Electron Transport-Dependent Chlorophyll-a Fluorescence Quenching by O₂ in Various Algae and Higher Plants

DOUG BRUCE, WILLIAM VIDAVER, KONRAD COLBOW, AND RADOVAN POPOVIC
Photobiology Group, Departments of Physics and Biological Sciences, Simon Fraser University,
Burnaby, British Columbia V5A 1S6 CANADA

ABSTRACT
A comparison of chlorophyll-a fluorescence in brown algae (Macrocytis integrifolia, Fucus vesiculosus), green algae (Scenedesmus obliquus, Ulva sp.) and higher plants (bean, corn) show differences in the relative fluorescence intensities and induction time courses which characterize each type of plant. These differences are not reflected in either the maximum fluorescence emission in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (Fₘₐₓ) or the nonvariable fluorescence (F₀). Constancy of F₀ and Fₘₐₓ suggests functional similarities of photosystem II and associated antenna pigments in the various classes of plants. The time course differences are observed only in the absence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea and appear, therefore, to be electron transport dependent. During induction, the peak in fluorescence (Fₛ) is much lower in all of the algae studied than in the higher plants. Exogenous O₂ strongly quenches Fₛ in all plants studied and our data indicate that the low Fₛ in the algae can be partially accounted for by endogenous O₂ quenching.

Photosynthetic plants can be classified according to the type of pigment system they possess. For example: red, brown, green algae are so designated because these colors result from the presence of phycocerythrin, fucoxanthin, and Chl-β as their respective accessory pigments. The brown algae also have Chl-c. Only green algae and the terrestrial green plants share a common pigment system. Despite this diversity in pigments, its significance is presumably limited to light capture and the basic energy conversion steps are believed to be similar for all O₂ evolving plants (1, 6). Chl-a is universally present in all plants which have the capacity for water splitting, and there is much evidence to indicate that the function of accessory pigments is to transfer excitation energy to Chl-a (4). Excitation energy transfer is a highly effective quencher of accessory pigment fluorescence, thus Chl-a fluorescence is indicative of light harvesting by the accessory pigments as well as by Chl-a.

In this work we compare the in vivo Chl-a fluorescence emission of two brown algal species, two green algae, and two higher plants in order to investigate possible differences in absorbed light energy utilization. We found major differences in both the relative fluorescence intensity and the dark adapted time courses. Using the technique of fluorescence quenching by hyperbaric O₂ (11, 12), we obtained results which suggest that some of the difference in fluorescence emission by the various plants can be accounted for by the influence of O₂ on electron transport.

MATERIALS AND METHODS
Fresh samples of mature Macrocytis integrifolia, Fucus vesiculosus, and Ulva sp. were collected weekly and kept in an aquarium at 15°C with a 16 h photoperiod. Phaseolus vulgaris and Zea mays were greenhouse grown under natural illumination; plants used were 2 to 4 weeks old. For Fₘₐₓ determinations in bean and corn, 8-mm leaf discs were floated on an aqueous solution of 1 µM DCMU in the dark for 1 h prior to measurement. To determine Fₘₐₓ in seaweed, 8-mm discs were suspended in a seawater solution of 1 µM DCMU for 1 h in the dark; kelp blade discs were first suspended in seawater for 30 min to allow partial removal of the mucopolysaccharides which interfere with DCMU inhibition. All seaweeds were kept at 10 to 15°C in seawater until used.

The fluorescence measuring apparatus, stainless steel pressure cell, and data acquisition technique have been previously described (12). Blue actinic light (Corning filter 4-96) was adjusted to 10 w m⁻² at the sample.

All fluorescence determinations were done using dark-adapted samples for more than 1 h. F₀ was determined with ≈80 ms flash of actinic light. Results from samples in an O₂ atmosphere were independent of the presence of 300 µl/l CO₂.

RESULTS
Typical fluorescence induction curves for bean are seen in Figure 1a. The upper trace shows Fₘₐₓ in the presence of DCMU; lower traces are for untreated leaves under increasing O₂ pressures (atm). The bottom trace is for F₀ in air, normalized to 1 relative fluorescent unit. Figures 1b and 1c show the same set of measurements for Ulva and M. integrifolia, respectively. It is readily apparent that although Fₘₐₓ = (Fₘₐₓ - F₀)/F₀ is similar in these plants, Fₛ = (Fₛ - F₀)/F₀ is lower in the seaweeds, especially so in M. integrifolia (Fig. 1d).

