Proceedings of the 1998 U.S. DOE Hydrogen Program Review

NREL/CP-570-25315

RENEWABLE HYDROGEN PRODUCTION BY PHOTOSYNTHETIC WATER SPLITTING

E. Greenbaum and J. W. Lee
Oak Ridge National Laboratory
P.O. Box 2008
Oak Ridge, TN 37831-6194

Abstract

This mission-oriented research project is focused on the production of renewable hydrogen. We have demonstrated (Greenbaum, 1980) that certain unicellular green algae are capable of sustained simultaneous photoproduction of hydrogen and oxygen by light-activated photosynthetic water splitting. It is the goal of this project to develop a practical chemical engineering system for the development of an economic process that can be used to produce renewable hydrogen. There are several fundamental problems that need to be solved before the application of this scientific knowledge can be applied to the development a practical process: (1) maximizing net thermodynamic conversion efficiency of light energy into hydrogen energy, (2) development of oxygen-sensitive hydrogenase-containing mutants, and (3) development of bioreactors that can be used in a real-world chemical engineering process. We are addressing each of these problems here at ORNL and in collaboration with our research colleagues at the National Renewable Energy Laboratory, the University of California, Berkeley, and the University of Hawaii.

Current Status of the Research

During the current year, we have made progress in understanding the limiting aspects of the production of molecular hydrogen and oxygen via light-activated microalgal water splitting. We have focused on item 1 above, an investigation of maximizing the net thermodynamic conversion efficiency of light energy into hydrogen energy. In particular, we have focused on the question of how many light reactions are required to split water to molecular hydrogen and oxygen. We recently reported (Greenbaum et al., 1995; Lee et al., 1996) that certain mutants of the green algal *Chlamydomonas reinhardtii* that lack detectable levels of the Photosystem I reaction center were capable of simultaneous photoproduction of molecular hydrogen and oxygen, photoassimilation of atmospheric carbon dioxide and photoautotrophic growth. Although the absence of PS I in mutants B4 and F8 for the data reported in the references was confirmed by physical, biochemical and genetic techniques, subsequent analyses in our own laboratories as well as those of colleagues to whom we have sent the mutants indicate that there is variability in the PS I content of the cultures depending on growth conditions. While some strains retain undetectable levels of P700, others contain variable (0-20%) amounts of wild-type P700. This property of mutants B4 and F8 has been communicated to the journals in which the results were initially published (Greenbaum et al., 1997a,b).

In his analysis of this work, Boichenko (1996) postulated a "leaky" model of the Z-scheme, illustrated schematically in Fig. 1. According to this model, under continuous high-intensity light PS I turns over with sufficient rapidity to accommodate reductant generated by multiple PS IIs thereby preserving a key requirement of the Z-scheme: only PS I is capable of generating low-potential reductant that can be used for hydrogen evolution or carbon dioxide fixation.

We are testing the hypothesis of Fig. 1. In these experiments, mutant Fud26 of the green alga *C. reinhardtii* that had a measurable ratio of Photosystem I to Photosystem II of 0.08 was used for our studies. Table 1 summarizes the properties of this mutant. Our preliminary results with the alga characterized in Table 1 suggests that the Z-scheme is not capable of quantitatively accounting for the pulsed yields of hydrogen and oxygen production or photosynthesis under light-limiting conditions. However, in order to be completely certain that this is the case, we feel that it is necessary to perform the Photosystem I, Photosystem II, and antenna size measurements side-by-side with the oxygen and hydrogen measurements rather that shipping the samples offsite for analysis.

We are in the process of acquiring a spectrometer that will enable us to perform our own measurements of Photosystems I and II. This instrument is scheduled for delivery on June 6, 1998. Acquisition of this capability will enable us to perform all measurements necessary to perform a quantitative test of the Z-scheme. This capability will enable us to address the criticism that perhaps the Photosystem I measurements in the mutants of our original work was degraded by the process of shipping or freezing the algae.

Table 1. Photosynthetic Parameters of Mutant Fud26*

Parameter	Value
PSI : PSII Ratio	0.081
PSI Antenna	150
PSII Antenna	320

*Measurements by A. Melis. Frozen samples were pelleted and shipped to the University of California by express courier for analysis.

There is an important practical motivation for determining if evidence can be adduced supporting the possibility that sustained simultaneous photoevolution of hydrogen and oxygen can be driven by a single light reaction because it can, in principle, lead to a doubling of the conversion efficiency of light energy into hydrogen energy. This result, combined with algae that linearize the light saturation curve and possess oxygen-tolerant hydrogenases, would be the appropriate test organisms for development in bioreactors that can be used in chemical engineering development processes.

Work Plan for FY 1999

It is anticipated that upon receipt of our own spectrometer we will be able to perform, a side-by-side basis, all the measurements that are necessary for performing a quantitative test of the Z-scheme for hydrogen and oxygen production and carbon dioxide assimilation. We will also address the question of how the accumulation of carbon reserves affects the ability of algae to photoproduce hydrogen by a Photosystem I-mediated reaction. This approach has the advantage of spatial separation of hydrogen and oxygen which avoids the oxygen-tolerance problem of the hydrogenase enzyme. We will also work on the important problem of linearizing the light saturation curve of photosynthesis.

References

1. Boichenko, V. A. 1996. "Can Photosystem I be a Photogenerator of Low Potential Reductant for CO_2 Fixation and H_2 Photoevolution?" *Photosyn. Res.*, 47:291-292.

2. Greenbaum, E., J. W. Lee, C. V. Tevault, S. L. Blankinship, T. G. Owens, and L. J. Mets. 1997a. "CO_2 Fixation and Photoevolution of H_2 and O_2 in a Mutant of *Chlamydomonas* Lacking Photosystem I." *Nature*, 376:438-441.

