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Flash activation kinetics and photosynthetic unit size for oxygen evolution using 3-nsec light flashes

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SUMMARY

The flash saturation yield of photosynthetic oxygen evolution in Chlorella is the same for 2-μsec flashes from a xenon flashlamp as for 3-nsec flashes from a dye laser pumped by a nitrogen laser. Following 5 min of darkness, oxygen is produced by the third flash from either source.

A brief light flash of saturating intensity excites each photosynthetic reaction center once and only once; a dark step must intervene before the reaction center can accept a second quantum of excitation energy. The flash saturation yield of oxygen evolution was shown early to be 1 O_2 molecule per flash per 2500 chlorophyll molecules, when sufficient dark time is allowed between flashes (for review, see ref. 1). Since the quantum requirement for oxygen evolution is roughly ten, this gave rise to the idea that 250 chlorophyll molecules work together to use a single photon. This became the operational definition of the size of the photosynthetic unit.

Later experiments showed that longer flashes give a higher flash saturation yield and hence a smaller apparent photosynthetic unit, because they permit more than one primary reaction center. These experiments (see, for instance, refs. 2 and 3), which span 25 years, are reviewed by Kok and Cheniae[1].

A separate line of experiments used brief light flashes of saturating intensity applied after a long dark period, during which time oxidized intermediates on the path from water to oxygen were presumed to have reverted to stabler reduced states. Joliot and co-workers[4,5] found that the third such flash produces much more oxygen than the long term average flash saturation yield. No oxygen at all is produced by the first or second flash[6]. The yield of subsequent flashes can be described mathematically as an oscillation with a basic period of four modified by damping terms.

These experiments naturally raise the question: how brief a flash is brief enough to ensure that the reaction center turns over only once? In other words, how fast is the dark process that 'sets up' the reaction center to accept another excitation quantum?

One of us has shown that the first few 27-μsec flashes from a xenon flashlamp following a long dark period gave substantially the same results as 40-nsec flashes from a Q-switched ruby laser under the same conditions[6]. Recently, Mauzerall[7] discovered an unexpected transient increase, with a rise time about 25 nsec, in the *in vivo* fluorescence quantum yield following a long dark period. This result raised the possibility that events in the 3–40 n-sec time range might be producing unsuspected 'double-hit' processes even during light flashes lasting less than 10 μsec, such as are now used in many laboratories[8–10].

The present work demonstrates that neither the flash saturation yield nor the flash activation kinetics of photosynthetic oxygen evolution change significantly when 3-nsec pulses from a dye laser are used instead of attenuated 2-μsec pulses from a xenon flashlamp. This brings this experiment to within a factor of 7.5 of the ultimate limit set by the 0.4-nsec lifetime of *in vivo* fluorescence emission[11].

Chlorella pyrenoidosa were grown in Myers medium[12] aerated with a 5% CO_2 air mixture. Growth flasks were shaken gently in a constant temperature water bath under fluorescent light.

Oxygen measurements were carried out on a bare stationary polarographic electrode similar to that of Joliot and Joliot[13], using a detection circuit adapted from theirs. The circulating medium was 0.1 M KCl buffered to pH 7.6 with 0.01 M phosphate. The output signal was displayed on an oscilloscope or a Sanborn recorder without averaging. White light flashes were obtained from a quenched Novatron 599 lamp by a model 422 micropulser, both obtained from the Xenon Corp., Medford, Mass. 2 μsec elapsed from onset through the peak and down to 1/3 maximum intensity. 20 μsec after the beginning of the flash, its tail had fallen to $2 \cdot 10^{-3}$ peak intensity; 20 μsec later it was down to 10^{-4} peak intensity. The light was focussed by thick glass lenses but was otherwise unfiltered.

Flashes of 460-nm light were obtained from a circulating dye laser of 7-dimethylamino-4-methylcoumarin, Eastman P5419 (practical). The dye solution was optically pumped by a focussed beam of 3371-Å light from a nitrogen laser, Avco Corp. Model C102 (refs. 14,15). These flashes lasted 3 nsec from onset through the peak and down to one-third peak height, as measured by an ITT model FW114A biplanar photodiode connected to a Tektronix 519 oscilloscope. Additional experiments were done directly with the output beam of the nitrogen laser itself. These flashes have a duration of approx. 10 nsec (full width at half height).

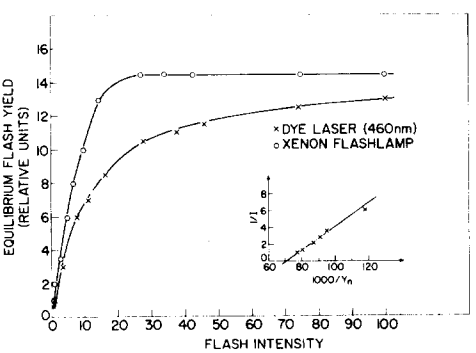
The optics were arranged so that the flashlamp or the laser could be focussed alternately on the same sample by adjusting a mirror and a few lenses. This made it possible to compare the signal produced by the same sample in response to the two kinds of flashes. (The construction of the electrode does not permit quantitative comparisons between different samples.) The diffusion time of oxygen out of an algal cell is long compared to the flash duration, so that the oxygen flash yield can be taken as proportional to the spike height for either flash duration. The shape of the signal produced by the microjet of oxygen is the same for the two kinds of flashes.

