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Minireview

Thermoluminescence in plants

S. Demeter and Govindjee

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Recently considerable progress has been achieved in the elucidation of the origin of thermoluminescence in chloroplasts. The assignment of 2 of the thermoluminescence bands, peaking at around +5°C (Q or D band) and +30°C (B band), to the recombination of charges, originating from the oxidized species of the oxygen evolving complex (the so-called S states) and to the reduced primary and secondary quinone acceptors Q_A and Q_B, respectively, has aided in the investigation of reactions involving both the electron donor and acceptor sides of photosystem II. In this paper we review recent thermoluminescence results concerning the deactivation of S states, temperature and pH dependence of S state transitions, and the activity of the water oxidizing system after removal of Cl^-, manganese or the 33 kDa protein. Reports on the use of thermoluminescence in investigations on the sites of action of herbicides and redox changes of Q_B conferred by herbicide resistance are also discussed. The effect of pH, bicarbonate, and Acceleration of Deactivation Reaction of enzyme "Y" (ADRY) reagents on the photosystem II reactions are presented in the light of thermoluminescence observations. Further possible applications of thermoluminescence promising better understanding of the photosynthetic processes are suggested.

Key words – Delayed light emission, electron transport, herbicide, photosystem II, thermoluminescence, water splitting.

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Introduction

Thermoluminescence (TL) is emission of light induced by heating of preilluminated materials. This emission reflects the charge recombination between a trapped electron and a positive charge (hole) separated during the preillumination. In plant photosynthesis, light energy absorbed by the reaction center chlorophyll *a* is converted into redox energy. In this process, positive and negative charges are separated from each other and subsequently stored in various components of the electron transport chain. Thus it can be expected that preilluminated photosynthetic organelles will exhibit TL emission upon heating. Arnold and Sherwood (1957) discovered this phenomenon in plants; it proved to be common to all photosynthetic organisms. It has been observed in leaves, chloroplasts, chloroplast fragments, algae and photosynthetic bacteria. In the last five years a considerable progress has been achieved in the understanding of TL in green plant materials, and the origin of several TL bands has been established.

Both the S states of the water oxidizing complex (WOC) and the primary (Q_A) and secondary (Q_b) quinone electron acceptors of photosystem II (PS II) contribute to the generation of TL. Since small changes in the redox properties of the electron transport components are reflected in the TL characteristics, the TL phenomenon has become an increasingly useful tool in photosynthesis research. The TL method has been applied in the investigation of the WOC (Inoue and Shibata 1979, 1982) and in the study of the electron acceptor side of PS II including the mode of action of herbicides (e.g., Droppa et al. 1981a, b, c, Horvath 1986) as well as herbicide resistance of plants (Demeter

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et al. 1985a). A general and excellent review by Sane and Rutherford (1986) summarizes most of the key results reported in the TL investigations until 1985. In order to avoid overlap with their review, we have attempted to discuss only the recent observations and have concentrated on selected topics, emphasizing those features of the method that make it advantageous in comparison with other methods in some selected areas of photosynthesis research.

Abbreviations – ADRY, acceleration of deactivation of reactions of the enzyme 'Y'; DCMU, commonly called diuron, 3-(3,4–dichlorophenyl)-1,1-dimethylurea; ΔH, enthalpy; ΔS, entropy; EPR, electron paramagnetic resonance; PS II, photosystem II; Q_A, quinone electron acceptor of PS II; Q_B, secondary quinone electron acceptor of PS II; TL, thermoluminescence; WOC, water oxidizing complex; DLE, delayed light emmission; PQ, plastoquinone.

The thermoluminescence process in photosynthesis

According to the present view, thermoluminescence is associated mainly with photosystem II, the system that oxidizes water to O_2 and reduces plastoquinone (water-plastoquinone oxidoreductase). In this system, P680, the reaction center chlorophyll *a*, located on two polypeptides D_1 and D_2 (of M_r 32 and 34 kDa; Fig. 1), transfers its electron to a pheophytin molecule within about 3 ps (e.g. Wasielewski et al. 1988) (Fig. 2). Within about 200 ps the reduced pheophytin transfers electrons to a one electron acceptor plastoquinone molecule, Q_A, suggested to be bound on D_2 (e.g. Govindjee 1984; Trebst 1987). Q_A, then, transfers electrons to a two electron acceptor, yet another plastoquinone molecule, Q_B, bound on D_1. Two turnovers of P680 lead to the reduction of Q_B, to Q_B^{2-} and the formation of plastoquinol (Q_BH_2) which then exchanges with another plastoquinone to form Q_B (see Velthuys 1981). The electrons lost from P680 are recovered, according to the current thinking, from Z, suggested to be a tyrosine molecule (tyrosine −161) in D_1 (Debus et al. 1988, Vermaas et al. 1988); this occurs within 20–50 ns (see review by Witt et al. 1986). Oxidized Z recovers electrons from WOC that involves several polypeptides, manganese, chloride and calcium, ions (e.g. Andersson and Åkerlund 1987). The WOC must accumulate 4 positive charges before water can be oxidized to molecular O_2: $2\,H_2O + 4\,\oplus \rightarrow O_2 + 4H^+$. The redox state of WOC is represented by the so-called S_n states (S_0, S_1, S_2, S_3, and S_4), where n represents the number of oxidizing equivalents on the WOC. In all likelihood, the charge accumulator is manganese.

