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PHOTOSYNTHETIC HYDROGEN AND OXYGEN PRODUCTION BY GREEN ALGAE

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Summary

An overview of photosynthetic hydrogen and oxygen production by green algae in the context of its potential as a renewable chemical feed stock and energy carrier is presented. Beginning with its discovery by Gaffron and Rubin in 1942, motivated by curiosity-driven laboratory research, studies were initiated in the early 1970s that focused on photosynthetic hydrogen production from an applied perspective. From a scientific and technical point of view, current research is focused on optimizing net thermodynamic conversion efficiencies represented by the Gibbs Free Energy of molecular hydrogen. The key research questions of maximizing hydrogen and oxygen production by light-activated water splitting in green algae are (1) removing the oxygen sensitivity of algal hydrogenases; (2) linearizing the light saturation curves of photosynthesis throughout the entire range of terrestrial solar irradiance — including the role of bicarbonate and carbon dioxide in optimization of photosynthetic electron transport and (3) the minimum number of light reactions that are required to split water to elemental hydrogen and oxygen. Each of these research topics is being actively addressed by the photobiological hydrogen research community.

Introduction

Photosynthetic hydrogen production by green algae was discovered in the pioneering experiments of Gaffron and Rubin (1942). This work was followed up by Gaffron and his colleagues in a series of seminal papers (Gaffron, 1960; Kaltwasser *et al.*, 1969; Stuart and Gaffron, 1971 & 1972;) as well as many others. From the point of view of renewable fuels and chemical feedstock production, it is light-activated simultaneous photoproduction of hydrogen and oxygen that is of primary interest. The pioneering experiments in this field were performed by Spruit (1958) who developed a novel two-electrode polarographic technique for the simultaneous measurement of hydrogen and oxygen transients by the green alga *Chlorella*. The principle conclusion he came to was that hydrogen and oxygen metabolisms are closely related and they derived from water splitting. Later work by Bishop and Gaffron (1963) indicated that light-dependent evolution of hydrogen appeared to require both photosystems.

Research on photosynthetic hydrogen production as a renewable energy source began in the 1970s (Gibbs, *et al.*, 1973; Lien and San Pietro, 1975, Mitsui *et al.*, 1977). Using the two-electrode technique Bishop *et al.* (1977) measured and interpreted hydrogen and oxygen production from a large group of green algae. However, due to the buildup of hydrogen and oxygen, with subsequent inhibition (*vide infra*) these reactions could be followed for only several minutes. Using a flow system that removed inhibitory oxygen, it was shown (Greenbaum, 1980) that sustained simultaneous photoproduction of hydrogen and oxygen could be observed for hours. In prior experiments using a glucose-glucose oxidase trap, Benemann *et al.* (1973) demonstrated hydrogen production from water by a chloroplast-ferredoxin-hydrogenase system. Measurement of the hydrogen analog of the Emerson and Arnold photosynthetic unit size (Greenbaum, 1977a, b) indicated that photogenerated reductant expressed as molecular hydrogen

was derived from the mainstream of the photosynthetic electron transport chain. Direct measurement of the turnover time of photosynthetic hydrogen production (Greenbaum, 1979 and 1982) demonstrated that this parameter was comparable to the turnover time of oxygen production. It was also shown (Greenbaum, 1988) that net conversion efficiencies of 5-10% could be achieved in the linear low-intensity region of the light saturation curve.

The Three Problems

Fig. 1 is a schematic illustration of the minimum number of components required for light-activated hydrogen and oxygen production in green algae. The hydrogenase enzyme is synthesized *de novo* under anaerobic conditions. In normal photosynthesis carbon dioxide is the preferred electron acceptor for photogenerated reductant from Photosystem I. However, direct kinetic competition between hydrogen evolution and the Calvin cycle can easily be observed (Graves *et al.* 1989; Cinco *et al.* 1993). The three scientific research problems associated with photosynthetic hydrogen and oxygen production are (1) oxygen sensitivity of hydrogenase; (2) antenna size, bicarbonate and the light saturation problem; and (3) the minimum number of light reactions required to split water to molecular hydrogen and oxygen.

