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DISCOVERY OF AN ALTERNATIVE OXYGEN SENSITIVITY IN ALGAL PHOTOSYNTHETIC H_2 PRODUCTION

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Abstract

Characterization of O_2-tolerance in H_2-producing photosynthetic organisms is essential to the development of this renewable energy source, since application of green algae for H_2 production is confronted with the problem of oxygen sensitivity. During current reporting period, we observed a new oxygen sensitivity in *Chlamydomonas reinhardtii* that is clearly distinct from the oxygen sensitivity of the hydrogenase. This distinct O_2 sensitivity indicates that there is an alternative electron transport pathway that can take electrons away from the hydrogenase-catalyzed H_2 production pathway to O_2. Our experiments demonstrated that this alternative mechanism is more sensitive to O_2 than the oxygen sensitivity of the hydrogenase. These findings redefine the meaning of "oxygen tolerance" in algal H_2 production. Future work will focus on mapping this alternative electron transport pathway and on developing a technique to control this pathway to enhance the production of H_2.

Introduction

Algal photosynthetic hydrogen (H_2) production is a potential future clean energy resource. In green algae, photoevolution of H_2 and O_2 occurs in the same cell where the photosynthetically produced O_2 can inhibit the production of H_2.[1] Therefore, application of green algae for H_2 production is confronted with the problem of oxygen sensitivity. In the past, this O_2-sensitive phenomenon was generally interpreted as the O_2-inhibition effect on hydrogenase activity.[2] During this reporting period, we discovered that this classic interpretation of O_2 sensitivity needs to be revised. In our recent experiments that characterized O_2-tolerance in H_2-producing wild-type *Chlamydomonas reinhardtii*, we observed a new oxygen sensitivity that is clearly distinct from the oxygen sensitivity of the hydrogenase. This distinct O_2 sensitivity indicates that there is an alternative electron transport pathway that can take electrons away from the hydrogenase-catalyzed H_2 production pathway to O_2. Our experiments demonstrated that this alternative mechanism is more sensitive to O_2 than the oxygen sensitivity of the hydrogenase. Therefore, these findings represent an important progress in algal H_2 production studies. This paper reports the detailed experimental results.

Materials and Methods

In our recent O_2-tolerance assays, we discovered a new oxygen sensitivity that is an alternative to the oxygen sensitivity of hydrogenase in wild -type *Chlamydomonas* stain 137c. The wild-type alga was grown under light intensity of about 20 $\mu E.m^{-2}. s^{-1}$ in minimal plus acetate medium. When the culture grew to a cell density of about10^6 cells/ml, the algal cells were harvested by gentle centrifugation (3000 RPM). It was then washed and re-suspensed in fresh minimal medium for O_2-tolerance hydrogen production assays. The O_2-tolerance assays were performed under atmospheres of research grade helium (purity >99.9999%, zero oxygen) and 0.1000% O_2 in helium using our unique dual-reactor-flow detection system. The 0.1000% O_2 in helium was a primary standard purchased from Matheson Gases and Equipment, Inc.

As illustrated in Fig. 1, the assays were conducted using a laboratory-built dual-reactor flow detection system.[3] For each assay, 35 ml of 137c algal sample (3 µg Chl/ml) was placed and sealed in each of the two reactors that are water-jacketed and held at 20°C with a temperature controlled water bath (Lauda RM6, Brinkmann Instruments, Germany). The algal sample was then purged by helium flow (50 ml gas /min) through the liquid reaction medium. This helium flow serves two purposes: 1) to remove O_2 from the algal sample to establish and maintain anaerobic condition that are necessary for induction of the algal hydrogenase synthesis and production of H_2; and 2) to carry the any H_2 gas product to the hydrogen sensors. After induction of hydrogenase and establishment of steady-state photoevolution of H_2 under the helium atmosphere (it normally took about 8 hr or more), the primary standard 0.1000% O_2 in helium was introduced into the reactors by replacing the pure helium at the same flow rate (50 ml/min) to characterize the oxygen sensitivity of photoevolution of H_2. The actinic illumination at 120 $uE.m^{!2}.s^{!1}$ (about 6% of the full LED intensity) for the H_2 photoevolution

