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ON THE O-J-I-P FLUORESCENCE TRANSIENT IN LEAVES AND D1 MUTANTS OF *CHLAMYDOMONAS REINHARDTII*

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1. THE O-J-I-P TRANSIENT

Chl a fluorescence transient is an excellent probe for measuring the effects of stress on the PSII photochemistry of plants and for selecting PSII mutants. Using a shutterless system (time resolution, 10 μs: Plant Efficiency Analyser (PEA) by Hansatech Instr., UK), we show (Fig.1) that the rise of this transient follows: O-J (2 ms)-I (30 ms)-P (200 to 500 ms) at saturating red (500 to 600 Wm^{-2}) light. All three steps J,I,P can appear as intermediate optima followed by minima or dips which are more or less pronounced depending upon the sample (Fig.2). It is shown here that what varies in the D1 mutants of *C. reinhardtii* is the P/J ratio, not the P/O ratio, as measured earlier by camera-shutter instruments [1]. This O-J-I-P sequence was first reported by the authors last year [2], and it was a clear extension of the described fluorescence transients discussed in reviews [3-5] as O-I-P-T (if the dip D is not mentioned).

2. DCMU AND PREILLUMINATION EFFECTS

Both DCMU and preillumination raise the "J" level (F_J). Placing a drop of DCMU (1mM) on the lower side of an attached pea leaf and measuring the fluorescence transient of that leaf from the upper side (measurements during 2 h) led to a transformation of the three step fluorescence rise OJIP into an O-J rise only (Fig.3). This O-J rise appears more or less sigmoidal on a linear time scale depending upon the preparation of the sample (e.g., sigmoidal in high salt, 10 mM $MgCl_2$, and exponential in low salt, 0.5 mM $MgCl_2$, chloroplasts). We conclude that the O-J-rise has the same origin as the fluorescence rise observed in low light in DCMU treated samples [6] or the fast rise under high light reported by Delosme [7] as "I" which, however, does not correspond to the I in the Chl a fluorescence transient nomenclature. We also think that the O-J-rise is different than the O-I_1-rise [8] observed at extremely high light intensity. As seen in Fig.1, a preillumination of 1s followed by a dark period of 5 seconds provoked a O-J-rise as high as the P-level while the I and P levels remained unchanged. The J-level decreased as the dark time between the two illuminations increased.

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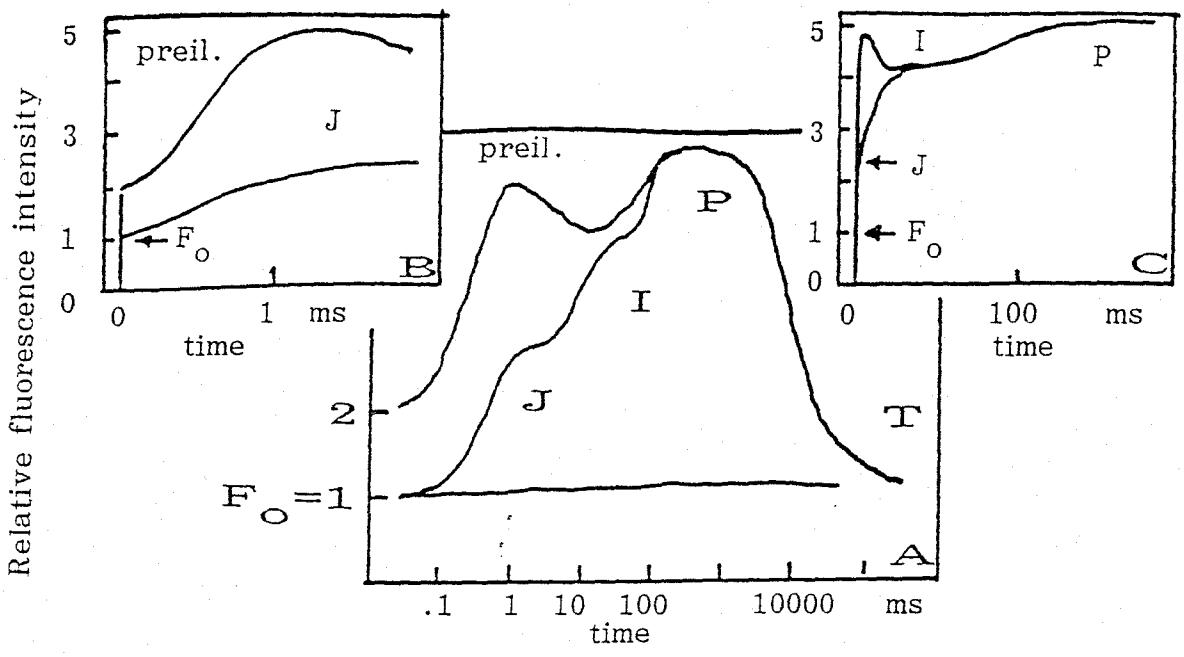

