Biochimica et Biophysica Acta, 681 (1982) 383–387
Elsevier Biomedical Press

BBA 41142

A SLOW COMPONENT OF DELAYED LIGHT EMISSION AS A FUNCTION OF TEMPERATURE MIMICS GLOW PEAKS IN PHOTOSYNTHETIC MEMBRANES

EVIDENCE FOR IDENTITY

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(Received March 2nd, 1982)

Key words: Delayed light emission: Thermoluminescence; Photosynthesis; Glow curve; (Chlorella, Euglena, Spinach)

Evidence for a correlation between a slow component of delayed light emission and thermoluminescence from photosynthetic membranes is presented. It was observed that the intensity of delayed light measured 2.5 s subsequent to illumination at different temperatures when plotted as a function of temperature reproduces the glow curve pattern. The slow component of delayed light emission is also quantitatively related to the yield of thermoluminescence, the sum of the two remaining constant.

Introduction

Energy storage associated with photosynthetic membranes during electron transport is represented by the delayed light emission. Although the delayed light emission accounts for a very small part of the total light energy lost by the photosynthetic membrane, it has proved to be an important indicator of many parameters associated with photochemical reactions [1–4]. Recent investigations on thermoluminescence from several laboratories [5–11] have suggested that the glow curves not only originate in the light-induced electron transport of both photosystems and represent energy storage by the photosynthetic membrane [5,12–15] but also share many common properties with the delayed light emission. For example, the delayed light emission and glow curve pattern are both affected by inhibitors of photosynthetic electron transport [1,2,5–8] and the oxidized states of the water-splitting complex [16–20]. This suggest that the delayed light emission and thermoluminescence may be related to each other and that some components of the delayed light emission may represent some of the glow peaks.

The possible correlation between the delayed light emission and thermoluminescence was further suggested by our recent analysis of glow curves [21]. Calculations following the theory of Randall and Wilkins [22] revealed that the lifetimes of electrons in the trapped states at their respective glow peak temperatures ranged between 35 and 50 s. This strongly indicated that the 'seconds component' of delayed light emission observable at the respective glow peak temperatures may be identical with the thermoluminescence. The evidence presented in this communication shows that a slow component (observed 2.5 s after illumination) of delayed light emission, when plotted as a function of temperature, traces a pattern that quantitatively mimics thermoluminescence (glow peaks). This establishes clearly, for the first time, the expected equivalence of the two phenomena.

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Abbreviation: DCMU, 3-(3,4)-dichlorophenyl)-1,1-dimethylurea.

0005-2728/82/0000–0000/$02.75

Materials and Methods

Glow curves of spinach leaf, algal cells and chloroplasts were recorded according to the procedure described earlier [7,10]. The cryostat employed in the study together with the temperature controller and programmer permitted heating of the sample at the desired linear rate or holding it at any temperature between 77 and 350 K [23].

The profile of intensity of delayed light emission as a function of temperature was obtained as follows: The sample, relaxed at room temperature in the dark for 5 min, was cooled to the desired temperature in the dark on the cryostat. It was then exposed to saturating white light [10] for 30 s and the intensity of delayed light emission 2.5 s after turning off the light was measured. The profile of intensity of delayed light emission was constructed by plotting the intensity thus measured against the temperature at which the sample was excited. The intensity of delayed light emission measured after 2.5 s was considered as the slow component.

For quantitative studies the relaxed sample at room temperature 298 K was excited in the cryostat with white light for 30 s. The decay of the intensity of delayed light emission, 2.5 s after switching off the light, was recorded for a known interval of time before warming the sample to 333 K to obtain the thermoluminescence still left in the material. The areas under the decay curve and the glow curve were measured separately. The time interval between recording of the delayed light emission and heating of the sample was varied from a few to several seconds. In each case, the delayed light emission and thermoluminescence yields were obtained separately.

In another experiment, after recording the decay of delayed light emission for some time the sample was quickly cooled to 77 K by pouring liquid nitrogen from the side of the photomultiplier housing in the dark. The thermoluminescence was obtained on subsequent warming of the sample to 333 K.

Results and Discussion

In Fig. 1 the glow curves (solid lines) of *Chlorella, Euglena*, spinach leaf and chloroplasts are compared with the profiles (open circles) of the intensity of 2.5 s delayed light emission obtained over the corresponding temperature range. In all cases, the delayed light emission profile matches the thermoluminescence pattern. Although the glow curve patterns of different materials differ, their delayed light emission profiles are found to match their respective glow curves invariably. This behaviour is further supported by the results presented in Fig. 2. It is known [5,6,8,10,11] that addition of DCMU to chloroplasts or algal cells considerably changes the glow curve pattern as some peaks are lost and some intensified. We note that even in these cases the delayed light emission profile resembles the glow pattern.

