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Flash-induced fluorescence estimation of Photosystem II electron-transport rates in light-adapted leaves

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A method is described for the measurement of fluorescence transients induced by a bright flash of light in the presence of constant illumination. The application of this technique to the estimation of Photosystem II electron-transport rates in vivo is described. The technique does not require leaf excision, gas tight enclosure or dark adaptation and is non-destructive. The relationship between flash-induced fluorescence and oxygen evolution is practically constant at light intensities from 40 to 400 $\mu E \cdot m^{-2} \cdot s^{-1}$ (the highest tested), at temperatures from 10°C to 25°C, in the presence of the Photosystem II inhibitor atrazine, in the presence of the uncoupler carbonyl cyanide *m*-chlorophenyl hydrazone and in material previously subjected to photoinhibitory conditions. This measurement technique permits easy, rapid estimation of Photosystem II electron transport activity in vivo with portable equipment.

Introduction

One important effect of stress conditions on plants is photoinhibition of PS II. Chilling, heat, drought, mineral stress, and SO_2 all bring about photoinhibition at this site [1]. Both the study of plant responses to stress and programs to improve plant responses to adverse conditions require that this phenomenon be readily measured in situ. For screening programs, it is particularly desirable that measurements be rapid, easily made, and easily interpreted. Present methods for assessing photosynthetic competence include gas-exchange methods such as infrared gas analysis (IRGA) determinations of CO_2 uptake and O_2 evolution measurements using the oxygen electrode [2]. Other methods include the photoacoustic technique [3] and fluorescence measurements. Fluorescence measurements include variable fluorescence in liquid nitrogen [4], variable fluorescence with herbicide blockage [5] and variable fluorescence [6] and related parameters [7] from Kautsky induction transients. Fluorescence induction transient analysis has been used to study tolerance to several adverse environmental conditions [8–12].

The present method, like other fluorescence based methods, detects activity of PS II because this is the source of the majority of variable fluorescence at room temperature. It is distinct from other fluorescence-based methods in that it is designed to detect electron transport during steady-state photosynthesis. Other methods require blockage of PS II either by extreme cold or poisons, or rely on the response of the plant to

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; FIF, flash-induced fluorescence; *I*, light intensity; P, steady state photosynthetic oxygen evolution; PS II, Photosystem II; Q_A, the primary quinone acceptor of Photosystem II; RC, reaction center; $\Delta\phi_F$, change in fluorescence quantum yield induced by a light flash; ϕ_{O_2}, apparent quantum yield of oxygen evolution; IRGA, infrared gas analysis.

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sudden transitions in light intensity.

It is well established that PS II is more fluorescent when Q_A is reduced than when it is oxidized [13]. The fluorescence decay during transition from the reduced to the oxidized state as a consequence of electron transport has been used to study oxidation-reduction events in PS II. Typcially, fluorescence decay transients are observed in dark-adapted material exposed to a bright light flash which results in reduction of Q_A. Studies of this kind have been used to characterize Q_A-to-Q_B electron transfer [14,15], effects of a herbicide resistance mutation on Q_A-to-Q_B transfer [16], and effects of pH on electron-transfer processes [17]. Flash-induced fluorescence of photosynthetic systems subjected to constant illumination has also been observed [18]. Falkowski et al. [19] use this technique to estimate the fraction of reaction centers with Q_A in the reduced state in algae at various light intensities.

The object of the present experiments was to assess the feasibility of the application of the flash-induced fluorescence technique to studies involving screening for stress tolerance. Such an application requires a simple, rapid measurement that is insensitive to ambient conditions of light and temperature. In addition, it is necessary that the proportionality between electron transport and flash-induced fluorescence be maintained even when the photosynthetic apparatus is partially damaged. Atrazine, CCCP and photoinhibition were used to explore the effects of loss of photosynthetic competence on the relationship between flash-induced fluroescence and oxygen evolution. This study shows that in light-adapted material a simple relationship exists between flash-induced fluorescence and oxygen evolution under most circumstances tested.

Since measurement is made on the illuminated steady state, the measuring light will not perturb the system so long as it is held constant throughout the time of measurement. (The use of bright continuous illumination is avoided for flash-induced fluorescence measurement on dark-adapted systems because it would result in alteration of reaction center states.) Fluorescence decay transients induced in a steady state distribution of reaction center states are identical on subsequent flashes, so signal averaging is easily done. Since the process being observed has decay half-times on the order of milliseconds or less, the time required for measurement, as opposed to sample preparation or positioning, is a negligible practical factor. An important advantage of measuring the illuminated steady state is that measurements can be made on actively photosynthesizing systems.

Materials and Methods

Plant material. Barley (*Hordeum vulgare* L. cv. boyer and *H. vulgare* cv. himalaya) was grown in the greenhouse in 4-inch pots in a commercial potting soil mix (Supersoil, Rod McLellan Co., South San Francisco, CA). Plants were grown without supplemental light. Plants were used between 1 and 2 months after sowing. Rice (*Oryza Sativa* L. cv. IR8 and cv. Tainan 3) was grown in the greenhouse in 6-inch pots with flooded clay soil supplemented with ammonium sulphate and potassium phosphate. Natural light was supplemented with 1000 W metal halide lamps. Plants were used between 2 and 3 months after sowing.

