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Low-pH-induced Ca^{2+} ion release in the water-splitting system is accompanied by a shift in the midpoint redox potential of the primary quinone acceptor Q_A

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pH-dependent regulation of Photosystem (PS) II (observed as 'high-energy quenching') has been characterized by chlorophyll fluorescence and thermoluminescence measurements in PS II particles, thylakoid membranes, alga cells and leaf tissue. Steady state redox titration of fluorescence yield performed at pH 6.5 revealed that the midpoint redox potential of the primary quinone acceptor, Q_A, is shifted towards positive direction from $E_m = -80$ mV to $E_m = +40$ mV (the absolute values for E_m were varying by about 40 mV between different preparations) after incubation of PS II particles at pH 4.2 for 15 min in the presence of the Ca^{2+} chelator, PP_i. The original midpoint potential was restored after the addition of 300 μM $CaCl_2$. Low-pH treatment (pH 4.6) of PS II particles also resulted in a decrease of the Q band of thermoluminescence (appearing between 10–14°C after DCMU addition) with a concomitant appearance or intensification of a high temperature band between 42–50°C (C band). In accordance with the results of the redox titration of fluorescence yield the C band is attributed to a low-pH-induced high potential form of Q_A. The interconversion of Q band into the C band was more pronounced in the presence of the Ca^{2+} chelator, EGTA. Addition of $CaCl_2$ to the low-pH-treated particles diminished the C band and restored the Q band. Light-induced acidification of the thylakoid lumen (ΔpH formation under illumination conditions of 'high-energy quenching') was also accompanied by a transformation of the Q band to the C band in isolated thylakoids, in the green alga, *Chlorella vulgaris* and in pea leaves. The phenomenon was completely reversed by abolishing the pH gradient with 10 mM NH_4Cl. Addition of the Ca^{2+}-channel inhibitor verapamil to the thylakoid suspension before the formation of a ΔpH suppressed the transformation of Q band into the C band. In contrast, when a ΔpH was first established and then verapamil was added, the ΔpH-induced change in the glow curve was irreversible and conversion of C band back to the Q band was prevented. It is suggested that the appearance of the C band is associated with Ca^{2+}-dependent reversible inactivation of the water-splitting system and with a shift in the redox potential of Q_A. We propose that pH-dependent Ca^{2+}-release is a physiological process which controls the electron transport of PS II in vivo.

Introduction

pH-dependent regulation of PS II (high-energy quenching) occurs in plants when photosynthesis is limited by the biochemical reactions of the carbon metabolism and excess light energy is absorbed. Under such conditions the ΔpH across the thylakoid membrane increases to values higher than that required for maximal ATP synthesis. A high ΔpH exerts control to PS II and ΔpH-dependent 'down-regulation' may adjust PS II activity to the actual requirement of electrons by carbon metabolism [1–3]. This 'energy-dependent' decrease in PS II activity is accompanied by a decline in chlorophyll fluorescence (high-energy

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Abbreviations: Chl, Chlorophyll; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; DMBQ, 2,5-dimethylbenzoquinone; EGTA, ethyleneglycolbis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Mes, 4-morpholinoethanesulfonic acid; Mops, 4-morpholinopropanesulphonic acid; PP_i, pyrophosphate; Q and C bands, thermoluminescence bands appearing at 10 and 45°C, respectively; Q_A and Q_B, primary and secondary quinone acceptor of PS II, respectively; PS, photosystem; S_2 and S_3, oxidation states of the water-splitting system; TL, thermoluminescence; Y_Z, a redox-active tyrosine that serves as the electron donor to the reaction center of chlorophyll *a*.

quenching, see Ref. 3), and excess light energy absorbed by PS II is dissipated probably as heat [4]. By this control process, the reaction center is protected against photodestruction; however, the mechanism of energy quenching is still unknown. There is a controversy in the literature, as to whether the quenching occurs in the antenna [5] or in the reaction center of PS II [1] (for a discussion, see Ref. 3). It has been proposed, that quenching in the antenna is related to the formation of zeaxanthin via the violaxanthin cycle [6] or aggregation of LHC II complexes [7].

