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Backward electron transport in photosystem 2 reaction center and temperature dependence of delayed luminescence characteristics

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Abstract

The temperature dependence of parameters of light-induced changes in millisecond delayed luminescence (half-width of the maximum, maximal and steady-state luminescence intensity) is studied within the temperature range from −23 to 45 °C in leaf segments of Chinese rose (*Hibiscus rosa sinensis*). Delayed luminescence (DL) is induced and registered by a homemade setup based on a Lewis–Kasha-type phosphoroscope. The temperature dependence of steady-state luminescence intensity is shown to have two maxima, at −10 and 35 °C. At room temperatures, the steady-state value of luminescence intensity is minimal, and its value correlates with the temperature tolerance of the plant. Depending on cooling and heating regimes, the DL steady-state value vs. temperature curves is found to be different. We suppose this effect to be caused by temperature-induced destructive changes in the structure of photosystem 2 reaction centre and probably by salting out.
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Keywords: Delayed luminescence; Cold and heat injury; Tolerant range

1. Introduction

The delayed luminescence (DL) phenomena can be described as light emission of photosynthetic organisms shortly after their illumination, but later than prompt luminescence emission. In a final step, DL is radiated during the same excited P680 (P680*) to P680 transition as prompt luminescence. In case of prompt luminescence, P680* is created directly by excitation, while for DL the P680* state results from recombination of products formed in a primary photochemical act. So unlike prompt luminescence which does not need more than one single chlorophyll molecule to be emitted, the entire entity of the photosynthetic apparatus is necessary for DL emission.

Using the theory of higher plant DL developed during last several years, it is possible to determine such characteristics of primary processes as rate constants for forward and activation energies for backward electron transport reactions in the photosystem (PS) reaction centre (RC), the charge location in the RC and the state of Calvin cycle [1–4]. Earlier it has been shown that DL is a sensitive test for the state of the photosynthetic system. Even the changes in the rate of transport through the phosphate translocator are clearly manifested in induction kinetics of delayed luminescence [4]. The aim of this work was to investigate the temperature dependence of delayed luminescence induction kinetics.

2. Materials and methods

For the measurements, we used the circular cuttings ($d=2$ cm) of the leaves of *Hibiscus rosa sinensis*. The plant was grown in the laboratory on the natural soil. Delayed luminescence measurements were performed on a homemade set [4], where the sample holder was modified to maintain the sample at necessary temperature in the range from −25 to 50 °C. The sample was cooled by the heat-absorbing side of a Peltie module to which a thermocouple was fixed; the heat-emitting side was fixed on the holder that served as a radiator. For better temperature contact the space between the holder and the Peltie module was filled with special heat-conducting paste. The holder was cooled by circulating water, the temperature of which could be kept constant by a thermostat in the range of 10–80 °C, or by ice cubes inside the holder. The Peltie module was connected to a power supply with voltage of 10 V and 5 A current

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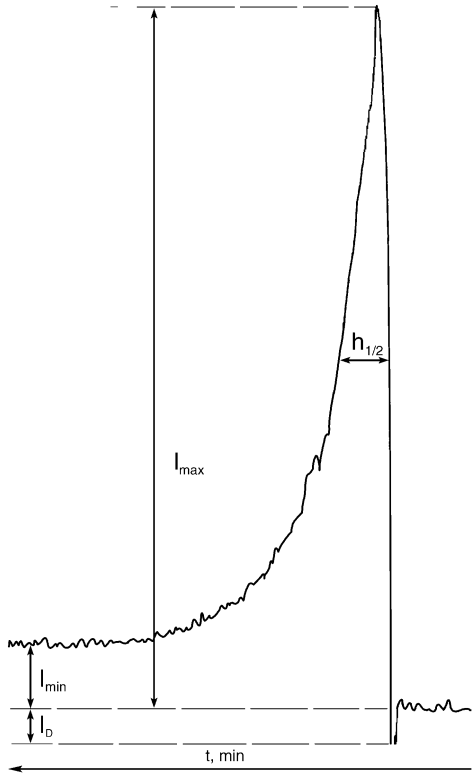

Fig. 1. Notations on typical DL induction curve. I_{max}—maximal DL intensity, I_{min}—its steady-state value, I_0—parasitic constant signal in the absence of sample, $h_{1/2}$—half-width of DL peak.

