Chapter 19

Thermoluminescence

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Summary

Thermoluminescence (TL) of photosynthetic membranes was discovered by William Arnold and Helen Sherwood in 1957. In the last half century, several studies have elucidated the mechanism of TL emission, which showed that the recombination of different charge pairs generated and trapped during pre-illumination are responsible for the observed light emission. Since most of the TL bands originate within Photosystem II (PS II), the technique of TL has become a useful complementary tool to chlorophyll *a* fluorescence to probe subtle changes in PS II photochemistry. The technique is simple and non-invasive; it has been successfully used to study leaf, cells, thylakoids and even reaction center preparations. The TL technique provides quick information about the redox potential changes of the bound primary quinone (Q_A) and the secondary quinone (Q_B) acceptors of PS II; TL has been extensively used to study the effects of photoinhibition, mutations, stresses and myriad responses of the photosynthetic apparatus during acclimation and adaptation. This chapter reviews crucial evidence for the identification of charge pairs responsible for the generation of different TL bands; the relationship of these bands to the components of delayed light emission; responses to excitation pressure arising out of environmental factors; methodology, and instrumentation. A model based on the detailed analysis of the redox shifts of the PS II electron acceptors Q_A and Q_B, explaining the possibility of non-radiative dissipation of excess light energy within the reaction center of PS II (reaction center quenching) and its physiological significance in photoprotection of the photosynthetic membranes has been suggested. Developments in the analysis of biophysical parameters and the non-adherence of photosynthetic TL to the analysis by the 1945 theory of J.T. Randall and M.H.F. Wilkins have been briefly reviewed.

Abbreviations: ATP – Adenosine triphosphate; CAM – Crassulacean acid metabolism; Cyt b_{559} – Cytochrome b_{559}; D1 – 32 kDa Photosystem II reaction center polypeptide; D2 – 34 kDa Photosystem II reaction center polypeptide; DCMU – 3-(3,4-dichlorophenyl)-l,l-dimethylurea; DHAP – Dihydroxyacetone phosphate; EGTA – Ethyleneglycol bis (beta-aminoethyl ether)-N,N,N',N'-tetra acetic acid; DLE – Delayed light emission; EPR – Electron paramagnetic resonance; LHCII – Light-harvesting complex of Photosystem II; MDA – Malondialdehyde; NAD(P)H – Nicotinamide adenine dinucleotide phosphate, the reduced form; P680 – Primary electron donor chlorophyll of Photosystem II; PGA – 3-phosphoglycerate; Pheo – Pheophytin; PS I – Photosystem I; PS II – Photosystem II; PQ – Plastoquinone; Q_A – Primary electron-accepting quinone in Photosystem II reaction centers; Q_B – Secondary electron-accepting quinone in Photosystem II reaction centers; S states – Oxidation states of the manganese cluster of PS II; TL – Thermoluminescence; T_M – Temperature of maximum thermoluminescence emission; Tris – Tris(hydroxymethyl) aminomethane; Y_D^+ – Redox active tyrosine-160 of the D2 protein; Y_Z^+ – Redox active tyrosine-161 of the D1 protein

I. Introduction

A. Definition

Thermoluminescence (TL) in semiconductors has been well studied and has been shown to originate in a recombination of the hole and the free electron that is generated during the exposure of the sample to electromagnetic radiation. Exposure of a dielectric specimen to ionizing or non-ionizing radiation results in the production of holes and free electrons that get trapped in the lattice of the material because of defects. When they acquire enough energy, the probability of escape of the trapped charge carriers is increased and their recombination produces electromagnetic radiation. Because the energy for the release of the electrons is provided by heating of the specimen the resultant luminescence is called *thermoluminescence*. When pre-illuminated oxygenic

photosynthetic membranes are cooled and then heated in the dark from low temperature, they emit light at distinct temperatures yielding what is known as the glow curve(s) or TL. The TL observed in photosynthetic membranes was initially explained on the basis of a mechanism in semiconductors. However, it became apparent that TL in photosynthetic membranes occurs as a result of a recombination between the oxidized donors and reduced acceptors produced in the preceding light exposure. Furthermore, there are several recombination events that are involved in generating the complex glow curves since several peaks are produced that are susceptible to electron transport inhibitors, modifiers of the water-oxidizing complex or compounds that interfere with the light-induced electron transport.

B. Historical

The discovery of delayed light emission (DLE) by photosynthetic organisms that later led to the study of TL was, in a way, a chance event. When Strehler and Arnold (1951) were attempting to demonstrate the photosynthetic formation of energy rich phosphate (ATP) (adenosine tri phosphate) using a firefly luminescent assay, they observed that light was given off by the chloroplasts even in the absence of the firefly extract. They studied different characteristics of this light emission and concluded that this DLE was a reflection of the reversibility of certain early reactions of photosynthesis. Several interesting observations were made and they were subsequently confirmed and extended by other studies. These included, e.g., the effect of inhibitors of photosynthesis, the action spectra and its dark decay.

Arnold and Sherwood (1957) discovered TL in dried chloroplasts and had used the term glow curve. Tollin and Calvin (1957) also showed that there are several components of DLE decaying with different half lives. Furthermore, they also showed TL from dried chloroplasts yielding two peaks, one between 50°C and 60°C and another between 140°C and 150°C. A mechanism involving the recombination of holes and electrons trapped during illumination was proposed by Arnold (1965). In subsequent studies, Arnold (1966) and Arnold and Azzi (1968) reported TL glow curves in green algal species belonging to the genera *Chlorella* and *Scenedesmus*, including *Scenedesmus* mutants. However, the first well resolved glow curves were published by Rubin and Venediktov (1969) and Shuvalov and Litvin (1969).

It was clear that not only are there several bands that appear at different temperatures but also that most of these originated in PS II. Furthermore, there were indications that TL and DLE are related to each other. These important observations were confirmed and further elaborated in the 1970s by P.V. Sane, V.G. Tatake and T.S. Desai using continuous illumination of the sample during freezing and by Yorinao Inoue and Kazuo Shibata using both flash and continuous illumination (see Inoue and Shibata, 1982; Sane and Rutherford, 1986). The flash excitation studies provided a greater insight into the mechanism of origin of PS II-related TL bands. These and subsequent studies during the 1980s by several research groups demonstrated the potential of the TL technique in probing PS II. In more recent years the bands that appear at somewhat higher temperatures have also been characterized. We now know that TL can be used not only to probe PS II but in addition it can provide information on several other physiological aspects. Several excellent reviews have appeared on these aspects (Inoue and Shibata, 1982; Sane and Rutherford, 1986; Demeter and Govindjee, 1989; Inoue, 1996; Vass and Govindjee, 1996). Vass (2003) has also provided an account of the history of photosynthetic TL that also includes photographs of the many researchers in the field of TL in photosynthetic systems. In this chapter we will emphasize those studies that have appeared since 1995 and were not covered by the previous reviews (cf., however, Ducruet and Vass, 2009; and Rappaport and Lavergne, 2009).

Earlier and subsequent studies (see previous reviews cited above for detailed discussion) from different laboratories have provided strong evidence to suggest that several recombination events emit light generating characteristic TL bands occurring at various temperatures during warming in darkness of the illuminated sample. Apart from the very low temperature peaks (the Z peaks) and those that appear at higher temperatures (above 50°C) all other bands are either sensitive to inhibitors such as 3-(3,4-dichlorophenyl)-l,l-dimethylurea (DCMU)

or to procedures that inhibit the water-oxidizing complex. Therefore, it is evident that these bands are all associated with recombination events occurring within PS II involving either of the two quinone (electron) acceptors (Q_A and Q_B) and one or the other S states of the water-oxidizing complex. The emission spectra of the TL bands are similar to the fluorescence spectra from chloroplasts (see e.g., Sonoike et al., 1991) and therefore the light emission must occur from the PS II antenna systems.

Light emission by plants and algae has provided a very valuable tool in our hands to probe the function of the light-associated reactions. Chlorophyll *a* fluorescence has been used for several decades to monitor the overall function of the photosynthetic apparatus, including that of PS II, quenching mechanisms and intersystem electron transport, as well as the effects of stress conditions (for reviews on chlorophyll *a* fluorescence see Papageorgiou and Govindjee, 2004). Thermoluminescence which provides information on the back reactions and recombinations of oxidized and reduced electron donors and acceptors of PS II has proven to be an excellent complement to the fluorescence studies. Although TL was discovered almost five decades ago its extensive use in probing PS II function has increased dramatically in the past three decades or so. With a better understanding of the mechanism and origin of different TL bands, its utility has been extended from merely probing the reactions occurring within PS II to understanding energy charge and stress related responses including lipid oxidation under in vivo conditions. It is emerging as a technique with some unique applications.

C. Instrumentation

Thermoluminescence measurements have usually been made by easily assembled, home-made instruments using a cold finger or sample holder that can be quickly cooled and heated at a predetermined rate of heating, a temperature sensor located in the sample holder for an accurate measurement of the sample temperature, and an end window type red sensitive photomultiplier equipped with high voltage power supply and signal amplifier. The apparatus is then coupled to a computer data acquisition and signal processing system that can display the signal from the photomultiplier and temperature sensor on Y and X, axes, respectively. To obtain the best signal, the photomultiplier is located as close as possible to the sample; the cooling of the photomultiplier tube reduces the noise further. In many instruments a strong lens is used to focus the light emitted from the sample onto the photomultiplier tube to improve the signal to noise ratio. The earlier versions of the set ups were somewhat cumbersome although quite sensitive with respect to the temperature of the sample (Tatake et al., 1971). With enormous improvements in electronics, compact PCs and very powerful software for signal processing, the present day TL instruments are very versatile and yet quite simple. Furthermore, if one is interested only in measuring PS II-related TL peaks, cooling of the sample to liquid nitrogen temperature is not needed and the cold finger can be even simpler. Our research group has been extensively using a simple set up described and built by Zeinalov and Maslenkova (1996) for routine measurements of glow curves of leaf and algal samples. It has a small heat capacity sample holder that can be very quickly cooled to −80°C and heated at a very fast rate of 36°C min^{-1}. Several samples per hour can be studied with such a set up. A versatile and efficient system for measurements of glow curves using flash excitation and luminescence decay has been described by Ducruet and Miranda (1992). Bhatnagar et al. (2002), Ducruet (2003) and Gilbert et al. (2004b) have described improved versions for specific uses. A flash lamp that can excite the sample with a single turnover saturating pulse is essential for flash induced TL studies. Such flash lamps are readily available commercially. The first commercially available computerized thermoluminescence system (Z700) has been introduced recently by Photon Systems Instruments (Brno, Czech Republic) and is distributed worldwide by Qubit Systems Inc. (Kingston, Ontario, Canada).

The use of programs such as THERMO Lite (Verion 1.5/2005) developed by Jean-Marc Ducruet (Ducruet and Miranda, 1992) permit deconvolution and resolution of the complex glow curves obtained. This is particularly important when glow curves are obtained from a sample that was illuminated continuously during freezing. The flash induced glow curves are usually simple and contain a single major peak. Numerical analysis of glow curves has been described by

Ducruet and Miranda (1992). Care should be taken during deconvolution of glow curves (Sane, 2004) so that one does not generate false peaks through deconvolution.

Samples such as leaf discs are directly placed on the sample holder while a suspension of algal cells, chloroplast or thylakoid membranes is usually spotted on a filter disc. Some laboratories use a plastic holder to place the leaf or filter disc to prevent damage from the plant/algal material to the cold finger. In all the cases one needs to know the difference in the actual temperature of the sample and the one indicated by the set up. Necessary corrections need to be applied to ensure that the corrected peak temperatures are reported. This is essential particularly when biophysical constants are to be calculated from the obtained data. The excitation of the sample either by continuous light during freezing of the sample or by flash excitation with a single flash, series of flashes or a combination of continuous light followed by flashes at specific temperature is done on the stage of the sample holder at a predetermined temperature prior to quickly cooling the sample. (For a review, see Ducruet and Vass, 2009)

II. Glow Curves and their Characteristics

A. Nomenclature

In their initial studies, different research groups had used different nomenclatures for the TL bands observed. This led to some confusion, but at present there is unanimity regarding the nomenclature of different TL bands. We will use the existing nomenclature in describing the TL bands (Inoue, 1996).