Fig.1. OJIP--T Chlorophyll *a* fluorescence transient of an attached pea leaf, excited with red (650nm) LED's giving an intensity of 650 W m^{-2}, are shown on a logarithmic (A) or on a linear (B,C) time scale. Fig.1A (middle curve) and Fig.1B and 1C were plotted from the same data points of a dark adapted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

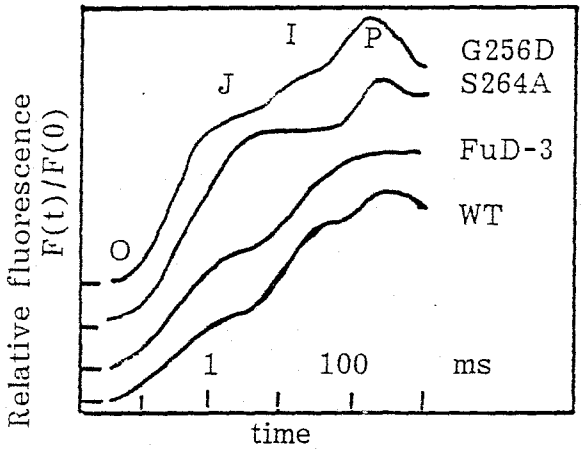

Fig.2. O-J-I-P fluorescence transients of *Chlamydomonas reinhardtii* cells. Measurements are on colonies directly on the petri dish. WT, wild type; FuD-3, PS I RC less mutant, and two D1 mutants S264A (DCMU-4) and G256D (AR 204). Curves are staggered to avoid overlap.

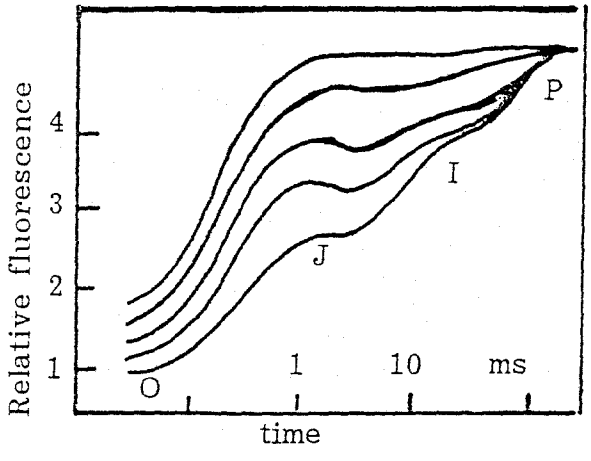

Fig.3. Fluorescence rise from Fo to F_{max} of an attached pea leaf on which a drop of 0.1 mM DCMU was applied. The different curves (from bottom to top) correspond to the time after application of DCMU: 0, 0.5, 1.0, 1.5, 2.0 hours. The same area of the leaf is measured.

3. DMQ - DCBQ EFFECTS

An experiment with dark adapted pea thylakoids in the presence or the absence of quinones DMQ and DCBQ is shown in Fig.4. The experimental conditions were the same as used by Cao and Govindjee [9]. We confirm that DMQ quenches down the F_{max} to the I-level of the control and DCBQ quenches the F_{max} level down close to the J-level (measured as F_{2ms}) of the control, (Fig.4A). The same trend was found whether 0.1 mM ferricyanide was present or not. The quantum yield of photochemistry (measured as $(F_{max}-F_o)/F_{max}$) was nearly unchanged by DMQ; however, it decreased in the presence of DCBQ (Fig.4B). The fractions V_J (at 2 ms), and V_I (at 30 ms) of the <u>total</u> (at 500 ms) variable fluorescence remained nearly unchanged in the presence of DMQ, while in presence of DCBQ the fractions V_J and V_I increased in parallel and the fractions $1-V_J$ and $1-V_I$ decreased.

