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Thermoluminescence study of charge recombination in Photosystem II at low temperatures. II. Oscillatory properties of the Z_v and A thermoluminescence bands in chloroplasts dark-adapted for various time periods

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The oscillations of the Z_v and A thermoluminescence bands were investigated in spinach chloroplasts which had been dark-adapted for various time periods and subjected to a series of flashes at +2°C before continuous illumination at various low temperatures. When excited with continuous light below −65°C, the Z_v band exhibited period-4 oscillation, with maxima on preflashes 0, 4 and 8. Above −65°C, the oscillation pattern depended greatly on the dark-adaptation period of the chloroplasts. In preilluminated samples (15 s light followed by 3 min dark), when the Q_B pool is half oxidized, the oscillation of the thermoluminescence intensity measured at −50°C was similar to that observed below −65°C. However, after the thorough dark-adaptation of the chloroplasts (6 h), when the major fraction of the Q_B pool is assumed to be oxidized, a binary oscillation appeared in the oscillation pattern, with maxima at odd flash numbers. Below −65°C, period-2 oscillation of the Z_v band could not be induced by the dark-adaptation of the chloroplasts, suggesting an inhibition of electron exchange between Q_A and Q_B. Upon excitation of the chloroplasts with continuous light at −30°C, the A band oscillated with a periodicity of 4 with maxima at preflash numbers 2 and 6. At pH 7.5, the period-4 oscillation was converted into a period-2 oscillation by thorough dark-adaptation of the chloroplasts (24 h). Model calculations of the oscillatory patterns suggest that the period-4 oscillations of the Z_v and A bands are determined by the concentrations $[S_0]+[S_1]$ and $[S_2]+[S_3]$, respectively, which are present after the preflashes prior to the low-temperature continuous illumination. The period-2 oscillations in the amplitudes of the Z_v and A bands reflect the changes occurring in the redox state of the Q_B pool in a sequence of flashes. The possible relationship between the characteristics of the Z_v and A bands and the temperature-dependence of the S state transitions was investigated. Comparison of the amplitudal changes of the B ($S_2Q_B^-$ and $S_3Q_B^-$ recombination) and Q ($S_2Q_A^-$ recombination) thermoluminescence bands as a function of the excitation temperature suggests that the $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_4$ transitions are blocked at about −65 and −40°C, respectively. It is also concluded that the thermoluminescence intensity emitted by the reaction center is about twice as high in the S_3 state as in the S_2 state.

Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; PS, Photosystem; Q_A, primary quinone electron acceptor of Photosystem II; Q_B, secondary quinone electron acceptor.

Introduction

It has been established in several studies that the number of positive charges stored in the pathway from water to P680 has a marked effect upon the light-induced reactions of PS II. After a series of flashes, the rereduction kinetics of the secondary donor Z^+ (Signal II_{vf}) [1] and $P680^+$ [2] depend on the S state of the oxygen-evolution system. The fluorescence yield is also influenced by the charge storage on the oxygen-evolving side of PS II [3,4]. The intensity of the various phases of delayed luminescence shows an oscillation of period-4 as a function of the excitation flash number, a phenomenon which is related to the S states [5,6]. It has recently been demonstrated that thermoluminescence and delayed luminescence are different manifestations of the same charge recombination phenomenon [7,8]. As a consequence, the Q (also called D [9]), B and C thermoluminescence bands appearing in the glow curve at about +10, +30 and +50°C, respectively, oscillate with a periodicity of 4 [10–13]. The oscillatory pattern depends greatly on the preconditioning of the chloroplasts. After the long-term dark-adaptation of chloroplasts, the period-4 oscillation is superimposed by a period-2 oscillation, indicating the contribution of the secondary acceptor, Q_B, to the charge recombination process [12–14].

