Biochimica et Biophysica Acta 809 (1985) 369–378
Elsevier

BBA 41842

Thermoluminescence study of charge recombination in Photosystem II at low temperatures. I. Characterization of the Z_v and A thermoluminescence bands

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(Received February 11th, 1985)
(Revised manuscript received May 15th, 1985)

Key words: Thermoluminescence; Photosystem II; Oxygen evolution; Electron transport; Trypsin; (Spinach chloroplast)

The characteristics of the Z_v and A thermoluminescence bands appearing in the glow curve at about −75 and −30°C, respectively, were investigated in spinach chloroplasts. Inhibitory concentrations of DCMU decreased the amplitude of the Z_v band by half and completely abolished the A band. On the other hand, after two preflashes at +2°C before freezing, the A band could be charged by low-temperature illumination even when the electron transport was interrupted between Q_A and Q_B by DCMU addition after the preflashes. Two-flash preillumination greatly enhanced the amplitude of the A band, but diminished that of the Z_v band. Tris washing and NH_2OH treatment, which inactivated the oxygen-evolving system, almost completely abolished the Z_v band, but did not affect the A band. Severe trypsin treatment, which also impaired the oxygen-evolving system, resulted in a very large intensification of the Z_v band. The half-times of the A and Z_v bands, determined by theoretical analysis of the thermoluminescence data, proved to be about 4 ms and 200–500 μs, respectively. These results, taken together with EPR data from the literature, suggest that the A band arises from charge recombination between a negatively charged acceptor located before the DCMU block (most probably Q_A^-) and the oxidized donor Z^+ (which accounts for the EPR Signal II_{vf} and Signal II_f). The electron carrier responsible for the Z_v band is also a component located prior to the inhibitory site of DCMU (Q_A^-); its interacting counterpart is an unidentified donor which is involved in charge exchange with the S states.

Introduction

The primary charge separation in PS II is rapidly stabilized by electron transfer reactions on the donor and acceptor sides of the reaction center. As a result of successive electron transfer steps, at room temperature the positive charge is stored in the S_2 state of the water-splitting system and the electron in the single-reduced state of Q_B. When the temperature falls, many electron transfer reactions slow down and become gradually inhibited. In the temperature range −10 to −40°C, oxygen evolution is impaired [1]; below −40°C, electron transfer from Q_A to Q_B is considerably slowed down [2,3]. Consequently, the length of the electron transport chain participating in charge stabilization decreases with decreasing temperature. As the normal pathway of the forward charge stabilization process is inhibited, the trapped charges interact in a luminescence back-reaction with in-

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

creasing probability [4,5]. Thus, upon heating one can expect the appearance of thermoluminescence bands at certain low temperatures determined by the redox span between the ultimate electron donor and acceptor in the shortened electron transport chain. The characterization of thermoluminescence bands may provide information about the electron transport components which are localized near the reaction-center chlorophyll, P-680, the study of which requires very sophisticated techniques at physiological temperatures, due to the rapidity of the primary electron transfer reactions.

In the glow curves of green plant materials below 0°C, five thermoluminescence bands have been distinguished by different research groups [6]. Ichikawa et al. [7] observed three bands and designated them Z (−160°C), Z_v (variable) and A (−10°C). Desai et al. [8] also found three peaks and used the terminology Z (−155°C), peak I (−36°C) and peak II (−12°C). Of these five bands, the Z band is not related to the photosynthetic activity [9], and peak II could be observed only in the presence of PS II inhibitors that block electron flow from Q_A to Q_B [10]. The A band and peak I can be considered identical on the basis of their similar characteristics [11]. Only two of the five low-temperature thermoluminescence bands, designated Z_v and A (peak I) bands in the present work, are closely related to in vivo photosynthetic electron transport [6,12]. Neither the Z_v nor the A band is present in the glow curves of etiolated leaves [6] and both of them are sensitive to heat treatment [6,12]. Remarkably, the Z_v band has the unique property that its peak temperature varies with the excitation temperature [7]. The location of charges participating in the generation of these bands is not known.

