

SHORT COMMUNICATIONS

BBA 43279

Time-dependent quenching of chlorophyll *a* fluorescence from (pigment) system II by (pigment) system I of photosynthesis in *Chlorella*

The quenching of System II fluorescence by System I light was discovered in 1960 (ref. 1) and was later investigated in detail²⁻⁴. DUYSSENS AND SWEERS³ suggested that System II reduces a compound "Q", and System I oxidizes this reduced Q back to its oxidized form. The Q in the oxidized, but not in the reduced, form is a quencher of fluorescence. We have measured the time dependence of this quenching of System II fluorescence by System I light in *Chlorella pyrenoidosa*. In the first few seconds of the fluorescence transient, however, we observe a stimulation, and only thereafter a quenching. The quenching effect attains a maximum value after 30-120 sec of System II illumination and then declines to a low level. A comparison of this data with the time-course of System II fluorescence suggests that additional assumptions are required to explain our data.

Chlorella pyrenoidosa (Emerson strain 3) was grown for 4 days in inorganic medium⁵ and resuspended in NaHCO₃-K₂CO₃ buffer, pH 9.2. Several cultures and at least twenty samples have been tested for the results presented here. Measurements were made with the instruments described earlier^{6,7}. Fluorescence was excited by intense blue light (λ peak, 480 nm; half-maximum band width, 100 nm; absorbed quanta, approx. $4 \cdot 10^{14}$ sec·cm²), and was measured at 685 nm (half-band width, 5 nm). Quenching was caused by 710 nm light (half-band width, 13 nm; absorbed quanta, approx. $5 \cdot 10^{11}$ sec·cm²). 546 nm light of similar band width and intensity caused no change in fluorescence yield. Filters were used to transmit fluorescence and to prevent nearly all the exciting light from entering the measuring monochromator. With 710 nm light alone, the light leak was very low, less than 2% of blue-excited fluorescence; it was measured and subtracted from the blue + 710 nm excited fluorescence.

The experimental procedure was a series of 5 min dark-5 min (blue) light cycles. Blue light preferentially excited System II. Typical fluorescence transients resulting from the dark-light cycles have been published previously^{7,8}. The System II fluorescence yield (observed at 685 nm) undergoes a slow induction labelled S, M and T (see Figs. 1 and 2). Interaction with System I was studied by adding 710 nm light at different times during the induction of fluorescence mentioned above.

The 710-nm light caused no fluorescence quenching in the first 5-10 sec of the (blue) light period (Figs. 1 and 2). Instead, the fluorescence yield increased slightly (1-5%). We considered the possibility of an apparent increase due to nonlinearity in the fluorescence (F) versus intensity (I) curve and the fact that about 10% of the 710 nm light quanta excite System II⁹. However, the intensity (I) of blue light was high enough so that the fluorescence (F) was in the second linear portion of the curve F versus I. This linearity was tested by adding some 546 nm light (absorbed almost equally in the two systems; the intensity of this light was adjusted to give the same

signal as 710 nm light). No change in the fluorescence yield was observed with 546 nm light in contrast to results with 710 nm light. The small increase in fluorescence yield caused by 710 nm light during the first 5–10 sec of System II light is therefore considered real. The absence of quenching during this period was observed in *Chlorella* cells suspended in their growth medium as well as in Warburgs buffer No. 9.

