

The quantum yield of photoinhibition is the same in flash light and under continuous illumination – implications for the mechanism

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Introduction

Several different mechanisms have been suggested to function in photoinhibition of PSII under visible [1-6] and ultraviolet (UV) light [7]. Furthermore, it has been suggested that the mechanism varies according to the intensity of visible light [4]. The three most popular hypotheses about the mechanism of visible-light-induced photoinhibition assume that the electron transfer reactions of PSII are the ultimate reason why PSII is photosensitive. In the 'acceptor-side' mechanism [2] photoinhibition begins with the reduction of the plastoquinone pool under high light, and the lack of oxidised Q_B then leads to double reduction of Q_A and enhanced formation of chlorophyll triplets. The 'low-light' mechanism [4] is based on the assumption that significant amounts of reactive singlet oxygen can be produced by triplet chlorophyll formed in the S_{2/3}Q_B⁻ and S_{2/3}Q_A⁻ recombination reactions. The third widely accepted hypothesis, 'donor-side photoinhibition' is based on the finding that PSII becomes very sensitive to light if the oxygen-evolving complex (OEC) is made inactive with hydroxylamine washing or chloride depletion [8]. The sensitivity results from the high oxidation potential of the oxidised primary donor P₆₈₀⁺ [6]. Long-range effects mediated by active oxygen produced outside PSII or by free chlorophylls have also been suggested to cause photoinhibition [3,9]. However, these suggestions cannot explain the fast rate of photoinhibition under anaerobic conditions [2].

A general problem with the acceptor and donor-side hypotheses is that they do not explain the fact that the initial rate of photoinhibition is directly proportional to light intensity [10-11]. The acceptor-side mechanism requires intense light to reduce the plastoquinone pool and cannot explain why photons of low light cause photoinhibition. The photon yield of the donor-side mechanism, in turn, would depend on the relative rates of electron transfer from P₆₈₀⁺ to the plastoquinone pool and from OEC to P₆₈₀⁺, and would thus depend on the rates of electron transfer, not on the rate of photon absorption. The third hypothetical mechanism, low-light photoinhibition, does not account for photoinhibition under moderate or high light.

Because photoinhibition rates predicted by the low-light, acceptor-side and donor-side mechanisms depend on PSII electron transfer rates, these hypotheses can be tested by varying the number of photons absorbed without varying PSII electron transfer rate. We did this by illuminating thylakoids with short saturating 'single turnover' flashes.

Materials and Methods

Pumpkin (*Cucurbita pepo* L.) thylakoids (50 µg Chl/ml) were suspended in a buffer solution containing 0.3 mM sorbitol, 50 mM Hepes-KOH, pH 7.6, 5 mM MgCl₂, 5 mM NaCl and 1 M glycine betaine. The thylakoids were enclosed in 200-µl, 1 cm dia. stirred cuvettes and sealed with a Schott GG400 UV-blocking filter. Flash illumination (1 flash/4.7 s) was given at 20 °C with a FX-200 Xenon flash lamp (EG&G) brought to 1 mm distance from the cuvettes. At regular intervals, the light-saturated oxygen evolution rate (H₂O to dichlorobenzoquinone) was measured from thylakoids from one flashed and one non-flashed cuvette. The flash intensity was varied with capacitors. The number of photons entering the cuvette per flash was measured by chemical actinometry at 475-610 nm [12]; the result was extended to the 400-700 nm range by comparison with the spectrum of the flash lamp. In some experiments, as indicated, a slide projector was used as a source of continuous light with the same cuvette system. The saturation curve for O₂ evolution under flash illumination was measured with a Hansatech oxygen evolution by using a fused silica rod both as a light guide and a stopper. The thylakoid sample (310 µl, 100 µg Chl/ml) was illuminated at 10 Hz flash frequency and the number of photons entering the oxygen electrode chamber was measured actinometrically. The ratio of

chlorophyll to PSII was calculated to be 480 from the maximum rate, assuming that 10 % of the flashes fail to advance the S-states. The rate constant of photoinhibition (k_{PI}) was obtained by fitting the photoinhibitory loss of light-saturated oxygen evolution activity to a first-order reaction, and subtracting the rate constant of dark inactivation. All experiments were repeated three times.

Results and discussion

Photoinhibition caused by Xenon flash illumination of pumpkin thylakoids was strictly of first order (Fig. 1), which made it possible to extract the rate constant, k_{PI} . The rate constant of the dark inactivation was subtracted to obtain the final k_{PI} . When the intensity of the flashes was varied, the rate of the reaction varied accordingly (Fig. 1).

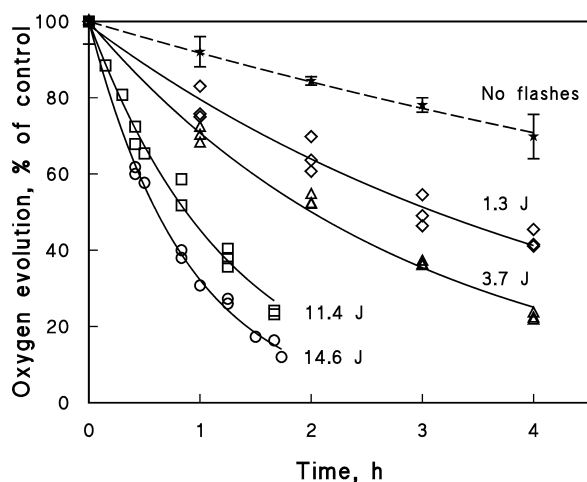


Fig. 1. Time course of photoinhibition of pumpkin thylakoids during illumination with 5- μ s Xenon flashes (1 flash/4.7 s) of 1.3 J (tilted squares), 3.7 J (triangles), 11.4 J (squares) or 14.6 J (circles). The upper curve (stars) shows dark inactivation. Each data point refers to an independent experiment; the dark inactivation data points represent the mean and SE of 6 experiments. The curves show the best fits to the first-order equation.

