 oscillation with maxima on the 2nd and 6th flashes can be simulated only with this

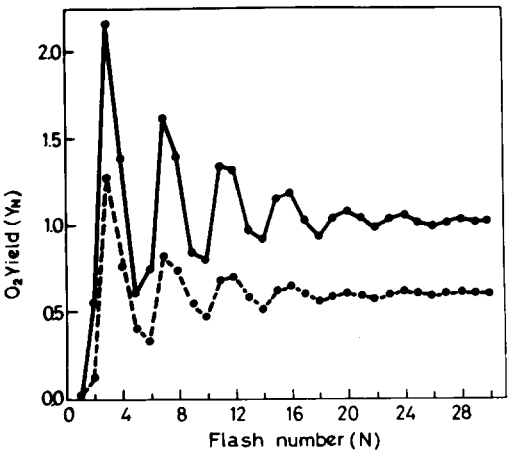

Fig. 3. O_2 flash yield sequence observed with isolated maize chloroplasts. (———) Chloroplasts were preilluminated at +30°C for 30 s by continuous white light, followed by 5 min dark adaptation and measurement of O_2 yield. (- - - - -) Chloroplasts were stored for 6 h in the dark at +6°C before measurement of O_2 yield.

assumption (Fig. 2d) which implies that after 5 min dark-adaptation of chloroplasts the deactivation of S states is already completed ($S_0 : S_1 = 30 : 70$), while the B pool is still near to the steady-state distribution ($B : B^- = 50 : 50$). With these assumptions using the parameters for misses and distribution of S states ($\alpha = 0.08$; $S_0 = 30$; $S_1 = 70$) and considering various B/B^- ratios satisfactory agreement was obtained between the observations and predictions by computer modeling (compare Fig. 2a–c with Fig. 2d–f).

The satisfactory fits of the experimentally observed oscillatory patterns by the calculated curves provide convincing support for the assumptions we have made in the model calculations. It deserves attention that in contrast to earlier suggestions [1,2] our results (Fig. 2f) indicate that the B pool can be almost 100% oxidized after long-term dark-adaptation of chloroplasts. This result is corroborated by a recent report of Robinson and Crofts [21].

To check whether the binary oscillation of thermoluminescence is caused by variations occurring during dark adaptation in the water-splitting system or not the O_2 flash yield sequence was determined by a fast polarographic method [16]. The O_2 yield pattern exhibits a period-4 oscillation in both freshly isolated and long-term dark-adapted chloroplasts thereby excluding the possibility that the appearance of the binary oscillation in the thermoluminescence yield is due to degradation effects at the donor side of PS II (Fig. 3). The steady-state level of the O_2 yield shows that chloroplasts lost 40% of their oxygen evolving capacity during 6 h of dark adaptation. The decrease in the amplitude of the thermoluminescence intensity after restoring the period-4 oscillation by preillumination is the result of this residual inactivation (Fig. 2a, dashed line).

The final conclusion drawn from the experimental and theoretical investigation of the oscillation of thermoluminescence intensity is that the B band originates from the charge recombination occurring between the singly reduced two-electron acceptor B^- and the S_2 and S_3 states of the water-splitting system (recombination of the S_2B^- and S_3B^- states). Our conclusion is in agreement with the recent suggestions of Rutherford et al. [10].

The results obtained by uninhibited chloroplasts were supported by experiments carried out in the presence of the PS II inhibitor DCMU. DCMU blocks the electron transfer from the primary acceptor Q to the secondary acceptor B. If the B band is related to the single-reduced secondary acceptor B^- the amount of B^- present can be characterized by the amplitude of the B band. In accordance with this, addition of increasing concentrations of DCMU to the dark-adapted chloroplast suspension gradually diminished the B band (Fig. 4). A plot of the amplitude of the B band as a function of DCMU concentration followed the same decay course as the rate of the electron transport from H_2O to *p*-benzoquinone (Fig. 5). The pI_{50} value of DCMU determined from the half-decay of the B band agrees well with the data obtained from Hill reaction measurements [23].

The decrease of the B band is accompanied by a concomitant increase of a thermoluminescence band appearing at about +10°C in the glow curve (Fig. 4). In the presence of DCMU only the primary acceptor is reduced (Q^-) and the water-splitting system can undergo one transition ($S_1 \rightarrow S_2$). Therefore it is natural to conclude, in agreement with earlier suggestions [10,24,25], that the thermoluminescence band at 10°C originates from the S_2Q^- state. Accordingly, this thermolumines-