The effect of the redox state of the water-splitting system on the photoinduced reactions of PS II is preserved at low temperatures between about 0 and −100°C [15]. Lowering of the temperature causes a strong dependence of the fluorescence yield [4,15,16] and the delayed luminescence yield [15,17] upon the number of light flashes given prior to freezing. A similar flash-dependence is exhibited by the Z_v and A thermoluminescence bands peaking at about −75 and −10°C, respectively [18,19]. An investigation of the oscillatory properties of thermoluminescence may help to clarify the origin of the thermoluminescence bands and relate them to electron donor and acceptor components. In order to determine the possible relationships between S states and low-temperature thermoluminescence, we have studied the oscillatory patterns of the Z_v and A thermoluminescence bands in chloroplasts dark-adapted for various time periods and excited at various temperatures. To explain the variations observed in the oscillations of the Z_v and A bands, the temperature-dependence of the S state transitions was also investigated.

Materials and Methods

Chloroplasts were isolated from spinach leaves as described in the accompanying paper [20]. The suspension of chloroplasts contained 0.4 M sorbitol/10 mM NaCl/5 mM $MgCl_2$/2 mM EDTA and 50 mM Hepes (pH 7.5) or phosphate (pH 6.0) buffer with 125 μg Chl/ml. In order to have the Q_B pool in steady-state distribution ($Q_B:Q_B^- = 50:50$), the samples were preilluminated with white light ($10\ W \cdot m^{-2}$) for 15 s at +30°C and kept in the dark for 3 min to allow the relaxation of the S states. Following this pretreatment, the chloroplasts were dark-adapted at +4°C for various time periods before thermoluminescence measurement. Thermoluminescence was excited by means of a series of xenon flashes (1 Hz) at +2°C, followed by continuous illumination with white light at various low temperatures. In some cases thermoluminescence was generated only by continuous white light or flashes. After excitation, the samples were quickly cooled down to a temperature 20–30 Cdeg lower than the excitation temperature. Thermoluminescence measurements were performed at a heating rate of 20 Cdeg/min with the same apparatus as described in Ref. 20. Mathematical simulation of the oscillatory patterns is based on the Kok model, using the matrix method of Lavorel [21]. The calculations were performed as reported earlier [12]. The parameters used in the calculations were $\alpha = 0.08$ for misses, $\beta = 0.04$ for double hits, and $S_0:S_1:S_2:S_3 = 30:70:0:0$ for the dark distribution of the S states.

Results and Discussion

Since the emission temperature of the Z_v band varies with the excitation temperature (see Fig. 2 in Ref. 20), and the contribution of a hidden band to the thermoluminescence intensity at around −50°C cannot be excluded, the oscillation properties of the band were investigated at both −70 and −50°C. In chloroplasts preilluminated with flashes at +2°C before low-temperature excitation at −70°C, the Z_v band underwent a period-4 oscillation, with maxima at the zeroth, fourth and eighth flashes (Fig. 1). According to the Kok model of oxygen evolution, after the zeroth, fourth and eighth flashes the water-splitting system is mainly in the S_0 and S_1 states. Consequently, the oscillation pattern of the Z_v band excited at −70°C is determined by the number of centers present in the S_1 and S_0 states before low-temperature excitation. At −70°C, the oscillation of the Z_v band was not influenced by the dark-adaptation period of the chloroplasts (Fig. 1a–c). Although the amplitude of the band decreased somewhat after a 24 h dark adaptation, the oscillation pattern was not altered.

When continuous illumination was applied at −50°C after flash preillumination at +2°C, the oscillation pattern displayed a great dependence on the dark-adaptation period (Fig. 2). In chloroplasts preilluminated for 15 s with continuous light (to induce the steady-state distribution of the Q_B pool) and kept in the dark for 3 min at +30°C (allowing the relaxation of the S states), the thermoluminescence intensity exhibited the same oscillation pattern as was observed at −70°C (Fig. 2e). However, after a 30-min dark-adaptation the oscillation pattern was changed into another period-4 oscillation, showing maxima at the 3rd and 7th flashes (Fig. 2f). Following a 2-h dark-adaptation of the chloroplasts, the period-4 oscillation was superimposed with another oscillation of period 2 (Fig. 2g), suggesting that at −50°C the two-electron acceptor, Q_B, plays an important role in the determination of the oscillatory behavior of thermoluminescence. Illumination of the dark-adapted chloroplasts (2 h) with weak continuous light for 15 s before flash excitation and low-temperature illumination restores the original steady-state distribution of the Q_B pool and in turn the period-4

