ENERGETIC EFFICIENCY OF HYDROGEN PHOTOEVOLUTION BY ALGAL WATER SPLITTING

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ABSTRACT Absolute thermodynamic efficiencies of conversion of light energy into chemical-free energy of molecular hydrogen by intact microalgae have been measured with an original physical measuring technique using a tin-oxide semiconducting gas sensor. Thin films of microalgae comprising of 5 to 20 cellular monolayers have been entrapped on filter paper, thereby constraining them in a well-defined circular geometry. Based on absolute light absorption of visible polychromatic illumination in the low-intensity region of the light saturation curve, conversion efficiencies of 6 to 24% have been obtained. These values are the highest ever measured for hydrogen evolution by green algae.

Among the intriguing aspects of modern quantitative biological physics are the determination of the conversion efficiency of light energy into chemical energy by green plants and the analytical shape of the light-saturation curve of photosynthesis. Photosynthesis saturates with increasing light intensity because it is comprised of serially-linked, light-driven, and thermally-activated electron-transfer reactions. It is well known that measurement of conversion efficiencies under strictly light-limiting conditions assesses the inherent capabilities of the photophysical machinery of photosynthesis independently of subsequent nonlight-dependent biochemistry (1).

Although obviously interrelated, maximum quantum conversion efficiency bears closely on molecular mechanism, whereas maximum energy conversion efficiency relates to net productivity. As reviewed by Pirt (2), there is, as yet, no clear consensus on values for maximum photosynthetic efficiency. However, measurements by Ley and Mauzerall (3) indicate a quantum efficiency for aerobic oxygen evolution by *Chlorella vulgaris* in the range of 9 to 11% for pulsed illumination at 596 nm. The limiting aspects of production of energy-rich compounds by photosynthetic and photochemical conversion and storage of light energy has been treated authoritatively by several authors (4–6). In particular Parson (7) has presented a lucid analysis of the thermodynamics of the primary reactions of photosynthesis by deriving expressions for the change in free energy that occurs when a photochemical system is illuminated. In this, as well as the earlier work of Ross, et al. (8), the important distinction was made between midpoint redox potentials, which are molecular properties, and the actual redox potential of a system which is determined by the midpoint potential and the ratio of the concentrations of oxidized and reduced molecules.

Energy conversion efficiency measurements based on continuous light-induced water splitting by algae are presented here. Unlike aerobic terrestrial photosynthesis whose terminal electron acceptor is atmospheric carbon dioxide and whose energy-rich photoproduct is a carbon dioxide fixation compound, the energy-rich photoproduct in this work is molecular hydrogen. As first discovered by Gaffron and Rubin (9) and reviewed by Weaver et al. (10) and Bishop and Jones (11), certain classes of eukaryotic algae are capable of evolving molecular hydrogen under appropriate physiological conditions: when placed in an oxygen-free atmosphere, certain green algae are capable of synthesizing the enzyme hydrogenase. Moreover, if the atmosphere is devoid of carbon dioxide, the normal terminal electron acceptor of photosynthesis, hydrogen ions can serve as the acceptor by being reduced to molecular hydrogen in a reaction catalyzed by the enzyme hydrogenase. Because it has been previously shown that eukaryotic green algae are capable of sustained simultaneous photoevolution of hydrogen and oxygen (12), hydrogen production by algal water splitting is strictly analogous to normal photosynthesis in that water can serve as the source of reductant and, as mentioned above, hydrogen is the energy-rich product. However, from a quantitative point of view, the analogy breaks down because with hydrogen ions as the terminal electron acceptor, the light-saturated rate of oxygen evolution is at best a few percent of the light-saturated rate of oxygen evolution with carbon dioxide as the terminal electron acceptor.

An absolute measurement of thermodynamic conversion efficiency of light energy into Gibbs free-energy of molecular hydrogen requires two measurements: (*a*) the absolute

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rate of light absorbed by the algal sample and (*b*) the absolute rate of hydrogen photoproduction. Fig. 1 is a schematic illustration of the chamber and flow system used to measure the light absorbed by the algae and to irradiate them during hydrogen and oxygen production. The main body of the chamber consists of a large glass O-ring cover plate pressing against a thin clear quartz backing plate. Two smaller O-ring connectors are used for inlet and outlet ports for gas flow to create a helium atmosphere for the algae. The algae are entrapped on filter paper and appressed to the thin quartz plate. The sensing element of a spectrally flat electro-optic radiometer, model 550 (EG&G) is positioned behind the sensor and is used for absolute measurement of transmitted light. In this apparatus, algae are entrapped in a well-defined circle of 36-mm diameter. Net light absorbed by the algae is determined by measuring the difference in transmitted light in the absence and presence of the algae. Measurement of the angular distribution of reflected and scattered radiation indicated that virtually all of the scattered radiation was forward-scattered and captured by the active area of the radiometer (13).

