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THERMOLUMINESCENCE STUDIES ON PHOTOSYNTHETIC ENERGY CONVERSION

I. EVIDENCE FOR THREE TYPES OF ENERGY STORAGE BY PHOTOREACTION II OF HIGHER PLANTS

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SUMMARY

Thermoluminescence was examined in isolated chloroplasts in the presence and absence of electron acceptors and inhibitors of electron transport, and in digitonin subchloroplast particles. Absence of emission from Photosystem I particles indicated that thermoluminescence originated from energy storage in Photoreaction II. Three distinct glow peaks were observed, suggesting that quantum conversion at Reaction Center II may involve three different types of energy storage with differing stabilization energies. Electron acceptors and inhibitors of electron transport had different effects on the three glow peaks. Although correlations can be drawn between the three storage states of the glow curves and components of delayed light emission at different dark times, we were unable to definitely associate any of the thermoluminescence peaks with delayed light emitted during a particular time-range of dark decay.

INTRODUCTION

Thermoluminescence is light emitted upon heating a sample which has first been illuminated and cooled. The measurement was originally used for the study of energy storage in inorganic crystals. Such light emission has been interpreted as resulting from the recombination of electrons and holes which were separated and trapped during illumination, frozen into position by cooling, and released by heating [1], but it could originate from any thermal reaction.

Arnold and Wilkins were the first to apply thermoluminescence measurements to photosynthetic systems, using dried chloroplasts [2] and *Chlorella* suspensions [3]. A number of Russian researchers [4–6] have studied thermoluminescence from algae

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and whole leaves, and Fleischman [7] has examined the thermoluminescence of photosynthetic bacteria and chromatophores. The phenomenon appears to be a thermal back reaction common to all photosynthetic organisms, and to reflect energy storage directly involved in stabilization of early products of photosynthetic quantum conversion.

In this paper we have investigated thermoluminescence from isolated chloroplasts and from Photosystem II and Photosystem I subchloroplast particles. We have also examined the effect of electron acceptors and photosynthetic poisons on the thermoluminescence of isolated chloroplasts.

EXPERIMENTAL

1. *Thermoluminescence apparatus*

Glow curves from chloroplasts and digitonin subchloroplast particles were measured with the apparatus shown in Fig. 1. The sample holder was a copper plug, which gave rapid heat transfer from the sample holder into the sample during heating. The sample of plant material had a 0.1 mm copper–constantan thermocouple embedded in it to monitor the temperature. The copper sample holder fitted inside an inconel tube connected to a pair of shutters. When open, shutter A allowed the sample to be illuminated; when closed, illumination was prevented. Shutter B had a mirror on the bottom surface. With shutter B closed and shutter A open, the exciting light was reflected off the mirror and onto the sample, so the photomultiplier was protected

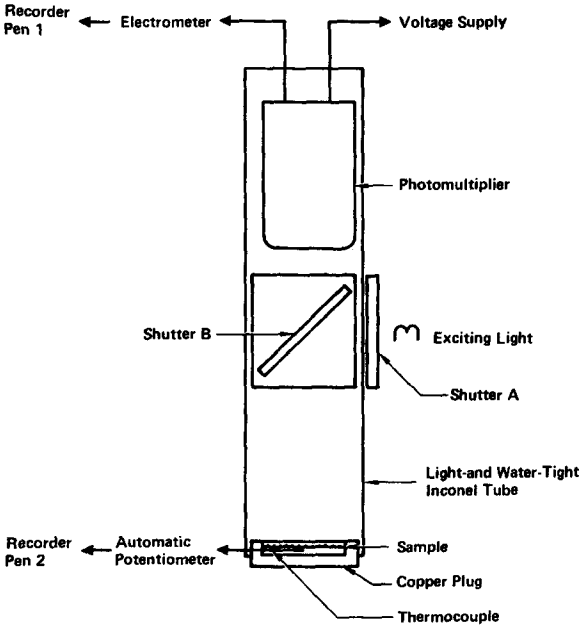

Fig. 1. Schematic diagram of the thermoluminescence apparatus. The shutters move in and out of the plane of the diagram. Shutter B has a mirror on its underside. When shutter A is on and B is closed the sample receives illumination from the light source, but the photomultiplier is protected. When shutter A is closed and B is open, the photomultiplier views the sample.

from the high intensity illumination by shutter B. Illumination was from a Unitron 500W microscope illuminator which gave an intensity of $5 \cdot 10^5$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1} of white light incident on the sample. When shutter A was closed and shutter B open, no exciting light struck the sample but the photomultiplier monitored any light emitted by the sample.