Sample pretreatments. For certain experiments, photosynthesis was inhibited with atrazine (Ciba-Geigy) or CCCP (Sigma). Inhibitors were applied by uptake into the transpiration stream of a detached leaf for between 30 min and 2 h. Flash-induced fluorescence was used to monitor the progressive inhibition of photosynthesis with uptake, and a leaf segment with an appropriate degree of inhibition was selected for further measurements. Atrazine was used at 1 to $5 \cdot 10^{-5}$ M, and CCCP at $5 \cdot 10^{-5}$ M. Photoinhibitory treatments were carried out in a growth chamber on attached leaves by exposure to 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ at 25°C for 10–20 h. Light was provided by a 400 W metal halogen lamp. Unless otherwise indicated, all samples were pretreated for half an hour with 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ from a Kodak 4000 slide projector after excision and inhibitory pretreatment, if any. Measurements were thus made on material that was uniformly light adapted.

Oxygen evolution. An oxygen electrode (Yellow Springs Instrument Co., model 53) was used with a chamber constructed in the laboratory consisting of an aluminum block drilled to accept the oxygen electrode probe with a gas tight seal, with ports for the admission of gas mixtures with needle

valve flow controls and channels for the flow of temperature controlled water. An aluminum block drilled to accept a fiber optic probe permitted the simultaneous measurement of flash-induced fluroescence and oxygen evolution from a 7.9 mm^2 portion of the leaf segment. The CO_2 buffer was 1% (w/v) tricine (pH 7), 1% (w/v) $NaHCO_3$. The net chamber volume was 400 µl.

Flash-induced fluorescence measurement. Flash-induced fluorescence transients were measured with a portable, battery-powered instrument built as described [20]. The light flash was provided by a flash lamp (EEG 9B1.5), and the steady illumination which served both to drive photosynthesis and measure fluorescence was provided by a 12 W halogen lamp (Sylvania model 1990). Both light sources were focussed with condensor lenses (Melles-Griote model 01 LAG 005) onto the surface of one branch of a bifurcated fiber optic light guide (Dolan-Jenner model ER836). Both steady and flashing lights were filtered through a Corning blue-green filter model 4-96. The common end of the fiber optic light guide was placed at the leaf surface for measurement. This arrangement results in a flash energy at the leaf surface of approx. 0.5 $J \cdot m^{-2}$, with a flash duration (width at half maximum) of 1 µs and a steady light adjustable from 10 to 400 $\mu E \cdot m^{-1} \cdot s^{-1}$. The second leg of the fiber optic led to a phototransistor which measured light emitted from the surface of the leaf. This light was filtered through a Kodak wratten filter type 70 'deep red'.

Electronic circuitry for the detection of flash-induced fluorescence was built as described [20]. A phototransistor provided a signal to a current sensing amplifier. The amplified signal was gated to two sample-and-hold circuits. The first of these sampled between 1 ms before flash triggering and the time of flash triggering. The output of the hold amplifier was fed back to the current sensing node in such a way that the output of the signal amplifier was adjusted to zero. From 225 to 275 µs after the flash, the signal was gated to the second sample-and-hold amplifier. The signal held by the second sample-and-hold was thus a measure of the difference in fluorescence induced by the light flash. The fluorescence signal in the 225–275 µs interval was about 85% of its value at the peak of flash-induced fluorescence.

Results and Discussion

Theory

The observation that provides the basis for the use of flash-induced fluorescence is that the photosynthetic apparatus with Q_A in a reduced state is more fluorescent than it is with Q_A oxidized (except when fluorescence is otherwise quenched, e.g., by P-680$^+$). Consequently, there is a component of the total fluorescence signal that is a result of reaction centers having a reduced Q_A.

A photosynthetic system with all Q_A in the oxidized state also has some probability of emitting a photon as fluorescence. Thus the change in fluorescence induced by Q_A reduction is related to the change in the probability of fluorescent emission by the photosynthetic system, i.e., the differential fluorescence yield between centers with Q_A reduced and those with Q_A oxidized. The magnitude of the change of the fluorescence signal resulting from the reduction of Q_A is dependent on the number of centers in which Q_A is reduced multiplied by the amount of light that they absorb and the differential fluorescence yield.

