The Photosynthetic Unit of Hydrogen Evolution

Abstract. *A study of the absolute photoproduction of hydrogen by autotrophically grown* Chlorella vulgaris *with single-turnover flashes of light indicates that (i) while the Emerson and Arnold photosynthetic unit has the value chlorophyll : oxygen ≈ 1700 : 1, the hydrogen analog of this unit has the value chlorophyll : hydrogen ≈ 1400 : 1, and (ii) whereas the yield of oxygen from dark-adapted algal cells is zero on the first flash and then undergoes damped oscillations of period 4 about the steady-state value, the corresponding yield of hydrogen is fixed at the steady-state value from the first flash onward. These observations suggest that in the molecular mechanism of photosynthetic hydrogen evolution (i) the light reaction is at least 60 percent as efficient as the corresponding oxygen evolution reaction as measured by the ability to utilize absorbed visible quanta, and (ii) there are no sequential, photoproduced, metastable intermediates as there are in the case of oxygen evolution. Therefore, a minimum of two reducing equivalents from two different photosystems must have access to a common pool in producing molecular hydrogen if these photosystems each produce one electron per single-turnover flash.*

A powerful technique for the study of photosynthetic reactions is the technique of illumination with single-turnover flashes. These are flashes intense enough to excite all available photoreactive centers, but short enough to cause only one photoact per center due to dark reactions. In a classical experiment, Emerson and Arnold (*1*) showed that the ratio of chlorophyll to oxygen molecules in *Chlorella* under a steady-state flash regime is about 2500, the classical photosynthetic unit size. This measurement and arguments by Gaffron and Wohl (*2*) slowly led to the modern concept of a photosynthetic unit, wherein many chlorophyll molecules are associated with a single reaction center. Several such centers are involved in the photoproduction of oxygen or hydrogen. In studying photosynthetic hydrogen evolution, one is logically led to compare and contrast it with the corresponding case of oxygen evolution. It is shown in this report that the molecular mechanism of photosynthetic hydrogen evolution is not analogous to that of oxygen evolution. However, the values of the photosynthetic unit size based on oxygen or hydrogen evolution are comparable.

By combining the technique of single-turnover flashes with improved methods of oxygen polarography, Joliot and coworkers (*3*) were able to demonstrate that the oxygen evolved from dark-adapted *Chlorella* or isolated chloroplasts undergoes a damped oscillation with period 4. Further work along these lines led to the linear, four-step model of photosystem II proposed by Kok and coworkers (*4*), which is the widely accepted model of photosynthetic oxygen evolution. Recently a new technique, using a flow system and a zirconium oxide high-temperature electrode as the sensing element, has been developed (*5*). Unlike the oxygen polarograph, used in all previous flash work, the flow apparatus is capable of absolute calibration and in addition has sufficient sensitivity to detect the oxygen or hydrogen evolved from photosynthetic organisms illuminated with single-turnover, saturating flashes. The recent results on oxygen evolution show disagreement with the accepted model (*4*), and a new model has been proposed (*6*). The results on hydrogen evolution are described in this report.

Figure 1 summarizes a set of experimental results. The left ordinate in Fig. 1 indicates the individually resolved, absolute yield of hydrogen per mole of chlorophyll per flash, and the abscissa indicates the flash number. It is clearly seen that when dark, anaerobically adapted *Chlorella vulgaris* are illuminated with single-turnover flashes of light in the absence of added redox reagents, the hydrogen yield on the first flash is nonzero and is essentially equal to the hydrogen yield per flash for the subsequent flashes. The fact that algae can evolve molecular hydrogen under anaerobic conditions was first reported by Gaffron and Rubin (*7*). Working with *Scenedesmus*, they showed that there is an induction period of several hours for the photoproduction of hydrogen. A similar induction period has been observed for *Chlorella*, as indicated in Fig. 2. The hydrogen data of Fig. 1 are for *Chlorella* that have been held under helium for 3 hours.

