Short Communication

Absence of Fluorescence Quenching in a Photosynthetic Mutant of Euglena gracilis

George K. Russell
Department of Biology, Adelphi University, Garden City, New York 11530

Harvard Lyman
Microbiology Division, Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973

Robert L. Heath
Biology Department, Brookhaven National Laboratory, Upton, New York 11973

Received December 31, 1968.

Substantial experimental evidence indicates that the level of chlorophyll fluorescence in algae and higher plants is controlled predominantly by the activity of photosystem II (PS II) of the photosynthetic electron transport chain (2). The overall fluorescence yield seems to depend on the oxidation/reduction state of Q, the presumed primary electron acceptor of PS II (7) (H₂O → → Y → Q → A → ? → P₇₀₀ → XH). Accordingly, Duysens' hypothesis (4), the oxidized form of Q quenches chlorophyll fluorescence, while the reduced form (QH) does not. We report here a photosynthetically deficient strain of Euglena which appears to be lacking the fluorescence quenching normally associated with the presence of Q.

Euglena gracilis (strain Z) was cultured as previously described (9). The pale green mutant P₄ is unable to carry out photosynthetic electron transport because of a block at or near light reaction II: detailed studies of this strain have been reported elsewhere (10).

Chloroplast fragments were prepared according to Katoh and San Pietro (6). Fluorescence was measured with the technique devised by Duysens (3). Whole cells or chloroplasts were illuminated with a modulated monochromatic light beam (λ = 435 nm, 270 cps), and the fluorescence emission was measured with a photomultiplier/amplifier system tuned to the chopping frequency. Provision was also made for illuminating the sample with a constant actinic beam (510 ± 40 nm; I = 5 × 10⁵ ergs cm⁻² sec⁻¹); fluorescence excited by this light was not detected. With this apparatus the relative fluorescence yield could be measured in the presence and absence of actinic light.

Fig. 1 illustrates the relationship between fluorescence and chlorophyll concentration for whole cells of Euglena. The fluorescence of P₄ was consistently 3 to 5 times higher than the wild type. We attribute this to the absence of fluorescence quenching in whole cells of the mutant strain. Wild type fluorescence could be increased 2 to 3-fold by the addition of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethyl urea] to a final concentration of 10 μM. Fluorescence emission spectra of wild type and P₄ at room temperature showed maxima at 685 nm.

The effect of actinic illumination on the relative fluorescence yield was studied in isolated chloroplast fragments. Illumination of wild type fragments with a very weak measuring beam (20 ergs cm⁻² sec⁻¹)
Fig. 2. The effect of actinic illumination on the relative fluorescence yield of wild type and P₁ chloroplasts. The modulated light intensity was 20 ergs cm⁻² sec⁻¹. The intensity of the actinic beam was 5 × 10² ergs cm⁻² sec⁻¹. The measuring beam was turned on at the arrow marked 1 above. The actinic beam was turned on at 2 and off at 3. A) Wild type chloroplasts. B) P₁ chloroplasts.

excited a low level of fluorescence (Fig. 2a). Subsequent illumination of the sample with actinic light increased the fluorescence yield by a factor of 2.5, presumably by bringing about higher steady state reduction of Q (Fig. 2a). Comparable experiments with P₁ chloroplast fragments clearly demonstrated the absence of fluorescence quenching in the mutant strain. The low intensity measuring beam induced a higher level of fluorescence than that seen in wild type, and the actinic light had no effect on the fluorescence yield (Fig. 2b).

The kinetics of fluorescence emission by isolated plastid fragments are shown in Fig. 3. The biphasic curve shown for wild type plastids (Fig. 3a) is typical of many photosynthetic systems (7). The initial rise (F₀) represents constant chlorophyll fluorescence which is unaffected by Q. The second (or variable) phase is related to the state of oxidation of Q. The time course shown in Fig. 3a demonstrates the attainment of a steady state in which Q is partially reduced. Addition of DCMU to a final concentration of 10 μM altered the kinetics of induction and increased the final yield (Fig. 3a). Since DCMU is believed to block electron transport between Q and A, the secondary electron acceptor (2), the increase of fluorescence with time in Fig. 3b represents the kinetics of Q reduction without subsequent reoxidation of QH₂ by photosystem I. The final fluorescence yield reflects almost complete absence of fluorescence quenching.

No initial rate of increase in fluorescence could be obtained with P₁ plastids even in very weak measuring light (Fig. 3c); maximum fluorescence was attained within the time required for shutter opening on the measuring beam (ca. 25 msec). In DCMU-treated wild type, 4 to 5 sec were required for attainment of maximum fluorescence (Fig. 3b). DCMU had no effect on either the fluorescence yield or the rate of attainment of the final yield in P₁ (Fig. 3c).

Within the context of the Duysens hypothesis, we conclude that 1) Q is missing altogether in P₁, or 2) Q is permanently reduced in P₁, or 3) the rate of Q reduction is many times higher in P₁, and the kinetics of reduction can not be detected under the conditions of our experiments, or 4) Q is present in P₁, but unable to quench chlorophyll fluorescence for various reasons (e.g. mutational alteration of chloroplast structure in P₁). Explanation 2 is rendered very improbable by the existence of pathways of QH₂ reoxidation in the dark even in the presence of DCMU (1). We have no evidence either to support or reject explanation 4. We favor the interpretation that the mutant strain may be missing Q, the primary oxidant of PS II of photosynthesis.

Several investigators have reported increased fluorescence yields in photosynthetic mutants of a variety of organisms. Mutant IIb of Oenothera recently described by Fork and Heber (5) shows constant high fluorescence, and many of the properties of this mutant are similar to P₁. The fluorescence of mutant II of Scenedesmus is unaffected by
actinic illumination (1) but the fluorescence kinetics have not been reported. Butler (2) has suggested that this mutant may be missing Q. Recently Lavorel and Levine (8) have described the characteristics of a mutant strain of *Chlamydomonas reinhardi* (*acr-115*) which appears to be lacking Q, but which also lacks detectable amounts of cytochrome 559; further investigations will be necessary to determine the exact nature of the lesion(s) in the mutant. The finding of mutant organisms apparently lacking Q opens the possibility of identifying the chemical nature of this substance.

**Literature Cited**


