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Effect of pH on the thermoluminescence of spinach chloroplasts in the presence and absence of Photosystem II inhibitors

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The glow curve of chloroplasts excited by continuous light of high intensity (500 $W \cdot m^{-2}$) at pH 7.5 during cooling from +2 to −80°C consisted of seven bands appearing at about −30°C (TL_{-30}), −15°C (TL_{-10}), +10°C (TL_{+10}), +30°C (TL_{+30}), +50°C (TL_{+50}), +65°C (TL_{+65}) and +85°C (TL_{+80}), in which TL stands for thermoluminescence. In the pH range from 5.5 to 9.0 the peak positions of the TL_{-30}, TL_{-10}, TL_{+50}, TL_{+65} and TL_{+80} bands were independent of pH. On the other hand the peak positions of the TL_{+10} and TL_{+30} bands were gradually shifted from +25 to −5°C and from +20 to +40°C, respectively, as the pH was decreased from 9.0 to 5.5. The same pH-induced shift (from +25 to −5°C) was observed for the TL_{+10} band when electron transport was inhibited by DCMU. In dinoseb-treated chloroplasts the peak position of the main thermoluminescence band also exhibited pH dependency, and shifted from +20 to −20°C upon lowering the pH from 9.0 to 5.5. After the water-splitting system had been inactivated by Tris or NH_2OH treatment no pH-induced shifts were observed in the peak positions of the thermoluminescence bands of DCMU and dinoseb-treated chloroplasts. The results suggest that the effect of pH on the thermoluminescence of untreated and inhibitor-treated chloroplasts is associated with protonation/deprotonation reactions occurring at the donor and acceptor sides of Photosystem II during the $S_1 \rightarrow S_2$ transition of the water-splitting system.

Introduction

Considerable progress has been achieved in the last few years in research into thermoluminescence of chloroplasts. It has been demonstrated that upon flash excitation the main thermoluminescence band appearing at about +30°C in the glow curve (B band), originates from the charge recombination of the $S_2Q_B^-$ and $S_3Q_B^-$ redox couples [1,2]. At pH 5.0 the B band is split into the B_1 ($S_3Q_B^-$ recombination) and B_2 ($S_2Q_B^-$ recombination) component bands peaking at about +20 and +45°C, respectively [3]. In the presence of Photosystem II inhibitors, which interrupt electron transport between Q_A and Q_B the B band is replaced by the so-called Q band [2] (also designated as the D band [4]). The Q band is assigned to charge recombination of the $S_2Q_A^-$ redox couple [2].

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Abbreviations: ADRY, acceleration of the deactivation reactions of the water-splitting enzyme system Y; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; dinoseb, 2,4-dinitro-6-isobutylphenol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Q_A, primary quinone electron acceptor of Photosystem II; Q_B, secondary quinone electron acceptor; TL, thermoluminescence.

In spite of our increasing knowledge of the thermoluminescence of chloroplasts there is some controversy in the literature, concerning not only the origin of certain thermoluminescence bands but even regarding the number of bands overlapping in the glow curve [5]. The situation is further complicated by the fact that the position of certain thermoluminescence bands depends on the pH of the medium [6,7]. The shift of the B_2 band to higher temperatures upon decreasing pH was explained by a protonation of the reduced secondary acceptor, Q_B^- at acidic pH values and by a lack of proton release in the $S_1 \rightarrow S_2$ transition [7]. The effect of pH on the peak position of the B band was theoretically analyzed in order to determine the pH induced change in the value of the redox midpoint potential of Q_B and in the equilibrium constant of the equilibrium $Q_A^- Q_B \rightleftharpoons Q_A Q_B^-$ [6]. In contrast to the above findings recently Vass et al. [8] observed a shift of the B band to lower temperatures with decreasing pH. Furthermore they also reported that in DCMU-treated chloroplasts the peak position of the Q band was shifted from +11°C to +30°C upon changing the pH from 7.5 to 8.8. The phenomenon was ascribed to an effect of pH on the redox potential of Q_A^-.

In the present work we investigated the effect of pH on the thermoluminescence of DCMU-, and dinoseb-treated chloroplasts in a wider pH range between pH 5.5 and 9.0. It was found that the rate of the backreaction of Q_A^- with the donor side of Photosystem II strongly depends on the pH between pH 6.5 and 9.0. After Tris or NH_2OH treatment the thermoluminescence of inhibitor-treated chloroplasts was independent of pH. This observation implies that the donor side of Photosystem II undergoes a deprotonation reaction during the $S_1 \rightarrow S_2$ transition of the water-splitting system.

