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INHIBITION OF OXYGEN EVOLUTION FOLLOWING ILLUMINATION OF *CHLORELLA* CELLS WITH FAR-RED LIGHT

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SUMMARY

The effect of brief far-red illumination on *Chlorella pyrenoidosa* cells has been investigated.

An inhibition of oxygen evolution occurs 20 to 30 s after the end of the far-red illumination. This inhibition occurs in a step following the initial charge separation process by the System II centers. It is reversible in the light through a purely photochemical process.

INTRODUCTION

Wavelength dependence of photosynthetic oxygen evolution has been a subject of interest for some time. In this paper, we will describe a new phenomenon through which brief far-red illumination of algae (< 1 min) induces an inhibition of oxygen evolution which can last for several minutes. This inhibition is reversed in the light through a purely photochemical process.

MATERIAL AND METHODS

(a) *Chlorella pyrenoidosa* are grown in Knop medium with Arnon's trace elements A_5 and B_6. Before use cells are resuspended in 0.05 M phosphate buffer (pH 6.4) containing 10 mM KCl.

(b) Oxygen flash yields are measured by a polarographic method as described by Joliot et al. [1]. A flash lamp (General Radio Strobotron) was used for these measurements (duration 3 μs at one-third of the peak intensity). Absorption changes were measured using a kinetic spectrophotometer as described by Joliot et al. [2].

RESULTS AND DISCUSSION

Oxygen emitted by a sequence of flashes following continuous illumination

Algae were preilluminated by far-red light (710 nm) for 30 s and a series of saturating flashes were given at the end of the preillumination. Fig. 1 shows typical

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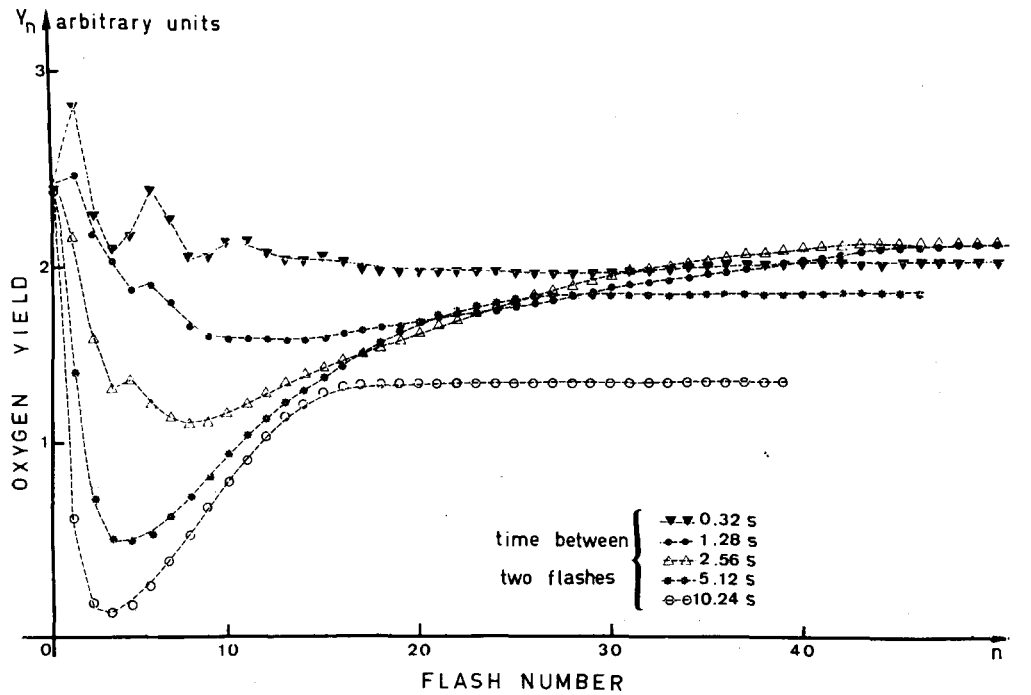

Fig. 1. Oxygen flash yield sequence following 30 s of illumination with far-red light (710 nm). Y_n stands for the amount of oxygen evolved after flash number n. Dark time between the saturating flashes is varied as indicated on the figure. Intensity of the far-red light is such that one photon is absorbed each second per System II center. The first flash of the series is synchronized with the extinction of the far-red beam.

