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MULTIPLE-FLASH DEVELOPMENT OF THERMOLUMINESCENCE BANDS IN DARK-GROWN SPRUCE LEAVES

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Summary

Spruce leaves greened in darkness were devoid of three of the five thermoluminescence bands found for mature leaves. These bands were developed rapidly by exposure of the dark-grown leaves to continuous light. The development of these bands was studied by illumination with repetitive flashes at varied intervals. Flashes at intervals of 1 s were the most effective in inducing these bands. Those at shorter or longer intervals were less effective. It was deduced from these data that the development of these bands is a multi-quantum process which involves at least two photo-events with a dark reaction between them.

Mature leaves of higher plants show five thermoluminescence bands emitted at different temperatures [1], which were denoted in previous papers [2—4] as Z_V, A, B_1, B_2 and C bands. The A, B_1, B_2 and C bands were emitted at specific fixed temperatures, whereas the Z_V band was emitted at a variable temperature dependent on the temperature for excitation of thermoluminescence by 1-min illumination with red light [3,4]. It was attempted in the previous study [3] to relate these bands to certain photosynthetic activities. The measurement of the Hill activities of heat-treated spinach chloroplasts in the presence and in the absence of an artificial electron donor such as diphenylcarbazide, suggested that emission of the A, B_1 and B_2 bands is linked to the presence of an intact water-splitting system for evolving oxygen or a site closely related to the system.

This view was further confirmed by the recent observations [4] on angiosperm leaves greened under intermittent illumination or dark-grown gymnosperm leaves lacking specifically the oxygen-evolving activity [5—9]. These leaves emitted only the Z_V and C bands, being completely devoid of

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the remaining three bands. It was demonstrated that these three bands of A, B_1 and B_2 were rapidly developed by brief exposure of such leaves to continuous light, and the development was accompanied by enhancement of delayed emission, appearance of fluorescence induction and generation of the oxygen-evolving activity. This strongly suggested that the development of these thermoluminescence bands is one of the characteristic phenomena accompanying the photoactivation of the latent water-splitting system in flashed angiosperm leaves [4] or in dark-grown gymnosperm leaves (unpublished), and that the three bands A, B_1 and B_2 developed originate from energy storage in the water-splitting system in Photosystem II, whereas the other two bands (the Z_v and C bands) are emitted from some other systems.

The process of photoactivation has been studied by means of multiple flashes with varied intervals by Radmer and Cheniae [10] for manganese deficient algal cells, and by Inoue [11] for flashed wheat leaves, and it was found that the process involves more than two consecutive photoreactions with a rate-limiting dark reaction between them. These authors measured the water-splitting activity or the delayed emission accompanying the activation.

It is demonstrated in the present study that the development of the above thermoluminescence bands is a multi-quantum process. Dark-grown spruce leaves were illuminated with flashes at varied intervals for this study, and the optimal dark interval between successive flashes required for maximal activation was determined.

Spruce seeds (*Picea abies* L.) were germinated and grown on moist vermiculite in darkness at 24 ± 1°C. The dark green needle-like leaves containing about 1 μg chlorophyll/mg fresh weight were picked from the 20 to 30-day old seedlings, spread on moist filter paper and then, exposed to 2-μs white xenon flashes repeated at uniform intervals from a Sugawara stroboscope model MSK-1A. The energy per flash on sample leaves was about $2 \cdot 10^2$ ergs/cm^2. The illuminated leaves were cooled to −45°C after 10 min of dark incubation at room temperature, and then illuminated with strong red light for excitation of thermoluminescence. After 1-min illumination with red light, the leaves were cooled to −196°C and heated at a rate of 0.5°C/s. The thermoluminescence from the leaves was measured by the same method as described previously [3,4] with a Jasco photon counter model KC-200 equipped with an EMI photomultiplier, 9659QB, and the digital photon count in every 32 Hz was recorded against leaf temperature on an X-Y recorder. Isolation of chloroplasts from spruce leaves and measurements of their Hill activity were carried out as described previously according to the method of Oku et al. [12].