The signal from the photomultiplier (RCA Quanticon No. 8852) was amplified by a picoammeter (Keithley 417) and recorded on one channel of a two channel recorder (Honeywell Electronik 19). The picoammeter received 10^{-8}–10^{-9} amps of signal from the photomultiplier. The thermocouple signal was monitored by an automatic potentiometer (Westronics) which converted the copper–constantan voltage into a signal proportional to temperature, and this converted voltage was recorded by the second recorder channel. Thus the light emitted by the sample, and the temperature of the sample, were monitored simultaneously on the same chart.

The sample was cooled by bringing liquid nitrogen in a Dewar flask into contact with the bottom of the sample holder. The sample was heated by bringing the copper sample holder into contact with 85 °C water. To prevent water condensation on the photomultiplier face during heating, dry nitrogen gas was blown continuously over the surface of the sample.

2. *Procedure for obtaining glow curves*

The following standard procedure was used in most experiments because it gave maximum emission and best repeatability. A 1-ml sample containing chloroplasts or subchloroplast particles equivalent to 60 μg chlorophyll was frozen to liquid nitrogen temperature, and illuminated with white light while cooling from 0° to 196 °C. The frozen sample had a thickness of about 0.4 mm. Once the sample reached liquid nitrogen temperature illumination ceased, and after 10–15 s in the dark at this temperature the sample was heated to 70 °C. The heating rate was 10–12 °C/s below 0 °C and 7–9/s above 0 °C. Use of a relatively thin sample uniform illumination, ensured uniform melting, and prevented large temperature gradients from the top to the bottom of the sample.

3. *Preparations of chloroplasts and subchloroplast particles*

Chloroplasts were isolated from greenhouse grown Good King Henry (*Chenopodium bonicus-henricus*), or from market spinach, following the method of Jagendorf and Avron [8]. Chlorophyll was determined according to Avron [9].

Subchloroplast particles were prepared from market spinach using Anderson and Boardman's method of digitonin incubation and differential centrifugation [10]. Pellets collected after centrifugation at $10\,000 \times g$ were called D-10 (System II); those collected after centrifugation at $144\,000 \times g$ were called D-144 (System I). The subchloroplast particles were resuspended with the aid of a teflon pestle in 1 M sucrose, 0.002 M tricine–NaOH (pH 8.0) [11].

RESULTS

Fig. 2 shows a typical thermoluminescence profile (a glow curve) of isolated chloroplasts. The emission had three peaks in the temperature range of −30 °C to +60 °C. The first appeared below 0 °C and we have called it peak 1 or storage state 1. The maximum of the second peak was between +20 °C and +35 °C. The third peak