Saturation curves were obtained with Wratten filters calibrated in a Cary 14 spectrophotometer. They were corrected for the emission spectrum of the lamp but not for the absorption spectrum of the sample. 20 saturating flashes were applied to algae before the 5-min dark period required in some experiments.

Fig. 1 shows the flash saturation yield curves obtained from dye laser and xenon flashes, spaced 1 sec apart, after a long series of flashes. The dye laser did not yield enough energy per pulse for complete saturation, but the reciprocal plot in the inset shows clearly that the two curves have the same asymptote. A log-log plot indicates that the differences in the shapes of the two curves are not due to corrections to the incident lamp intensity and deserve further study. The focussed beam of the nitrogen laser was sufficient to saturate the oxygen signal to the point where inserting a filter of 50% transmission did not change the signal height. The saturation value under these conditions was the same as that obtained from the xenon flashes. These ultraviolet laser flashes inactivated the oxygen evolution system so fast, however, that we were unable to obtain a complete saturation curve.

Fig. 2 shows the flash activation kinetics obtained from xenon flashes spaced 0.32 sec apart, after a dark period of 5 min. The unattenuated xenon flashes produced a small yield of oxygen on the second flash, followed by oscillations similar to those observed by Joliot. (A small yield on the second flash was often observed with super-saturating 20-nsec flashes[6].) The flash yield pattern is in only fair agreement with the prediction of the model proposed by Kok *et al.*[9], using $\alpha = 0.23$ and $\beta = 0.015$.

As these flashes are attenuated, the yield of the second flash disappears, the yield of the third flash diminishes relative to that of the fourth, and the dramatic oscillation in



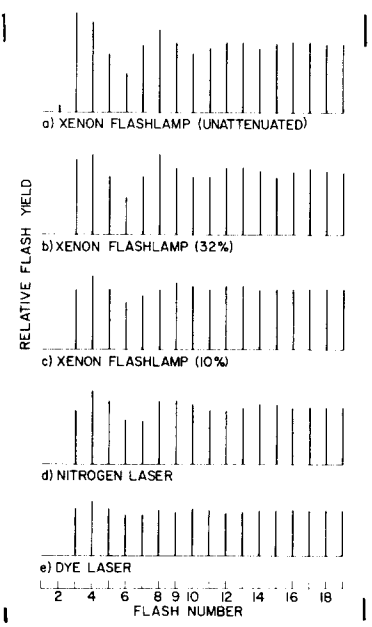


Fig. 1. Saturation curve for oxygen evolution from 2-μsec xenon and 2-nsec dye laser flashes. Flash separation, 1 sec. Intensities of light from the two sources are not to the same scale. Inset shows double reciprocal plot for laser flashes. Extrapolation to infinite intensity gives $Y_n = 14.3$.

Fig. 2. Oxygen yields from first 15 flashes following 5 min dark period. a – c, 2-μsec xenon flashes with various attenuations; d, 10-nsec nitrogen laser; e, 2-nsec dye laser.

the yields of subsequent flashes is reduced to a ripple. Still, Fig. 1 shows that the flashes are sufficient to give a steady-state flash yield within 10% of saturation. These experiments indicate that considerably more light is needed to saturate the third flash than is needed to saturate the steady state signal, at least in Chlorella under our experimental conditions. This observation does not agree with the model of Kok *et al.*[9].

Fig. 2d shows the flash activation kinetics obtained with the nitrogen laser. The pattern of flash yields is very similar to that produced by the attenuated xenon flash of Fig. 2c. The laser flash gave a steady-state signal which was reduced by less than 5% by a 50% attenuation with pyrex microscope slides. Fig. 2e shows that the same activation kinetics were obtained with dye laser flashes of intensity sufficient to produce 69% of the saturating signal as previously illustrated in Fig. 1.

Until we have a high repetition rate source of nanosecond flashes considerably more intense than the ones now available, it will be impossible to say for certain that full-fledged oscillations will occur in response to nanosecond flashes. However, the present data do not support a substantial difference between the activation of photosynthetic oxygen evolution under nanosecond and attenuated microsecond flashes.

We therefore restate the earlier conclusion[6] that the oxidized and reduced primary products of Photosystem II take much longer than 27 μsec (the length of the flashes of ref. 6) to separate to such an extent that the reaction center can accept another quantum of excitation energy and use it for photochemistry.

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