Materials and Methods

Chloroplasts were isolated from spinach leaves using a procedure described earlier [9]. After isolation the chloroplasts were suspended in a medium containing 0.4 M sorbitol/10 mM NaCl/1 mM $MnCl_2$/5 mM $MgCl_2$/2 mM EDTA and 50 mM Hepes (pH 7.5) to yield 2–3 mg chlorophyll/ml and were kept on ice in darkness. Before thermoluminescence measurements the chloroplasts suspension was diluted further with the suspension medium of appropriate pH to give a chlorophyll concentration of 125 μg Chl/ml and incubated in the dark for 10 min.

DCMU and dinoseb were added to the samples in the dark and the samples were incubated in the presence of the inhibitors for 10 min before thermoluminescence measurements.

For Tris treatment, chloroplasts were suspended in 0.8 M Tris-HCl (pH 8.8) to yield 2 mg Chl/ml and incubated for 20 min under room light at +4°C before being pelleted. After washing of the chloroplasts twice, they were resuspended in the standard suspension medium and stored in the dark at +4°C until use. NH_2OH treatment was carried out by incubating the chloroplasts in the presence of 5 mM NH_2OH for 20 min in the dark at +4°C, followed by 5 min centrifugation at 2000 × *g*. The pelleted chloroplasts were washed twice and resuspended in the suspension buffer to give a concentration of 125 μg Chl/ml.

For thermoluminescence measurements 0.4 ml aliquots of samples were excited by white light during continuous cooling from +2 to −80°C. In flash experiments samples were excited either +2°C or in some experiments at −80°C by xenon flashes (General Radio, Stroboslave, 3 μs, 0.5 J). The flashes were given at 1 s intervals. After flash excitation at +2°C the samples were quickly cooled down to a temperature 20–30°C lower than the excitation temperature. Thermoluminescence was measured in an apparatus similar to that described by Tatake et al. [10] at a heating rate of 20°C/min.

Results

Dependence of the shape of the glow curve on the excitation conditions

It has been reported by several authors that the excitation conditions greatly affect the shape of the glow curve [5,11,12]. Sane et al. [12] investigated the effect of different light intensities on the glow curve and came to the conclusion that all of the peaks are present at very low light intensities. However, at pH 7.5 at low light intensities (less

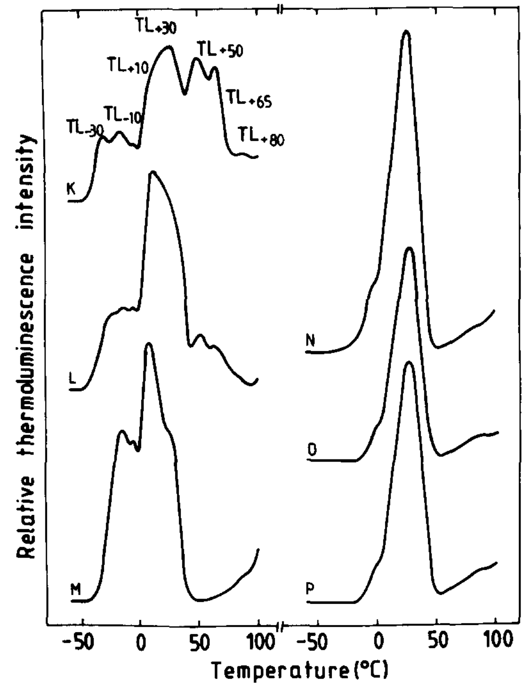

Fig. 1. Thermoluminescence of spinach chloroplasts excited at various light intensities. Samples were illuminated at pH 7.5 either by white light during cooling from +2 to −80°C (curves K, L, M, N, O) or by a single flash given at +2°C (curve P). K: 1000 W·m^{-2}; L: 500 W·m^{-2}; M: 1 W·m^{-2}; N: 0.1 W·m^{-2}; O; 0.01 W·m^{-2}. The distortion appearing at 0°C in the glow curves is caused by the solid-liquid phase transition of water.

than 1 W · m^{-2}) or at flash excitation we could charge only the B band at +30°C and the number of the excitable bands increased with the excitation light intensities (Fig. 1). At high light intensities (500–1000 W · m^{-2}) seven thermoluminescence bands could be charged in the glow curve appearing at about −30°C (TL$_{-30}$), −15°C (TL$_{-10}$), +10°C (TL$_{+10}$), +30°C (TL$_{+30}$), +50°C (TL$_{+50}$), +65°C (TL$_{+65}$) and about +85°C (TL$_{+80}$).

