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Thermoluminescence from Photosynthetic Membranes

P V SANE, T S DESAI* and V G TATAKE*
National Botanical Research Institute, Lucknow 226 001
**Bhabha Atomic Research Centre, Trombay, Bomay 400 085*

The continuous illumination and flashing light studies have established relationship between thermoluminescene, "S_i" states and redox pairs responsible for luminescene. Tl can be used for *in situ* measurement as a probe of PS II photochemistry. It can be conveniently used for studying temperature dependence of H_2O oxidation, deactivation of "S_i" states, herbicide effects, function of ADRY reagents and electron transport in thermophilic organisms. The relationship between Tl peaks and components of delayed light emission should enable us to study PS II photochemistry at room temperature under field conditions simply by studying the components of delayed light emission.

KEy Words : Thermoluminescence, Photosynthetic membrane, Delayed light emission

Introduction

The measurement of light emission or luminescence in photosynthetic organisms provides valuable information about the functioning of the photosynthetic membranes. Luminescence is generated by charge recombination primarily in photosystem (PS) II but also in PS I. the microsecond or faster components reflect information on the charge movement within or close to the reaction centre. The slower components arise as a result of a back-flow of electrons from somewhat distant components of the photosynthetic apparatus (See Lavorel et al. 1986). The slow components of delayed light can be conveniently examined by a study of glow curves or thermoluminescence. As will be shown later a one to one correspondence between glow peaks and components of delayed light emission has been convincingly demonstrated. A large body of data gathered so far on thermoluminescence from photosynthetic membranes have enabled us to identify the charge pairs responsible for luminescence.

In this presentation, we provide a brief summary of research work on thermoluminescence with emphasis on the work done by our group.

Materials and Methods

In principle for a study of glow peaks, what is done is that a photosynthetically active preparation is illuminated to induce charge movement at a specified temperature. The sample is then quickly cooled to low temperature to freeze the redox states of the components as also the conformation change acquired by the membrane. The sample is then heated gradually, preferably, at a predetermined rate of heating. As the temperature rises the electrons and holes trapped in the redox components start moving permitting back reactions and finally recombinations to emit light by reexcitation of the chlorophyll molecule. The emitted light which is spectrally similar to chlorophyll fluorescence is detected by an appropriate detector. A plot of the intensity of light emitted as a function of temperature yields glow curves. A few variations of this basic system for obtaining glow curves have been used by different investigators (Sane & Rutherford 1986).

Results and Discussion

Nomenclature: Figure 1 gives an idealized thermoluminescence curve from photosynthetic membranes. A total of 8 peaks appearing at −160, −70 (variable), −37, −12, +10, +25, +48, and +70 °C could be seen. These have been designated as Z, Zv, I, II, III, IV, V and VI respectively. All the peaks cannot be observed in one preparation but using appropriate conditions, each of the peaks can be seen distinctly (see Desai et al. 1975, Sane et al. 1977, Tatake et al. 1980, Sane et al. 1984, Desai et al. 1982).

Origins of different peaks: Of the 8 peaks, the Z originates in chlorophylls but is unrelated to photosynthetic electron transfer (Arnold & Azzi 1968, Shuvalov & Litvin 1969, Sane et al. 1974).

The Zv band is peculiar in many respects (See Ichikawa et al. 1975) and its independent identity was questioned by Desai et al. (1977). The data in the literature are confusing but Demeter et al. (1985) suggested that it may result from a recombination between Q_A^- and an unknown donor D_Z^+.

The peak VI is unrelated to the functional electron transport and has been related to chemiluminescence (Desai et al. 1982).

The glow peaks from peak I to peak V are related to

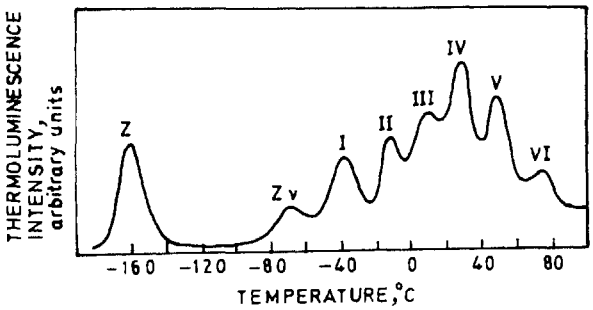

Figure 1 An idealized thermoluminescence curve from plant photosynthetic membranes

the electron transport and functional photosynthetic membrane. The precise species involved in the recombination have been worked out. What follows is a summary of the approaches used and the conclusions that can be drawn from the results obtained. The nomenclature used for components of electron transport is identical to that commonly used by others.