Assuming that the differential fluroescence yield is substantially constant, it is possible to estimate the electron-transport rate through PS II from the magnitude of the flash-induced fluorescence transients. The total fluorescence emitted by a leaf or other photosynthetic preparation can be measured immediately before a brief saturating flash of light, and then again immediately after the light flash. The difference in fluorescence yield before and after the flash is a consequence of the conversion of reaction centers from the Q_A oxidized (open) to the Q_A reduced (closed) state. If reaction centers already have Q_A reduced, or if they are not photochemically active, then they will not contribute to this change in fluorescence.

$$\mathrm{FIF} = \Delta\phi_F I \frac{RC_O}{RC_T} \tag{1}$$

where FIF is the flash-induced increase in fluorescence intensity, $\Delta\phi_F$ is the differential fluorescence yield as defined above, I is the intensity of the continuous light, and RC_O/RC_T is the fraction of open, active reaction centers. The PS II electron transport rate is:

electron transport rate is:

$$P = \phi_{O_2} I \frac{RC_O}{RC_T} \quad (2)$$

and

$$P = \mathrm{FIF} \frac{\phi_{O_2}}{\Delta\phi_F} \quad (3)$$

where P is the rate of oxygen evolution and ϕ_{O_2} is the apparent quantum yield of oxygen. For example, for ϕ_{O_2} of 0.05 and $\Delta\phi_F$ of 0.00005, a flash-induced fluorescence of 2.0 $pE \cdot cm^{-2} \cdot s^{-1}$ implies an oxygen evolution rate of 2000 $pmol \cdot cm^{-2} \cdot s^{-1}$ or 120 $nmol \cdot cm^{-2} \cdot min^{-1}$. For simplicity, Eqns. 1 and 2 neglect the non-linearity in the relationship between fluorescence and reaction center closure [21]. Since the same non-linearity is expected for flash-induced fluorescence and electron transport, Eqn. 3 is exact. In the presence of photorespiration, Eqn. 3 represents the total electron transport through PS II rather than oxygen evolution.

There are possible sources of error in the estimation of electron transport from flash-induced fluorescence. Previous studies have shown that quenching of fluorescence has a large non-photochemical component [22,23]. The differential fluorescence yield may not be constant, but decreases with increasing light intensity, resulting in a decrease in the ratio of flash-induced fluorescence to electron transport. Thus the linear relationship described may be an approximation valid only for the light-adapted state at light intensities below saturation [24]. It is necessary to address this problem experimentally, since variation in differential fluorescence yield could result in systematic errors in the estimation of electron transport, or might require a more complex theoretical analysis if there is a non-linear relationship between flash-induced fluorescence and electron transport.

Another possible error occurs because the fluorescence signal from portions of the leaf distant from the surface is attenuated. Thus fluorescence measurement is more surface biased than is oxygen evolution. When the measurement light approaches saturation, fluorescence-detected electron transport may saturate before electron transport detected by gas exchange.

Experimental

The apparatus used for measurement of flash-induced fluroescence is shown diagrammatically in Fig. 1. The fiber optic probe provides both flashing and constant illumination to the leaf surface, and carries the fluorescence signal to the photodetector. Fluorescence is detected immediately before the flash and approx. 250 μs after the flash. The fluorescence transient, measured with a digital storage oscilloscope (Nicolet model 4094), declines by approx. 15% in the time between the flash and the measurement (data not shown). The flash-induced fluorescence increase (the difference between the measurements before and after the flash) is amplified, filtered, and recorded on a chart recorder. Constant illumination is switch selectable at intensities ranging from 10 to 400 $\mu E \cdot m^{-2} \cdot s^{-1}$. The flash energy used in these experiments is sufficient to give 70–75% of the maximum signal inferred from curve fitting. An example of data used for this determination is shown in Fig. 2, flash-induced fluorescence vs. flash energy for a rice leaf with constant illumina-

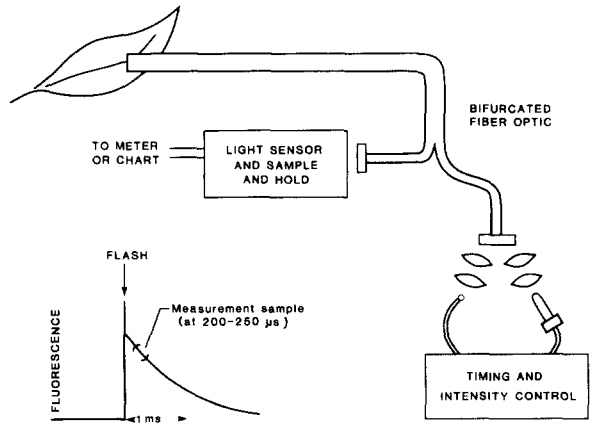

Fig. 1. Flash-induced fluorescence instrument. Two light sources, a flash lamp and a constant intensity lamp, are blue filtered and focussed on one branch of a bifurcated fiber optic. The common bundle of the fiber optic carries light to the sample and fluorescence to the photodetector via the other branch. A red filter at the photodetector reduces the non-fluorescence signal. The fluorescence immediately before and after the flash is compared electronically. The inset shows the general shape of the flash-induced fluorescence transient.