As described above, the light energy captured by the reaction center chlorophyll *a*, P680, is quickly converted into the free energy of the charge pair formed in a charge separation act. The separated positive and negative charges are quasipermanently stored on various components of the electron transport chain (Fig. 2). The reversal of charge separation, that is back reaction of charges resulting in charge recombination, requires overcoming an activation energy barrier; the free energy of activation involved here is the sum of free energy losses built up in the successive charge separation steps along the electron transport chain. If the temperature of the sample is increased, there is some probability that the trapped charges can overcome the barrier of the activation energy and migrate back to the reaction center chlorophyll *a* where they undergo charge recombination (Fig. 3). The energy released in the recombination is transferred back to the bulk chlorophyll and emitted as a thermoluminescence photon or converted into heat in a radiationless transition. If the reversal of the photoinduced charge separation can take place at the same ambient temperature at which the light excita-

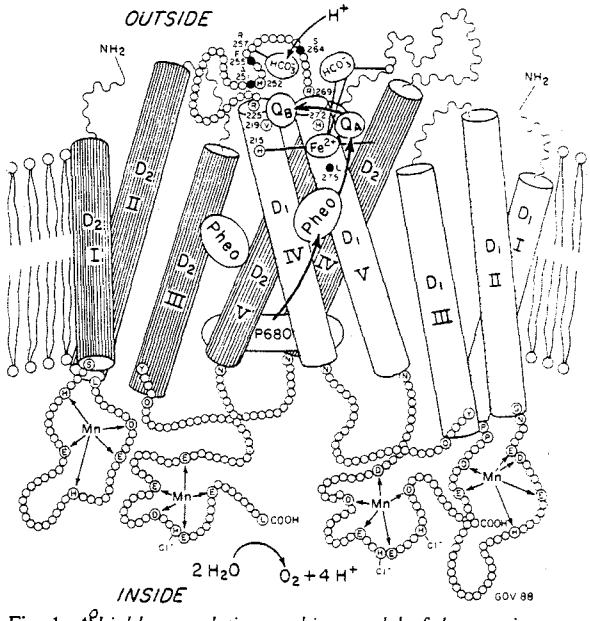

Fig. 1. *A highly-speculative working model of the reaction center of photosystem II, the site of origin of most of thermoluminescence.* D_1-I through V, equivalent to the L-subunit of the bacterial reaction center, is the 32 kDa herbicide binding protein. D_2-I through V, equivalent to the M-subunit of the bacterial reaction center, is the 34 kDa polypeptide. Approximate locations of the functional chlorophyll *a* P680, the functional and non-functional pheophytin (Pheo), bound plastoquinone Q_A (on D_2), Fe^{2+} and bound plastoquinone Q_B (on D_1) are shown. By analogy with photosynthetic bacteria, Q_A and Fe^{2+} are about at the same distance in the membrane as Q_B. The N-terminals of D_1 and D_2 face the outside, whereas, the C-terminals face the inside of the membrane. Suggested sites of HCO_3^- on arginine-257, and on Fe^{2+} are also shown. The 'Y' in D_1-III is now suggested to be 'Z', the electron donner to P680. Amino acids that are known to be involved in the herbicide niche are shown by solid dots; mutations at these sites create herbicide resistance. Possible sites for Mn and Cl^- binding are suggested to be on the lumenal portions of the D_1 and D_2 polypeptides. Manganese cannot be at all the 4 places since 4 Mn atoms are within 3.3 Å of each other. (after Govindjee 1988, unpublished).

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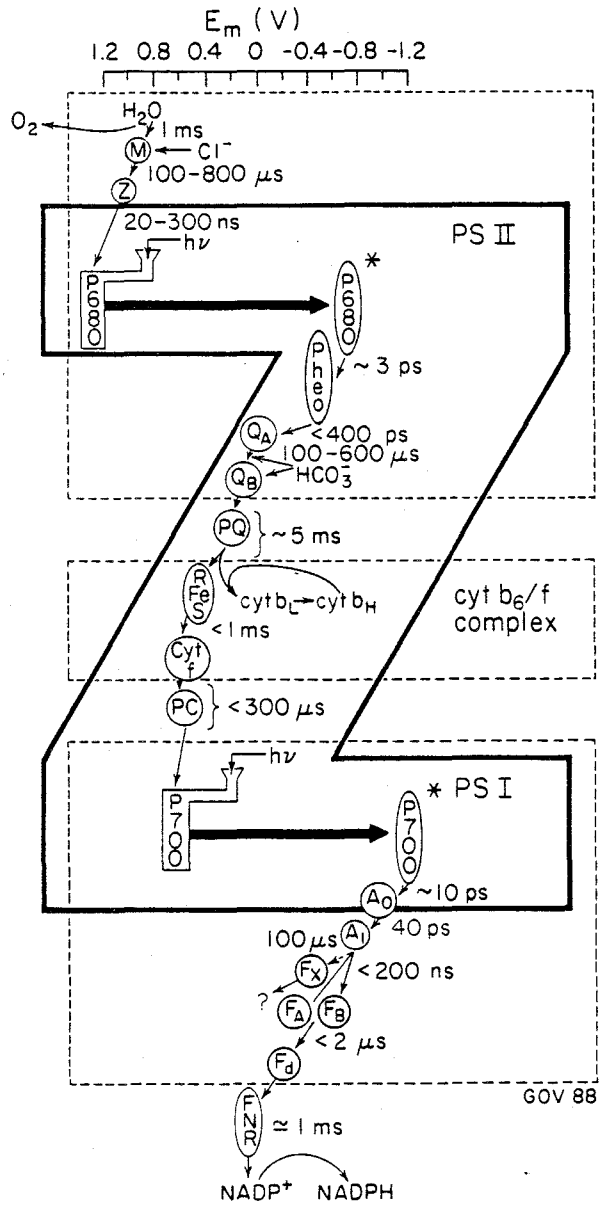

