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I. COOPERATION AND LOSS OF + CHARGES IN O₂ EVOLUTION

In the first year covered in this report, we finished two papers on the "Cooperation of Charges in Photosynthetic O₂ Evolution" (1,2). So far, the hypotheses described in these papers have stood up and been accepted by Joliot and co-workers, (3, 4), who are also active in this area. It is obvious, however, that the present model is only a starting point and a long list of questions and alternatives can be compiled. We have investigated some of these:

1) One possible hypothesis to explain the high yield of the third flash (Y₃) given after a long dark period is the assumption that the first flash adds two oxidizing equivalents to the O₂ generating system, while all other flashes add a single one. This assumption is not entirely arbitrary: after long dark the fluorescence behavior is anomalous - the rise curve in the presence of DCMU can reveal a quadratic (2 quantum) character. (As we pointed out in the enclosed manuscript (5), Joliot's early explanation for this behavior - energy transfer between photosynthetic units - might not be satisfactory.) A careful study of the dependence of Y₃ upon the intensity of each of the first two flashes did not show a quadratic intensity dependence, i. e., the use of 2 photons.

2) Another aspect we have studied concerns the loss of O₂ precursors in dark reactions. This loss seems nearly always second order, the (first) half time has been observed to vary between 100 seconds (after illumination with slowly flashing light in the presence of an electron acceptor) and 0.6 seconds (after System II light in absence of exogenous electron acceptor).

Study of this rapid deactivation is complicated: Firstly, in the absence of electron acceptor the steady state rate (Mehler reaction) is low which prevents very precise measurements of its decay. Secondly, in the dark the acceptor pools of System II (Q+A) become oxidized which leads to an increase of the O_2 rate. Adequate analysis, therefore, required several assumptions (see sketch).

We assumed that in light the pool was depleted in the same first order manner, initially (where activation prevents observation of the true photochemical rate - dashed line) as was observed later (solid trace). Data of this type showed a decay of the O_2 rate (V_i) which resembled the decay of fluorescence yield in the presence of DCMU. Bennoun (6) had reasonable grounds to assume that the return of the fluorescence yield in the presence of DCMU reflected a back reaction between the photooxidant and reductant of System II. Possibly, therefore, the same back reaction occurs also in the absence of DCMU: In both cases, primary acceptor "A" (either because of the poison or because the A is in the reduced state).

We have attempted to interpret the interplay of the many parameters involved in these experiments: the rates of pool restoration

and deactivation in dark, the O_2 quantum yield, equilibration constants between Q, A_2 and A_1 (7, 8) and the appearance of the flash yield oscillation. To account for these other parameters and also the overall rate limitation by System I (due to the absence of an electron acceptor), Dr. Radmer has expanded the computer program of Forbush et al. Using reasonable rate and equilibrium constants, the model could predict the observations with surprising fidelity. One interesting point again stressed in this modelling, was the proportionality of the System II quantum yield with the oxidation state of a primary electron acceptor pool of about four equivalents (Q's). Before publishing this material, we intend to perform some sensitivity tests to check how unique the assumptions of the model are.

3) Some other projects, still in progress, might also be mentioned: a rapid loss of O_2 rate (S_3 states) can also be observed in the presence of System I electron acceptors and might not be due entirely to an unfavorable redox state of Q and A. For instance, by adding 650nm background lights on a weak long wave modulated beam, we could measure both "enhancement" and "deactivation". It was possible to estimate the redox state of the quinone pool and conclude that additional reductants in the chloroplast can remove oxygen precursor.

In addition to these laborious kinetic approaches, we have also tried to directly observe the effect of added chemical reductants. Our aim is, of course, to observe not only the loss of S_3 , known to be

unstable, but to chemically induce changes of the flash yield pattern. Specifically, we tried to reduce the presumably stable S_1 state ($S_1 \rightarrow S_0$), which would cause a lowering of yield #3 with a simultaneous increase of yield #4. So far, our success has been meager. Some agents such as uncouplers, poisons, low salt media, etc. lower the photochemical yields but cause no essential changes in the O_2 yield oscillation. Also, anaerobic conditions, and the addition of reduced ferredoxin and/or NADPH seem to induce a transitory loss of System II efficiency rather than acting on the S_1 state. Unfortunately, our present (bare) platinum electrode can only be used with a few exogenous reductants.