In the present study we have carried out a detailed characterization of the A and Z_v thermoluminescence bands in order to determine the electron transport components responsible for their appearance. A preliminary report on part of this work has appeared elsewhere [13].

Materials and Methods

Chloroplasts were prepared from 1–2-month-old spinach grown in a green-house. The leaves were cut into small pieces and ground in a buffer (pH 6.5) comprising 0.4 M sorbitol/10 mM NaCl/1 mM $MnCl_2$/5 mM $MgCl_2$/2 mM EDTA/2 mM sodium ascorbate/0.4% bovine serum albumin/50 mM Mes. The slurry was filtered through four layers of cheesecloth and centrifuged at $1000 \times g$ for 1 min. The pellet was discarded and the intact chloroplasts of the supernatant were collected by 5-min centrifugation at $2000 \times g$. The chloroplasts were resuspended 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) or phosphate (pH 6.0) buffer to give a concentration of 125 μg Chl/ml and were kept on ice in darkness.

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 $2000 \times g$. The pelleted chloroplasts were washed twice and resuspended in the suspension buffer to give a concentration of 125 μg Chl/ml. 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. For heat treatment, samples in test tubes were dipped into warm water and kept at the desired temperatures for 1 min in the dark. Trypsin digestion of chloroplasts was carried out in the suspension medium at pH 7.0. Chloroplasts were incubated with trypsin (4 μg trypsin/1 μg Chl) for 10 min in the dark at room temperature.

The rate of oxygen evolution was measured at saturating light intensity by using a Clark-type electrode in a temperature-controlled cell at +25°C. The assay medium contained 0.1 M sorbitol/10 mM K_2HPO_4/20 mM NaCl/4 mM $MgCl_2$/2 mM EDTA/50 mM Hepes (pH 7.5)/2 mM ferricyanide and chloroplasts carrying 50 μg Chl in a final volume of 3.0 ml. Thermoluminescence was measured in an apparatus similar to that described by Tatake et al. [14]. The termoluminescence light emitted by the sample during heating was measured by a red-sensitive photomultiplier (EMI 9558B) and the signal was amplified by a home-made differential amplifier and fed to an *X-Y* recorder. The temperature of the sam-

ple holder was monitored using a platinum resistor thermometer placed below the samples. 0.4 ml aliquots of a dark-adapted chloroplast suspension containing 50 μg Chl were excited for 30 s by white light from a NARVA 650 W halogen lamp. The exciting light was passed through a heat-absorbing water filter (thickness 10 cm) and a Balzers neutral density filter giving an illumination intensity of 10 $W \cdot m^{-2}$. In flash experiments, samples were excited by xenon flashes (General Radio, Stroboslave, 3 μs, 0.5 J). The flashes were given at 1 s intervals. 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. Computer analysis of glow curves and determination of half-times and free energies of activation were carried out as described in Ref. 15.

Results and Discussion

In order to determine the number of thermoluminescence bands appearing below 0°C, the glow curves of isolated spinach chloroplasts were excited at various low temperatures. It has been reported that the peak temperature of the Z band is about −155°C [9]. However, we found that with decreasing excitation temperature the peak position of the band gradually shifted to lower temperatures, with a concomitant increase in its emission intensity (Fig. 1). This observation suggests that the actual peak temperature of the band could only be determined by excitation of thermoluminescence below liquid nitrogen temperature. The excitation of the Z band at very low temperatures resulted in a partial charging of another band, peaking at about −75°C (Fig. 1). When the chloroplasts were excited below −90°C, the peak position of the band remained constant, but its amplitude progressively decreased, while its half-bandwidth increased with decreasing excitation temperature (Figs. 1 and 2). Above −90°C, the emission temperature of the band varied with the excitation temperature (Fig. 2). We identified this band with Ichikawa's Z_v band [7]. Further increase of the temperature of excitation revealed the existence of a third band, at about −30°C (Fig. 3). As will be convincingly verified below,

