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REVIEW

Thermoluminescence: experimental

Jean-Marc Ducruet · Imre Vass

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Abstract Thermoluminesence measurements are useful for the study of Photosystem II electron transport in intact leaves, in algal and cyanobacterial cells, as well as in isolated membrane complexes. Here an overview of the experimental approaches is provided. In the present review, instruments and the experimental procedures for measuring thermoluminescence emission from photosynthetic systems of various origins are summarized and discussed. Major pitfalls frequently encountered in measurements with isolated membranes, suspensions of intact organisms or solid leaf samples are highlighted. Analytical and numeric methods for the analysis of measured thermoluminescence curves are also discussed.

Keywords Photosystem II · Instrument · Signal analysis · Thermoluminescence

Abbreviations

DCMU	3-(3,4-Dichlorophenyl)-1,1-dimethylurea
PS II	Photosystem II
Q_A and Q_B	Primary and secondary quinone electron acceptors in PS II
PID	Proportional integral derivative
S_0, S_1, S_2, S_3	Oxidation states of the manganese oxygen-evolving complex
TL	Thermoluminescence
TTL	Transistor–transistor logic
T_M	Temperature at the maximum of a TL band

J.-M. Ducruet (✉)
Groupe de Biospectroscopie Végétale, Ecologie Systématique Evolution (ESE), Université Paris-Sud-Orsay & Institut National Recherche Agronomique (INRA), Bâtiment 362, 91400 Orsay, France
e-mail: jean-marc.ducruet@u-psud.fr

I. Vass
Institute of Plant Biology, Biological Research Center, Temesvari ktr. 62, 6726 Szeged, Hungary

Introduction

Thermoluminescence (TL) is a weak light emission stimulated by warming of irradiated samples; it results from a recombination of preexisting ± charge pairs, that had been stabilized in potential wells. Warming increases the vibrational energy that allows charge recombination to occur by overcoming the activation energy barriers. TL can be observed in various materials, including solid states, minerals, amino acids and complex biological systems, and is widely used in mineralogy, archeology, radiation dosimetry, detection of irradiated food, with dedicated commercial instruments which are able to detect light emission well above the ambient temperature (McKeever 1988). However, using TL to investigate the stabilization of light-separated charge pairs in the photosynthetic systems requires very specific instrumental features, i.e., the possibility to cool the sample well below 0°C, while performing fast temperature jumps or drops combined with complex illumination patterns from various light sources.

Thermoluminescence of photosynthetic systems was first observed by Arnold and Sherwood (1957). Several reviews are available (Sane and Rutherford 1986; Demeter and Govindjee 1989; Vass and Inoue 1992; Vass and Govindjee 1996), some emphasizing theoretical aspects (Tyystjärvi and Vass 2004) or application to leaf discs (Ducruet 2003). An interesting historical review, with photographs of people, is that by Vass (2005). Photochemically separated charge pairs are stabilized on electron carriers by activation energy

barriers that limit back reaction, i.e., charge recombination, at physiological temperatures. TL originates from Photosystem II (PS II) via thermally stimulated delayed light emitted by singlet excited chlorophylls, generated by the recombination of the $S_2Q_A^-$, $S_2Q_B^-$ and $S_3Q_B^-$ charge pairs (Rutherford et al. 1982; Demeter and Vass 1984). Besides radiative recombination, the non-radiative charge recombination pathways play an important role in TL emission (Vass and Demeter 1984; Rappaport et al. 2005; Cser and Vass 2007; Rappaport and Lavergne, this issue). The rate of recombination can be made negligibly small by cooling the sample, before or immediately after an illumination, to temperatures where thermally stimulated reversal of charge separation becomes negligible. Then a progressive warming increases the recombination rate, revealing thereby the different types of charge pairs as successive TL bands.

