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# IDENTIFICATION OF FAR-RED-INDUCED RELATIVE INCREASE IN THE DECAY OF DELAYED LIGHT EMISSION FROM PHOTOSYNTHETIC MEMBRANES WITH THERMOLUMINESCENCE PEAK V APPEARING AT 321 K

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**The relative increase in the decay of delayed light emission from photosynthetic membranes following far-red light excitation is shown to be related to thermoluminescence peak V appearing at 321 K. Peak V and the relative increase in the decay of delayed light emission show parallel changes in their intensities following far-red excitation.**

The delayed light emission from photosynthetic membranes [1] has been shown to consist of several components [2–5]. Those components that decay faster have been well characterized [6–8] as they provide information about the fate of energy absorbed by photosynthetic membranes. The study of slow components decaying in seconds or minutes has received much less attention although they probably provide information on temporary energy storage during photosynthetic electron transport. Most studies on delayed light emission suggest that it originates in a back-reaction in the PS II reaction center. However, PS I delayed light emission has also been reported [9–11].

Bertsch and Azzi [12] reported interesting studies on delayed light emission resulting from excitation of several algal cells by far-red light which preferentially excited PS I chlorophylls. It was demonstrated that a slow component of delayed light emission decaying in 0.5–60 s shows a relative maximum in the intensity; clear peaks were observed in red and blue-green algae. Further investigations by Bjorn [13] of this phenomenon showed that small amounts of phenazine methosulfate accelerated the emission of a long-lived afterglow in the 40 s range. These studies have thus shown that excitation of PS I contributes towards slowly decaying delayed light emission. A relative maximum increase in a slow component of delayed light emission was observed during developmental stages [14] and may be related to the phenomena discussed here.

Recent studies from our laboratory [15] have demonstrated the identity of slow components of delayed light emission with thermoluminescence (for a review on thermoluminescence, see Ref. 16). Considering our earlier observation [17,18] that peak V appearing at 321 K arises in PS I and can be excited by far-red light, we investigated whether the relative increase in the delayed light emission following excitation by far-red light could be related to peak V. The studies reported in this communication show that the relative increase in delayed light emission following far-red illumination is indeed associated with a postillumination

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Abbreviations: Chl, chlorophyll; PS, photosystem.

increase in the intensity of peak V. It is concluded that the buildup of peak V following far-red excitation is related to the (relative) increase in the decay of delayed light emission.

Experiments were carried out using freshly plucked leaves of the money plant (*Pothos aurea*) grown inside the building in diffuse light. A leaf disc (1.5 cm in diameter) placed in the stainless-steel planchet was irradiated at room temperature (298 K) using a 500 W tungsten lamp and the following two Schott interference filters as necessary: red, $\lambda_{T_{max}}$ 649.2 nm, $T_{\lambda_{max}}$ 39.5% and bandwidth at half maximum of 2.4 nm; far-red, $\lambda_{T_{max}}$ 710 nm, $T_{\lambda_{max}}$ 47.5% and bandwidth at half maximum of 3.4 nm. The incident intensity was saturating for both delayed light emission and thermoluminescence. The decay of delayed light emission was monitored continuously at room temperature by placing the planchet on a sample holder of the thermoluminescence measuring equipment [19]. Glow curves were recorded as described previously [17]. The excitation spectrum was obtained by illuminating the leaf disc with different wavelengths of the light from the monochromator of an Aminco-Bowman spectrophotofluorometer and by plotting the yield of relative increase in the decay of delayed light emission against wavelength of excitation. In experiments involving quenching of delayed light emission at different predetermined stages following illumination, the leaf disc was irradiated and its delayed light emission monitored. At the desired stage during the decay of delayed light emission, liquid nitrogen was poured on the sample. This immediately quenched the delayed light emission. The quenched sample was then used to measure the glow curves.

The decay patterns of delayed light emission following red and far-red excitation of money plant leaf discs are presented in Fig. 1A. When excited by far-red light the delayed light emission first decreases in intensity but after about a minute it starts rising (curve with open circles), giving a peak at about 2 min after excitation. Thereafter, the delayed light emission intensity continuously decreases. However, if the leaf is excited with red light or white light the delayed light emission intensity decreases continuously without showing a relative increase (curve with solid circles).

The far-red excitation of a fresh leaf from other plants growing in bright sunlight does not show the relative increase in delayed light emission as observed for money plant leaf grown in shade. The leaves of all the plants grown in shade, however, demonstrate the relative increase in delayed light emission following far-red excitation. The data in Fig. 1B show that a leaf of canna plant growing in full sunlight, if kept in the dark overnight, also exhibits the relative increase in delayed light emission (curve with open circles). Interestingly, a leaf plucked about an hour after sunrise from a plant growing outdoors shows a tendency to exhibit the relative increase in delayed light emission (curve with crosses) but as the leaf is exposed for longer time to sunlight this tendency is lost. Thus, a leaf plucked at noon has almost lost the ability to show this phenomenon (curve with solid circles).

