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Study of polyphasic fluorescence induction in *Lemna minor* in the presence of methyl viologen (MV) and duroquinone (DQ)

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Introduction

Kautsky fluorescence induction is characterized by a polyphasic rise of the chlorophyll *a* (Chl *a*) fluorescence. The change of the fluorescence intensity indicates the variation of the number of PSII open reaction centres considered to be capable in performing the primary photochemical act and electron transport toward to PSI (Duysens and Sweers, 1963). The fluorescence induction of plant exposed to normal conditions shows different transitory states, beginning with Fo, the so called constant or dead fluorescence corresponding to the minimal fluorescence yield originated mostly from Chl *a* of the PSII light-harvesting complex (Munday and Govindjee, 1969; Butler, 1978). A maximum fluorescence, Fm, is reached when all PSII primary electron acceptor Q_A are reduced. The quantum yield of PSII photochemistry is then evaluated as ϕPSII = Fm-Fo/Fm = Fv/Fm (Genty et al., 1989). Following the maximum level, also noted as F_P transient, the fluorescence yield decreases rapidly by attaining a steady state that corresponds to the balance of the light induced PSII-PSI electron transport and reflects the change of oxido-reduction state of the PSII-PSI electron transport carriers (Briantais et al., 1986). The rapid fluorescence induction obtained by saturating light revealed two fluorescence transitional states denoted as J and I appearing before the maximum fluorescence level F_P (Neubauer and Schreiber, 1987; Schreiber and Neubauer, 1987). Actually, it is accepted that O-J-I-P reflects the successive but overlapping filling-up PSII electron acceptor pools as Q_A, Q_B and PQ whose oxido-reduction states are closely controlled by PSII functions (Govindjee, 1995). The inflection J may represent the maximum of Q_A^- and reflects also the functioning of the water-splitting system (Hsu, 1993). The following fluorescence rise, the J-I transient, characterizes the closure of the remaining PSII open centers resulting in an accumulation of $Q_A^-Q_B^-$ (Strasser et al., 1995, Barthélemy et al., 1997). The maximum yield of fluorescence can be only reach when the PQ pool become reduced, therefore the I-P fluorescence step is a consequence of $Q_A^-Q_B^{2-}$ accumulation (Strasser et al., 1995). These two transients has been suggested to reflect the heterogeneous rates of PQ reduction by showing a fast and slow reduction of PQ pool from two different PSII populations (Krause and Weis, 1991; Strasser et al., 1995).

In this study we demonstrated the dependency between the different dynamic steps of the fast Chl *a* fluorescence rise and the electron transport event related to PSII-PSI activity. In the most earlier studies the use of normalized fluorescence yield was necessary to compare results from different experiments which induced additional difficulties to see the real change of

kinetics and its significance. To avoid this problem we took the advantage by using an adapted optical system combining at the same time the measuring process of PEA (Plant Efficiency Analyzer) and PAM (Pulse Amplitude Modulated) fluorometers. Therefore we were able to analyze different fluorescence induction of the same plant under identical optical set-up conditions. In this investigation, we studied the change of the rapid fluorescence transients in *Lemna minor* exposed to continuous illumination in absence or presence of the exogen electron acceptors, methyl viologen (MV) and duroquinone (DQ). We succeeded to observe a rapid fluorescence transient "inside" of the Kautsky fluorescence induction.

Materials and methods

Lemna minor was cultured in an inorganic growth medium and exposed at 21 °C under constant cool white light at an intensity of 100 µmol. m^{-2} s^{-1}. As PSII and PSI electron acceptors, DQ and MV were used respectively (Henrysson and Sundby, 1990; Dodge, 1982). DQ was dissolved in ethanol at a final concentration of 0.15 % inducing no inhibition of the photosynthetic process (data not shown). By employing combined PEA and PAM fluorometers (Hansatech, England) we measured rapid fluorescence transient with a 1 s PEA saturating flash before, during and after Kautsky fluorescence induction when *Lemna minor* was exposed to actinic light monitored by a PAM fluorometer. The PEA saturating flash was provided by an array of six light-emitting diodes giving a maximum emission at 650 nm with an intensity of 600 $W.m^{-2}$. The fluorescence induction was measured from 10 µs to 1 s and the fluorescence yield at 40 µs was considered as the true Fo value. The rise of the fluorescence