For the anaerobic conditions of Fig. 1

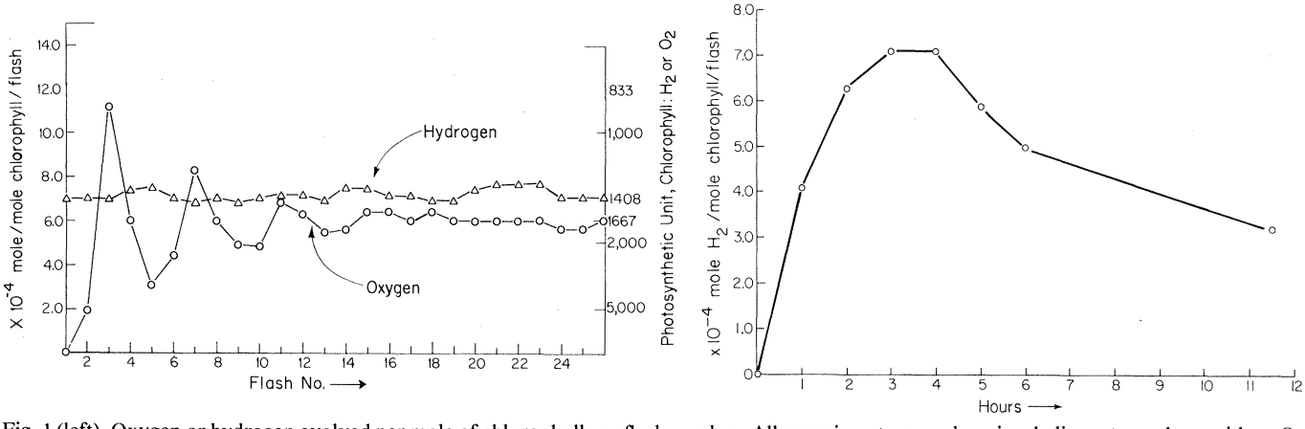

Fig. 1 (left). Oxygen or hydrogen evolved per mole of chlorophyll per flash number. All experiments were done in a helium atmosphere with an O_2 content of 10 ppm. The time between flashes was 10 seconds. The concentration of *p*-benzoquinone for the oxygen curve was 1.0 m*M*. *Chlorella vulgaris* were grown on a mineral medium at a light intensity of 3×10^3 erg cm^{-2} sec^{-1}. The gas detection system consisted of a flow apparatus designed and built in our laboratory. The sensing element was a zirconium oxide high-temperature Nernstian electrode. Absolute calibration of the apparatus was achieved by placing an electrolysis cell in tandem with the reaction cuvette containing the algae. The flash lamps were two General Radio Stroboslaves, type 1539A. These delivered saturating flashes with a half-time of 4 μsec. The timing for the flash lamps was provided by Tektronix series 160 pulse generators. The chlorophyll content of the algae was determined spectrophotometrically by extraction into methanol. Results of control experiments on hydrogen evolution with 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) present were the same as those without DCMU. Production of hydrogen was distinguished from uptake of the trace oxygen in the helium carrier gas by observing that removal of the hydrogen by catalytic scrubbing downstream from the reaction cuvette caused abolition of the measured signal ascribed to hydrogen. Fig. 2 (right). Hydrogen induction curve of autotrophically grown *C. vulgaris*. The induction curve for *Scenedesmus* D_3 has a similar shape, but the plateau region extends for several hours.

(hydrogen evolution) the components of the electron transport chain linking photosystem I and photosystem II are fully reduced (*8*). Consequently, oxygen evolution is not observed for anaerobically adapted algae in the absence of chemical oxidants. If *p*-benzoquinone is added to the anaerobic *Chlorella* suspension, the same experimental procedure measures the oxygen yield per flash. Under these oxidizing conditions (although still strictly anaerobic at an oxygen concentration of 10 parts per million) there is no evidence for evolution of hydrogen. This dichotomy in the local chemical conditions required for hydrogen or oxygen evolution lies at the heart of the problem of biophotolysis of water (*9, 10*).