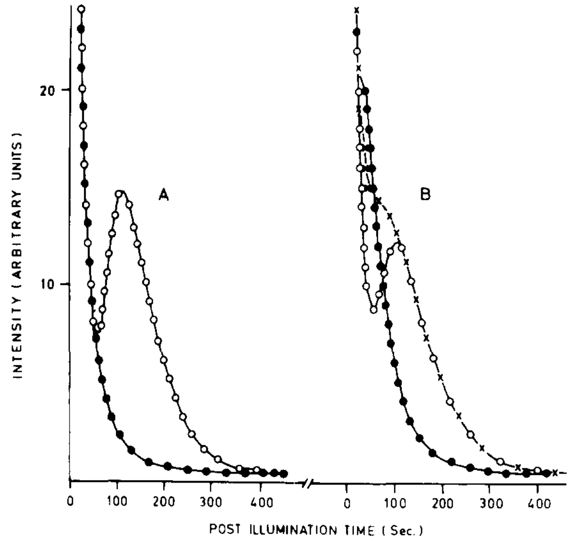


Fig. 1. Delayed light emission decay curves. (A) (●——●) Money plant (*Pothos aurea*) leaf disc excited for 2 min with a 500 W tungsten lamp with a red (650 nm) light interference filter on the sample. (○——○) Same as above except that the excitation was with a far-red (710 nm) light interference filter. (B) Canna leaf disc excited as in A with far-red light interference filter on the sample; (×——×) fresh leaf plucked 1 h after sunrise; (●——●) fresh leaf plucked at noon and studied immediately, and (○——○) the same after 24 h in darkness.

The excitation spectrum of the far-red induced increase in the decay of delayed light emission was studied using the money plant leaf. After correction for the emission characteristics of the lamp

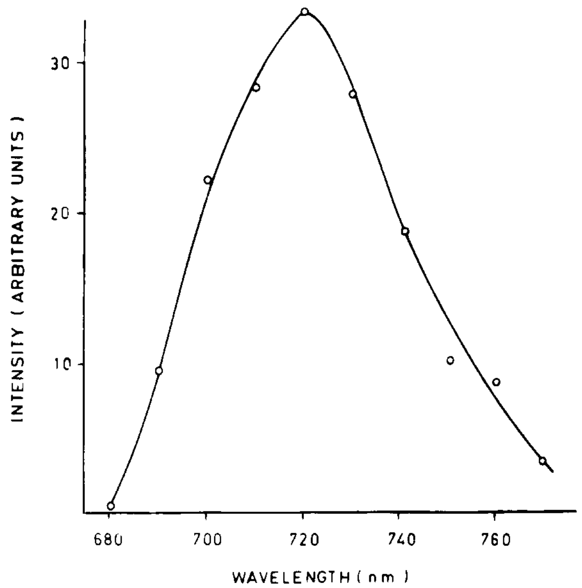


Fig. 2. Corrected excitation spectrum of the relative increase in the decay of delayed light emission.

the excitation peak was found to be around 720 nm (Fig. 2) and the spectrum shows that Chl *a* absorbing in the long-wavelength region (mostly belonging to PS I) is responsible for the phenomenon.

The relative increase in the intensity of delayed light emission can only be due to an absolute increase in the yield of one of its components. In view of the fact that glow peaks are related to the slow components of delayed light emission and peak V appearing at 321 K is preferentially excited by far-red light [17], we attempted to identify the component responsible for showing relative increase in the delayed light emission by studying glow curves and their yield at different points during the course of decay of delayed light emission. The delayed light emission of leaf after far-red excitation was monitored (Fig. 3A, inset) and then the delayed light emission was quenched at three different points (by quickly cooling three different samples to liquid nitrogen temperature): (1) 65 s