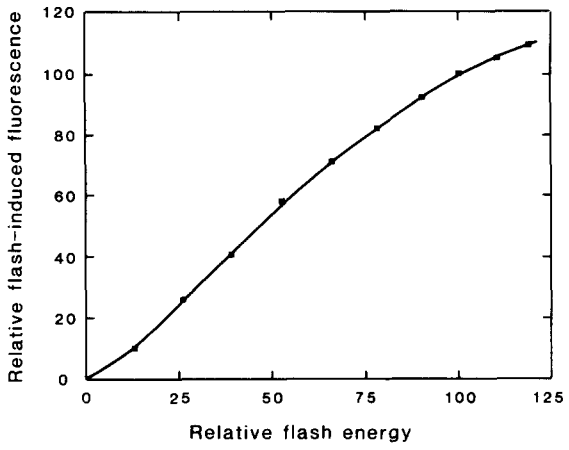

Fig. 2. The dependence of flash-induced fluorescence on flash energy. Flash-induced fluorescence was measured in a light adapted rice leaf at 200 $\mu E \cdot m^{-2} \cdot s^{-1}$. Flash energy and signal size are expressed as percentages of their values when flash energy is approx. 0.5 $J \cdot m^{-2}$. Flash-induced fluorescence under these conditions is about 1.7 $pE \cdot cm^{-2} \cdot s^{-1}$ in detached rice leaves. Flash-induced fluorescence data were measured alternately at 0.5 $J \cdot m^{-2}$ and at a test energy, and the data normalized to the adjacent 0.5 $J \cdot m^{-2}$ data to compensate for small changes in signal size. The curve shown is the best fit of the form $Y = Y_{max} + ae^{-bx} + ce^{-dx}$; with $Y_{max} = 138$, $a = -568$, $b = -0.0213$, $c = 430$, $d = -0.0269$. Signal size at 0.5 $J \cdot m^{-2}$ is approx. 72% of Y_{max}.

tion at 200 $\mu E \cdot m^{-2} \cdot s^{-1}$. The non-linear relationship between fluorescence and reduced Q_A is evident from the sigmoidicity of this curve noticeable at low flash energy.

Since it is possible to perturb the photosynthetic process sufficiently that differential fluorescence yield is not constant, it is necessary to establish the range of conditions in which valid estimates of electron transport can be made. The experiments that follow were designed to determine whether the relationship between flash-induced fluorescence and electron transport estimated from oxygen evolution is constant over a range of measurement conditions. The effect of variation of light intensity and temperature and the effect of reduced photosynthetic electron-transport capacity resulting from photoinhibition and treatment with atrazine and CCCP were investigated. Oxygen concentrations were maintained at 2% or less to minimize photorespiration. The data were corrected for dark respiration.

Oxygen evolution and flash-induced fluorescence were measured simultaneously, with a chamber built for the purpose. This eliminates artifacts of variation in electron-transport capacity with position on the leaf. Flash-induced fluorescence is between 0 and 30% lower in material that is inserted in the chamber and exposed to less than 2% O_2 and about 5% CO_2, compared to the same leaf segment previously measured in open air. Flash-induced fluorescence recovers at least partially when the leaf segment is removed from the chamber. Insertion in the chamber may result in changes in electron-transport rate, differential fluorescence yield, or both.

Light adaptation

Flash-induced fluorescence measurements were made on leaf segments that were light adapted by treatment with 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 30 min before insertion into the oxygen electrode chamber. If flash-induced fluorescence measurements are made on a leaf segment that is dark-adapted, the flash-induced fluorescence signal undergoes a complex evolution to a steady-state level. The kinetics of flash-induced fluorescence changes during the period of light adaptation depend on the plant type and the previous history of the sample. Two examples of measurements of flash-induced fluorescence during adaptation to light of dark-adapted material are shown in Fig. 3.

Light adaptation is known to influence strongly the fluorescence yield. Conceivably, adaptation to different light levels might affect differential fluorescence yield, so the effect of light intensity on the relation between flash-induced fluorescence and oxygen evolution was investigated. The ratio of flash-induced fluorescence to oxygen evolution is nearly constant throughout the range of light intensities used. Fig. 4 shows the response of (a) O_2 evolution and (b) flash-induced fluorescence to light intensity in a barley (cv. himalaya) leaf segment at 25°C. Flash-induced fluorescence and oxygen evolution respond similarly to light intensities from 40 to 400 $\mu E \cdot m^{-2} \cdot s^{-1}$. 40 $\mu E \cdot m^{-2} \cdot s^{-1}$ was the lowest light level for which oxygen evolution rates could be easily measured, and 400 $\mu E \cdot m^{-2} \cdot s^{-1}$ the highest intensity available from the instrument. Oxygen evolution at 400 $\mu E \cdot m^{-2} \cdot s^{-1}$ was approximately half of the light saturated rate determined with a different light source

