

BBA Report

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Thermoluminescence in spinach chloroplasts and in *Chlorella*

TED MAR and GOVINDJEE

Departments of Botany and Physiology and Biophysics, University of Illinois, Urbana, Ill. 61801 (U.S.A.)

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SUMMARY

Preilluminated spinach chloroplasts and *Chlorella pyrenoidosa*, when subjected to a quick temperature jump of about 15°, emit light. This thermoluminescence is observed both in normal and 3(3,4-dichlorophenyl)-1,1-dimethylurea-treated samples, but is absent when hydroxylamine is added to either of these samples. These results are explained in terms of a back reaction of System II of photosynthesis.

Isolated chloroplasts will emit light when subjected to acid–base pH transitions¹ and salt induced ionic transitions^{2,3}. In both cases light emission by the chloroplasts requires preillumination. This indicates that the pH and ionic transitions may be acting as a trigger to release stored light energy. We find that with a temperature jump of about 15°, spinach chloroplasts that have been preilluminated will also emit light. Similar results have been found for the green alga *Chlorella pyrenoidosa*. The differences between our thermoluminescence experiments and those by Arnold and Sherwood⁴ are discussed in this paper. We explain our results in terms of a back reaction of System II leading to light emission.

Chlorella was grown as previously described⁵. Chloroplasts were isolated from spinach by the method outlined in ref. 2. The instrument assembled to measure the thermoluminescence was similar to that used by Mayne and Clayton¹ for measuring chemiluminescence. A red cut off filter was placed before the photomultiplier to transmit chlorophyll emission. A 1-cm² glass cuvette was placed in a light tight box in front of a photomultiplier (EMI 9558 B). Two small holes were made on top of the box to allow two hypodermic needles to extend to the bottom of the cuvette. An opening was made on the side of the box for the illumination of the sample. The intensity of the illuminating white light was $3.8 \cdot 10^5$ ergs · cm⁻² · sec⁻¹. The signal from the photomultiplier was displayed on a Tektronic 502 oscilloscope and was recorded on Polaroid 3000 film. The measurements

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

were made as follows. 1 ml of the sample (chlorophyll concn., $10 \mu\text{g}/\text{ml}$) was placed in the cuvette inside the light tight box. The sample was illuminated for 10 sec. After a dark time of 10 sec, 1 ml of hot water was injected into the cuvette. The resultant luminescence signal was then recorded. Temperature was measured with a TRI-R electronic thermometer. The temperature of the chloroplasts before the hot water was injected was 16° ; for *Chlorella*, 26° . The temperature of the hot water itself was 53° . After 1 ml of the hot water was injected into 1 ml of sample, the temperature was 33° for chloroplasts and 38° for *Chlorella*.

Both chloroplasts and *Chlorella* gave off light when 1 ml of warm water was injected. No light was given off unless the sample was preilluminated. When cold water (16°) instead of warm water, was injected after preillumination, no light was given off. Hence the light emission we observed is not due to an osmotic change but a temperature change. Fig.1 shows the light emitted by normal chloroplasts (A), by chloroplasts with the addition of $1 \cdot 10^{-5}$ M 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (B), with $1 \cdot 10^{-2}$ M hydroxylamine (C), and with both $1 \cdot 10^{-2}$ M hydroxylamine and $1 \cdot 10^{-5}$ M DCMU (D). Results with *Chlorella* are similar except that there is a greater decrease in the signal upon the addition of DCMU.