Thermoluminescence bands observable in photosynthetic samples, and their origins, are presented in Table 1: more detailed information can be found in reviews cited therein. Briefly, B bands are those found in control photosynthetic material after one or a few flash(es); the Zv, A, A_T, Q and C bands also originate from recombination of charge pairs stored on PS II electron carriers and appear after special treatments (e.g. the inhibitor DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), which displaces plastoquinone from its binding niche Q_B, induces a Q instead of a B band). The Z band is related to low temperature photochemistry of a pigment. The afterglow (AG) band is a B-type recombination that depends on the back transfer of electrons from stroma to the acceptor side of PS II. High temperature TL bands (HTL) are chemiluminescence emissions observed without previous illumination and enhanced by warming, which reflect the radiative thermolysis of peroxidized compounds. The peak temperatures (T_M) may, however, vary over a wider range since they depend on the heating rate of the samples, and are influenced by some specific conditions examined below, e.g. use of thermophilic organisms (Govindjee et al. 1985). Thermoluminescence provides specific information on Photosystem (PS II), some being unattainable by other methods. Furthermore, like chlorophyll fluorescence, TL allows comparative studies to be made at various integrative levels, i.e., on PS II particles, thylakoid membranes, intact chloroplasts, protoplasms, algal cells and leaf pieces. [For details on PS II, see Wydrzynski and Satoh (2005), and for chlorophyll fluorescence, see Papageorgiou and Govindjee (2004)]. Photosynthetic thermoluminescence has been developed using set-ups built in specialized laboratories, which has restrained the use of TL in the wider domain of ecophysiological research even though commercial instruments are now available (www.psi.org, www.fan-gmbh.de). We examine here the experimental aspects of TL measurements and we will point out some experimental pitfalls.

Table 1 Origin of thermoluminescence bands

Name	T_M range	Origin	Origin PS II	Comments
Z	−160°C	Pigments	−	Low temperature pigment photochemistry
Zv	−70 to −100°C	(P_{680}^+ Q_A^-?)	+	Tm depends on illumination temperature
A_T	−10 to −20°C	$TyrZ^+Q_A^-$	+	Damage to Mn oxygen-evolving complex (TyrZ is the functional donor to PS II center)
A	∼−15°C	$S_3Q_A^-$?	+	
Q	+2 to 10°C	$S_2Q_A^-$	+	Damage to secondary Q_B quinonic acceptor or inhibition by DCMU-like herbicides
B	30 to 38°C	$S_{2/3}Q_B^-$	+	Lumen pH > 7
B2	28 to 32°C	$S_2Q_B^-$	+	Lumen pH < 7
B1	20 to 30°C	$S_3Q_B^-$	+	Lumen pH < 7
AG	+45°C (→ +35°C)	$S_2/S_3Q_B + e^-$	(+)	Electron from stroma, in intact chloroplasts or cells
C	+52/55°C	$TyrD^+Q_A^-$	+	Minor band, increased by DCMU or damage (TyrD is the non functional donor to PS II center)
HTL1	60 to 85°C	?	−	Different bands of unknown origin, without illumination
HTL2	120 to 140°C	Lipid peroxides	−	Thermolysis: –C–O–O– → *C=O + Chl → *Chl

T_M values are given for data obtained with a 0.5°C/s TL heating rate

More detailed information on Z, Zv, A_T, A, Q, B, C bands can be found in Sane and Rutherford 1986; Demeter and Govindjee 1989; Vass and Inoue 1992; Vass and Govindjee 1996; Tyystjärvi and Vass 2004. For afterglow (AG) and high-temperature thermoluminescence (HTL) bands, see Ducruet 2003

Instruments for photosynthetic thermoluminescence

Measuring luminescence

Luminescence emitted by PS II has an emission spectrum similar to that of chlorophyll fluorescence, with two emission bands (Sonoike et al. 1991), a sharp one at 685/695 nm and a broader one at 735 nm that becomes prominent at high chlorophyll concentrations, as a result of reabsorption of luminescence at shorter wavelengths. Detection requires red-sensitive photomultiplier tubes (PMT) protected by a shutter during actinic illumination. Recent avalanche photodiodes (APD) with large areas are sensitive enough to detect luminescence, while being more tolerant to high light intensities. The detector current is amplified by a DC transimpedance amplifier (typically 10 V/μA). Photon counting is not necessary but offers the possibility to calculate a χ^2 in order to assess the goodness-of-fit of a simulation of a TL signal ($\chi^2 \rightarrow 1$ for a perfect fit).

An efficient collection of light emitted by the sample can be achieved by placing the PMT windows close to the sample (Fig. 1), with a protective window or a lens with a short focus (e.g., aspherical). Alternatively, a light guide allowing the PMT to be placed at a distance can be used especially when extreme temperatures are reached in the sample holder.

Light collection must be geometrically compatible with pre-illumination of the sample by different light sources (flash, white light or far-red light). The light sources and detector(s) can be held on a sliding or a rotating runner, and placed successively in front of the sample (Ichikawa et al. 1975; Vass et al. 1981). A multifurcated light guide, although it causes some signal loss, is convenient to connect light sources and detectors to a common end facing of the sample, thus allowing a fully automated measuring cycle to be programmed.

