6

Thermoluminescence in Chloroplast Thylakoid

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1. Introduction

1.1 Historical perspective

Luminescence occurs in almost all the materials absorbing photon energy and is a phenomenon of light emitting process. There are various types of luminescence, e.g. fluorescence, phosphorescence, delayed luminescence, chemiluminescence and thermoluminescence. In these cases, light is emitted when a photo- or chomo-excited molecules is deexcited to its ground state. During this deexcitation process, radiationless internal conversion and heat dissipation occurs, which reduces the quantum yield of light emission. Luminescence can be also thermally induced and enhanced by heating the sample in the dark. This process is called thermoluminescence (TL) and describes the emission of light at characteristic temperatures from samples containing chemiluminescent active species, radical pair states or electron hole pairs (Ducruet, 2003; Misra et al., 2001). Many minerals heated at very high temperature emit luminescence and so TL has been used initially in geology, archeological dating and radiation dosimetry. The theory of charge recombination in these processes was first worked out for such minerals (Randall & Wilkins, 1945). Thermally induced photon emission by a pre-irradiated chloroplast or thylakoid or by leaf samples in darkness is known as thermoluminescence (TL) (Misra et al., 2001; Misra & Ramaswamy, 2001). This is characteristic of solid states (semi-conductors) under thermally activated recombination of electrons and positive holes that are generated by particle radiation or electromagnetic field at room or low temperature prior to their heating in dark (Chen & McKeever, 1997). TL signals were first detected in dried chloroplasts samples (Arnold & Sherwood, 1957; Tollin & Calvin 1957). Photosynthetic systems in dried chloroplasts are supposed to be severely damaged. TL emission was also recorded in intact leaves and algal cells (Arnold & Sherwood, 1957). Arnold (1966) proposed a model of recombination of free holes in PS I and of free electrons from PS II as the sources of thermally induced luminescence from algae (Chlorella) cells in darkness. However, Arnold & Azzi (1968) refuted the role of free holes from PS I. Based on further evidence on the generation of charges in irradiated chloroplast, in the new model for TL charge recombination the positive and negative charge traps were proposed to reside within PS II (Arnold & Azzi, 1968). Further, biophysical studies and availability of photosynthetic

mutants, paved the way to the charaterisation and conformation of the origin of TL peaks between the temperature range of -40°C to +50°C is unequivocally from the charge recombinations in PSII. This finding has been further corroborated by subsequent experimentation by several workers using bundle sheath chloroplasts of C-4 plants that apparently lack PSII and, therefore, show very weak TL. The inhibition of PSI activity by $HgCl_2$ does not affect the glow peak yield of isolated chloroplast (Sane et al., 1977; Horvath et al., 1978). Several other studies using herbicides and inhibitors that interact with PSI electron flow supports this conclusion.

Photosynthetic TL could be defined as an emission of light at characteristic temperatures from pre-illuminated photosynthetic samples (leaves, isolated chloroplasts or algae) during warming in the dark starting from low temperature (Sane, 2004). A set of different bands in TL emission curves appear as a result of recombination of different charge pairs. Even small changes in the redox properties of radical pairs affect the intensity and the peak position of TL bands. This complexity of information of TL emission curves can be used for selective monitoring of the effects of various biotic and abiotic stress factors. Thermoluminescence emission from freshly detached unfrozen leaves is defined as secondary or "afterglow" (AL) emission with T_m at 45°C and proves to be a sensitive test of energetic imbalance in the chloroplasts during various stress conditions (Ducruet, 2003). High-temperature thermoluminescence (HT1, HT2 and HT3 bands with T_m above 60°C) appears as a result of accumulation of lipid peroxides and can be used as a simple and efficient tool to monitor oxidative stress in leaves.

Randall & Wilkins (1945) considered that the charge recombination of fixed +/- charge pairs obeys a first order kinetics. Vass et al. (1981) postulate to photosynthetic TL studies. The probability of recombination *L(T)* for eachTL band at every measured temperature can be calculated, using the Arrhenius equation (Ducruet, 2003):

$$L(T)=N^{R}PT\Delta t\exp\left(-E_A/k_BT\right)$$

Where:

N = number of charge pairs able to recombine at temperature T.
R = order of reaction (R=1, as charge pairs are not exchangeable between PSII centres).
P = Pre-exponential factor related to the Arrhenius frequency factor s as P=*K(T)* x s, *K(T)* being an unknown factor; Δt sampling duration (~1 s); EA is the activation energy, and *kB* is the Boltzmann constant.