Dissipation of excess energy in the reaction center has been discussed as a recombination process (see, e.g., Refs. 8–11). On the basis of time-resolved fluorescence spectroscopy, ΔpH-induced quenching of excited singlet states has been explained by regular photochemical charge separation followed by recombination between P680$^+$ and Q_A^- to the ground state [11]. Charge recombination at the reaction center is stimulated by a donor side limitation of PS II [8–10]. It is well documented, indeed, that the oxygen evolving system is impaired at low pH and that the ns-phase of P680$^+$ reduction is replaced by a μs-phase (see, e.g., Refs. 12–16). This donor-side limitation seems to be initiated by a pH-dependent release of Ca^{2+} ions. It was found by Boussac and co-workers [17] that photooxidation of the donor Y_Z is inhibited in Ca^{2+}-depleted PS II. Ono and Inoue [18] reported that incubation of PS II-enriched membranes at pH 3.0 causes a release of Ca^{2+} ions and inhibition of the $S_2 \rightarrow S_3$ transition of the water-splitting system. In contrast, Shen and Katoh [19] claimed recently that pH dependent inactivation of oxygen evolution is not directly caused by extraction of Ca^{2+} ions. In a previous study we have suggested that Ca^{2+}-release and inactivation of PS II may occur in a physiologically relevant pH-range as well [10].

Barr and co-workers [20] demonstrated the existence of another Ca^{2+}-selective binding site at the acceptor side of PS II between the acceptor sites of silicomolybdate (Q_A site) and 2,5-dimethylbenzoquinone (Q_B site). Indeed, the survey of literature (see, e.g. Refs. 11–14,17) indicates that exposure of isolated PS II to low pH can influence its donor and acceptor side.

Since the phenomenon of thermoluminescence (TL) can reflect changes occurring simultaneously at the donor and acceptor sides of PS II, we applied this technique to investigate pH-induced inactivation of PS II. pH effects on thermoluminescence glow curves have already been investigated before in the pH-range from 5.5 to 9.0 [21]. It was observed that at low pH the amplitude of the Q thermoluminescence band ($S_2Q^-{}_A$ charge recombination in the presence of DCMU) considerably decreased with a concomitant increase of a band around 50°C (C band). Ono and Inoue [22,23] demonstrated that treatment at very low pH (pH 3.0), when the activity of the water-splitting system is inhibited, evokes a drastical upshift of the Q thermoluminescence band from 13 to 45°C. These authors attributed the shift to altered redox properties of the donor side. On the basis of the similar peak positions and behaviour it can be assumed that the shifted Q band appearing at 45°C is identical with the C band [24]. Within a certain limit the temperature of TL after low pH-treatment varies with the treatment conditions. Binding of the 24 kDa protein may influence the exact position of that TL band [25].

In this paper we have investigated thermoluminescence at moderately low pH (pH 4.6) in BBY-particles and during light-induced internal acidification in isolated thylakoid membranes, algae and intact leaf tissue. By comparing TL with redox titration of Chl fluorescence, we attribute the observed reversible shift of the Q band from about 10 to 45°C to a concerted alteration of the water-splitting system and shift in the redox potential of the PS II acceptor side quinone, Q_A, both induced by pH-dependent Ca^{2+}-release at the lumen side. We discuss the significance of these changes for pH-dependent regulation in vivo.

Materials and Methods

Thylakoids were isolated from spinach and incubated in a medium containing 0.3 M sorbitol, 50 mM KCl, 7 mM $MgCl_2$, 15 mM Hepes (pH 7.6). To build up a ΔpH thylakoids were illuminated for 3 min with white light at a light intensity of 10 W/m^2 in the presence of an electron acceptor (50 μM methylviologen). The final chlorophyll concentration was 10–20 μg Chl/ml for measuring fluorescence or oxygen-evolution and 50 μg for measuring thermoluminescence. The thylakoids were uncoupled by 10 mM NH_4Cl or 2 μM nigericine. The transthylakoidal pH gradient was calculated from 9-aminoacridine fluorescence [26].

PS II particles (BBY) were prepared from spinach as described in Ref. 27. The preparations contained no detectable contamination with PS I as tested by gel electrophoresis and fluorescence spectroscopy at 77 K. The rate of oxygen evolution measured from water to DMBQ was about 550 μmol O_2/mg Chl per h. pH-dependent quenching was obtained by incubating the particles in a medium containing 0.3 M sorbitol, 50 mM KCl, 5 mM $MgCl_2$ and 30 mM of different buffer substances: glycylglycine (pH 4.0–4.5), succinic acid (pH 4.5–5.0), Mes (pH 5.0–6.5), Mops (pH 6.5–7.0). The particles (100 μg Chl/ml) were incubated for 15 min in the different media (5 min in weak light, 8–10 μmol quanta/m^2 per s, and 10 min in darkness) and transferred back to pH 6.5 (the 5-fold volume of the buffer pH 6.5 was added) before starting a measurement. For fluorescence the particles were measured directly at the pH value of the incubation medium. The light treatment was necessary because pH-induced

Ca^{2+} release appears at higher S-states of the Mn cluster.