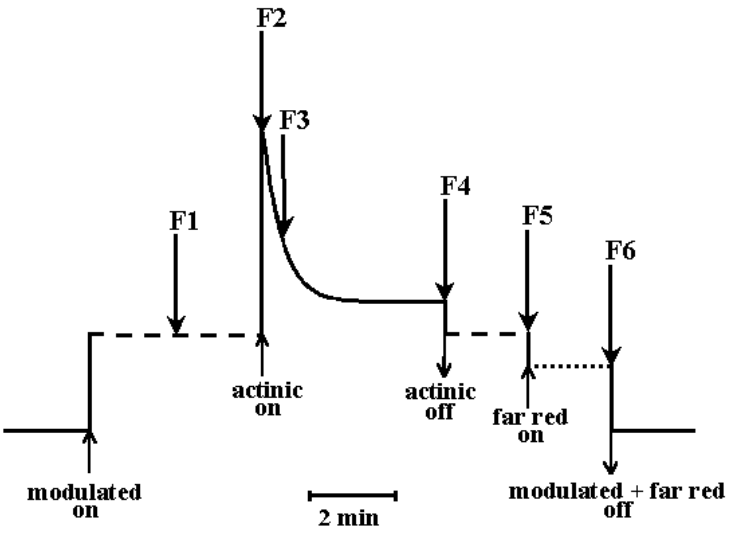

Fig. 1: Scheme of the 1 s PEA flash distribution when *Lemna minor* was exposed to continuous light maintained by a PAM fluorometer.

yield was plotted on a logarithmic time scale allowing the visualization of the rapid transients. Before the fluorescence induction measurement, *Lemna minor* was dark adapted for 30 min and placed in the growing medium containing MV or DQ at concentration of 200 and 50 µM respectively. We measured six independent rapid fluorescence inductions following the protocol shown in fig. 1. When *Lemna minor* was exposed every 2 min to a saturating flash of 1 s, no difference were found by comparing the maximum fluorescence yield and the O-J-I-P fluorescence transients. In our experiments the relaxation time of 2 min was respected except between 2^{nd} and 3^{rd} flash, because time from 3^{rd} flash was conditioned by the time of the fluorescence decay after Fp transient.

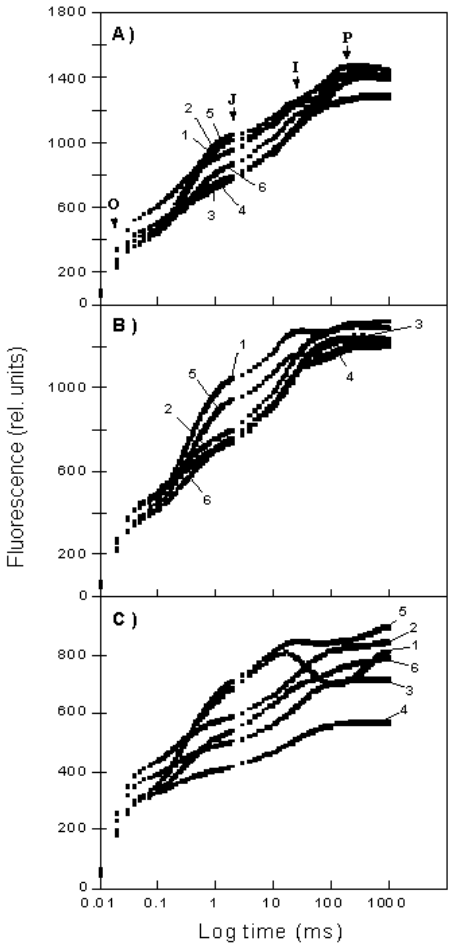

Fig 2. Chl *a* fluorescence induction transients of 30 min dark-adapted Lemna minor. (A) Control; (B) 200 µM MV; (C) 50 µM DQ and exposed to 6 saturating flashes. Vertical arrows indicate fluorescence transition J, I and P.