However, charge recombination in PSII varies from that of the minerals. So, DeVault et al. (1983) and DeVault & Govindjee (1990) proposed a theoretical calculation of photosynthetic TL (cf. Tyysjarvi & Vass, 2003).

Further, developments in the TL techniques and biophysical probing revealed that the charge arise as a reversal of the primary photochemical processes in PS II (Misra et al., 2001a, b; Sane, 2004). Therefore they are also in some relation with characteristics of fluorescence induction and emission (Ducruet, 1999; Setikova et al.,1999). There are reviews on thermoluminescence use in herbicide research (Horvath, 1986) and general reviews on TL in chloroplasts or photosynthetic organisms (Sane & Rutherford, 1986;, Inoue, 1996; Vass & Govindjee, 1996). In the present chapter, we focus mainly on the use of TL in studies of

primary photochemical processes in chloroplast thylakoid membranes particularly in PSII. The use of TL technique in the studies of thylakoid development, and the impact of environmental stresses are discussed.

TL measurements need excitation of the sample and the cooling of the sample at low temperature (liquid nitrogen), which is then followed by heating in the darkness and simultaneous recording the luminescence emitted during heating. There are several types of TL apparatus assembled and built so far (Tatake et al., 1971; Manche, 1979; Zeinalov & Maslenkova, 1996; Gilbert et al., 2003; Ducruet, 2004). For the measurement of steady-state TL, a sample such as a section of leaf, chloroplast, or algal material is placed on the sample holder and is illuminated by white light or light of a particular wavelength through a monochromator. A heater coil placed under the cupperplanchet (sample holder) slowly and linearly heats up the sample. A peltier cooling and heating system is used recently. The ultra weak TL emission is amplified several fold and is measured with a red sensitive photomultiplier. The emission(maxima same as prompt fluorescence at about 730 nm in leaves) recorded against temperature gives rise to the TL glow curve or a TL band as shown in Fig. 1. The shape of the glow curve depends on the excitation temperature, time period of excitation, heating and cooling rate, and intensity and wavelength of excitation light. The sample heating varies from 0.5 to 18°C/sec. A single flash illumination or continuous light illumination at low temperature gives rise to one TL band where as continuous illumination during sample cooling gives rise to multiple components as shown in Fig. 1. Luminescence emission from a leaf disc is sufficiently strong to be recorded with an analogue photomultiplier tube (PMT) positioned very close (about 1.5 cm above) to the sample by a light-proof holder that can slide laterally to an illumination position where a light guide comes in front of the sample whilst the PMT is protected from strong actinic light (Ducruet et al., 1998). Alternatively, luminescence can be conveyed to the detector by a light guide, which prevents heating of the PMT. In the conventional TL apparatus, the sample is frozen by liquid nitrogen in order to ensure that no recombination occurs before TL recording. A complete study on the effect of freezing on TL emission has been reported by Homann (1999), who reported few artifacts during freezing. So before the start of experiments, a comparison between TL emission in frozen and unfrozen samples be done, to ascertain the quality of TL signals.

2. Charge recombinations

In a leaf, algae, cyanobacteria, intact chloroplasts, isolated thylakoids, a brief (5 ms) flash induces one charge separation per PSII centre as shown in Fig. 1. This separated charges recombine to stabilize to S_2Q_B pair, and is able to emit luminescence with a exponential decay phase with $t_{1/2}$~40 s at 20 °C, or the B band with a maximum temperature T_m between 30°C and 40°C. When a sequences of 1 to 10 flashes are triggered before TL recording, the B band intensity oscillates with a period of 4 with maxima at two and six flashes, corresponding to the maximum S_2+S_3, the only two states able to yield luminescence upon recombination with Q_A^- or Q_B^-. The S_1 state is unable to recombine.