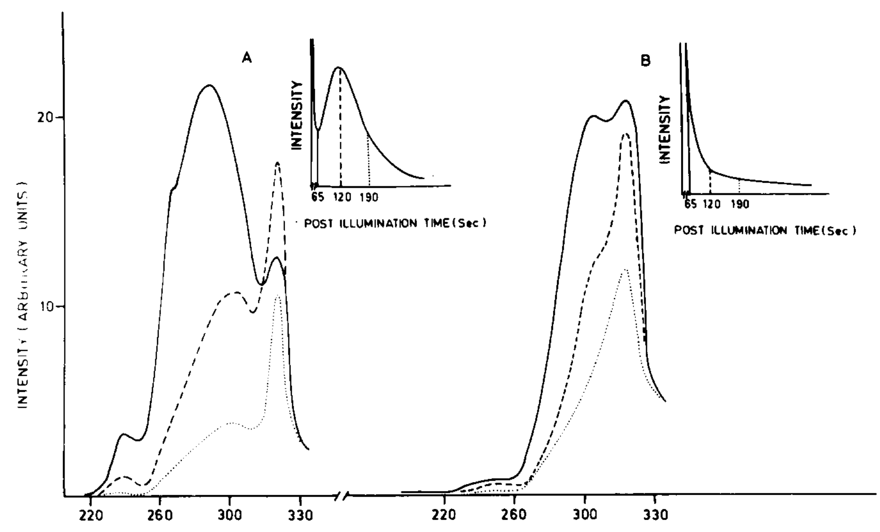


Fig. 3. Glow curves from money plant leaf discs after being cooled to 77 K at predetermined stages during the course of the decay of delayed light emission. (A) Glow curves of leaf discs cooled to 77 K at (i) 65 s (———), (ii) 120 s (- - - - -) and (iii) 190 s (· · · · · ·) following far-red light excitation. (Inset) Decay of far-red-light-induced delayed light emission. The delayed light emission was quenched by cooling the sample to 77 K. The vertical lines indicate the times after excitation at which different samples were quenched for subsequent glow curves: (———) 65 s, (- - - - -) 120 s, (· · · · · ·) 190 s. (B): Same as in A except the leaf disc was excited with red light. (Inset) Same as inset in A except that the leaf disc was excited with red light.

following excitation which corresponds to the lowest intensity of delayed light emission before it starts increasing, (2) 125 s following excitation which corresponds to the peak of relative maximum in the intensity of delayed light emission and (3) 190 s after excitation which corresponds to a point when a large part of the delayed light emission has decayed. The quenched samples were then used for obtaining glow curves (Fig. 3A). A similar experiment was repeated for a leaf exposed to red light. At 65, 125 and 190 s following excitation with red light the delayed light emission was quenched (Fig. 3B, inset) and glow curves of these samples were measured (Fig. 3B). The data presented in Fig. 3A and Table I for far-red excited sample show glow peaks appearing at 261 (II), 283 (III), 298 (IV) and 321 K (V) and that the yields of all the glow peaks except peak V decrease with time. The yield of peak V was 50% higher after 125 s than that after 65 s, and its relative contribution to the total yield continued to increase thereafter.

In the case of red light excitation (Fig. 3B), however, there was not only a decline with time in the total yield of glow peaks but the yield of each peak, including peak V, also decreased. These observations suggest that if there is a relative increase during the decay of delayed light emission following far-red excitation then only is there an increase in peak V intensity. We therefore propose that the relative increase in delayed light emission seen after far-red excitation is due to the buildup of peak V. Thus, the slow (probably the slowest) component of delayed light emission at room temperature, which is accounted for by peak V, is responsible for the relative increase in delayed light emission. In view of our theoretical calculations (Ref. 20, see also Ref. 21) this component should have a half-life equivalent to a few minutes at room temperature.

TABLE I

CHANGES IN THE YIELD OF GLOW PEAKS OBTAINED FROM SAMPLES COOLED TO 77 K DURING THE DECAY OF DELAYED LIGHT EMISSION (DATA CALCULATED FROM FIG. 3A)

Yields are expressed in arbitrary units.

| Time interval between excitation and cooling to 77 K | Total yield of all the glow peaks | Yield of peak V appearing at 321 K | Yield of peak V as a percent of total yield |
|---|---|---|---|
| 65 s | 120 | 20 | 17 |
| 125 s | 70 | 30 | 43 |
| 190 s | 26 | 14 | 50 |

The total yield of glow peaks as well as the total yield of delayed light in both far-red or red excited leaf decreases with time and they decrease quantitatively in a similar fashion as expected from the identity of delayed light emission with glow peaks suggested earlier [15].

The relative increase in the delayed light emission following excitation by far-red light but not by white or red light observed by us is similar to that reported earlier [12,13] for other materials. The new information brought out by the present studies is that this peculiar behavior is seen in leaves of plants grown in the shade or in a leaf kept overnight from a plant grown in full sunlight. The leaf of a plant growing outdoors if plucked in the morning has a tendency to show the relative increase in the delayed light emission (Fig. 1B) but the same leaf after exposure to bright daylight loses this tendency.

The presence of this phenomenon in plants grown in shade suggests that the phenomenon may be related to the organization of the thylakoid membrane. Considering the effects of far-red and red light, it is tempting to suggest a role of ATP produced during electron transport and also the 'state changes' undergone by the photosynthetic membranes by light 1 and 2 in this phenomenon. These possibilities are under investigation.

The most interesting new information presented here is that the relative increase in the decay of delayed light emission leading to a peak following far-red excitation is due to the buildup of glow peak V under these conditions. Our results also bring out the involvement of PS I Chl *a* in contributing towards delayed light and consequently to thermoluminescence. This, once again, confirms the relationship of the slow component of delayed light emission with thermoluminescence [15]. The question of why peak V builds up following only far-red excitation of leaves of a plant grown in shade needs to be answered and is currently under investigation.

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