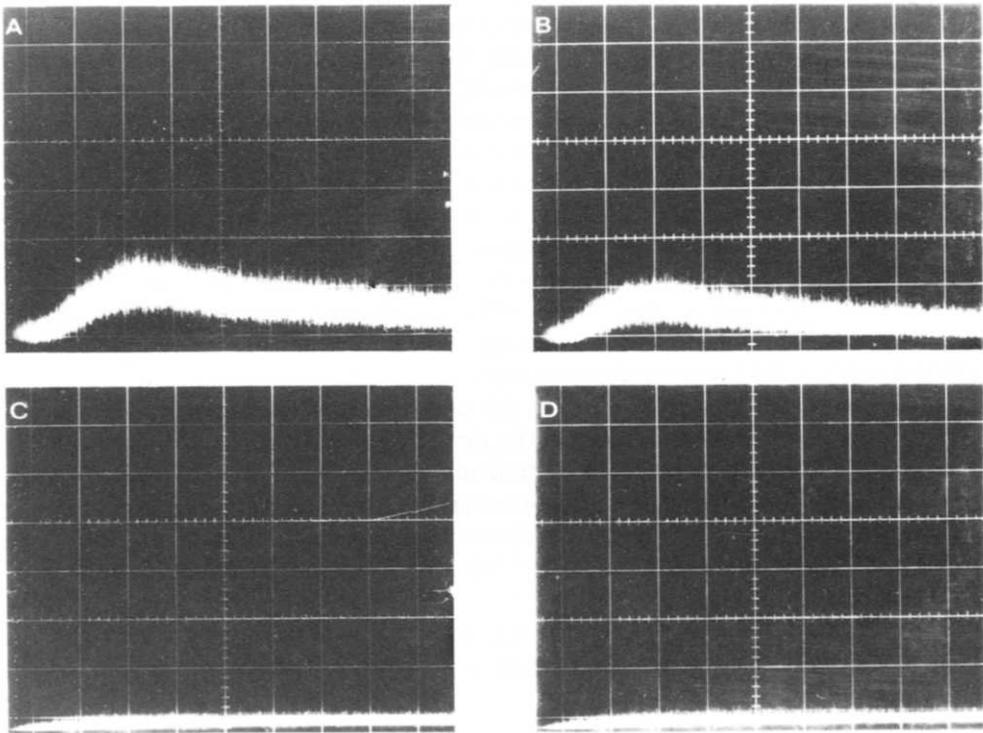


Fig.1. Time-course of the luminescence induced by a temperature jump in spinach chloroplasts. The temperature jump is induced by adding hot water at 53° to the chloroplasts at 16° ; the final temperature was 33° . Curve A is the signal induced in normal chloroplasts; B, in chloroplasts with $1 \cdot 10^{-5}$ M DCMU; C, with $1 \cdot 10^{-2}$ M hydroxylamine; D, with both $1 \cdot 10^{-2}$ M hydroxylamine and $1 \cdot 10^{-5}$ M DCMU. Oscilloscope setting: 0.5 sec/div.; 20 mV/div.

A similar decrease in the intensity of the emitted light, in the presence of DCMU, was observed by other workers² when they induced light emission by an acid-base change or ionic change. This similarity may be interpreted to mean that the emitted light due to a temperature jump may have the same underlying mechanism as the emitted light due to pH or ionic changes. However, the real reason for the decrease in light emitted, with DCMU present, is unclear.

The amount of light emitted may depend on the concentration of the oxidized form of the primary donor of System II (Z^+). Figs. 1C and 1D show that the addition of hydroxylamine inhibits any light emission due to a temperature jump. Following the procedure of Mayne and Clayton¹, we found that hydroxylamine also inhibits any light emission due to a pH change. Since there is evidence⁶⁻⁹ indicating that hydroxylamine quickly reduces Z^+ , a stable species^{10,11} in the normal cell, the amount of light emitted by a temperature jump may depend on the concentration of Z^+ . The light emission is suggested to originate by a "back reaction" of Z^+ with a reduced entity on the reducing side of System II. We are not sure what this entity is.

This experiment is different from the thermoluminescence experiments of Arnold and Sherwood⁴. In our experiments, temperature is made to change quickly (17° within fractions of seconds) whereas the rate of temperature change was $14^\circ/\text{min}$ in Arnold and Sherwood's experiment. Increasing the temperature slowly in the range of temperature used in our experiments will not increase the intensity of light emission, since any increase in the rate of dissipation of the chemically stored energy due to the increase of temperature will be negated by the continual decrease in the amount of stored energy. The intensity of light emission depends on the rate of dissipation and the amount of energy stored. Arnold and Sherwood's experiments were designed to see if there is energy that can be stored in states of high activating barrier that low activating energy cannot surmount. Since in our experiments, the temperature jump is only 17° , the energy change is calculated to be only $1.4 \cdot 10^{-3}$ eV. This small change in energy will not cause any energy stored in states of high activation barrier to be converted back to light. Thus, Arnold and Sherwood's explanation may not apply to light emission observed here.

Thermoluminescence reported in this paper may have the same mechanism as that of delayed light emission observed right after the incident light is turned off. Arnold and Azzi¹², and Bertsch¹³ have proposed electron-hole recombination to explain delayed light, whereas Lavorel¹⁴ and Stacey *et al.*⁹ suggested an alternate theory in which triplets are formed by a back reaction of System II followed by triplet-triplet "fusion" to give a singlet. The deexcitation of which produces delayed light. We favour the latter but we cannot yet distinguish between the two theories.

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