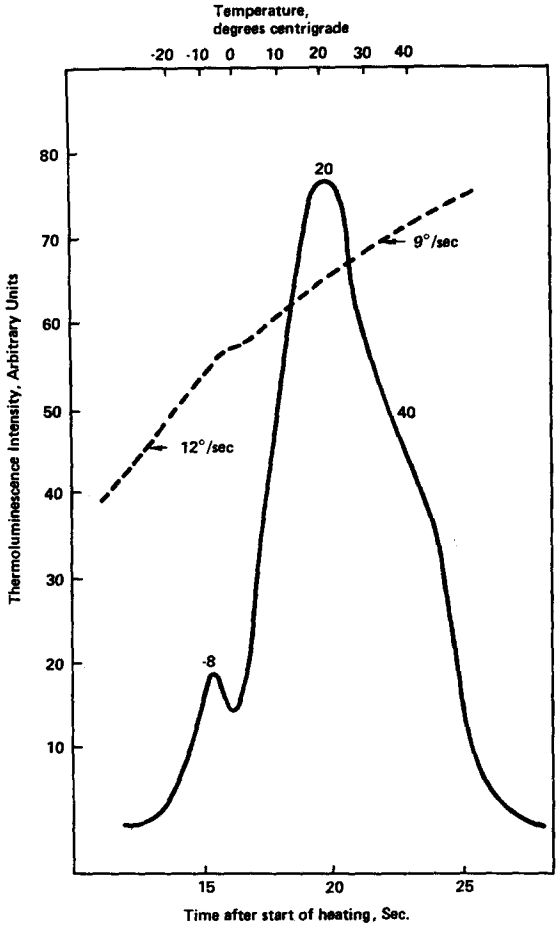

Fig. 2. Thermoluminescence of isolated chloroplasts. Reaction mixture consisting of 1 ml containing 65 μg chlorophyll, 0.05 M tricine (pH 7.8), 0.02 M NaCl, 0.005 M $MgCl_2$. The temperature of each peak maximum is indicated. The time scale refers to time after the beginning of heating. The dotted line is a tracing of the heating curve.

was a shoulder on peak 2, appearing between +40 °C and +50 °C.

The addition of an electron acceptor to a chloroplast sample prior to freezing affected the three thermoluminescence peaks of isolated chloroplasts differently. Figs 3a and b show that addition of the electron acceptor ferricyanide enhanced peak 1 and decreased peaks 2 and 3. The electron acceptor dichlorophenolindophenol (DCPIP), or readdition of the natural acceptor NADP plus ferredoxin to isolated chloroplasts, gave the same effect as ferricyanide.

Figure 3c shows the effect on thermoluminescence of adding the electron transport inhibitor 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) to chloroplasts prior to freezing. This compound reduced peak 1 and enhanced peaks 2 and 3. Fig. 3d shows that ferricyanide had no effect in the presence of DCMU; the profile is the same as that of DCMU alone. The effect of the electron transfer inhibitor ortho-phenan-

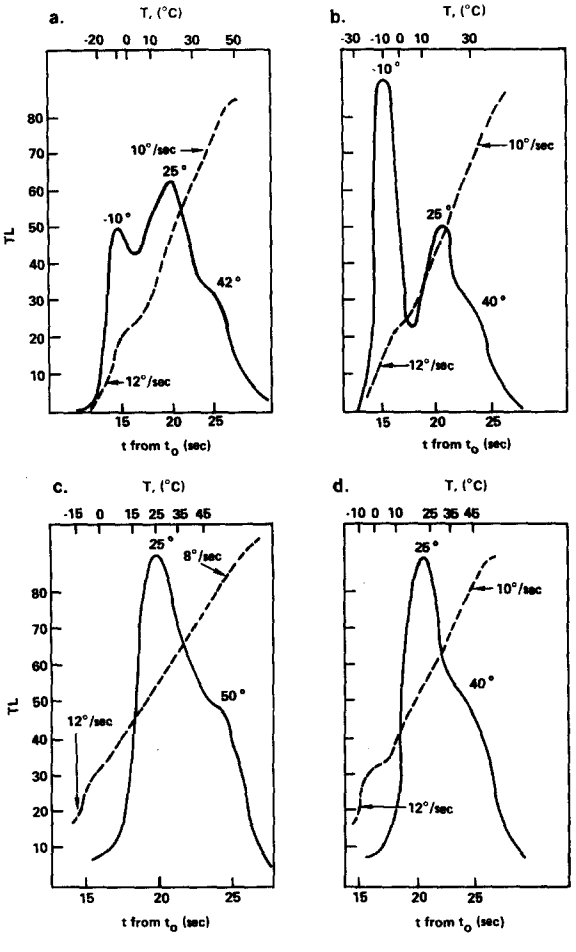

Fig. 3. Thermoluminescence of isolated chloroplasts in the presence and absence of ferricyanide and DCMU. Reaction mix: 0.025 M tricine (pH 7.8), 0.02 M NaCl, 0.005 M $MgCl_2$, 65 μg chloroplasts/ml. a has no addition, b has $5 \cdot 10^{-4}$ M ferricyanide, c has $2 \cdot 10^{-6}$ M DCMU, d has DCMU and ferricyanide. The temperature of each peak maximum is indicated above the peak. The time scale refers to time after start of heating. The dotted line is tracing of the heating curve. Ferricyanide reduction was measured in an Aminco Chance Spectrophotometer in the single beam mode, reading 420 against 480 nm.

throline was the same as that of DCMU.