Fig. 2. *The Z scheme of electron transport in photosynthesis.* *Dashed rectangles:* Three major multiprotein complexes (PSII, photosystem II; cyt *b/f*, cytochrome b_6/f complex; and PS I, photosystem I), located in the thylakoid membrane, and containing the photosynthetic components required for electron flow from H_2O to $NADP^+$.
Primary reactions: The electron carriers are placed horizontally according to their midpoint redox potentials at pH 7.0 (Em,7) Electron flow is initiated when a photon or exciton reaches the reaction center chlorophyll *a* P680 (in PS II) and P700 (in PS I) (see hν going into the funnel). P680* and P700* (see ovals) indicate the first singlet excited states of P680 and P700. The *first* reaction of P680* is the conversion of excitonic energy into chemical energy: charge separation, i.e., the formation of the cation $P680^+$ and the anion pheophytin$^-$ ($Pheo^-$) within ~3 ps. However, the first reaction of P700*, the charge separation into $P700^+Ao^-$, may need ~10 ps. Here, Ao is a special chlorophyll *a* molecule. The $P680^+$ recovers its lost electron from Z, now thought to be tyrosine-160 of the D_1 polypeptide of PS II.
Secondary reactions of PSII. The positive charge on Z is then transferred to the charge accumulator M, or the water oxidizing complex (WOC). It is suggested that M is nothing other than a Mn-cluster located on the lumenal portions of the D_1 and D_2 polypeptides of PS II (see Fig. 1). The *intrinsic* binding site of Cl^- lies somewhere in this region. Water oxidation seems to require another polypeptide, an extrinsic 33 kDa polypeptide, and this is where *extrinsic* binding sites of Cl^- may exist. Four positive charges must accumulate before an O_2 molecule is evolved. The $Pheo^-$ delivers the extra electron to a primary (plastoquinone) electron acceptor, Q_A, located on the D_2 polypeptide of PS II; Q_A delivers its electron to a secondary (plastoquinone) electron acceptor Q_B, located on the D_1 polypeptide of PS II (See Fig. 1) Bicarbonate ions seem to be involved in the Q_A – Fe – Q_B region, where Fe is an iron atom (Fe^{2+}) between Q_A and Q_B. After reduction to plastoquinol, i.e. after two turnovers of the reaction center P680, Q_B (H_2) exchanges with a mobile plastoquinone (PQ) molecule.
Reactions of the cyt b_6/f complex. Plastoquinol (PQH_2) delivers one electron to the Rieske Fe-S center (R-Fe-S), and the other to a cytochrome *b* (b_L). The electron on R-Fe-S reduces cytochrome *f* (cyt. *f*) and the one on cyt. b_L is transferred to cytochrome b_H (cyt. b_H), returning back in a cyclic process (called the Q-cycle).
Secondary reactions of PS I, Reduced cyt. *f* delivers its extra electron to a copper protein, plastocyanin (PC), which delivers the electron to $P700^+$ (produced in the primary PS I reaction). On the other hand, A_0 passes its electron to A_1 (perhaps a phylloquinone). The rest of the electron carriers are: Fx (an iron sulfur center X), F_B (an iron sulfur center B). F_A (an iron sulfur center A), Fd (ferredoxin) and FNR (ferredoxin-$NADP^+$ reductase).
Reaction times. The diagram shows either measured or estimated times of the various reactions in the Z-scheme, except for the production of P680* and P700* that occur in femtosecond time scale. The bottleneck reaction is of the order of 5 ms and it involves the total time involved in the exchange of $Q_B(H_2)$ with PQ, diffusion of PQH_2 to the cyt. b_6/f complex, and the reoxidation time of PQH_2. [The diagram does not show the steps involved in H^+ uptake and release.] (After Govindjee 1988, unpublished.)

tion of the sample has occurred, the emitted light is referred to as delayed light (see Jursinic 1986 for a review). However, if the separated charges are quasipermanently trapped and thermal excitation is required to induce the recombination of charges, the phenomenon is called thermoluminescence.