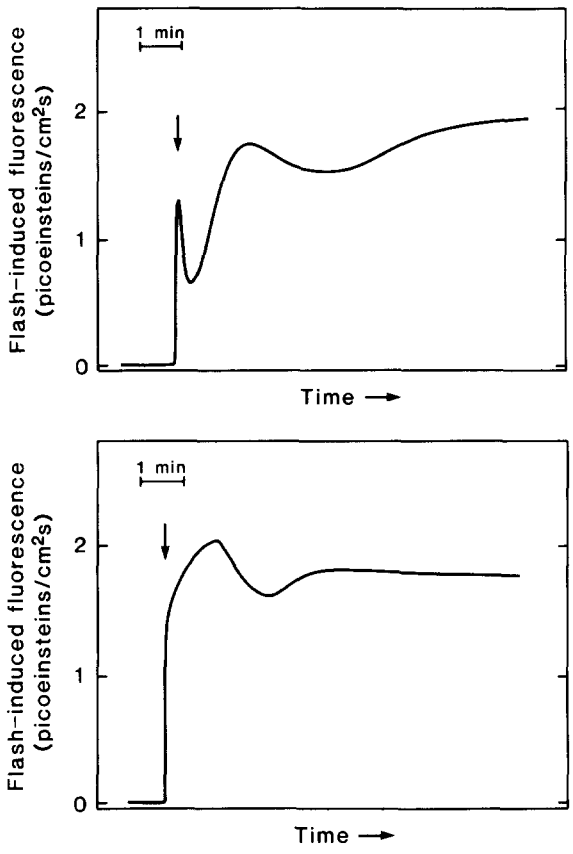

Fig. 3. Flash-induced fluorescence during light adaptation. An excised leaf of barley (a) and of rice (b) were dark adapted for 0.5 h and then flash-induced fluorescence measured at a light intensity of 300 $\mu E \cdot m^{-2} \cdot s^{-1}$. The start of illumination is indicated by an arrow.

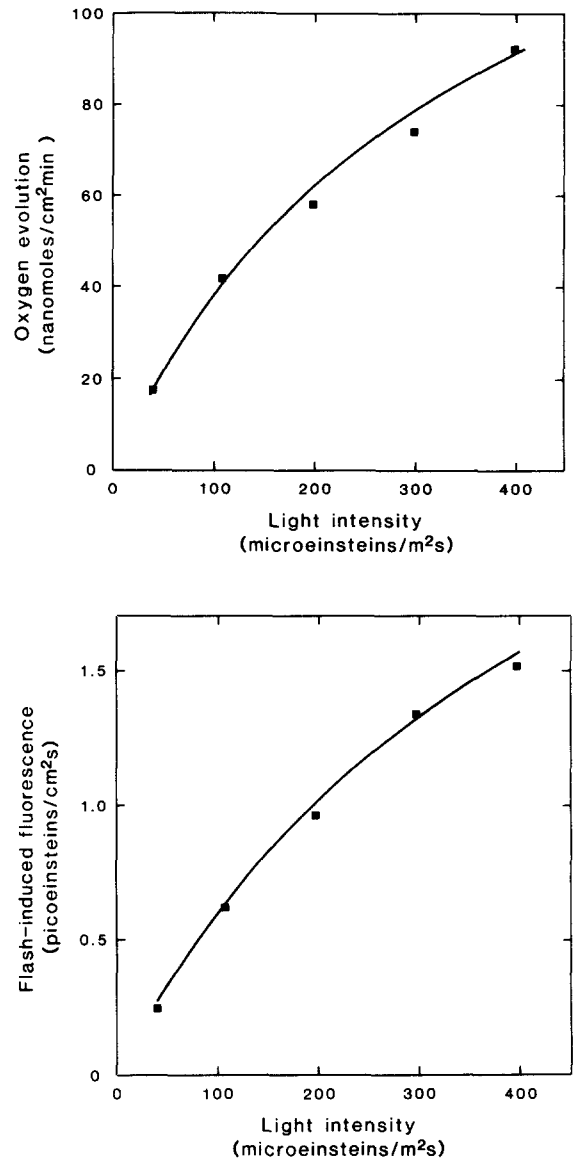

Fig. 4. The response of oxygen evolution (a) and flash-induced fluorescence (b) to light intensity. A segment of barley leaf was placed in the oxygen-electrode chamber and oxygen evolution and flash-induced fluorescence measured concurrently at several light intensities from 40 to 400 $\mu E \cdot m^{-2} \cdot s^{-1}$.

(Dolan-Jenner Fiberlite model 180). Different leaf samples of the same cultivar showed some variation in the rate of O_2 evolution and size of flash-induced fluorescence signal, but the ratio of flash-induced fluorescence to O_2 evolution rate is constant. Fig. 5 shows the correlation of flash-induced fluorescence and O_2 evolution for several leaf samples from two different rice cultivars, IR8 and Tainan 3, at a range of light intensities up to 400 $\mu E \cdot m^{-2} \cdot s^{-1}$. The two strains exhibited a very similar relationship of flash-induced fluorescence to oxygen evolution.