In order to identify the redox components responsible for the generation of the glow peaks,two approaches have been used. In one approach chemicals that act as inhibitors of electron transport at specific sites are used to block the electron flow beyond a certain component. This permits accumulation of reducing equivalents on certain components. Simultaneously the positive charges located on the "S" or water-oxidizing complex could be calculated. On this basis the components associated in a back reaction leading to luminescence can be identified. Similarly using either far red light or red light one can isolate reactions of PS I and PS II. Advantage can also be taken of the purified PS I and PS II fractions. These approaches have been used very effectively by us.

Another approach which provides somewhat more precise information about the positive charges on the "S" state and negative charges on the acceptors of PS II viz., Q_A, Q_B relates to the use of the short duration intense flashes. This approach has been used by Inoue et al. (Inoue & Shibata 1982) and Rutherford et al. (Sane & Rutherford 1986). The two approaches together have yielded rich information on the origin of each of the glow peaks.

The effect of DCMU, DCCD, CCCP, tris washing and TNM on the pattern of glow curves is presented in figures 2–5. The temperatures have been given in K and their equivalent have been stated in table 1. The characteristics of the treatments used are as follows:

DCMU: Blocks electron flow between Q_A and Q_B; permits only one turnover of PS II in its presence thus creating S_2 and Q_a^- when a relaxed sample is illuminated in its presence.

Table 1 *Characteristics of glow peaks related to photosynthetic electron transport*

Peak	Temp.	Observations	Possible origin
I	−37°C (236K)	Sensitive to DCMU; Unaffected by DCCD, CCCP, TNM and Tris treatment; "S" states not involved; not excited by far red; present in a PS II fraction but not in PS I.	$Z^+ Q_B$
II	−12°C (261K)	DCMU enhances; Not sensitive to DCCD; Inhibited by TNM, CCCP and Tris washing; "S" state necessary; not excited by far-red, present in PS II fraction.	$S_2Q_A^-$ S_2 because DCMU can permit only one turnover hence S_1 of the relaxed sample can become S_2 but not S_3 or S_4
III	+10°C (283K)	Sensitive to DCMU; DCCD enhances; TNM and tris inhibit, not excited by far red; present in PS II fraction	$S_3Q_B^{2-}$
IV	+25°C (298K)	Abolished by DCMU; inhibited by DCCD; TNM and Tris completely inhibit the peak, CCCP also inbibits; present in a PS II fraction.	S_2Q_B
V	48°C (321K)	Enhanced by DCMU; unaffected by DCMU, TNM, CCCP or Tris; present in a PS I fraction; excited by far-red light.	Arises in PS I

DCCD: In uncoupled chloroplasts blocks reduction of the pool of plastoquinone but permits reduction of Q_B.

CCCP: Accelerates deactivation of the S states and hence does not permit build up of S_2, S_3, S_4, etc.

TNM: Blocks functioning of the water oxidizing complex and hence build up of S_2, S_3, S_4, etc. is not possible.

Tris: Same as TNM.

Studies using far-red excitation and isolated PS I and PS II fractions showed that peak V appearing at 321 K (48°C) arises exclusively in PS I whereas for the appearance of peaks I, II, III and IV, electron transport in PS II is essential (Sane et al. 1977).

In table 1 a summary of observations using chemicals and preferential excitation is presented together with the