3. Greenbaum, E., J. W. Lee, C. V. Tevault, S. L. Blankinship, T. G. Owens, and L. J. Mets. 1997b. "Photosystem I Measurements in Mutants B4 and F8 of *Chlamydomonas*," *Science*, 277:167-168.

4. Greenbaum,E., J. W. Lee, C. V. Tevault, S. L. Blankinship, and L. J. Mets. 1995. "CO_2 Fixation and Photoevolution of H_2 and O_2 in a Mutant of *Chlamydomonas* Lacking Photosystem I." *Nature*. 376:438-411.

5. Greenbaum, E. 1980. "Simultaneous Photoproduction of Hydrogen and Oxygen by Photosynthesis." *Biotechnol. Bioeng. Symp.*, 10:1-13.

6. Lee, J. W., C. V. Tevault, T. G. Owens, and E. Greenbaum. 1996. "Oxygenic Photoautotrophic Growth Without Photosystem I." *Science*, 273:364-367.

Figure

Fig. 1 Schematic illustration of the "leaky" Z-scheme of photosynthesis. It is postulated that under saturating illumination PS I undergoes multiple turnovers such that it can accommodate all output generated by Photosystem II.

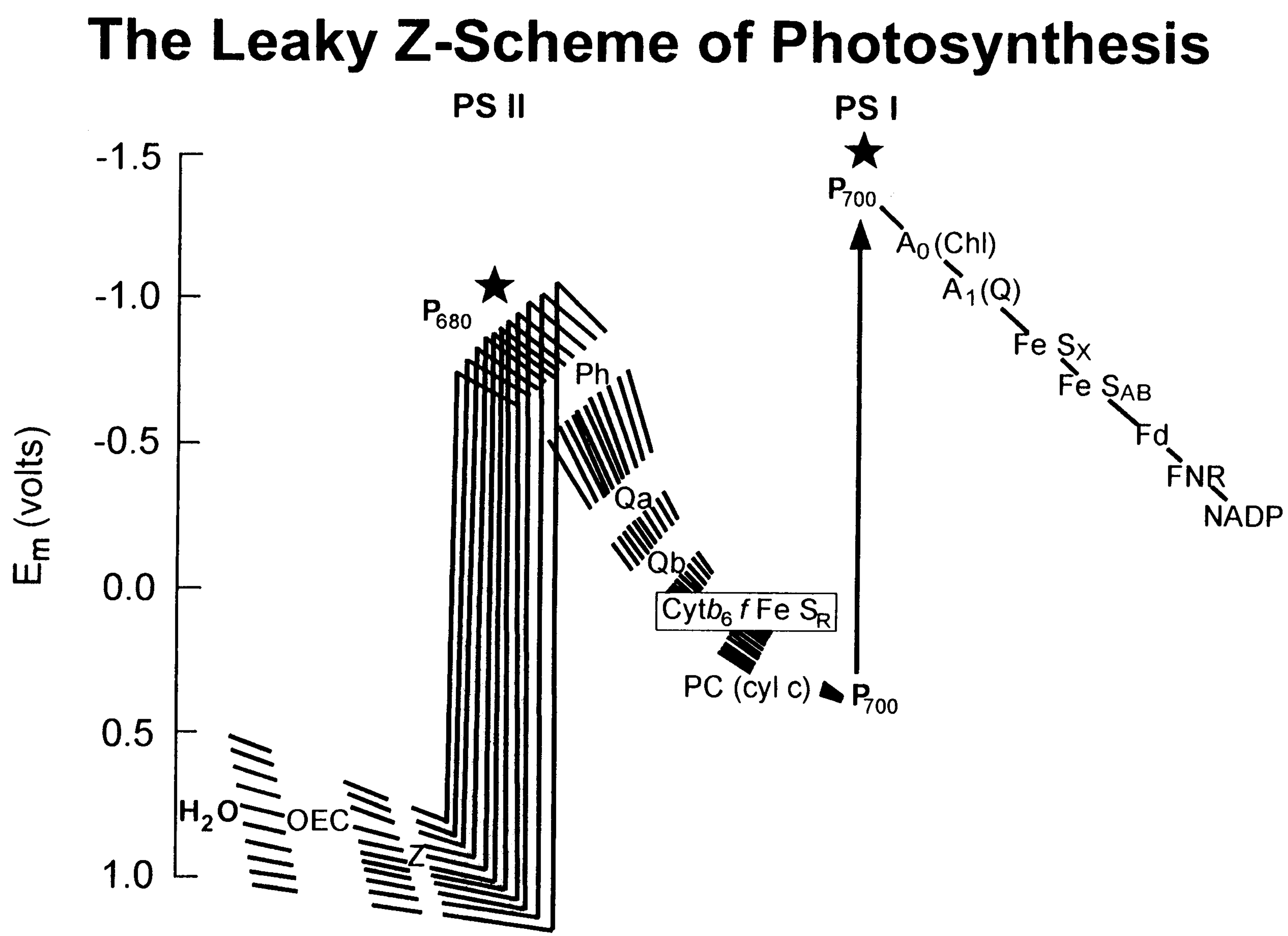
The Leaky Z-Scheme of Photosynthesis
PS II
PS I
E_m (volts)
-1.5
-1.0
-0.5
0.0
0.5
1.0
H_2O
OEC
Z
P_{680}
Ph
Qa
Qb
Cytb_6 f Fe S_R
PC (cyl c)
P_{700}
P_{700}
A_0(Chl)
A_1(Q)
Fe S_X
Fe S_{AB}
Fd
FNR
NADP

Figure 1