The simplest equation to describe the process of formation of a single TL band, due to the Randall-Wilkins theory, is, in differential form:

$$I = -\varphi \frac{dn}{dt} = \varphi nk = \varphi nse^{-E/k_BT} \qquad (1)$$

where I is the intensity of the glow, φ is a proportionality constant, n is the number of the trapped electrons, k is the reaction rate constant, s is the preexponential frequency factor, E is the activation energy, k_B is the Boltzmann constant and T is the absolute temperature.

The luminescence intensity as a function of temper-

ature goes through a maximum due to two opposing processes: an increase in the recombination rate and a decrease in the trapped charge population. The TL intensity plotted vs. temperature is called a glow curve (Fig. 3, right hand side). The glow curve of chloroplasts is the superposition of several TL bands corresponding to different charge separation states. In order to determine the parameters in equation (1), it is necessary to fit simultaneously the whole glow curve with the sum of TL bands using multicomponent curve fitting methods (Vass et al. 1981).

The activation entropy ($\Delta S^{\ddagger}$) of a charge separation state can be related to the frequency factor by:

$$\Delta S^{\ddagger} = k_B \ln (sh/k_B T_m) \quad (2)$$

where h = Planck's constant and T_m is the average temperature for the glow curve of a single peak. This assumes absolute reaction rate theory.

The peak position of a TL band is dependent on several factors including the rate of heating. It seems logical that the peak position of a TL band be also affected by the redox distance between the donor and acceptor pair undergoing charge recombination. Any change in the redox potential of either the donor or acceptor component may be reflected in a shift in the peak position of the corresponding TL band.

Vass et al. (1981) and DeVault et al. (1983) pointed out that the rate of recombination is determined not only by the rate-limiting back reaction step but also by earlier steps, which affect the concentration of charges that are ready for the rate-determining step. It was suggested that ΔH (enthalpy) of the earlier steps could add on to the activation energy, and ΔS (entropy) of the earlier steps to the activation entropy of the back reaction step. The Q or D band was explained (also see Rutherford et al. 1982) as due to charge recombination of S_2 and Q_A^- through intermediates S_1 and Z, and the B band as due to charge recombination of S_2 and Q_B^- through intermediates S_1 and Q_A^-.

TL in chloroplasts has been investigated in the temperature region from −196°C to 100°C. At least 13 TL bands exist in this temperature region peaking at around −180, −75, −55, −30, −15, 10, 20, 30, 45, 50, 65, 75, and 85°C, respectively. The peak positions of the bands depend on the measuring conditions, heating rate, excitation temperature and most of all on the mode of preillumination. The sample can be excited by flashes at a fixed temperature or by continuous illumination either during cooling or at a certain low temperature. Due to the different measuring conditions, the equivalence of the bands observed by different researchers is uncertain. The inconsistent designation of bands found in the literature enhances the confusion further. In the present review an alphabetical nomenclature introduced first by Arnold and Azzi (1968) will be used. The well known major TL bands are: Z (−180°C), Zv (−75°C), A (−10°C), D (~5°C) (in the presence of diuron), B (30°C), and C (55°C) (Fig. 4).

The main aim of the TL research has been the identification of the acceptor-donor pairs responsible for the generation of these TL bands. However, until now, the origin of only three TL peaks, the so called, A, D (or Q) and B bands, can be considered to be satisfactorily established.

The B band

The most characterized TL band of chloroplasts appears at around 30°C at pH 7.5 and is designated as the B band. The B band consists of two overlapping bands, the B_1 and B_2 bands. On the basis of flash experiments the B_1 and B_2 bands can be accounted for by the $S_2Q_B^-$ and $S_3Q_B^-$ charge recombination, respectively (Rutherford et al. 1982, Demeter and Vass 1984).

Model calculations confirmed this assignment. The charge recombination involves several steps: the electron on Q_B^- must go uphill to the first singlet excited state of the reaction center P680 with heat quanta, and the electron in the ground state of P680 must go uphill to the redox S_2^{2+} state of the WOC, converting it into the S_1^+ state of the WOC. This creates an excited state of P680 (P680*) by dark chemical back reactions; the excition, thus created here, migrates to an antenna chlorophyll *a* molecule and is released as a light quantum ($h\nu$) as noted earlier.

The oscillatory patterns of the B band intensity measured experimentally as a function of exciting flash number (maxima at 2nd and 6th flashes) agrees well with the calculated amount of $S_2Q_B^- + S_3Q_B^-$ states, especially if it is considered that the S_3 state is more efficient than the S_2 state in producing TL. The distribution of $Q_B : Q_B^-$ and $S_0 : S_1$ in dark-adapted materials also controls the flash number dependence (e.g. Rutherford et al. 1984a, b, Demeter and Vass 1984). Recently firm

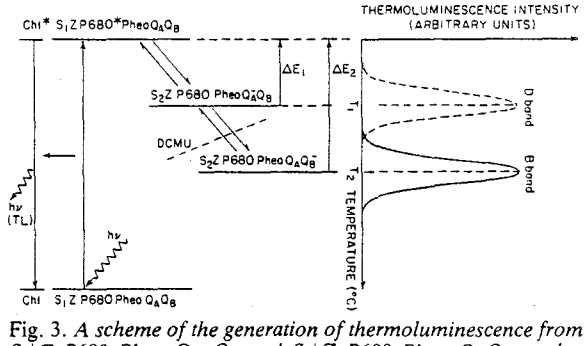

Fig. 3. *A scheme of the generation of thermoluminescence from S_2^+Z P680 Pheo $Q_A^-Q_B$ and S_2^+Z P680 Pheo $Q_AQ_B^-$ redox states.*

ΔE_1, and ΔE_2 are free energy losses in the charge separation steps; T_1 and T_2 are peak temperatures of the thermoluminescence bands in the glow curve, $h\nu$ is the energy of the absorbed photon, and $h\nu$ (TL) is the energy of the emitted thermoluminescence photon (after S. Demeter 1988, unpublished).