Following a light period that creates an equipartition of S_0, S_1, S_2, and S_3, the states S_0 and S_1 remain stable during a dark adaptation whilst S_2 and S_3 are converted to S_1, resulting in a 1/4 S_0 3/4 S_1 distribution in dark-adapted material. In leaves, approximately 40% of Q_B is reduced (Rutherford et al., 1984a), so that the Q_B^-/Q_B ratio weakly oscillates with a period of

2 according to flash number. In isolated thylakoids at low pH (<6.5) the B band, induced by two flashes, splits into two bands B_1 ($S_3Q_B^-$) and B_2 ($S_2Q_B^-$), because S_3 is more destabilized by protonation than S_2 (Joliot & Joliot, 1980; Rutherfordet al., 1984b; Demeter & Sallai, 1986; Miranda & Ducruet, 1995b). Upon treatment by a PSII-inhibiting herbicide (diuron, atrazine) which blocks the Q_A to Q_B electron transfer, the electron is stored as Q_A^-, a less stabilized state (i.e. the activation barrier is smaller) than Q_B^- and produces a Q-band peaking at a lower temperature (around 5 °C at neutral pH) than the B band upon recombination with S_2/S_3. This band is associated with a C-band at about 55 °C due to $D^+Q_A^-$ (Johnson et al., 1994), D^+ being the oxidized form of Tyrosine D on the inactive branch of PSII. The functional electron donor to the PSII P_{680} centre is Tyrosine Z: $Z+P_{680*}$ $Tyr_Z^+ + P_{680}$.

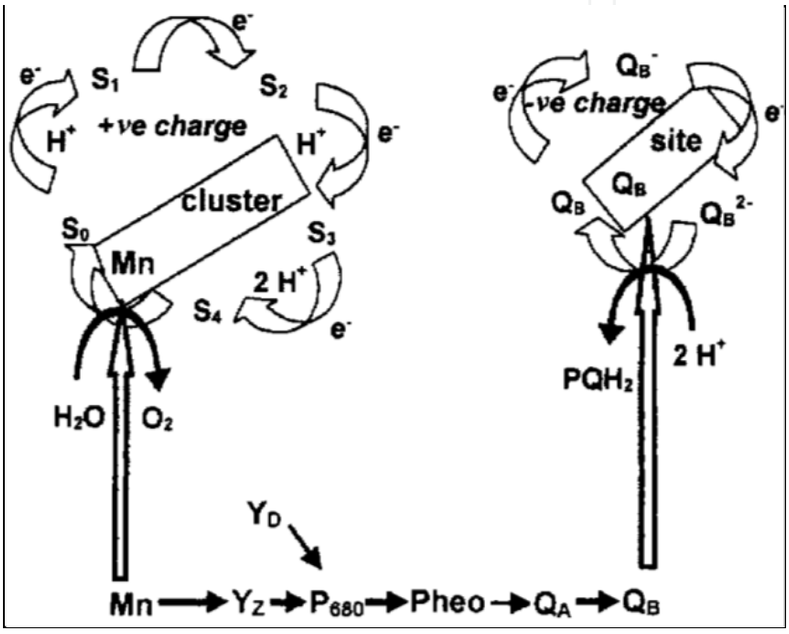

Fig. 1. Schematic view of the +ve and -ve charge formation in PS II. (Adapted from Misra et al., 2001a,b).

A fast rereduction of Z^+ by S states occurs, except when the oxygen-evolving manganese complex is damaged, which leads to the emergence of A band $Z^+Q_B^-$ at about -15 °C. Other TL bands exist at lower temperatures which are not fully explained and will not be considered here since they have been of little interest in plant biology. Twenty years ago, thermoluminescence brought a conformation of the charge stabilization model of PSII. It remains a valuable technique that gives a global view complementing more analytical tools and it is particularly well adapted to the study of PSII mutants. Furthermore, like chlorophyll fluorescence, TL is relevant to various levels of integration, from subchloroplast particles to algal cells or leaves (Farineau, 1993; Homann, 1999). However, applications to leaf photosynthesis have been relatively few until now, due to instrumental constraints and to a lack of understanding of in vivo signals. Several reviews already exist on photosynthesis TL (Sane & Rutherford, 1986; Vass & Inoue, 1992; Misra et al., 2001; Tyysjarvi & Vass, 2003). The present chapter will be focused on TL emission by leaves and will address the instrumental, theoretical and practical issues of measuring TL in leaf discs. Taking advantage of new and recently available components, simple instruments designed for leaf studies can now be built.