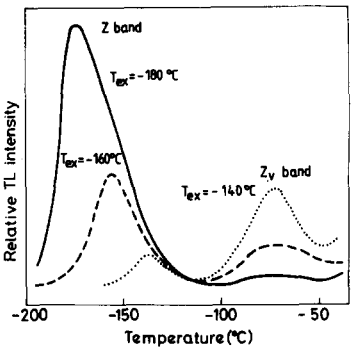

Fig. 1. Excitation of the Z and Z_v thermoluminescence bands at various low temperatures, indicated by T_{ex} on each curve. Samples were illuminated with white light of 10 $W \cdot m^{-2}$ for 30 s and heated at a rate of 20 Cdeg/min. For more details, see Materials and Methods and the text.

this band must be identical with peak I of Sane et al. [11] and the A band of Inoue [17]. Accordingly, this band will be referred to as the A band throughout this paper.

It has been suggested that the Z_v band consists of the high-temperature tail of the Z band when excited below −100°C, but when excited at higher temperatures it is a part of peak I, which appears at −36°C [16]. However, our observations (Figs. 1–3) demonstrate that the Z_v band is not part of the Z band or peak I, but an independently existing thermoluminescence band.

Although the peak positions of the Z_v and A thermoluminescence bands (−75 and −30°C, respectively) are positioned relatively far from each

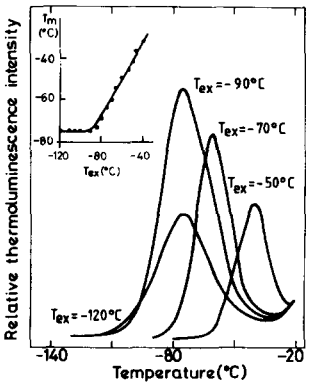

Fig. 2. Dependence of the amplitude of the Z_v thermoluminescence band on the temperature of excitation. The insert shows the shift of peak temperature (T_m) as a function of excitation temperature (T_{ex}).

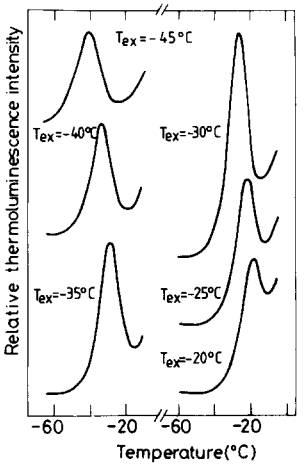

Fig. 3. Variations of the height and peak position of the A thermoluminescence band as a function of excitation temperature (T_{ex}).

other, the thermoluminescence intensity remains surprisingly high when excited at intermediate temperatures. To clarify the possible existence of another hidden thermoluminescence band in the -60 to -40°C temperature region experiments are in progress.

The origin of the Z_v and A bands has scarcely been investigated. Inoue [17] ascribed the A band to the S_4 state of the water-splitting system. Since low concentrations of DCMU abolished both the Z_v and A bands, it was concluded that the electron carrier responsible for these bands is the reduced secondary quinone, Q_B^- [10,18]. However, the inhibitory effect of DCMU on the generation of the Z_v band can hardly be explained by the interruption of the electron transport between Q_A and Q_B, since the Z_v band can be excited below -70°C when electron transfer is blocked, even in the absence of DCMU [12]. In a recent paper [13], we pointed out that the ethanol used to dissolve DCMU exerts a strong inhibitory effect on the charging of the Z_v band. Similarly, ethanol also decreased the amplitude of the A band (not shown). The Z_v and A bands were completely abolished by 2.5 and 1% (v/v) ethanol, respectively. A similar effect of ethanol was observed in delayed luminescence experiments carried out at -40°C [19]. Luminescence, measured at 40 ms after flash excitation, was strongly inhibited by 1% (v/v) ethanol. Glycerol and ethylene glycol also strongly diminished the amplitudes of the Z_v and A bands (not shown). This phenomenon is in agreement with the observations of Joliot and Joliot [20] according to which high concentration of glycerol markedly decreases the oxygen-evolving capacity of chloroplasts and influences the fast rise of fluorescence induction curves [20].