Oxygen Sensitivity of Hydrogenase

In the application of intact unicellular green algae for hydrogen production one is confronted with the problem of oxygen sensitivity of the hydrogenase enzyme. Hydrogenase is synthesized under anaerobic conditions and, at present, must be kept that way in order to preserve its functionality. As illustrated in Fig. 1 oxygen and hydrogen by green algae are coproduced in the same volume. Therefore, a way must be found to prevent inhibition of

hydrogenase activity by the photosynthetically produced oxygen. This challenging problem is the focus of research at the National Renewable Energy Laboratory (Ghirardi *et al.*, 1997) and is reported elsewhere in these proceedings.

Antenna Size, Bicarbonate and the Light Saturation Problem

Schematic illustrations of the relationship of the light saturation curves of photosynthesis and the light-harvesting antennas to the photosynthetic electron transport chains are presented in Figs. 2 and 3. In full sunlight, $\approx 1000 \text{ W/m}^2$, there exists a kinetic imbalance between the rate of photon excitation of the reaction centers and the ability of the thermally-activated electron transport chains to process photogenerated electrons. Whereas the reaction centers can receive photoexcitations at the rate of $\approx 2000 \text{ sec}^{-1}$, movement through the electron transport chain is of the order of 200 sec^{-1} or less (Gibbs *et al.*, 1973). Therefore, normal photosynthesis saturates at much less than full sunlight, typically $\approx 10\%$. This is illustrated in the lower parametric curve of Fig. 2 labeled 200:1 implying 200 chlorophylls per reaction center. The actual number varies, of course, depending on alga and growth conditions. (Melis, 1989, Melis *et al.*, 1996) This is the situation that is illustrated schematically on the left-hand side of Fig. 3 where 200 chlorophylls service a single reaction center.

Since there is little opportunity to increase the rate of thermally-activated electrons through the photosynthetic electron transport chain, an alternate strategy is illustrated in the right-hand side of Fig. 3. Kinetic balance between the rate of photon excitation and rate of photosynthetic electrons can, in principle, be balanced, even at full sunlight, by reducing the absolute antenna size per reaction center. Under optimum conditions the idealized response is linear, illustrated by the parametric curve labeled 20:1. If such a response could be achieved in a

real-world system, photosynthetic productivity on a per chlorophyll basis would increase and high solar irradiances would be converted to useful biomass energy. Linearization of the light saturation curve of photosynthesis was demonstrated by Herron and Mauzerall (1972). Melis *et al.* (1998) have demonstrated linearization of the light saturation curve for high-light grown cultures of *Dunaliella*. These results indicate that the concept is technically correct.

An additional complication of the light saturation problem involves the requirement of bicarbonate to optimize electron transport through Photosystem II. Since carbon dioxide/bicarbonate are the exclusive sink for photosynthetically generated reductant they need to be removed so that the flow of electrons produces hydrogen rather than carbon dioxide fixation compounds. Complete removal of carbon dioxide, however, impairs electron transport in Photosystem II and further reduces the saturating light intensity for sustained simultaneous photoproduction of hydrogen and oxygen by about factor of 10. Qualitatively speaking, light saturation occurs at about 10 W/m². One strategy to overcome this limitation is to take advantage of the differential affinity of CO₂/bicarbonate between the Photosystem II binding site and the Calvin cycle. Such an approach has been explored by Cinco *et al.* (1993) in which light-activated hydrogen and oxygen evolution as a function of CO₂ concentration in helium were measured for the unicellular green alga *Chlamydomonas reinhardtii*. The concentrations were 58, 30, 0.8 and 0 ppm CO₂. The objective of these experiments was to study the differential affinity of CO₂/HCO₃⁻ for their respective Photosystem II and Calvin cycle binding sites vis-à-vis photoevolution of molecular oxygen and the competitive pathways of hydrogen photoevolution and CO₂ photoassimilation. The maximum rate of hydrogen evolution occurred at 0.8 ppm CO₂. The key result of this work was that the rate of photosynthetic hydrogen evolution can be increased, at least partially, by satisfying the Photosystem II CO₂/HCO₃⁻ binding site requirement