The need for a membrane covered platinum electrode was felt long ago. Electrodes of the standard Clark type have a much too slow time response. B. Forbush has spent some time to analyze the "design parameters" of improved electrodes. He succeeded in obtaining a time response of a few tenths of a second, but the fragility of his instrument did not allow routine use. Some recent promising ideas have not yet materialized in an operational instrument.

Photon Transfer Between Pigment Units of System II

Joliot and Joliot (9) observed a nonlinear relation between the flash yield and the quantum yield of O_2 and a non first order fluorescence rise curve in very strong light or in the presence of DCMU. These observations underly Joliot's hypothesis that photon transfer occurs between pigment units of Photosystem II. The validity of the

observations as well as the interpretation have been challenged recently by Myers and Graham (10), who made O_2 flash yield studies and by Doschek and Kok (manuscript included) who studied fluorescence. A valid objection to the measurement of flash yields "on top" of a background light has been raised by Wang and Myers (pers. comm.). They present arguments that measurements of this type can result in a distortion of the flash yield.

This objection, however, does not apply for the type of measurement we introduced during the past year in which flashes were alternated with (10 ms) rate pulses, separated by dark periods (0.1 sec). These measurements still yielded non linear O_2 rate vs. yield plots in normal chloroplasts. However, low salt conditions (and possibly the presence of Antimycin A) induced linearity. This variable appearance of non-linearity - also observed in the fluorescence behavior (5) made us search for alternate explanations. On the one hand, a paper by Stiehl and Witt (11) showed a second order time course for the light driven reduction of plastoquinone. On the other hand, we have obtained evidence that a chromatic effect might be involved; we plan to pursue this possibility further. An improved polarograph illumination system designed for this purpose is nearly completed.

II. INTERACTION BETWEEN SYSTEMS I AND II, EFFECT OF MAGNESIUM, ETC.

A paper by Marsho and Kok (12) submitted in this reporting period described a number of observations concerning the relative concentrations of the electron carriers between the two photoacts and their interactions. For each P_{700} , there appeared to be 12 equivalents in the System II reductant pool (probably 6 plastoquinone molecules), one cytochrome f and one plastocyanin. The behavior of the intermediate which was presumed to be plastocyanin showed true equilibrium with P_{700} ($K \approx 20$). The interaction between cytochrome f and P_{700} , however, was complex, suggesting a high equilibrium constant in darkness and a low constant in the light ($K \approx 5 - 10$). Earlier (13), it was observed in this laboratory that the overall reaction chain between the photoacts ($Q \rightarrow P_{700}$) showed a similar change of the (apparent) equilibrium constant.

Until his departure, Dr. Marsho further pursued this phenomenon, possibly linked to phosphorylation or a high energy state. He noticed that a low salt (Mg) concentration in the suspension medium or the presence of the uncouplers CCCP and Antimycin A, prevented a significant reduction of the A+Q pool by weak System II light. Normally, (i. e., in the presence of $>3 \text{ mM Mg}^{++}$ or $>100 \text{ mM K}^+$) 650 illumination reduces most of the pool - as can be observed in a subsequent transient of the P_{700} signal. Strong rate saturating light, however, reduces the pool in either condition.

Quantum yield measurements were made to explore the cause of this salt effect (a loss of System II efficiency, a change of

equilibrium constant, or - as suggested by Murata (14) - a change of quantum distribution between the two photosystems. The results indicated only a slight effect of low salt on electron transport from H_2O to ferricyanide or viologen or from DCIPH to viologen (isolated System I in presence of DCMU). However, "low salt" considerably decreased the yield of NADP reduction either with water or DCIPH as the electron donor. (These results do not agree with Murata's and only partially with those of Avron and BenHayim (15). Furthermore, measuring O_2 with the modulated polarograph, using NADP as the electron acceptor, we found no enhancement of System II by long wave light. (This same observation was recently reported by Sun and Sauer (13). One effect of low salt thus seems to be specific inhibition of NADP reduction. Recently we studied the effect of "low salt" on the enhancement of the rate of viologen reduction in modulated long wave light by background illuminations of 650 m μ . In weak intensities, we observed the same (relative) stimulation with or without added Mg^{++} . This argues for invariance of the quantum distribution between the systems.

III. HIGH TIME RESOLUTION MEASUREMENTS

Many of the important processes in photosynthesis occur rather rapidly. These include O_2 evolution, the stabilization of charge separation in the traps, and the recovery of these traps. We, therefore, have developed instrumentation to make optical measurements with rather high time resolution (times less than one millisecond).