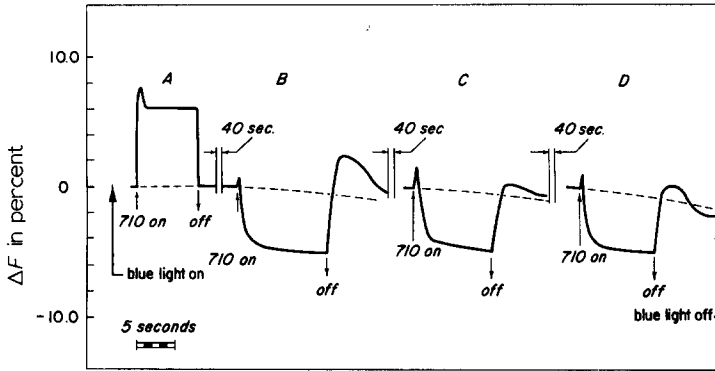


Fig. 1. Percent changes in fluorescence yield in *Chlorella pyrenoidosa* caused by supplementary illumination of 710 nm. Fluorescence at 685 nm excited by blue light. (A): far red light given after 5 sec of blue illumination; (B–D): given after successive periods of 40 sec. The level of fluorescence prior to 710 nm illumination was adjusted to read zero; the signal due to 710 nm light alone has been subtracted (see text).

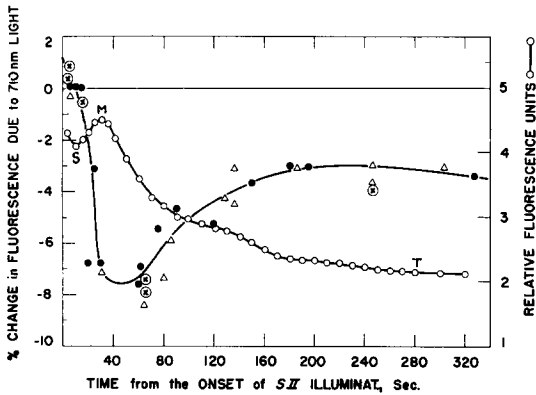


Fig. 2. Percent changes in the fluorescence yield due to 710 nm light at various times during the fluorescence transient. Experiments with three different cultures (thick line with open triangles, solid circles and crossed circles) are shown: the scale to the left. For comparison the fluorescence transient for the cultures used in these experiments is represented by the curve with a thin line and small open circles: the scale to the right. (For definition of S, M, T, see ref. 8.)

After 10 sec of blue light, however, 710 nm light caused quenching (Fig. 2). The amount of quenching increased and became maximal between 30 and 120 sec; it then decreased slowly until about 3–5 min (Fig. 2). The maximum amount of quenching varied from culture to culture, between 7 and 18 %, but remained constant in a given sample. When the maximum quenching was 7 %, it occurred between 30 and 60 sec, declined to 3 % by 3 min. However, when the maximum quenching was 14–18 %, it occurred between 1 and 2 min and the quenching declined more slowly.

Upon cessation of 710 nm light, the fluorescence yield increased (1–4%) and then declined to the original steady state (Fig. 1). This rise was completed within 1–2 sec, and the half-time of its decline was greater than 5 sec. This confirms the observations of MURATA¹⁰ in *Porphyridium* and those of BONAVENTURA AND MYERS¹¹ in *Chlorella* (see also ref. 12).

When the intensity of System II light was decreased, the amount of quenching by System I light declined. And, when the intensity was lowered to the 24% of that used in the experiments in Figs. 1 and 2, no quenching was observed.

DCMU (3-3',4'-dichlorophenyl)-1, 1-dimethyl urea; $1 \cdot 10^{-5}$ M), the well known poison of photosynthesis, abolished quenching. Most of the transitory yield increase after the cessation of System I light was also abolished by DCMU. However, during the 5–10 sec of fluorescence induction, a 1–2% increase remained even in the presence of DCMU.

The time dependence of the quenching of fluorescence and its comparison with the time-course of fluorescence indicate that System II fluorescence yield does not entirely depend on the amount of reduced Q or the "light state" of the chloroplast (see ref. 11). In the 50–200 sec range, the quenching remains high although fluorescence continues to decline sharply. Also, for the same level of fluorescence (*i.e.*, for the same concentration of QH or for the same state of chloroplast) the percentage of quenching is higher in the M → T decline phase than in the S → M rise phase. The explanation of these results requires further study. However, one working hypothesis is to suggest that System I light may trigger two types of changes that have opposing effects on the fluorescence yield. (For some recent ideas on the interaction of two systems see refs. 13, 14.)

We gratefully acknowledge the support of the National Science Foundation.

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Received May 11th, 1970

Revised manuscript received August 7th, 1970