Since the Z_v and A bands are very sensitive to ethanol, the effects of DCMU were investigated through the addition of various concentrations of DCMU in 0.025% (v/v) ethanol (Fig. 4). The inhibitory concentration of DCMU (approx. 1 μM) completely abolished the A band, while the amplitude of the Z_v band decreased only by half and remained at this level at higher DCMU concentrations, too. The resistance of the Z_v band to DCMU treatment indicates that the negatively charged acceptor participating in the generation of the Z_v band is located before the DCMU block. In contrast to the Z_v band, the A band was abolished by DCMU, suggesting that the electron trap for the emission of this band could be Q_B^- [10,18]. However, this conclusion had to be revised after a thorough investigation of the properties of the A band. It was observed by Inoue [17] that the glow curves of chloroplasts suspended in a medium of pH 5.0 show a higher A band than that measured at neutral pH. In order to find the optimum conditions for investigation of the A and Z_v bands, we carried out a detailed study of the effects of pH on the amplitudes of the two bands (Fig. 5). The A and Z_v bands exhibited a complementary relationship as a function of pH. Whereas at low pH the charging efficiency of the A band was higher than

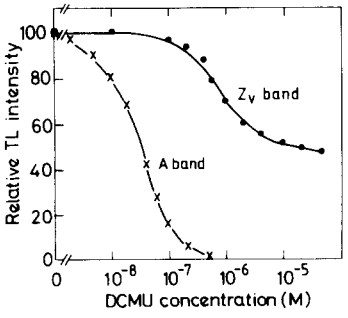

Fig. 4. Effects of DCMU on the amplitudes of the Z_v and A thermoluminescence bands. After DCMU addition, the ethanol content of the sample was 0.025% (v/v). The Z_v and A bands were excited at -80 and -30°C, respectively.

that of the Z_v band, at high pH the Z_v band predominated. The behavior of the Z_v and A bands is closely correlated with the pH-dependence of the delayed luminescence components and with the reduction kinetics of the reaction-center chlorophyll, P-680 [21,22]. A common feature of these phenomena is that in the micro- to millisecond time region at low pH the slowly-decaying components predominate, while with increasing pH the contribution of the fast component is higher. In accordance with this general behavior, the amplitude of the A band ($t_{1/2} \approx 4$ ms calculated for 25°C) was higher at the lower end of the pH range studied (pH 4.0–7.5), while the Z_v band ($t_{1/2} \approx 200$–400 μs at +25°C) exhibited a high emission intensity between pH 7.5 and 10.0 (Fig. 5). It is important to note that, although the amplitudes of the Z_v and A bands are greatly influenced by pH changes, their peak positions do not depend on the pH indicating that the midpoint potentials of the donors and acceptors assigned to these bands are also pH-independent or have the same pH-dependence preserving the redox distance between the interacting donor-acceptor couples. Although low pH is favorable for charging the A band, at pH 4.0 a considerable decrease could already be observed in its amplitude, indicating the inactivation of a species responsible for its appearance. Interestingly, the disappearance of the EPR-detectable component, Signal II_f [23], also occurs at about this pH, suggesting the involvement of the same species in the generation of the A band and Signal II_f. Remarkably, at extreme pH values (4.0 and 10.0), when the oxygen evolution is already inhibited [23–25], a new thermoluminescence band appeared in the glow curves between 0 and −10°C (Fig. 5). Probably this band was observed previously in chloroplasts treated with phenolic herbicides [26,27] and chaotropic agents [28]. The −10°C band is presumably identical with the peak II of Sane et al. [10,11].