Temperature

Measurements were made at several temperatures to determine whether there is any temperature effect on the relationship of flash-induced fluorescence and oxygen evolution. Flash-induced fluorescence measured at 15°C in the oxygen evolution chamber saturated at approx. 200 $\mu E \cdot m^{-2} \cdot s^{-1}$, but oxygen evolution did not. This indicates that differential fluorescence yield decreases

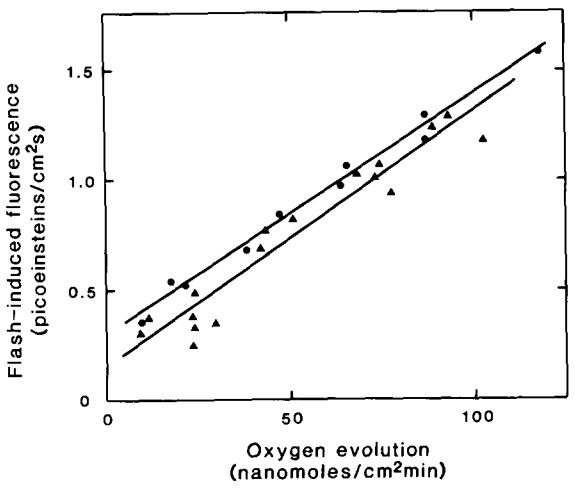

Fig. 5. Correlation of flash-induced fluorescence and oxygen evolution in rice. Flash-induced fluorescence and oxygen evolution were measured concurrently in several leaf samples of rice cultivars IR8 (▲) and Tainan 3 (●) over a range of light intensities from 40 to 400 $\mu E \cdot m^{-2} \cdot s^{-1}$. Linear regression lines to the two data sets are shown. The non-zero intercept is probably due to errors in estimation of the respiratory rate. Slopes are IR8, 0.0112; Tainan 3, 0.011; with correlation coefficients of 0.93 and 0.98 respectively.

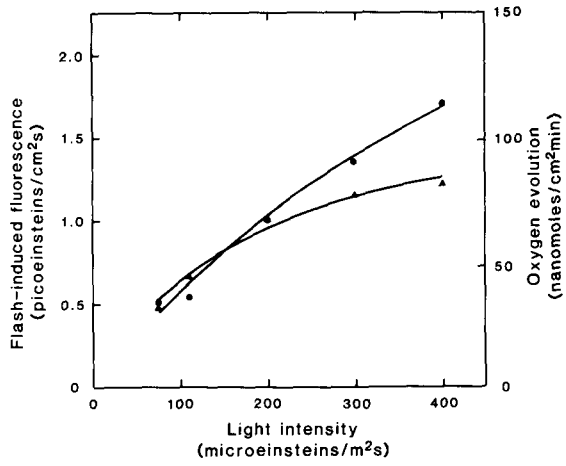

Fig. 6. Flash-induced fluorescence response to light intensity at 15°C. A leaf segment of barley cv. boyer was placed in the oxygen electrode chamber and flash-induced fluorescence (▲) and oxygen evolution (●) measured concurrently at light intensities from 40 to 400 $\mu E \cdot m^{-2} \cdot s^{-1}$.

at light intensities above 200 $\mu E \cdot m^{-2} \cdot s^{-1}$, under these conditions. Fig. 6 shows an example of flash-induced fluorescence and oxygen evolution vs. light intensity at 15°C. Flash-induced fluorescence was also measured before placement into the chamber, with the leaf segment on a temperature-controlled plate. Flash-induced fluorescence did not saturate at the available light intensities when measured in ambient air at 15°C. Similar results were obtained with whole plants in short term growth chamber studies. This indicates that the saturation of the flash-induced fluorescence response is due to an interaction of low temperature and chamber insertion. The decrease in differential fluorescence yield is similar to that seen in algae at light intensities resulting in near-maximal rates of oxygen evolution [19].

The effect of temperature on the relationship between flash-induced fluorescence measured before chamber insertion and subsequent oxygen evolution at the same temperature is shown in Fig. 7a. The ratio of flash-induced fluorescence to oxygen evolution is increased by approx. 20% with a 10°C drop in temperature. In concurrent measurement experiments the proportionality between flash-induced fluorescence and oxygen evolution is similarly affected for light intensities below the point where flash-induced fluorescence plateaus. This is shown in Fig. 7b, which is a compilation of data from experiments with different leaf samples done at temperatures ranging from 10°C to 25°C.

Inhibition of Photosystem II

In order to examine the effect of loss of PS II activity on the correlation of flash-induced fluorescence with oxygen evolution, the effect of the PS II inhibitor atrazine was studied. Flash-induced fluorescence was measured before and during oxygen evolution measurement. Flash-induced fluorescence measurements in the oxygen electrode chamber were less sensitive to atrazine poisoning than oxygen evolution, as illustrated in Fig. 8a. The linear relationship between flash-induced fluorescence measured in ambient air and subsequent oxygen evolution is maintained, as shown in Fig. 8b. The formation of the pH gradient that results in decrease in the differential fluorescence yield may be especially susceptible to atrazine poisoning in the conditions of the oxygen-electrode chamber.