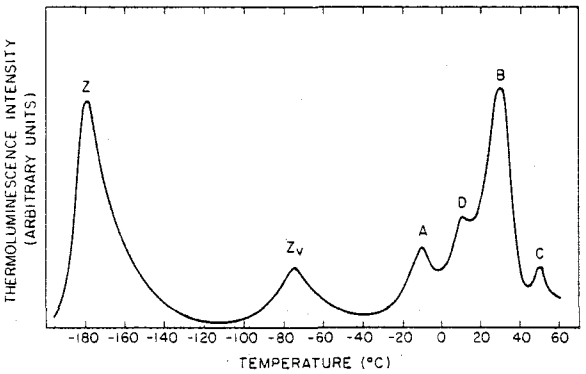

Fig. 4. *Idealized glow curves of chloroplasts representing the main thermoluminescence (TL) bands.*
Usually, these bands cannot be excited simultaneously in the same sample under a single experimental condition (after S. Demeter 1988, unpublished).

experimental evidence has been provided for the participation of Q_B^- in the generation of the B band (Wydrzynski and Inoue 1987). The removal of the secondary quinone molecules using a heptane/isobutanol extraction procedure resulted in a reversible abolition of the B band. At low pH (pH 5.5), the band splits into B_1 and B_2 bands as a consequence of the shift of the B_2 band to higher temperatures due to protonation of Q_B^- (Inoue 1981). The peak position of the B_1 band does not change, since the stabilization effect of the Q_B^- protonation is cancelled by the effect of a deprotonation event occurring in the $S_2 \rightarrow S_3$ transition (Rutherford et al. 1984b). The pH dependence of the positions of the B_1 and B_2 bands allows the determination of the pK values for the protonation of Q_B and Q_B^-. The corresponding values of pK 6.4 and 7.9 obtained by the TL method (Vass et al. 1986) are in good agreement with the values obtained by Robinson and Crofts (1984) by fluorescence methods.

The D (or Q) band

The inhibition of the electron transport chain between Q_A and Q_B by DCMU abolishes the B band with a concomitant appearance of a new band between 0 and 10°C. This band is attributed to $S_2Q_A^-$ recombination and is named D (see review in Sane and Rutherford 1986) or Q band (Demeter and Vass 1984). The temperature of this glow peak is shifted to a lower temperature, because the activation energy needed for this recombination reaction is lower than that for the $S_2Q_B^-$ reaction, as discussed by DeVault et al. (1983) (also see Fig. 3).

The A band

Under special measuring conditions, charge recombination can also be observed from the $S_3Q_A^-$ redox couple in normal samples. According to Koike et al. (1986) the A band of TL, which is emitted at around −10°C, arises from recombination between the negative charge on the primary quinone acceptor of PS II (Q_A^-) and the positive charge on the S_3 state of the O_2-evolving system.

Applications of thermoluminescence in the investigation of photosystem II

A. S-State transitions in the water splitting system

It is well known that the redox state of the oxygen complex undergoes the following reactions:

$$S_1 \text{ (dark state)} \xrightleftharpoons{^1h\nu} S_2$$

$$\text{(observed by low temperature EPR)} \xrightleftharpoons[H^+]{^2h\nu}$$

$$S_3 \xrightarrow[2H^+]{^3h\nu} S_4 \xrightarrow[O_2]{^4h\nu} S_0$$

The assignment of the B and D (Q) TL bands to the $S_2/S_3Q_B^-$ and S_2/Q_A^- recombination, respectively, opens up a new perspective in the study of the photosynthetic processes with regard to either the water-splitting system or the primary and secondary quinone acceptors. The changes in the amplitudes and peak positions of the B and D (Q) bands can be easily followed by TL technique, providing information about the changes in the redox states of the respective electron transport components. Since the S states participate in the generation of the B band, its amplitude exhibits a period-4 oscillation as a function of flash number (Inoue and Shibata 1982). The oscillatory behavior of the B band provides an unique opportunity to investigate the S state transitions even if the O_2 evolution is inhibited.

