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A New Perspective on Hydrogen Production by Photosynthetic Water-Splitting

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A NEW PERSPECTIVE ON HYDROGEN PRODUCTION BY PHOTOSYNTHETIC WATER-SPLITTING

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Abstract

Present energy systems are heavily dependent on fossil fuels. This will eventually lead to the foreseeable depletion of fossil energy resources and, according to some reports, global climate changes due to the emission of carbon dioxide. In principle, hydrogen production by biophotolysis of water can be an ideal solar energy conversion system for sustainable development of human activities in harmony with the global environment. In photosynthetic hydrogen production research, there are currently two main efforts: 1) Direct photoevolution of hydrogen and oxygen by photosynthetic water splitting using the ferredoxin/hydrogenase pathway; 2) Dark hydrogen production by fermentation of organic reserves such as starch that are generated by photosynthesis during the light period. In this chapter, the advantages and challenges of the two approaches for hydrogen production will be discussed, in relation to a new opportunity brought by our recent discovery of a new photosynthetic water-splitting reaction [*Nature*, **373**, 438-441 (1995)], which, potentially, has **twice the energy efficiency** of conventional water-splitting via the two light reaction Z-scheme of photosynthesis.

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Hydrogen is a versatile, clean and environmentally acceptable energy carrier. It can be produced by photolysis of water, an inexpensive and inexhaustible raw material. Photolysis can be performed using either inorganic systems such as semi-conductors or living organisms such as cyanobacteria or green microalgae. It is now clear that green algae are probably better for H_2 production than cyanobacteria, since the latter use the more energy-intensive enzyme, ATP-requiring nitrogenase for production of H_2 . Based on a recent feasibility analysis (1), H_2 production by green algae can be more cost-effective than semi-conductor photovoltaic-electronics. The discussion in this article is focused on H_2 production by photosynthetic water splitting.

Fundamentals of photosynthesis and H_2 production

Photosynthesis is the fundamental biological process that converts the electromagnetic energy of sunlight into stored chemical energy that supports essentially all life on Earth. In green algae as in higher plants, photosynthesis occurs in a specialized organelle, the chloroplast. Light energy captured by the photosynthetic reaction centers is stored predominately by reduction of CO_2 , using water as the source of electrons. As illustrated in Fig. 1, the key components of the photosynthetic apparatus involved in light absorption and energy conversion are embedded in thylakoid membranes inside the chloroplast. They are two chlorophyll (chl)-protein complexes, Photosystem I (PSI) with a reaction center, P700, and Photosystem II (PSII) with another distinct reaction center, P680. According to the current and prevailing concept of oxygenic

photosynthesis, the Z-scheme, first proposed by Hill and Bendall (2) and now described in many textbooks (3-7), PSII can split water and reduce the plastoquinone (PQ) pool, the cytochrome (Cyt) b/f complex, and plastocyanin (PC), while PSI can reduce ferredoxin (Fd)/nicotinamide adenine dinucleotide phosphate (NADP⁺) and oxidize PC, the Cyt b/f complex, and the PQ pool. As a result, the electrons derived from water splitting are transferred to Fd/NADP⁺ which provide the reducing power for reduction of CO₂ to carbohydrate in the stromal region of the chloroplast by a series of enzymatic reactions collectively called the Calvin cycle. Electron transport in the membrane is coupled with proton transport from the stroma into the lumen, generating a proton gradient across the thylakoid membrane. The proton gradient drives phosphorylation through the coupling factor CF_o-CF₁ to make essential adenosine triphosphate (ATP) for the reduction of CO₂. This is the common description of oxygenic photosynthesis.

In many green algae, such as *Chlamydomonas*, there is a hydrogenase that can be induced under anaerobic conditions (8, 9). The hydrogenase can catalyze the reduction of protons to produce H₂ using electrons from the reduced Fd as shown in Fig 1. Since protons are also produced by water splitting at PSII, the net result of this Fd/hydrogenase pathway is simultaneous photoevolution of H₂ and O₂ using water as the substrate and light energy as the driving force.