Temperature regulation

Most photosynthetic TL bands (A/A_T, Q, B, AG, C; Table 1) are located between −20°C and +55°C and can therefore be resolved by a temperature scanning from −30°C to +70°C. The very low temperature Z and Z_V bands are seldom used in photosynthetic research. Of more practical interest is the illumination of sample at 77 K to produce an inversion $Q_B^- \leftrightarrow Q_B$ in order to determine the Q_B^-/Q_B ratio (Rutherford et al. 1982, 1984a); this can, however, be performed in liquid nitrogen without temperature regulation.

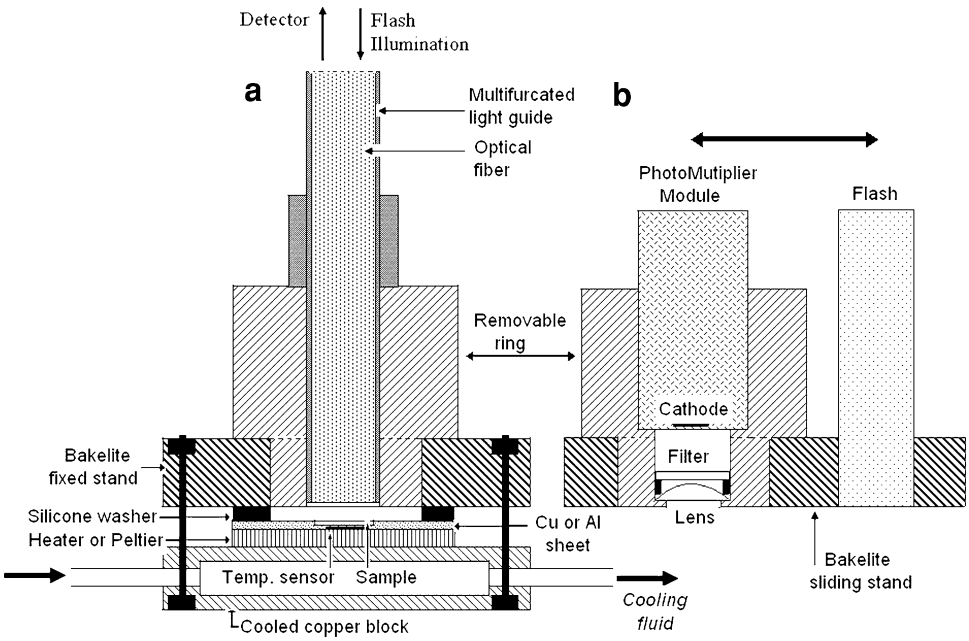

Fig. 1 Thermoluminescence measuring heads. The temperature-regulated sample holder (bottom) is similar for **a** and **b** (not shown). The cooling fluid may be water, water and antifreeze, cold gas, or liquid nitrogen for fast cooling. The sample cuvette is for liquid suspensions. Leaf discs are placed on a flat surface with a washer pressing on the edges, its central hole defining the measuring area. **a** For samples with high TL emission (leaves), a mutifurcated light guide conveys both exciting light from flash or lamp toward the sample and the luminescence toward the detector. Sample can be accessed by removing the light guide holder. **b** For samples with low TL emission (cyanobacteria), a lens (aspheric) focuses the emitted light on the detector surface through an optional red filter (RG665). The sliding stand allows to place alternatively the flash and the detector in front of the sample

A flexible regulation, allowing fast temperature changes, must be synchronized with complex illumination pattern(s) (Table 2). Liquid nitrogen, in a Dewar flask beneath the sample holder, is classically used for cooling while warming is performed by an electrically insulated resistor. Several set-ups dedicated to photosynthetic TL have been described. A mobile and vertical sample metal blade bearing a cuvette containing the sample (see e.g. Ichikawa et al. 1975; Ducruet and Miranda 1992) can be pushed up and down, allowing a fast cooling; this arrangement may, however, generate a temperature gradient in the cuvette, which can be alleviated by inserting a short intermediary metal plate between the longer cooling blade and the sample. Alternatively, a fixed and horizontal sample holder on top of a copper rod dipping in liquid nitrogen (Vass et al. 1981) provides a better temperature homogeneity at the expense of a slower cooling. The fast cooling problem can be solved by using a pressurized tank for liquid nitrogen (Fig. 1), with some cold gas in the sample holder for temperature regulation and rapid injection of gas or liquid nitrogen by activating a solenoid switch for fast cooling (Prasil et al. 1996; Noguchi et al. 2002).