The TL$_{-30}$, TL$_{+10}$ and TL$_{+30}$ bands are probably identical to the A, Q (or D) and B bands which have already been described in the literature [1,2,5,13]. The TL$_{-10}$ band is probably equivalent to peak II of Sane et al. [13,14]. As to the exact number of thermoluminescence bands in the high temperature region of the glow curve considerable confusion exists in the literature [5,14–18].

The general consensus is that the high-temperature part of the glow curve consists of only one so-called, C band, superimposed at about +50°C on a quickly rising black body radiation of the sample holder [5]. The present work provided experimental evidence to show that by choosing appropriate excitation conditions three thermoluminescence bands contribute to the high-temperature region of the glow curve. One band peaking at about +85°C (TL$_{+80}$) may be related to chemiluminescence of chlorophylls [15]. Another band appearing at about +65°C (TL$_{+65}$) may correspond to peak VI of Desai et al. [15]. It is of note that Klimov et al. [19] observed this band in the glow curve of manganese free Photosystem II particles. The TL$_{+50}$ band is identical to the C band of Inoue and Shibata [5] and peak V of Sane et al. [12,14].

The effect of pH on the thermoluminescence of isolated spinach chloroplasts

The shape of the glow curve depends not only on the excitation conditions but is considerably influenced by the pH of the suspension medium, too. We have investigated the effect of pH on the thermoluminescence bands between pH 5.5 and 9.0. Thermoluminescence was excited both at low (1 W · m^{-2}) and high (500 W · m^{-2}) light intensities.

Fig. 2 shows that in agreement with previous results [9] the TL$_{-30}$ band (A band) is not considerably influenced by pH. The TL$_{-10}$ band seems to vanish at both low pH and high light intensities, but can be clearly distinguished as a shoulder on the descending side of the TL$_{-30}$ band upon excitation of chloroplasts at low light intensities. The peak positions of the TL$_{+50}$, TL$_{+65}$ and TL$_{+80}$ bands do not depend on pH. On the other hand the peak positions of the TL$_{+10}$ (Q) and TL$_{+30}$ (B) bands show a marked pH dependence. At pH 8.5 the two bands cannot be distinguished because they overlap each other. At pH 9.0 they apparently cross each other and the TL$_{+10}$ band appears at a little higher temperature (+25°C) than the TL$_{+30}$ band (20°C). At pH 7.5 the two bands become gradually separated. The TL$_{+30}$ band appears as a shoulder on the descending side of the TL$_{+10}$ band. Between pH 7.5 and 5.5 the separation of the two bands increases. While the TL$_{+30}$ band is shifted higher, the TL$_{+10}$ band is

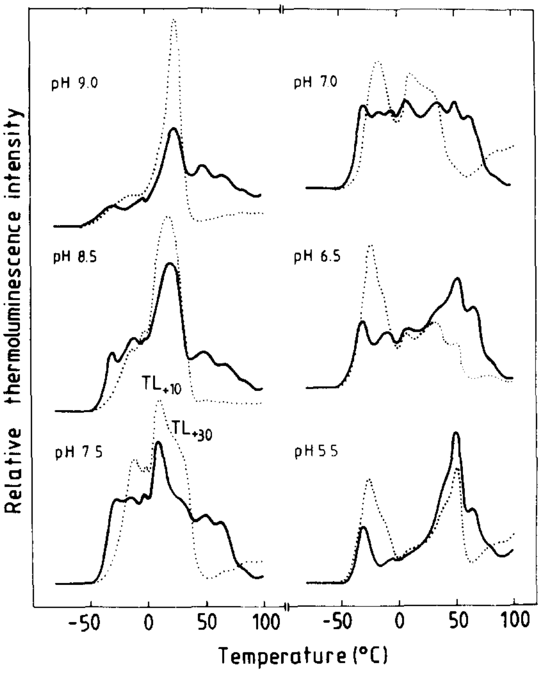

Fig. 2. Effect of pH on the thermoluminescence of spinach chloroplasts. Chloroplasts were incubated in the dark for 10 min at the appropriate pH before thermoluminescence measurements. Thermoluminescence was excited by white light of either high (500 $W \cdot m^{-2}$) or low (1 $W \cdot m^{-2}$) intensity during cooling the samples from +2 to −80°C. Continuous and dotted curves represent high and low intensity light, respectively.

shifted to lower temperatures. Simultaneously, the emission intensity of the TL_{+10} band gradually decreases until it can be barely observed at pH 5.5. At pH 5.5 the peak positions of the TL_{+10} and TL_{+30} bands appear at −5 and +40°C, respectively. The pH-induced shift of the TL_{+30} band has been described earlier [6,7]. It was interpreted by a protonation of Q_B^- with decreasing pH. An explanation for the disappearance of the TL_{+10} band can also be suggested. The TL_{+10} band is probably related to Q_A^- [1,2]. During the course of thermoluminescence measurements, due to the slow heating rate of the sample, the electrons can proceed from Q_A to Q_B decreasing the amount of Q_A^- and consequently the amplitude of the TL_{+10} band. With decreasing pH the midpoint potential of Q_B becomes more positive and the leakage of electrons increases due to the increasing value of the equilibrium constant of the $Q_A \cdot Q_B^- / Q_A^- \cdot Q_B$ equilibrium [6].