The properties of TL bands already characterized in thylakoids or subchloroplast particles are modified *in vivo* by the cellular environment, particularly when leaves are kept unfrozen during the cooling step. Different types of stresses also modify the shape and intensity of the existing bands or enhance minor bands. Furthermore, a TL `afterglow' band observable only in intact systems (intact chloroplasts, algae and leaf fragments) reflects the flow of electrons from reducing compounds present in the stroma to the plastoquinone pool and the quinonic acceptors of PSII. This back-transfer follows one of the pathways involved in the cyclic/chlororespiratory electron flow and appears to be a sensitive indicator of photosynthetic metabolism. Although PSII is totally destroyed at about 60 °C, huge chlorophyll TL bands can be observed at higher temperatures.

They correspond to a heat-enhanced chemiluminescence from molecular species generated by radical forms of oxygen, such as lipid peroxides, which accumulate in stress situations. Despite the fact that the mechanisms of high-temperature TL emission (HTL) is completely different from photosynthetic TL, recorded through a single temperature scan from 0 °C to 160 °C on the same leaf disc. Both the photosynthesis TL bands and the oxidative stress HTL bands proves to be of practical interest.

3. TL glow peaks: Nomenclatures and classifications

There are several classifications of TL glow bands. However, more acceptable are 'Roman number system – I, II, III, IV etc' (Table 1) or 'Alphabetic form - A, B_1, B_2, C, Z, Z_v or M_o' (Fig. 2, Misra et al., 2001) (Table 1). TL glow peaks that are well characterized are- A, AG AT, B, C, M_o, Q,Z and Z_v bands (Table 1). The TL band Z and Zv arise at -169˚C and at -80˚C to -30˚C (Inoue, 1996). These two bands are assigned to charge recombination between charged pigment molecules. The Chl^+Chl^- charge recombination give rise to Z-band and $P680^+$ Q_A^- charged recombination gives rise to Z_v- band of the TL spectra. However alteration in the PS II RC is mainly studied through the changes in the TL bands A, AG, AT, B, C, M_o, or Q. So a detailed characteristic of the charge pairs and the factors responsible for such glow peaks are explained.

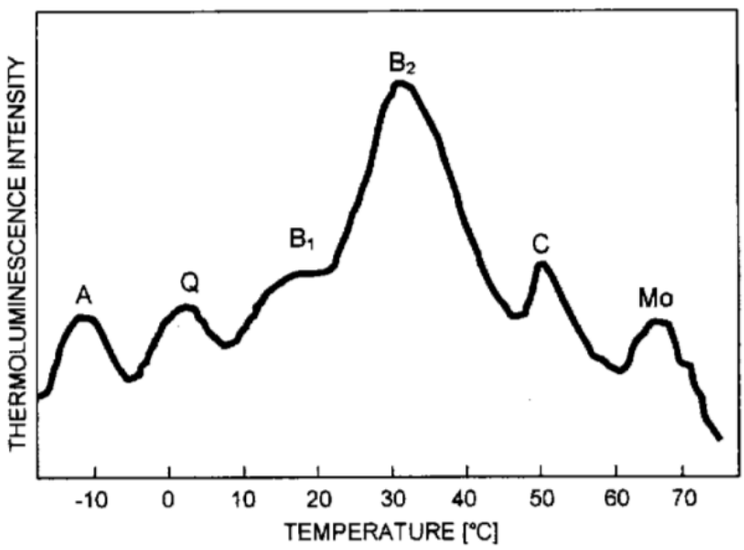

Fig. 2. Theoreticel picture of thermoluminescence (TL) glow peaks of photosynthetic materials.

3.1 A-band (II band)

A TL glow peak at -10˚C originates by photo-exiting photosynthetic samples by two flashes at room temperature, then cooling of samples to 77K, and further continuous irradiation of the sample (Laufer et al., 1978; Inoue, 1981; Tatake et al.,1981; Demeter et al., 1985). This band is designated as A-band. The charge recombination of $S_3Q_A^-$ is responsible for the origin of the A-band (Koike et al., 1986).