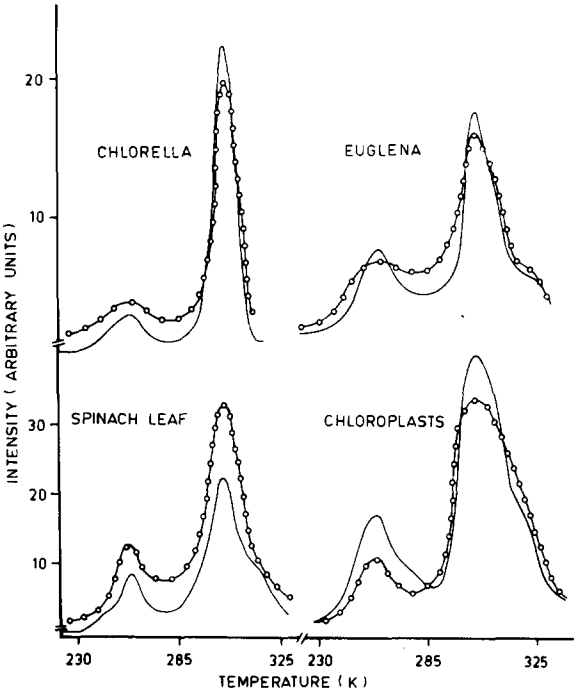

Fig. 1. Comparison of the glow curve pattern with the delayed light emission pattern observed in different photosynthetic materials. The delayed light emission pattern was obtained by plotting the intensity of delayed light emission observed 2.5 s after excitation at different temperatures. (———) Thermoluminescence (O——— O) delayed light emission.

The quantitative relationship between thermoluminescence and the slow (seconds) component of delayed light emission is demonstrated by the data shown in Fig. 3. In this experiment the decay of the slow component of delayed light emission at

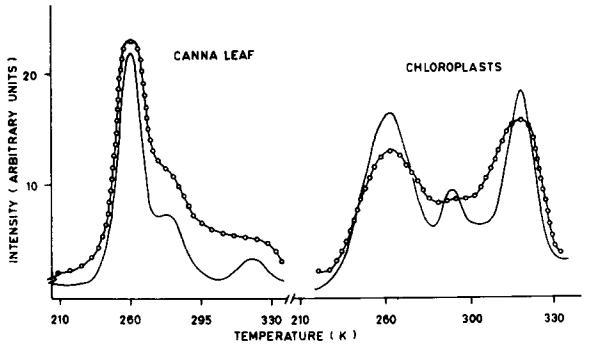

Fig. 2. Same as in Fig. 1 except that the material was Canna leaf infiltrated with 10 μm DCMU and chloroplasts from spinach treated with 10 μm DCMU. (———) Thermoluminescence (O———O) delayed light emission.

298 K was recorded for some time before heating the sample to 333 K. Instead of decreasing, the intensity of delayed light emission started increasing on heating the sample, resulting in a glow peak with a temperature maximum at about 321 K (Fig. 3A). Interestingly, the area under the glow curve matches the yield of delayed light emission obtained by extrapolating the decay curve till its intensity becomes negligible. Stimulation of light emission of the decaying delayed light emission by a temperature jump was reported earlier [24–26]. However, it was not possible for those authors to look into the quantitative aspects that are reported above. In another set of experiments, the samples were chilled to 77 K after recording the delayed light emission for some time. Lowering of the temperature quenched the delayed light emission completely. Subsequent warming of these samples to 333 K resulted in thermoluminescence with a glow peak at about 321 K. In this case, also the yield of thermoluminescence was equal to the yield of delayed light emission that would have been obtained if the sample were allowed to emit delayed light emission instead of being quenched by cooling to liquid nitrogen temperature. This behaviour was observable at any time during the entire course of delayed light emission decay (data not presented). The extrapolation of delayed light depletion decay to zero intensity in these experiments was obtained by varying the time over which the decay was observed prior to quenching as was also done by allowing the decay of delayed light emission to completion in a sample which was not cooled to liquid nitrogen temperature. This experiment clearly demonstrates that the slow component of quenched delayed light emission of the sample is converted into thermoluminescence. However, it was noticed that the sample did not show thermoluminescence when it was allowed to lose delayed light emission totally prior to cooling to 77 K. This is the first time that total quenching of the decaying slow component of delayed light emission by cooling to 77 K and its re-emission as thermoluminescence on warming up as well as their quantitative correlation have been demonstrated.