Figure 4 shows the effect of different concentrations of DCMU on peaks 1 and 2. Peak 1 was not measurable at DCMU concentrations higher than 10^{-8} M, while peaks 2 (and 3) showed some enhancement at 10^{-8} M. The maximum enhancement of these two peaks was at the same concentration that photochemistry, measured as ferricyanide reduction, was completely inhibited. At higher concentrations of DCMU thermoluminescence from all three peaks is inhibited, and at 10^{-3} M DCMU no emission was observed.

We compared thermoluminescence of digitonin subchloroplasts particles en-

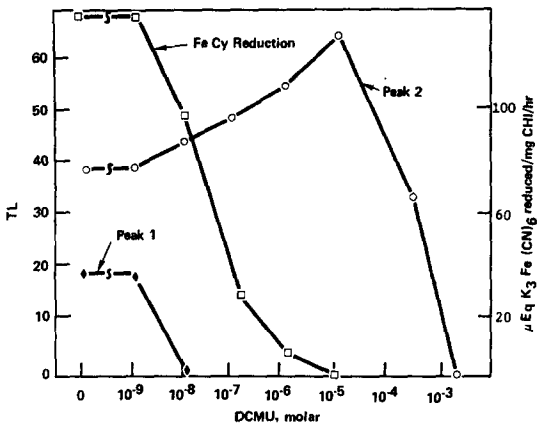

Fig. 4. The effect of increasing concentrations of DCMU on thermoluminescence and ferricyanide reduction. The graph plots the height of peaks 1 and 2 and extent of ferricyanide reduced as a function of increasing DCMU concentration. (Chl = chloroplasts).

riched in either Photosystem II or Photosystem I to that of isolated chloroplasts. Fig. 5 shows D-10 particles which were enriched 40% Photosystem II activity and D-144 particles which contained only 10% Photosystem II. The D-144 particles gave very little emission. The D-10 particles gave more emission per unit chlorophyll than chloroplasts. This would be the expected results if thermoluminescence originated only in Photosystem II.

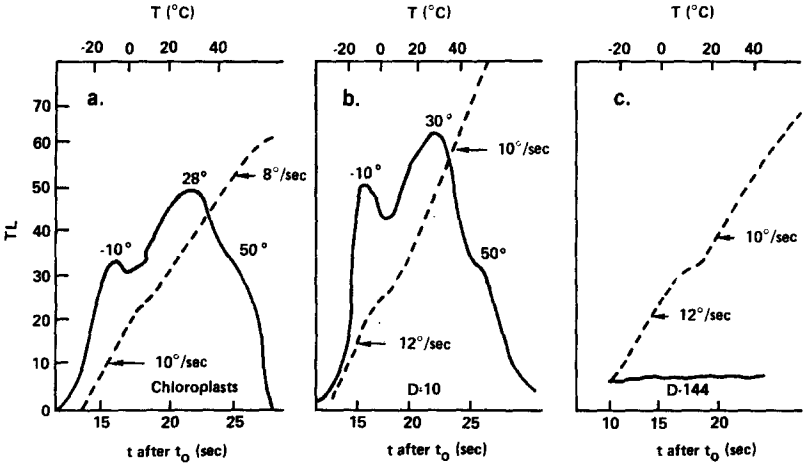

Fig. 5. Thermoluminescence of chloroplasts and subchloroplast particles. Reaction mix: 0.025 M tricine (pH 7.8), 0.02 M NaCl, 0.005 M $MgCl_2$, 50 μg chloroplasts/ml. The temperature of each peak maximum is indicated above the peak and the time scale refers to time after the start of heating. The dotted line is a tracing of the heating curve.

DISCUSSION

The glow curve emission from isolated chloroplasts was similar to that of whole leaves of *Trianea bogotensis* examined by Shuvalov and Litvin [4], except that they did not observe our peak 3. We suspect this disagreement may be attributed to their relatively slow heating rate (0.01–1 °C/s compared to our 8–12 °C/s), which would probably result in insufficient resolution of the peak 3 shoulder from the falling side of peak 2.