3.2 AG- band

An after glow (AG) or delayed luminescence rise is induced by far-red radiation (Bertsch & Azzi, 1965). This emission was reported by only in intact systems such as intact chloroplasts (Hideg et al., 1991), leaves (Bjorn, 1971), or protoplasts (Nakamoto et al., 1988), and could not be detected in isolated thylakoids or PS II RC complexes. This AG glow peak at 45˚C could be resolved by slow heating of leaf samples (Miranda & Ducruet, 1995a,b; Ducruet et al., 1997). The AG-band is suppressed by the PS II electron transport inhibitor diuron (Miranda & Ducruet, 1995a,b) and by the PS I cyclic electron transport inhibitor antimycin or uncouplers (Bjorn, 1971). This suggests that in addition to PS II, also the cyclic electron transport and/or the trans-thylakoid proton gradient are involved in the generation of AG glow peaks in leaves. Sundblad et al. (1988) assigned the AG glow peak primarily to the back reaction of $S_2/S_3Q_B^-$ charge recombination. The occurrence of AG peaks was also assigned to the presence of high concentrations of ATP and/or NADPH in the plant cells (Inoue et al., 1976).

3.3 A_T-band

The Tris-washed PS II particles show a TL glow peak similar to A-band (Inoue et al., 1977; Rozsa & Demeter, 1982). Tris washing depletes functional Mn cluster from PS II particles. So the generation of S-states is impaired. Unlike the A-band, this band at -10˚C arises due to charge recombination of $His^+Q_A^-$ (One & Inoue, 1991; Kramer et al., 1994). This positive charge is assigned to His195 residue and His190 residue of the D1 polypeptide (Kramer et al., 1994). This was confirmed by the impaired A_T-band in *Chlamydomonas reinhardtii* mutants substituted for the above His residues (Kramer et al., 1994).

3.4 B-band (III (B1), IV (B2))

This is the best-characterised TL glow peak in photosynthetic materials. The B-band arises usually at around 30-35˚C. It is correlated to the water-oxidising complex of PS II (Inoue, 1976; Rozsa & Demeter, 1982). Charge recombination between $S_2Q_B^-$ (band III or B_1 band) and $S_3B_Q^-$ (band IV or B_2 band), together generate the B-band (Rutherford et al., 1982). In dark-adapted chloroplasts, the distribution of S_0 and S_1 are 25% and 75%, respectively. Thus, the maxima obtained after the second flash indicate the participation of the S_3 state in the generation of peak IV (B band). Based on several studies it has been concluded that peak III (B_1 band) originates from $S_3Q_B^-$ and peak IV (B_2 band) originates from the recombination of $S_2Q_B^-$.

The peak V (C band) is not related to the photosynthetic electron chain (Table 1). Peak V (C band) was first observed in DCMU treated chloroplasts and in etiolated leaves (Vass et al. 1981). Since in etiolated leaves the oxygen evolving system is inactive, it has been suggested

that peak V is not related to the water splitting enzyme. However, several studies have shown that this peak also undergoes a period four oscillation and it has been proposed that this band may be originating from the charge recombination of the $S_0Q_A^-$ and $S_1Q_A^-$ redox couple (Demeter & Govindjee, 1989; Sane et al., 1983).

Peak	Approximate Temperature (°C)	Origin	Mean Lifetime (t, sec)	Remarks
Illuminated samples				
Z	-160	Chl^+Chl^-		0.2
Z1	-70	$P680^+Q_A^-$	1.3	
II (A)	-10	$S_3Q_A^-$	-	Mn oxygen-evolving complex
Q	+5°C	$S_2Q_A^-$	-	Secondary Qb, quinone acceptor (induced by herbicides)
III (B1)	+20	$S_3Q_B^-$	-	After one flash
IV (B2)	+30	$S_2Q_B^-$	29	After three flashes
V (C)	+50	$Y_D^+ Q_A^-$	1062	
AG	+45	S_2/S_3Q_B	1.3	
Oxidative bands(do not depend on preillumination)				
HTL1 (=Mo band*)	65 to85 °C	Aldehydes+H2O		A pseudo-HTL2 occurs in wet samples
HTL2	120 to140 °C	Lipid peroxides		dry samples
HTL3	> 160°C	Induce by warming		Oxidative band increases during TL warming

Notes: Peaks Z1, II (A), IV (B_2), and V (C) oscillate with flash number and the maxima differs between S_3 for peak II, S_2/S_3 for peak IV (B), and S_1 for peak V (C). Some peaks oscillate when diuron was added after excitation, for example, peaks II and V (C). *Misra et al. (2001)