Fig. 2 also shows that at pH 6.5 and pH 5.5 the TL_{+30} and TL_{+50} bands overlap each other, due to the shift of the TL_{+30} band to higher temperatures. In addition, it can be seen that the amplitude of the TL_{+50} band is considerably enhanced at low pH.

The effect of pH on the thermoluminescence of DCMU and dinoseb-treated chloroplasts

Fluorescence induction experiments demonstrate that in chloroplasts excited by continuous light not only the plastoquinone and the secondary acceptor pools are filled up by electrons but the primary acceptor molecules (Q_A) are also gradually reduced. In agreement with this, if the chloroplasts are illuminated by weak light or flashes, only the TL_{+30} band, which is associated with Q_B^-, appears in the glow curve (Fig. 1). However, excitation of thermoluminescence by high intensity light charges the TL_{+10} band, too. According to earlier reports [1,2,5,11] excitation of chloroplasts in the presence of Photosystem II inhibitors, which block electron transport between Q_A and Q_B and result in the accumulation of Q_A^-, also gives rise to the appearance of a thermoluminescence band at about +10°C (Q band). Thus it can be assumed that the TL_{+10} and the Q bands are equivalent to each other and are associated with the reduced primary acceptor, Q_A^-. In the following we will designate the TL_{+10} and TL_{+30} bands as Q and B bands, respectively.

Since in the presence of DCMU the leakage of electrons from Q_A^- is inhibited during the course of the thermoluminescence measurement it can be expected that DCMU treatment represents favourable conditions for charging the Q band. Indeed, DCMU addition resulted in an enlargement of the Q band as compared to the TL_{+50}, TL_{+65} and TL_{+80} bands (Fig. 3). At pH 9.0 the Q band appeared at +25°C. Decreasing the pH to 6.5 resulted in a shift of the band to approx. −5°C and no additional shift occurred upon further decrease of the pH.

In the glow curve of DCMU-treated chloroplasts the amplitude of the TL_{+50} band increased with decreasing pH similarly as it occurred in untreated chloroplasts.

DCMU addition abolished both the B and TL_{-30} bands indicating that these bands can be

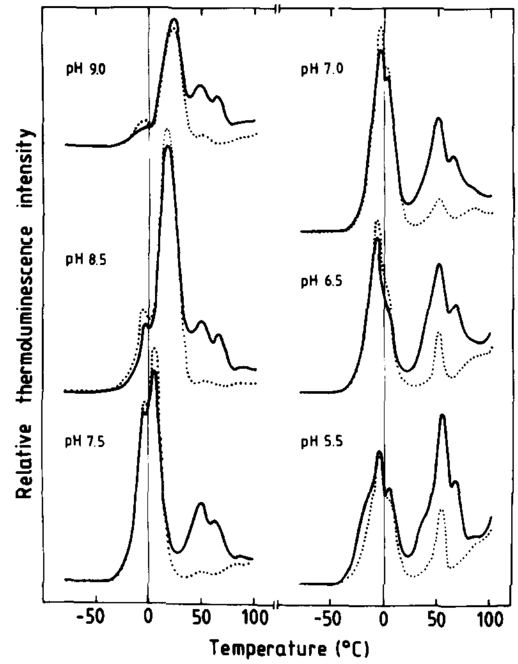

Fig. 3. Effect of pH on the thermoluminescence of DCMU-treated spinach chloroplasts. Prior to thermoluminescence measurements chloroplasts were incubated in the dark for 10 min at the appropriate pH in the presence of 10 μM DCMU. Excitation conditions are the same as were described in Fig. 2.

associated with Q_B^- [1,2,14] (but see also Ref. 9). However, at pH 5.5 and at high light intensities the TL_{-30} and the B bands appeared in the glow curve as small shoulders at the rising side of the Q and TL_{+50} bands, respectively (Fig. 3). This observation implies that at low pH and at high excitation intensities some of the electron-transport chains remained uninhibited even in the presence of 10 μM DCMU.