Since the amplitude of the A band is considerably enhanced at low pH, we investigated its properties at pH 6.0. In agreement with earlier observations [29], the A band could be excited at −30°C

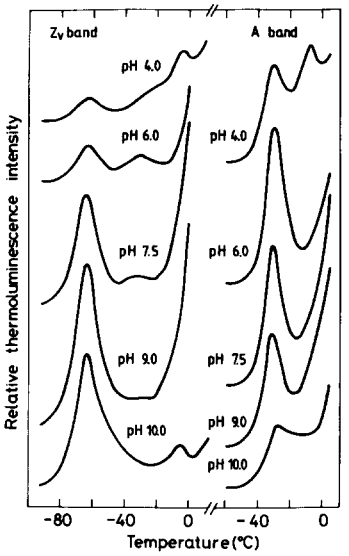

Fig. 5. Effects of the pH of the medium on the amplitudes of the Z_v and A thermoluminescence bands. For pH treatment, chloroplasts were suspended in phosphate buffer of appropriate pH and incubated at room temperature for 10 min in the dark before thermoluminescence measurements. The Z_v and A bands were excited at −80 and −30°C, respectively.

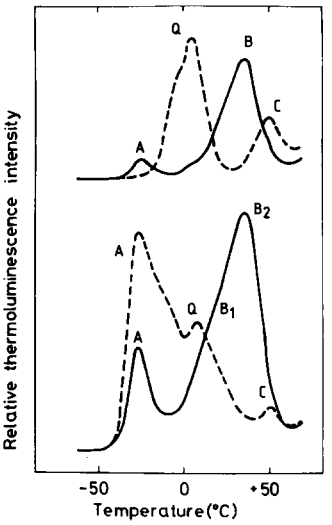

Fig. 6. Effect of flash preillumination on the height of the A thermoluminescence band. Upper part: Dark-adapted chloroplasts suspended in 50 mM phosphate buffer (pH 6.0) were excited with continuous white light for 30 s at −30°C. Lower part: Chloroplasts were preilluminated with two flashes at +2°C, cooled in the dark to −30°C and illuminated with white light. Solid and dashed lines represent the untreated and 10 μM DCMU-treated samples, respectively. DCMU was added either to the dark-adapted sample (upper part) or immediately after flash preillumination before freezing (lower part).

(Fig. 6, upper part), but illumination at −80°C proved to be inefficient in filling up the trap (Fig. 7, upper part). When chloroplasts were preilluminated by two flashes before freezing, the amplitude of the A band increased considerably ('A band-enhancing effect' [17]) and it could be charged to high intensity, even at −80°C (Figs. 6 and 7, lower parts). The similar behavior of our A band peaking between −25 to −30°C and Inoue's A band at −10°C strongly suggests that the two bands are identical. DCMU abolished both the A and B (B_1 and B_2 [7]) bands and resulted in the appearance of the Q and C bands [30,31]. However, after two preflashes the A band could be charged, even in the presence of DCMU both at −30°C and at −80°C (Figs. 6 and 7, lower parts). It can therefore be assumed that the inhibitory effects of DCMU and low temperature on the appearance of the A band are indirect and do not occur on the acceptor side of PS II, as previously suggested [10,18], but on the donor side of the reaction centers. Since only the $S_1 \rightarrow S_2$ transition can proceed in the presence of DCMU or at low temperature, the A band cannot be excited. However, after the water-splitting enzyme system is transformed into the S_3 state by two preflashes, the A band can be charged, even if the electron transfer from Q_A to Q_B is inhibited by DCMU or low temperature. Consequently, the reservoir of electrons responsible for the A band is not Q_B [10,18] but Q_A or another acceptor located before the inhibitory site of DCMU. It is noteworthy that DCMU addition after two preflashes considerably intensified the emission intensity of the thermoluminescence between −30 and 0°C. In addition, the intensity of the Q band decreased and a shoulder appeared at the descending side of the A band (at about −10°C), thus indicating the overlapping of the A band with a thermoluminescence band hidden under the envelope of the glow curve (Fig.6, lower part). The behavior of the Z_v band upon flash preillumination was opposite to that of the A band (Fig. 7). Its amplitude was higher in dark-adapted chloroplasts than in preilluminated ones. Addition of DCMU to the preilluminated samples did not inhibit the effect of flash preillumination (Fig. 6 and 7). Since the amplitudes of the Z_v and A bands depend greatly on the oxidation state of the water-splitting system, it is natural to assume that the S states are the reservoirs of positive charges responsible for these bands. However, thermoluminescence measurements carried out with trypsinized chloroplasts raise questions concerning this assumption.