Under anaerobic conditions and darkness, H₂ may be produced by fermentative metabolic degradation of organic reserves such as starch (Fig. 1). This fermentative metabolic process has been well studied (10, 11). Since the substrate of the fermentative pathway is generated photosynthetically by reduction of CO₂, the net result of the sequential process, photosynthesis and fermentation, is still splitting water to H₂ and O₂, with CO₂ as an intermediate.

Hydrogen Production by Direct Photosynthetic water splitting

The discovery of photosynthetic H_2 production is based on the classic work of Gaffron and Rubin in 1942 (12). However, only since 1973 at the time of the energy crisis, photosynthetic H_2 production has been investigated as a potential source of energy (13-35). Sustained photoevolution of H_2 and O_2 by microalgae was first demonstrated by Greenbaum in 1980 (15). Under anaerobic conditions, sustained photoevolution of H_2 and O_2 in microalgae can be readily demonstrated using a reactor-flow-detection system (Fig. 2). Figure 3 presents a typical measurement of H_2 and O_2 production in *Chlamydomonas* under helium atmosphere using the reactor-flow-detection system. The data clearly demonstrate that photoevolution of H_2 and O_2 can occur stably with a stoichiometric ratio of H_2 to O_2 of nearly 2:1 as expected for water-splitting. Photoevolution of H_2 and O_2 can be sustained for weeks.

The advantage of the simultaneous H_2 and O_2 photoevolution is that it can potentially have a high energy conversion efficiency since electrons energized by the light reactions are used directly in the reduction of protons to produce H_2 by the Fd/hydrogenase pathway (Fig. 1). However, H_2 production by this mechanism requires gas product separation since both H_2 and O_2 are produced simultaneously in the same volume. Furthermore, until an O_2 -insensitive hydrogenase is developed, the O_2 concentration in the algal suspension has to be kept low to maintain H_2 production since the hydrogenase is sensitive to O_2 . Therefore, an efficient and inexpensive technique to separate and remove gas products is needed.

Another important aspect is that the photoevolution of H_2 and O_2 in microalgae is often saturated at a relatively low actinic intensity. This is probably due to three factors:

- 1) Accumulation of a back-proton gradient because of the limited permeability of thylakoid

membrane to protons and loss of ATP utilization due to the inactivation of the Calvin cycle; 2) Partial inactivation of PSII activity owing to loss of CO₂ binding at a regulatory site on the PSII reaction center in the absence of CO₂; 3) The normal non-linear response of the light saturation curve of photosynthesis. Therefore, the potential still exists to improve the efficiency of photosynthetic H₂ production. Further research is needed to eliminate these limiting factors. The first limitation may be solved on a short basis by using an appropriate proton uncoupler that dissipates the proton gradient across the thylakoid membrane (36), whereas the second could be overcome by eliminating the requirement of CO₂ binding through molecular engineering. The third limitation can, in principle, be overcome by reducing the antenna size of the photosynthetic reaction centers.

To avoid gas product separation and to increase efficiency, we have previously proposed a PSI and PSII reactor system that can potentially produce H₂ and O₂ in two separate compartments (32). This reactor system is based on the structure and function of isolated PSI and PSII reaction centers, and on the catalytic activity of metallic platinum and osmium for H₂ production (37-41). As illustrated in Figure 4, O₂ and protons are produced by water splitting by an array of PSII reaction centers in the PSII compartment. The electrons acquired from water splitting are wired to the reducing side of a PSI array where the electrons are energized again by PSI photochemistry. The PSI-energized electrons are then used to evolve H₂ by platinum (or osmium)-catalyzed reduction of protons that come from PSII compartment through a proton conducting channel. As described previously, this system should be able to operate continuously since the number of protons and electrons generated can be balanced with the number of them consumed. We believe, this is an important direction for future research. In this laboratory,

research progress has been made in this direction (42-44). Recently, we have constructed a two dimensional spatial array of PSI reaction centers on a gold surface at nanometer scale by a platinization anchoring technique (44).