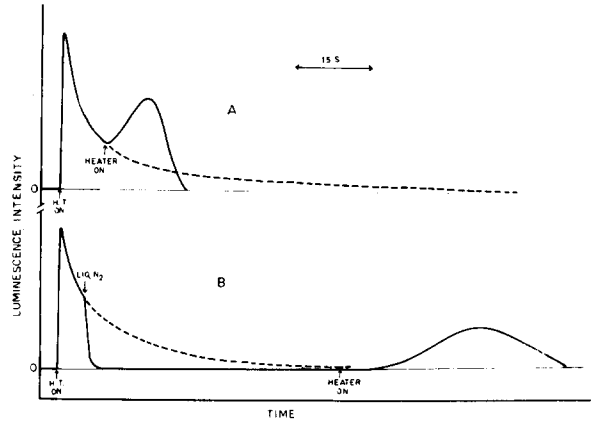

Fig. 3. Decay of delayed light emission and the residual thermoluminescence. (A) The decay of delayed light emission 2.5 s after excitation was recorded for some time. The heating of the sample was started subsequently (indicated by the arrow) and the temperature was raised to 333 K to obtain the glow curve at the same chart speed. The broken line represents extrapolation to zero intensity of the decay curve of delayed light emission without heating. H.T., high tension applied to photomultiplier. (B) Same as A except the sample was cooled to 77 K during the course of decay at a given time shown by arrow. The broken line represents the delayed light emission decay extrapolated to very low intensity. The glow curve was obtained at the same chart speed by heating the sample (shown by arrow) from 77 to 333 K.

It may be pointed out that the slow component of delayed light from the photosynthetic material can be seen if it is excited at any temperature above 230 K. If, however, one quenches the delayed light emission by cooling to 77 K following excitation at different temperatures, the glow curve pattern obtained on subsequent heating is different. The relative intensities of glow peaks undergo

a change. The maximum intensity is demonstrated by that glow peak which appears immediately above the temperature of excitation. For example, if the sample relaxed at room temperature is excited at 253 K before cooling to 77 K, it shows peaks II, III, IV and V appearing at temperatures of 261, 283, 298 and 321 K, respectively [7,10]. The maximum intensity is shown by peak II. It may be mentioned that if this sample is allowed to lose delayed light emission at 253 K following excitation the intensity of peak II decreases faster than that of other peaks.

On the basis of the results presented above (Fig. 3A and B), it is argued that if the glow peaks and the decay of delayed light emission seen after 2.5 s are identical quantitatively, then, if we allow the sample to lose delayed light emission for some time and then study the thermoluminescence of the sample, the thermoluminescence yield should be less than expected. However, the addition of the delayed light emission and thermoluninescence yields should be equal to thermoluminescence yield measured without allowing any loss of delayed light emission. Both these points are demonstrated in Fig. 4. With time (irrespective of temperature between 273 and 308 K), the integrated delayed light emission increases and concomitantly the thermoluminescence yield remaining in the sample decreases. They bear an inverse relationship as expected if the hypothesis that the slow component of delayed light emission that we are monitoring is identical to thermoluminescence. We therefore suggest that the thermoluminescence under our experimental protocol is identical to the slow component of delayed light emission observed at the given temperature.

An earlier attempt to associate different components of delayed light emission with different glow peaks [27] was questioned on several grounds by others [6,13]. However, the data on temperature jump delayed light which in some respects is similar to thermoluminescence [25,26] suggested a relationship between delayed light emission and temperature jump delayed light. In view of the fact that both delayed light emission and thermoluminescence exhibit similar quadruple oscillatory behaviour a correlation between them has been also recently speculated upon [18,20]. The present report is the first one which comprehensively demonstrates the relationship between a slow component of delayed light emission and thermoluminescence and also convincingly shows their identity.

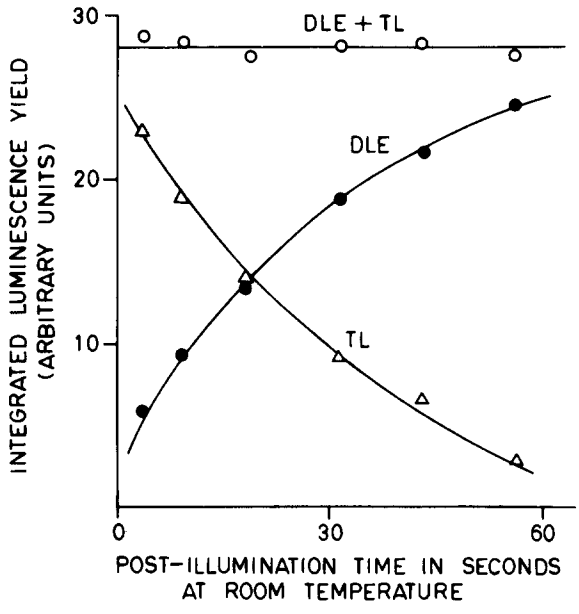

Fig. 4. Relationship between the integrated yields of delayed light emission and thermoluminescence. The decay of delayed light emission 2.5 s after excitation was recorded at a fast chart speed for known time intervals before heating the sample to 333 K to obtain the glow curve at the same chart speed. The time interval between recording of delayed light emission and heating of the sample was varied from a few to several seconds. Individual integrated yields of delayed light emission (●——●) and thermoluminescence (△——△) and their sum (○——○) were plotted as a function of time interval between recording of delayed light emission and heating of the sample.

Acknowledgements

The authors wish to thank Professor Govindjee of the University of Illinois, Urbana, U.S.A., for suggesting improvements in the manuscript during his visit to India under UNDP'S TOKTEN program.

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