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Simultaneous analysis of variable and delayed chlorophyll fluorescence during induction period in photosynthetic apparatus

V Goltsev [1], I Yordanov[2], D Stefanov[3,2], I Zahareiva[1], P Lambrev[1], R Strasser[4]

[1]*Dept. Biophysics and Radiobiology, Fac. Biology, Univ. Sofia, e-mail: goltsev@biofac.uni-sofia.bg, fax: (+3592) 656-641*
[2]*Inst. Plant Physiology, Sofia, Bulgaria;* [3]*Dept. Biol., Grad. School Sci., Osaka Univ., Osaka, Japan;* [4]*Lab. Bioenergetics, Univ. Geneva, Switzerland*

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Introduction

Luminescent methods based on registration of emitted by the photosynthetic apparatus quanta of prompt and delayed chlorophyll fluorescence (PF and DF) are one of most perspective biophysical methods for express analysis of the plant state. Although light quanta are emitted by the same population of molecules, the two types of fluorescence contain information about different fundamental processes of the photosynthetic apparatus (Vesselovski and Vesselova 1990; Krause and Weis 1991). The simultaneous registration and analysis of both luminescence types enriches our knowledge and understanding about the current state of the investigated object.

In the present work we describe equipment for synchronized registration of PF and DF induction kinetic curves (IKC) in the micro- and millisecond time range, as well as for analysis of photoinduced changes in the kinetics of delayed fluorescence dark relaxation.

Materials and Methods

Plant material

Leaves from wild type barley and its chlorophyll *b*-less mutant (*chlorina f2*^{2800}) were used as experimental objects. Before measurements whole plants were dark-adapted for 1 hour. Leaf segments were transferred in the measuring chamber and kept for 1 min in the dark at the experimental temperature of 20°C.

FL-2006 – fluorometer for simultaneous registration of prompt and delayed chlorophyll fluorescence

The registration of the PF and DF was carried out using an FL-2006 fluorometer, manufactured by TEST (Krasnoyarsk, Russia). It allows the simultaneous registration of several essential types of luminescence properties on a single object: i) IKC of PF and DF; ii) kinetic curves of the dark decay of millisecond DF at any given time of the induction period; iii) temperature curves (thermograms) of both types of light emission.

A Bequerel-type disc phosphoroscope separates the PF and DF. The time period of the phosphoroscope work-cycle is 11.3 ms, including 5.5 ms “light period”, when the PF is detected, another 5.5 ms “dark period” for registration of DF and two non-detection intervals of 0.35 ms between them. The disc rotates at ca. 2000 rpm so that the time needed to fully open/close the working aperture of the phosphoroscope does

not exceed 0.2 ms. The fluorescence emitted during the "light period" is detected by a FEU-79 photomultiplier with a multialkaline photocathode type S20. In order to prevent any scattered actinic light to hit on the photomultiplier, a cross-filter system is set up – a blue-green ($\lambda \leq 660$ nm) glass filter before the light source and a red ($\lambda \geq 680$ nm) glass filter before the photomultiplier. During the "dark period" a second photomultiplier detects the emitted DF. The anode signals are registered by a two-channel 10-bit ADC every 40 μs.

A typical recording of the combined PF and DF signal obtained in the first 0.5 s after illumination onset is presented on Fig. 1.

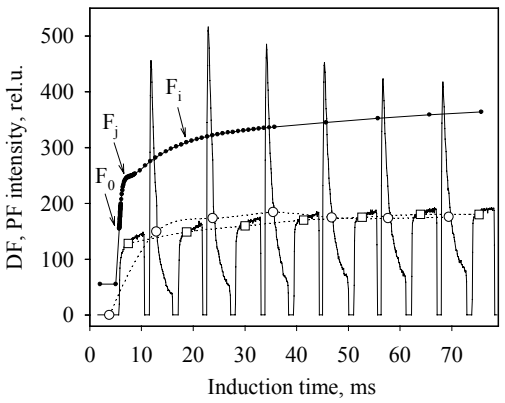

Fig. 1. Combined recording of PF and DF registered through both ADC channels. The signal is a sequence of alternating PF and DF measurements (120 points each), separated by dead intervals. The averaged values form both integral induction curves (PF – square, DF – circle). Integrated curves of PF and DF are plotted to the same scale. Upper curve presents the PF induction trace, registered using Handy PEA (Hansatech Instruments Ltd.), with approximately the same excitation light intensity (1200 $\mu mol.m^{-2}.s^{-1}$). It has been shifted to prevent overlapping.