Table 1. Nomenclature of TL Glow Peaks in Plants

3.5 High temperature (HTL) bands

Strong TL bands are observed even in dark adapted samples above physiological temperatures >50/60 °C. These bands are ascribed to chemiluminescence bands or HTL, and are few oxidative bands unrelated to PS II. Such TL bands in algae or leaves were reported by Venediktov et al., (1989), Vavilin et al., (1991) and Merzlyaket al., (1992). A HTL band around 60-75°C was also reported by Hideg & Vass (1993), Stallaert et al., (1995) and Marder et al., (1998). This band is reported to be due to the accumulation of lipidperoxides (Vavilin & Ducruet, 1998). When the samples were kept in water while heating to prevent desiccation a band at 130 °C was observed and this was proposed to be due to the hydrolysis of lipid peroxides (Ducruet & Vavilin, 1999) or an indicator of oxidative stress (Havaux & Niyogi, 1999). Similar HTL bands around 60-75°C is reported by other authors also (Skotnica et al., 1999; Havaux & Niyogi, 1999; Ducruet & Vavilin, 1999).

4. Thylakoid organization and changes in TL glow peak characteristics

In a healthy leaf, the B band at about 35 °C is prominent, although other bands the Q-band, AG or C-bands can also be detected. Oscillations were also seen in the case of TL bands, especially peaks III B_1), IV (B_2) and V(C) (Bhagawat & Bhattacharjee, 2005). The oscillation of peak IV (B band) of the TL band is the best-characterized band showing maxima at 2, 6, 10, etc., flashes with a periodicity of four (Misra et al., 1998). Manganese oxidation states S_2/S_3 were found to be the most luminescent states. The different TL peaks attributed to $S_2Q_A^-$ and $S_2Q_B^-$ reflects different activation energies for the recombination reaction to take place in each of these states. This energy difference may, in part, reflect a different midpoint potential between the Q_A/Q_A^- and Q_B/Q_B^- redox couple.

Increase of the Q-band (T_m ~5 °C), and the associated C-band (~55 °C), reflects a damage to Q_B of PSII e.g. during photoinhibition (Misra et al., 1996; 1998; Janda et al., 1992). The A band (~ +15 °C) is prominent in thylakoid samples or leaf with damaged oxygen evolving complex. However, frozen samples show Q and A bands, which are reported to be artefacts (Homann, 1999).

5. Stress induced changes in TL glow peaks

TL is a useful tool for the study of photosynthetic electron transfer both at the acceptor and the donor sides of PS II (Misra et al., 2001a, b; Misra & Ramaswamy, 2001) as well from the water–oxidase complex to PSI (secondary quinone acceptor) (Bhagawat & Bhattacharjee, 2005).

The effects of various abiotic and biotic stress factors that influence PSII activity, such as UV, high light, high temperature, drought, viral infection, hormonal effect, have also been studied. Major TL bands are missing in etiolated leaves. Intermittent illumination to greening leaves which does not develop Mn clusture properly do not show the TL bands (Inoue et al., 1976; Sane et al., 1977). Misra et al. (1998b) reported a gradual increase in the Q and B band from base to apex of that wheat leaves greening under continuous illumination, which confirms a developmental gradient across the wheat leaf lamina and a gradual development and organization of the photosynthetic PSII complexes. Leaf aging under continuous light or under continuous darkness resulted in a decrease in Q and B bands (Joshi et al., 1993). Biswal et al. (2001) showed through an elegant TL experiment a block in the electron flow from Q_A to Q_B during leaf aging and also showed a decrease in the quinone pools in PS II.

Salt stress affects differentially the Q and B band (Biswal et al. 2002; Sahu et al. 1998; Misra et al. 1998c). Relatively the B band is more affected than the Q band. These are dose and time dependant. Zurita et al. (2005) showed that not only the B band is affected, but there is a back flow of electrons in the salt stressed photosynthetic system.

Mineral supplementation of N, P, K or their mixtures induce a shift in the A band from nearly -13°C to 8°C, suggesting the accumulation of S_4 states in the mineral supplemented leaf (Soltnev et al., 1998). Heavy metals such as Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+} affects B band only (Mohanty et al., 1989). Inert gases (N_2, He, Ar, Xe) and anoxic environment reduces the B and C band intensities in leaves (Soltnev et al., 1999). Halogens like I^- quenches the TL peaks probably by donating electrons to the S-states (Solntsev et al., 1995). Drought stress shifts the

B band to a lower temperature, which is explained by the redox changes in the thylakoid membranes changing the redox pool in the chloroplast (Ducruet & Vavilin, 1999; Janda et al., 1999).