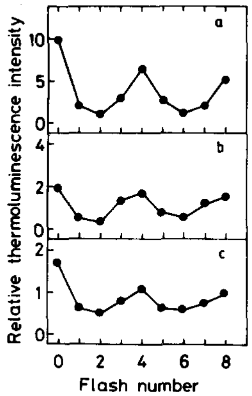

Fig. 1. Oscillation of the Z_v thermoluminescence band in spinach chloroplasts dark-adapted for various time periods and excited with a series of flashes at +2°C prior to continuous illumination for 30 s at −70°C. (a) Chloroplasts were preilluminated with continuous light for 15 s at +30°C and kept in the dark for 3 min before flash excitation and thermoluminescence measurement. (b) Chloroplasts were stored for 2 h in the dark at +4°C before flash excitation. (c) Chloroplasts were stored for 24 h in the dark at +4°C before flash excitation.

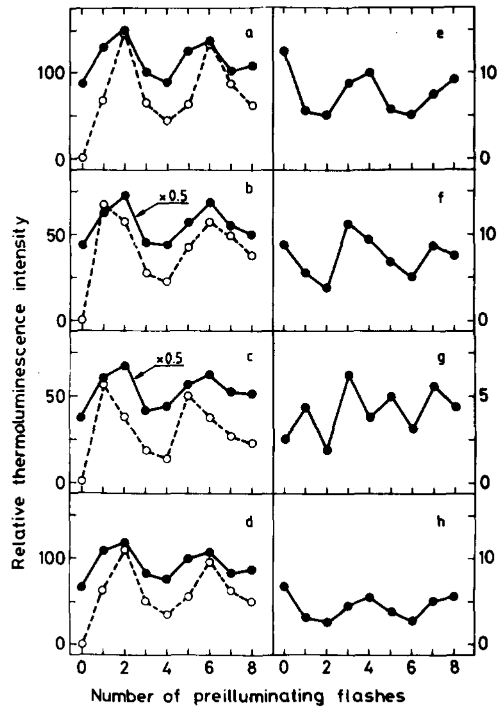

Fig. 2. Oscillation of the B (left side) and Z_v (right side) thermoluminescence bands in spinach chloroplasts dark-adapted for various time periods and excited with a series of flashes at +2°C prior to continuous illumination for 30 s at −50°C. Dashed lines represent the oscillatory patterns of the B band excited only with a sequence of flashes at +2°C. Solid lines illustrate the glow curves induced by additional continuous illumination at −50°C. (a) and (e) Chloroplasts were preilluminated at +30°C for 15 s with continuous light followed by a 3 min dark adaptation before flash excitation and thermoluminescence measurement. (b) and (f) Chloroplasts were stored for 30 min in the dark at +4°C before flash excitation. (c) and (g) Chloroplasts were stored for 2 h. (d) and (h) Chloroplasts stored for 2 h in the dark were reexcited with continuous light for 15 s and kept in the dark for 3 min before flash excitation.

oscillation (Fig. 2h). The decreased amplitude of the thermoluminescence yield after restoral of the period-4 oscillation by preillumination is caused by the inactivation of a small percentage of the water-splitting enzyme, due to the prolonged dark-storage of the chloroplasts. The B band (appearing at +30°C), measured in parallel with the oscillation of thermoluminescence at −50°C, exhibited a period-4 oscillation, with maxima at flash numbers 2 and 6 (Fig. 2a, solid line). The oscillation pattern was independent of the dark-adaptation period of the chloroplasts (Fig. 2a–d). However, when excitation was performed only with flashes at +2°C, the oscillatory pattern of the B band, like that of the low temperature thermoluminescence, was greatly dependent on the dark-adaptation period of the chloroplasts (Fig. 2 a–d, dashed line), in agreement with earlier observations [11,12]. It was surprising that the variations of the oscillatory patterns of the B band and that of the thermoluminescence intensity at −50°C followed different time courses. While at −50°C the thermoluminescence intensity already displayed period-2 oscillation after a 2-h dark adaptation, binary oscillation of the B band could be observed only after a 6 h [12] or sometimes a 24 h dark adaptation (unpublished result). Since period-2 oscillation of the Z_v band cannot be induced at −70°C, even in thoroughly dark-adapted chloroplasts, it can be concluded that the electron exchange is blocked between Q_A and Q_B at this temperature. An attempt was made to determine the threshold temperature for the interruption of electron transfer between Q_A and Q_B. The oscillation of thermoluminescence was measured at various temperatures in chloroplasts dark-adapted for 30 min (Fig. 3). At −64°C, the oscillatory maxima occurred at flash numbers 3 and 7, but were shifted to the zeroth, fourth and eight flashes after excitation of thermoluminescence at −66°C. Consequently, the electron exchange between Q_A and the oscillating secondary quinone, Q_B is interrupted at about −65°C. However it cannot be excluded either that at −65°C the electron transport is diverted from Q_B to an alternative non-oscillating acceptor which is back-reacting with an oxidized donor of PS II.