The PF rise in the first registering cycle reflects the initial phase of the induction transient, that corresponds to the OJ rise observed on high resolution fluorometers (Strasser et al. 1995) (for comparison see Fig. 1). Since the time needed to open the shutter is 0.2 ms and registration starts afterward, the exact value of F_o cannot be measured. Instead of it, the average of the first 10 points is stored as F_o. The DF emitted during the dark period changes remarkably during the registration period and the data stored as an induction point for each period is an average of all collected points.

Results and discussion

Induction kinetics

Upon illumination of photosynthetic objects initially adapted for 1 h in the dark, both PF and DF undergo typical changes, referred to as induction transients (Krause and Weis 1991; Radenovic et al. 1994). Fig. 2 represents a 3D view of the PF and DF time course of barley leaves. The DF induction curve, registered in a 3 min time period, encompasses two major phases – a fast one, taking place in the first second of induction, and a slow one, that can last for minutes. The IKC as a whole can be regarded as the combined result of 6 superimposed peaks, denoted in order of their occurrence on the timescale as $I_1 \div I_6$. The I_2, I_4 and I_6 peaks correspond to the B, C and D peaks in the nomenclature of Radenovic et al. (1994).

The DF is assigned to charge recombination in PS II reaction centre (RC). The $Z^+P_{680}Q_A^-$ states, generated during the previous period of the sample illumination, have a major contribution to the DF emitted in the millisecond time range. Thus the DF intensity at each moment of the IKC is proportional to the relative concentration of the "open" RC $ZP_{680}Q_A$, which are capable to produce "light emitted" states upon illumination, as well as to the radiative recombination quantum efficiency. On the other hand, the quantum efficiency of the radiative recombination is dependent on the thylakoid membrane energization (Wraight and Crofts 1971) and on the redox state of

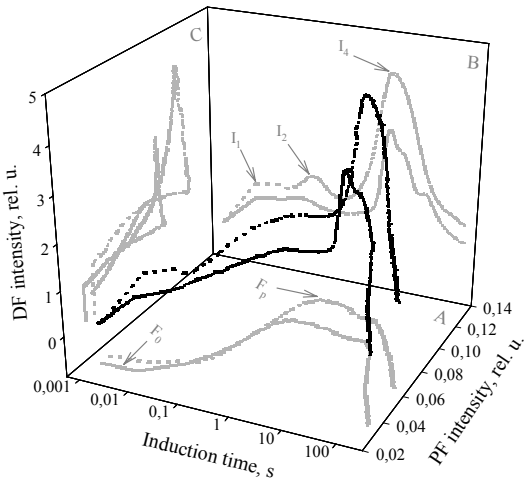

Fig. 2. 3D presentation of the time course of simultaneously recorded integral prompt and delayed chlorophyll fluorescence of leaf disks from barley - wild type (solid) and *chlorina f2* mutant (dotted). Projections (2D) of the experimental points: on plane A – PF induction curves, on B – DF induction curves and on C – phase diagrams (presenting function DF(PF)). Actinic light intensity was 1200 μmol.m^{-2}.s^{-1}.

the next electron carrier Q_B. Therefore one can consider $ZP_{680}Q_AQ_B$, $ZP_{680}Q_AQ_B^-$ and $ZP_{680}Q_AQ_B^=$ as "light emitting" states, whereas $ZP_{680}Q_A^-Q_B^=$ can be marked as "non-emitting". The earlier changes in the DF intensity are related to transitions between these states (Goltsev and Yordanov 1997). The maximums observed in the DF intensity at the millisecond time range (denoted as I_1 and I_2) coincided with the moment of maximal rate of $F_j - F_i$ and of $F_i - F_p$ increase in PF, and their amplitudes reflect the redox transitions in the acceptor side of PS II (Goltsev and Yordanov 1997). The appearance of the late maximums in DF (I_4 and I_5) in the second time range of the IKC can be related to the photoinduced proton gradient as well as to the initiation of the photosynthetic dark reactions (Wraight and Crofts 1971; Gaevski and Morgun 1993). A well-pronounced dip, D_2, is usually observed between the fast (I_1-I_2) and slow (I_4-I_5) phases of the DF induction curves and coincides with the maximal level (F_p) of PF. It may be a result of closure of the RCs of PS II during the PQ pool reduction (Goltsev and Yordanov 1997). I_6 appears in the slow phase, usually at temperatures above 30°C. It can be supposed that I_6 is a result of activation of the Calvin cycle, which is initiated by ATP and NADP.H accumulation.