through a variable resistance, permitting to keep the Peltie element at the voltage from 0 to 10 V and thus regulating the temperature difference between the heat-absorbing and heat-emitting side in the range from 0 to -25 °C. The temperature of the sample from 0 to -25 °C was obtained with Peltie module on the ice-cooled holder, from 25 to 0 °C—with Peltie module on a holder cooled by circulating 25 °C water and from 25 to 50 °C—by circulating water of corresponding temperature with Peltie switched off. The usual experimental protocol was as follows. The sample was illuminated by red light for 30 s, then dark adapted for 5 min; during this time, the necessary temperature was established. Delayed luminescence was excited by series of light flashes of 1.8 ms duration repeated with 100 Hz frequency and the luminescence intensity was measured in the middle of the pause between the flashes for 1.8 ms. For every new experiment, a new piece of leaf was taken; series of experiments were performed either on the cuttings from the same or from different leaves. For registration of temperature dependence of steady-state DL intensity at different cooling regimes one sample usually was used: when the DL intensity reached its steady-state value, the temperature was changed and in 5 min after the new temperature value was established the measurement was repeated with the same sample. Two regimes of heating were used for registration of temperature steady-state DL temperature dependence. In the first the sample was gradually cooled from the room temperature, in the second the sample was primarily cooled to -23 °C and then slowly heated up to room temperature. I_0, the constant signal of holder without any sample, was measured before each series of measurements and subtracted from registered DL intensities.

3. Results and discussion

Fig. 1 shows a typical delayed luminescence induction curve, taken at 37 °C. The following parameters were used for analysis of the DL induction curves: I_{max}—maximal intensity of DL, $h_{1/2}$—half-width of DL peak, I_{min}—steady-state DL level. The temperature dependences for these parameters are given in Figs. 2–4. In these figures, each point without error intervals corresponds to a new sample for two full series, while the points with error bars result from averaging over all series.

One can see that maximal intensity of DL differs from zero at temperatures between -23 and 50 °C (Fig. 2), half-width of DL maximum has a single peak at about -10 °C (Fig. 3). Fig. 4 shows the temperature dependence of steady-state DL intensity. The existence of two peaks on this temperature dependence can be explained in the following way. At temperatures lower than -23 °C some of the forward electron transport reactions, especially the diffusion ones, are much slowed and the backward electron transport is inhibited by low temperatures. As the temperature increases, the forward reactions are still slow, while the rate of backward reactions in the PS 2 RC increase as $\exp(-E_A/kT)$, where E_A is the activation energy, k is the Boltzmann constant and T is temperature and we have a maximum around -7 °C. With increasing temperature, the forward electron transport overgrows the backward ones, that is why at physiological temperatures the DL luminescence intensity is lowest that corresponds to most effective utilisation of light energy. At 30–40 °C, we suppose that the Calvin cycle

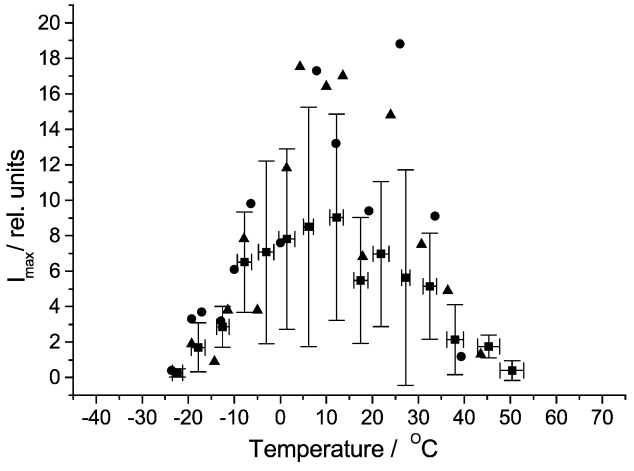

Fig. 2. The temperature dependence of I_{max} maximal DL intensity. ●, ▲—series measured on the pieces of one leaf, ■—average over all experiments.

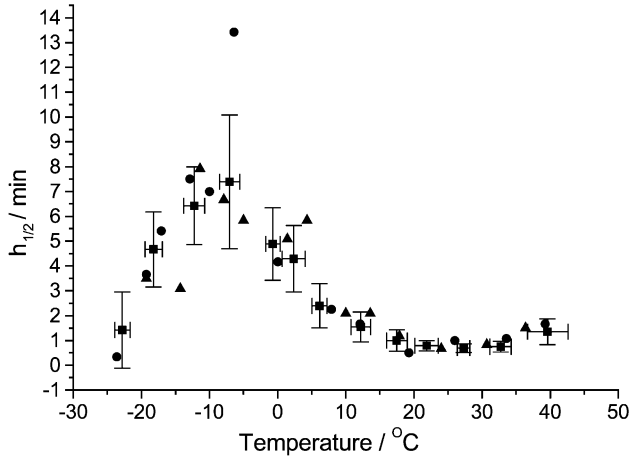

Fig. 3. The temperature dependence of $h_{1/2}$ half-width of DL peak. ●, ▲—series measured on the pieces of one leaf, ■—average over all experiments.

activity decreases, causing the increase in backward electron transport and hence in DL intensity. At 45–50 °C, destructive changes in the PS 2 RC take place, making both forward and backward electron transport in the RC impossible and resulting in the fall of DL intensity. The position of maxima on steady-state DL intensity vs. temperature curve is reported to correlate with heat and cold-tolerance of the plant [1].