Role of chloride. Chloride ions function on the electron donor side of photosystem II (PS II) (see Fig. 2 and Homann 1987, Coleman and Govindjee 1987). TL measurements demonstrated that Cl^- depletion inhibits the $S_2 \rightarrow S_3$ transition (Ono et al. 1987, Rozsa and Demeter 1987, Vass et al. 1987b) and increases the redox spans of both $S_2Q_B^-$ (B band) and $S_2Q_A^-$ (Q band) charge pairs by about 60–80 mV, thus increasing the stability of these charge pairs against recombination (Vass et al. 1987b). The parallel increase in stabilization of $S_2Q_B^-$ and $S_2Q_A^-$ by Cl^- depletion can be explained by assuming the formation of an abnormal EPR multiline signal-silent S_2 (Ono et al. 1987) with a lowered (50–80 mV) redox potential.

Replaced of Cl^- by various anions in the S_2 state of the water splitting system resulted in an upward shift in the peak position of the B band indicating a decrease in the midpoint potential of the S_2 state (Ono et al. 1987). The extent of the shift of the TL peak position due to anion substitution changed in agreement with the order of effectiveness of these anions ($Cl^- \sim Br^- > NO_3^- >$

I^-) on O_2 evolution (cf. Critchley et al. 1982). Chloride may act to stabilize the oxygen evolving complex, adjusting its redox properties and/or participating in the extraction of protons from water.

In Cl^--depleted PS II particles, I^--binding to the D1 (herbicide-binding) protein reversed the shifting in the peak position of the B band, thus producing a normal peak (Ikeuchi et al. 1988). It was suggested that I^- associates with the Cl^--binding site and upon illumination donates electrons to the abnormal S_2 formed in the absence of Cl^- (Homann et al. 1986). From the iodination of D1 in both Mn-depleted and in Mn-retaining (but Cl^--depleted) PS II membranes, it was concluded that D1 protein bears a domain for Cl^--binding, and a Mn-binding site. (See also Coleman and Govindjee 1987, where Cl^- and Mn-binding sites are suggested to occur on both D_1 and D_2 polypeptides.)

The 33 kDa extrinsic popypeptide. This polypeptide is among the three extrinsic polypeptides (18, 24 and 33 kDa) involved in O_2 evolution. It is, however, the most important of this extrinsic group for its catalytic role. Contrary to the effect of Cl^- extraction, the removal of the 33 kDa extrinsic protein inhibits S_3 to S_4 transition (Ono and Inoue 1985) and selectively affects the stabilization of the $S_2Q_A^-$ redox couple with no effect on the $S_2Q_B^-$ charge pair (Vass et al. 1987a, b). Depletion of the 33 kDa protein increases the redox distance between S_2 and Q_A^- by 50–55 mV. The most plausible interpretation of the $S_2Q_A^-$ specific stabilization by 33 kDa protein removal is modifications in the redox potentials of both S_2 and Q_B^-, which compensate each other and result in an almost unchanged redox span of $S_2Q_B^-$ and an increased redox span of $S_2Q_A^-$.

Similar to the removal of 33 kDa protein, a short-term alkaline incubation of thylakoids reversibly inhibits the functioning of the water-oxidizing system by interruption of S-state transition from S_3 to S_4 (Vass et al. 1985). The primary event of inactivation by high pH is the release of 33 kDa peripheral protein, which facilitates Cl^- depletion and subsequently leads to the release of functional Mn as a secondary event.

Role of Mn. It is well known that 4 Mn ions per PS II reaction center are required for O_2 evolution. As expected, complete removal of Mn ions from the PS II particles abolishes all of the TL bands related to the oxygen-evolving system (Ono and Inoue 1985, Klimov et al. 1985). Readdition of $MnCl_2$ to Mn-depleted particles does not reactivate these TL bands.

B. Temperature dependence of the S state transitions

Since the thylakoid membranes undergo various molecular rearrangements accompanying S-state conversions, it can be expected that the S states exhibit different temperature dependencies. Direct observation of the S_2 multiline EPR signal allows the study of the temperature dependence of the $S_2 \rightarrow S_3$ transition. However, for the investigation of the other S state conversions, only the TL method is suitable. Following the changes in the oscillation pattern of the B band, Inoue and Shibata found that the $S_2 \rightarrow S_3$ transition is blocked at −35°C and the $S_3 \rightarrow S_4$ step at −20°C (see review by Inoue and Shibata, 1982). Somewhat different values were obtained from the comparison of the temperature dependencies of the amplitudes of the B and D (Q) bands, namely, −65°C for the inhibition of the $S_2 \rightarrow S_3$ transition and −40°C for the block of the $S_3 \rightarrow S_4$ transition (Demeter et al. 1985a, b).

Recently, by TL measurements, a low-temperature sensitive intermediate precursor state, denoted as PS_3, has been suggested to exist between S_2 and S_3 in the photosynthetic water oxidation (Koike and Inoue 1987). The PS_3 state is suggested to be an S_3 state, which is formed by a one electron abstraction from S_2 with no H^+ release due to low temperature.

The temperature dependence of the electron transport between the primary quinone (Q_A) and the secondary (Q_B) was also investigated by TL. A threshold temperature of −65°C was obtained for the interruption of electron transfer between Q_A and Q_B (Demeter et al. 1985b).

