THE Fo AND THE O-J-I-P FLUORESCENCE RISE IN HIGHER PLANTS AND ALGAE

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Abstract. The variable chlorophyll (Chl) a fluorescence yield is related to the photochemical activity of photosystem II (PS II) of the oxygen evolving organisms. The kinetics of the fluorescence rise from the minimal yield Fo to the maximal yield Fm is a monitor of the accumulation of net reduced Qa with time in both active (Qb-containing) and inactive (non-Qb) PS II centers. The measurements of true Fo and that of the complete fluorescence transient from true Fo to Fm are useful in obtaining a kinetic picture of PS II activity. Using a shutter-less system (Plant Efficiency Analyzer, Hansatech, UK) that is capable of providing the first measured point at about 20 microseconds and that allows data accumulation over several orders of magnitude of time, we have measured the complete fluorescence transient in low and moderate (up to 700 W m^{-2}) light intensities in several photosynthetic systems (higher plant leaves and chloroplasts; and the cell suspensions of green alga Chlamydomonas reinhardtii and several of its herbicide-resistant mutants, altered in single amino acids in its D1 protein). In all cases, the fluorescence transient follows a regular pattern of O-J-I-P--T, where two intermediate inflections J (at about 2 ms) and I (at about 20 ms) appear between Fo and Fm levels. Furthermore, the ratio of Fm to Fo is about 5 in all cases and the lowered published ratio in several cases is suggested to be due to the J level being mistaken for Fo. We also present data on the effects of varying the dark times between preillumination and measurements of the transient, on the intensity dependence, and on the effect of the addition of diuron. The relationship of the O-J rise to the fast fluorescence rise observed by other investigators will also be discussed.

Chlorophyll (Chl) a fluorescence transients provide information on the photochemical efficiency of photosystem II (PS II) of the oxygen evolving organisms[1,2,3,4]. Fo (or the O level) level is the "instantaneous" low fluorescence when photochemical efficiency is maximal; here, the concentration of the electron acceptor Qa is maximal. Measurement of true Fo level is not a trivial problem in instruments that use camera shutters. Most mechanical shutters have a full opening time of one or more ms. On the other hand, light emitting diode has the advantage that no shutter is needed and the time resolution can be as short as 500 ns.

With a new commercial instrument (Plant Efficiency Analyzer,

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Hansatech, UK) Chl a fluorescence transient was recorded in a time span ranging from 20 microseconds to 20 minutes. The data was then plotted on a logarithmic scale covering 6 orders of magnitude. All photosynthetic samples tested showed a typical transient from the "O" to the "P" level with two intermediate inflections that we call J and I, respectively. Thus the regular transient is OJIP, not simply OIP.

Under moderate light intensities (200-700 W m^{-2}), the first rise from O to J is highly sigmoidal and levels off at about 2 ms, the time at which most camera shutters open. However, the I phase levels at about 20 ms, and the peak P at about 200 ms (the times being dependent upon light intensities). Fig. 1A shows our data for pea leaves on a log scale, and fig. 1B,C shows data on two linear time scales. A preillumination of 1 s followed by 5 s darkness before the onset of the continuous exciting light shows an increased Fo and a much faster fluorescence rise to the J level followed by a distinct decline and then an increase to the P level. After several minutes in light, the fluorescence reaches the terminal level T. Interrupting the light for 2 s after T reveals the absence of variable fluorescence in this state.

In the green alga Chlamydomonas reinhardtii, that had been measured by instruments with camera shuttters to have hight Fo (indicated as F at 4 ms) yields (see e.g.[5]), our experiments show that both the wild type and the herbicide-resistant D1 mutant DCMU-4 (S264A) had high variable fluorescence; the ratio of Fm to true Fo was 5 as in leaves and chloroplasts (fig. 2). In S264A mutant, there is a rapid Fo to J rise. It appears that instruments using camera shutters often mistake J level to be true Fo level unless "O" level is marked by calculation from measurements at low light intensities.