without fully activating the Calvin-Benson CO₂ reduction pathway. These preliminary experiments suggest that mutants of *Chlamydomonas reinhardtii* that have a genetically engineered low CO₂ affinity for the Calvin cycle and relatively higher affinity for the PS II CO₂/HCO₃⁻ binding site may be good candidates to explore for relieving the CO₂ part of the light saturation constraint.

The Minimum Number of Light Reactions

It has recently been reported (Greenbaum *et al.*, 1995; Lee *et al.*, 1996) that mutants of the green alga *Chlamydomonas reinhardtii* that lack detectable levels of the Photosystem I (PS I) reaction center are capable of sustained simultaneous photoevolution of H₂ and O₂, CO₂ reduction and photoautotrophic growth. The data indicated that under some circumstances PS II alone is sufficient to generate reductant capable of driving hydrogen evolution and CO₂ fixation, not of course that the Z-scheme is universally wrong. 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). The fact that mutants B4 and F8 can synthesize fluctuating levels of PS I raises the possibility that small amounts of this reaction center might exclusively generate reductant that can drive hydrogen evolution and carbon dioxide fixation.

In his analysis of this work, Boichenko (1996) postulated a "leaky" model of the Z-

scheme, illustrated schematically in Fig. 4. According to this model, under continuous high-intensity light PS I turns over with sufficient rapidity to accommodate reductant generated by multiple PS II's 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. Based on comparative rates of photosynthesis with wild-type *Chlamydomonas reinhardtii* and known (Melis *et al.*, 1996) properties of PS I antenna size it was felt that the earlier results were not consistent with this model even if PS I were present, but undetectable. Preliminary data (Greenbaum *et al.* 1997b), testing the leaky Z-scheme model of Fig. 4 on direct measurement of absolute single-turnover saturating flash yields of hydrogen and oxygen production in mutant Fud26 as well as photosynthesis measurements under light-limiting conditions (O_2 evolution with CO_2 as electron acceptor) suggest that the leaky Z-scheme model cannot quantitatively account for the measured amounts of photosynthesis. Therefore, in addition to the Z-scheme the pathway, there is, in mutant Fud26, an alternative oxygenic photosynthetic pathway.

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Scenedesmus. *Planta* (Berlin) 100: 228-243

Figure Legends

- Fig. 1 Schematic illustration of the process of photosynthetic hydrogen and oxygen evolution in green algae. The hydrogenase is synthesized under anaerobic conditions. In the absence of CO_2 , the natural terminal electron acceptor, photogenerated reductant can be expressed as molecular hydrogen catalyzed the hydrogenase enzyme.
- Fig. 2 Schematic illustration of the relationship between photosynthetic antenna size and saturating light intensity. The parameters labeling each curve are the stoichiometric ratios of chlorophyll antenna pigment to photosynthetic reaction center.
- Fig. 3 Schematic illustration of the relationship of chlorophyll antenna size, photosynthetic electron transport chains and conversion efficiency.
- Fig. 4 The "leaky" Z-scheme model of photosynthesis.

Fig. 1

PARAMETRIC CHARACTERIZATION OF THE RATE OF PHOTOSYNTHESIS vs SOLAR RADIATION

VARIABLE CHLOROPHYLL: RC RATIOS

Fig. 2

Antenna Size and Photosynthetic Efficiency

Fig. 3

The Leaky Z Scheme of Photosynthesis

Fig. 4