O₂ reversibly quenched Fₛ in all plants studied (Fig. 1a, b, c). The O₂ quenching curves (Fig. 2) show similar half-quenching O₂ pressures of 7 atm for bean, 6 atm for Ulva, and 5 atm for M. integrifolia, suggestive of similar mechanisms for O₂ quenching; Fₛ is lower in Ulva than in bean and lowest in M. integrifolia at all O₂ pressures.

Half-quenching with DCMU present required more than 40 atm O₂ in all three plants, and O₂ quenching of Fₛ fits the same curve for all three (Fig. 2). The result is similar to that previously reported for bean, Scenedesmus obliquus, and chloroplasts from spinach and lettuce (11, 12).

In Table I, values of Fₛ and F₀ for various higher plants, unicellular algae, and seaweeds are compared; Fₛ is similar for all of the plants, yet F₀ is distinctly lower in the algae than in corn or bean.

1 Abbreviations: Fₘₐₓ, maximum in variable fluorescence in the presence of DCMU; F₀, 0 level fluorescence; Fₛ, peak in variable fluorescence during induction of photosynthesis.
**FLUORESCENCE QUENCHING BY O₂ IN ALGAE AND HIGHER PLANTS**

**FIG. 1.** Fluorescence induction curves. a, bean; b, Ulva sp.; c, M. integrifolia; d, a comparison of the bean, Ulva sp., and M. integrifolia fluorescence induction curves in air. For a, b, and c, Fₘₐₓ was measured in the presence of DCMU, the control curve is labeled air, and in the lower traces the numbers refer to O₂ pressures in atmospheres. The F₀ trace in air is shown on an expanded time scale.

**FIG. 2.** O₂ quenching of fₘ (solid line) and fₚ (dashed line) in bean (○), Ulva sp. (□), and M. integrifolia (△).

**DISCUSSION**

Interpretation of the data is made under the following assumptions (7).

(a) The measured F₀ is the minimum fluorescence yield resulting from the maximum possible rate of energy transfer from antennae pigments to PSII.

(b) DCMU changes the absolute fluorescence yield by the prevention of Q oxidation via electron transport.

(c) During the initial induction of photosynthesis (5–10 s), the variable fluorescence yield is determined by changes in the redox state of Q.

With the above assumptions, the constancy of fₘ observed in the diverse plants studied is indicative of a functional similarity in PSII and associated antennae pigments which permits in vivo comparisons of the relative redox state of Q. However, as fₘ is a relative measure, similarities in absolute energy transfer efficiencies from antennae to PSII are not implied. In algae not inhibited with DCMU, variable fluorescence was seen to be highly quenched. As fₘ was similar in all the plants, the low fₚ in the algae suggests that during induction Q is relatively more oxidized in the algae than in the higher plants. Either less efficient water-splitting, more efficient electron transport to PSI, or higher oxidation losses from electron transport would result in a lower fₚ. A major electron transport loss involves reduction of O₂ and the subsequent production of H₂O₂ (2, 3, 5, 9). We have previously described the quenching of variable fluorescence by O₂ in higher plants and unicellular algae and suggested that this is most likely due to oxidation of electron carriers near PSI by O₂ (11, 887).
12). It has been reported that Scenedesmus obliquus shows higher rates of O₂ uptake (O₂ reduction) than spinach or soybean (2, 5). The low variable fluorescence yield of S. obliquus (Table 1) supports these results if O₂ is assumed to be the quencher of \( f_o \). We have shown the endogenous quenching of \( f_o \) to be greater in the brown algae than in higher land plants. As O₂ is a potent quencher of \( f_o \) in all the plants, it is conceivable that the endogenous quencher is O₂. If so, higher rates of O₂ reduction and presumably H₂O₂ production are to be expected in the brown algae. In a separate report, evidence supporting this idea is presented from experiments comparing chloroplasts isolated from higher plants and various brown algae (8).

Although electron transport may be more sensitive to O₂ in algae than in higher plants (2, 5), it is also possible that internal concentrations of O₂ are higher in algae. Dissipating endogenously evolved O₂ may be more difficult for aquatic algae than for higher land plants as boundary-layer resistances to gas exchange are much higher in an aqueous environment. For example, CO₂ uptake has been shown to be limited by boundary-layer thickness in Macrocystis pyrifera (13). Increased electron transport to endogenous O₂ in seaweeds and other algae may partially account for the low light saturation of photosynthesis observed in the brown algae (10, 14).

LITERATURE CITED