Uncoupling of ATP synthesis

Since the stability of the chloroplast pH gradi-

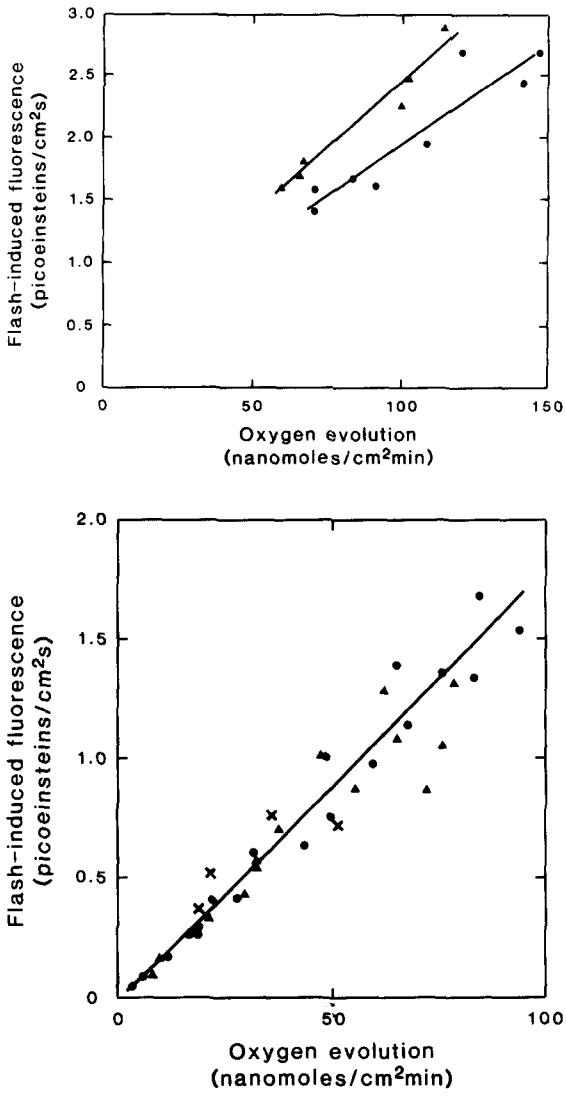

Fig. 7. Effect of temperature on flash-induced fluorescence in barley. (a) Flash-induced fluorescence and oxygen evolution were measured sequentially in leaf segments of barley cv. boyer, at 25°C (●) and at 15°C (▲). Oxygen evolution data is compensated for the lower sensitivity of the electrode at 15°C. Measurements were made at 200 and 400 $\mu E \cdot m^{-2} \cdot s^{-1}$. Slopes are 25°, 0.016; 15°, 0.02; correlation coefficients are 0.86 and 0.95, respectively. (b) Flash-induced fluorescence and oxygenevolution were measured concurrently in leaf segments of barley cv. himalaya at 25°C (●) at 15°C (▲) and at 10°C (×). Oxygen evolution data were compensated for the lower sensitivity of the electrode at 15°C and 10°C. The 25°C data were used to fit the regression line. The slope is 0.018, the correlation coefficient is 0.96. Flash-induced fluorescence at 10°C and 15°C plateaus when oxygen evolution is greater than 500–750 $\mu mol \cdot m^{-2} \cdot min$, which results in data points to the right of the 25°C line.

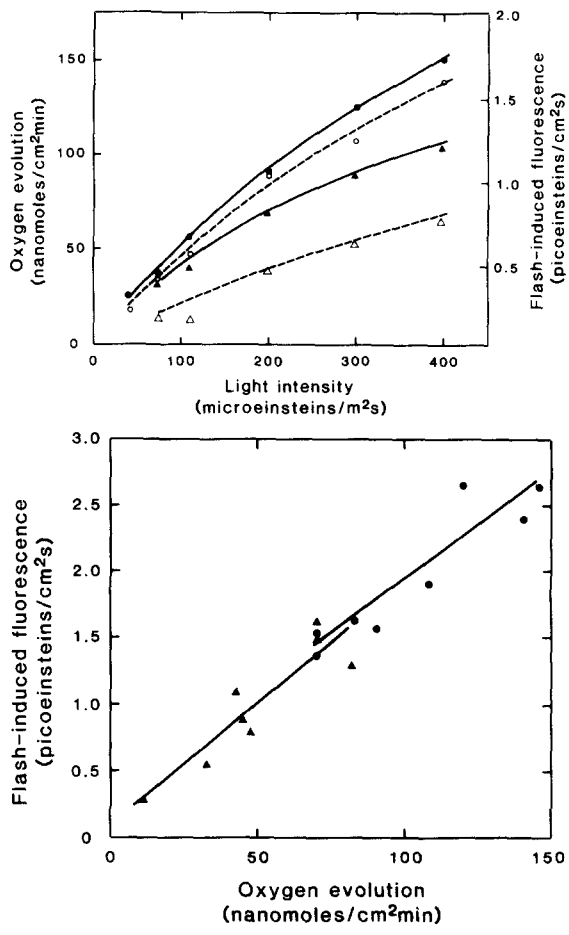

Fig. 8. Effect of atrazine on the correlation of flash-induced fluorescence and oxygen evolution. (a) Flash-induced fluorescence of leaves partially poisoned with atrazine (▲) and unpoisoned (●) and oxygen evolution of partially poisoned (△) and unpoisoned (○) leaves were measured concurrently in barley cv. boyer leaf segments for a range of light intensities. (b) Flash-induced fluorescence was measured before placement of leaf segments in the oxygen electrode chamber for oxygen evolution measurements in barley cv. boyer that was partially poisoned (▲) or unpoisoned (●). Regression line slopes are control, 0.016; poisoned, 0.018; with correlation coefficients of 0.86 and 0.82, respectively.

ent may be important to the relationship between flash-induced fluorescence and oxygen evolution, the effect of the uncoupler CCCP was investigated. Leaf segments were allowed to take up CCCP in the transpiration stream, and concurrent measurements of oxygen evolution and flash-induced fluorescence were made. The relationship between flash-induced fluorescence and oxygen evolution was not affected by treatment with

CCCP, even though respiration was increased and photosynthetic oxygen evolution was inhibited by up to 50% (data not shown).