Dark relaxation kinetics

In each registration cycle the level of DF intensity decreases exponentially with time (Fig. 1). The data measured in the interval between 0.2 and 5 ms after illumination stops can be described analytically as a sum of exponents: $DF(t) = \sum L_i \cdot e^{-t/\tau_i}$. Deconvolution of experimental points gives two exponential decays: $DF(t) = L_1 \cdot e^{-t/\tau_1} + L_2 \cdot e^{-t/\tau_2} + L_3$, i.e. the DF intensity during the dark period of registration can be presented as a sum of three exponentials – a sub-millisecond component with amplitude L_1 and lifetime $\tau_1 \approx 0.4 \div 0.9$ ms, a millisecond component with amplitude L_2 and lifetime $\tau_2 \approx 1.2 \div 3.5$ ms, and a third component with lifetime much greater than the time of the registration period (which is presented as a constant). The parameters obtained by fitting the exponential decay reflect the kinetics of PS II electron transport reactions, related to the accumulation/depletion of "light-emitting" temporary states of the RCs. The parameters' values can be calculated at various times of the induction period (see Fig. 3).

In the time course of the amplitudes maximums similar to those of DF IKC recorded in integral regime are found. In the millisecond time range the dynamics of L_1 and L_2 changes follows the course of DF induction kinetics. The changes of amplitudes of all components are in correlation during slow induction phase (I_4, I_5). The changes of the sub-millisecond and the millisecond decay component amplitudes are associated with the dynamics of different "light emitting" states of PS II reaction centres in the initial

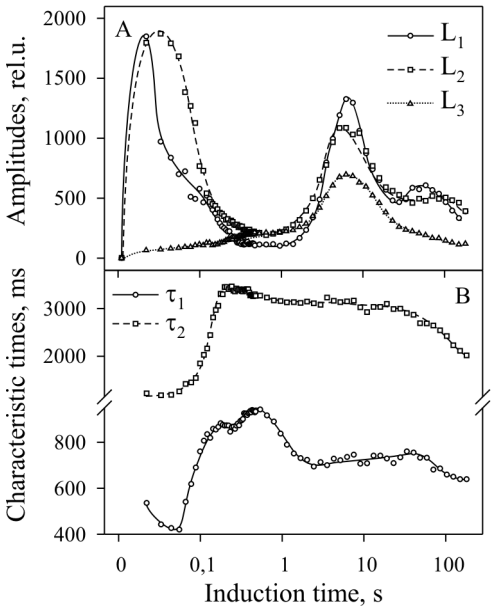

Fig. 3. Time courses of dark relaxation parameters of delayed chlorophyll fluorescence in leaf disks from barley wild type. The parameters were calculated by fitting of experimental DF decay kinetics using equation $DF(t) = L_1 \cdot e^{-t/\tau_1} + L_2 \cdot e^{-t/\tau_2} + L_3$. A – amplitudes L_1, L_2 and L_3. B – lifetimes, τ_1 and τ_2, of the kinetic components.

part of IKC (up to 1 s). During slow phase (1 ÷ 10 s) this is probably a consequence of transmembrane proton and electric gradients (Schmidt and Schneckenburger 1995). Both τ_1 and τ_2 grow within the first 300 ms of IKC. The change of the first component supposedly correlates with reduction of Q_B to Q_B^- and of the second – with the PQ pool reduction and the closure one of the ways leading to disappearance of "light emitting" states. The following acceleration of the DF decay for the fast component occurs in the period 1–5 s, simultaneously with the accumulation of transmembrane proton gradient. The acceleration of the slow component occurs after 20–30 s, when the photosynthetic dark reactions are activated.

The emission, decayed in the submillisecond domain is associated with the recombination of $Z^+P_{680}Q_A^-$, and the lifetime (τ_1) is determined by the probabilities for direct and back electron transport in this state. The millisecond characteristic time (τ_2) represents emission of PS II reaction centres in Z^+P_{680} $Q_A^-Q_B^=$ state, and L_2 reflects the moment concentration of the PS II reaction centres in $Q_AQ_B^=$ state (Goltsev et al. 1998; Zaharieva et al. 1998).

In conclusion, luminescence measuring equipment is presented, allowing the simultaneous registration of the variable and delayed fluorescence, and providing a wide variety of parameters, characterizing the transition of the photosynthetic apparatus from dark-adapted to light-adapted state.

Acknowledgments:

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