results of such experiments for various dark times between flashes. When this time is long enough the pattern of the oxygen yield displays two phases. During the first phase, the oxygen yield decreases to a minimum value. During the second one, it increases and reaches a stationary value. The transitory reduced flash-yield of oxygen corresponds to a deficit in oxygen production of 4–7 equivalents per System II center (one to two molecules of oxygen per center). This peculiar pattern of the oxygen yield is specifically observed after far-red illumination. As shown Fig. 2, no biphasic pattern of the oxygen yield is observed following illumination of algae with red light exciting System II at the same rate. The two phases of the oxygen yield pattern observed following far-red light display distinct properties. In the experiment shown on Fig. 3, we compared the pattern observed with saturating or nonsaturating flashes exciting only 10 % of the System II centers. The dark time between the flashes is the same in both cases. One observes that the time required to reach the minimum yield is not dependent upon the number of absorbed photons following far-red light. This minimum occurs about 20 s after the end of the preillumination. On the other hand, the recovery phase is purely dependent upon the number of absorbed photons following far-red illumination. The recovery phase lasts 10 times longer when nonsaturating flashes are used. Half of the recovery is observed when about 8 photons have been absorbed per System II center.

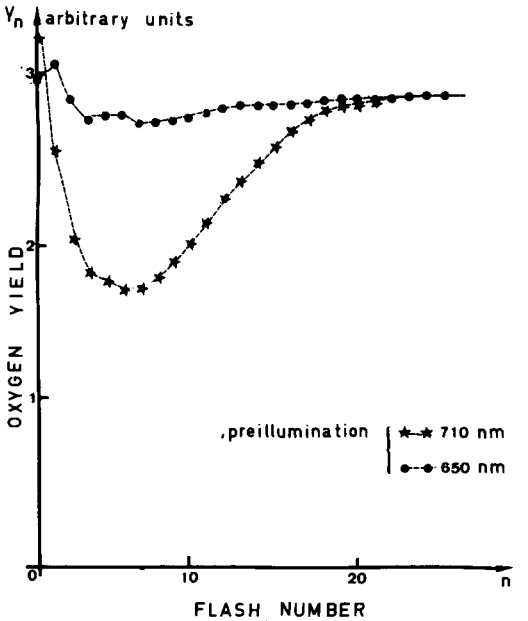

Fig. 2. Oxygen flash yield sequence following 30 s of illumination with either red or far-red light. Intensity of red and far-red light is adjusted so that in both cases one photon is absorbed each second per System II center. Dark time between saturating flashes is 2.5 s. Y_n, see Fig. 1.

The dependence of the oxygen flash yield pattern upon the duration of the far-red illumination is shown in Fig. 4. One observes that after 3 s of far-red light most of the phenomenon has developed. Longer preillumination times only slightly shorten the time at which the minimum yield is observed. No changes in the pattern are detectable for preillumination longer than 60 s.

Origin of the inhibition of oxygen evolution following illumination with far-red light

Evolution of oxygen requires several successive events: (a) charge separation by the System II centers, (b) stabilisation of the charges, (c) accumulation of positive equivalents to give rise to the S_4 state of Kok et al. [3], (d) evolution of oxygen from S_4.

Experiments were performed in order to understand at which level the far-red induced inhibition takes place.

Charge separation: It was shown that the increase in absorbance at 520 nm observed 400 μs after one flash is proportional to the number of charge separations induced by the flash [2, 4]. With *Chlorella* cells, System II centers contribute at least 25 % of the electric field generated. In the experiment shown Fig. 5, we measured the absorbance change at 520 nm induced by a series of saturating flashes following far-red illumination. No detectable variation of the absorbtion occurs in the course of the series. Therefore the inhibition of oxygen evolution following far-red illumination is not due to the appearance of photochemically inactive System II centers.

Accumulation of charges: The amount of oxygen evolved by four saturating flashes given a few hundred milliseconds apart permits an estimation of the amount of the four S states [3]. In the experiment shown Fig. 6, we plot the change in yield of