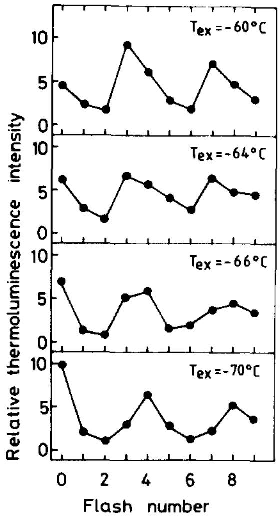

Fig. 3. Changes in the oscillatory pattern of the thermoluminescence intensity, depending on the excitation temperature (T_{ex}). Chloroplasts were dark-adapted for 30 min at +4°C before thermoluminescence measurements. After flash excitation at +2°C, the samples were cooled down and excited with continuous light for 30 s at the indicated temperatures.

The oscillatory behavior of the A band was also investigated as a function of the dark-adaptation period of the chloroplasts (Fig. 4). In agreement with the results of Inoue and Shibata [19], at pH 7.5 chloroplasts excited with a series of flashes at +2°C prior to continuous illumination at −30°C showed a damped period-4 oscillation, with maxima after the second and sixth flashes (Fig. 4a). However, the oscillation pattern varied from preparation to preparation (and was sometimes even inverted) and could be satisfactorily reproduced only in chloroplasts isolated from plants grown in bright sunlight. On the other hand, at pH 6.0 the oscillation of the A band was more pronounced and easily reproducible (Fig. 4c). Interestingly, the pH of the medium influences the oscillation pattern of the A band. At pH 6.0, binary oscillation could not be induced by long-term dark-adaptation of the chloroplasts (Fig. 4d), but at pH 7.5 the thermoluminescence yield oscillated with a periodicity of 2 after a 24 h dark storage (Fig. 4b, solid line). It has been shown that at low pH the protonation of Q_B^- increases the redox distance and inhibits the effective electron exchange between Q_A and Q_B [22,23]. An altered charge equilibrium between Q_A and Q_B, like a temperature-induced inhibition at −65°C, may account for the lack of binary oscillation of the A band at pH 6.0.