We have found that the temperature dependence of steady-state DL intensity is very sensitive to the cooling or heating regime we use in the experiments. At heating of a piece of *H. rosa sinensis* leaf, primarily chilled to −23 °C, up to 25 °C, only a weak maximum of steady-state DL intensity is observed around −10 °C. When the steady-state DL luminescence intensity is measured during the cooling of the cutting from the same leaf from 25 to −23 °C, a wide maximum is observed (see Fig. 5). We suppose that during the cooling the structure of PS 2 RC is irreversibly changed, leading to impaired forward electron transport.

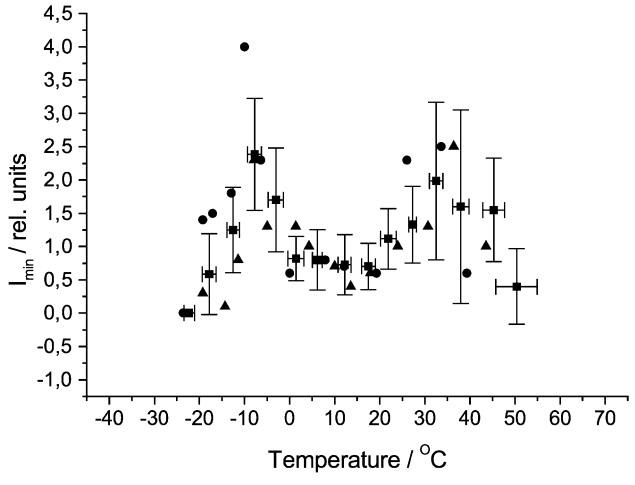

Fig. 4. The temperature dependence of I_{min}. DL steady-state value. ●, ▲—series measured on the pieces of one leaf, ■—average over all experiments.

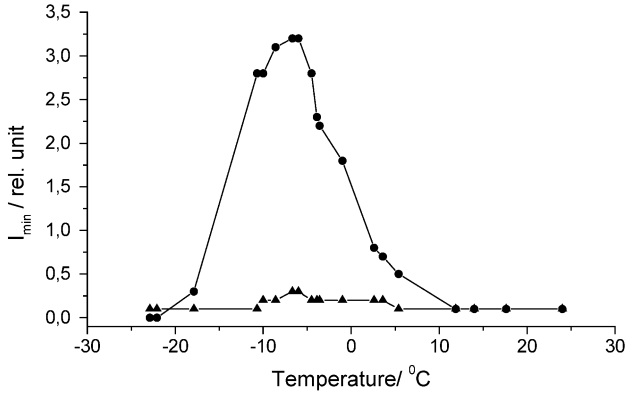

Fig. 5. Temperature dependence of steady-state DL intensity (measured on the same sample) for different cooling and heating regimes. ●—gradual cooling from room temperature to −23 °C, ▲—fast cooling to −23 °C and then gradual heating to room temperature and measurement.

Observations of structural rearrangements in the PS 2 RC resulting in change of electron transport rate may be found in literature. The study of characteristic times of electron transport in bacterial reaction centres has shown the 1.5–2-fold increase in this rate when the samples was chilled from room temperature to liquid helium [6], and this observation remains unexplained. In the work of Stowell et al. [7], the changes in the rate of electron transfer in the region of plastoquinone for *Rhodobacter sphaeroides* between different cooling regimes were observed. Ducruet [8] reports several artefacts in thermoluminescence measurements, connected to the cooling of the sample. Finally, Harnischfeger [9] has shown that the fluorescence spectrum of the sample depends on the cooling rate. It can be supposed that this effect is caused by the physico-chemical phenomena of salting out, the protein condensation in the solution with high ion concentrations. This supposition can also explain the non-monotone changes of the so-called "work integral" with cooling, discovered in Ref. [10].

4. Conclusions

In the present work, the temperature dependences of delayed luminescence parameters were determined for the leaves of *H. rosa sinensis*. Temperature dependence of steady-state delayed luminescence intensity shows that even at leaf cooling to −23 °C some destructive changes in the PS 2 RC may start, leading to abrupt decrease in the rate of forward electron transport and hence both in prompt fluorescence and delayed luminescence intensity.

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