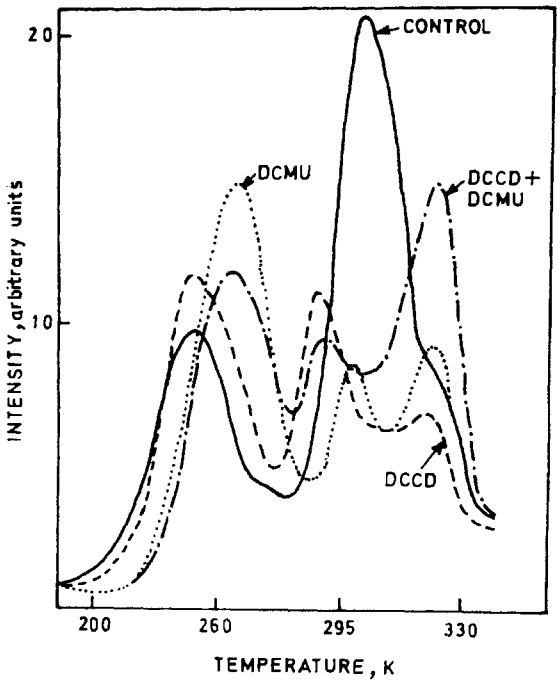

Figure 2 Glow curve pattern of spinach chloroplasts in the presence of different compounds. Control (———); Dicyclohexylcarbodimide (DCCD), 1 mM (---); 3-(3,4, dichlorophenyl)-1, 1-dimethyl urea (DCMU), 10 uM (:....): DCCD, 1 mM + DCMU, 10μM (—●—●—●—)

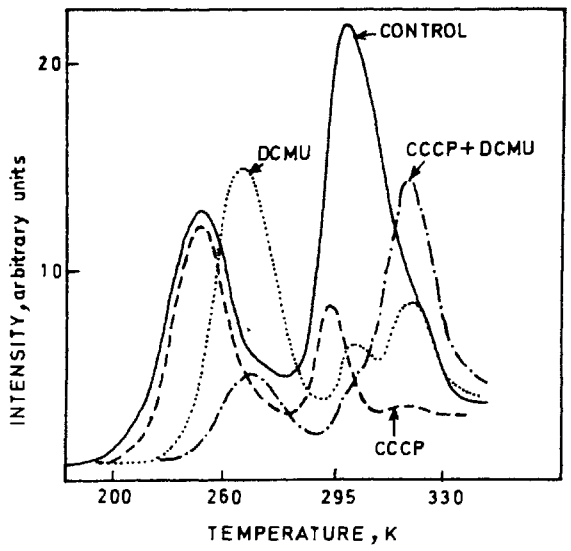

Figure 3 Glow curves of spinach chloroplasts in the presence of different compounds. Control (———); carbonyl cyanide- m-chlorophenlhydrazone (CCCP), 20 μM (----); DCMU, 0.1 mM (....); CCCP, 20 μM + DCMU, 0.1 mM (—●—●—●—)

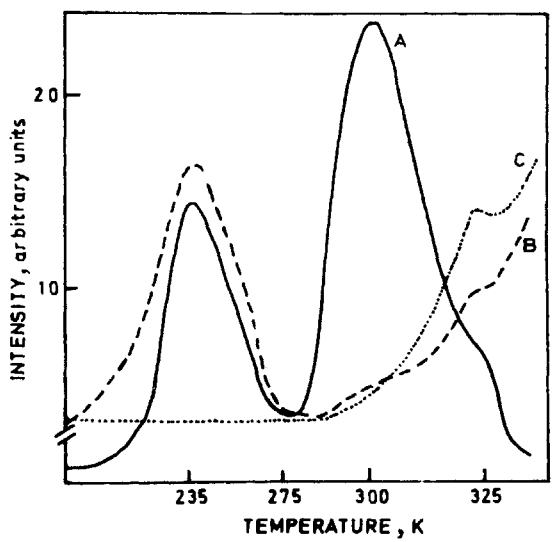

Figure 4 Glow curves of spinach chloroplasts. A-control; B-tris treated; and C- tris treated + DCMU (10μM)

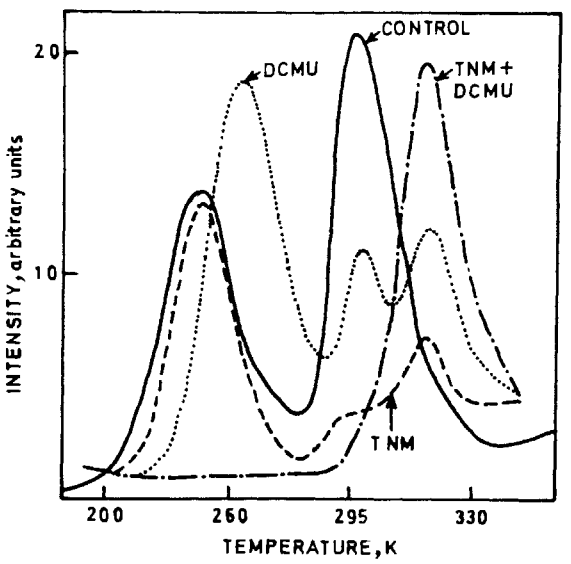

Figure 5 Glow curves of spinach chloroplasts in the presence of different compounds. TNM-tetranitromethane

most logical conclusions concerning the origins of peaks I to V.