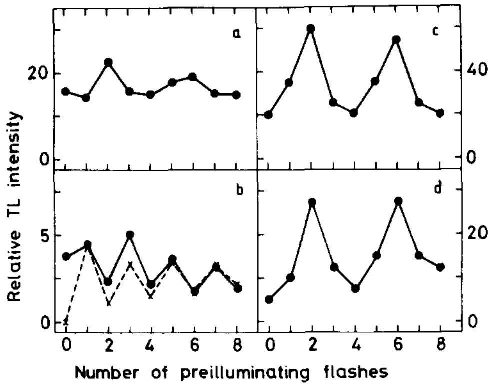

Fig. 4. Oscillation of the A thermoluminescence band as a function of the flash number in spinach chloroplasts suspended in Hepes (pH 7.5) (left side) and phosphate (pH 6.0) (right side) buffers and dark-adapted for various time periods. Flash excitation of the samples occurred at +2°C and was followed by continuous illumination for 30 s at −30°C. (a) and (c) Chloroplasts were preilluminated at +30°C for 15 s with continuous light and kept in the dark for 3 min before flash excitation. (b) and (d) Chloroplasts were stored for 24 h in the dark at +4°C before flash excitation. The dashed line in (b) represents the computer-simulated oscillation of the A band on the assumptions that its intensity is determined by the sum of reaction centers present in the $S_2Q_B^-$ and $S_3Q_B^-$ states prior to low-temperature continuous illumination, and that the thermoluminescence yield in the S_3 state is twice that emitted in the S_2 state. The parameters for misses and double hits are $\alpha = 0.08$ and $\beta = 0.04$, respectively. The initial distribution of the S states is $S_0:S_1:S_2:S_3 = 30:70:0:0$. The Q_B pool is completely oxidized ($Q_B:Q_B^- = 100:0$) and the initial distribution of reaction centers is $S_0Q_B:S_0Q_B^-:S_1Q_B:S_1Q_B^- = 30:0:70:0$.

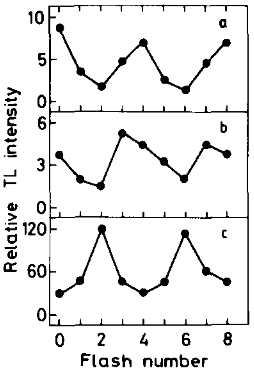

Fig. 5. Effects of DCMU on the oscillations of the Z_v and A thermoluminescence bands. The Z_v and A bands were measured at pH 7.5 (50 mM Hepes) and pH 6.0 (50 mM phosphate), respectively. Dark-adapted chloroplasts (30 min at +4°C) were preilluminated with flashes at +2°C. After the addition of 10 μM DCMU, the suspension was mixed for 20 s and cooled down quickly in the dark. Thermoluminescence was generated with continuous light for 30 s at various temperatures: (a) −70°C; (b) −50°C; and (c) −30°C.

It was shown in the preceding paper [20] that the reservoir of electrons participating in the generation of the Z_v and A bands is the primary acceptor, Q_A. In accordance with this, the oscillatory patterns of the two bands were not influenced by DCMU added after flash preillumination of the chloroplasts (Fig. 5a–c). On the other hand, DCMU addition increased the amplitude of the A band considerably (cf. Fig. 4c with Fig. 5c): in the presence of DCMU, the leakage of electrons from Q_A towards the plastoquinone pool is inhibited during the course of thermoluminescence measurements.

In order to account for the experimentally observed results, we tried to correlate the measured oscillatory patterns with the populations of the S states as a function of the exciting flash number. It was found that the oscillation of the Z_v band when excited below −65°C, strikingly correlated with the concentration $[S_0]+[S_1]$ present before the low-temperature continuous illumination (cf. Fig. 1 with Fig. 6a).

The oscillation of the A band closely resembled those of the B and Q thermoluminescence bands. Since the oscillations of the B and Q bands are

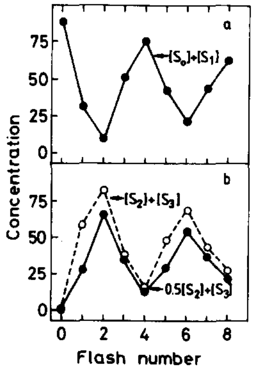

Fig. 6. Concentrations $[S_0]+[S_1]$ and $[S_2]+[S_3]$ as functions of the flash number, calculated via the Kok, model. The populations of the S states were computed by using the probabilities $\alpha = 0.08$ for misses and $\beta = 0.04$ for double hits. The dark distribution of the S states before the first flash was assumed to be $S_0:S_1:S_2:S_3 = 30:70:0:0$.With regard to the lower yield of thermoluminescence in the S_2 state as compared to the S_3 state (see Fig. 7 and the text), the oscillation of the sum $0.5[S_2]+[S_3]$ is also presented.

determined by the S_2 and S_3 states [10–12,14], we assumed that the same two S states play role in the generation of the A band. Indeed, a good correlation was obtained between the oscillation of the A band and the concentration $[S_2]+[S_3]$ reached after the past preilluminating flash prior to the continuous illumination (compare Fig. 4c with Fig. 6b, dashed line). As the thermoluminescence yield in the S_2 state was found to be approximately half of that observed in the S_3 state (see Fig. 9 and the related discussion), an even better correlation could be obtained between the oscillation of the A band and the concentration $0.5\ [S_2]+[S_3]$ (cf. Fig. 4c with Fig. 6b, solid line).