The instrumentation has features designed to eliminate problems more or less specific to photosynthetic systems.

In these measurements the system is perturbed by a short ($\sim 3\mu\text{sec}$) saturating xenon flash. The recovery of the system is then monitored by a weak measuring beam which does not further perturb significantly.

Because of the severe interferences by the direct fluorescence of the flash and by the delayed light emission and because of the rapidity of recovery, ΔOD and fluorescence measurements in the red spectral region are very difficult. To circumvent this, the measuring beam is modulated at 14 mc. A tuned detection system is used to discriminate this signal from the unmodulated delayed light and flash fluorescence. When it is further necessary to prevent the fluorescence of the flash from reaching the photomultiplier (to avoid saturation), a rotating disc is used, arranged as a phosphoroscope. This prevents the flash and the direct fluorescence it produces from reaching the photodetector; it, however, introduces a time delay between flash and observation (60 μsec in our present apparatus, 10 μsec in a new one, nearly completed).

Since the signals are very small and rapid, the signal to noise ratio is poor; this necessitates extensive averaging: (Our present signal averages have 0.2 μsec time resolution). To further enhance the signal to noise ratio, attempts are made to make the measuring light as strong as possible without perturbing the system. Since such perturbations depend on the duration of the measuring light, this duration is kept short.

A flow through sample cuvette or pulsed sample renewal is used to allow rapid measurements on chloroplasts either dark adapted or otherwise preconditioned by light.

Delayed light measurements are made with the same apparatus, with the measuring light and detector tuning removed.

These precautions allow unambiguous observations on chloroplast particles which have well defined and controlled activity.

Delayed Light Emission

Since a detailed account of many of our observations on delayed light are published (16), only a brief account will be given.

Ever since the observation that green plants show chlorophyll luminescence at times longer than the intrinsic fluorescence lifetime (17), this delayed light has been studied in the hope that such studies could be used to elucidate some of the processes of photosynthesis. We studied the yield of delayed light from 65 to 800 μ seconds after short ($\sim 3 \mu$ sec) flashes in the hope that at these short times the delayed light might reflect some of the initial steps in the O_2 evolving process. Following the lead of Barberi et al. (18), who showed that in a sequence of flashes the longer delayed light (~ 20 msec) oscillates with a period of four out of phase with the oscillation of the oxygen yield, we studied the delayed light from a similar sequence of flashes given to dark adapted chloroplasts and algae. We observed three new, short lived ($t_{1/2}$ approximately 10, 35 and 200 μ seconds) components of the delayed light. The two slower components

oscillate with a period of four in phase with oxygen evolution.

The three components do not result from changes in fluorescence yield; they indicate the behavior of some basic processes occurring in System II. The time limitation of the instrument permits us to say little about the fastest (10 μ sec) component except that it does not oscillate in the same manner as the slower components and that the state leading to this component of delayed light is not formed in the presence of DCMU. The slowest component ($\sim 200 \mu$ sec) is also eliminated by DCMU, probably because more than one photoact is needed for its production.

Since the other components are eliminated by DCMU, the 35 μ sec. component of delayed light can be isolated. It decays in a first order manner. The component can only be formed from traps active prior to the flash. Its yield saturates at about 10^{-4} quanta per trap.

The 200 μ second component appears to be associated with trap recovery which will be discussed later. The 35 μ second component seems to involve some intermediate step. In both cases, the delayed light does not seem to involve a simple recombination of charge from donor and acceptor. Chemical treatment that alters the oscillatory yield of oxygen also alters that of the 35 and 200 μ second components of delayed light. However, methylamine in a concentration that alters both the oscillatory yield of O_2 and the 200 μ second, markedly changes the behavior of the 35 μ second component: a direct

correspondence between the oscillations of O_2 evolution and those of the 35 μ second component of delayed light does not exist.

Fluorescence

In another attempt to understand some of the early processes in System II, fluorescence measurements were made in the time range of 0-1msec. Two types of measurements were made: The first is of the changes in fluorescence yield during short (3-10 μ second) flashes; the flashes being used as both actinic and measuring light. The second (using the apparatus described above) is the fluorescence after brief 3 μ sec actinic flashes, measured using a weak modulated light beam. Some new results obtained from these two types of measurements are described below.