It has been reported that Photosystem II inhibitors, which block electron transport between Q_A and Q_B, can be classified into two groups on the basis of their properties [20,21]. These inhibitors are designated as DCMU and phenolic type inhibitors. The two groups can also be distinguished by thermoluminescence measurements [22]. While at pH 7.5 the peak position of the main thermoluminescence band appears between 0 and +10°C in the presence of DCMU-type inhibitors, chloroplasts inhibited by phenolic type inhibitors exhibit the main band between 0 and −10°C. Accordingly, in dinoseb (a phenolic herbicide) -treated chloroplasts at pH 7.5 the Q band can be observed at about −10°C (Fig. 4). We have tested whether in dinoseb treated chloroplasts the pH can also affect thermoluminescence or not. Changing the pH from 9.0 to 6.5 the Q band is shifted from +20 to −20°C. The phenomenon was similar to the one observed after DCMU addition, except that the peak position of the Q band appeared at lower temperatures in dinoseb-treated chloroplasts than in DCMU-treated ones. Interestingly, the amplitude of the band diminished considerably with decreasing pH indicating that dinoseb shows ADRY reagent characteristics at low pH [23,24]. This conclusion is consistent with the earlier observation of Renger [25] according to which 2,4-dinitrophenol exhibits ADRY effect.

In DCMU- and dinoseb-treated chloroplasts the reaction center can undergo only one turnover. However, at low pH (pH 5.5) and at high light intensity, continuous illumination may cause the transfer or more than one electron through the

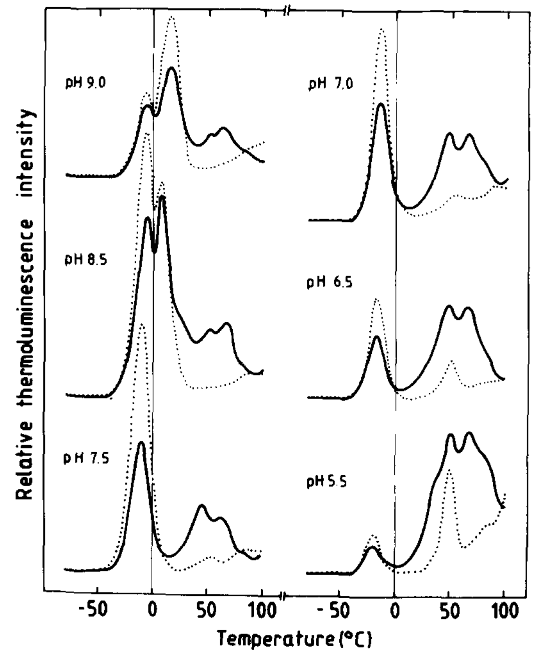

Fig. 4. Effect of pH on the thermoluminescence of dinoseb-treated spinach chloroplasts. Before thermoluminescence measurements chloroplasts were incubated in the dark for 10 min at the appropriate pH in the presence of 20 μM dinoseb. Other measuring conditions are the same as described in Fig. 2.

reaction center. In order to determine whether one turnover of Photosystem II is enough to observe pH induced shift in the peak position of the B and Q bands chloroplasts were excited by a single flash at $-80\,^\circ C$. In these measuring conditions only the $S_1 \rightarrow S_2$ transition can proceed [26] and the probability of double hits is negligible.

The pH dependence of the flash-induced thermoluminescence in untreated and inhibitor-treated chloroplasts was very similar to the previously described continuous-light-induced thermoluminescence (Fig. 5). In untreated chloroplasts the B band was shifted from $+20$ to $+40\,^\circ C$ upon changing the pH from 9.0 to 5.5. In DCMU- and dinoseb-treated chloroplasts the peak position of the Q band was shifted from about $+25$ to $-5\,^\circ C$ and from $+20$ to $-20\,^\circ C$, respectively. Consequently, the results of flash experiments are in excellent agreement with those obtained by the use of continuous illumination. It can be concluded that the pH induced shifts of the B and Q bands are associated with the singly oxidized and reduced state of the donor and acceptor sides of Photosystem II, respectively.

The effect of pH on the peak position of the B band has been interpreted by a protonation of Q_B^- with decreasing pH [6,7]. The shift of the Q band to lower temperatures by decreasing pH indicates an increase in the rate of backreaction between the negatively charged donor and positively charged acceptor molecules which participate in the generation of the band. This conclusion is supported by the results of fluorescence yield experiments. According to Vermaas et al. [27] the half time of fluorescence decay in the presence of DCMU ($S_2Q_A^-$ recombination) decreased from 1.15 s at pH 7.6 to 0.73 s at pH 6.0. Robinson and Crofts [28] also reported that the rate of backreaction from Q_A^- to S_2 shows a marked pH dependence, i.e., it increases rapidly with decreasing pH.