B. Description of Different Thermoluminescence Bands

There are over 13 TL bands that have been detected and described under different conditions. Of these, eight that appear between −50°C and +50°C are most important as these originate within PS II. A set of three bands that appear at higher temperatures are associated with lipid oxidation and provide an indication of the oxidative damage to the thylakoid membrane in response to various stress conditions. A list of these bands is given in Table 19.1 along with their respective DLE components.

1. Low Temperature Bands

The low temperature bands that appear below 77 K and the one appearing at 110–120 K have been designated as Z bands. Those that appear at 20, 50 and 70 K have been studied and described by Noguchi et al. (1993) by exciting photosynthetic pigment protein complexes and purified pigments at liquid helium temperatures and are called Z-α, Z-β and Z-γ, respectively. They do not originate in charge separation or electron transport reactions but are associated with the charge storage in chlorophyll aggregates and their interactions with their ligands. These bands therefore

Table 19.1. Thermoluminescence peak temperatures (T_M), charge pairs responsible for their emission, and related DLE components

Peak	Approximate peak position T_M (°C)	Charge pairs	Related DLE component
Very low temperature peaks	⁻250, ⁻220, ⁻200	Aggregated chlorophylls	
Z peak	⁻160	Chl^+Chl^-	
High temperature bands	+50 to +160	Oxidative chemiluminescence	
Photosystem II related bands			
Zv (variable)	−80 to −30	$P680^+Q_A^-$	Approx. 150 μs
A band	−10	$S_3Q_A^-$	4 ms
A_T band	−10	$His^+Q_A^-$	4 ms?
Q band	+5	$S_2Q_A^-$	2 s
B1 band	+20	$S_3Q_B^-$	30 s
B2 band	+30	$S_2Q_B^-$	60 s
C band	+50	$TyrD^+Q_A^-$	10 min
A_G band	+ 40 to 50	$S_2/S_3Q_B^-$ (see text)	

are of little importance for understanding the photochemistry of PS II.

Of the low temperature TL bands, the Z band that appears at −160°C has been well studied (Arnold and Azzi, 1968; Shuvalov and Litvin, 1969; Sane et al., 1974; Sonoike et al., 1991). Under continuous illumination at liquid nitrogen temperature this band is more efficiently excited by blue than by red light. It emits with a peak around 730–740 nm and was suggested to arise in chlorophyll triplets (Sane et al., 1974), or possibly in charge pairs between two Chl molecules (Sonoike et al., 1991). This band can also be excited by gamma rays. The band arises in isolated chlorophylls and is not important in understanding photosynthetic electron transport.

2. Bands Related to Photosystem II

There are at least eight bands that seem to arise within PS II. The reducing equivalents for all these bands are contributed by the two quinone acceptors of PS II, namely Q_A and Q_B. The oxidizing species involved contain the two charged S states, namely the S_2 and S_3 states, and the three electron donors closer to the reaction center II. These eight bands have proved very useful in understanding the function of the PS II complex.

a. The Variable Z_v Band

This band is called Z_v as it occurs at a variable temperature depending upon the temperature of excitation. Ichikawa et al. (1975) showed that this band appears at a temperature that is about 10–20°C higher than the temperature of its excitation. It appears in a sample preparation that has no active water-oxidizing complex (Vass et al., 1989) indicating that S states are not involved in its origin. The oxidizing equivalent for its origin is suggested to be $P680^+$. Studies by Chapman et al. (1991) using reconstitution experiments showed that the reducing entity involved is the reduced primary quinone electron acceptor Q_A^-. It appears that the charge pair responsible for its origin is $P680^+Q_A^-$. However, it is not clear why its temperature of emission is variable. Desai et al. (1977) proposed that the band may arise from charging of both the Z band and the A band and this could explain its dependence on the temperature of excitation. Klevanik (1995), using isolated PS II reaction center preparations, suggested that the charge pair responsible is oxidized tyrosine Tyr^+ and a reduced plastoquinone.

b. The A Band

A band is known to appear between −10°C and −40°C when continuous illumination is used during freezing of the sample (see Sane and Rutherford, 1986). The recombining charge species appears to reside in PS II as the band is excited by red but not far-red light (absorbed in PS I only). It appears in a PS II fraction but not in a PS I fraction and is absent in mutants lacking PS II (Arnold and Azzi, 1968; Shuvalov and Litvin, 1969; Lurie and Bertsch, 1974; Desai et al., 1975; Ichikawa et al., 1975; Sane et al., 1977). Based on its sensitivity to DCMU, Sane et al. (1977) suggested that the A band either may arise in a recombination of charges involving an acceptor beyond Q_B with the S_3 state or it may arise in the Q_A^- and S_3 state.

Demeter et al. (1985a) assigned this A band to the charge recombination of Q_A^- with the oxidized donor Z^+ based on its insensitivity to Tris-washing and NH_2OH treatment but it was present in DCMU-treated thylakoids that were exposed to two pre-flashes before the addition of DCMU and subsequent illumination at low temperature. The involvement of the S_3 state was explained by Koike et al. (1986). They used flash excitation at 15°C to create different S states followed by the addition of DCMU. The sample was then cooled to liquid nitrogen and exposed to continuous illumination. The excitation at liquid nitrogen in the presence of DCMU reduced Q_A by oxidizing Cyt b_{559} without changing the previously created S states and without reducing Q_B. The results and oscillations obtained for this band using the above approach suggested that this band arises in $S_3Q_A^-$.

c. The A_T Band

Another band that is insensitive to inhibitors of the water-oxidizing complex also appears at around −10°C (Inoue et al., 1977; Demeter et al., 1979; Sane et al., 1983a). It is different from

the A band since the S states do not participate in its production. However, it does arise in PS II fractions. Using chemical modification of histidine in Mn–depleted PS II preparations, Ono and Inoue (1991) suggested that the band arises in samples with oxidized histidine and Q_A^-. Using PS II particles from site-directed mutants of *Chlamydomonas reinhardtii* which involved His195 and His190 of the PS II D1 polypeptide, Kramer et al. (1994) demonstrated that modification of His190 to phenylalanine completely abolished the A_T band. Modification of His195 did change the intensity of the peak but did not shift the peak temperature. These data established that the positive charge was donated by His190.

d. The Q Band

The Q band appearing around 5°C has been very well studied and is related to the recombination of $S_2Q_{A.}^-$. The intensification of this band by DCMU addition (Rubin and Venediktov, 1969; Lurie and Bertsch, 1974; Desai et al., 1975; Ichikawa et al., 1975) suggested that the reducing equivalents may originate in Q_A^-. Desai et al. (1975) demonstrated that the decrease in fluorescence yield of the sample during warming was associated with the appearance of this band suggesting that the oxidation of Q_A^- was responsible for its production. This was probably the first direct evidence that related the oxidation of Q_A^- with the appearance of a TL band. The requirement of a functional water-oxidizing complex and hence S states was evident from the observations that the band was sensitive to mild heating (Sane et al., 1977) and that it was lost upon treatment of the thylakoids by Tris-washing or tetranitromethane that destroy the S states (Sane et al., 1983b). The involvement of S_2 was most clearly demonstrated by Rutherford et al. (1982) who showed that this peak appears in DCMU-treated thylakoids upon excitation by a single flash at −15°C. In the presence of some of the herbicides there is a shift in the temperature (T_M) of its peak. Droppa et al. (1981) observed that depending upon the type of herbicide used the Q band appeared at three different temperatures namely +5°C, 0°C or –14°C. They attributed this variation to the various redox states of the quinone acceptors due to the binding of the herbicides.

e. The B Bands

The most investigated bands that have provided valuable information on the function of PS II are the B bands arising in $S_2/S_3Q_B^-$. These bands are lost in the presence of inhibitors such as DCMU that block electron transport between the primary (Q_A) and secondary (Q_B) quinone acceptors (Arnold and Azzi, 1968; Rubin and Venediktov, 1969; Desai et al., 1975; Ichikawa et al., 1975). The band appearing around 30°C is also lost if the water-oxidizing complex is either destroyed or absent (Inoue, 1976; Rosza and Demeter, 1982; Sane et al., 1983a). Furthermore, this band was shown to oscillate with a periodicity of four (Inoue and Shibata, 1977a, b). These observations were clear indications that the B bands arise in a recombination reaction between the oxidized S states and Q_B^-. The involvement of Q_B^- has been confirmed by several workers (see reviews by Sane and Rutherford 1986; Inoue, 1996; and Vass and Govindjee, 1996; Rappaport and Lavergne, 2009). Although the bands appearing around 30°C were earlier assigned to $S_2/S_3Q_B^-$ it became clear that there are two bands, one arising in $S_2Q_B^-$ and another in $S_3Q_B^-$. Inoue (1981) showed that at pH values lower than 6 the two bands appear as distinct TL glow peaks at 20°C and 35°C respectively, while at higher pH values the bands appear as one peak at 35°C. The $S_3Q_B^-$ peak has been designated as the B1 band while the $S_2Q_B^-$ peak has been designated B2. The B1 band appears at lower temperatures (8–10°C lower than the $S_2Q_B^-$ band). If a dark-relaxed leaf is excited by one short saturating (single turnover) flash, a major band related to $S_2Q_B^-$ is seen, while with two flashes a composite B band ($S_2Q_B^- + S_3Q_B^-$) is seen. The emission intensity of the $S_3Q_B^-$ recombination has been shown to be 1.7–2 times higher than that arising from $S_2Q_B^-$ for reasons still not well understood (Demeter et al., 1985a, b; Rutherford et al., 1985). Since these two bands involve the participation of the S_2 and S_3 states and the two-electron acceptor quinone (Q_B), they provide information on important changes in the PS II reaction centers. Thus, numerous studies on photoinhibition and stress responses have monitored the changes in the intensities and shifts in the T_M or peak emission temperatures of these two bands to understand and probe the effects on PS II and electron transport

between the two photosystems. These aspects will be discussed later.

Although the usual temperature activating recombination of the $S_2Q_B^-$ state is around 30°C, the characteristic T_M of this band is highly variable and depends upon the growth conditions, species, and abiotic or biotic stress (see: Tables 19.2 and 19.3). For example, in *Synechosystis* sp. PCC 6803 grown at 30°C the $S_2Q_B^-$ band was reported at 36°C (Minagawa et al., 1999), while for the same strain also grown at 30°C but in another laboratory the $S_2Q_B^-$ signal appeared at 24–26°C (Shen et al., 1998). These variations are due to the use of different equipment, changes in growth conditions other than temperature and different heating rates. Similar variations can be seen in other bands also. Govindjee et al. (1985) have shown that in thermophilic cyanobacteria the Q and B bands appear at much higher temperatures of 35°C and 50–55°C, respectively. Therefore, the T_M of the peak alone does not reflect the charge pair responsible for its emission. One must provide additional evidence to ascertain the charge pair responsible for the band under consideration.

f. The C Band

In spinach leaves and cells from a species belonging to the genus *Euglena*, Desai et al. (1975) observed a prominent band appearing around 45–50°C that could be excited by far-red light and enhanced by DCMU (Ichikawa et al., 1975). The band was suggested to arise in PS I in view of its excitation by far-red light, its enrichment in a PS I fraction and its insensitivity to tetranitromethane and Tris treatment (Desai et al., 1975; Sane et al., 1977; Inoue et al., 1977) that destroy the water-oxidizing complex and hence no S-states are detected.