Delosme (19) had previously observed oscillations in fluorescence yield in a sequence of brief saturating flashes (measurements of the first kind). These oscillations are 180° out of phase with oxygen and therefore also out of phase with the 35 μ second component of delayed light. We studied the behavior of this fluorescence under various conditions in an attempt to gain further insight into the origin of the phenomena. As with the 35 μ second component of delayed light, chemical additions that altered the O_2 oscillatory yield of oxygen also change that of the fluorescence seen in the flash. This fluorescence is also changed by methylamine at a concentration that does not affect O_2 markedly. (Fig. 1).

The kinetic behavior of the fluorescence yield during the flash is complex. There appears to be a rise in fluorescence yield followed by a decay (19). When the traps are kept closed by weak background light and DCMU, a flash induces more than a 50% decrease in fluorescence yield. This decrease, which is related to System II, ~~seems to be a trap rather than a bulk chlorophyll related phenomenon,~~ since it is produced by about one quantum per trap. (Fig. 2).

Only preliminary measurements have been made concerning the kinetics for open traps. These indicate a light induced increase in the fluorescence yield, followed by a light induced decrease. The maximum increase in yield observed in such a short saturating flash represents less than a third of the total variable part of the fluorescence obtainable under other conditions. Further measurements are necessary to relate the oscillations to the kinetic components of fluorescence, and to relate the kinetics to function. If we indeed have two sequential photo-induced acts (a light induced rise, followed by a decrease), the first probably represents the formation of some intermediate state related to the charge separation usually envisioned in photosynthesis, while the second probably does not.

By using weak modulated light, we can observe relaxation of the fluorescence after a brief, saturating, perturbing flash. The rotating mechanical shutter does not at present allow measurements of this nature at times shorter than about 60 μ seconds after the flash. The decay that we observe can be described by a 200 μ second component followed by a slower (~ 1 msec) component; the decays being dependent on the S states.

We had previously obtained similar times for the recovery of the O_2 activity of System II after brief saturating flashes (1), indicating that the fluorescence decays parallel recovery of the traps. This can be seen in Figure 3; the times for System II recovery and fluorescence decay are both longer after a second flash than after a first. Superimposed on this decay of fluorescence yield is an amplitude factor related to the state of the system.

Under certain conditions we observed a rise of the fluorescence yield in the dark, rather than a decay. The half time for the rise is about 50 and 100 μ seconds at room temperature and $4^\circ C$ respectively. (Fig. 4). The rise is not observed if the traps are inactive (e.g., inactivated by DCMU and prior illumination) before the flash. Although the rise can clearly be seen (for dark adapted chloroplasts) in the presence of DCMU, for which no decay is present, the time resolution and opposing decay would make it at present impossible to detect any such rise under unpoisoned conditions, if such a rise actually exists. However, at $4^\circ C$, for which the rise is slower, a definite delay in the fluorescence decay is observed (Fig. 5). This decay oscillates with a period of four. The kinetics observed at $4^\circ C$ can be fit either by a 100 μ second rise followed by a decay, or by a delay followed by a decay.

The 50 μ second rise in fluorescence may be related to the 35 μ second component of delayed light; the times agree within experimental error. Further measurements are necessary to

refine the limits of error, mainly those in fluorescence. Assuming the rise takes place in active systems, it is possibly related to a dark step prior to reactivation of the traps. Such a dark step has been suggested by measurements of System II recovery of chlorella at low temperatures (20). Our fluorescence measurements would then indicate that the delay is dependent on the S states.

IV. FUNCTION OF MANGANESE IN PHOTOSYSTEM II

For many years it has been speculated that this transition metal is involved in photosynthesis. The basis of this speculation largely was two fold: 1) autotrophic growth of algae required much higher concentrations of this metal than heterotrophic growth: 2) Mn depletion by growth caused decreased quantum efficiencies of O₂ evolution (System II) but did not affect the quantum efficiency of Photosystem I partial reactions. Considering the susceptibility of System II complex to photobleaching, etc., and the often used long durations of growth to achieve Mn depletion, we scrutinized the validity of these conclusions and sought other means to access the function of manganese in System II. Our studies as well as others (21, 22) have given new insight into the site(s) and function of this metal. We also encountered a need for "photoactivation" of the O₂ evolving Mn enzyme - a hitherto unrecognized phenomenon of photobiology.

Firstly, our studies have indicated that a total pool of 6 Mn/ System II trap function on the H₂O splitting side of System II.