As can be seen from Fig. 1, a very high flash intensity (> 1 J flash energy) was required to clearly distinguish photoinhibition from dark inactivation. Because the low-light photoinhibition hypothesis that was earlier used to explain flash photoinhibition [4] predicts that the number of recombining $S_{2/3}QA^-$ or $S_{2/3}QB^-$ charge

pairs determines the rate of flash-induced photoinhibition, we next checked whether the number of these charge pairs depends on flash intensity. The inset of Fig. 2 shows that oxygen evolution in these thylakoids was fully saturated with flashes of less than 1 J energy. Thus, the number of $S_{2/3}QA^-$ or $S_{2/3}QB^-$ charge pairs produced per flash was independent of flash intensity in all experiments shown in Fig. 1. The result falsifies the low-light-hypothesis as an explanation flash photoinhibition. A basically similar flash-intensity dependence was actually noticed by Keren et al.[4] but the numbers of recombining charge pairs were not measured.

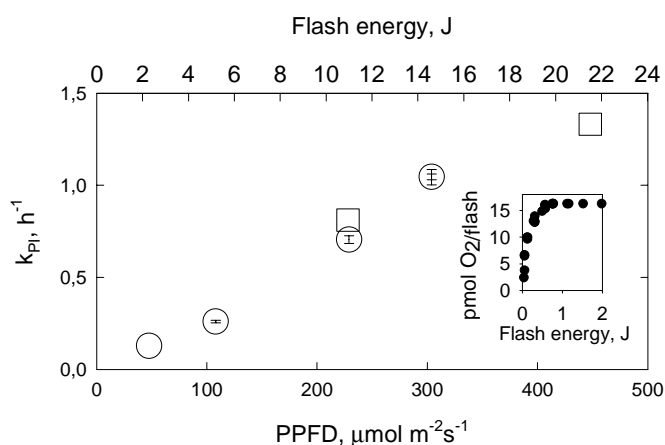


Fig. 2. The rate constant of flash photoinhibition, calculated from the data of Fig. 1 (circles), and the rate constant of photoinhibition under continuous light (squares), as a function of mean photon flux density (PPFD). The flash energy scale is shown on the top. The inset shows the dependence of the rate of oxygen evolution when thylakoid samples were illuminated with the same flash lamp in an oxygen electrode. The flash energies in the inset are calculated on the basis of actinometric photon counting measurements in the oxygen electrode cuvette and they can be directly compared to flash energy values used in the photoinhibition experiments.

To compare light intensities of the four different flash treatments, we measured the number of photons entering the cuvette during a flash, using chemical actinometry [11]. As expected, the flash photon content was directly proportional to flash energy (data not shown). A plot of k_{PI} versus flash energy (Fig. 2, circles) shows a direct proportionality, which is quite unexpected also in the frameworks of the acceptor and donor-side photoinhibition mechanisms. The acceptor-side mechanism cannot explain inhibition by flashes fired at 4.7 s intervals, because this rate of electron transfer is far too low to cause any significant reduction of the plastoquinone pool. The donor-side mechanism, in turn, does not explain what made the oxidised primary

donor more damaging when more light was used to create it. Because all flashes used for photoinhibition were oversaturating for PSII electron transfer (Fig. 2, inset), the time-averaged concentration of oxidised P_{680}^{+} did not depend significantly on flash intensity in the experiments shown in Figs. 1 and 2.

For comparison with continuous light, we next calculated a mean photon flux density (PPFD) value for each flash energy value by multiplying the number of photons per flash by the flash frequency and dividing by cuvette area. These mean PPFD values allow us to compare the photoinhibitory efficiency of a photon of continuous light to the efficiency of a photon of flash light. A slide projector lamp was used to produce light with roughly similar spectral quality as the UV-filtered Xenon flash (spectra not shown), and thylakoids enclosed in the same cuvette system as used in Fig. 1 were treated with moderate continuous light. Comparison of the k_{PI} values obtained under continuous light (Fig. 2, squares) and under flash light (Fig. 2, circles) shows that flash light and continuous light have equal photoinhibitory efficiency. The similarity of the quantum yield of flash-light-induced and continuous-light-induced photoinhibition shows that flash illumination is not a relevant model for dim continuous light in photoinhibition experiments.

Because the acceptor-side, donor-side and low-light mechanisms are incompatible with the experimental data in Figs 1 and 2, a new hypothesis for the molecular mechanism of photoinhibition is needed. The action spectrum of photoinhibition [3,10] suggests that photoinhibition is actually an ultraviolet-light phenomenon although its action spectrum has a low-efficiency tail extending to the visible. The action spectrum resembles the absorption spectra of Mn(III) and Mn(IV) gluconates [13] used as models for the Mn ions of OEC. We suggest that photoinhibition actually starts with photon absorption by these Mn ions of the OEC. The excited Mn leaves its site, rendering PSII susceptible to oxidation by P_{680}^{+} . Our observations (Tyystjärvi et al., in preparation) indeed suggest that Mn is released to the thylakoid lumen during photoinhibition and that under very high light, inhibition of OEC precedes the inhibition of OEC-independent PSII functions. The increase in k_{PI} with the length of the dark delay between laser flashes [4] can be explained by noting that the S_1 state, to which OEC relaxes in the dark, contains two Mn(III) and two Mn(IV) [14].

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