The availability of powerful thermoelectric elements (typically 15 V, 5A, 75 W), in which an electric current causes a cooling on one face and warming on the other face (Peltier effect), offers another way to regulate temperature. One of the faces must be temperature-controlled by a fan or, more efficiently, by water flowing through a copper block (Miranda and Ducruet 1995a; Andree et al. 1998; Gilbert et al. 2004) or a copper bar with the bottom immersed in ice or liquid N_2 (Scott-Townsend et al. 1998). Cooling or warming on the opposite, sample-bearing face can be achieved by inverting the current; the Peltier and Ohm effects contribute differently to cooling and warming; excessive current intensities should be avoided for efficient cooling. The lowest temperature attainable, by the above method, is practically about −30°C below the temperature of the water bath whereas 140 to 200°C, depending on the characteristics of the Peltier, can be reached by inverting the current intensity and stopping the water flow.

Hence, simple temperature regulation devices, using resistors or Peltier elements, are sufficient to record the A, Q, B, AG, and C photosynthetic TL bands, and even the high-temperature TL bands appearing at about 130°C. The latter band is due to thermolysis of peroxides; for such measurements, the sample holder is built with heat-resistant materials (e.g. bakelite).

Temperature is regulated by providing variable current (or voltage) to the resistor heater or to the Peltier element. In the case of a Peltier element, heating or cooling is selected by a current inverter triggered by the computer (Miranda and Ducruet 1995a) or by a comparator to which the theoretical temperature is transmitted by the computer (Scott-Townsend et al. 1998). For an efficient temperature regulation, the

Table 2 Characteristics of the main components of a TL setup

	Functions	Characteristics	Remarks
PMT module	Thermoluminescence emission	Red-sensitive PMT with built-in high voltage and transimpedance amplifier	For example, Hamamatsu H5701-50 (10 V/μA 2 kHz), +15/−15 V power supply
Heaters	Temperature control	Electrically insulated resistors,	For example, Thermocoax
		Peltier elements	For example, Marlow XLT single-stage
Analog to Digital converter A/D ≥12 bits	Temperature measurement	1 kHz, ≤ 100 μV/bit	Temperature signal linearized in mV/°C
	Thermoluminescence signal	10 Hz, ≤ 100 μV/bit	PMT → current to voltage amplifier
	Other signal source	Depends on source	e.g., for Chl. fluor. or P700 kinetics
Digital to Analog converter D/A ≥12 bits	Proportional temperature regulation	0 to +5 V or 0 to +10 V	PID or set temperature for Comparator (Peltier)
	PMT voltage	0 to +5 V or 0 to +10 V	Modules with built-in high voltage
	Intensity of light source	0 to +5 V or 0 to +10 V	
Digital output (TTL)	Power enabling for heater/Peltier	0 V (low) or +5 V (high)	TTL can trigger directly solid-state relays
	Detector shutter		
	Flash		
	Light source 1		
	Light source 2		
	Current inverter (Peltier)		
	Water flow for cell holder or Injection of liquid N2 from tank		

Digital output: voltage pulse from 0 to 5 V (TTL). Proportional temperature regulation (PID) by a variable power supply to the heater

PID Proportional integral derivative, *PMT* Photomultiplier tube, *TTL* Transistor–transistor logic

temperature sensor (thermocouple, thermistor, platinum wire) must respond fast and be located close to the heater. Fast-responding thermocouples and analog converters should be preferred to slower digital thermometers. The temperature within the sample can be checked by a second thermocouple or by an infra red (IR) thermometer for measurements on leaves, but this raises technical problems and the result can be misleading. We note that measuring temperature between a leaf disc and the heating plate is more reliable than measuring it above the leaf in contact with air. Our major goal is in getting signal reproducibility, within about ±1°C. Using a small sample volume in close contact with the heater/thermometer element, through a thin copper or aluminium sheet, and warming rates not above 0.5°C/s allow for temperature equilibration: then, the temperature in the sample is approximately the same as that of the heating plate, which can be taken as a reference. Leaves are gently pressed against the warming plate by a washer, taking care that no protruding vein breaks the thermal contact; this is done by placing a drop of water or vaseline or paraffin oil underneath it. We note that silicone and glycerol are not as good for this purpose for leaves, as judged from long-term symptoms (G Cornic, personal communication).

A critical step is the solid to liquid transition, when warming from temperatures below freezing of water to higher temperatures; it produces artifacts since the Q band appears as two bands, one peaking below and the other above 0°C, or there is an apparent up-shift of the Q band to higher temperatures. This problem can be alleviated by (i) using concentrated samples with a small volume of water, or preferably vaseline to insure a thermal contact when working with leaves; (ii) overheating around 0°C to speed up the melting of ice; (iii) adding to buffer a compound, such as glycerol, which decreases and broadens the ice melting temperature range; however, possible effects on the charge recombination must be also considered in evaluating these data (Krieger et al. 1998).