C. Deactivation of the S_2 and S_3 states

In darkness, the only stable states are the S_0 and S_1 states, with S_1 being the most stable state; the S_4 state is short-lived and quickly converted to S_0, whereas S_2 and S_3 deactivate to the S_1 state. TL has proved to be a powerful method to investigate the deactivation of these S states. The deactivation of the S_2 state in the S_2Q_B centers ($t_{1/2}$ = 150 s) was much slower than in the $S_2Q_B^-$ centers ($t_{1/2}$ = 20–30 s) (Rutherford et al. 1984a). This suggests that Q_B^- is the major source of electrons responsible for the deactivation of S_2 in centers in the state $S_2Q_B^-$. The source of electrons responsible for deactivation of S_2 in the S_2Q_B centers is not known. As suggested by Rutherford et al. (1984b), it could be plastoquinol (PQH_2) according to the reaction:

$$S_2Q_B + PQH_2 \rightarrow S_1Q_B^- + PQ + 2H^+.$$

To follow the deactivation of S_3 by TL is rather complicated because S_3 decays via S_2. Thus, the deactivation of the S_2 and S_3 states are superimposed in the TL intensity, and it is difficult to separate them. In a thermophilic alga *Synechococcus vulcanus,* the $S_2Q_B^-$ and $S_3Q_B^-$ states proved to be more stable than in chloroplasts or leaves (Govindjee et al. 1985b). The peak position of the B band appeared at 55°C. The half-times of the deactivations, at room temperature, of the $S_2Q_B^-$ and $S_3Q_B^-$ states, as measured by both TL and O_2 polarography, were 200 and 75 s, respectively.

In agreement with the results obtained by other methods, TL observations demonstrated that in the presence

of ADRY reagents the deactivation of the S_2 and S_3 states is accelerated (Renger and Inoue 1983).

D. Effects of herbicides

A large number of photosynthetic herbicides block the electron transport of PS II between the primary and secondary quinone acceptors. In all likelihood, these herbicides work by displacing the Q_B from its binding site on the D_1 protein. These herbicides have their binding niche on this protein (see Trebst 1987). Since Q_A^- and Q_B^- contribute to the generation of the D (Q) and B bands, it can be expected that any herbicide-induced change in the state of these acceptors will be reflected in the amplitude and peak position of the related TL bands. Indeed, in the presence of DCMU-type herbicides that have a common structural element, the $-\overset{\|}{C}-N-$ group, the peak position of the D (Q) band appears between 0 and 10°C (Vass and Demeter 1982). On the other hand, phenolic herbicides give rise to a band between 0 and −15°C. These TL observations indicate, in agreement with earlier reports, that in addition to the inhibition of electron transfer out of Q_A^-, phenolic herbicides have an action site on the donor side of PS II as well. The TL results were confirmed by DLE investigations (Hideg and Demeter 1986). In the presence of phenolic herbicides, the decay of DLE was considerably faster than in the presence of DCMU-type herbicides. On the basis of the peak position of the D (Q) band, the PS II herbicides were classified into two main groups (Vass and Demeter 1982). Since the effects of DCMU- and phenolic-type herbicides on the peak position of the D (Q) TL band are different, their displacement in the chloroplast membrane can be followed by TL measurements (Droppa et al. 1981a, b, c, Demeter et al. 1982). In contrast to the use of expensive, radioactively labelled herbicides, TL proved to be a new and simple technique to monitor the displacement of dinitro-o-cresol (DNOC) by atrazine and DCMU, or the displacement of bromoxynil by DCMU.

The presence of the D (Q) band in the TL glow curve of inhibitor-treated chloroplasts is indicative of an inhibitory action at the level of Q_B. This TL characteristic was applied to demonstrate that high concentrations of trifluralin, disalicydenepropanediamine (DSPD) and 2-heptyl-4-hydroxyquinoline-N-oxide (HQNO) block the electron transport between Q_A and the PQ pool (Droppa et al. 1981b). The application of TL in the herbicide research is reviewed in detail elsewhere (Horvath 1986).

E. Herbicide resistance

Herbicide resistance is due to an alteration in the herbicide/quinone binding protein environment, which binds the secondary quinone acceptor, Q_B (Fig. 1). Single amino acids are changed in these resistant plants. The membrane alteration responsible for the decreased herbicide affinity at the herbicide binding site is accompanied by a change in the functioning of electron carriers on the reducing side of PS II. Since any change in the state of the Q_A and Q_B acceptors is reflected in the positions of the D (Q) and B bands, TL becomes a suitable method for studying possible modifications in the redox states of Q_A and Q_B conferred by herbicide resistance.

Comparison of TL of chloroplasts from triazine-susceptible and -resistant plants (Demeter et al. 1985c) showed that the D (Q) band, attributed to the charge recombination of the S_2 and Q_A^- redox states, appeared at about the same temperature in both biotypes, indicating that the triazine resistance is not accompanied by an alteration in the midpoint potentials of the S_2/S_3 and Q_A/Q_A^- redox couples. On the other hand, the position of the B band was shifted from +30 to about +15°C in the glow curve of resistant chloroplasts as compared to the sensitive ones.

