PHOTOSYNTHETIC HYDROGEN AND OXYGEN PRODUCTION BY GREEN ALGAE

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Summary

Photosynthesis research at Oak Ridge National Laboratory is focused on hydrogen and oxygen production by green algae in the context of its potential as a renewable fuel and chemical feed stock. 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 transpor;t and (3) constructing real-world bioreactors, including the generation of hydrogen and oxygen against workable back pressures of the photoproduced gases.

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, 197 1 & 1972;) as well as many others. From the point of view of renewable fuels and chemical feedstock production, it is light-activated simultaneous photoproduction ofhydrogen and oxygen that is ofprimary interest. The pioneering experiments in this field were performed by Spruit (195 8) 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.

Research Problems

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. In one approach, 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).

Antenna Size, Bicarbonate and the Light Saturation Problem

In full sunlight, ≈ 1000 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 -2000 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 that full sunlight, typically -10%.

Since there is little opportunity to increase the rate of thermally-activated electrons through the photosynthetic electron transport chain, an alternate strategy is to reduce the antenna size. 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. If such a response could be achieved in a real-world system, photosynthetic productivity on a per chlorophyll basis would increase and high solar n-radiances would be converted to useful biomass energy. Linearization of the light saturation curve of photosynthesis was demonstrated by Herron and Mauzerall(l972). 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 (PSII). 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 PSII 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/m^2. One strategy to overcome this limitation is to take advantage of the differential affinity of CO_2/bicarbonate between the PSII 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 CO, concentration in helium were measured for the unicellular green alga *Chlamydomonas reinhardtii.* The concentrations were 58, 30, 0.8 and 0 ppm CO,. The objective of these experiments was to study the differential affinity of CO_2/HCO_3^- for their respective PSII and Calvin cycle binding sites vis-a-vis photoevolution of molecular oxygen and the competitive pathways of hydrogen photoevolution and CO, photoassimilation. The maximum rate of hydrogen evolution occurred at 0.8 ppm CO,. The key result of this work was that the rate of photosynthetic hydrogen evolution can be increased, at least partially, by satisfying the PSII CO_2/HCO_3^- binding site requirement without fully activating the Calvin-Benson CO, reduction pathway. These preliminary experiments suggest that mutants of *Chlamydomonas reinhardtii* that have a genetically engineered low CO, affinity for the Calvin cycle and relatively higher affinity for the PSII CO_2/HCO_3^- binding site may be good candidates to explore for relieving the CO, part of the light saturation constraint.

Thermodynamic Driving Pressure of Photosynthetic Hydrogen Production

We have shown that the thermodynamic driving pressure of hydrogen production in the green alga *Scenedesmus* D_3 is equal to or greater that one atmosphere. This was accomplished by measuring the rate of photosynthetic oxygen production by *Scenedesmus* in one-atmosphere of pure hydrogen. The practical significance of this work is that it helps to minimize the amount of pump work required to deliver hydrogen at a useable pressure. At a minimum, the amount of energy saved is $\Delta W = RT\ln(P_{final}/P_{initial})$. Since this is the reversible equilibrium thermodynamic value, the actual value for real gases, including irreversible processes, will be several times this. The amount of energy saved can be calculated to be in the range 68,500-l 14,200 J mol^{-1} (ΔG_P^o for the reaction, $H_2O \rightarrow H_2 + \frac{1}{2}O_2$, is 237 200 J mol^{-1}). Another significant aspect of this result is that it demonstrates for the first time that the oxygen evolution enzyme is insensitive to the presence of high concentrations (i.e., pure) hydrogen. That is to say, unlike the well-known sensitivity of the hydrogenase enzyme to even low concentrations of oxygen, the oxygen evolving complex of photosynthesis is unaffected by the presence of hydrogen. The indirect method of measuring oxygen was necessary because it is experimentally impossible to measure photosynthetically produced hydrogen against a background carrier gas of pure hydrogen since the gas sensitive semiconducting detectors that are used for the hydrogen analysis are saturated in pure hydrogen. Isotopic labeling experiments are currently under way for direct measurement of hydrogen production.

[Note: Much of the text of this abstract has been previously published. Please see E. Greenbaum and J. W. Lee, "Photosynthetic Hydrogen and Oxygen Production by Green Algae: An Overview," in *BioHydrogen*, 0. Zaborsky, Ed. pp.235-242 (1998).

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