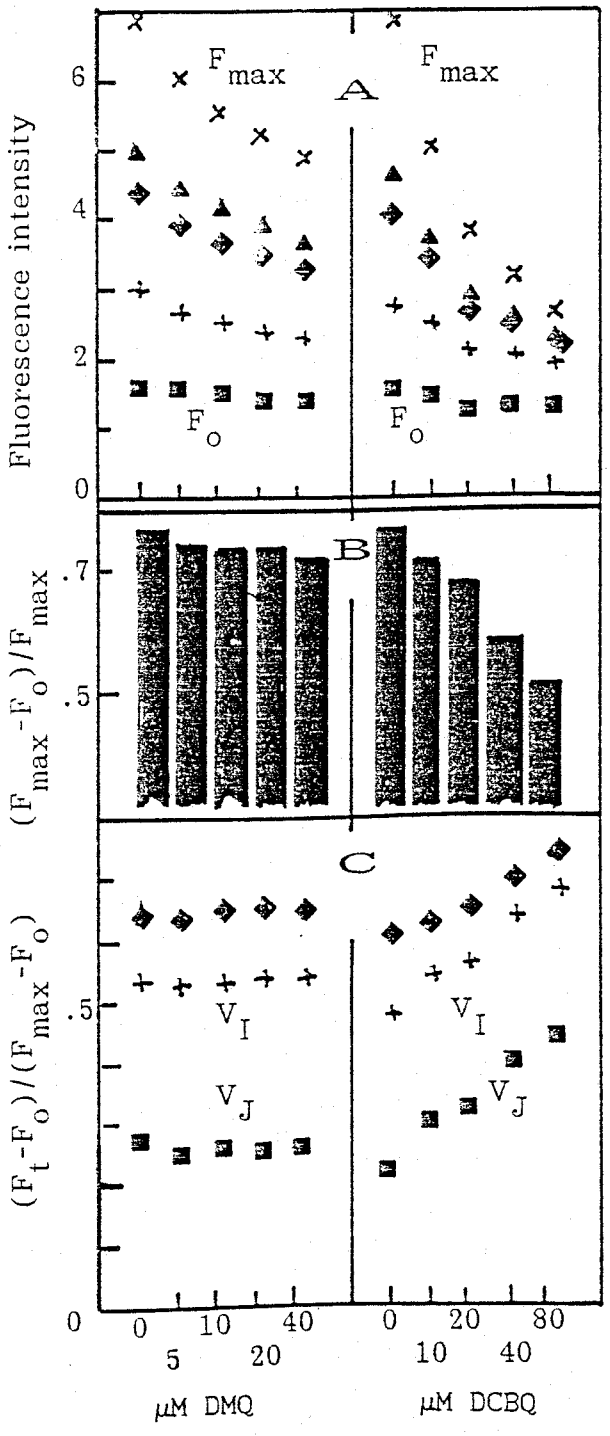

Fig.4. The effect of DMQ (left) and DCBQ (right) on pea thylakoids at 20°C. Reaction medium: 0.4 M sorbitol, 50 mM KH_2PO_4, and 4 mM $MgCl_2$. The Chl concentration was 40 μg/ml.

(A) Fluorescence yields during the rise from 20 μs (■ ; O); to 2 ms (+ ; J); to 30 ms (◆ ; I); to 50 ms (▲) and to F_{max} (✕).

(B) Maximum quantum yield of photochemistry measured as $(F_M-F_O)/F_M$.

(C) Relative variable fluorescence yields at different times of the rise from Fo to F_{max}:

$V_J = (F_{2ms}-F_O)/(F_{max}-F_O)$

$V_I = (F_{30ms}-F_O)/(F_{max}-F_O)$

$V_{50ms} = (F_{50ms}-F_O)/(F_{max}-F_O)$

DMQ stands for 2,5-dimethyl-*p*-benzoquinone.

DCBQ stands for 2,6-dichloro-*p*-benzoquinone.

4. CONCLUDING REMARKS

Under continuous illumination, all observed increases in fluorescence yield are associated with increases in the net $[Q_A^-]$ which belong to all different types of PS II units, according to the grouping concept [5], such as e.g. active (fast or slow) and inactive centers. The total $[Q_A^-]$ can, during the O-P-rise, even decrease, as reflected in fluorescence depressions (or dip) after J and I-levels.

A Working Hypothesis: In a dark adapted sample placed under continuous light, the following events are possible:

O-level as:	$Q_A\,\square + Q_AQ_B$	light ----->	J-level as:	$Q_A^-\square + Q_A^-Q_B$
Dip after J as:	$Q_A^-\square + Q_AQ_B^-$	------>	I-level as:	$Q_A^-\square + Q_A^-Q_B^-$
Dip after I as:	$Q_A^-\square + Q_AQ_B^{2-}$	------>	P-level as:	$Q_A^-\square + Q_A^-Q_B^{2-}$

Ignoring protonation, exchange of PQ with Q_B^{2-} determines the formation of PQH_2. A preilluminated sample shows a very different fluorescence rise because at the beginning of the second illumination a mixture of open and of closed centers is present.We believe that the proper kinetic analysis of the O-J-I-P transient will become a tool in calculating the different equilibria constants between the different types of open and closed reaction centers. Also, it will become a tool to study the dynamics of heterogeneity of photosynthetic units under in vivo conditions. We have observed that the O-J-I-P transient is universal for control samples and that it shows predictable changes depending upon the stress given to samples. For environmental research, we believe, the analysis of the O-J-I-P transient which takes only 1 second will replace the time consuming analysis of the whole transient from O-I-P to T. Therefore many samples (more than 100 per 1 hour) can be screened easily.

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5. REFERENCES

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