A COMPUTERISED EQUIPMENT FOR THERMOLUMINESCENCE INVESTIGATIONS

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Summary. Plant materials (intact leaves, chloroplasts or chloroplast particles) preilluminated at room temperature and rapidly cooled to -196 °C and on raising temperature are capable to emit light quanta (luminescence) the number of which is temperature depending. Such kind of thermoluminescence could be measured as function of temperature, by means of sensitive photo-electron counting technique. In the present work a computerised set for thermoluminescence investigation equipped in our laboratory is described.

Key words: photosynthesis, photosystem II, thermoluminescence, equipment

Introduction

The delayed fluorescence from green plant materials was discovered by Arnold and Sherwood (1957) who found that algae and leaves can store some of the light energy absorbed by chlorophylls at temperature below 0°C. The stored energy is re-emitted upon heating the samples. This luminescence has been interpreted as the result of the recombination of electrons and holes which were trapped in a frozen state during illumination followed by rapid cooling.

Four luminescence bands emitted at different temperatures was found by Arnold and Azzi (1968) in dried chloroplasts. Rubin and Venediktov (1969) found that the glow profile is greatly dependent on the system of illumination; continuous illumination of leaves during cooling yielded a profile with four peaks, whereas illumination of leaves at a low temperature around -50°C yielded a profile with two peaks.

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Fig. 1. The setup diagram of the equipment

Arnold and Azzi (1968) have calculated the activation energy, and Shuvalov and Litvin (1969) have shown some correlation of glow peaks to delayed fluorescence components with different decay constants. Sane et al. (1974) arrived at the conclusion that the band at 118 K (Arnold's Z band) is an emission from the triplet states of chlorophylls *in vivo*.

In the present study, we describe an computerised equipment for thermoluminescence measurements equipped in our laboratories.

The block-diagram of the thermoluminescence equipment is presented on Fig. 1, where 1 is the holder of the sample, 2 – dewar with a window, 3 – photomultiplyer, 4 - preamplifier, 5 - radiation measuring set 20 046 (VEB Robotron-Messelectronic -Otto Schön, DDR), 6 – analogue digital converter, 7 – IBM compatible computer, 8 - bridge amplifier for the microthermoresistor and 9 - transformer 220V/10-14V. The electric pulses obtained as a result of the thermoluminescence photons on the photomultiplyer anode are amplified by preamplifier 4, which is situated in the housing of the photomultiplyer and after which are translated to the pulse counting device (radiometer) -5. The output signal from the radiometer, which is proportional to the frequency of the thermoluminescence photons emission and the output signal from a bridge amplifier, proportional to the temperature of the holder (sample), are connected to the two different channels of the analogue digital converter in an IBM compatible computer. A special programme composite in C and machine languages reads and transfers data to the memory of computer every 2 ms and allows their simultaneous graphical presentation on the computer display. The mathematical treatment of the about 50 000 pairs of numbers - temperature and thermoluminescence (adjiance averaging, drawing of graphics and copying on the paper) is carried out by the help of programme package Origin 3.0.



An important part of the equipment is the holder of the samples, presented on Fig. 2. It is made by aluminium and consists from two parts. The first one which is the real sample holder is equipped with the resistance wire -3 Ohm/10 A, for the heating and microthermoresistor (FMF 2101, UMWELT SENSOR TEHKNIK) for the temperature measurement, and the second – extensor with handle. The first part should be made as thin as possible. The thermoresistor should be placed exactly on the surface of the metal block so that the sample (leaf disk or filter paper moistened with chloroplast suspension) should be in contact with it. The heating rate should be in the range 0.3-1 °C.s⁻¹.

On Fig. 3 is presented the electric diagram of the preamplifier the input of which is connected to the anode of the photomultiplyer and the output to the input of the photon counting device.



Fig. 3. The electric diagram of the preamplifier

On Fig. 4 is presented the principal scheme of the bridge amplifier used for temperature registration. The microthermoresistor is connected to the points A_1 and A_2 and the output is directed to the one of the channels of the analogue digital converter.

Fig. 5A represents an illustration of the recorded thermoluminescence curve, obtained with phaseolus leaf disk (with 8 mm of diameter), after one flash, given at 10 °C and after cooling in liquid nitrogen, which could be seen on the monitor of the computer during the heating between -25 °C and 45 °C. The data of this figure after adjiance averaging with 100 points are presented on Fig. 5B. The maximum in the



Fig. 4. The principal scheme of the bridge amplifier

thermoluminescence curve, obtained at 25 °C represents the so-called "B"-bands, reflecting the quantity of the oxygen evolving centres in S₂-states according to the model of Kok et al. (1970). In the frame of the hypothesis of the Rhutherford et al. (1982) and Demeter and Vass (1984) the thermoluminescence "B"-bands under such conditions is a result of the recombination of the S₂²⁺ and Q_B⁻ pairs.



Fig. 5. Thermoluminescence curves, obtained with phaseolus leaf disk (For explanation see the text)

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References

- Arnold, W., H. Sherwood, 1957. Are chloroplasts semiconductors? Proc. Natl. Acad. Sci. USA, 43, 105–114.
- Arnold, W., J. R. Azzi, 1968. Chlorophyll energy levels and electron flow in photosynthesis. Proc. Natl. Acad. Sci. USA, 61, 29–35.
- Demeter, S., I. Vass, 1984. Charge accumulation and recombination in photosystem II studied by thermoluminescence. I. Participation of the primary acceptor Q and secondary acceptor B in the generation of thermoluminescence of chloroplasts. Biochim. Biophys. Acta, 764, 24–32.
- Kok, B., B. Forbush, M. McGloin, 1970. Cooperation of charges in photosynthetic O₂ evolution. I. A linear four-step mechanism. Photochem. Photobiol., 11, 457–475.

- Rubin, A. B., P. S. Venediktov, 1969. On light energy storage by photosynthesizing organisms at low temperatures. Biofizika, 14, 105–109 (In Russ.).
- Rutherford, A. W., A. R. Crofts, Y. Inoue, 1982. Thermoluminescence as a probe of photosystem II photochemistry. The origin of the flash-induced glow peaks. Biochim. Biophys. Acta, 682, 457–465.
- Sane, P. V., V. G. Takate, T. S. Desai, 1974. Detection of the triplet states of chlorophylls *in vivo*. FEBS Lett., 45, 290–294.
- Shuvalov, B. A., F. F. Litvin, 1969. The mechanism of delayed light emission of plant leaves and energy storage in reaction centers of the photosynthesis. Mol. Biol. (Kiev), 3, 59–73 (In Russ.).
- Strehler, B. L., W. Arnold, 1951. Light production by plants. J. Gen. Physiol., 34, 809-820.