In order to determine whether the donor or acceptor side of Photosystem II is responsible for the pH dependence of the thermoluminescence of inhibitor treated chloroplasts the water-splitting system was irreversibly inactivated either by Tris or NH_2OH treatment. In chloroplasts treated with

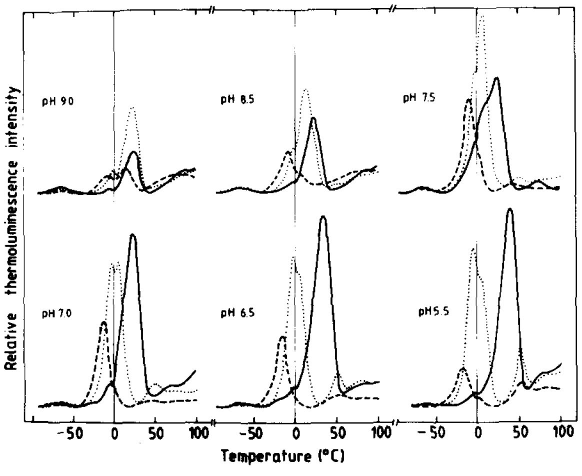

Fig. 5. Effect of pH on the flash-induced thermoluminescence of untreated (———), DCMU treated (······) and dinoseb treated chloroplasts (— — —). For inhibitor treatment chloroplasts were incubated in the dark for 10 min at the appropriate pH in the presence of either 10 μM DCMU or 20 μM dinoseb. Thermoluminescence was excited by a single flash at $-80\,^\circ C$.

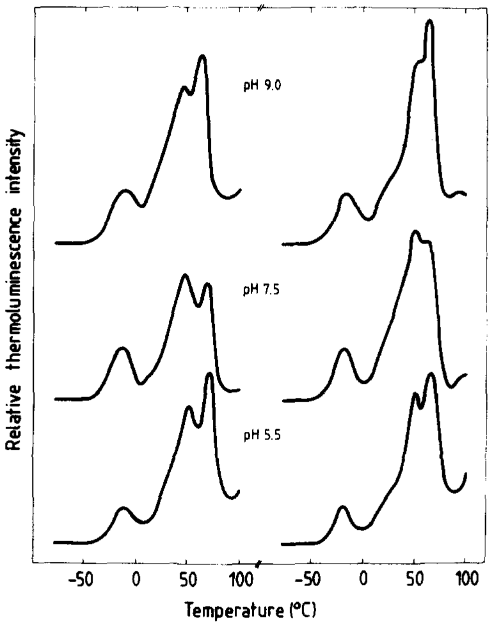

Fig. 6. Effect of pH on the thermoluminescence of Tris-treated (left side) and NH_2OH-treated (right side) chloroplasts in the presence of 10 μM DCMU. Tris washing and NH_2OH treatment were carried out as described in the Materials and Methods section. After Tris or NH_2OH treatment chloroplasts were incubated in the dark for 10 min at the appropriate pH in the presence of 10 μM DCMU before thermoluminescence measurements. Thermoluminescence was excited by continuous white light of 500 $W \cdot m^{-2}$ during cooling the sample from $+2$ to $-80\,^\circ C$.

1 μM DCMU, after the inhibition of the donor side of Photosystem II by Tris or NH_2OH treatment, only the TL_{-30}, TL_{+50}, TL_{+65} and TL_{+80} bands could be excited (Fig. 6). All of the bands observed in Tris- and NH_2OH-treated chloroplasts in the presence of DCMU were pH independent in the peak position. A similar observation was made if the chloroplasts having an inactivated water-splitting system were treated by 20 μM dinoseb (not shown).

Discussion

The peak position of a thermoluminescence band is determined by the redox span between the positively charged donor and negatively charged acceptor molecules undergoing charge recombination. Any change in the midpoint potentials of the interacting donor and acceptor components should be reflected in the peak position of the appropriate thermoluminescence band. If the reduction or oxidation of an electron transport component is associated with a protonation/deprotonation reaction the midpoint potential of the component should exhibit a pH dependence. Thus investigation of the effect of pH on the peak positions of the thermoluminescence bands can provide information as to whether certain electron-transport steps are accompanied by proton release/uptake or not.