Our thermoluminescence results are consistent with several other observations, according to which PS II behaves differently when the water-splitting system has accumulated charges (S_2 and S_3 states) as compared to the dark-adapted state (traps in the S_0 and S_1 states) [2,3,15–17,24–26]. This behavior led Joliot and Joliot [4] to suggest that each PS II center contains two donor-acceptor couples, one operative in states S_0 and S_1 and the other in states S_2 and S_3. A review of PS II donors by Bouges-Bocquet [27] also favored a parallel scheme for the description of the donor side of PS II. On the other hand, Brettel et al. [2] recently proposed a kinetic model of PS II based on a linear electron transfer from the oxygen-evolving complex to P-680^+ via two carriers, D_1 and D_2.

In a linear arrangement, for energetic reasons, the donor associated with the Z_v band (appearing at −75°C) should precede the donor responsible for the A band (peaking at −30°C). Naturally, in this case the inhibition of the Z_v band should be accompanied by the disappearance of the A band. In our thermoluminescence measurements, inactivation of the water-splitting system by Tris or NH_2OH treatment almost completely abolished the Z_v band, while the A band was not affected at all [20]. Consequently, the inactivation experiments cannot be reconciled with a linear model, but can be interpreted in the framework of Bouges-Bocquet's model. Thus, a donor (Z_1), which operates when the water-splitting system is in the S_0 and S_1 states, gives rise to the Z_v band, while another donor (Z_2), which transfers electrons from the S_2 and S_3 states towards P-680, is responsible for the A band. In agreement with our thermoluminescence results, recent EPR studies likewise indicate that the donation path of S_1 to P-680 differs from that of S_2 to P-680 [28].

Although the S states control the period-4 oscillations of the Z_v and A bands, they cannot account for the binary oscillation of these bands. It is natural to assume that the changes in the oscillatory patterns during the dark-adaptation of chloroplasts are associated with the redox changes of the Q_B pool. However, the reservoir of negative charges responsible for the Z_v and A bands is the primary acceptor, Q_A [20]. Since Q_A and Q_B are in charge equilibrium with each other, it can be inferred that the redox state of the Q_B pool influences that of the Q_A pool and in turn the oscillation pattern. During the dark-adaptation of chloroplasts, the Q_B pool is gradually oxidized from the initial equilibrium state ($Q_B:Q_B^- = 50:50$) to the completely oxidized state ($Q_B:Q_B^- = 100:0$). Hence on the assumption that the thermoluminescence intensity at −50°C is determined

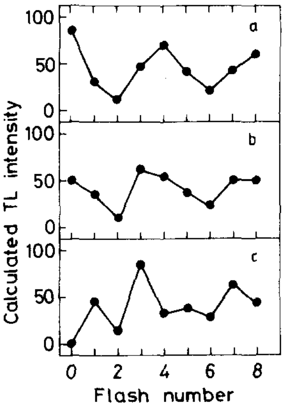

Fig. 7. Computer-simulated oscillations of the Z_v thermoluminescence band for various oxidation states of the secondary acceptor pool. The oscillatory patterns were calculated by assuming that the intensity of the Z_v band is determined by the sum of reaction centers present in the $S_0Q_B^-$ and $S_1Q_B^-$ redox states prior to low-temperature continuous illumination. The parameters used for the probability of misses and double hits are $\alpha = 0.8$ and $\beta = 0.04$, respectively. The dark distribution of the S states prior to the first flash is $S_0:S_1:S_2:S_3 = 30:70:0:0$. (a) The Q_B pool is 50% oxidized ($Q_B:Q_B^- = 50:50$) and the initial distribution of centers is $S_0Q_B:S_0Q_B^-:S_1Q_B:S_1Q_B^- = 15:15:35:35$. (b) The Q_B pool is 70% oxidized ($Q_B:Q_B^- = 70:30$) and $S_0Q_B:S_0Q_B^-:S_1Q_B:S_1Q_B^- = 21:9:49:21$. (c) The Q_B pool is 100% oxidized ($Q_B:Q_B^- = 100:0$) and $S_0Q_B:S_0Q_B^-:S_1Q_B:S_1Q_B^- = 30:0:70:0$.

by the number of centers in the $S_0Q_B^-$ and $S_1Q_B^-$ states, the variations in the oscillatory patterns could be satisfactorily simulated (Fig. 7).