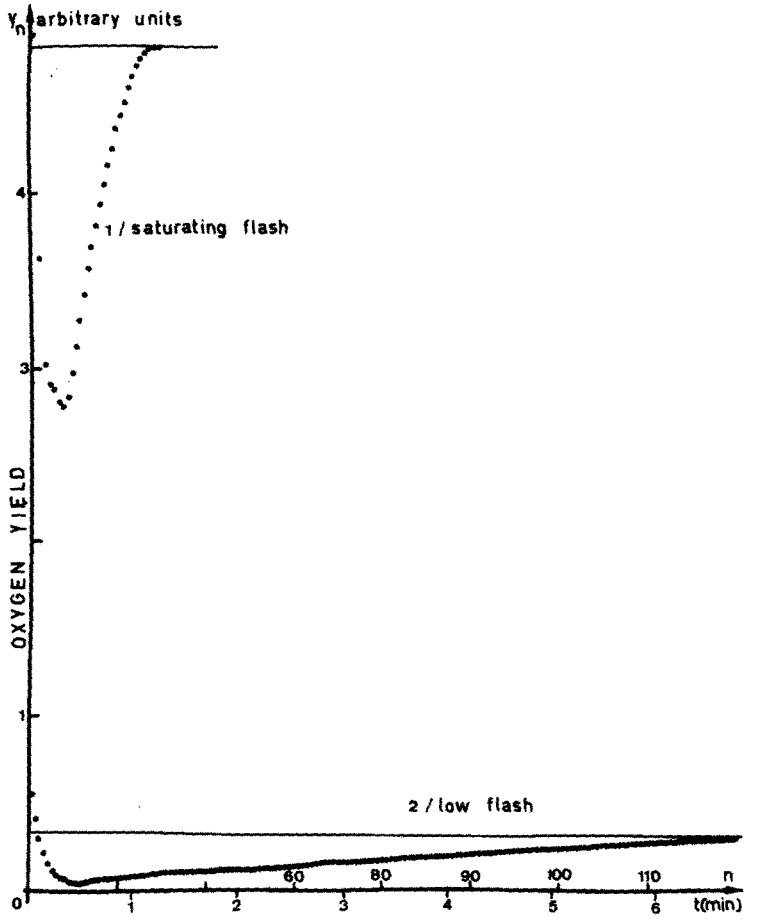

Fig. 3. Oxygen flash yield sequence following 30 s of illumination with far-red light as in Fig. 1. Dark time between flashes is 2.5 s. Curve 1, saturating flashes; curve 2, flashes exciting only 10 % of the System II centers. Y_n, see Fig. 1.

oxygen for each of four flashes (Y_1, Y_2, Y_3 and Y_4) as a function of the dark time following far-red illumination. Y_1 and Y_2 both decline, while Y_3 and Y_4 both increase but not by an amount sufficient to compensate for the decline in Y_1 and Y_2. Compare with the pattern on p. 293 of ref. 5, (after 2 flashes) where the decline in Y_1, is compensated for by an increase in Y_2. So, the decline in Y_1, after far-red illumination (which measured the amount of S_3) is not likely to be totally due to a decay of S_3 into the less charged state S_2, S_1 or S_0. However, if such a decay occurs one is forced to admit that the less charged S states produced do not participate in oxygen evolution when subjected to an illumination with three flashes.

Following illumination of algae with far-red light, an inhibition of oxygen evolution is observed. This inhibition can last for several minutes and is reversed in the light through a photochemical process. This inhibition is not due to the appearance of photochemically inactive System II centers. An intriguing aspect of this phenome-