Returning to the oxygen curve of Fig. 1, we see that the yield of oxygen on the first flash, unlike the corresponding observation for hydrogen, is zero. There is a small yield of oxygen on the second flash, which can be increased by adding ionic oxidants or increasing the flash frequency (*5*). The third flash produces the maximum oxygen yield, and the subsequent yields can be described as a damped oscillation with period 4. Oxygen oscillations have formed the experimental underpinning of the theories concerning the molecular mechanism of oxygen evolution (*3–6*). These oscillations indicate a role for photoproduced, sequential, metastable intermediates which are serially involved in the formation of oxygen. Apparently, the four oxidizing equivalents necessary to produce a molecule of oxygen from water cannot be produced in a single flash. It requires at least two flashes to produce some oxygen. This is not the case for hydrogen: one flash is sufficient. Thus, the molecular mechanism of hydrogen evolution must be such that no photoproduced, metastable intermediates are serially involved as they are in the case of oxygen evolution. Moreover, since two reducing equivalents are required to make one molecule of hydrogen, it follows that in the photoproduction of molecular hydrogen the reducing equivalents from at least two photosystems are fed into a common pool (probably ferredoxin), following the common assumption that the photosystems form one equivalent per single-turnover flash. Once in the pool, the two equivalents combine in the dark to produce hydrogen through hydrogenase. An alternate, although less probable, mechanism is one in which the first reducing equivalent is supplied by a dark reaction and the second by a light reaction (or vice versa). The sensitivity of the detection apparatus is sufficient to determine that less than 10 percent of the reaction centers are evolving hydrogen between flashes in a dark reaction.

The size of the photosynthetic unit for hydrogen evolution in *Chlorella* is chlorophyll : $H_2 \approx 1400 : 1$. The photosynthetic unit size for oxygen evolution (the Emerson and Arnold unit) for *Chlorella* coupled to benzoquinone is chlorophyll : $O_2 \approx 1700 : 1$. These data allow a determination of the ratio of hydrogen to oxygen, both normalized to the chlorophyll content of the algae. The ratio of H_2 to O_2 is 1.2. Were the movement of electrons by chlorophyll as efficient for hydrogen as for oxygen, the stoichiometric ratio would be 2. These data indicate that with regard to the photophysical apparatus of photosynthesis, the ability to utilize absorbed visible quanta for the light-driven reaction is at least 60 percent as efficient for photosynthetic hydrogen evolution as for photosynthetic oxygen evolution. One can think of several reasons why the ratio of H_2 to O_2 is only 1.2 rather than 2. One possibility is that not all the reducing equivalents are captured by the hydrogenase. Measurements on *Scenedesmus* D_3 give a unit size of chlorophyll : $H_2 \approx 800 : 1$. However, attempts to measure the oxygen unit size have resulted in a very low yield of O_2, most likely caused by losses of intermediates.

The concept of photosynthetic unit stems from the pioneering work of Emerson and Arnold (*1*). The experiments described in this report are, to my knowledge, the first determination of the photosynthetic unit size based on hydrogen evolution. This unit size is comparable to the size based on oxygen evolution.

Note added in proof: Since the initial submission of this report flash experiments identical to those performed on *Chlorella* as described above have been performed on *Chlamydomonas reinhardtii*. For *Chlamydomonas* the O_2 oscillations are essentially the same as for *Chlorella*. However, the H_2 yield is larger, such that the ratio of H_2 to O_2 is 1.9. This is close to the theoretical maximum.

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References and Notes

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11. The *Chlamydomonas reinhardtii* used in this work was obtained through the courtesy of Drs. N.-H. Chua, G. Schmidt, and K. S. Matlin. I express my deep appreciation to D. Mauzerall for valuable discussions and critical reading of the manuscript. I also thank S. Beale, F. Hong, N. Kagan, and R. Piccioni for their comments. Supported in part by NSF grant PCM74-11747.

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Competition Between Seed-Eating Rodents and Ants in Desert Ecosystems

Abstract. *Three kinds of evidence indicate that desert rodents and ants compete for seeds: (i) extensive overlaps in diet, (ii) reciprocal increases when one taxon is experimentally excluded, and (iii) complementary patterns of diversity and biomass in geographic gradients of productivity. The effect on seed resources and annual plants seems to be similar whether rodents, ants, or both are foraging.*

A primary challenge of contemporary ecology is to understand the processes that determine the diversity, organization, and stability of natural ecosystems. Competition between species for food and other resources is thought to be an important determinant of ecosystem structure and dynamics. Empirical support for this conclusion comes almost exclusively from field and laboratory studies of a small number of closely related species (*1*). Taxonomic specialization has prevented most ecologists from recognizing and investigating the significance of competition among distantly related organisms. Several recent studies indicate that such distantly related taxa as insects, birds, and mammals eat similar foods and are potentially important competitors (*2–4*). We now report competition between seed-eating desert rodents and ants that affects the structure and dynamics of ecosystems.

Seeds play a major role in the ecology