The Q band observed in the presence of DCMU and dinoseb is associated with the $S_2Q_A^-$ redox couple [2]. The peak position of the band is shifted to lower temperatures with decreasing pH. This indicates a decrease in the redox distance between the S_2 state and Q_A. Accordingly, either the redox potential of the S_2/S_1 couple should increase or that of the Q_A/Q_A^- redox pair should decrease when the pH is lowered. It has been reported that the midpoint potential of Q_A exhibits a −59 mV per pH unit dependence [29]. However, an increase in the midpoint potential of Q_A with decreasing pH should result in a shift of the peak position of the Q band in a direction opposite to the one observed. Furthermore, it has been shown by Conjeaud and Mathis [30] that the rate of backreaction from Q_A^- to $P\text{-}680^+Z^+$ is not affected by pH. In a recent work [9] we have reported that the peak position of the Z_v and A bands, which are assigned to Q_A^-, do not depend on pH. On the basis of these observations it can be inferred that the effect of pH on the peak position of the Q band cannot be attributed to a change in the midpoint potential of Q_A. This conclusion is substantiated by thermoluminescence measurements carried out with Tris- and NH_2OH-treated chloroplasts. Tris washing and NH_2OH treatment, which affect the action of the donor side of Photosystem II but do not influence the functioning of the Q_A and Q_B acceptors, completely removed the pH dependency of the thermoluminescence of DCMU-treated chloroplasts suggesting that the effect of pH on the Q band is associated with the donor side of Photosystem II. It can be assumed that the pH induced shift of the Q band is due to an increase in the redox potential of the S_2 state. If an S-state transition involves a proton release the midpoint potential of the S_{n+1}/S_n redox couple depends on the pH of the medium by −59 mV/pH unit. Thus one can conclude that during the $S_1 \rightarrow S_2$ transition the water-splitting system is deprotonized. This assumption can hardly be reconciled with the pH independence of the multiline EPR signal of the S_2 state [31] and with the pH dependence of the absorption changes due to electrochromic band shift of carotenoids [32]. The results of delayed luminescence [33], EPR [34] and pH [35] measurements also contradict proton release for the $S_1 \rightarrow S_2$ transition. Therefore another explanation can be offered if it is assumed that in inhibitor treated chloroplasts the proton release is due not to the water-oxidizing complex itself but to a protolytic reaction occurring at the level of other electrons donors in Photosystem II. Hong et al. [36] observed a cyclic protolytic reaction around Photosystem II in DCMU-poised chloroplasts which was not associated with water oxidation. Furthermore, in Tris-washed inside-out thylakoids the light-induced oxidation of the intermediate donor D_1 (Z) was found to be coupled with a H^+ release [37].

On the basis of our thermoluminescence results obtained by excitation of inhibitor-treated chloroplasts at −80°C it can be inferred that the deprotonation event at the donor side of Photosystem II proceeds at low temperatures, too. Thus, it can be concluded that the component undergoing a deprotonation is not associated with the electron-transport chain directly but could be a protein group close to a functionally active electron donor. This membrane group is deprotonated in the dark either before the sample is cooled down or during heating of the sample after thermoluminescence has been induced by flash excitation at −80°C. However, it must not be overlooked that according to the recent investigation of Jursinic and Stemler [38], NH_2OH in addition to inhibiting the water-splitting system, also has a site of action on the reducing side of Photosystem II. An inhibitory effect of NH_2OH on the acceptor side of Photosystem II may also affect the protonation properties of the primary and secondary quinone acceptors. Thus our experiments carried out with NH_2OH do not completely exclude the occurrence of a protonation step at the acceptor side of Photosystem II in inhibitor-treated chloroplasts. Moreover, it has been shown that the S_2 state participates in the generation of both the Q($S_2Q_A^-$ recombination) and B($S_2Q_B^-$-recombination) bands. Thus a deprotonation event occurring at the donor side of Photosystem II during the $S_1 \rightarrow S_2$ transition should influence similarly the peak positions of these bands. In contrast to this expectation the Q and B bands exhibit opposite pH dependencies. While the Q band is shifted to lower, the B band moves to higher temperatures with decreasing pH. This discrepancy cannot be resolved at present. Thus, it is possible that a pH-induced change at the acceptor side of Photosystem II also contributes to the shifts of the Q band. It has been reported by Rutherford and Zimmermann [39] that the primary quinone acceptor has two interconvertable EPR forms. While the protonated form ($g = 1.82$) appears at low pH or in the presence of dinoseb, high pH is favorable for the appearance of the deprotonated form ($g = 1.90$). The Q band may represent the charge recombination of the two EPR forms of Q_A with the S_2 state. It can be assumed that depending on the pH of the medium the midpoint potential of the dominating form of Q_A determines the peak position of the Q band. Parallel EPR and thermoluminescence measurements could test the validity of this second interpretation.