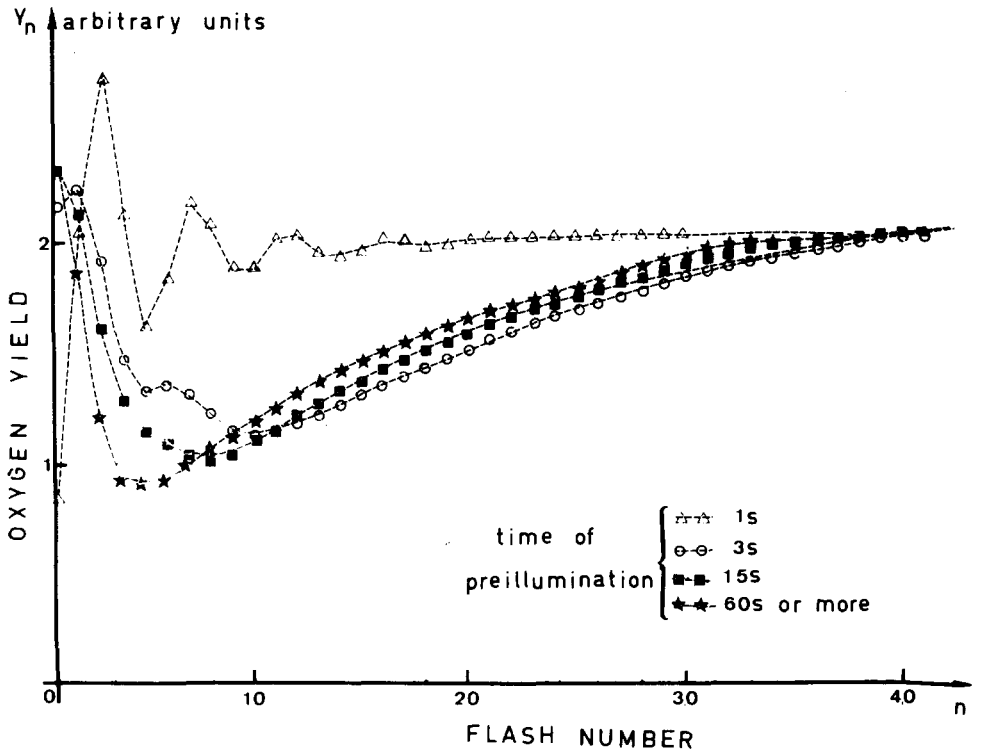

Fig. 4. Oxygen flash yield sequence following illuminations of various durations with far-red light. Dark time between flashes is 2.5 s. Other conditions as in Fig. 1.

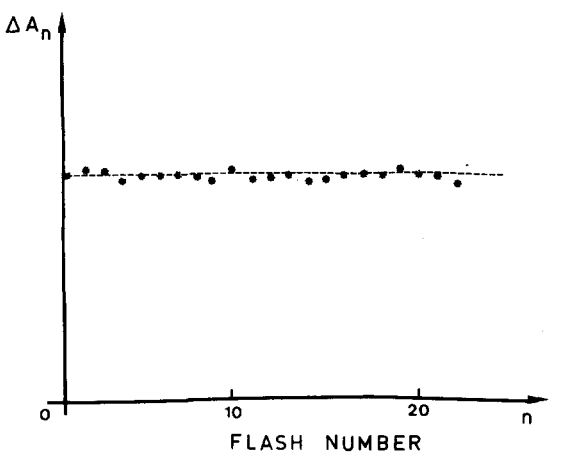

Fig. 5. Absorption increase at 520 nm induced by a series of saturating flashes following 30 s of illumination with far-red light. Absorption changes are measured 350 μs after each flash of the series. Intensity of the far-red light is the same as in Fig. 1. The first flash of the series is synchronized with the extinction of the far-red beam. Dark time between flashes is 2.5 s.

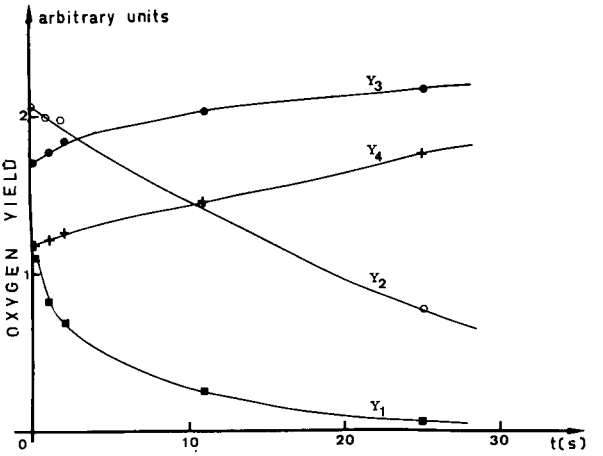

Fig. 6. Algae are illuminated for 30 s with far-red light as in Fig. 1. After a dark time t a series of four saturating flashes is given. Dark time between the flashes is 320 ms. The amount of oxygen evolved after each flash (Y_1, Y_2, Y_3 and Y_4) is measured.

non is that the inhibition is not observed immediately after the far-red illumination. A period of 20–30 s is necessary after the preillumination for the inhibition to develop. Also, good integrity of the photosynthetic apparatus is required in order to observe this phenomenon, as it was not detectable in isolated spinach chloroplasts. One hypothesis is that a pool of reductant is accumulated through Photoreaction I during the preillumination. This pool would become slowly accessible to the donor side of System II centers. Once this pool had become accessible to System II centers, it could react rapidly with the positive equivalents formed by the System II centers before the formation of the S state.

Another possibility would be that the far-red illumination induces an inactive state for a fraction of the System II centers which prevents their participating in oxygen evolution. More information is needed in order to understand the nature of the inhibition of oxygen evolution reported in this work.

REFERENCES

1 Joliot, P. and Joliot, A. (1968) Biochim. Biophys. Acta 153, 625–634
2 Joliot, P. and Delosme, R. (1974) Biochim. Biophys. Acta 357, 267–284
3 Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475
4 Junge, W. and Witt, H. T. (1968) Z. Naturforshc. 23b, 1284–1287
5 Joliot, P., Joilot, A., Bouges, B. and Barbieri, G. (1971) Photochem. Photobiol. 14, 287–305