The observations made by using flashing light have enabled Inoue and his coworkers (Inoue & Shibata 1982) and Demeter and his associates (Sane & Rutherford 1986) to identify the precise "S" state and the component which provides an electron for recombination. The peak IV which is a dominant peak in isolated chloroplasts was observed to oscillate with a period of four (Inoue & Shibata 1978a, b). The maxima occurred after the 2nd, 6th, 10th, etc. flashes. From this and subsequent work by Rutherford et al. (Sane & Rutherford 1986) it appears that S_2/S_3 may be involved in the recombination reaction with Q_B^-. Similarly peak II could be demonstrated to be due to $S_2Q_A^-$ as in the presence of DCMU it oscillates. The $S_3Q_A^-$ recombination cannot be ruled out as the peak maxima coincides with S_2 and S_3.

There is some confusion regarding the involvement of "S" state in generating peak V. Demeter et al. (1984) observed oscillations related to S_0/S_1 state when DCMU is added subsequent to excitation. Since DCMU induces this peak, Q_A^- is likely to be the most logical candidate recombining with S_0/S_1. Sane and Rutherford (1986) have attempted to explain these results in comparison to those obtained by inhibitor studies.

Relationship with delayed light emission: Delayed light emission (DLE) has long been known to show oscillations. There are several reasons to suggest that both thermoluminescence and DLE may be related and in fact DLE could be an expression of thermoluminiscence. The earlier work of Shuvalov and Litvin (1969) provided first evidence of such a relationship. Our work (Desai et al. 1982) on different photosynthetic material showed that a plot of delayed light intensity observed 2.5 seconds after excitation at different temperatures (figure 6) mimics the pattern of glow peaks. Work involving flashing light by Rutherford et al. (1984) provided further evidence for relating DLE with thermoluminescence. In fact, an analysis of glow peaks to determine activation energies, frequency factors and life times of the states responsible for glow peaks by Tatake et al. (1980) showed that different components of DLE could be an expression of different glow peaks. Using various conditions, inhibitors and different treatments Sane and his coworkers identified different components of delayed light responsible for each of the glow peaks (Rane & Sane 1985). Table 2 shows the relationship between the glow peaks and components of delayed light emission.

This relationship should permit us to obtain information about the functioning of PS II photochemistry merely by studying the decay of delayed light at room temperature.

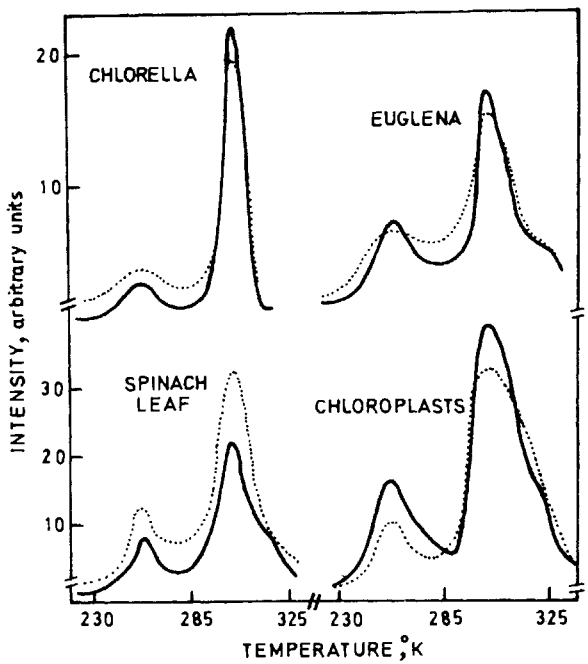

Figure 6 Comparison of T1 curves (———) with delayed light emission (....) observed in different photosynthetic materials. The delayed light emission pattern was obtained by plotting the intensity of delayed light emission observed 2.5 sec after excitation at different temperatures

Table 2. *Relationshio of different glow peaks with the components of delayed light decaying with different life times*

Peak	Origin	Life time ($t_{1/2}$) of equivalent delayed light component at	
		23°C	3°C
II	$S_2Q_A^-$	6 sec	12 sec
III	$S_3Q_B^{2-}$ or $S_3Q_B^-$	35 sec	80 sec
IV	$S_2Q_A^-$	40 sec	90 sec
V	PS I ? or $S_1Q_A^-$?	50 sec	115 sec

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