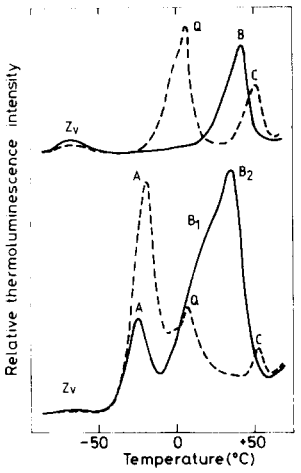

Fig. 7. Effects of flash preillumination on the heights of the A and Z_v thermoluminescence bands. Upper part: Dark-adapted chloroplasts suspended in 50 mM phosphate buffer (pH 6.0) were excited with white light for 30 s at −80°C. Lower part: Chloroplasts were preilluminated with two flashes at +2°C, cooled in the dark to −80°C and illuminated with white light. Solid and dashed lines represent the untreated and 10 μM DCMU-treated samples, respectively.

It has been shown that severe trypsin treatment not only interrupts the functional connection between Q_A and Q_B [32], but also leads to deterioration of the oxygen-evolving capacity [33,34]. Accordingly, in chloroplasts incubated in the presence of trypsin (4 μg trypsin/1 μg Chl) for 10 min at room temperature, the electron transport from water to ferricyanide was completely inhibited (not shown), indicating an impairment of the water-splitting system by trypsin. With the loss of water-splitting activity, the B band related to the active oxygen-evolving system disappeared and a very large Z_v band appeared in place of it at about −75°C (Fig. 8). In the glow curve of strongly trypsinized chloroplasts excited at −80°C, the A band could barely be seen, and the −10°C band which had been observed at extreme pH levels (Fig. 5) appeared as well. With increasing excitation temperature the amplitude of the Z_v band gradually decreased, with simultaneous intensifica-

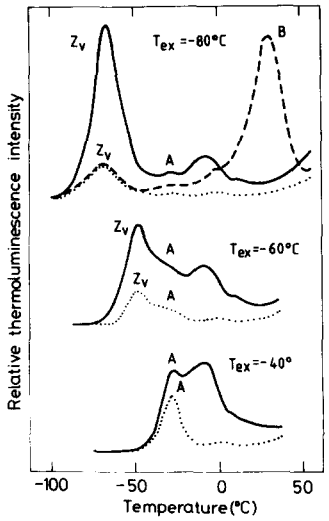

Fig. 8. Effects of trypsin treatment on the charging capacities of the Z_v and A thermoluminescence bands. Chloroplasts were digested with trypsin (4 μg trypsin/1 μg Chl) for 10 min in the dark at room temperature and thermoluminescence was excited at temperatures indicated by T_{ex}. Solid and dashed lines show the glow curves of trypsin-digested and control chloroplasts, respectively. The dotted line represents the glow curve of trypsin-treated chloroplasts after the addition of 50 μM ferricyanide.

tion of the A band and the −10°C band. Low concentration of ferricyanide abolished the band at −10°C, and the A band appeared as a distinct band after the excitation of chloroplasts at −40°C. Thermoluminescence measurements on trypsin-digested chloroplasts indicate that generation of the A and Z_v bands does not require an active water-splitting system.