In the alga *Chlorella*, Arnold [3] found no peak 1, but peaks 2 and 3 were similar to ours. We have confirmed his findings. In examining glow curves from *Euglena*, *Scenedesmus*, *Chlamydamonas* and the blue green alga *Anacystis*, we found that peak 1 was absent in all cases. It is possible that this storage state is only found in vascular plants.

We observe, in agreement with other workers [3–6] a low temperature emission peak which is not due to light absorption by chlorophyll, and which apparently is not related to photosynthesis [3]. This fourth peak (Shuvalov and Litvin's "L_1") is emitted between −180 and −140 °C, is excited by blue light but not by red light [3, 4], and is present in all green plant material which we examined. We found this peak to be present even after boiling for 5 min. We also found the low temperature peak to be unaffected by any photosynthetic reagent (DCMU, acceptors, etc.) which we utilized. This peak might be due to energy storage by carotenoids, because Arnold observed a similar thermoluminescence peak in carrot tubers. This idea, however, is not supported by the emission spectrum reported by Shuvalov and Litvin [4], in which the low temperature glow had a 740 nm maximum. Regardless of the origin of this low temperature peak, we doubt that it plays a significant role in photosynthetic quantum conversion.

Three separate lines of evidence indicate that the three glow peaks in the −20° to +80 °C range originate in Photosystem II. We found that electron acceptors and electron transport inhibitors of Photosystem II affect thermoluminescent glow curves. Photosystem I subchloroplast particles show very little thermoluminescence in this temperature range, while Photosystem II subchloroplast particles show enhanced light emission per unit chlorophyll as compared to chloroplasts. Arnold and Azzi [12] found, and we have confirmed, that *Scenedesmus* mutant 11 (with non-functional Photosystem II) emitted no measurable thermoluminescence in this temperature range.

Shuvalov and Litvin [4] concluded that their peak L_3 originated in Photosystem I. This conclusion was based on their observation that 700 nm light excited their L_3 (emitted at +20 °C) more effectively than their L_2 (emitted at −15 °C), although 640 nm excited both peaks more effectively than did 700 nm. We equate their L_2 with our peak 1, and their L_3 with our peaks 2 and 3, on the basis of emission temperatures and DCMU effects. We have confirmed the observation that all three peaks are more effectively excited by red wavelengths below 670 nm than by wavelengths above 680 nm. Furthermore, we observed that peaks 2 and 3 ("L_3") require less total exciting energy to reach saturation than does peak 1 ("L_2"). It thus seems possible that Shuvalov and Litvin's result was due to the relatively low chlorophyll absorption at 700 nm. In their experiment, peaks 2 and 3 may have been nearly saturated by the excitation, while peak 1 was barely excited. In any case, the weight of

evidence would seem to lead to the conclusion that all three peaks are from Photosystem II.

It would be useful to correlate the glow peaks with another measurement reflecting energy storage at the reaction center of Photosystem II. Two possibilities are absorption shifts and delayed light emission. Fleischman [7] was able to correlate the disappearance of glow peaks in bacteria when illuminated below −80 °C with a decrease in light induced P895 oxidation (the bacterial reaction center). We have examined the effect of illumination at different temperatures on the glow peaks of chloroplasts, and found below −50 °C illumination did not fill trap 1, but light at temperatures down to −196 °C allowed some filling of traps 2 and 3. Unfortunately, absorption shifts have not yet been unambiguously associated with the Photosystem II reaction center in the steady state, so a correspondence between the disappearance of peak 1 and an absorption shift cannot be made. Doring et al. [13] observed a shift at 692 nm in flash experiments, which on the basis of decay kinetics could be separated from the shift caused by P700 at that wave length, and which they identified with the Photosystem II reaction system. It is not known whether at low temperatures this absorption shift would be altered, correlating with a loss of one of the glow peaks.

Butler [14] has found absorption shifts at liquid nitrogen temperature. He observed reduction of a component called c550 and oxidation of cytochrome b559. The latter he feels at liquid nitrogen temperature acts as the primary electron donor to Photoreaction II and the former as the primary acceptor. It is possible that these may correspond to our peaks 3 and 2.