It is worth remembering that frozen sucrose-containing buffers emit white light flashes (triboluminescence) that appear as huge spikes on the TL signal.

Experimental procedures

Light excitation and flash sequences

Illumination of photosynthetic material rapidly causes an equilibration of S_0 to S_3 states and of Q_B and Q_B^-. In the dark, S_3 and S_2 states are converted to S_1, resulting in a dark-stable 75% S_1 25% S_0 distribution, with some exceptions in cyanobacteria, where stable fractions of S_2 and S_3 could be present even after long dark adaptation (Bader et al. 1983).

The Q_B^-/Q_B ratio is close to 50% after short (minutes) dark adaptation. It decreases only slightly in intact organisms even after long (hours) dark adaptation (about 40% Q_B^- in leaves; Rutherford et al. 1984a; also see Rutherford et al. 1982 for isolated thylakoids). However, in isolated thylakoids or PS II particles the Q_B^-/Q_B ratio decreases further during prolonged storage in the dark (Demeter and Vass 1984), and can be decreased to almost zero by preincubation with oxidants, such as ferricyanide (see Rutherford et al. 1984b). Continuous illumination, however, induces other changes, depending on light intensity and spectrum: (i) a reduction of the plastoquinone pool, which may extend to an overeduction of the plastoquinone acceptors (Q_A and Q_B) under high light intensities (Vass et al. 1992); (ii) a proton uptake into the lumen favoring slightly the $S_2 \rightarrow S_1$ and more the $S_3 \rightarrow S_2$ transitions (Lavergne and Jünge 1992), producing thereby a downshift of the B bands toward lower temperatures, unless the system is uncoupled either by a chemical or by freezing; (iii) high light intensities cause photoinhibitory damage leading to the loss of functional PS II centers, as well as modification of the acceptor side of PS II, which can explain the loss of Q and B bands (Vass et al. 1988), as well as the shift of the B band to lower temperatures (Ohad et al. 1990).

A major tool in TL studies is the use of single turn-over flashes, powerful and short enough (typically <5 μs) to induce one, and only one, charge separation in every PS II center. Flash saturation can be checked by the intensity of the Q band (Table 1) that should be maximum after the first flash (not increasing after 2 flashes). Sequences of 1, 2, 3 et seq single turn-over flashes, followed by TL recording, however, result in B bands oscillating with a period of 4, with a maximum at the second flash, which generates 25% S_2 and 75% S_3, e.g. 100% luminescence-emitting states, starting from the dark-stable S_0 and S_1 states (Fig. 2). However, this happens only when the Q_B^-/Q_B ratio is close to 50:50%, otherwise the oscillations with a period 2 of the Q_B side must be taken into account by calculating the proportion of luminescence-emitting pairs $S_{2/3}Q_B^-$ at every flash sequence (Rutherford et al. 1982; Demeter and Vass 1984). The Q_B^-/Q_B ratio can be experimentally determined by comparing the B band intensity induced by flashes with or without a prior illumination of the sample at 77 K, which inverts the ratio by electron transfer from cyt b559 (Rutherford et al. 1982; 1984a). Discrepancies between actual and calculated oscillations may be ascribed to a higher yield of TL from $S_3Q_B^-$ as compared to $S_2Q_B^-$ (Rutherford et al. 1984b).

Almost 100% of PS II centers in the S_1 state can be generated by one preflash producing S_1 and S_2, followed by a dark period at 20°C to allow the relaxation of S_2 into S_1. Then 1 or 2 flashes generate S_2 or S_3, respectively, in all