The top curve in Fig. 1a shows the thermoluminescence profile of mature spruce leaves measured after excitation at −45°C; the excitation temperature was fixed at −45°C throughout this paper, except when otherwise specified. The profile shows a strong B_2 band around +35°C with a B_1 shoulder around +10°C and weak Z_v and C bands around −35 and +70°C, respectively. The bottom curve in Fig. 1a is the profile of dark-grown spruce leaves, which contrasts strikingly to that of mature leaves. In spite of a considerable content of chlorophylls (30% of the content in mature leaves) in the dark-grown

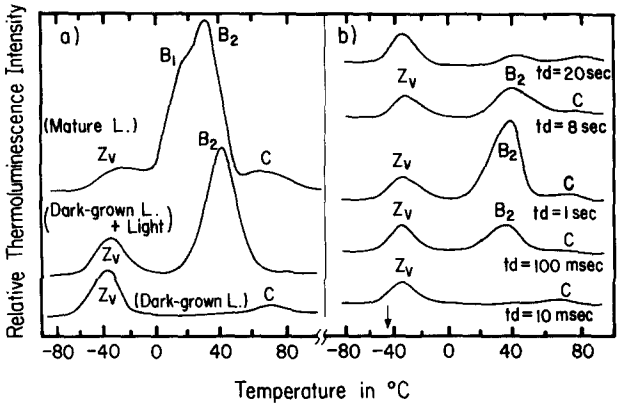

Fig. 1. (a), Thermoluminescence profiles of spruce leaves; mature leaves (top profile), dark-grown leaves (bottom profile) and illuminated dark-grown leaves (middle profile). The excitation was made by 1-min illumination with red light (⩾ 630 nm, 600 μW/cm²) at −45°C. The dark-grown leaves were illuminated for 30 min with weak red light (⩾ 630 nm, 28 μW/cm²) at room temperature before measurement of the middle profile. (b) Development of thermoluminescence bands induced by flashes at varying intervals. Dark-grown spruce leaves were exposed to 600 flashes (2-μs duration, $2 \cdot 10^2$ ergs/cm² per flash) at room temperature and the interval indicated as t_d on each profile was varied between 10 ms and 20 s. Measurements of thermoluminescence profiles were carried out under the same condition as the experiments described in (a).

leaves, their thermoluminescence profile showed only the Z_v and C bands and was devoid of the B_1 and B_2 bands. The middle curve, which is the profile obtained after exposure to the continuous light for 30 min, shows development of a strong B_2 band around +40°C. The same illuminated leaves, when observed by excitation at a higher temperature, emitted the B_1 band as a shoulder of the B_2 band. The A band could not be found in the profiles in Fig. 1a, observed at the excitation temperature of −45°C adopted throughout this experiment. A separate experiment at a higher excitation temperature showed that this band is emitted from mature or shortly illuminated dark-grown leaves but not from dark-grown leaves.

The curves in Fig. 1b are the profiles obtained for dark-grown spruce leaves exposed to the same number of flashes repeated at various intervals (indicated as t_d on each profile). The flashes at an interval of t_d = 1 s enhanced the B_2 band maximally, as seen from the middle profile, while those at longer (t_d = 8 s) or shorter (t_d = 100 ms) intervals enhanced the band much less and those at even longer (t_d = 20 s) or shorter (t_d = 10 ms) intervals showed practically no enhancement. The Z_v and C bands remained unchanged during this enhancement. The curve drawn through solid circles in Fig. 2 shows the dependency of the development of the B_2 thermoluminescence band on the flash interval. The height of the B_2 band thus developed by 600 flashes at uniform intervals was plotted against the dark interval. The height became maximal around t_d = 1 s, and dropped steeply at shorter intervals and gradually at longer intervals. This result indicates that the development of thermoluminescence B_2 band is a multi-quantum process involving at least two photoreactions. The steep drop at shorter intervals implies that the process involves a rate-limiting dark reaction, and the gradual drop at