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References

1 Rutherford, A.W., Crofts, A.R. and Inoue, Y. (1982) Biochim. Biophys. Acta 682, 457–465

2 Demeter, S. and Vass, I. (1984) Biochim. Biophys Acta 764, 24–32

3 Inoue, Y. (1981) Biochim. Biophys. Acta 634, 309–320

4 Inoue, Y. (1983) in The Oxygen Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Sato, K., eds.), pp. 439–450

5 Inoue, Y. and Shibata, K. (1982) in Photosynthesis I. Energy Conversion by Plants and Bacteria (Govindjee, ed.), pp. 507–533, Academic Press, New York

6 Demeter, S., Vass I., Hideg, E. and Sallai, A. (1985) Biochim. Biophys. Acta 806, 16–24

7 Rutherford, A.W., Renger, G., Koike, H. and Inoue, Y. (1984) Biochim. Biophys. Acta 767, 548–556

8 Vass, I., Koike, H. and Inoue, Y. (1985) Biochim. Biophys. Acta 810, 302–309

9 Demeter, S., Rózsa, Zs., Vass, I. and Sallai, A. (1985) Biochim. Biophys. Acta 809, 369–378

10 Tatake, V.G., Desai, T.S. and Battacharjee, S.K. (1971) J. Phys. E. Sci. Instr. 4, 755–757

11 Rubin, A.B. and Venediktov, P.S. (1969) Biofizika 14, 105–109

12 Sane, P.V., Desai, T.S., Tatake, V.G. and Govindjee (1977) Photochem. Photobiol. 26, 33–39

13 Sane, P.V., Desai, T.S., Tatake, V.G. (1983) Indian. J. Exp. Biol. 21, 396–400

14 Sane, P.V., Desai, T.S., Tatake, V.G. (1983) Indian. J. Exp. Biol. 21, 401–404

15 Desai, T.S., Tatake, V.G. and Sane, P.V. (1982) Photosynthetica 16, 129–133

16 Demeter, S., Vass, I., Horváth, G. and Läufer, A. (1984) Biochim. Biophys. Acta 764, 33–39

17 Mustárdy, L.A., Rózsa, Zs. and Faludi-Dániel, Á. (1981) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I. pp. 665–672, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands

18 Inoue, Y., Furuta, S., Oku, T. and Shibata, K. (1976) Biochim. Biophys. Acta 449, 357–367

19 Klimov, V.V., Allakhverdiev, S.I., Shafiev, M.A. and Demeter, S. (1985) Biochim. Biophys. Acta 809, 414–420

20 Oettmeier, W. and Trebst, A. (1983) in The Oxygen Evolving System of Photosynthesis (Inoue Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.), pp. 411–420, Academic Press Japan, Tokyo

21 Pfister, K. and Schreiber, U. (1984) Z. Naturforsch. 39c, 389–392
22 Vass, I. and Demeter, S. (1982) Biochim. Biophys. Acta 682, 496–499
23 Rutherford, A.W., Zimmermann, J.L. and Mathis, P. (1984) FEBS Lett. 165, 156–162
24 Renger, G. and Inoue Y. (1983) Biochim. Biophys. Acta 725, 146–154
25 Renger, G. (1972) FEBS Lett. 23, 321–324
26 Inoue, Y. and Shibata, K. (1978) FEBS Lett. 85, 193–197
27 Vermaas, W.F.J., Renger, G. and Dohnt, G. (1984) Biochim. Biophys. Acta 764, 194–202
28 Robinson, H.H. and Crofts, A.R. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 477–480, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands
29 Knaff, D.B. (1975) FEBS Lett. 60, 331–335
30 Conjeaud, H. and Mathis, P. (1980) Biochim. Biophys. Acta 590, 353–359
31 Damoder, R. and Dismukes, G.Ch. (1984) FEBS Lett. 174, 157–161
32 Saygin, Ŏ. and Witt, H.T. (1984) FEBS Lett. 176, 83–87
33 Bowes, M.H. and Crofts, A.R. (1981) Biochim. Biophys. Acta 637, 464–472
34 Willie, B. and Lavergne, J. (1982) Phtoobiochem. Photobiophys. 4, 131–144
35 Förster, V. and Junge, W. (1985) Photobiochem. Photobiophys. 41, 183–190
36 Hong, Y.Q., Förster, V. and Junge, W. (1981) FEBS Lett. 132, 247–251
37 Renger, G. and Voelker, M. (1982) FEBS Lett. 149, 203–207
38 Jursinic, P. and Stemler, A. (1983) Plant Physiol. 73, 703–708
39 Rutherford, S.Q. and Zimmermann, J.L. (1984) Biochim. Biophys. Acta 767, 168–175