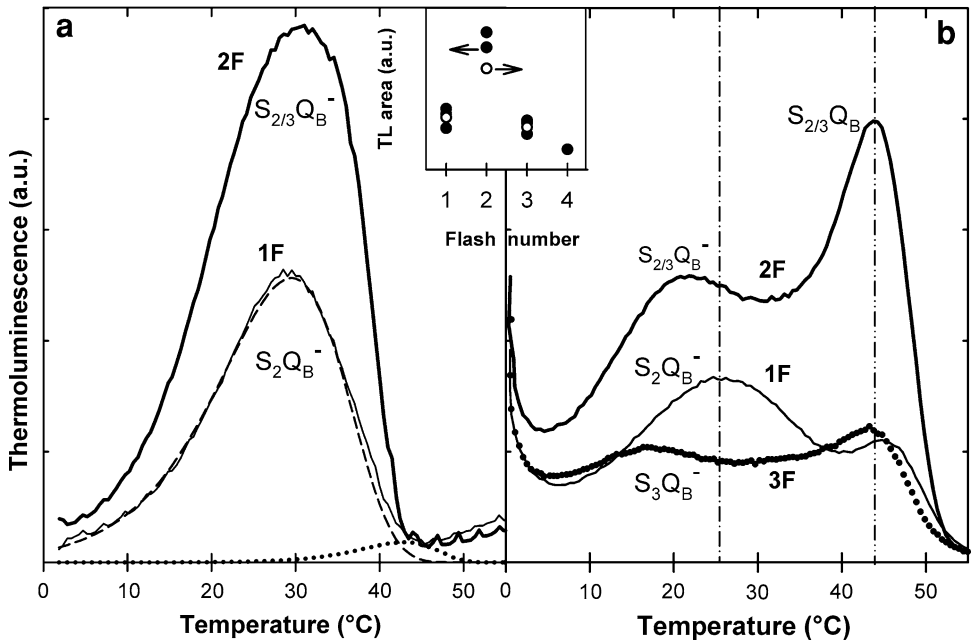

Fig. 2 Thermoluminescence emission from *Arabidopsis thaliana* unfrozen leaves. 1 flash (*thin line*) generates 75% S_2 and 25% S_1 (only S_2 and S_3 produce TL, not S_1), 2 flashes (*thick lines*) 25% S_2 and 75% S_3, 3 flashes (*dots*) 25% S_3. **a** Control plants. After 1 flash, the TL B band corresponds to $S_2Q_B^-$ (B_2, see Table 1) and is well fitted by one component. After 2 flashes, 3 components, $S_2Q_B^-$ (B_1), $S_3Q_B^-$ (B_2) and a residual one, are needed (not shown). **b** Mildly dehydrated plants. The B band is downshifted, at a greater extent for S_3 than for S_2, indicating a dark-stable acidic pH of lumen. The 45°C band (afterglow) results from a heat-induced electron transfer from stroma reductants to Q_B in the $S_{2/3}Q_B$ centers, enabling them to emit luminescence: its increase indicates a strong assimilatory potential NADPH + ATP (see Ducruet 2003). Insert: integrated TL area according to the number of flashes; *closed symbols*: Control (**a**); *open symbols*: Dehydrated (**b**). (M. Roman and J. M. Ducruet, unpublished, 1998)

the PS II centers. Dark relaxation of S_2 occurs within 2 or 3 min, when we have $S_2Q_B^-$ but requires about 10 min when we have S_2Q_B (Rutherford et al. 1984c).

Xenon flashes, currently used in many experiments, have the drawback of having a long lasting light glow, or "flash tail", which generates two charge separations in some PS II centers (double hits). Laser flashes can reduce, albeit not suppress, double hits. A more affordable solution is now possible with LEDs (light emitting diodes) that are becoming powerful enough to saturate all PS II centers by high intensity 5–10 μs square pulses.

Effects of warming rate

The apparent amplitude of the TL signal increases with the warming rate. However, what matters is the integrated intensity (amplitude × recording time), corresponding to the total number of emitted photons, so that a higher amplitude at a high warming rate is automatically compensated by a shorter recording time per temperature unit. However, the integrated TL intensity is not constant for photosynthetic TL bands; instead, it increases with increasing heating rate (Vass and Demeter 1984; Cser and Vass 2007). This effect arises from the contribution of non-radiative temperature-insensitive recombination pathways (tunneling), which take a greater share at slower warming rates where the temperature dependent recombination rates are small. Indeed, the integrated intensity increases with the warming rate until it reaches a plateau at about 20°C/min (0.33°C/s) for B bands, 25°C/min (0.4°C/s) for Q bands in spinach choloroplasts (Vass and Demeter 1984); however, the integrated intensity keeps increasing in the cyanoacterium *Synechocystis* 6803 up to 60°C/min (1°C/s) (Cser and Vass 2007). Warming rates from 20°C/min to 30°C/min (0.3°C/s to 0.5°C/s) are optimal to resolve photosynthetic TL bands.

Thermoluminescence bands are shifted toward lower temperatures when the warming rate is decreased, due to a simple mechanistic effect. A TL band results from two factors, which change in opposite directions when the temperature increases: (i) an increased rate of recombination, i.e., leading to increased number of photons emitted; and (ii) a decreased number of charge pairs available for recombination. At slower warming rates, more time elapses during the same temperature increase allowing more recombination events to occur, so that the charge pairs are eliminated faster and the TL maximum is reached at lower temperatures. As a consequence, the warming rates must be taken into account, when comparing published T_M of TL bands.

