TWO QUANTUM-HIT REQUIREMENT FOR DELAYED LIGHT EMISSION FROM PHOTOSYNTHETIC GREEN ALGAE

By Larry W. Jones*

Biology Division, Oak Ridge National Laboratory,† Oak Ridge, Tennessee

Communicated by William Arnold, May 15, 1967

The transformation of quantum energy to chemical energy in photosynthesis at some point must involve the separation of charges. To attain high quantum yields at low exciting-light intensities, these charges must be efficiently stabilized to prevent their back-reaction and mutual annihilation. Using energy considerations and evidence from delayed light and fluorescence, Arnold1 has proposed an electron-hole model of the photosynthetic unit in which charge stabilization is realized by the chemical use of only one of the charges from any absorption act. The opposite, unused charges reunite with a probability of emitting a light quantum which may appear as fluorescence or delayed light. In his model, two quanta are required to produce both the oxidizing and reducing moieties at their respective reaction centers.

This study shows that long-term delayed light emission by dark-adapted algae at room temperature is a two quantum-hit process—delayed light being emitted only after the second hit. Delayed light emission varies as the square of intensity of flashes of low-energy exciting light.

Materials and Methods.—The delayed light apparatus is shown schematically in Figure 1. Except for the additional light sources, it is similar to the pumping system of Strehler and Arnold.2 Algae are pumped through several elements connected by black rubber tubing (RT in Fig. 1). The algal suspension was first equilibrated with 5 per cent CO₂ in air and the desired continuous preillumination light (P) for at least four minutes in a 2-liter lollypop (L). When the pump was started, the algae were plunged into darkness as they were pulled into the rubber tubing. At given distances down the tubing the algae passed through short lengths of glass tubing into which the preillumination (Fr) and/or assay (Fₐ) lights could be introduced. These continuous lights through the glass tubing appeared to the algae as flashes whose duration depended upon the length of the glass tube exposed and the rate of flow. The algae then passed through additional black tubing into a 20-cm coil of glass tubing (S) which was taped to the face of a photomultiplier (PM), through the gear pump, and into a large vessel which terminated their passage through the system. The signal from the photomultiplier was fed to a vibrating reed amplifier (Amp) and recorded (R) as delayed light.

As the highest pumping rate (305 cm sec⁻¹), 2 liters of algal suspension were pumped through the system in 27 seconds. An RCA 7102 photomultiplier was held at liquid nitrogen temperature to reduce thermal noise. The over-all time response of the electronics was about 1 second. The intensity of unfiltered tungsten exciting lamps was varied by moving them known distances from the lollypop or by using neutral density filters. The cell concentration of the algae in the apparatus was always less than 0.2 μl/ml.

Chlorella pyrenoidosa (Indiana Culture Collection No. 252) and N. I. Bishop's oxygen mutant 8 of Scenedesmus obliquus were grown as previously described,3,4 and were suspended in their inorganic basal media in the apparatus. No change in
delayed light emission from either alga was noted over eight-hour working periods at room temperature.

Delayed light from the assay flash was combined with that from preillumination. By chopping the assay light beam ($F_A$) with periods of five to ten seconds, the delayed light brought about by preillumination alone could be determined separately from that caused by both preillumination and the assay flash.

**Results.**—Intensity of delayed light emission by dark-adapted *Chlorella* cells (no preillumination) varied as the square of the intensity of the exciting flash. Curves 1 in Figures 2 and 3 show this relationship for delayed light emitted 0.14–0.25 seconds after a 1.7-millisec flash of white light in a linear and log-log plot. Curve 4 in Figure 3 shows the same relationship for an 11.7-millisec flash where delayed light was measured 0.28–0.39 seconds after the flash. Giving two exciting flashes sequentially ($F_P$ and $F_A$ of Fig. 1) resulted in a larger intensity of delayed light emission than the sum of the intensities of the two flashes given separately. The combined delayed light emission from the two above flashes (highest intensity of the 11.7-millisec flash given 0.14 sec before various intensities of the shorter flash) is shown in curve 3 of Figure 2.

The increase in delayed light emission above that expected from the sum of emission from the two flashes given separately is assumed to be caused by an increase in the efficiency of delayed light production from the second flash. The intensity of delayed light emission from the combined flashes can therefore be divided into two components: (1) the intensity of delayed light produced by the first flash alone and attributed to it, and (2) all additional delayed light which is attributed to the second flash. Curves 2 in Figures 2 and 3 plot the component of delayed light attributed to

---

**Fig. 1.**—Schematic diagram of delayed light apparatus. See Materials and Methods.
the second flash, which was calculated simply by subtracting the first flash component. The delayed light component attributed to the second flash varied directly with the intensity of the second flash and extrapolated very close to zero when two flashes were given sequentially.