Oxygen-evolution was measured with a Clark-type oxygen-electrode, using 1 mM DMBQ as acceptor for PS II at saturating light intensities ($I = 3000$ μmol quanta/m^2 per s). Stationary fluorescence was measured with a pulse modulation fluorometer (PAM 101, Walz). The F_m level of fluorescence (with reduced Q_A) was recorded during short (1–2 s) saturating light pulses. Actinic light from a halogen lamp was filtered through Calflex C, K 65 (Balzers) and RG 630 (Schott) filters. Illumination was started with a Compur-photoshutter. The difference between the F_m level and the F_o level (with oxidized Q_A) is noted as variable fluorescence.

Redox potentials were titrated in an O_2-free chamber with a Pt-calomel-electrode (Ingold) and a pH/mV-meter (Knick). Reductive titrations were carried out by addition of small amounts of $Na_2S_2O_4$ and oxidative titrations by addition of $K_3[Fe(CN)_6]$. 10 μM methylviologen was added as a redox mediator. The redox state of Q_A was followed by fluorescence measurements with the PAM fluorometer (Walz) using a modulated weak measuring light ($I < 1$ μmol quanta/m^2 per s, so that no photoreduction of Q_A could occur.

Thermoluminescence was measured as previously described [28]. Samples (thylakoids and algae) were excited at room temperature with continuous white light ($I = 40$ W/m^2) for 3 min, cooled down to 2–4°C, incubated at this temperature for 30 s with 20 μM DCMU (or a mixture of 20 μM DCMU and uncoupler) and cooled down quickly to −80°C, where they were again excited for 30 s. Glow curves (TL intensity as a function of temperature) were recorded at a heating rate of 20 C°/min.

In experiments with BBY-particles DCMU was added immediately after the incubation. They were cooled down directly to −80°C. Illumination conditions were as for thylakoids and algae.

Thermoluminescence measurements were carried out on pea leaves, which were illuminated for 3–5 min at a light intensity of 400 W/m^2 and were than infiltrated after the illumination with a 5 mM DCMU-solution for 20 s. For uncoupling, the leaves were incubated for 20 s in a mixture of 10 mM NH_4Cl, 2.5 mM nigericin and 5 mM DCMU.

Results

When a high ΔpH is created in the light and the pH in the lumen decreases below 5.5, the activity of PS II measured by oxygen evolution decreases in parallel to the variable part of Chl fluorescence. The apparent pK value of this inhibition is about 4.8 [11,29]. A similar pH response of fluorescence was obtained when isolated BBY particles were directly incubated in media of various pH. The pH response of oxygen evolution and fluorescence indicates a 1 H^+ transition with an apparent pK value of about 4.7 [3,9,10,11,18,29,30].

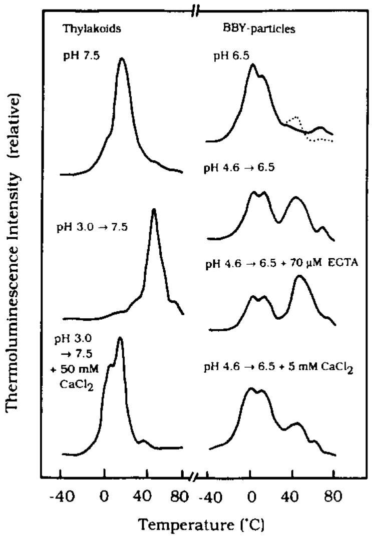

Fig. 1. Effect of low-pH treatment on thermoluminescence of thylakoids and BBY particles. Left: thylakoids were incubated at pH 3.0 for 5 min in weak light, the thylakoids were transferred to pH 7.5 and 10 μM DCMU was added before thermoluminescence measurement. Right: BBY particles were incubated at pH 4.6 (5 min in weak light and 10 min in darkness), 10 μM DCMU was added before the TL measurement at pH 6.5. TL was excited at −80°C by white light ($I = 40$ W/m^2) for 30 s and measured at a heating rate of 20 C°/min. Chlorophyll concentration of the samples was 50 μg Chl/ml.