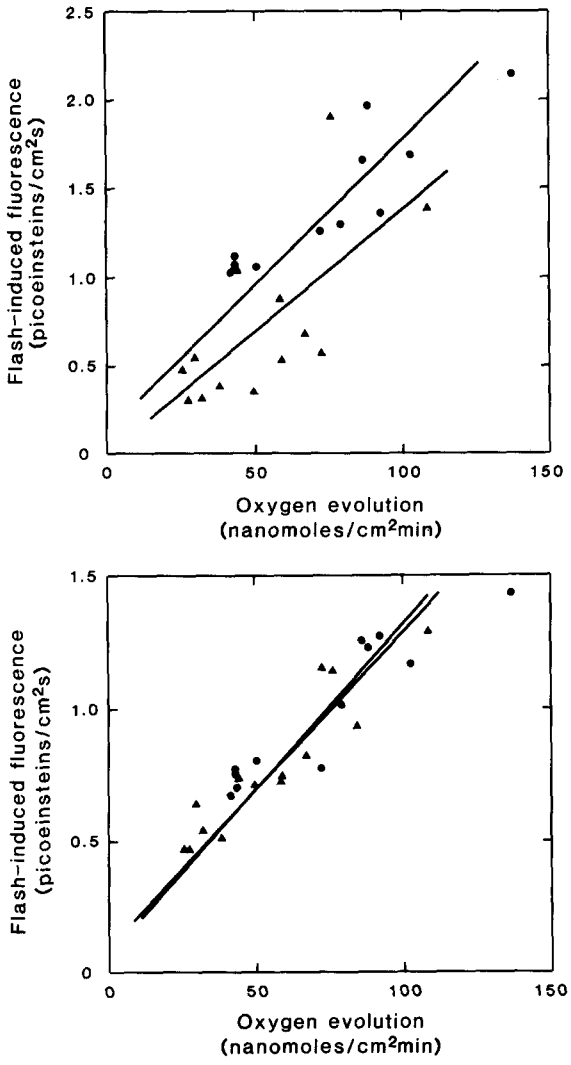

Fig. 9. Effect of photoinhibition on the correlation of flash-induced fluroescence and oxygen evolution. (a) Flash-induced fluorescence was measured before placement of the leaf segments in the oxygen electrode chamber. Leaf segments were not photoinhibited (●) or photoinhibited to varying degrees (▲). Measurements were made at 200 and 400 $\mu E \cdot m^{-2} \cdot s^{-1}$. Regression line slopes are control, 0.016; photoinhibited, 0.014; with correlation coefficients of 0.72 and 0.69 respectively. (b) Concurrent measurements of flash-induced fluorescence and oxygen evolution were made on leaf segments of rice cv. IR8 that were not photoinhibited (●) or that had been previously photoinhibited to varying degrees (▲). Regression line slopes are control, 0.012; photoinhibited, 0.0125; with correlation coefficients of 0.77 and 0.85 respectively.

Photoinhibition

Because the quantitative determination of photoinhibition is of particular interest, the correlation between oxygen evolution and flash-induced fluroescence was studied in photoinhibited material. Photoinhibition has essentially no effect on the relation between flash-induced fluorescence and oxygen evolution. Photoinhibited leaf segments of rice cv. IR8 were utilized for measurement of flash-induced fluorescence both before (Fig. 9a) and during (Fig. 9b) measurement of oxygen evolution. Non-concurrent measurements show considerable scatter, possibly due to variation in photosynthetic activity with either time or position. With concurrent measurement, photoinhibition has essentially no effect on the relationship of flash-induced fluorescence to oxygen evolution.

In summary, the relation between oxygen evolution and flash-induced fluorescence is substantially constant under a range of conditions that cause very strong perturbations in the net photosynthetic electron transport rate in barley and rice. A single measurement at a flash energy of 0.5 $J \cdot m^{-2}$ with a constant illumination at 200 $\mu E \cdot m^{-2} \cdot s^{-1}$ can be used to estimate the light-limited electron-transport rate of light-adapted leaves. Flash-induced fluorescence provides a means for rapidly and easily estimating the PS II electron-transport rate in vivo, with potential applications to studies of stress-induced photoinhibition and herbicide inhibition of electron transport. The use of flash-induced fluorescence for estimating PS II electron transport in vitro, and the inference of distribution of reaction center states by analysis of the flash-induced transient decay kinetics are currently being investigated.

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