Leaves irradiated with 725 nm illumination showed B- and C- bands, but A- band was missing (Mirand & Ducruet, 1995a). Probably this is due to photoabsorption by P_{700} pigment in PSI and not by PSII. Increasing the duration of the illumination decreased the peak temperature of B-band, probably due to a progressive lumen acidification.

Photoinhibitory treatment which usually result in the decreased quantum efficiency of PSII and degradation of D1 polypeptide, do not affect the S-states but affects B-band with least effect on C-band (Misra et al., 1997; 1998a). Isolated thylakoids showed a parallel decrease in Q- and B-band (Vass et al., 1988) suggesting both Q_A and Q_B is affected by photoinhibition. Ohad et al. (1990) reported a band shift of B-band to lower temperature, suggesting lumen acidification and redox changes in PSII. Misra et al. (1997, 1998a) reported Q- and B-band intensity decreased with high temperature in pothos and spinach leaves. Janda et al. (1999) reported membrane leakiness and grana destacking might be the reason for the changes in AG bands both at high temperature and freezing.

Viral infection of the plant shifts the B-band to a higher temperature with a decrease in its intensity (Rahoutei et al., 1999). There appears a new peak at 70°C, which is suggested to be due to membrane lipid peroxidation due to hypersensitive reactions in the leaf cells (Stallaert et al., 1995).

6. Conclusion and future perspective

The phenomenon of thermoluminescence in photosynthetic materials has been used routinely in characterizing the redox reactions of PSII in thylakoid membrane both in isolated systems and intact leaves. TL methods are also used as a non-invasive method for the detection of genetic lessions or genetic modifications in the plants (Lurie & Bertsch, 1974; Ichikawa et al., 1975; Debus et al., 1988; Minagawa et al., 1999). Also, the screening of photosynthetic mutants from these genetically modified organism, led to the confirmation of the origin of TL bands. This simple instrument can be fabricated in a laboratory with minimum infrastructure facility. The charge pair interactions giving rise to TL bands of characteristic peaks is used routinely for the stress characterization both donor side and acceptor side of PS II in photosynthetic materials. The oscillation pattern of TL can denote the ''S'' state transition and can be used for a titre of the Mn clusture of oxygen evolving complex.

TL characteristics may help in identifying new site(s) of action of herbicides and other chaotropic agents. However, TL studies are only confined to PS II and the method has such limitations or confines. The other limitations are the shift in the peak temperature of TL bands due to differences in the instrumentation, illumination temperature, and several other parameters that are obscure. The phenomenon of HTL is now a days used as an useful tool for understanding oxidative changes in photosynthetic systems. Also this system is now used in 'sensors' for developing 'biosensors' (Zhang et al., 2007). However, before this proposition comes true, the instrumentation needs more precission and miniaturization.

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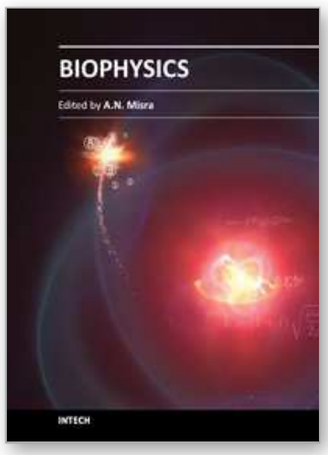

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Biophysics is a vast cross-disciplinary subject encompassing the fields of biology, physics and computational biology etc in microbes, plants, animals and human being. Wide array of subjects from molecular, physiological and structural are covered in this book. Most of these chapters are oriented toward new techniques or the application of techniques in the novel fields. The contributions from scientists and experts from different continents and countries focuss on major aspects of biophysics. The book covers a wide range of topics reflecting the complexity of the biological systems. Although the field of biophysics is ever emerging and innovative, the recent topics covered in this book are contemporary and application-oriented in the field of biology, agriculture, and medicine. This book contains mainly reviews of photobiology, molecular motors, medical biophysics such as micotools and hoemodynamic theory.

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