Hydrogen Production by Dark Fermentation of Photosynthetic Product

An advantageous feature of fermentative H_2 production is its temporal separation from photosynthesis (Figure 1). That is, O_2 evolution and CO_2 photoassimilation by photosynthesis occurs during the day, whereas fermentative H_2 production by degradation of photosynthetic product (starch) can occur during the night. By taking advantage of this temporal separation between O_2 evolution (day) and H_2 production (night), one can potentially develop an algal H_2 production technology that avoids the problem of gas product separation. This approach is a major project by a group of Japanese scientists (22, 29, 33, 34). Important progress has been made. Miura et al (34) have demonstrated algal fermentative H_2 production that is temporally separated from photosynthesis (CO_2 fixation and O_2 evolution) using a combination of green algae and photosynthetic bacteria comprising over 100 L of green algae.

The challenge, as always, in this fermentative H_2 production approach is the efficiency. Figure 5 presents a typical measurement of photosynthesis and fermentative H_2 production in wild-type *Chlamydomonas* in helium atmosphere in the presence of CO_2 under cycles of 12 hr of moderate actinic illumination (PAR, $200 \mu E \cdot m^{-2} \cdot s^{-1}$) and 12 hr of darkness, using the flow-detection system (Fig. 2). From the data, it can be clearly seen that the rate of fermentative H_2 production is very slow --- less than 5% of the rate of oxygen evolution during the day. Therefore, enhancing the rate of dark fermentative H_2 production is the key challenge.

New Opportunities Brought by a Recent Discovery

In this laboratory, our current research has focused on improving the energy efficiency of photosynthetic hydrogen production. Using mutants of *Chlamydomonas* that lack PSI but contain PSII, we have demonstrated a new type of photosynthesis. That is, photoevolution of O₂ and H₂, and photoassimilation of CO₂ by PSII light reaction alone (35). Based on studies of electron transport pathway (Lee and Greenbaum, 1996, unpublished), the newly discovered water-splitting reaction for H₂ and O₂ production (1) or for CO₂ fixation and O₂ evolution (2) may require only half the number of photons of conventional Z-scheme photosynthetic reactions (3) and (4).

Newly Discovered PSII photosynthesis:

Energy efficiency = 67.4% ($\lambda = 680 \text{ nm}$)

Energy efficiency = 68.2% ($\lambda = 680 \text{ nm}$)

Conventional Z-scheme Photosynthesis:

Energy efficiency = 33.7% ($\lambda = 680 \text{ nm}$)

Energy efficiency = 34.1% ($\lambda = 680 \text{ nm}$)

Both reactions (1) and (2) have a significantly large negative value of ΔG° . They should be able to occur spontaneously. Therefore, although the discovery is surprising and novel, it still obeys

the laws of thermodynamics. Since reactions (1) and (2) require half number of photons of reactions (3) and (4), the discovery can potentially lead to a H_2 production and/or CO_2 fixation technology with twice the energy conversion efficiency of conventional Z-scheme photosynthesis.

The demonstration of photosynthesis by a single light reaction proved that a single light reaction can span the potential difference between water oxidation and proton reduction for sustained evolution of H_2 and O_2 , which was previously thought to be impossible (45). Therefore, we can now propose a new reactor system containing biometallocatalysts that requires only a single type of photochemical reaction center (Fig. 6), but is able to perform the same function of the reactor system in Fig. 3. As illustrated in Fig. 6, when water is split to O_2 and protons by PSII, electrons from the reducing side of PSII should, neglecting resistive loss, be able to reduce protons on a platinum catalyst surface to evolve H_2 in a separate compartment without PSI.

At low light intensity and under ideal laboratory conditions, the maximum sunlight to H_2 energy conversion efficiency for Z-scheme photosynthesis has been measured to be about 10% (46). From a practical point of view, application of "PSII photosynthesis" can potentially double the sunlight conversion efficiency from 10% to 20% (35). This potentially higher efficiency (20%) can put photosynthetic H_2 production in a much more competitive position vis-à-vis other solar technologies. Moreover, since PSII photosynthesis can also photoassimilate CO_2 , it should also be able to improve the energy efficiency of photosynthesis (CO_2 fixation) in general. Therefore, the discovery also provides a new opportunity to improve energy efficiency for production of H_2 by the photosynthesis/fermentation combined system of Miura et al (34).