As indicated in Fig. 2, when the light is turned on, hydrogen and oxygen that are evolved in the semisolid environment of the algal film diffuse into the helium gas phase created by the helium carrier and are then swept downstream to oxygen and hydrogen detectors. Calibrations of the oxygen and hydrogen sensors were achieved with an electrolytic cell. Although the electrochemical oxygen sensor is linear over the range of gas phase concentrations studied, the tin oxide semiconducting hydrogen sensor is not. Calibration curves for both hydrogen and

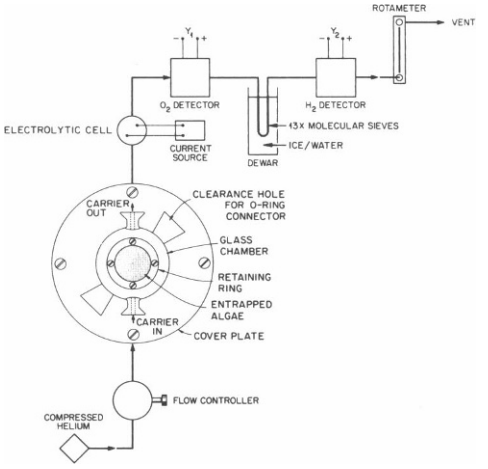

FIGURE 1 Schematic illustration of experimental apparatus used to measure absolute light absorption and hydrogen and oxygen production by filter-paper entrapped microalgae. The chamber is an integral part of a helium-carrier flow system with connecting ports as indicated. Hydrogen and oxygen sensors are located downstream from the chamber. Absolute calibration of these sensors is achieved using Faraday's law of electrochemical equivalence with an electrolysis cell that is located in tandem with the chamber.

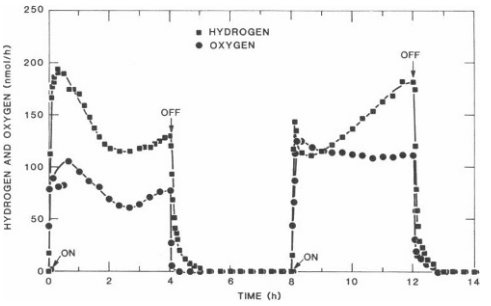

FIGURE 2 Simultaneous photoevolution of hydrogen and oxygen from the anaerobically adapted green alga *Scenedesmus* D_3. The algae are irradiated with a projector lamp at normal incidence to the plane defined by the filter-paper entrapped algae. Two 4-h intervals of illumination are indicated. The values of hydrogen evolution used in the energy efficiency calculation were those at the end of each 4-h interval.

oxygen were constructed by observing steady-state direct current deflections for sequential electrolysis currents of 5, 10, 25, 50, and 100 μA. Using a Hewlett-Packard 85 microcomputer, (Hewlett-Packard Co., Palo Alto, CA) least squares fitting routines were used to generate analytical expressions for the calibration curves. It was determined empirically that good fits to the calibration data were obtained by fitting the oxygen data to a straight line and the hydrogen data to a rectangular hyperbola, $y = (\alpha x)/(1 + \beta x)$, where x is the rate of hydrogen generation, y is the sensor response and α and β are constants, the numerical values of which were determined by the least squares fitting routine (14). The accuracy of the gas sensors is $\pm 5\%$ or better. The accuracy for the overall efficiency measurement is estimated to be $\pm 15\%$ as is the statistical variability. The uniformity of the algal film was $\sim 5\%$. This value was determined by scanning the fluctuation of transmitted light with a EG&G Lite-Mike, model 560B. Since the active area of this photodetector is only 0.051 cm^2 it is much smaller than the active area of the algal film. By sampling a small fraction of the transmitted light at numerous points behind the algal film the optical uniformity was determined.

Table I summarizes the energy conversion efficiencies based on net absorbed photosynthetically active radiation. This efficiency was computed using Faraday's law of electrochemical equivalence to determine the rate of hydrogen production by the electrolysis calibration cell and the associated Gibbs free-energy content ($\Delta G° = 237$ kJ/mol) of hydrogen. The efficiency entries for Table I were computed for the actual conditions under which the experiments were run: hydrogen and oxygen simultaneously coevolved in the same volume and transported to downstream gas sensors by the helium carrier at relatively dilute concentrations. Energy expenditures for hypothetical processing steps such as separation and compression to multiatmosphere values, which will decrease effective efficiencies, are not included in Table I.