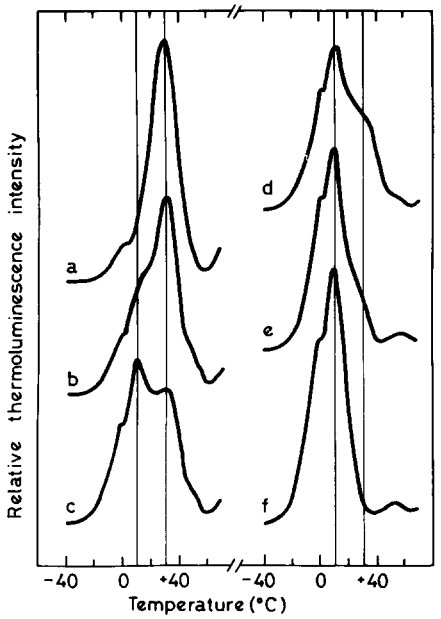

Fig. 4. Effect of DCMU on the thermoluminescence of isolated maize chloroplasts preilluminated at +30°C for 30 s by continuous light, followed by 5 min dark adaptation. Various concentrations of DCMU were added to the dark-adapted chloroplasts and the samples were cooled down to −60°C. Glow curves were excited by continuous light of 10 W/cm^2 for 2 min at −60°C. (a) No addition; (b) 50 nM DCMU; (c) 75 nM DCMU; (d) 100 nM DCMU; (e) 200 nM DCMU; (f) 800 nM DCMU.

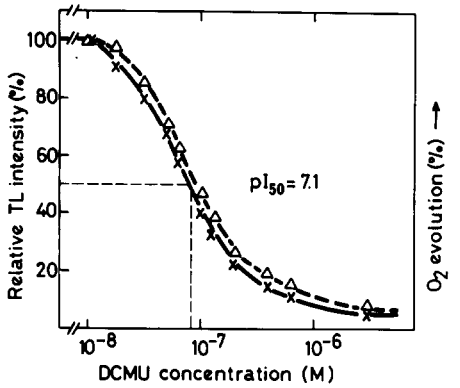

Fig. 5. (×——×) Amplitude of the thermoluminescence band appearing at 30°C (B band) and (△——△) the rate of O_2 evolution ($H_2O \rightarrow$ *p*-benzoquinone) as a function of DCMU concentrations. Thermoluminescence was measured as in Fig. 4. Glow curves were decomposed into the Q and B bands by a computer-assisted curve resolution program and the amplitude of the B band was plotted as the percentage of the amplitude obtained by measuring the untreated sample. The rate of oxygen evolution for untreated chloroplasts was 120 μmol O_2 per mg Chl per h.

cence band will be called Q band in the paper.

In a sequence of flashes given after DCMU addition the amplitude of the Q band remained almost constant regardless of the number of exciting flashes (Fig. 6, right side). On the other hand, after flash preillumination followed by DCMU addition the oscillation of the Q band exhibited a similar pattern as that of the B band (Fig. 6, left side). It has been reported that addition of DCMU after preilluminating flashes induces a backflow of electrons from B^- to Q [1,26]. As a result after DCMU addition the redox state of B prior to DCMU addition is reflected in the redox state of Q. It is assumed that in chloroplasts kept in dark for 5 min after 30 s illumination the secondary acceptor pool is in 50% single-reduced state, whereas the water-splitting system is completely relaxed. Consecutive flashes do not change the redox state of the B pool. Consequently, after DCMU addition the percentage of Q reduced is

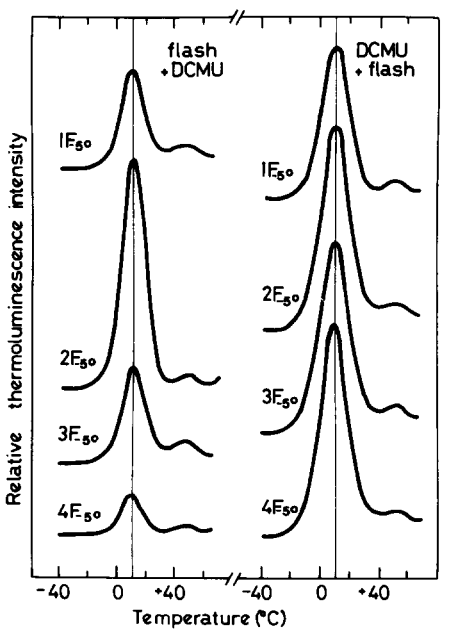

Fig. 6. Glow curves of isolated maize chloroplasts excited by flashes before (left side) and after (right side) DCMU addition. Flash excitation of the samples occurred at −5°C, and it was followed by the addition of 10 μM DCMU. The suspension was mixed for 10 s and quickly cooled down to −40°C (left side). Samples treated by 10 μM DCMU before flash excitation were subjected to the same procedure (right side). All of the samples contained 30% glycerol.