Inactivation of the donor side at low pH has already been investigated (see, e.g., Refs. 12,15,16). Ono and Inoue [18,22] were the first who observed a reversible inactivation of PS II associated with Ca^{2+} release at very low pH. They also found that after incubation of PS-II-enriched membrane fragments at pH 3.0, in presence of DCMU, the peak position of the Q thermoluminescene band ($S_2Q_A^-$ charge recombination) was shifted from 13 to 45°C. By addition of 50 mM $CaCl_2$ the abnormal TL band was shifted back to the original position at 13°C. At the same time low pH induced a smaller reversible shift in the peak position of the B band ($S_2Q_B^-$ charge recombination) from 32 to 45°C. We have been able to repeat these experiments with thylakoids (Fig. 1, left). Low pH had similar effects on the thermoluminescence of DCMU-treated BBY particles (Fig. 1, right). A more moderate pH (pH 4.6 instead of pH 3.0) was chosen, because it is close to that obtained upon acidification of the thylakoid lumen under physiological conditions in presence of a high ΔpH. Compared to thylakoids, the peak temperature of the Q band of BBY particles is shifted by 5°C to lower temperatures most likely due to an altered surrounding of the Q_A acceptor (compare the left and right sides of Fig. 1). We note that the trough in the Q band at 0°C is an artifact caused by the solid-liquid phase transition of water [31]. During incubation at pH 4.6 the fluorescence was quenched by about 50% (data not shown, but see Refs. 10,11), suggesting that approx. 50% of the PS II centers are inactivated. After preincubation at this pH (but measured at pH 6.5) we observed in the thermoluminescence signals a decrease in the intensity of the Q band (10°C) by about 40% and a related appearance of the C band (42–44°C), which was nearly absent in the control (Fig. 1, right, second curve from the top). No change in the total intensity of thermoluminescence was observed. After incubation at pH 4.6 in presence of EGTA the Q-band (measured at pH 6.5) was further reduced (about 50%), as Ca^{2+} ions were complexed and could not rebind any more; (Fig. 1, right, second curve from the bottom). In presence of EGTA the decrease of the Q band was similar to the decrease of Chl fluorescence and O_2-evolution at the related pH (not shown). A small C band could even be seen in the control after the addition of EGTA (Fig. 1, right, top curve, dotted line). By the addition of 5 mM $CaCl_2$ the intensity of the Q-band in the control could be largely restored, and only a low emission peak remained at 45°C (Fig. 1, left, bottom curve). In most measurements we also observed a small peak at about 65°C [21], which, however, was not influenced by pH treatment.

Fig. 2 shows the dependence of the Q and C band (intensity at 45°C) in BBY particles on the pH of a pretreatment. An inverse correlation was found between the intensity of these two bands. The apparent p*K* value of the decrease in the amplitude of the Q band and the simultaneous increase of that of the C band (p*K* about 4.4; see Fig. 2), was similar to that of fluorescence quenching obtained under comparable conditions. When the treatment was carried out in presence of EGTA the apparent p*K* was shifted to somewhat higher values (not shown).

An upward shift in the peak temperature of a thermoluminescence band by 30°C suggests stabilization of the recombining charge pair, i.e., an increase in the difference of the redox potentials between the interacting oxidized donor and reduced acceptor. This was confirmed by redox titrations of fluorescence yield, which are supposed to reflect the redox state of the acceptor side quinone, Q_A (Fig. 3). As fluorescence was measured with a very dimmed light ($I \leq 1$ μmol quanta/m^2 per s), photochemical reduction of Q_A was prevented. Oxidative and reductive titrations have been carried out by addition of small amounts of ferricyanide and dithionite, respectively, at pH 6.5 with BBY particles previously incubated at pH 4.2. When PP_i was added as a chelator to prevent rebinding of Ca^{2+} (previously released at low pH) during the transition to high pH, a high potential form of Q_A (E_m about +40 mV) was titrated. Addition of 300 μM $CaCl_2$ (to reactivate the water-splitting complex) restored the normal low potential form (E_m about −80 mV). The dashed lines in Fig. 3 represent redox titrations carried out in the absence of PP_i and Ca^{2+} ions at pH 4.2 and pH 6.5 without pretreatment (the original values are shown in Ref. 11). Under such conditions, the midpoint potentials titrated from fluorescence were about +40 mV at pH 4.2 ('high-potential form') and about −120 mV ('low-potential form') at pH 6.5. Between different samples the titrated redoxpotentials could vary within a range of about 40 mV. The high potential form of Q_A was also obtained after inactivation of the water-splitting complex by Tris or NH_2OH treatment, and this form did not show any pH dependence [11]. A similar phenomenon could be observed in thermoluminescence: in Tris- and hydroxylamine-treated chloroplasts the Q band was replaced by the C band with a pH-independent peak position at around 50°C [21].