This conclusion was confirmed by thermoluminescence measurements on isolated chloroplasts in which the oxygen-evolving apparatus had been inactivated by various chemical and physical treatments (Fig. 9). In Tris-washed and NH_2OH-treated chloroplasts, which are completely deprived of their oxygen-evolving capacity, the B band associated with the intact water-splitting system disappeared and the Z_v band was greatly diminished (Fig. 9, curves L, M). The amplitude of the A band did not change considerably. This observation suggests that our A band is identical with the peak I of Sane et al. [10,11]. Peak I, like our A band, was not sensitive to Tris treatment but was abolished by DCMU. In the glow curve of Tris-washed and NH_2OH-treated chloroplasts the −10°C band was also clearly discernible (Fig. 9, curves P, Q). It is noteworthy that the C band could also be charged in Tris- and NH_2OH-treated chloroplasts [31]. The results of heat treatment on the thermoluminescence of chloroplasts incubated for 1 min at +55°C were consistent with the changes induced by Tris and NH_2OH treatments. The B band was completely abolished, but the Z_v, A and C bands could clearly be observed (Fig. 9, curves N, R). The results of inactivation experiments and experiments on trypsin-treated chloroplasts suggest that the traps of positive holes responsible for the emission of the Z_v and A bands are not the S species, but some other electron donors. This view is consistent with the observation that the Z_v and A bands are emitted from partially purified PS II particles isolated by Triton treatment, which are no longer equipped with the S system [7].

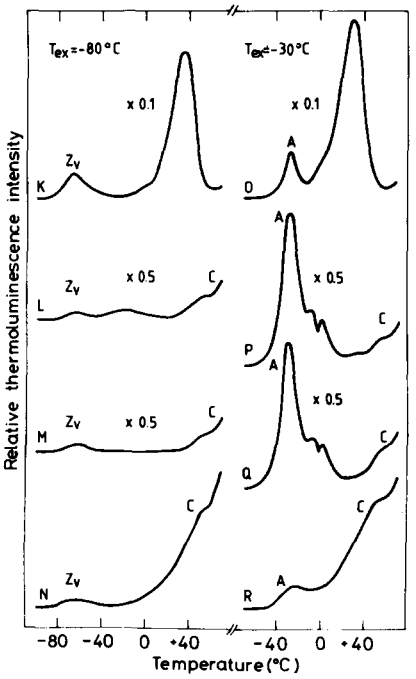

Fig. 9. Effects of inactivation of the oxygen-evolving system on the emission intensities of the Z_v and A thermoluminescence bands. K, O; untreated samples; L, P, 0.8 M Tris-HCl (pH 8.8); M, Q, NH_2OH-treated chloroplasts; N, R, heating for 1 min at +55°C. Curves K,L,M,N, chloroplasts excitation at −80°C. Curves O,P,Q,R, excitation at −30°C. The sizes of the upper and middle curves have been reduced by factors of 10 and 2, respectively. The trough at 0°C in curves P and Q was a result of the solid/liquid phase transition of water.

In order to facilitate identification of the Z_v and A bands with electron transport as well as with delayed luminescence and fluorescence induction components, the free energies of activation (ΔF) and half-lives ($t_{1/2}$) were calculated for the two bands by the procedure applied earlier [15]. The A band was fitted by a first-order Randall-Wilkins theoretical curve and the resulting calculations for +25°C gave $\Delta F \approx 0.624$ eV and $t_{1/2} \approx 4$ ms. The Z_v band could be fitted only by a second-order curve, and its parameters varied somewhat from one chloroplast preparation to another. Its half-life varied between 200 and 400 μs, and the free energy of activation between 0.500 and 0.600 eV.

The effects of flash preillumination on the amplitudes of the Z_v and A bands indicate that the donors which give rise to the appearance of these bands participate in charge exchange with the water-splitting system. Although our knowledge about the molecular intermediates involved in oxygen production is rapidly increasing [35,36], it is not easy to relate the A and Z_v bands to known electron transport components. A possible donor which could account for the generation of the Z_v and A bands is cytochrome *b*-559. This possibility is eliminated by experiments performed in the presence of ferricyanide. 1 mM ferricyanide, which completely oxidized cytochrome *b*-559 [37], had a negligible effect on the amplitude of the Z_v band. Although the amplitude of the A band decreased by half on the addition of 1 mM ferricyanide (not shown), one can exclude the participation of cytochrome b_{559} in charging of the A band, for at −30°C, where the A band appears, cytochrome *b*-559 cannot compete efficiently with the secondary donor, Z, in electron donation to P-680 [38,39]. In addition the midpoint potential of cytochrome *b*-559 is not positive enough to account for the low activation energies calculated for the Z_v and A bands.