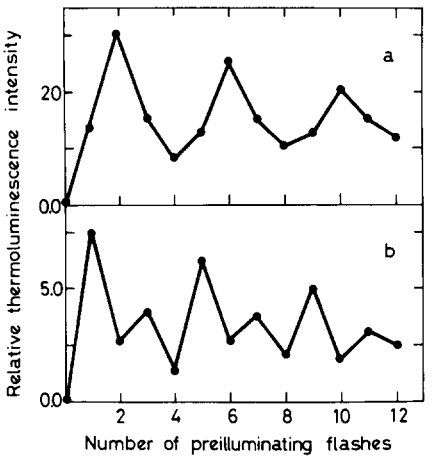

Fig. 7. Oscillation of the intensity of the thermoluminescence band appearing at +10°C (Q band) after a variable number of preilluminating flashes. All of the samples contained 30% glycerol. Flash excitation occurred at −5°C and it was followed by the addition of 10 μM DCMU. The suspension was mixed for 10 s in dark and quickly cooled down to −40°C before thermoluminescence measurement. (a) Before flash excitation the chloroplasts were preilluminated at +30°C for 30 s by continuous white light of 10 W/m^2 followed by 5 min dark adaptation; (b) as (a), except that chloroplasts were stored for 6 h in dark at +6°C before flash excitation and DCMU addition.

independent of the flash number thus the oscillatory pattern of the Q band is determined only by the four successive S states. The amplitude of the Q band exhibits a period-4 oscillation with maxima after the 2nd, 6th and 10th flashes indicating the participation of the S_3 state in the charge recombination reaction (Fig. 7a).

The results obtained by DCMU-treated chloroplasts suggest that the Q band is generated by charge recombination of either the S_2Q^- or S_3Q^- state depending on the redox state of the water-splitting system.

In long-term dark-adapted chloroplasts the redox-state of the B pool oscillates with a periodicity of two between the half-reduced and fully reduced (oxidized) states as a function of the exciting flash number. After DCMU addition this period-2 oscillation in the redox state of the B pool is reflected in the redox state of Q and as expected a binary oscillation appeared in the oscillatory pattern of the Q band (Fig. 7b).

It is again remarkable that the peak position of the Q band remained unchanged regardless of whether the oxidizing side of PS II was in state S_2 or in state S_3 (Fig. 6, left side). This implies that the redox potentials of the reactants involved in the thermoluminescence reaction do not change as a function of the number of flashes given in the presence or absence of DCMU. Similar results were obtained in delayed luminescence measurements. Delayed luminescence originating from the S_3Q^- state proved to be quite similar in luminescence yield and rate of decay to that from the S_2Q^- state [1].

To explain these interesting observations in delayed luminescence and thermoluminescence one may suggest that the midpoint potentials of the S_2 and S_3 states are approximately the same. Moreover, the change in the amplitude of thermoluminescence in a sequence of flashes indicates that the S states may exert a direct (e.g., electrostatic) effect on the excitation yield of the radiative charge recombination reaction as assumed in [6,27]. Although a direct effect of the S states on the excitation yield is hard to reconcile with the recent observation of De Grooth and Van Gorkom, according to which the delayed luminescence yield is not dependent on the presence of an electric field [28].

A different approach to the problem was considered by Velthuys who proposed a model of charge accumulation in the water-splitting system on the basis of delayed luminescence investigations [1,29]. According to his model, the water-splitting system can exist in six different redox states and the S states can be rewritten in terms of formally specified donors so that the scheme $MZ \rightarrow M^+Z \rightarrow M^{2+}Z \rightarrow M^{2+}Z^+ \rightarrow M^{3+}Z^+ \rightarrow M^{4+}Z^+$ represents $S_{-1} \rightarrow S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_4$. Oxidized Z^+ only is responsible for charge recombination and luminescence and its concentration oscillates with the so-called S state of PS II. This model can explain the oscillation of the amplitudes of the B and Q bands in a series of flashes and the independence of their band positions on the flash number. The validity of this model for the $S_2 \rightarrow S_3$ transition has been recently confirmed. Absorbance change measurements in the ultraviolet region of the spectrum showed that the redox potential of the oxidized donor (Z^+), in our opinion the oxidized substrate of thermoluminescence,

remains unmodified during the $S_2 \rightarrow S_3$ transition [30].

An alternative mechanism which cannot be overlooked is that a side-carrier of stable redox potential which can reversibly exchange charges with the S states [31] participates in the generation of thermoluminescence. Since its redox potential does not change with the advancement of S states, the peak positions of the B and Q bands do not change either in a sequence of flashes.