The binary oscillation of the A band could also be successfully simulated on the assumption that the Q_B pool is in the completely oxidized state (Q_B:Q_B^- = 100:0) and that the amplitude of the band is proportional to the sum of the centers present after each flash in the $S_2Q_B^-$ and $S_3Q_B^-$ states. Considering that the thermoluminescence yield is about twice as high in the S_3 state as in the S_2 state (see the discussion of Fig. 9), satisfactory agreement has been obtained between the measured and calculated oscillatory patterns (compare the solid and dashed lines of Fig. 4b).

It is rather perplexing that, in spite of our conclusion that neither the water-splitting system nor the secondary acceptor participates directly in the generation of the Z_v and A bands [20], we observed both the period-4 and the period-2 oscillation of these bands. The oscillations could arise by two possible mechanisms:

(i) We can assume a direct electrostatic effect of the accumulated charges on the luminescence yield [29,30].

(ii) The electron donor and acceptor molecules interacting in a charge recombination reaction are in charge equilibrium with the water-splitting system and the secondary acceptor, respectively.

Our observations favor the second alternative (ii). In the glow curve of dark-adapted chloroplasts the conversion of the period-2 oscillatory pattern of thermoluminescence into a period-4 oscillation with decreasing excitation temperature (approx. −65°C) cannot be interpreted simply by a change in the electrostatic effect of negatively charged Q_B on the thermoluminescence yield. It is more plausible to assume that the charge exchange is inhibited between Q_A and Q_B at −65°C.

The results described in the present work demonstrate that the period-4 oscillation patterns of the low-temperature thermoluminescence depend on the excitation temperature (Fig. 3). It has been reported that the S state transitions of the water-splitting system are also temperature-dependent [19,31]. We may assume that the two phenomena are related. In order to test this assumption, we investigated the temperature-dependence of the S-state conversions. It has been reported that both S_2 and S_3 states contribute to the generation of the B band (recombination of $S_2Q_B^-$ and $S_3Q_B^-$) appearing at about +30°C [10–12]. In a sequence of flashes given at +2°C, the B band showed a maximum after the second flash, indicating that the emission intensity is highest in the S_3 state (Fig. 8, solid line). When an additional low-temperature illumination was applied, as also performed in the excitation of the Z_v and A bands, the oscillation pattern varied with the temperature of illumination. When chloroplasts were excited with continuous light at −20°C after two flashes at +2°C, the amplitude of the B band was smaller than that observed without continuous illumination, and it was almost constant, independently of the number of preilluminating flashes (Fig. 8, dashed line). In contrast, continuous illumination at either −40 or −60°C after two preflashes only slightly affected the B band height, consistent with the observation of Läufer et al. [32] (Fig. 8, alternating dot-dash line and the dotted line). It can be inferred that continuous illumination at −20°C results in several turnovers of the water-splitting system, causing an almost equal distribution of the S states. To confirm this interpretation, the oscillation of the B band was excited with a series of flashes at −20°C (not shown). A damped, but clear period-4 oscillation could be induced, with maxima at the second and sixth flashes, suggesting that the water-splitting system can complete several cycles at −20°C. On the other hand, after two preilluminating flashes, continuous illumination of the chloroplasts at −40 and −60°C did not change the amplitude of the B band (Fig. 8), indicating that the S_3 state cannot advance further upon continuous illumination, since the $S_3 \rightarrow S_4$ transition is inhibited at these temperatures. This conclusion was corroborated by experiments in which the B band was excited only with continuous light at various temperatures, without flash preillumination (Fig. 9). With decreasing excitation temperature, the emission intensity of the B band gradually increased, reaching a maximum at about −40°C; this was followed by a sharp decline, ending in an almost constant level below −70°C. In agreement with our previous conclusions, the temperature-dependence of the B band height can also be explained by assuming a gradual inhibition of the $S_3 \rightarrow S_4$ transition with decreasing temperature. Since the emission intensity of the B band is the highest in the S_3 state, the inhibition of the $S_3 \rightarrow S_4$ transition in an increasing number of electron transport chains can account for the progressive intensification of the B band. At −40°C, the $S_3 \rightarrow S_4$ transition is completely blocked and the B band exhibits a maximum. At temperatures lower than −40°C, the $S_2 \rightarrow S_3$ transition is also gradually inhibited, causing a concomitant decrease in the amplitude of the B band.