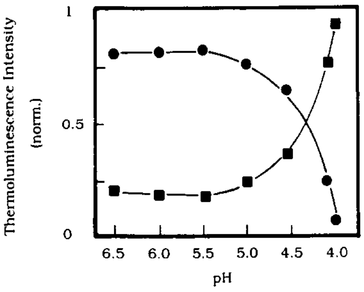

Fig. 2. pH dependence of the Q (●, 10°C) and C (■, 42°C) thermoluminescence bands in BBY particles. Thermoluminescence intensities were normalized to the amplitudes of the Q and C bands measured at pH 6.5. Other measuring conditions as in Fig. 1.

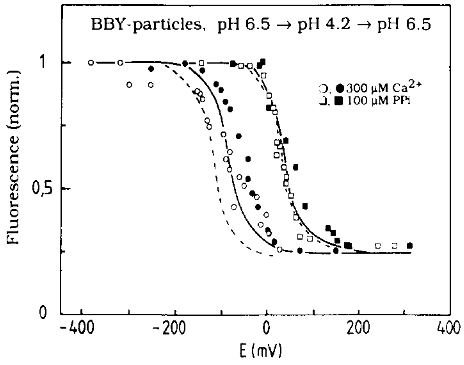

Fig. 3. Redox titration of fluorescence yield in low-pH-treated and control BBY particles. BBY particles were preincubated at pH 4.2 in weak light and then transferred to pH 6.5. Titrations have been carried out at pH 6.5. Addition of 100 μM PP_i: oxidative titration (■), reductive titration (□). Addition of 300 μM $CaCl_2$: oxidative titration (●), reductive titration (○). Reductive titration was carried out by addition of small amounts of $Na_2S_2O_4$ and oxidative titration by addition of $K_3[Fe(CN)_6]$. 10 μM methylviologen was added as a redox mediator. Dashed line: Nernst-curve ($n = 1$) for redox titrations carried out at pH 6.5 (right curve) or pH 4.2 (left curve). Original values see Ref. 10.

The results suggest that the occurrence of a high potential form does not depend primarily on low pH but is associated with an impaired donor side.

Our in vitro experiments with BBY particles demonstrated that incubation of PS II particles at low pH converts Q_A from its regular form to a high potential form. With thylakoids we could show that it is indeed the internal acidification of the lumen which causes Ca^{2+}-release and shift in the redox potential of Q_A. Fig. 4 shows the effect of light-induced internal acidification on the thermoluminescence in thylakoids. Formation of a ΔpH leads to a decrease of the Q band between 10 and 15°C and to a respective appearance of the C band at 43°C (Fig. 4, left, middle curve), similarly to what we observed after incubation of PS II particles at pH 4.6 (Fig. 1, right). This light-induced change was completely reversed by addition of the uncoupler NH_4Cl (Fig. 4, left side, bottom curve). When the Ca^{2+}-channel inhibitor verapamil was added before illumination the thermoluminescence became insensitive to the acidification and only a small change was seen in the glow curves even in the presence of a ΔpH (Fig. 4, right side). In contrast, when a ΔpH was first established and then verapamil was added, the ΔpH-induced change in the glow curve was irreversible and recovery by uncouplers was prevented (Fig. 4, bottom curve). The acidification of the thylakoid lumen is not influenced by verapamil (data not shown). Inhibition of electron transport at the donor side of PS II by verapamil and other Ca^{2+}-channel inhibitors have been shown by Semin and coworkers [32]. The results shown here suggest that verapamil can inhibit both Ca^{2+} release by acidification and Ca^{2+} rebinding after uncoupling [11].