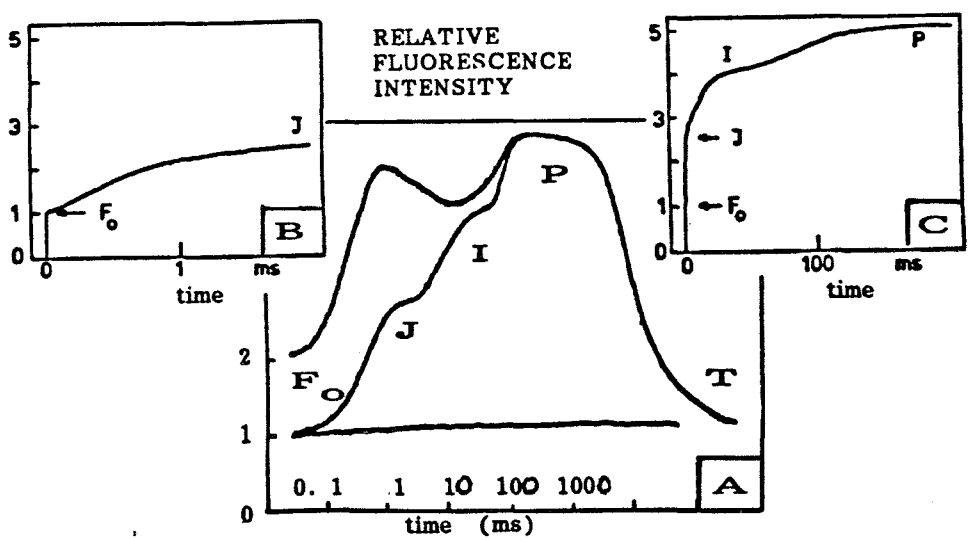

Fig. 1. OJIP--T Chlorophyll a fluorescence transient of an attached pea leaf, excited with red (650nm) LED's giving an intensity of 650 W m^{-2}, are shown on a logarithmic (A) or on a linear (B,C) time scale. Fig. 1A middle curve and Fig. 1B and 1C were plotted from the same data points of a dark adapted sample. Fig 1A upper curve shows a dark adapted sample which was preilluminated for 1s followed by 5s dark before the onset of continuous light. Fig. 1A lower curve shows the fluorescence trace when the light in the steady state T was turned off for 2s.

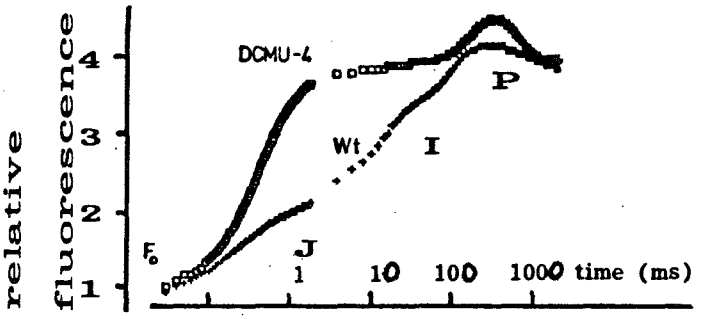

Fig. 2. OJIP Chlorophyll a fluorescence transient of Chlamydomonas reinhardtii (wild-type wt) and the herbicide-resistant D1 mutant DCMU-4 (S264A).

To gain an insight into the dynamics of the O-J-I-P transient in pea leaves, three sets of experiments were performed: (1) a preillumination (the same as measuring light) of 1 s was given to dark-adapted pea leaves followed by a variable dark time before the transients were measured; (2) after the pea leaves were adapted to continuous light (several minutes) and the fluorescence had reached the terminal level T, a variable dark time was given before a succeeding set of transients were measured; and (3) fluorescence transients were measured in dark-adapted leaves upon excitation with different light intensities. Our results showed that the first O to J phase is very sensitive to preillumination and to the dark time between preillumination and measurements, whereas the slower I to P phase was not. On the other hand, in the light-adapted state, the fast OJ rise is regenerated in one or two minutes dark, whereas the P level required about 20 minutes. Furthermore, the J level requires much higher light intensities than the P level to saturate. As expected, the addition of diuron, that blocks electron flow beyond Qa, provoked a fast fluorescence rise from the O to the J level. Studies on the heterogeneity of photosynthetic units have been reported earlier by one of us[12,13] on fluorescence transient curves in the presence of diuron. They can be deconvoluted in two exponential and one hyperbolic functions. According to the grouping (cooperativity) concept[14], these fluorescence kinetics were attributed to big (with LHCII's), small (without LHCII's) and grouped (with cooperativity) PSU's.

The O to J rise measured here at moderate light intensities is obviously related to the fast fluorescence rise measured at high light intensities by the use of specialized methods to get a shutter opening time in μs range[6,7,8] or using, like we do, a shutterless system and fast date acquisition[9]. By the use of modulated fluorescence methods the appearence of a new peak in the fluorescence transient has been reported[10,11] and called I_1. However, no true kinetics of the OI_1 phase has been measured and this I_1 appeared as a peak only at extremely high light intensities (up to about 15.000 W m^{-2}), whereas in our experiments a short preillumination of moderate light (e.g. 1s and 300-600 W m^{-2}) was enough to create the OJ phase as a peak as high as the peak fluorescence P. Further research is needed to correlate the OI_1 phase of high light conditions to the regular OJIP-transient seen in all analyzed plants.

In conclusion, the method used in the current study, that allows the measurement of the complete transient from the O level to the T level over several orders of time scale from microseconds to minutes, provides the

measurement of the true Fo level and the kinetics of all the transient changes (OJIP) in moderate light intensities. We can see the entire OJIDP--T-transient at one look when plotted on a logarithmic time scale.

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