The threshold temperature for the complete inhibition of the $S_2 \rightarrow S_3$ transition can be determined from a comparison of the temperature-dependences of the B and Q thermoluminescence bands (Fig. 9). The Q band can be induced by the DCMU treatment of the chloroplasts, and appears at about +10°C in the glow curve [10–12]. While both S_2 and S_3 states participate in the generation of the B band, the Q band is associated only with the S_2 redox state [10–12]. Above −65°C, the amplitudes of the B and Q bands exhibited different temperature-dependences (Fig. 9). Below −65°C, however, the amplitudes of the two bands were the same and followed the same temperature-dependence, suggesting that the $S_2 \rightarrow S_3$ transition is completely blocked at about −65°C and that the remaining portion of the B band is related only to the S_2 state. It is noteworthy that the maximum intensity of the B band observed at −40°C was twice that of the Q band. This observation suggests, in agreement with recent conclusions [33,34], that the thermoluminescence yield is about twice as high in state S_3 as in state S_2.

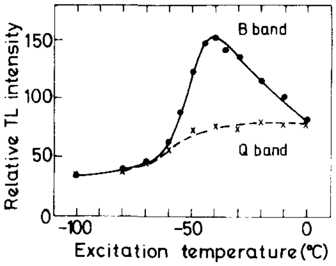

Fig. 9. Changes in amplitude of the B (+30°C) and Q (+10°C) thermoluminescence bands as functions of the excitation temperature. The B and Q bands were excited with continuous light for 30 s in untreated and 10 μM DCMU-treated chloroplasts, respectively. Chloroplasts were preilluminated with continuous light for 15 s at +30°C and kept in the dark for 3 min before thermoluminescence measurements ($Q_B:Q_B^- \approx 50:50$).

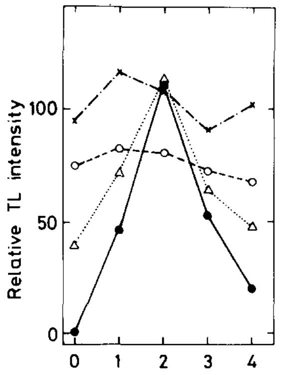

Fig. 8. Effect of low-temperature continuous illumination on the oscillation of the B thermoluminescence band induced by a variable number of flashes at +2°C. Isolated spinach chloroplasts were excited with a series of flashes at +2°C (———), followed by continuous illumination for 30 s at various low temperatures: −20°C (— — —); −40°C (·–·–·) and −60°C (······).

The threshold temperatures obtained in this work for the individual S state transitions differ significantly from those determined by Inoue and Shibata [19] from thermoluminescence measurements. On the other hand, the temperature-dependence of the EPR multiline signal associated with the S_2 state [31] lends support to our result that the threshold temperature for the $S_2 \rightarrow S_3$ transition is about −65°C. Inoue and Shibata investigated the temperature-dependence of S state transitions with flash excitation, while we applied continuous illumination. It has been demonstrated that the threshold temperature for generation of the multiline signal with continuous light is lower than the threshold temperature for generation of the signal with a single flash [31]. Thus, the discrepancy between our results and those of Inoue and Shibata may be resolved by taking into account the different illumination conditions. We emphasize that the threshold temperatures of the S state transitions were determined from the behavior of the B band, which reflects only the final state of the water-splitting system, reached after warming-up of the sample. However, it may occur that on low-temperature excitation the positive charge is trapped on an intermediate donor, and is transferred to the water-splitting system only upon warming, inducing an advancement of the S state system. Thus, it is not known from our measurements whether, at a given threshold temperature, an S state transition itself or some precursor state is inhibited.

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