It is possible to make a qualitative comparison between delayed light and thermoluminescence. Delayed light is made up of different components. At no one time of measurement does the delayed light exhibit a simple exponential decay; the microsecond [15], millisecond [16, 17] and second [18] delayed light all contain two or more exponentially decaying components. These components may be different storage states in the reaction center itself, or back reactions from components along the electron transport chain, or, as Bertsch and Lurie [19] suggested, delayed light may reflect both types of states.

The presence of electron acceptors or photosynthesis inhibitors affects the delayed light decay differently at different time spans. DCMU depresses delayed light at 10^{-3} s and enhances delayed light at 10^{-1} s. Ferricyanide, on the other hand, enhances delayed light at 10^{-3} s and depresses delayed light at 10^{-1} s. Thus, a possible speculation would be that peak 1 corresponds to a component contributing to 10^{-3} second delayed light, while peaks 2 and 3 correspond to a component contributing to 10^{-1} sec delayed light.

One difficulty with this speculation is that 10^{-8} M DCMU depresses 1 ms delayed light by less than 10 % [20], while completely abolishing thermoluminescence peak 1 (Fig. 4). Furthermore, we found that thermoluminescence peak 1 is not excited by light flashes shorter than about 1 s, nor is it excited by temperatures below −50 °C. Millisecond delayed light, on the other hand, is excited by single flashes even at liquid nitrogen temperatures [21]. Thus, we are unable to clearly associate the individual glow peaks with a specific time-component of delayed light.

Nevertheless, the three emission peaks indicate that Photoreaction II is capable of at least three distinct types of energy storage in chloroplasts of higher plants. This suggests that quantum conversion involves three stabilization energies at Photo-

reaction II of vascular plants.

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REFERENCES

1 Randall, J. F. and Wilkins, M. H. F. (1945) Proc. Royal Soc. 184, 366–389
2 Arnold, W. and Sherwood, H. (1959) J. Phys. Chem. 63, 2–4
3 Arnold, W. (1966) Science 154, 1046–1049
4 Shuvalov, V. A. and Litvin, F. F. (1969) Molek. Biol. 3, 59–73
5 Litvin, F. F. and Shuvalov, V. A. (1969) Dokl. Acad. Nauk. USSR 181, 733–736
6 Rubin, A. B. and Venediktov, P. S. (1969) Biofizika, 14, 105–109
7 Fleischman, D. E. (1971) Photochem. Photobiol. 14, 65–70
8 Jegendorf, A. T. and Avron, M. (1959) Arch. Biochim. Biophys. 80, 246–257
9 Arnon, D. I. (1949) Plant Physiol. 24, 1–14
10 Anderson, J. M. and Boardman, N. K. (1966) Biochim. Biophys. Acta 112, 403–421
11 Lurie, S., Cohen, W. and Bertsch, W. (1971) in II Intl. Congr. on Photosyn., p. 197–205 (Forti, ed.), Stresa, Italy
12 Arnold, W. and Azzi, J. R. (1968) Proc. Natl. Acad. Sci. 61, 29–36
13 Doring, G., Renger, G., Bater, J. and Witt, H. T. (1969) Z. Naturforsch. 24B, 1139–1143
14 Butler, W., Vissney, I. and Simons, H. (1973) Biochim. Biophys. Acta 292, 140–151
15 Zankel, K. (1971) Biochim. Biophys. Acta 245, 373–385
16 Ruby, R. H. (1968) Photochem. Photobiol. 8, 299–308
17 Bonaventura, C. and Kindergan, M. (1971) Biochim. Biophys. Acta 234, 249–265
18 Bertsch, W. F. and Azzi, J. R. (1965) Biochim. Biophys. Acta 94, 15–26
19 Bertsch, W. and Lurie, S. (1971) Photochem. Photobiol. 14, 251–260
20 Bertsch, W., West, J. and Hill, R. (1971) Photochem. Photobiol. 14, 241–250
21 Tollin, G. and Calvin, M. (1957) Proc. Natl. Acad. Sci. U.S. 43, 895–908