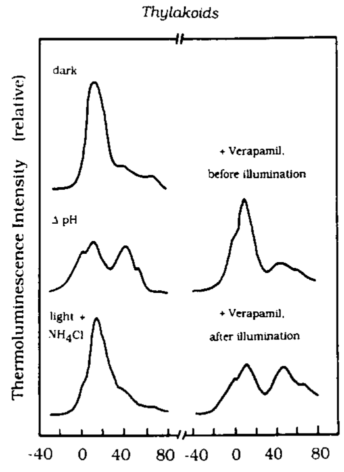

Fig. 4. Effect of light-induced ΔpH on thermoluminescence of thylakoids. To build up a ΔpH, thylakoids were illuminated for 3 min with white light at a light intensity of 400 W/m^2 in presence of 50 μM methylviologen, 0.3 M sorbitol, 50 mM KCl, 7 mM $MgCl_2$ and 15 mM Hepes (pH 7.6). Immediately before measurement of thermoluminescence 10 μM DCMU was given. The light-induced ΔpH was abolished by the addition of 10 mM NH_4Cl. The right side of the figure illustrates the effect of the Ca^{2+}-channel inhibitor verapamil (25 μM). Other measuring conditions as in Fig. 1.

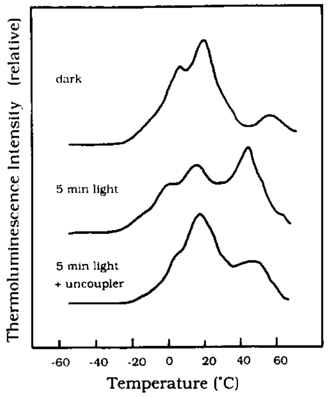

Fig. 5. Thermoluminescence glow curves of dark-adapted and preilluminated algae (*Chlorella vulgaris*). The same medium was used as for thylakoids. Illumination and measuring conditions see Fig. 4. A mixture of NH_4Cl (10 mM) and nigericin (2 μM) was used as uncoupler.

Illumination of algae (*Chlorella vulgaris*) does also lead to an appearance of the C band and a decrease of the Q band, as can be seen in Fig. 5 (curve in the middle compared to upper curve). The total emission is independent of preillumination. The light-induced changes in thermoluminescence are largely reversible by the addition of uncoupler (Fig. 5, bottom curve). Compared to thylakoids the Q band and the C band were shifted to higher temperatures in this algae (Q band at 18°C and C band at 48°C).

Light-induced changes in thermoluminescence were also measured in intact leaves under conditions of 'high-energy quenching' of fluorescence. Fig. 6 shows thermoluminescence curves of pea leaves. A normal Q band (11°C) and a small C band (46°C) was observed in predarkened leaves. A 'high-energy state' of the leaf (see Refs. 1,3) was obtained by 3 min illumination (400 W/m^2) of attached leaves previously kept in darkness for 1.5 h. In these leaves the intensity of the Q band was reduced and that of the C band increased to the same extent (Fig. 6, right side, top curve). After predarkening the plant for 6 h followed by 5 min illumination the effect was more pronounced (Fig. 6, right side, bottom curve). The Q band was then virtually absent and replaced by the C band. The light-induced shift was completely reversed by infiltration of the leaf with uncouplers (Fig. 6, left side, bottom curve).

Discussion

It was observed by Ono and Inoue [18,22,23] that inactivation of the water-splitting complex by low-pH-induced Ca^{2+}-release is associated with a shift in the

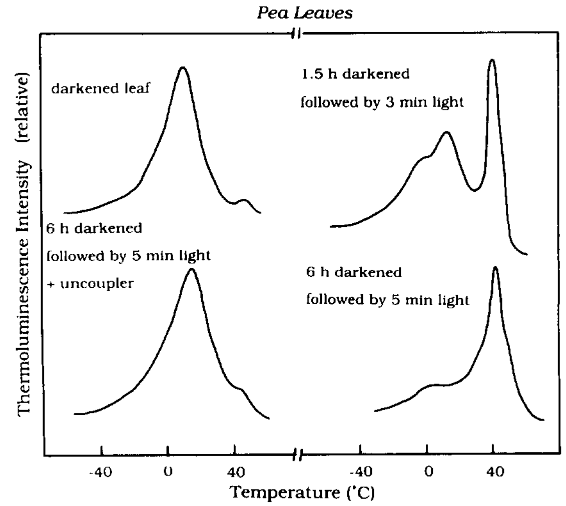

Fig. 6. Thermoluminescence glow curves of dark-adapted and preilluminated pea leaves infiltrated with DCMU. Left side, upper part: leaf was dark-adapted for 1.5 h. Right side, upper part: after 1.5 h dark adaptation the leaf was illuminated for 3 min at a light intensity of 400 W/m^2. Right side, lower part: leaf was kept in the dark for 6 h and illuminated for 5 min. Left side, lower part: leaf was infiltrated with NH_4Cl and nigericin after 6 h dark and 5 min illumination. Conditions for measuring thermoluminescence were as in Fig. 1. The intensities of thermoluminescence are not directly comparable, because different leaves were used for each measurement.