DLE investigation of triazine-resistant and susceptible chloroplasts in the seconds to minutes time scale led to results similar to those obtained by TL, confirming the common origin of the two phenomena (Hideg and Demeter 1986). The decay of DLE was considerably faster in the resistant biotype than in the susceptible one. The shift in the peak position of the B band and the acceleration of the decay of DLE in resistant chloroplasts can be accounted for by a decrease in the midpoint potential of Q_B^- in resistant chloroplasts as compared to the susceptible ones (Demeter et al. 1985c). The difference in the decay time of DLE and in the peak position of the B band in susceptible and resistant plant biotypes enables one to apply DLE and TL analysis as a new technique for a quick determination of triazine resistance in plants.

Comparison of TL and DLE of urea-, triazine- and phenolic-type herbicide-resistant *Nicotiana plumbaginifolia* mutants showed that the acceleration of the decay of DLE and the shift in the peak position of the B band depended rather on the degree than on the type of resistance (A. Cséplö, and E. Hideg, personal communication). The phenomenon is more pronounced in plants with a greater degree of resistance.

F. Role of bicarbonate

As shown in Fig. 1, HCO_3^- may be bound to the PS II very near the binding niche of the herbicides. Bicarbonate has been suggested to be required for the operation of the electron acceptor side of PS II: i.e., on the Q_A-Fe-Q_B complex (Govindjee and Van Rensen 1978, Govindjee and Eaton-Rye 1986). It is shown that HCO_3^- reverses the inhibitory effect of formate (HCO_2^-) on the Q_A^- to $Q_B^{(-)}$ reaction, and on the exchange of Q_B with the PQ pool. The absence of HCO_3^- seems to slow down the reduction of the plastoquinone pool by slowing down the arrival of electrons as well as of protons to PQ (Govindjee and Eaton-Rye

1986, Blubaugh and Govindjee 1988, Eaton-Rye and Govindjee 1988, Van Rensen et al. 1988).

Bicarbonate depletion resulted in (a) a shift of the B band to higher temperatures indicating, a stabilization of the $S_2/S_3Q_B^-$ charge pair; (b) the elimination of the flash number-dependent oscillations in TL intensity, indicating a blockage in the electron flow at the two-electron gate Q_B (Govindjee et al. 1984). This establishes the site of HCO_3^- action at PS II by the TL method; the same site has been shown to exist in leaves by this method (Garab et al. 1988).

Concluding remarks

TL originates from recombination of positive and negative charges (Fig. 3) separated in the photosynthetic light energy conversion process and subsequently stored on various components of the electron transport chain (Fig. 2). A shift in the peak position of a TL band indicates a change in the redox distance between the interacting positively charged donor and negatively charged acceptor. Comparison of TL in triazine susceptible and resistant chloroplasts demonstrated a lowering in the midpoint potential of Q_B^- in the resistant chloroplasts as compared to the susceptible ones. It was also found that the removal of the 33 kDa protein or Cl^- from the water-splitting system resulted in an increase in the redox span of the $S_2Q_A^-$ redox couple. Similar stabilization of the $S_2Q_A^-$ state could also be observed as a result of the effect of high pH.

The oscillation in the amount of oxidized donor or reduced acceptor molecules undergoing charge recombination can be followed from the flash-dependent amplitude changes in TL intensity. On the basis of the oscillation pattern of TL, it was concluded that removal of Cl^- from the water-splitting system inhibits the $S_2 \rightarrow S_3$ transition. On the other hand, extraction of the 33 kDa protein, or short term incubation at high pH, results in an inhibition of the $S_3 \rightarrow S_4$ transition. Analysis of the TL oscillation of chloroplasts as a function of temperature allowed the determination of threshold temperatures of S state transitions.

The disappearance of one TL band with a concomitant intensification of another one indicates the interruption of the electron transport chain and accumulation of charges on new components located before the blocking site. This characteristic of TL allowed the determination of a new site of action of trifluralin and disalicydenepropanediamine (DSPD) between the Q_A and the PQ pool. The existence of an additional site of action of phenolic herbicides at the donor side of PS II could also be verified by TL.

It can be expected that in the future, the application of TL in photosynthesis research will become broader in scope. Parallel TL, EPR, and absorption change measurements will be helpful in the identification of TL bands of unknown origin. The TL method can be fruitful in the investigation of the heterogeneity of the acceptor and donor side of PS II. At low temperature (77K), the oxidation of alternate donors to P680 [carotene, a slow electron donor D (that resembles Z), cyt b_{559}, and a component responsible for the $g = 4.2$ EPR signal] suggests that these donors can be resonsible for some of the TL bands. Since Q_A is not connected to the PQ pool in the so-called PS II_β centers (located in the stroma lamellae), TL could make a distinction between these and PS II_α centers (the major PS II centers, located in the grana). TL may provide new information about the redox reactions of a component, labeled as Q_{400} and originating in Fe of the Q_A-Fe-Q_B complex C (Petroleas and Diner 1986). Furthermore, TL may also prove to be a useful method to probe the impairment of PS II during photoinhibition of chloroplasts at high light intensities.

At present the application of the TL method is almost completely restricted to the photosynthesis of chloroplasts. The introduction of TL into the investigation of bacterial photosynthesis would open a new promising field for research. For a description of bacterial reaction center, see Michel and Deisenhofer (1988).

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