While studying the flash excitation of this band appearing at 50°C in maize inside-out thylakoid membranes, Demeter et al. (1984) suggested that the C band may arise in a recombination between $S_0Q_A^-$ or $S_1Q_A^-$. The involvement of Q_A^- was inferred because of the enhancement of the band in the presence of DCMU. Neither the S_0 nor the S_1 states are known to carry a positive charge and hence they cannot be involved in a recombination reaction. In a subsequent study, Demeter et al. (1993) compared the TL and Electron Paramagnetic Resonance (EPR) measurements in DCMU-treated PS II particles. They concluded that the C band seemed to arise in a recombination of the g=1.82 form of $Q_A^-Fe^{2+}$ and the oxidized tyrosine Y_D^+ (of D_2) responsible for the EPR signal II. Krieger et al. (1993) showed that low pH treatment of PS II particles caused a decrease in the $S_2Q_A^-$ signal with a concomitant increase in the intensity of the C band. The interconversion of the $S_2Q_A^-$ band and C band was more pronounced in the presence of the Ca^{2+} chelator, ethylene glycol tetraacetic acid (EGTA). The $S_2Q_A^-$ band was restored upon the addition of Ca^{2+} with a decrease in the C band. Based on redox titration of the fluorescence yield they suggested that the C band was due to the low pH-induced high midpoint redox potential form of Q_A. The origin of the C band was further investigated by Johnson et al. (1994) using thylakoid membranes. The decay of the C band, enhanced by DCMU and illumination at 77 K, paralleled the disappearance of the EPR signal attributable to Q_A^- and the decay of the free radical Y_D^+. It therefore appears that the C band arises in a recombination of the $Q_A^-Y_D^+$ pair. These workers also investigated low pH Ca^{2+}-depleted PS II preparations but did not find a change in the redox potential of Q_A. Ducruet (1999) suggested that a back transfer of an electron from the secondary quinone acceptor (i.e., Q_B^-) to the primary acceptor Q_A of PS II was responsible for the luminescence-emitting recombination with Y_D^+. This band has been suggested to arise in PS II Q_B – non-reducing centers by Andree et al. (1998).

g. The A_G Band

A detailed investigation on the far-red light-induced band arising around 40–50°C (Desai et al., 1983) was made by Miranda and Ducruet (1995b). Several features of this band have been brought out by their studies. These authors suggested that although the band arises in $S_2/S_3Q_B^-$ recombination it is distinct from the B1/B2 bands since the Q_B^- is created as a result of a back transfer of an electron from the PQ pool in the dark during warming. The stable S_2/S_3 states created as a result of far-red illumination participate in the recombination reaction. Miranda and Ducruet (1995b) argued that far-red excitation oxidizes the PQ pool and randomizes the S_2/S_3 states. This produces stable S_2 and S_3 in the dark. The absence of Q_B^- resulting from oxidation of Q_B^- by far-red

light does not allow S_2/S_3 Q_A^-/Q_B^- recombination thus preventing deactivation of S_2/S_3 states. Far-red excitation could also result in cyclic electron flow around PS I and ATP production that may contribute to the back transfer of electrons from the PQ pool to Q_B, thus generating Q_B^- in the dark. This Q_B^- recombines with the stable S_2/S_3 states to yield an A_G (afterglow) band. This band is the expression of the far-red light-induced after glow and shares common properties with it. Since cyclic ATP synthesis participates in the emission of this band, the band is indicative of the metabolic state of the leaf/sample.

A band appearing at 46°C in a *Mesembryanthemum crystallinum* exhibiting facultative crassulacean acid metabolism (CAM) was induced by salt (Krieger et al., 1998). This band could be excited by a single turnover flash, oscillated with a periodicity of four and its intensity was related to the changes in the ratio of dihydroxyacetone phosphate to phosphoglyceric acid – an indicator of the energy status of the chloroplasts. Krieger et al. (1998) suggested that the band arose in the oxidized S_2/S_3Q_B condition in which Q_B becomes reduced by a reversed electron flow or by electron flow from NAD(P)H/PQ oxidoreductase. Soon thereafter, Janda et al. (1999) suggested that the far-red excited band peaking at 45°C is an indicator of abiotic stress in plants. Roman and Ducruet (2000) used this band as an indicator of the NADPH+ATP energetic potential in plants. Ducruet et al. (2005) have used the A_G band to monitor the cyclic electron flow around PS I. These latest studies indicated the presence of a 45°C peak designated as the A_G band in the stroma lamellae fraction. Its excitation by far-red light, and its association with cyclic electron flow around PS I, seem to confirm the earlier observations (Sane et al., 1977), but they equally demonstrate that it does not originate in PS I, although ATP generated by PS I cyclic electron transport may contribute to its appearance. We point out that the C band and A_G band are two different bands that could occur in the same temperature range but are distinguishable by their different characteristics.

3. High Temperature Bands

Arnold and Sherwood (1957) in their earlier experiments had shown light emission peaking at 125°C in irradiated dried thylakoids. However, its origin was not discussed. A TL band that appears at a temperature of about 73°C was described by Desai et al. (1982b). On the basis of its excitation and emission characteristics the origin of this band was assigned to chemiluminescence from chlorophyll upon the destruction of the membrane. The band was not related to functional electron transport. Kafarov et al. (1988) demonstrated that the intensity of this high temperature peak is correlated with the concentration of malondialdehyde (MDA), a product of lipid peroxidation indicating that this band is related to lipid peroxidation. Bohne et al. (1986) showed that chlorophylls in chloroplasts or micelles can efficiently detect electronically excited species generated in enzyme reactions through red emission.

In a study of photoinhibition in natural phytoplankton of the Black Sea, Matorin et al. (1992) observed peroxidation of the thylakoid membrane lipids associated with the appearance of a high temperature chlorophyll TL; the authors suggested that this high temperature TL band could be used to assess the extent of irreversible cell damage during photoinhibition. Further elucidation of this high temperature TL peak has come from the work of Hideg and Vass (1993) who suggested that this band arises in temperature enhanced interaction of molecular oxygen and the thylakoid membrane probably involving lipid peroxidation.

Solntsev (1995) demonstrated that the band appearing at 50–70°C is not affected by the spectral composition of irradiation. The variation in the T_M of this peak reported by different research groups has been explained by Stallaert et al. (1995) who showed that the T_M depends upon the rate of heating during TL measurement; this band showed a temperature maximum of 90°C if the rate of heating was 30°C min^{-1}, while the T_M appeared at 70°C if the rate of heating was 3°C min^{-1} The fungal elicitor cryptogein that induces lipid peroxidation also results in the increased high temperature TL band (Stallaert et al., 1995). Further elucidation and characterization of this band was done by Vavilin and Ducruet (1998) who studied this high temperature TL band in samples that were allowed to dry. Existence of three bands appearing at 62–75°C, 114–128°C and 151–158°C was observed. Treatments causing oxidative damage to membrane lipids produced a

small 62–75°C band but a significant rise in the 114–128°C peaks. Since this band did not change upon treatment with quenchers of active oxygen species or scavengers of free radicals or replacement of oxygen by argon, Vavilin and Ducruet (1998) proposed that the 114–128°C band arises as a result of thermal decomposition of lipid cyclic peroxides. In contrast, the 151–158°C bands were not related to thermolysis of lipid peroxidation products. The stress-induced appearance of this band has been also confirmed (Vavilin et al., 1998; Marder et al., 1998; Skotnica et al., 1999). Havaux (2003) has reviewed the studies on the use of high temperature TL for quantifying oxidative stress in plants. He has suggested that the TL bands appearing in the range of 80–150°C could be used for detecting and quantifying both lipid peroxidative damage and oxidative stress in plants.

III. Mechanism and Parameters of Light Emission

A. Mechanism

The emission spectra of the TL bands are similar to the fluorescence spectra from chloroplasts and therefore the light emission must occur from the PS II antenna system, as it does for fluorescence (Sonoike et al., 1991; Papageorgiou and Govindjee, 2004). When the light is absorbed by the chlorophylls of PS II, charge separation in the reaction center produces the excited singlet state ^{1}P680*. The electron from this excited singlet is transferred to pheophytin (Pheo) generating the charge separated state $P680^+Pheo^-$. The energy gap between $P680^+$ and $Pheo^-$ is about 1.8 V. The separated charge pair is stabilized by the production of an oxidized donor D^+ and a reduced acceptor A^-. The recombination of the oxidized donor and reduced acceptor is prevented because part of the energy absorbed is lost during the charge stabilization process. In order to produce delayed fluorescence or TL, it is necessary that the singlet excited P680* is regenerated. This requires generation of the singlet radical pair $^1(P680^+Pheo^-)$. The reducing equivalents required for generating the radical pair seem to arise from the reductants (Q_A^- and/or Q_B^-) and the oxidizing equivalents are apparently contributed by the S states. Since only the S_2 and S_3 states possess positive charges (because of the 1:0:1:2 proton release pattern during sequential oxidation of the Mn complex) they are the only logical donors of positive charges (see Fig. 19.1).

The recombination of the positive charges on the electron donors and the negative charges on the electron acceptors can occur through a series of back reactions culminating in the production of the $P680^+Pheo^-$ radical pair. A schematic representation of generation of the positively and negatively charged pairs as a result of photosynthetic electron transport in PS II leading to TL emission upon recombination is depicted in Fig. 19.1. In fact, many studies have already identified the reducing and the oxidizing species that participate in the recombination reaction leading to TL (see e.g., Vass and Govindjee, 1996; Inoue, 1996). In some cases besides the S states, other oxidized donors such as His^+ or Y_D^+ could also participate in the recombination reaction. These two donors are involved in charge recombination when the water-oxidizing complex is unable to reduce these donors for one reason or the other.

A modified version of an earlier energetic scheme of photosynthetic TL emission arising from charge recombinations in PS II (Vass and Govindjee, 1996; Inoue, 1996) is presented in Fig. 19.2. (For a review of PS II, see Govindjee et al., 2010.) Light absorption by the photosynthetic pigments results in charge separation produced upon charge stabilization, reduced acceptors Q_A^-/Q_B^- and the oxidized donors S_2 and S_3 (Fig. 19.1). The primary electron donor P680 accepts excitation energy and produces charge separation via the first singlet excited state (for a discussion of excitation energy transfer, see Clegg et al., 2010). Excitation of P680 results in the formation of the radical pair $P680^+Pheo^-$ (P^+Pheo^-). This is followed by the electron transfer from $Pheo^-$ to the first stable electron acceptor A (Q_A). The donors (D) and acceptors (A) of PS II generate stabilized charge pairs in generic terms as normal ($[D^+A^-]n$), shallower ($[D^+A^-]s$) and or deeper ($[D^+A^-]d$) traps, respectively and the TL peak position is determined by the free activation energy (ΔG^*) for the respective radiative charge pair. Various modifications of the molecular and electrostatic environment of the charge trapping species in both the donor (denoted as the water-oxidizing complex – WOC in Fig. 19.2) and acceptor (primary and secondary quinone acceptors – Q_A and Q_B) side of PS II can induce deeper or shallower stabilization of the charge pair accompanied by