temperature of the Q and B thermoluminescence bands from about 13 and 30°C, respectively, to 45°C. A temperature upshift in the peak position of a thermoluminescence band indicates, that the redox span between the interacting acceptor and donor has become larger and the separated charges are more stabilized. Ono and Inoue [18,22,23] correlated the upward shift of thermoluminescence with a modification of the S_2 state. They proposed a stabilization of the S_2 state by lowering its redox potential after Ca^{2+} depletion. In contrast to these observations Boussac et al. [33,34] observed in a Ca^{2+}-depleted system a modified and stabilized S_3 EPR signal, which they assigned to a histidine radical. They assumed an inhibition of the $S_3 \rightarrow S_4$ and not of the $S_2 \rightarrow S_3$ redox transition of the water-splitting system. Since low-pH treatment replaced both the Q ($S_2Q_A^-$ charge recombination, peaking at 10 to 14°C) and B band ($S_2Q_B^-$ charge recombination, peaking at about 30°C) the effect may not be accounted for only by a shift in the redox potential of the S_2 state. A redox shift in S_2 should result in a similar shift in both bands.

In this paper we present evidence that the redox potential of the Q_A acceptor is changed after Ca^{2+} depletion (Fig. 3). By steady-state redox titration we found that the midpoint redox potential of Chl fluorescence was shifted from $E_m = -80$ mV (at pH 6.5) to $E_m = +40$ mV (at pH 6.5 after preincubation for 5 min at pH 4.2). A rather low concentration of Ca^{2+} ions (300 μM) was required to reverse the redox shift. The high-potential form of Q_A [11] and a C band of TL [21,24] were also observed in Tris- or NH_2OH-treated samples. Tris- and hydroxylamine treatment are known to modify the S-state cycle and inactivate oxygen evolution (see, e.g., Refs. 16,35,36). Obviously, inactivation of the water-splitting complex is accompanied by a modification of the acceptor side of PS II. The manganese cluster and quinone acceptors are situated on different sides of the membrane-spanning polypeptides D1 and D2. Alteration on one side could be mediated through the polypeptide to the other side.

There might also be the possibility, that Tris, hydroxylamine and low pH could influence the donor and acceptor sides of PS II simultaneously. It has been suggested that, in addition to its effect on the water-splitting system, Tris treatment inactivates electron flow through the Q_B site [37] and hydroxylamine treatment reduces the affinity of atrazine to its binding site [38]. In low-pH-treated chloroplasts electron transport is inhibited not only at the donor side [12–16], but is also inhibited at the reducing side of PS II between the acceptory site of silicimolybdate and 2.5-dimethylbenzoquinone. The existence of a Ca^{2+}-binding site between Q_A and the plastoquinone pool was suggested [20]. Therefore, at least in the experiments with BBY particles, it can not be completely disclosed that besides a donor side change, Ca^{2+} release at the acceptor side of PS II may also contribute to the observed shift in the redox potential of Q_A, which in turn is reflected as a shift in the peak position of the Q TL band.

On the other hand, the similarity in the thermoluminescence shift seen upon acidification of BBY particles with those observed upon internal acidification in thylakoids or intact cells strongly suggests that primarily the donor side is affected by low pH and that the Q_A shift on the acceptor side is a secondary effect induced by a modification on the donor side. In illuminated thylakoids, where only the donor side is exposed to acidification, the interconversion of the Q and C bands could also be influenced by the Ca^{2+}-channel inhibitor verapamil, suggesting that Ca^{2+} is involved in the ΔpH-induced transition.