All of the algae used for the experiments of Table I were

TABLE I
ENERGY CONVERSION EFFICIENCIES OF GREEN ALGAE FOR HYDROGEN AND OXYGEN PRODUCTION

Alga	Absorbed light	Light on	H_2	Efficiency (PAR)
	$\mu W/cm^2$	no.*	nmol/h	%‡
Scenedesmus D_3	5.1	1	126	16
		2	181	23
C. reinhardtii (sup)	2.2	1	44	13
		2	54	16
		3	61	18
		4	64	19
		5	71	21
		6	71	21
		7	61	18
C. reinhardtii (UTEX 90)	8.4	1	78	6
		2	104	8
		3	104	8
C. moewusii	9.1	1	337	24
		2	309	22
		3	253	18

*The entries in this column correspond to the ordinal number of successive periods of illumination. The light was on for either a 3- or 4-h period, after an equal period of darkness.

‡Conversion efficiency based on absorbed photosynthetically active radiation. PAR = photosynthetically active radiation. Based on repeated measurements and calibrations, it is estimated that the experimental error in these measurements is, at most, ±15%. The efficiencies were computed for the rates of hydrogen evolution at the end of the period of illumination when the algae were in a steady (or nearly steady) state.

grown photoautotrophically on minimal solution with atmospheric carbon dioxide as the sole carbon source. Therefore, all of the reductant that is expressed as molecular hydrogen is ultimately derived from water splitting. However, due to the presence of alternate electron acceptors (11) and endogeneous reductants (15, 16), the stoichiometric ratio of hydrogen to oxygen at any given moment is not necessarily equal to 2; this ratio is, however, usually close to 2. The *Scenedesmus* D_3 experimental data for the light on-off cycles used to calculate the corresponding entries for Table I are illustrated in Fig. 2. Similar data were recorded for the other entries.

Under light-limiting conditions, energy conversion efficiencies based on absorbed photosynthetically active radiation vary from 6 to 24% (Table I). With increasing light intensity and under nonlight-limiting conditions, however, net conversion efficiencies decrease. For example, at an incident light intensity of 100 mW/cm^2, conversion efficiencies are well below 1%. However, the range of efficiencies indicated in Table I implies that under appropriate experimental conditions a major fraction of the reductant that is generated by photosystem I can be expressed as molecular hydrogen. Working with a liquid phase-reconstituted chloroplast system, Gisby and Hall (17) measured hydrogen conversion efficiencies from 0.26 to 0.80%. One possible explanation for the lower efficiencies observed with this reconstituted in vitro system is the poorer coupling between and spatial separation of the three components.

The kinetically limiting aspects of photosynthesis have been discussed by Kok (18) and Clayton (19). Photosynthesis saturates with increasing light intensity because of the inability of thermally activated biochemical reactions to keep pace with the increasing rate of quantum excitation of the photosynthetic reaction centers at the higher intensities. As previously demonstrated, this kinetic limitation expresses itself mechanistically in terms of the number of apparent functional photosynthetic units present in the algae (20). The present results on energy efficiencies are completely consistent with the analysis of Kok (18) and Clayton (19) who have described a rational approach to overcoming this inherent kinetic limitation of photosynthesis. By working with photosynthetic systems of smaller photosynthetic unit sizes (i.e., smaller optical cross sections), it should in principle, be possible to preserve a kinetic balance between the rate of quantum excitation and the rates of thermally-activated electron-transfer reactions at higher light intensitites. For example, the structure of photosynthetic tissue is such that each reaction center is served by ~200 molecules of antenna chlorophyll. These antenna chlorophyll molecules do not participate directly in the photochemistry; they absorb light and deliver the energy to the reaction centers. If every quantum absorbed by the antenna in full sunlight were utilized photochemically at the reaction centers, electrons would be flowing through the complete chain from water to ferredoxin (or hydrogenase) at a rate of ~2,000 per s. However, the electron-transport chain can transport no more than ~200 electrons/s, so that in full sunlight only 1/10 of the incoming quanta can be utilized. The prospect of increasing the rate at which the electron-transport system can operate is limited. However, if each reaction center were served by an antenna of only 20 chlorophyll molecules rather than 200, quanta would be delivered to the reaction centers at a rate of 200/s in full sunlight rather than 2,000/s. The electron-transport machinery could then keep pace. The practical implication of preserving this kinetic balance is that the high conversion efficiencies which have been measured in this work at low incident light intensities will be preserved at higher incident light intensities. The logical upper limit for these higher intensities is, of course, the maximum value of terrestrial solar irradiance.

Success in this endeavor will contribute not only to a further understanding of the biophysics of photosynthesis, but also to the solution of a practical problem in the realm of fuels and chemical synthesis from renewable resources.

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