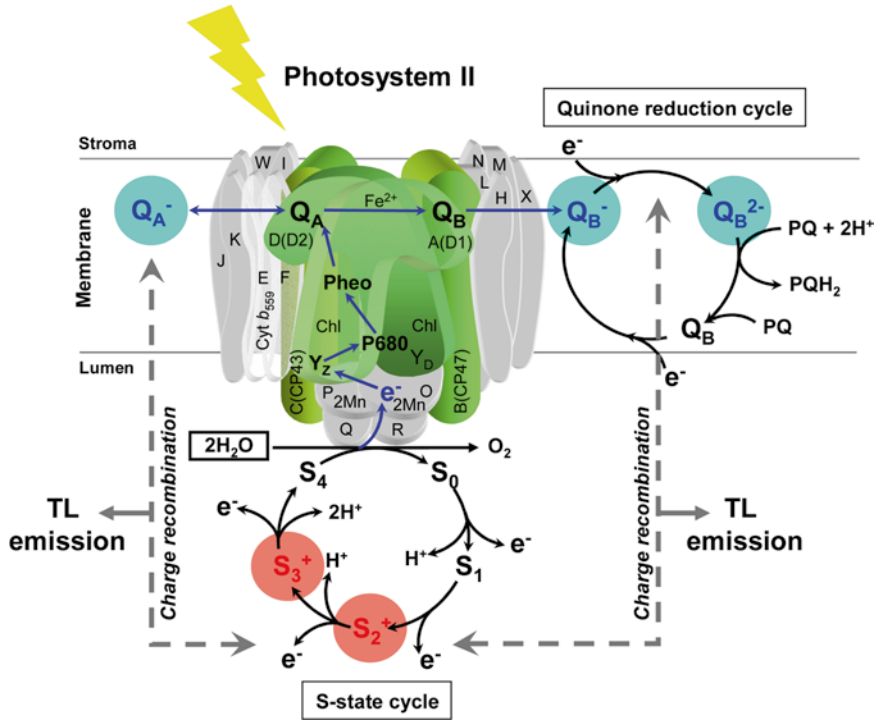

Fig. 19.1. Diagram of photosystem II (PS II) representing the polypeptide composition, electron transport carriers and light-driven photosynthetic electron transport reactions (*blue solid arrows*) from Mn to the secondary quinone electron acceptor (Q_B) in PS II resulting in water oxidation by the Mn cluster at the donor side of PS II. Electron transport in PS II generates light-dependent turnover of positive charges via the S-state cycle (Kok's cycle) by withdrawing e^- and H^+ from H_2O. The electron transport also generates positively charged $P680^+$, Y_Z^+ and Y_D^+, thus reducing the primary quinone electron acceptor (Q_A) to Q_A^-. Consequently, Q_A^- donates an electron to Q_B and the negative charges at the acceptor side of PS II are generated by the quinone reduction cycle forming semiquinol or quinol molecules. Charge recombinations (*grey dashed lines*) between different combinations of negatively and positively charged species result in thermoluminescence (TL) emission which is characteristic for each redox pair.

higher or lower ΔG* resulting in up-shifted or down-shifted TL emission peaks, respectively.

The energy barrier can be overcome by a supply of thermal energy enabling the charge pairs to recombine generating the $P680^+Pheo^-$ singlet radical pair and finally P680*. When P680* returns to the ground state, it can emit light as TL (the probability of which is very low due to its low abundance), but most of the energy is transferred to the more abundant antenna chlorophyll molecules that emit light as TL. Since there are four distinct charge pairs ($S_2Q_A^-$, $S_3Q_A^-$, $S_2Q_B^-$ and $S_3Q_B^-$) generated within PS II, the detection of four TL bands appearing at characteristic peak temperatures (T_M) is to be expected. Furthermore, the T_M of each of these peaks strongly depends on the redox potential difference between the recombining charge species. Therefore, if there is a change of the redox potential in any of the participating components this will be reflected in a shift in the T_M of the peak generated by the recombining charge pair. Thus, the same recombining species can have an up-shifted or a down-shifted peak. Extending this explanation further we can argue that a back flow of electrons from another component to the oxidized acceptor, e.g., Q_A/Q_B could give rise to a reduced acceptor that was not present earlier and if the oxidized donor such as S_2 or S_3 was present this will permit recombination of the charge pairs. However, in this case the energy required for overcoming the energy barrier will depend upon the energy needed for the back transfer of the electron from a component such as reduced plastoquinone. The resultant peak in this case will have a different T_M although it is still generated by the same charge pair.

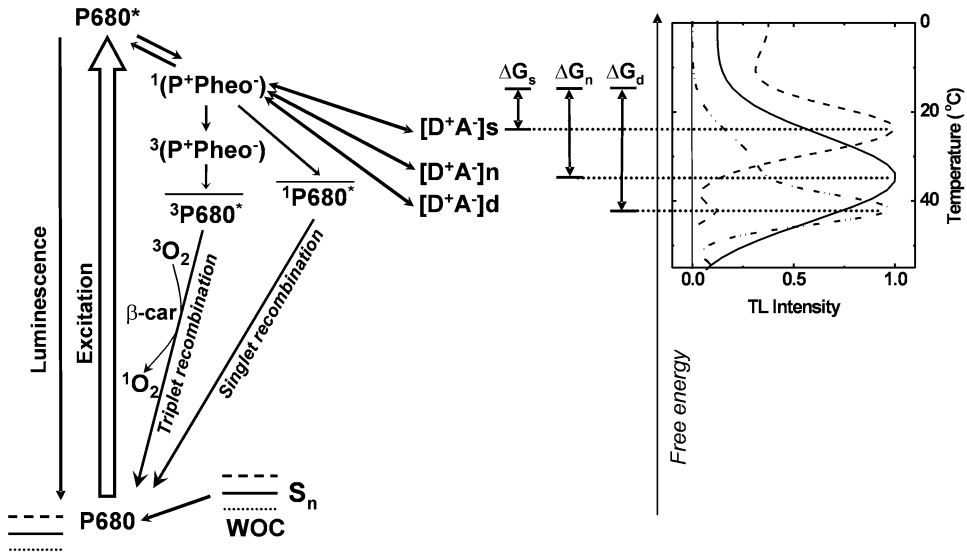

Fig. 19.2. Energetic scheme of photosynthetic TL emission arising from charge recombinations in PS II. The primary electron donor P680 accepts light excitation energy and the charge separation via the first singlet excited state results in the radical pair $P680^+Pheo^-$ (P^+Pheo^-) followed by the electron transfer from $Pheo^-$ to the first stable electron acceptor A (Q_A). *D* and *A* represent the donors and acceptors of PS II and $[D^+A^-]n$, $[D^+A^-]s$ and $[D^+A^-]d$ indicate the stabilized charge pairs with normal (*n*) shallower (*s*) and deeper (*d*) traps, respectively. The free activation energy for the radiative charge pair (ΔG*) determines the *TL* peak position. Various modifications of the molecular and electrostatic environment of the charge trapping species in both donor (water-oxidizing complex - WOC) and acceptor (primary and secondary quinone acceptors – Q_A and Q_B) side of PS II can induce deeper or shallower stabilization of the charge pair accompanied by higher or lower ΔG* resulting in up-shifted or down-shifted TL emission peaks, respectively. In some cases, back electron transfer from Q_A^- to Pheo may result in a singlet $^1(P680^+Pheo^-)$, or by spin de-phasing a triplet $^3(P680^+Pheo^-)$, radical pair. Non-radiative charge recombination pathways via singlet recombination $[^1(P680^+Pheo^-)]$ to the ground state P680 or the first singlet excited state $^1P680^*$ and via triplet recombination $[^3(P680^+Pheo^-)]$ resulting in the triplet excited state $^3P680^*$, which may be quenched by β-carotene via singlet oxygen, are shown.

The reductants produced during electron transport are the reduced forms of the two plastoquinone electron acceptors of PS II. The Q_A species is a single electron acceptor and is reduced by $Pheo^-$. It immediately transfers this electron to the two electron acceptor Q_B to first produce Q_B^-. If Q_B^- was already there then the transfer of electron to Q_B^- from Q_A^- produces Q_B^{2-}, which is immediately protonated. The plastoquinol (Q_BH_2) thus formed, leaves the PQ-binding site and is replaced by another plastoquinone molecule from the PQ pool. Thus, when the PQ pool is in an oxidized state, Q_A^- is not expected to accumulate and hence it cannot contribute to the reducing equivalents for a recombination reaction. However, if for some reason the electron transport from Q_A to Q_B is restricted, then Q_A^- would accumulate and provide reducing equivalents for TL. Another situation that could result in substantial accumulation of Q_A^- is if the PQ pool is fully reduced and Q_B^- cannot be oxidized. In this situation Q_A^- accumulation could also take place. Such a situation does occur in nature when the PQ pool is kept reduced by chlororespiration under certain environmental conditions.

Under normal conditions the redox potential difference between Q_A and Q_B is such that the equilibrium constant is vastly in favour of Q_A^- oxidation. However, if the redox potential of Q_A or Q_B changes such that the redox potential difference between the two is considerably reduced then a large proportion of Q_A could remain in a reduced condition under a steady state situation. There are several examples where the redox potential difference between these two plastoquinone acceptors is reduced and the peaks arising from recombination involving Q_A^- or Q_B^- appear at almost the same temperature. In a later section these aspects are discussed in more detail with specific examples.

B. Oscillations of the B Band(s)

Most TL studies are currently being carried out using flash excitation of dark-adapted samples. As noted above, the positively charged oxidizing equivalents arise primarily in S_2 and S_3 states of the water-oxidizing complex. In dark-adapted samples, only S_1 and S_0 are present and these advance to higher oxidized states under flash illumination. Dark-adapted samples contain about 25% S_0 and 75% S_1 and the distribution of Q_B and Q_B^- has been shown to be 50 : 50 (see, e.g., the review by Inoue, 1996). In general, the Q_A reduced by flash excitation will immediately reduce Q_B within ~200 μs. Assuming the above distribution of the $Q_B : Q_B^-$ and $S_0 : S_1$ in a dark-adapted sample, we can predict the intensity of the TL bands as a function of flash numbers. The distribution of different charge pairs (S-states and Q_B) and the predicted TL emission following consecutive flash excitation is depicted in Fig. 19.3. In a dark-adapted sample the proportion of the charge pairs is expected to be as follows (Fig. 19.3a):

S_0Q_B	:	$S_0Q_B^-$	:	S_1Q_B	:	$S_1Q_B^-$
1	:	1	:	3	:	3

None of the S-states in this situation carry a positive charge and hence there is no possibility of any TL emission although 50% of Q_B is in a reduced state. The actual glow curve shows that there is a general rise above 40°C that, in fact, yields a TL peak unrelated to PS II (Fig. 19.3a). However, there is no TL arising in PS II.

Excitation of the sample by a single saturating turnover flash will advance S_0 to S_1 and S_1 to S_2, respectively and simultaneously convert Q_B to Q_B^- and Q_B^- to Q_B^{2-}. The deposition of the second charge on Q_B^- will cause protonation of the Q_B^{2-} and it will be replaced by a new plastoquinone molecule Q_B. Under these conditions the expected combination of S-states and Q_B is (Fig. 19.3b):

$S_1Q_B^-$	:	S_1Q_B	:	$S_2Q_B^-$	:	S_2Q_B
1	:	1	:	3	:	3

Only $S_2Q_B^-$ will produce TL in proportion to its presence. The accompanying TL trace in Fig. 19.3b shows the expected $S_2Q_B^-$ peak appearing at 40°C in Scots pine (A.G. Ivanov, P.V. Sane, N.P.A. Hüner and G. Őquist, unpublished results).

If the dark-adapted sample was excited by two successive flashes the second flash will convert S_1 to S_2, S_2 to S_3 and Q_B^- to Q_B and Q_B to Q_B^- and the distribution of the S-states and Q_B would be as follows (Fig. 19.3c):

S_2Q_B	:	$S_2Q_B^-$	:	S_3Q_B	:	$S_3Q_B^-$
1	:	1	:	3	:	3

Such a distribution of the S-states and Q_B will result in producing 3 arbitrary units of TL intensity from $S_3Q_B^-$ plus one arbitrary unit of TL intensity from $S_2Q_B^-$ (Fig. 19.3c). If one presumes that $S_3Q_B^-$ is 1.8 times more luminescent than $S_2Q_B^-$ (Rutherford et al., 1985), the expected total luminescence (TL intensity) will be about 6.4 units. It should be noted that the major TL in this case will be emitted due to $S_3Q_B^-$ charge recombination. The characteristic peak (T_M) of $S_3Q_B^-$ appears at lower temperatures than $S_2Q_B^-$ and in fact, the TL peak appeared at 35°C after two flashes in the experiment shown (Fig. 19.3c). Apparently, there was some TL emission peaking around 0°C arising from $S_2Q_A^-$ indicating that not all of the Q_A^- produced was oxidized by Q_B. Furthermore, the total luminescence was not twice that produced after one flash, indicating that theoretically predicted values are not always seen in the experiment. However, the trends are as predicted.