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Literature Cited

1. Benemann, J. R. *Xth Proc. World Hydrogen Energy Conf.* **1995** pp. 931-40.
2. Hill R., and Bendall, F. *Nature* **1960**, 186, 136-137.
3. Stryer, L. *Biochemistry* (W. H. Freeman and Company, New York, ed. 4, 1995), pp. 617-618, 658-664, 676-677.
4. Salisbury, F. B., and Ross, C. W. *Plant Physiology* (Wadsworth Publishing Company, Belmont, CA, ed. 3, 1985), pp. 191-194.
5. Goodwin, T. W., and Mercer, E. I. *Introduction to Plant Biochemistry* (Pergamon Press, Oxford, UK, ed. 2, 1986), pp. 117-124.
6. Alberts et al., *Molecular Biology of the Cell* (Garland Publishing, Inc., New York, 1983), pp. 517-519.
7. Darnell, J., Lodish, H., Baltimore, D. *Molecular Cell Biology* (Scientific American Books, Inc., New York, 1986), pp. 901-903.
8. Roesler, P.G. and Lien, S. *Plant Physiol.* **1984** 76, 1086-1089.
9. Boichenko, V. A. and Hoffmann, P. *Photosynthetica* **1994**, 30, 527-552.
10. Gfeller, R. P. and Gibbs, M. *Plant Physiol.* **1984**, 75, 212-218.

11. Chen, C. (1992), *Carbon Dioxide and Hydrogen Metabolism in Cells and Isolated Chloroplasts of Chlamydomonas reinhardtii*, PhD Dissertation, Brandeis University, Waltham, MA, pp. 99-109.
12. Gaffron, H. and Rubin, J. *J. Gen. Physiol.* **1942**, 26, 219-240.
13. Greenbaum, E. *Science* **1977**, 196, 879-878.
14. Bishop, N. I., Frick, M., and Jones, L. W. (1977), in *Biological Solar Energy Conversion*, Mitsui, A., Miyachi, S., San Pietro, A., and Tamura, S. eds. Academic Press, New York, pp. 2-22.
15. Greenbaum, E., *Biophys. J.* **1980**, 54, 365-368.
16. Bamberger, E. S., King, D. Erbes, D., and Gibbs, M. *Plant Physiol.* **1982**, 69, 1268-1273.
17. Greenbaum, E. and Ramus, J. *J. Phycol.* **1983**, 19, 53-57.
18. Greenbaum, E., Guillard, R.R. L, Sunda, W. G. *Photochem. and Photobiol.* **1983**, 37(6), 649-655.
19. Hoopen, H. T., Snellink, L. J., Gemert, J. V, Fuchs, A., Roels, J. A. *Prog. Ind. Microbiol.* **1984**, 20, 191-196.
20. Greenbaum, E. *Photobiochem. Photobiophys.* **1984**, 8, 323-332.
21. Aparicio, P. J., Azuara, M. P., Ballesteros, A., and Fernadez, V. M. *Plant Physiol.* **1985**, 78, 803-806.
22. Miura, Y., Ohta, S., Mano, M., Miyamoto, *Agric. Biol. Chem.* **1986**, 50(11), 2837-2844.
23. Mahro, B. and Grimme, L. H. *Arch Microbiol.* **1986** 144, 25-28.
24. Greenbaum, E. (1988), in *Algae And Human Affairs* Lembi, C. A., and Waaland, R. J. eds. Cambridge University Press, Cambridge, pp. 283-304.
25. Grave, D. A., Reeves, M. E. and Greenbaum, E. *Plant Physiol.* **1988**, 87, 603-608.
26. Grave, D. A., Tevault, C. V., Greenbaum E. *Photobiochem. Photobiophys.* **1989**, 50, 571-576.
27. Greenbaum, E. *Adv. Hydrogen Technology* **1990**, 2, 743-754.
28. Grave, D. A., Spladlin, G. M., Greenbaum, E. *Photobiochem. Photobiophys.* **1990**, 52, 585-590.