The properties of the A and Z_v bands prompted us to assign these bands to delayed luminescence decay components and ESR transients. The behavior of the A band is well correlated with that of the delayed luminescence component measured at 40 ms after a flash given at −40°C [40]. Similarly to the A band, the 40 ms component was abolished by the addition of DCMU. Further, after two-flash preillumination DCMU did not inhibit the appearance of either the A band or the ms delayed luminescence.

In the ms time region the intensity of delayed luminescence measured as a function of temperature exhibits a maximum at −30 to −40°C [40–42], i.e., the temperature region where the intensity of the A band is maximum. Moreover, at −35°C the luminescence of chloroplasts preilluminated with two flashes at +2°C before cooling was about 15-times higher than that of dark-adapted chloroplasts [19,40]. This phenomenon is also observed in thermoluminescence (Figs. 6 and 7) as an 'A band-enhancing effect' [6,29]. From these common characteristics, we conclude that the 40-ms delayed luminescence and the A band correspond to each other and are probably of the same origin.

Assignment of the A band to the donor denoted Z (or D_1 [43]), which gives rise to the EPR component Signal II_{vf} under normal physiological conditions [44] or to Signal II_f when oxygen evolution is blocked [45], is also very convincing. The amplitude of Signal II_{vf} is highest after excitation of chloroplasts by two flashes [46], a phenomenon reminiscent of the 'A band-enhancing effect' [6]. Both the A band and Signal II_f can be excited in Tris-washed and NH_2OH-treated chloroplasts possessing an inactivated water-splitting system [44]. On the other hand, neither the A band nor the Signal II_{vf} can be excited in the presence of DCMU. Like the A band, Signal II_{vf} cannot be induced efficiently by the illumination of chloroplasts below −40°C. Both the A band and Signal II_{vf} decay via first-order kinetics. The half-time of Signal II_{vf} (approx. 0.9 ms [44]) is comparable with that of the A band (approx. 4 ms). The excellent agreement between the properties of the A band and the fast components of Signal II lead us to suggest that the A band arises from charge recombination between a negatively charged acceptor located before the DCMU block (Q_A^-) and the oxidized donor, Z^+ (denoted D_1 in [43]), which also accounts for the EPR Signal II_f and Signal II_{vf}.

The question arises of the identity of the donor responsible for the Z_v band. A new EPR signal has recently been detected at $g = 4.1$; this could be excited at about −73°C but gradually disappeared

upon warming of the sample to $-53°C$ [47,48]. The $g = 4.1$ signal could not be excited in the presence of NH_2OH and was considered to be either a 'pre-S_3' [48] or an intermediate state between the S_1 and S_2 states [47]. Since the Z_v band exhibits a maximum and can be permanently trapped at about $-75°C$, while it is also greatly diminished by NH_2OH, it is tempting to assign the Z_v band to the $g = 4.1$ signal. However, the relationship between the Z_v band and the $g = 4.1$ EPR signal could be substantiated only by comparative thermoluminescence and EPR measurements. Since the behavior of the Z_v and A bands upon Tris and NH_2OH treatment closely resembles that of Signal II_{vf} and Signal II_f, it cannot be completely excluded that the Z_v and A bands correspond to Signal II_{vf} and Signal II_f, respectively. However, the observations that only the Z_v band is present in intermittently illuminated angiosperm leaves [6], and that the Z_v and A bands differ in behavior upon heating [7], indicate that, unlike Signal II_{vf} and Signal II_f, different donors are responsible for these two bands. At any rate, our results provide substantial support for the conclusion that the electron carrier responsible for the Z_v band is a component located prior to the inhibitory site of DCMU (Q_A^-) and its interacting counterpart is an unidentified donor which is involved in charge exchange with the S states.

Acknowledgements

We are grateful to Mr. G. Kiss and Mr. I. Horváth for development and maintenance of the thermoluminescence apparatus. This work was supported by the Hungarian Academy of Sciences under Contract No. 366/82/1.6.

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