If the relaxed sample was excited by three successive flashes the distribution of the S states and Q_B would be as follows (Fig. 19.3d):

$S_3Q_B^-$	:	S_3Q_B	:	$S_0Q_B^-$	:	S_0Q_B
1	:	1	:	3	:	3

Only the $S_3Q_B^-$ will produce TL and its intensity will be about 1.8 TL units (Rutherford et al., 1985). The accompanying TL trace (Fig. 19.3d) shows a smaller peak appearing at about 35°C characteristic of $S_3Q_B^-$ recombination, e.g., in Scots pine. Thus, the theoretical prediction seems to match the experimental observations. The flash-induced predicted changes in the charge pair generation do not make allowance for the retention of some charge on Q_A. However, as seen in Fig. 19.3 the peak representing $S_2Q_A^-$ is seen around 0°C and this happens because the samples were illuminated at a temperature that slows down the movement of charge from Q_A^- to Q_B.

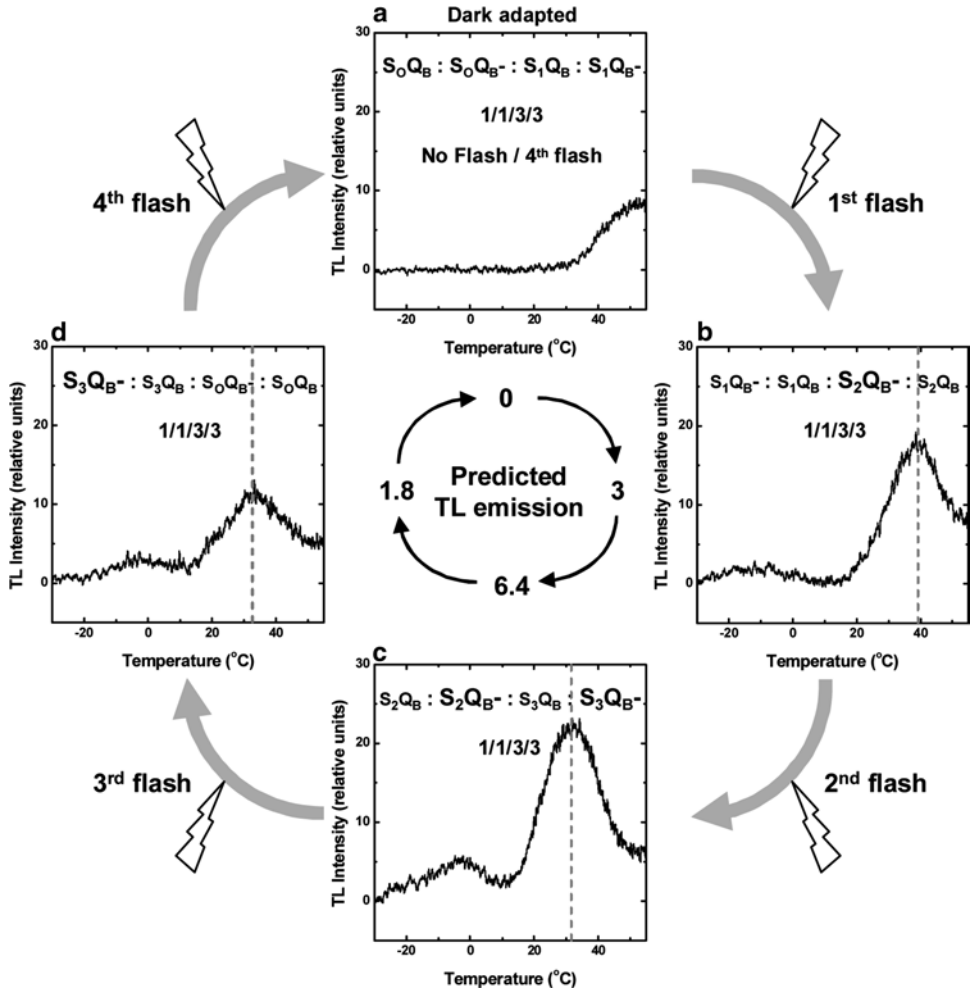

Fig. 19.3. Predicted distribution of S states and TL emission intensity after excitation with successive single turnover saturating flashes of white light. (**a**) Considering that TL emission is generated only in $S_2Q_B^-$ and $S_3Q_B^-$ recombinations in dark-adapted photosynthetic membranes (cells, leaf tissue, chloroplasts) TL is not observed. Under such a dark-adapted condition the only S states observed are S_0 and S_1. (**b**) After one single saturating flash the distribution of S states will change and the appearance of three $S_2Q_B^-$ charge recombinations will result in TL emission of three relative units. (**c**) If the dark relaxed sample is exposed to two successive single saturating flashes the distribution of S states will change again and the four luminescent charge pairs (one $S_2Q_B^-$ and three $S_3Q_B^-$) will increase the TL emission to 6.4 relative units. (**d**) After three successive saturating flashes only one luminescent charge pair is produced ($S_3Q_B^-$) and the relative TL emission drops to 1.8 relative units. After four flashes the distribution of S states will be the same as existed in the dark-adapted sample and no TL emission will be produced (TL glow curve not shown). The representative TL glow curves were recorded in intact Scotch pine (*Pinus sylvestris* L) needles. Note that when the emission is primarily from $S_3Q_B^-$ the T_M is lower by 8–10°C compared to when it is primarily from $S_2Q_B^-$.

The excitation of the sample by four successive flashes should result in the production and distribution of charge pairs identical to those in the dark-adapted sample with zero TL emission. However, numerous experimental studies of S_2Q_B oscillations have demonstrated that TL emission is rarely zero after four successive flashes and this happens because of double hits, misses and other reasons such as heterogeneity in PS II centers, as well as recombination and decay of some of the charge pairs. The predictions suggest that $S_2Q_B^-$ recombination alone could be observed only after one flash, while application of two consecutive flashes will result in a composite peak consisting primarily of $S_3Q_B^-$ TL emission. The assumption that in the dark-adapted samples $Q_B : Q_B^-$ will be present in 50:50 proportion may also not be always true (see Inoue, 1996). However, even if

the proportion of $Q_B : Q_B^-$ in the dark is shifted to 75:25, one flash will still produce TL primarily from $S_2Q_B^-$. Thus, irrespective of the dark-adapted state the TL emission following the first flash will always be due to $S_2Q_B^-$.

Explanations for flash-induced TL bands that are induced by illumination at different temperatures (even after freezing the samples to liquid nitrogen temperatures) have been offered.

For example, if the reduced PQ pool reduces Q_B in S_2Q_B or S_3Q_B they would contribute to TL. Krieger et al. (1998) have used such considerations to explain the occurrence of a 46°C band in CAM plants, suggesting that it arises in S_2/S_3Q_B upon reduction of Q_B in the dark through reversed electron flow from PQ or the reduction of PQ pool by NAD(P)H/PQ oxidoreductase.

After flash excitation at low temperatures (77 K) the following event takes place: Cyt b_{559} is oxidized and Q_A is reduced, and subsequently at an appropriate temperature Q_B is reduced forming $S_2Q_B^-$ and $S_3Q_B^-$ leading to TL (Rutherford et al., 1984). If $S_2Q_B^-$ or $S_3Q_B^-$ were generated after the flash excitation, the illumination at 77 K would make them non-luminescent by introducing one more electron on Q_B to produce Q_B^{2-} that would get protonated and replaced by oxidized Q_B. We know that low temperature illumination does not change the distribution of the S states but merely changes the redox state of Q_B. Thus, shuffling the charge pairs can be manipulated by changing the illumination regime to which the sample is subjected (Rutherford et al., 1984).

If the electron transport in the sample has been blocked by DCMU (or a similar compound) then the charge by illumination at low temperature will be located on Q_A rather than Q_B. With such manipulations it is possible to observe oscillations even of $S_2/S_3Q_A^-$ (Koike et al., 1986).

C. Biophysical Parameters

The phenomenon of TL occurring in photosynthetic membranes is somewhat similar to the one that occurs in semiconductors/inorganic crystals. The electrons trapped in metastable states during exposure to electromagnetic radiation get released during subsequent warming of the sample and migrate to a stable location emitting energy as luminescence. The theoretical framework for calculating different physical parameters from TL curves, developed by Randall and Wilkins (1945), had assumed that the de-trapping process involved a single temperature dependent rate constant with Arrhenius behavior. Constants such as activation energy, frequency factor, and lifetime of the electron in the trapped state, could be calculated using the Randall-Wilkins theory. Using this theory of a single step de-trapping mechanism, the photosynthetic glow curves were analyzed in the late 1960s (Arnold and Azzi, 1968 and Shuvalov and Litvin, 1969). In these early calculations an arbitrary frequency factor was assumed. Subsequently, the activation energies were first calculated by using the initial rise method, and then, using the calculated values of the activation energies, other physical parameters of the glow curves were determined (Tatake et al., 1981). The initial rise method requires that each of the glow peaks should be without any overlap. Tatake et al. (1981) obtained well-separated glow peaks and discovered that the values obtained for frequency factors were too large and concluded that Randall-Wilkins theory as such was not applicable in analyzing the photosynthetic glow curves. At the same time Vass et al. (1981) used computer-assisted programs to resolve the glow peaks and calculated the physical parameters using Randall-Wilkins theory after introducing a small modification to accommodate absolute rate theory. Vass et al. (1981) further noted that even after the modifications that they introduced, the activation energies as well as the lifetime of the trapped states were unrealistically larger than expected. They suggested that in photosynthetic TL the recombination involves back reactions or reversal of the electron transport steps.

To explain the discrepancies between the observed results and the predictions of the existing theory, DeVault et al. (1983) modified the Randall-Wilkins theory by postulating temperature dependent equilibria between two or more electron carriers acting as traps for electrons or holes. By this modification the abnormally large apparent activation energies and frequency factors could be partly explained; they suggested that the rate-limiting back reaction step is not the only one that determines the rate of recombination but earlier steps that affect the concentration of charges are also important and need to be factored in. The newer concepts were further extended by DeVault and Govindjee (1990) who related

the free energies with the redox potentials of the intermediates involved in TL emission. Earlier work of Demeter et al. (1985b) reporting shifts of T_M of the B band in triazine-resistant biotypes of *Erigeron canadensis* had suggested that the shifts of the peak temperatures, T_M, were proportional to the changes in the midpoint potentials of Q_B. The studies of DeVault and Govindjee (1990) demonstrated that changes in T_M are proportional to changes in free energy and hence changes in midpoint potentials. This study provided strong theoretical support for inferring redox midpoint potential changes from shifts in peak temperature, T_M. Several research groups including the authors of this chapter have extensively used the T_M shifts to indicate changes in redox potentials of charge pairs involved in recombination leading to TL. Krieger et al. (1993) have provided confirmation of such a relationship in their studies which determined the redox potential of Q_A by redox titrations and related it to the shift of the T_M of the band involving Q_A.

Vidyasagar et al. (1993) argued that the methods used above have not considered the probability of re-trapping during the process of de-excitation while there was evidence for a re-trapping possibility in the reversal of electron transport during photosynthetic TL. They applied the general order kinetic model and calculated the physical parameters. These calculations certainly showed lower values of activation energies for the high temperature peaks but they did not improve the values of activation energies for low temperature peaks. However, this method provided much smaller and acceptable values of frequency factors for the high temperature peaks.