Effects of pH

In isolated thylakoid membranes or PS II particles, the observed TL curve is highly dependent on the pH of the buffer. At increasing pH from 6.5 to 8.5, the Q band due to $S_2Q_A^-$ is upshifted from about +2°C to 25°C, the $S_2Q_B^-$ band is downshifted from 40°C to 30°C and the S_3 Q_B^- stays always around 30°C (Rutherford et al. 1982; Vass and Inoue 1986). These pH effects can be explained by proton uptake from the lumen for $S_2 + 0.5\ H^+ \rightarrow S_1$ and $S_3 + H^+ \rightarrow S_2$ (Lavergne and Jünge 1992) or proton release in the stroma for $Q_B^- \rightarrow Q_B + H^+$ and $Q_A^- \rightarrow Q_A$. In intact chloroplasts, cells or leaves, the pH of stroma is buffered around 7.5 (Held et al. 1973), but the pH of lumen is decreased to 5.0–5.5 due to proton pumping. This effect favors the reverse transition of the S states, hence faster recombination rate (Joliot and Joliot 1980), resulting in a downshift, larger for the B1 band ($S_3Q_B^-$) than for the B2 band ($S_2Q_B^-$) (Miranda and Ducruet 1995a, b; Fig. 2b). Therefore, the downshifted position of the $S_3Q_B^-$ B1 band induced by 2 or 3 flashes in cells or leaves is an indicator of the dark stable ΔpH.

Effects of freezing

Freezing in intact cells occurs below 0°C. In progressively cooled leaves, water stays in a supercooled liquid state until it rapidly freezes, at a nucleation temperature indicated by a brief temperature rise, generally around −5°C. Formation of ice crystals disrupts thylakoid membranes, producing an uncoupling, which corresponds to a relaxation of ΔpH effects on the TL signal, i.e., the B band downshifts and the "afterglow" emission occurs due to a heat-induced electron transfer to Q_B in $S_{2/3}Q_B$ centers (Miranda and Ducruet 1995a; Ducruet et al. 2005; Fig. 2b). This is the first effect of freezing that can be used as a tool to uncouple thylakoids, without infiltrating leaves with uncouplers, or in some poorly permeable algal cells.

However, freezing may also produce gross signal distortions in certain plant species (Ducruet et al. 1998; Homann 1999; Janda et al. 2004). This happens mainly in species rich in polyphenolic compounds (e.g., in grapevine) that are sequestered in the vacuoles and released by frost-induced membrane disruption, not in species selected for low bitterness (e.g., spinach, pea, lettuce).

Signal analysis

Charge recombination is an electron transport reaction, which can be described by the Marcus theory (Marcus and Sutin 1985). A simplified version of the rate equation is given by the Eyring formula:

$$k(T) = k_B/h\kappa T \exp\left(-\Delta G^{\#}/k_B T\right),$$

where k_B is the Boltzmann's constant, h is the Planck's constant, κ is the so called transmission factor, T is the temperature, and $\Delta G^{\#}$ is the free energy of activation which represents the height of the activation barrier. By separating $\Delta G^{\#}$ into its enthalpy ($\Delta H^{\#}$) and entropy ($\Delta S^{\#}$) components ($\Delta G^{\#} = \Delta H^{\#} - T\ \Delta S^{\#}$) and replacing $\Delta H^{\#}$ with the more commonly used E_a activation energy one obtains:

$$k(T) = sT\exp(-E_a/k_B T),$$

where s is the so called pre-exponential factor, $s = k_B/h\kappa \exp(\Delta S^{\#}/k_B)$.

Thermoluminescence from a stable charge separated state arises via a series of thermally activated equilibrium reactions among charge separation intermediates ($P^+Phe^- \leftrightarrow P^+Q_A^- \leftrightarrow Tyr\text{-}Z^+Q_A^- \leftrightarrow S_iQ_A^- \leftrightarrow S_iQ_B^-$) until the actual charge recombination ($P^+Phe^- \leftrightarrow P^*$) occurs. Kinetic description of these events is largely complicated by the reversibility of each step in the process, as well as by the possibility of direct non-radiative charge recombination to the ground state from each of the charge separation states. The situation can be simplified by some critical assumptions: (i) the series of equilibrium reactions between the stable charge separated state and P* is replaced by a single step process, whose activation parameters are additive; (ii) non-radiative destabilization of the charge separated state by forward electron transport and direct charge recombination can be neglected; (iii) the probability of retrapping, i.e., formation of the original charge separated state once the recombination reaches P*, is negligible. With these assumptions the kinetic equation is simplified to the so called first order case (Randall and Wilkins 1945),