This pool size first was estimated in this laboratory from measurements of flash yields of O_2 as affected by Mn depletion by growth. Later we analyzed the effect of extraction of chloroplast Mn by Tris (23) or NH_2OH (24) on O_2 evolution and on System II sensitized artificial electron donor photooxidations. Such studies showed:

- 1) Each System II trapping and O_2 evolving center contains 6 Mn atoms;
- 2) O_2 evolution capacity is abolished with the loss of 4 Mn; O_2 evolution capacity in weak or strong light proved to be linear with this Mn. Such results indicated that complete O_2 reaction centers are inactivated by such extractions. We tentatively equate this pool of 4 Mn with the O_2 yielding "S" state complex of Kok et al (1).
- 3) With Mn removal, the capacity for donor (ascorbate (25) or p-phenylenediamine (23)) photooxidation in strong light declines in a very similar fashion as the O_2 evolving capacity. However, the quantum yield of these photooxidations remains unaffected by Mn removal. Such results showed that for the photooxidation of these donors, the larger of the two System II pools is not essential; however, the results imply two different sites of electron entry of these donors into System II with different rate constants;

- 4) Studies of the fluorescence yield and of photooxidations of artificial electron donors, suggested that the smaller, more tightly bound Mn pool amounting to 2 Mn/System II trap, also was essential for System II;
- 5) We concluded that of the 6 Mn associated with the oxidant side of System II, two are linked more closely to trapping center events than the other four. While all six are essential for O₂ evolution, only four correlate with O₂ evolving capacity;
- 6) Such studies incidentally specified one of the sites affected by Tris or NH₂OH. One inhibitory site of NH₂OH, a compound long known to be an inhibitor of O₂ evolution, therefore was identified.

Further studies in our laboratory on the effects of NH₂OH on System II revealed the following:

- 1) Illumination of chloroplasts in the presence of NH₂OH results in destruction of all System II activities without affecting System I activity;
- 2) Incubation of algae or chloroplasts with this agent in the dark causes release of only the larger System II Mn pool with simultaneous loss of O₂ evolving capacity -- the rate of destruction being proportional to the concentration of O₂ centers and NH₂OH; this destruction showed a temperature dependency ($Q_{10}=2.43$);

- 3) The pH dependency of the NH_2OH induced loss of O_2 centers suggested that unprotonated NH_2OH is the effective species;
- 4) The effectivity for inducing loss of O_2 centers and Mn was markedly altered by N- and O-substitution. Generally, this effectivity paralleled the effectivity for donating electrons to the photooxidant of System II; however, hydrazine, an isostere of NH_2OH and an effective donor to System II (26, 27) was ineffective for O_2 center destruction.
- 5) The effect of NH_2OH on O_2 center destruction also was observed with intact cells of several green algae and the blue-green alga, *Anacystis*. This enabled us to extend our studies on the photoactivation of O_2 evolving centers.

We have defined photoactivation as that process in which System II trapping events induce (1) incorporation of Mn into the O_2 evolving centers; and (2) the capacity to evolve O_2 . Initially, studies of this process were pursued in this laboratory using algae which were depleted of Mn (and thus O_2 evolving centers) by growth on a Mn-deficient medium.

Subsequently, after we recognized that Mn could be extracted with NH_2OH , studies of photoactivation were extended with NH_2OH treated cells. Also we reasoned that if photoactivation is fundamental to O_2 evolving organisms, cells capable of synthesis of the photosynthetic apparatus in darkness would contain trapping centers and

the interconnecting electron transport chains but would lack the large Mn pool and thus not be capable of O_2 evolution. Evidence supporting this hypothesis was obtained and therefore we examined the photoactivation of O_2 evolution of Chlorella and Chlamydomonas which had been cultured in darkness on media (containing Mn^{+2}) supplemented with organic carbon sources.

Because of the similarity of results of photoactivation obtained with cells either depleted of Mn by autotrophic growth, or by NH_2OH extraction, or by heterotrophic growth, we summarize these studies of photoactivation together:

- 1) In any given illumination, the time-course of appearance of O_2 evolving centers proved first order, the rate being proportional to the number of inactive O_2 evolution centers.
- 2) The action spectrum proved to be identical to that of System II. The process was independent of System I and occurred in Scenedesmus Mutant #8 devoid of this System (28).
- 3) The quantum efficiency was optimal over a small range of intensities and declined at both lower and higher intensities. The efficiency was half maximal at either 1 or 10 hits per System II trap per second.
- 4) Kinetic data obtained with flashing or continuous light showed that the activation of O_2 evolution centers is a multi (minimally two) quantum process: the product of the first

photoact relaxes to a new photosensitive state of limited stability which is converted by light into a stable O_2 evolution center.