The positively-charged donor component participating in the generation of the high-temperature TL band at 45°C can not be identified from our measurements. Ono and Inoue [22,23] suggested that a modified S_2 state is responsible for the 45°C band which can not be excited below -40°C. However, the 45°C band could be charged even at -80°C in our measurements (not shown). Moreover, the band also appeared in Tris- and hydroxylamine-treated chloroplasts [21,24] where the S_2 state of the water-splitting system is inactivated [35,36]. Consequently, we suggest that either a modified S_2 state [21] or another donor [34] is the positively charged counterpart of the modified Q_A responsible for the 45°C band in DCMU-treated samples. From the TL measurements we can estimate the redox potential of this donor. The free energy of activation associated with the Q (at 10°C) and C band (at 45°C) in DCMU-treated samples are 790 mV [39] and 940 mV [31], respectively. Thus, the 35°C temperature difference between the Q and C bands corresponds to a redox potential difference of about 150 mV. Since redox titration of fluorescence yield indicates a 120–160 mV shift of Q_A (Fig. 3), it follows that the midpoint redox potential of the recombining donor is very close to that of the unmodified redox potential of the S_2 state and could differ from it by only about 30 mV. This suggests that a ΔpH-induced upward shift in the peak temperature of TL emission is caused mainly by a redox change occurring at the acceptor side of PS II and only a small and possibly not very significant change could be associated with a modification of the donor side.

A heterogeneity of the Q_A acceptor has already been described in the literature [40]. In EPR experiments two semiquinone-iron forms of the primary quinone acceptor, Q_A-Fe^{2+}, were distinguished which were interconvertable with changing pH [41]. It has been suggested that two tightly-bound iron quinones exist in the PS II-acceptor complex [42]. Redox titrations of the fluorescence yield in thylakoid membranes showed two transitions indicating the existence of a low (Q_L, E_m is about -300 mV) and high-potential form (Q_H, E_m is about 0 mV) of the primary acceptor [40,45] which seem to be both associated with the PS II_α centers. The redox potential of Q_A also seems to be different in PS II_α and PS II_β centers [40,43]; the slow β-phase of fluorescence induction could be titrated at about $+120$ mV [44,45]. It should be noted, however, that there still exists a certain discrepancy between the absolute values of the redox potential of Q_A published in the literature [45–47] and we do not want at this point to ascribe the high-potential form found in our study upon Ca^{2+} depletion to one of the 'high-potential Q_A' described in the literature.

The results shown here demonstrate that PS II exists in two states which are interconvertible: one active state is characterized by a high fluorescence yield and the Q band in thermoluminescence and an apparent E_m for fluorescence of about -80 to -120 mV. The other, (Ca^{2+} depleted) inactive state is characterized by a low fluorescence yield of the C band in thermoluminescence and a shift in the redox potential of Q_A to a high-potential form (E_m about $+40$ mV). The distribution between the two states depends on the pH in the lumen. A small TL band at about +45°C can always be observed in the TL glow curve of chloroplasts, indicating that a certain amount of Q_A is in the high-potential form even in presence of an uncoupler. In hydroxylamine-treated thylakoids redox titration of fluorescence exhibits the high-potential form of Q_A [10] and a large C band appears in thermoluminescence [24].

As shown in Fig. 3, an inverse correlation exists between the intensity of the Q and C bands in dependence on pH. Consequently, after a suitable calibration the amplitude of the C band could be used to estimate the pH in the thylakoid lumen. Since according to our present results the C band is associated with the high-potential form of Q_A it might also be possible to estimate the amount of centers with Q_A in the high-potential form from the area of the C band in comparison with that of the Q band. High-energy quenching is associated with a decrease in the Q band and a concomitant intensification of the C band, while the overall emission is not significantly affected by pH (see Figs. 1, 4 and 5). It suggests that in the high-energy state no significant quenching of singlet excited states occurs in the antenna and, despite a large shift in the redox potential, a charge pair can be formed at the reaction center. In contrast to high-energy quenching, photoinhibition was shown to be accompanied by an overall decrease in TL intensity with no large changes in the emission temperature [48–51].

We suggest that Ca^{2+} is a regulatory cofactor of the oxygen-evolving system which is involved in the ΔpH-dependent 'down regulation' of PS II: strong acidification of the lumen which occurs when ATP-consuming reactions limit photosynthesis causes Ca^{2+} release and impairment of the water-splitting system in a certain percentage of the reaction centers depending on ΔpH. Inactivation of the donor side is accompanied by conversion of a high potential form of Q_A. The idea that there exists a pH-dependent equilibrium between an active and an inactive ('high energy') state agrees well with a recently proposed two-state model of photosynthetic control of PS II in leaves [1,29].

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