We have shown that in uninhibited chloroplasts after excitation with a few flashes only the B band, characterized by a small half-bandwidth, can be observed in the glow curve at +30°C (Fig. 1) (or at higher temperatures depending on the heating rate [10]). However, in earlier publications the main thermoluminescence band of uninhibited chloroplasts has been observed as a broad band appearing between +20°C and +25°C [15,18,32]. We tried to find the reason for this discrepancy. It was found that after excitation of chloroplasts by weak continuous light of short period only the B band shows up at +30°C (Fig. 8a). However, with prolonged excitation of chloroplasts the Q band

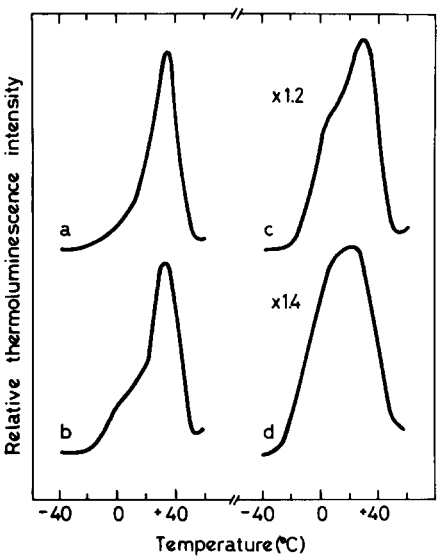

Fig. 8. Effect of the duration of excitation on the shape of the glow curve. All of the samples contained 30% glycerol. Excitation occurred at −10°C by continuous white light of 0.3 W/m^2 for various time periods. After excitation the samples were quickly cooled down to −40°C and thermoluminescence was measured. The duration of excitation was: (a) 5 s; (b) 15 s; (c) 30 s; (d) 60 s.

also appeared in the glow curve and gradually increased next to the B band resulting in a broad band between +15°C and +25°C in which the component bands could not be discerned any longer (Fig. 8). Fluorescence induction measurements have demonstrated that prolonged illumination of chloroplasts results in a gradual reduction of the plastoquinone pool and Q^- accumulates even in the absence of DCMU [33]. Therefore, we can assume that after prolonged excitation of chloroplasts when a major part of the plastoquinone pool is in the reduced state both the primary (Q^-) and secondary acceptors (B^-) contribute to the backreaction of charges resulting in thermoluminescence.

Using the theory of thermoluminescence [18] the free energies of activation and the half-times of the individual Q and B bands have been calculated and the data are given in Table I. Taking into account that according to our observations the primary acceptor Q^- and secondary acceptor B^- recombine with both the S_2 and S_3 states and these states cannot be distinguished in the charge recombination reaction, the redox potential difference: $E_m(B/B^-) - E_m(Q/Q^-) = 70$ mV. If the midpoint potential of the couple Q/Q^- is −30 mV [34,35], the midpoint potential of the couple B/B^- would be about +40 mV. A value of −300 mV as suggested in Ref. 36 for the couple Q/Q^- would result in $E_m = -230$ mV for the couple B/B^-.

The half-times calculated for the backreactions of Q^- and B^- at +25°C are 3.0 and 48 s, respectively. Similar values (1.5 s and 22 s) have recently been obtained by Robinson and Crofts [21] in fluorescence yield measurements. From the half-times of the charge recombination reactions of Q^- and B^-, the equilibrium constant (K_1) for the sharing of one electron between Q and B, that is for the equilibrium $Q^-B \overset{K_1}{\rightleftharpoons} QB^-$, can be calculated. The ratio gives a value for $K_1 = 16$, which is in good agreement with the value of 15–20 reported by Diner [37] and by Robinson and Crofts [21] as well.

The results presented in the paper demonstrate that contrary to Refs. 7 and 11 the redox state of the acceptor side of PS II has a definite role in the determination of the thermoluminescence characteristics of chloroplasts. Both the reduced

TABLE I

CHARACTERISTICS OF THE INDIVIDUAL Q AND B THERMOLUMINESCENCE BANDS

The B and Q bands were excited at +1°C and −5°C, respectively, by 2 flashes each. Other measuring conditions are given in Figs. 1 and 6. Characteristics of the bands were obtained by a least-squares fitting of the glow curves as described in Ref. 18. T_m, temperature at the maximum of a thermoluminescence band: ΔF, free energy of activation; $t_{1/2}$, half-time.

Designation of bands	T_m (°C)	ΔF(25°C) (eV)	$t_{1/2}$(25°C) (s)
Q	10	0.790	3
B	30	0.860	48

primary and secondary acceptors interact with the S states of the water-splitting enzyme and their contribution to the generation of thermoluminescence depends on the redox state of the plastoquinone pool. When the water-splitting system is in the S_2 state the thermoluminescence bands appearing at +10°C (Q band) and +30°C (B band) originate from the charge recombination of the S_2Q^- and S_2B^- states, respectively. In the S_3 state of the water-splitting system the species responsible for the Q and B bands are S_3Q^- and S_3B^-, respectively.

The result opens up a new perspective in the study of the photosynthetic processes which involve the primary and secondary acceptors. The changes in the amplitudes and peak positions of the Q and B bands can be easily followed by the thermoluminescence technique providing information about the changes in the redox states of the primary and secondary acceptors.

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