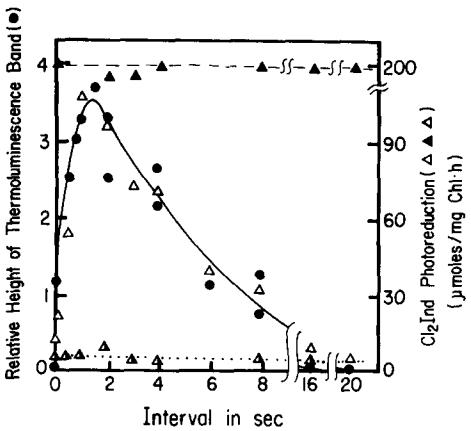

Fig. 2. Effects of the dark interval on the development of thermoluminescence bands and the degree of generation of the Hill activity in dark-grown spruce leaves during flash illumination. Solid circles along the bell-shaped solid curve show the relative height of the B_2 band generated in dark-grown leaves after exposure to 600 flashes at varied intervals, and estimated on the profiles shown in Fig. 1b. △ ——— △, the Hill activity of the chloroplasts isolated from the leaves exposed to flashes. ▲— — —▲ and ▲. . . .▲ show the activity of Cl_2Ind photoreduction in the presence of diphenylcarbazide (0.5 mM) and diphenylcarbazide plus DCMU (50 μM), respectively.

longer intervals indicates that an intermediate generated by the first photoreaction decays during the dark interval unless it is further converted to the final active state by the next flash.

A similar experiment was carried out by measuring the Hill activity of the chloroplasts. The chloroplasts isolated from dark-grown spruce leaves showed considerable activity (200 μmol/mg chlorophyll · h) of Cl_2Ind (2,6-dichlorophenolindophenol) photoreduction with diphenylcarbazide as electron donor, but showed a very weak activity with water as electron donor to split water to evolve oxygen (25 μmol/mg chlorophyll · h). This indicates that Photosystem II in such chloroplasts bears some defects in its water-splitting site, while the reaction center has been developed almost completely. In fact, the water-splitting activity was rapidly generated by brief exposure of the dark-grown leaves to continuous light or to repetitive flashes. In the experiment with flashes, the water-splitting capacity was generated in parallel with the flash number and reached saturation above 2000 flashes (dark interval, t_d = 1 s). The activity on the saturation level was about 200 μmol/mg chlorophyll · h, which is similar to the activity with diphenylcarbazide found for dark-grown chloroplasts. The open triangles in Fig. 2 show the dependency of the yield of the photoactivation of the water-splitting capacity on the flash interval. The activity generated by 600 flashes was plotted at an appropriate relative scale against the dark interval between successive flashes. Almost all of the triangles thus plotted are located on the curve drawn from the data of thermoluminescence, showing a bell-shaped response with a maximum around t_d = 1 s. Similar agreement between water-splitting activity and thermoluminescence was obtained previously for intermittently flashed wheat leaves [2,11], which indicated that also here the process involves

more than two consecutive photoreactions with a rate-limiting dark reaction between them.

It was confirmed from these data that the process of development of the thermoluminescence B_2 band in dark-grown spruce leaves is a multi-quantum process as well as the process of photoactivation of their latent water-splitting system. Judging from the similar responses of thermoluminescence, delayed emission, fluorescence variation and the Hill activity to repetitive flashes found in the present and previous papers [8,12], these four phenomena may originate from the same structural changes induced by light in the water-splitting system via a multi-quantum process. Structural or valency changes in manganese catalyst in the water-splitting system have been suggested by Cheniae and Martin [14—16] to be involved in the process of photoactivation in manganese deficient algal cells, and the process has also been shown to involve two quantum reaction. All these results lead us to conclude that the activated manganese catalysts are a possible trapping site for the emission of the thermoluminescence B_2 band observed by excitation at —45°C and also for the A and B_1 bands observed at higher excitation temperatures.

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