Rappaport et al. (2005) considered the $S_2Q_A^-$ recombination in PS II with a more complete theory that took into account the various competing recombination routes. However, they realized that such a more complete theory could not account for the location of the TL bands that occurs at much higher temperatures than predicted from the overall rate and temperature dependence of the $S_2Q_A^-$ recombination. They suggested that this happens due to the presence of kinetic heterogeneity of PS II recombination reactions. Using mutants of *Chlamydomonas reinhardtii* the role of membrane potential on the T_M and the intensity of the $S_2Q_A^-$ TL band was elaborated (Rappaport et al. 2005). The E130L mutant in which residue 130 of the D1 subunit was changed from glutamate to leucine was specifically chosen for this purpose because it has an enhanced $S_2Q_A^-$ band that appears at much higher temperature. This allowed Rappaport et al. (2005) to resolve the entire TL band without going below −10°C. In the absence of an uncoupler, the $S_2Q_A^-$ TL band appeared at a much lower temperature (around 20°C in the E130L mutant) as compared to its appearance at almost 30°C in the presence of the uncouplers nonactin and nigericin. The shift to higher temperature was accompanied by a decrease in its amplitude. They argued that while the modification of the energy barrier for recombination should shift the TL band along the temperature scale in the Randall-Wilkins scheme, the integral of the band should not have changed since the recombination reaction coincides with the radiative pathway. Their data, they argued, 'falsifies' this prediction of Randall-Wilkins theory. (for a review on the theory of TL, see Rappaport and Lavergne, 2009.)

Rantamäki and Tyystjärvi (2011) applied Arrhenius, Eyring and Marcus theories to analyze $S_2Q_A^-$ charge recombination measured as Q band and the decay of chlorophyll fluorescence yield after a single turnover flash at different temperatures in the presence of DCMU. They observed that all of the three theories gave the correct Q band position. However, Marcus theory gave a better fit for the rising part of the curve while both Eyring and Marcus theories gave good fits for the decreasing part of the TL curve. This paper discusses these theories in detail and provides information on their applicability in analyzing TL and fluorescence resulting from more than one competing route. In a previous paper these authors (Tyystjärvi and Rantamäki, 2009) have discussed retrapping in photosynthetic TL.

The effects of transmembrane electrochemical potential on TL bands were addressed by Farineau (1996) and it was shown that its presence reduced the activation energies of B bands. Upon addition of uncouplers of phosphorylation the bands revert to their normal T_M values and hence have increased activation energies. It is now obvious that the Randall-Wilkins theory for the calculation of the different physical constants from TL in phototsynthetic membranes as such cannot be applied and modifications, as initially suggested by DeVault et al. (1983) and subsequently elaborated upon

by others, must not be ignored. Rose et al. (2008) have combined the ideas of Rappaport et al. (2005) and of DeVault and Govindjee (1990) in explaining changes in the T_Ms, and thus in the redox potentials of Q_B/Q_B^- bands, in D1-Arg275 mutants in *Chlamydomonas reinhardtii*. Further research is needed to establish a final definitive model.

IV. Thermoluminescence and Delayed Light Emission

Both the TL and delayed light emission (DLE; also called delayed fluorescence; discovered by Strehler and Arnold, 1951) of photosynthetic membranes are related phenomena. Shuvalov and Litvin (1969) associated the TL bands they observed with different phases of delayed fluorescence. Indications that the delayed light and TL may be related to each other were also apparent from the temperature jump experiments of Mar and Govindjee (1971), Jursinic and Govindjee (1972) and Malkin and Hardt (1973). The fact that TL bands and components of DLE not only originate in light-induced electron transport but share many other common properties such as oscillations and inhibition by inhibitors of electron transport suggested that DLE and TL may be related to each other. Furthermore, the analysis of different TL bands by the application of Randall and Wilkins theory suggested that the lifetimes of the electrons in the trapped states at their respective glow peak temperatures were in a time scale of seconds (Tatake et al., 1981) indicating that DLE components that decay on a time scale of seconds may be related to the TL bands. A detailed study of the possibility that the two are identical was investigated by Desai et al. (1982a) who elegantly showed that a slow component of DLE mimics glow peaks and that the DLE and TL are quantitatively related and represent the expression of the same phenomenon. In a subsequent study, Rane and Sane (1985) identified specific components of DLE with four major TL peaks associated with photosynthetic electron transport. The relationship between TL and DLE was also shown in the studies of Rutherford et al. (1984) and Hideg and Demeter (1985). Several subsequent studies have strengthened the relationship between TL bands and the components of DLE (Vass et al., 1988). While each TL band is associated with one or the other DLE component there are some DLE components that do not have a corresponding TL band. However, there are advantages in using TL rather than DLE under some conditions as the components of DLE are much better resolved by TL as each separate band corresponds with one or the other DLE component.

V. Thermoluminescence and Photoinhibition

Photoinhibition has been extensively studied by TL in cyanobacteria, algal cells, higher plants and isolated thylakoids (Ohad et al., 1988, 1990; Briantais et al., 1992; Mäenpää et al., 1995; Ono et al., 1995; Andree et al., 1998). In all the cases a loss in the intensity of the $S_2Q_B^-$ peak has been seen as indicative of the degradation of the D1 polypeptide. Most studies have indicated that the rate of electron transfer from the reduced Q_A to Q_B is decreased. Besides these expected results TL studies have indicated shifts of $S_2Q_B^-$ and $S_2Q_A^-$ recombination that cannot be attributed to the shift of redox change in the donor components that contribute oxidizing equivalents, specifically the S states. The obvious conclusion drawn from these studies is that the redox species on the acceptor side (Q_A^- and Q_B^-) contributing to the charge recombination seem to undergo changes. The redox shifts are such that the redox gap between Q_A and Q_B is considerably reduced. Another interesting observation made is that these shifts occur only in intact systems and not in isolated thylakoids suggesting that the shifts are associated with the dynamic nature of the cell components other than membranes themselves. An additional observation made is the intensification of the high temperature peak related to the lipid peroxidation that invariably occurs under many stress conditions including light stress (Havaux, 2003).

Studies on *Chlamydomonas reinhardtii* cells by Ohad et al. (1988) suggested a shift of $S_2Q_B^-$ to lower temperature by about 15°C that was associated with an increase in the minimal (F_o) fluorescence intensity, when the cells were photoinhibited at intensities of 300–1,000 W m^{-2}. Concurrently, $S_2Q_A^-$ intensity decreased to the extent of 30–40% along with a decrease in maximal variable

chlorophyll *a* fluorescence ($F_m - F_o$) in the presence of DCMU. These changes were attributed to a light-dependent turnover of D1 protein. In a subsequent study, Ohad et al. (1990) showed a decrease in the activation energy of $S_2/S_3Q_B^-$ in the cells and also in isolated thylakoids. In *Synechocystis* sp. PCC 6803 cells in which the D1 polypeptide was modified by substitution of glutamine 241 to histidine (the CA1 mutant), Mäenpää et al. (1995) observed that photoinhibition resulted in the shift of $S_2/S_3Q_A^-$ recombination to a higher temperature by 10°C. Expectedly there was an increase in the $t_{1/2}$ of chlorophyll fluorescence decay in the presence of DCMU. Mäenpää et al. (1995) suggested that a modification of the redox couple Q_A/Q_A^- had occurred and this rendered the reoxidation of Q_A^- more difficult. Similar shifts in $S_2Q_B^-$ have been observed in *Synechococcus* sp. PCC 7942 cells by Sane et al. (2002) when the cells growing at 36°C were shifted to 25°C. The shifts were reversed when the cells were shifted back to 36°C. The reduction in growth temperature results in increased excitation pressure causing conditions similar to photoinhibition. The cells responded by substitution of D1:1 by D1:2 – a characteristic response of these cells to various stress conditions. Upon shifting to low temperature the $S_2Q_B^-$ recombination occurred at a temperature similar to that of $S_2Q_A^-$. This had reduced the gap between the redox potentials of Q_A and Q_B to almost zero. The redox state of the donors (S states) was not changed.

In higher plants, the $S_2Q_B^-$ band has also been observed to shift to low temperature during photoinhibition, demonstrating a more negative redox potential of Q_B, closer to that of Q_A (Briantais et al., 1992). It appears that the S states had not undergone a change in their redox potential since the $S_2Q_A^-$ band had not shifted. Sane et al. (2003) studied responses of cold acclimated *Arabidopsis thaliana* plants to high light using TL and chlorophyll fluorescence measurements. They observed that the $S_2Q_A^-$ band was shifted to a higher temperature while the $S_2Q_B^-$ band was shifted to a lower temperature in cold acclimated plants. Sane et al. (2003) suggested that the narrowing of the gap in the T_Ms of these two bands was related to the increased redox potential of Q_A and decreased redox potential of Q_B in cold acclimated plants: these effects resulted in an increased reaction center quenching, i.e., enhanced probability for non-radiative $P680^+Q_A^-$ radical pair recombination facilitated by an increased population of reduced primary electron-accepting quinone (Q_A) in PS II. The expected enhanced reaction center quenching was confirmed by in vivo chlorophyll *a* fluorescence quenching analysis. The enhanced dissipation of excess light within the reaction center of PS II (reaction center quenching) must have been the reason for resistance of cold acclimated plants to photoinhibition. Ivanov et al. (2003) and Huner et al. (2006) have suggested how redox potential changes of the two plastoquinone acceptors could explain the increased reaction center quenching and hence improved tolerance to photoinhibition. Furthermore, Ivanov et al. (2006) have shown similar shifts resulting in the narrowing of the gap in the redox potential of Q_A and Q_B in barley and rye grown under high excitation pressure. The reaction center quenching concept proposed by Sane et al. (2003) could explain, in part, the resistance or decreased photoinhibition in high light acclimated or low temperature acclimated plants. Probably the same explanation can be extended to understand the stress response of the plants to various stresses as explained later.

Interestingly the photoinhibition of isolated thylakoids/membranes demonstrate somewhat different results than observed in intact leaves or cells. Vass et al. (1988) working with isolated spinach thylakoids did not observe any shifts either in the Q or the B bands or in the half times of related delayed fluorescence. Similarly Andree et al. (1998) using grana, stroma and margin fractions did not observe any shifts of Q or B bands although the TL intensities decreased. Photoinhibition under aerobic conditions using oxygen-evolving membrane preparations from spinach showed an expected decrease in the intensity of the $S_2Q_B^-$ peak but there was no temperature shift (Ono et al., 1995).