- 5) The rate constants involved for a given alga were very similar irrespective whether depletion of Mn had occurred by autotrophic growth in Mn deficient media or by NH_2OH extraction of cells.
- 6) The time-course of reappearance of O_2 evolution capacity was correlated with the time-course of reappearance of the Mn pool which we correlate with the O_2 yielding catalyst.
- 7) Micromolar concentrations of compounds known to chemically reduce Mn valency states $> +2$ and/or to reduce the light generated photooxidant of the System II trapping center proved effective reversible inhibitors.

We concluded from these studies with the NH_2OH extracted algae that the model (29) previously proposed to explain the complex sequence of reactions in the photoactivation of Mn deficient cells also appears applicable to photoreversal of NH_2OH extracted cells. The hypothesis (29) has been made that Mn^{+2} photooxidation to Mn^{+4} is required for the insertion of Mn into the O_2 yielding center. The strong and reversible inhibition by compounds (hydrazine, hydroquinone) known to either chemically reduce Mn valency states $> +2$ and/or to reduce the light generated photooxidant of System II tend to support this hypothesis. Our results do not necessarily

imply that the ground state valency of Mn in such O₂ evolving centers is $> +2$. It is entirely possible that the presumed photooxidation of Mn⁺² to Mn⁺⁴ is required only for the insertion of Mn into its yet unidentified O₂ evolving complex. Clearly, these questions remain to be resolved.

We are well aware that our data showing the absence of O₂ evolution by heterotrophic cultured cells is at variance with the few studies reported in the past (30, 31). It should be noted however that in almost all of the earlier studies the assessment of capacity to evolve O₂ was determined by measurements of photosynthesis. In this measurement, induction periods for the CO₂ enzymes are common and thus may have concealed the photoactivation phenomenon. Our methodology excludes such troubles and therefore we feel confident with our data.

Still a huge enigma to us is our inability to achieve significant photoactivation with cell-free preparations. Despite a few varied attempts our success has been minimal. Curiously, cross-linking of proteins in the cell with mild concentrations of glutaraldehyde or formaldehyde leads to abolishment of photoactivation. Yet a 10-fold higher concentration of these fixatives cause less than 20 percent decrease of O₂ evolution of photoactivated cells. Clearly, we are still far-removed from understanding all the events occurring in photoactivation. Similarly, we still are lacking critical information concerning the chemical environment of the Mn we associate with the O₂ evolving enzyme.

Fig. 1. Variable part of the fluorescence yield (arbitrary units) produced by saturating flashes, measured at the peak of each flash, as a function of the number of flashes given to dark adapted chloroplasts. The variable part is the fluorescence yield above that of dark adapted chloroplasts. The variable part of the DCMU, closed trap, fluorescence yield (measured with weak light) is 240 units. The solid line is for chloroplasts, no additions; the dashed line, 100 mM methylamine added. The average of 32 measurements (each on a new sample) was used for each curve to lessen errors due to fluctuations of the flashes.

FLUORESCENCE YIELD, arbitrary units

ABSORBED QUANTA, arbitrary units

Fig. 3. Fluorescence yield as a function of time after a saturating flash. Solid line, after the first flash; dashed line after the second flash given to dark adapted chloroplasts. Closed traps as measured by the ability of subsequent flashes to produce oxygen as a function of time after a saturating flash. Closed circles, after the first flash; squares, after the second flash. F_0 is the fluorescence yield of dark adapted chloroplasts. The curves are normalized at 100 and 2000 μseconds. Fluorescence curves were obtained from 512 averages, using a new sample each time.

Fig. 4. Fluorescence yield as a function of time after a saturating flash given to dark adapted chloroplasts at 4°C in the presence of DCMU. F_0 is the fluorescence yield before the flash. The result of 512 averages, using a new sample each time.

Fig. 5. Fluorescence yield as a function of time after a saturating flash. Solid line after the first flash, dashed line after the second flash given to dark adapted chloroplasts. F_0 is the fluorescence yield of dark adapted chloroplasts at 4°C . The result of 512 averages, using a new sample each time.

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