This nonlinear portion of the intensity curve near zero should also appear in continuous exciting-light intensity curves, but at intensities below the sensitivity of the apparatus (e.g., Fig. 4, upper portion). Continuous preillumination does affect the intensity of the component of delayed light attributed to the assay flash (Fig. 4, lower portion). Except for effects of the dark time in the tube between the two light sources, the component of delayed light produced by the low-energy flash should approach the first derivative of the delayed light intensity curve for continuous preillumination \(dDL/dI\). Two apparent deviations from this derivative are seen in Figure 4, lower portion. First, low preillumination intensities increase the component of delayed light emission from the assay flash exactly opposite of the expected derivative. This increased emission (attributed to the assay flash) is predicted by the \(I^2\) dependence of delayed light emission by dark-adapted algae; i.e., the preillumination intensity curve must also show this increase in delayed light efficiency at preillumination intensities below the sensitivity of the apparatus (is an
S-shaped curve). Second, at preillumination intensities which saturate delayed light emission, the derivative approaches zero instead of the constant positive value found. That the flash-delayed light component is not zero at these preillumination intensities is brought about by a recovery of the ability of the algae to emit delayed light during the dark time in the tube between the two exciting lights. The assay flash response, then, does approach the predicted first derivative of the continuous preillumination intensity curve when the $I^2$ dependence and the dark time in the tube are considered, thus supporting the above assumption.

When saturating continuous preillumination is turned off, the efficiency of delayed light emission by the assay flash increases to a maximum in less than a second, and then begins a slow decline to the low value typical of dark-adapted algae. Two
to four minutes are necessary for delayed light efficiency to drop halfway from the maximum value to the low-efficiency dark-adapted level. Incomplete dark adaptation gives nonlinear intensity curves with slopes intermediate between 1 and 2 on log-log plots. Complete dark adaptation for clear \( I^2 \) dependence requires at least 15 minutes of complete darkness. Intensity curves with slopes higher than about 2 on log-log plots were not found after up to eight-hour periods of darkness.

Dark-adapted Scenedesmus mutant 8 cells display \( I^2 \) dependence identical to that of Chlorella (curve 8, Fig. 3). However, several hours of darkness are necessary to establish the dark-adapted state for either mutant 8 or the "wild-type" parent Scenedesmus. The dark time necessary to establish the dark-adapted state therefore varies greatly between algal species.

Discussion.—The \( I^2 \) dependence of delayed light resulting from the assay flash in dark-adapted green algae and the great increase of delayed light emission efficiency brought about by low-intensity preillumination are interpreted as a requirement for two quantum-absorption acts by each functional photosynthetic unit before delayed light is emitted. That similar kinetics are found in dark-adapted Scenedesmus mutant 8 cells, which do not contain an active photosystem I, places the requirement for both quantum hits in photosystem II (the oxygen-evolving system) and precludes the involvement of both photosystems.

Direct evidence for a two-hit mechanism in photosystem II has been elusive because of the stability of the single-quantum-hit state. The effect described can be observed directly only with very low-energy exciting light and relatively long periods of dark adaptation which allow the single-quantum-hit state to decay. The assay flash was incorporated into the delayed light apparatus in this study to measure the efficiency of delayed light emission at fixed times after prior illumination. The energy of the flash was made as low as possible so that on an average less than one quantum in the flash would be absorbed per reaction center.

Indirect evidence for this effect has been reported by Joliot from experiments on the induction of fluorescence and oxygen evolution. The model presented by Joliot adequately incorporates the data presented here, but treats the first quantum-absorption act as a photochemical "activation" reaction which must take place before photosystem II is active—oxygen evolution then taking place via a single-hit mechanism. His data, and those presented here, do not rule out the possibility of a need for two hits for the continuous activity of photosystem II or oxygen evolution.

The synergistic effects of two sequential light flashes on delayed light emission is similar to that found for oxygen evolution in Wittingham's laboratory, although their second flash was made saturating. They interpreted their results as classical enhancement brought about by interactions of the two photosystems. It remains to be seen whether the two phenomena are related.

Arnold's electron-hole model of the photosynthetic unit uniquely predicts the data presented. Although the interpretation of these data is not unequivocal, this model warrants further consideration.

Summary.—Long-term (0.1–1.0 sec) delayed light from a brief flash of exciting light was studied as a function of prior illumination in whole cells of Chlorella and of Bishop's mutant 8 of Scenedesmus.

Without prior illumination, delayed light varied as the square of intensity of
the exciting flash. With prior illumination, delayed light attributed to the flash varied directly with flash intensity. Mutant 8, which lacks photosystem I, gave similar results.

The intensity-squared dependence is interpreted as a requirement for two absorption acts before delayed light is emitted. The fact that mutant 8 gave similar results places the two-hit requirement in photosystem II, the oxygen-evolving step of photosynthesis. Evidence is given that the curve for delayed light intensity with continuous exciting light is not linear to zero, but is S-shaped at very low intensities of exciting light.

The author is grateful for the opportunity to work in W. Arnold's laboratory and the kindness which he was given.

*Permanent address: Botany Department, University of Tennessee, Knoxville 37916.
†Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.
3 Bertach, W. F., J. R. Azzi, and J. B. Davidson, "Delayed light studies on the oxygen-evolving steps of photosynthesis, I. Identification of the oxygen-evolving photoreaction as the delayed light emitter in mutants of Scenedesmus obliquus," to be published.