Photoinhibition seems to result in oxidative damage and this can be monitored by TL. Matorin et al. (1992) observed an increased high temperature band indicative of lipid peroxidation when microalgal cells of Black Sea phytoplankton were exposed to high light. Thus, this high temperature band could be used to assess the extent of irreversible damage of cells following photoinhibition. Havaux and Niyogi (1999) studying the *npq1* mutant of *Arabidopsis thaliana* lacking violaxanthin

Table 19.2. Shifts of $S_{2/3}Q_B^-$ and $S_2Q_A^-$ bands in response to various environmental stress conditions

Stress	Plant material	$S_{2/3}Q_B^-$ T_M	$S_2Q_A^-$ T_M	References
High light	*Chamydomonas reinhardtii* cells	Downshift	NC	Ohad et al., 1988
	Chlamydomonas reinhardtii cells	Downshift	NC	Ohad et al., 1990
	Pea leaves	Downshift	NC	Janda et al., 1992
	PS II membranes	Downshift	NM	Ono et al., 1995
	Barley leaves	Downshift	NC	Walters and Johnson, 1997
Cold stress/ Acclimation	Spinach leaves	Downshift	NM	Briantais et al., 1992
	Maize leaves	Downshift	NM	Janda et al., 2000
	Synechococcus sp. PCC 7942 cells	Downshift	NC	Sane et al., 2002
	Scots pine needles	Downshift	NC	Ivanov et al., 2002
	Arabidopsis thaliana leaves	Downshift	Upshift	Sane et al., 2003
	Barley and Rye leaves	Downshift	Upshift	Ivanov et al., 2006
Water stress	Bean leaves	Downshift	NM	Metwally et al., 1997
Desiccation	Barley leaves	Downshift	NM	Skotnica et al., 2000
	Spinach leaves	Downshift	Upshift	Peeva and Maslinkova, 2004
CO_2 depletion	*Chlamydobotrys stellata* cells	Downshift	Upshift	Wiessner et al., 1992
	Chlamydomonas reinhardtii cells	Downshift	NC	Wiessner et al., 1992
	Chlamydobotrys stellata cells	Downshift	Downshift	Demeter et al., 1995
	Synechococcus sp. PCC 7942 N5 mutant	Downshift	NM	Marco et al., 1993
Copper stress	Spinach thylakoids	Downshift	Upshift	Horváth et al., 1998
Mercury stress	*Chlorella kessleri* cells	Downshift	NC	El-Seekh, 1999
UV-B stress	Barley leaves	Downshift	Upshift	Gilbert et al., 2004a
Ozone stress	Barley leaves	Downshift	NM	Skotnica et al., 2003
	Barley leaves	Downshift	NM	Skotnica et al., 2005

NC no change, *NM* not measured

de-epoxidase (and which therefore cannot form zeaxanthin in strong light) observed an increased high temperature band reflecting lipid peroxidation. The use of high temperature TL bands to study and assess the extent of lipid peroxidation under in vivo conditions is another use of the TL technique. Havaux (2003) has discussed methods and quantification of the oxidative damage using high temperature TL bands.

VI. Thermoluminescence and Stress

Both biotic and abiotic stresses alter the photosynthetic characteristics and metabolic status of photosynthetic photoautotrophs, which may be reflected in specific modifications of PS II. TL patterns have been used as a probe for stress responses in intact organisms. Among the environmental factors temperature, light, salt, and drought are some of the important stresses that plants experience under natural conditions and many of these have been studied by the TL technique (see reviews by Misra et al., 2001 and Ducruet, 2003). Since PS II activity is usually reduced under severe stress conditions a decrease in all the TL bands that arise in PS II is expected, as has been reported (Ivanov et al., 2001; Sane et al., 2002). However, when the stress is relatively less severe, the band related to $S_2Q_B^-$ has been shown to shift to lower temperatures under numerous stress conditions (Table 19.2 and the references cited therein). Further, induction of a TL band appearing at a higher temperature in response to high temperature stress that is associated with lipid peroxidation has been demonstrated. Some studies have also demonstrated changes in the A_G band under photoinhibitory conditions. The most significant feature of the stress, irrespective of its kind, seems to be the decrease in the emission temperature of the $S_2Q_B^-/S_3Q_B^-$ band. This shift is also commonly seen during low temperature acclimation as well as acclimation to high light intensity.

The responses to high light causing photoinihibition have been discussed above.

A. Temperature

Both high and low temperature stress have been studied using TL. Down-shift of the B band ($S_2Q_B^-$) has been reported in cold acclimated spinach leaves (Briantais et al., 1992). Janda et al. (2000) have reported a down-shift in the T_M of the B band in low temperature grown maize. Similar observations have been made by Sane et al. (2002) in cold-stressed *Synechococcus* sp. PCC 7942 cells, and in cold acclimated *Arabidopsis thaliana* (Sane et al., 2003). There are also similar data available on barley and rye leaves grown at low temperature (5°C) (Ivanov et al., 2006) and on cold acclimated Scots pine needles (Ivanov et al., 2002; Sveshnikov et al., 2006). In maize subjected to low temperature (0°C) for 4 h, the shift of the B band to low temperature was associated with induction of the A_G band (Janda et al., 2000). In all the studies mentioned above, besides the down-shift of $S_2Q_B^-$ peak temperature, the overall reduction of the B band TL emission was also observed.

Exposure of barley plants to high temperature induced a high temperature TL band appearing between 70°C and 120°C (Havaux, 1998). Havaux and Niyogi (1999) observed an increase in high temperature TL in the *npq1* mutant of *Arabidopsis thaliana* that lacks the violaxanthin de-epoxidase enzyme.

B. Salt Stress

Salt stress has been found to reduce the intensity of $S_2Q_B^-$ band more than that of $S_2Q_A^-$ band (see Misra et al., 1999), but there were no shifts in the peak positions. In *Chlorella vulgaris,* El-Seekh (2004) showed that the S_2 to S_3 transition of the water-oxidizing system was inhibited by NaCl; in addition the intensities of both Q and B bands were reduced.

C. Heavy Metals

Studies on intact thylakoids that were treated with a 500 μM concentration of copper showed a down-shift of $S_2Q_B^-$ band to 15°C (Horváth et al., 1998). These results were explained as donor side effects. However, the A band ($S_3Q_A^-$) had shifted to a higher temperature. Mercury at 100 μM did not affect the $S_2Q_A^-$ charge recombination in *Chlorella kessleri*, while $S_2Q_B^-$ recombination was inhibited and shifted to lower temperature (El-Seekh, 1999)

D. Desiccation and Water Stress

In the initial stages of leaf desiccation both $S_2Q_B^-$ and $S_3Q_B^-$ bands have been observed to shift to lower temperatures, while an up-shift of the $S_2Q_A^-$ band was observed under the same conditions (Skotnica et al., 2000). The shifts of Q_A and Q_B related bands in opposite directions suggest that the electron donor side had not undergone a change in redox characteristics, rather the electron acceptors Q_A and Q_B came closer to each other in their redox potential values. Although the authors did not argue in terms of the increased possibility for reaction center quenching during the desiccation as a response to increased radiation stress we suggest that the redox properties of the photosynthetic apparatus were modified to accommodate the high excitation at least partially through the reaction center quenching mechanism as proposed by Sane et al. (2003) and Ivanov et al. (2003). The shift of the $S_2Q_B^-$ band to lower temperature from 32°C to 25°C has also been observed in water stressed bean plants (Metwally et al., 1997). Interestingly, the down-shift of the $S_2Q_B^-$ band was not seen in a desiccation tolerant plant (*Haberlea rhodopensis*) while in spinach at 10% relative water content the shift of $S_2Q_B^-$ was as much as 10°C in the direction of a lower temperature (Peeva and Maslinkova 2004).

E. Ozone and UV

Exposure of tomato and barley plants to ozone resulted in a concentration dependent down-shift of the $S_2Q_B^-$ band from 23°C to 15°C (Skotnica et al., 2003). Surprisingly, the chlorophyll fluorescence parameters F_v (variable fluorescence, F_m minus F_o), F_m (maximal fluorescence) and F_o (minimal fluorescence) did not change under the same conditions, thus indicating that PS II photochemistry in these leaves was still as efficient as in leaves not exposed to ozone. This could be explained by the possibility that the $S_2Q_B^-$ shift to lower temperature protected the reaction centers through the reaction center quenching mechanism

Table 19.3. Shifts of $S_2Q_A^-$ and $S_{2/3}Q_B^-$ bands in response to various mutations and treatments

Plant material	Mutations/Treatment	$S_2Q_A^-$ (T_M)	$S_{2/3}Q_B^-$ (T_M)	References
Synechocystis sp. PCC 6803 E189Q mutant cells	Replacement of D1-Glu189 with Gln	Upshift to 24°C	NM	Kimura et al., 2005
Synechocystis sp. PCC 6803 A1K mutant cells	Replacement of D1 with D1'	Upshift to 28°C	Upshift to 33°C	Sicora et al., 2004
Synechocystis sp. PCC 6803 D1-H332D mutant cells	Replacement of His332 in D1	Upshift to 24°C	Upshift to 47°C	Allahverdieva et al., 2004
Synechocystis sp. PCC 6803 D1-A244G mutant cells	Replacement of D1-Ala244 with Gly	Upshift by 5°C	Upshift by 5°C	Mizusawa et al., 2004
Synechocystis sp. PCC 6803 Y112L mutant cells	Replacement of D1Tyr112 with Leu	NS	Downshift to 20°C	Tal et al., 1999
Synechocystis sp. PCC 6803 NDSF mutant cells	Mutations in the PEST region of D1	NC	Downshift to 28°C	Minagawa et al., 1999
Synechocystis sp. PCC 6803 I6 mutant cells	Mutations in the C-terminal of D1	Upshift to 16°C	NC	Minagawa et al., 1999
Synechocystis sp. PCC 6803 CA1 mutant cells	Mutations in the D-de loop of D1	Upshift by 10°C	Upshift by 7°C	Mäenpää et al., 1995
Chlamydomonas reinhardtii D1 mutants	D1-arginine257 mutants	NC	Downshift by 10°C	Rose et al., 2008
Synechocystis sp. PCC 6803 C7-3 mutant cells	Mutations in the CD-loop of D2	Upshift by 9°C	NM	Vavilin and Vermaas, 2000
Synechocystis sp. PCC 6803 IC7 mutant cells	*psbH* null mutant	NC	Downshift to 27°C	Mayers et al., 1993
Tobacco Δ*psbJ* mutant cells	*psbJ* deletion mutant	Upshift by 10°C	Downshift by 4°C	Regel et al., 2001
Costata-2/133 mutant of pea	Reduction of LHCII oligomerization	Upshift by 5°C	Downshift by 4°C	Ivanov et al., 2005
Spinach PS II membranes	Removal of 16, 23 and 33 kDa proteins	Upshift by 20°C	Downshift by 5°C	Vass et al., 1987
Spinach PS II membranes	Removal of 33 kDa protein	Upshift by 20°C	NC	Vass et al., 1987
Synechocystis sp. PCC 6803 *ΔpsbO* mutant	*psbO deletion mutant*	Upshift to 26°C	Upshift to 39°C	Burnap et al., 1992
Spinach thylakoids	High pH	Upshift	Downshift	Vass and Inoue, 1986
Spinach thylakoids	Dark ΔpH	Downshift	Downshift	Miranda and Ducruet, 1995a
PS II membranes	Ca^{2+} depletion	Upshift	NM	Homann and Madabusi, 1993
Euglena cells	Diuron resistant mutant	NM	Downshift	Farineau and Laval-Martin, 1995
Synechocystis sp. PCC 6803A263P/mutants	Herbicide resistant mutants	NC	Downshift	Dalla Chiesa et al., 1997

NC no change, *NM* not measured, *NS* not shown

as proposed by Sane and co-workers (Sane et al., 2003; Ivanov et al., 2003). These results also indicate that in some cases TL can be a far more sensitive technique than fluorescence in monitoring environmental effects. The shift was reversed within 24 h upon shifting back the plants to an ozone free atmosphere. A similar, but smaller shift in the $S_2Q_B^-$ was observed in tomato and barley plants exposed to UV and this effect was associated with changes in high temperature peaks (Gilbert et al., 2004a).

F. Shifts of Q and B Bands

Table 19.2 summarizes reports that have shown shifts of $S_2/S_3Q_B^-$ and $S_2Q_A^-$ under different stress conditions. Shifts of the B and Q bands have also been documented by several research groups in response to various mutations and treatments (Table 19.3). It is apparent from Table 19.2 that the shift of the characteristic T_M of the B bands towards lower temperature is a general response of the photosynthetic apparatus to most of the

stress conditions studied. An up-shift of the $S_2Q_A^-$ band has been documented in some of the cases although this response is not as general as the shift of the B bands. In a series of studies, Ivanov et al. (2002, 2005, 2006); Sane et al. (2002, 2003) and Pocock et al. (2007) have demonstrated the down-shift of the B band and in some cases an up-shift of the Q band. These shifts were in the opposite directions and hence could not be attributed to the changes in the redox state of the electron donor side of PS II. Therefore, Ivanov et al. (2002) and Sane et al. (2003) suggested that the shifts resulted in the reduction of the redox gap between the two plastoquinone acceptors (Ivanov et al., 2002; Sane et al., 2003). This should result in the modification of the rate constants in the electron flow between Q_A and Q_B such that the electron will have a higher probability of being retained on Q_A.