$$I(t) = -c\mathrm{d}N/\mathrm{d}t = ck(T)N(t),$$

where $N(t)$ is the concentration of the charge separated state, $I(t)$ is the intensity of luminescence, and c is a constant factor. By using this first order kinetic assumption together with the Eyring formula, the shape of the TL curve can be obtained analytically (Vass et al. 1981), which describes a non-symmetric bell shaped curve and can be used to resolve a complex TL curve into components. [For theories on the origin of TL, see e.g., Vass et al. (1981); DeVault et al. (1983); DeVault and Govindjee (1990); Rappaport et al. (2005); Cser and Vass (2007); review of Rappaport and Lavergne (this issue); cf. application of a theory by Rose et al. (2008), which combines the ideas of DeVault and Govindjee (1990) and those of Rappaport et al. (2005).]

Instead of using an analytical solution of the kinetic equation, numeric methods can also be used. A numerical simulation of a TL band (Ducruet and Miranda 1992; Ducruet 2003) consists of calculating iteratively for each

sampling step at temperature T (K) the luminescence (or photons) $L(T)$ emitted during the time interval Δt, then subtracting it from N, the number of charge pairs remaining available for recombination (N_0 at start corresponds to the band area):

$$I(T) = NsT\Delta t\exp(-E_a/k_BT),$$

where E_a is activation energy (enthalpy), and k_B is Boltzmann constant.

Curve analysis, by both analytical and numerical methods, provides the three characteristic parameters of a TL band (component): the integrated area (N_0), the apparent activation energy (E_a) and a pre-exponential factor (s). From the pre-exponential factor an apparent activation entropy ($\Delta S^{\#} - k_B\ln(\kappa)$) can be calculated. However, the value of κ is usually <1, which leads to overestimation of $\Delta S^{\#}$. Further complication arises from the non negligible effect of retrapping and non-radiative pathways (Vass et al. 1981; Rappaport et al. 2005; Rappaport and Lavergne, this issue). In spite of the limitations imposed by the above assumptions, the shape of TL curves can be described reasonably well with the first order kinetic function in case of isolated thylakoids (Vass et al. 1981), as well as in intact systems, e.g. leaves in the absence of a dark-stable proton gradient (Ducruet and Miranda 1992; Fig. 2a), for the B band, albeit not for the Q band (Tyystjärvi and Vass 2004; Rappaport et al. 2005).

Potential users of TL analysis must be made aware of the following limitations: (i) in cyanobacteria, the shape of TL curves usually differs from that expected from the first order kinetic model. Further, even in these cases, TL curves can not be fitted with Gaussian components, as attempted in some cases in the literature, since none of the kinetic models leads to Gaussian shapes; (ii) estimation of $\Delta S^{\#}$ from the pre-exponential factor is limited by unknown contributions arising from $\kappa < 1$, the presence of non-radiative processes, and of non first order kinetics, from the initial concentration of the charge separated state; (iii) the presence of non-radiative charge recombination reactions decrease TL intensity (Rappaport et al. 2005; Cser and Vass 2007). Therefore, comparison of TL intensity (of Q and B bands) measured in different species must be made only after normalization to the same amount of active PS II centers, e.g., by using the initial amplitude of flash induced chlorophyll fluorescence arising from the $S_2Q_A^-$ state (Cser et al. 2008). However, TL intensity can be used to quantify active PS II under different experimental conditions in the same species or the same type of preparation.

Luminescence (see also delayed light emission, i.e., modulated luminescence, also called delayed fluorescence in the literature, Goltsev et al. this issue), and thermoluminescence (which is preferred to luminescence decay at constant temperature for its greater resolving power) have played a major role along with chlorophyll fluorescence (Papageorgiou and Govindjee 2004) in exploration of Photosystem II electron transport processes. These studies have resulted in a detailed characterization of charge stabilization on PS II electron carriers in the past. Thermoluminescence remains an essential tool in this domain, especially for studying mutants, with the advantage of being applicable at various integration levels, from PS II particles to whole cells (algae, protoplasts) or leaves. Beyond PS II, as a complement to chlorophyll fluorescence, thermoluminescence is an intrinsic probe of the photosynthetic metabolism, providing original information about the dark-stable lumen pH, the influx of electrons from stroma toward the intersystem chain and plastoquinones, the cyclic/chlororespiratory pathways, and the NADPH + ATP assimilatory potential. Provided that sufficient care is taken of the experimental conditions, thermoluminescence, among other optical methods, provides specific information regarding the metabolism of integrated photosynthetic systems.

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