Results and Discussion

The fluorescence patterns of the rapid transients induced with six separated flashes were different as seen by comparison of the transients obtained under actinic light (fig. 2A, flash 2, 3, 4) with those induced when modulated and far red light were on (fig. 2A, flash 1, 5, 6). It was evident that the oxido-reduction state of Q_B and PQ and probably the charge distribution among PSII primary and secondary electron donors/acceptors, including water-splitting system, have a determining role on the dynamic of Q_A reduction and on the recombination process at PSII reaction center which reflects the variation of the fluorescence yield at J transient (Strasser et al., 1995). The visible similarity between control and MV rapid transients showed that in both cases the linear electron transport was dominant (fig. 2A, B). It should be noticed that the fluorescence transient induced by first flash when *Lemna minor* was treated with MV had a fluorescence yield at I transient very closed to the one at P level. This indicates that the increase of the proton gradient inside of the thylakoid lumen, induced by MV, slows down the electron transport via Q_B and PQ pool (fig. 2A, B). However, DQ proposed as an electron acceptor at Q_A (Henrysson and Sundby, 1990) showed a much more interaction complexity as indicated by the rapid fluorescence transients when compared to those affected by MV. We should emphasize that in the presence of DQ, the fluorescence transient induced by the first flash indicates a rapid Q_A and Q_B reduction and then a rapid Q_B reoxydation process via endogen electron acceptor. This transient does not support the fact for DQ to be only an electron acceptor at Q_A site. The pronounced I transient and following deep

suggest for DQ to participate at Q_B as an electron acceptor. The participation of the cyclic electron transport via cytochrome b_6/f cannot be excluded (fig. 2C, flash 1; tab. IC). We also noticed a decrease of the F_o fluorescence and φPSII in *Lemna minor* treated with DQ when compared to the control. The decrease of the variable fluorescence at J transient may indicate the diminution of the number of PSII active reaction centres. The quenched Fo yield of fluorescence by 50 μM DQ may give evidence that DQ interfere in the excitation energy transfer from light-harvesting complex and in this way contribute as a competitive process in the excitation energy transfer to PSII reaction center (tab. IC).

When *Lemna minor* was exposed to actinic light, the membrane energization process takes place, therefore the fluorescence yield, induced by the analytical flash 2, 3 and 4, was progressively quenched indicating the formation of dissipative PSII reaction centres (fig. 2A, tab. IA). We may interpret that the decreased number of active PSII reaction centres will induced a diminished electron transport activity and energy storage process via Q_A reduction. However, when plant is exposed to a weak modulated light the recovery of rapid transition was noticed (flash 5). This shows that DQ during the dark recovery oxidize the PSII electron carriers. Nevertheless, the quenched fluorescence transients under far-red light may show that DQ does not alterate the electron transport communication between PSII and PSI since the quenching effect indicate the contribution of PSI to the kinetics of the fluorescence induction (fig 2C; tab. IC). *Lemna minor* treated with 200 μM MV showed a diminished variable fluorescence at J transient indicating the decrease of active PSII reaction centres participating in primary photochemistry. We also noticed that the fluorescence induction induced by flash 2, 3 and 4 do not show a distinguishable I transient. Nevertheless, when MV and DQ were not used as an electron acceptors (control plant), Fo under an actinic light was increased dramatically followed by a decrease of φPSII (tab. IB, flash 2, 3 and 4). This indicates that in non-treated plants under actinic light, the efficiency of energy transfer from antenna complex to PSII reaction center was decreased.

Table I : Parameters of the Chl *a* fluorescence transient of *Lemna gibba* exposed to 6 analytical 1s saturating flash: A, control; B, 200 μM MV; C, 50 μM DQ.

	Flash	Fo	Fm	ϕPSII	Fv_J	Fv_I	Fv_P
	1	319	1476	0.78	734	944	1157
	2	506	1434	0.65	601	-	928
A	3	420	1293	0.68	534	-	873
	4	386	1291	0.70	544	-	905
	5	318	1422	0.78	722	910	1104
	6	333	1408	0.76	558	866	1075
	1	313	1324	0.76	753	952	1011
	2	413	1296	0.68	-	-	883
B	3	414	1248	0.67	359	-	834
	4	404	1228	0.67	368	-	824
	5	312	1215	0.74	669	848	903
	6	326	1204	0.73	522	799	878
	1	267	810	0.68	443	539	543
	2	385	842	0.54	214	-	457
C	3	355	717	0.51	155	-	362
	4	301	570	0.47	124	-	269
	5	271	893	0.70	473	578	622
	6	280	786	0.64	276	418	506

We may conclude that the rapid fluorescence transients J, I and P reflects an integrating multifactor effects coming from the charge distribution within PSII complex and water splitting system. Under the condition where DQ and MV were used as electron acceptors the

kinetics of the rapid fluorescence transient reflects the charge distribution between water-splitting system, P680, Q_A, Q_B and PQ.

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