What is the extent of the redox shifts when the temperature gap between $S_2Q_A^-$ and $S_2Q_B^-$ peaks decreases by say 10°C in the 15–30°C range? In *Synechocystis* sp. PCC 6803, Minagawa et al. (1999) estimated an equilibrium constant K_{AB} of 26 in control cells with a gap in the T_M of Q and B bands equivalent to 26°C. In *Synechocystis* sp. PCC 6803 site-directed mutants with modified PS II, the gap in the T_M was reduced to about 20°C and the K_{AB} was reduced to 8–9. Similarly, narrowing the T_M gap between Q and B bands by 6–8°C resulted in a calculated decrease of the redox potential difference of 22 mV between the two quinone acceptors. Rose et al. (2008) using D1 mutations in *Chlamydomonas reinhardtii* analyzed the TL data theoretically, based on the recombination model of Rappaport et al. (2005). They concluded that the observed shift of the B band to lower temperature by 8–10°C due to mutations in D1 corresponded with the reduction in the free energy difference by 20–40 mV for the recombination of S_2 with Q_B^-. This difference was interpreted to be due to a lowering of redox potential of Q_B/Q_B^-. The apparent equilibrium constant (K'_{app}) values were almost one-third in the mutants as compared to the wild type.

We suggest that under stress conditions, changes in equilibrium constant and redox potential difference of similar magnitude, if not more, occurs as the T_M gap has been reduced in the same or even greater temperature range. Such a condition will favor and increase the probability for non-radiative recombination of $P680^+Q_A^-$ that will not generate the triplet chlorophylls and the accompanying production of reactive oxygen species responsible for the destruction of PS II. One expects a reduction in the radiative pathway that should be reflected in the decreased luminescence as TL. In fact, a reduction in the TL yield to 40% was observed when the T_M gap was reduced in cold-stressed *Synechococcus* sp. PCC 7942 cells and cold acclimated *Arabidopsis thaliana* leaves (Sane et al., 2002, 2003). The increased difference in the redox potential of Q_A and Pheo has been suggested to result in a lower probability of $P680^+Pheo^-$ recombination that yields chlorophyll triplets (Johnson et al., 1995; Krieger and Rutherford, 1997; Krieger-Liszkay and Rutherford, 1998).

Based on the above studies, we have advanced a proposal for reaction center quenching (non-photochemical) that explains in part the resistance of the plant/leaf to increased excitation pressure including photoinhibition (Sane et al., 2003; Ivanov et al., 2003, 2006; Huner et al., 2006).

A schematic model for explaining the reaction center quenching mechanism associated with the shifts in the T_M of $S_2Q_B^-$ and $S_2Q_A^-$ bands is presented in Fig. 19.4. Under unstressed conditions the T_M gap between the acceptors Q_A and Q_B is higher and this encourages increased luminescence yield and lower non-radiative dissipation of energy. Under stress conditions the T_M gap decreases due to the shifts of $S_2Q_B^-$ and $S_2Q_A^-$ bands towards each other. This increases the k_2 (see Fig. 19.4) retaining the electron on Q_A. The redox potential difference between Q_A and P680* is increased restricting the back transfer of the electron to $P680^+$ to regenerate P680*. Instead the Q_A^- recombines with $P680^+$ through a non-radiative pathway. The TL yield is therefore lowered. This increases the probability for non-radiative dissipation of excitation energy within the PS II reaction center (reaction center quenching) with reduced destruction of PS II (Fig. 19.4b).

The use of the TL technique has brought forth these explanations and it appears that any stress that plants experience results in increased excitation pressure. The reduction in the redox potential gap of the acceptors is a response that helps plants to overcome the excitation stress. The response appears to be universal: it is present in cyanobacteria, algae, higher plants and

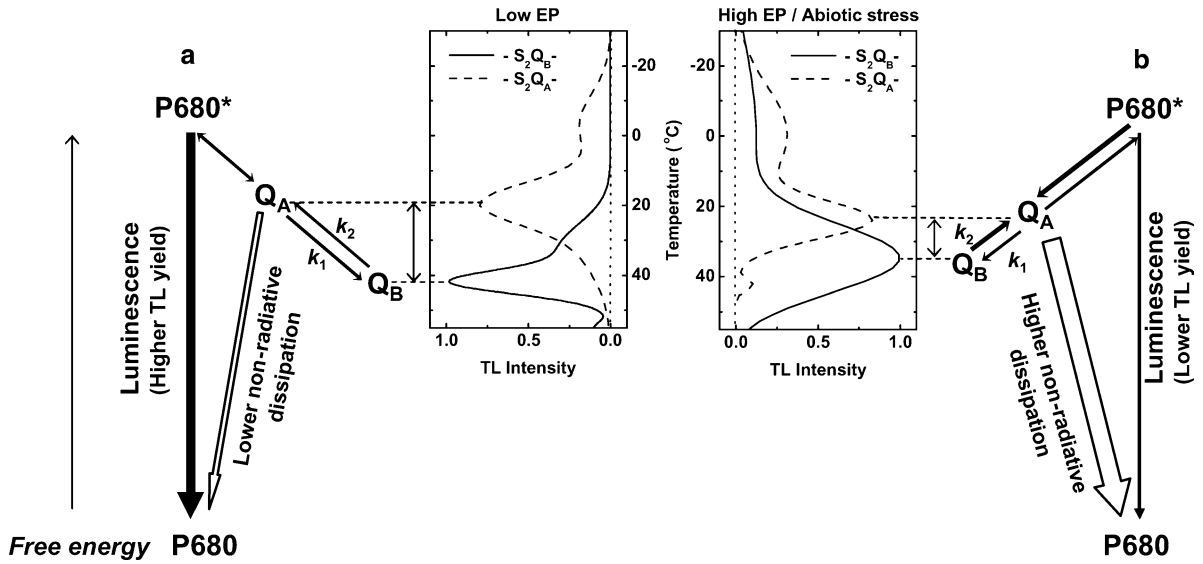

Fig. 19.4. Schematic diagram of the free energy levels explaining the differences in radiative vs non-radiative energy dissipation pathways in unstressed plants acclimated to low excitation pressure (EP) (**a**) and plants acclimated to high EP/stress conditions (**b**). (**a**) In unstressed plants (low EP) the free energy gap between $P680^+$ and Q_A^- would predominantly favor the radiative energy dissipation pathway characterized by higher TL yield (*thick black arrow*). When P680* returns to the ground state the energy is transferred to the antenna chlorophylls that emit light as TL. (**b**) In plants acclimated to high EP/stress conditions the increased free energy gap between $P680^+$ and Q_A^- determined by the higher TL peak emission temperature of $S_2Q_A^-$ charge recombination (see the TL glow curve of $S_2Q_A^-$ charge recombination under high EP conditions) would decrease the probability for a charge recombination pathway involving $P680^+Pheo^-$ and will cause stabilization of the $S_2Q_A^-$ pair. In addition, shifting the redox potential of Q_B toward Q_A determined by the lower TL peak emission temperature of $S_2Q_B^-$ charge recombination (see the TL glow curve of $S_2Q_B^-$ charge recombination under high EP conditions) favours the k_2 rate constant and also results in an increased steady state proportion of reduced Q_A. It is proposed that this will increase the probability for direct non-radiative recombination (*empty arrow*) of Q_A^- with $P680^+$ via the non-radiative pathway resulting in low TL yield without generating the chlorophyll triplet. In both types of plants the radiative charge recombination occurs, but is proportionally less in plants acclimated to high EP. The model is based on the assumption that redox properties of the donor side were not modified. The representative TL glow curves for $S_2Q_A^-$ (*dashed lines*) and $S_2Q_B^-$ (*solid lines*) were recorded in intact leaves of rye (*Secale cereale* L.) plants grown under low (20°C/50 μmol photons m^2 s^{-1}) and high (20°C/800 μmol photons m^2 s^{-1}) EP conditions.

even perennial trees and may be a complementary mechanism to the antenna quenching but more fundamental as it is present in even those organisms that have no antenna quenching possibilities (Ivanov et al., 2008). While the reduction in redox gap of acceptors in some cyanobacteria occurs through the replacement of D1:1 by D1:2 (as for example in *Synechococcus* sp. PCC 7942*)*; this D1 exchange mechanism is not possible in eukaryotes since they possess one *psbA* gene. However, we suggest that modifications in the lipid components of the chloroplast thylakoid membrane closely associated with PS II may regulate the redox gap between Q_A and Q_B. This may, in part, explain why isolated thylakoids do not show the $S_2Q_B^-$ shift while the intact tissue does.

VII. Concluding Remarks

Although the non-invasive TL technique is an attractive, simple technique to study the functioning of different aspects of PS II including studies of S-state turnover, modifications of D1 and D2, herbicide binding, function of the extrinsic proteins and cofactors related to the water-oxidizing complex and acclimation to various environmental conditions and stresses, the technique by itself is inadequate. It is often necessary to complement the data obtained using the TL technique with other biophysical and biochemical analyses. However, it does provide valuable and quick information on the relative redox state of important charge pairs that no other simple technique can. The TL peaks are indicative of a back reaction involving a

set of charged pairs but the confirmation of which charge pairs are involved must come from the use of other known perturbations.

DeVault and Govindjee (1990) provided a basis for relating the change in total free energy arising from the shifts in the T_Ms of the glow peaks to the changes in the redox potentials of the participating charge pairs. However, this relationship allows one to infer changes in the mid-point potentials of the redox carriers but not their actual redox potentials. These have to be determined by some other procedures such as redox titrations as has been done in some laboratories. Thus, the T_M shifts provide a basis for quickly assessing even the subtle changes taking place in the functional modifications of both the donor side and the acceptor side of PS II. In addition, it is now clear that TL can throw light on cyclic phosphorylation by PS I and the lipid peroxidation associated with stress-related responses. However, other procedures need to be used to understand the precise changes that have taken place in the energy transducing photosynthetic membranes.

Several laboratories have reported changes in TL characteristics following pretreatments of the sample material. The effect of freezing of the sample following the excitation was investigated by Homann (1999) and it was suggested that depending on the type of photosynthetic material the modifications in the TL characteristics could be interpreted as artefacts. Although this has to be kept in mind while conducting experiments, it is possible to determine whether such artefacts are occurring in one's experimental procedures. In most TL studies the data are used to compare two types of treatments and recombining species are identified using inhibitors or some other experimental treatments and not just by the temperature of their emission. Furthermore, the temperature range of recombining species is now well documented. In addition, the shifts in T_M are reversible. Thus, the generation of artefacts can be easily ascertained and overcome. However, we must be aware that potential artefacts could arise and those could lead to unwarranted interpretations.

The characteristics of different TL bands are very sensitive to the metabolic state of the cells that results in poising the redox state of components participating in the recombination reaction. During dark adaptation of the sample, depending upon the reduction status of the PQ pool and the possibility of its reduction through stromal enzymatic activities, the electron transport components such as Q_A and Q_B may become reduced and influence the TL patterns obtained in subsequent flash-induced studies. Moreover, the transmembrane electrochemical potential could also be influenced by the dark metabolic activity of the sample. As discussed in this chapter, the presence of a membrane potential down-shifts the T_M of some of the bands. In our opinion, precise control of growth conditions, developmental stage and the metabolic status of the organism is critical for the proper assessment and interpretation of changes in TL emission patterns.

The information now available about the mechanism and characteristics of different glow peaks has made the TL technique popular to monitor not just PS II function, but also to obtain information on energy charge, oxidative stress and several other responses of the photosynthetic apparatus. In some respects it is preferable to DLE as the components are better resolved in this TL method. We expect that the TL technique will be used as a routine tool in understanding of subtle but important changes that PS II may undergo during acclimation to an ever-changing environment.

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