29. Miura, Y., Saitoh, C. Matsuoka, K., *Biosci. Biotech. Biochem.* **1992**, 56, 751-754.
30. Cinco, R. M., MacInnis, J. M., and Greenbaum, E. *Photosyn. Res.* **1983**, 38, 27-33.
31. Lee, J. W., Blankinship, S. L., Greenbaum, E. *Appl. Biochem. Biotechnol.* **1995** 51/52, 379-385.
32. Lee, J. W. and Greenbaum, E. *Appl. Biochem. Biotechnol.* **1995**, 51/52, 295-305.
33. Miura, Y. *Process Biochemistry* **1995**, 30, 1-7.
34. Miura, Y., Akano, T., Fukatsu, K., Miyasaka, H., Mizoguchi, T., Yagi, K., Maeda, I., Ikuta, Y., Matsumoto, H. *Energy Convers. Mgmt* **1995**, 36, 903-906.
35. Greenbaum, E., Lee, J. W., Tevault, C. V., Blankinship, S. L., Mets, L. J. *Nature*, **1995**, 376, 438-441.
36. Nicholls, D. G., and Ferguson, S. J. *Bioenergetics* (Academic Press Inc., California, ed. 2, 1992) pp. 31-33.
37. Greenbaum, E. *Science* **1985**, 230, 1373-1375.
38. Greenbaum, E. *J. Phys. Chem.* **1988**, 92, 4571-4574.
39. Greenbaum, E. *J. Phys. Chem.* **1990**, 94, 6151-6153.
40. Greenbaum, E. *J. Phys. Chem.* **1992**, 96, 514-516.
41. Lee, J. W., Tevault, C.V., Blankinship, S. L., Collins, R.T., Greenbaum, E. *Energy & Fuels.* **1994**, 8(3), 770-773.
42. Lee, J. W., Lee, I., Laible, P. D., Owens, T. G., and Greenbaum, E. *Biophys. J.* **1995**, 69, 1-8.
43. Lee, I., Lee, J. W., Warmack, R. J., Allison, D. P., and Greenbaum, E. *Proc. Natl. Acad. Sci. USA* **1995**, 92, 1965-1969.
44. Lee, J. W., Lee, I., and Greenbaum, E. *Biosensors & Bioelectronics* **1996**, 11, 375-387.
45. Bolton, R. J. *Science* **1978**, 202, 705-711.
46. Greenbaum, E. *Biophys. J.* **1988**, 54, 365-368.

Caption for Figures

Fig. 1. Conventional photosynthetic pathway based on the Z-scheme.

Fig. 2. Schematic illustration of a dual-reactor-flow system for simultaneous detection of H_2 , O_2 and CO_2 .

Fig. 3. Sustained photoassimilation of H_2 and O_2 in *Chlamydomonas* 137c under anaerobic conditions and in the absence of CO_2 [E. Greenbaum, J. W. Lee, C. V. Tevault, S. L. Blankinship, L. J. Mets, *Nature*, 376, 438 (1995)].

Fig. 4. A photosynthetic reactor system made of PSI and PSII electro-optical cells for production of H_2 and O_2 in separate compartments.

Fig. 5. Photoassimilation of CO_2 , evolution of H_2 and O_2 by *Chlamydomonas* 137c in the presence 700 ppm CO_2 in helium.

Fig. 6. A proposed biometallocatalytic reactor system for production of H_2 and O_2 in separate compartments by a single light reaction (PSII).

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Fig. 1
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Fig. 2
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PRODUCTION OF H_2 AND O_2 BY *Chlamydomonas*
137c ($3 \mu\text{g chl/mL}$) IN PURE HELIUM

Fig. 3
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Fig. 4
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 ORNL DWG 93A-865

Fig. 5
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ORNL DWG 96-77R

Fig. 6
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 ORNL DWG 96A-88R