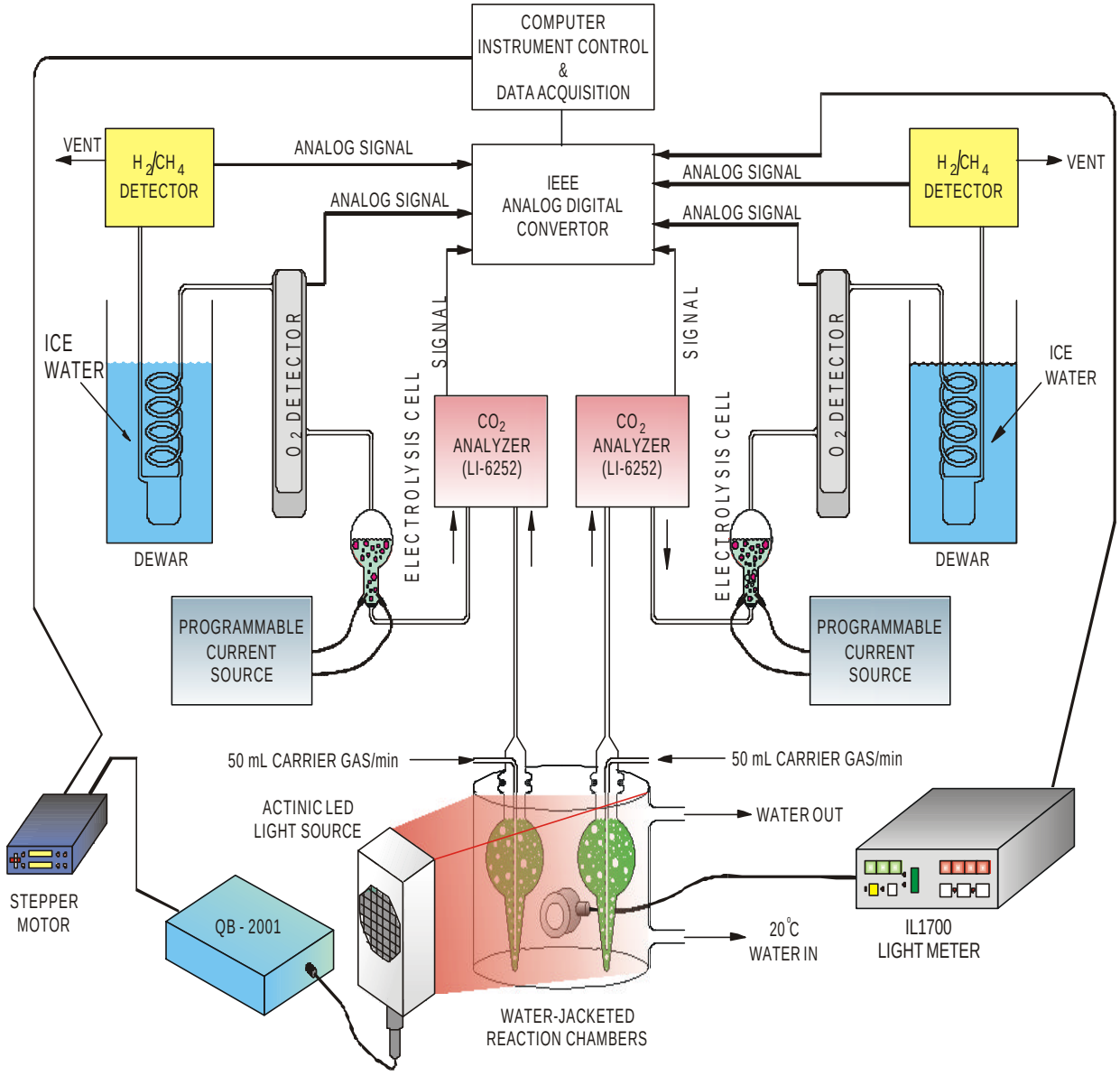

Figure 1. Schematic of a Dual-Reactor Flow Detection System for Simultaneous Detection of CO_2, H_2, and O_2.

assay was provided by an electronically controlled LED light source with its full (100%) intensity of about 2000 uE.m^{-2}.s^{-1} at 670 nm. The actinic intensity was measured with a IL-1700 light meter. Both the rate of H_2 production and the actinic intensity were recorded simultaneously by a PC computer.

Results and Discussion

The results of the assays are very intriguing. As illustrated in Fig. 2, introduction of 0.1000% O_2 dramatically reduced the rate of algal H_2 photoevolution. The steady-state H_2 production rate in the presence of 0.1000% O_2 was 0.33 ∶moles H_2 mg chl^{-1}Ahr^{-1} which is only about 2.8% of the full steady-state rate (12 ∶moles H_2 mg chl^{-1}Ahr^{-1}) before the introduction of the 0.1000% O_2. In the past, this type of H_2 production decay was commonly interpreted as the inhibition of O_2 on hydrogenase activity. Our experimental results have now proved that this classic interpretation of oxygen sensitivity on algal H_2 production is not consistent with the data. According to the classic interpretation, the reduction of H_2 production after the introduction of 0.1000% O_2 is due to O_2 inhibition on hydrogenase per se. That is, hydogenase activity would be the limiting factor for the rate of H_2 photoevolution. If this interpretation were correct, one would expect the rate of H_2 photoevolution to be no higher than the inhibited rate (0.33 ∶moles H_2 mg chl^{-1}Ahr^{-1}) after a brief dark period in the presence of 0.1000% O_2. However, the experimental data turned out to be very different from the classic expectation. As shown in Fig. 2, there was a surge of H_2 photoevolution after a 2-hr dark period *in the continued presence of 0.1000% O_2*. The peak rate of H_2 photoevolution was about 15 ∶moles H_2 mg chl^{-1}Ahr^{-1} which is about 45 times higher than the classically predicted rate (0.33 ∶moles H_2 mg chl^{-1}Ahr^{-1}). This assay has now been repeated for more than 6 times. All the assay results were consistent with the observation presented in Fig. 2.

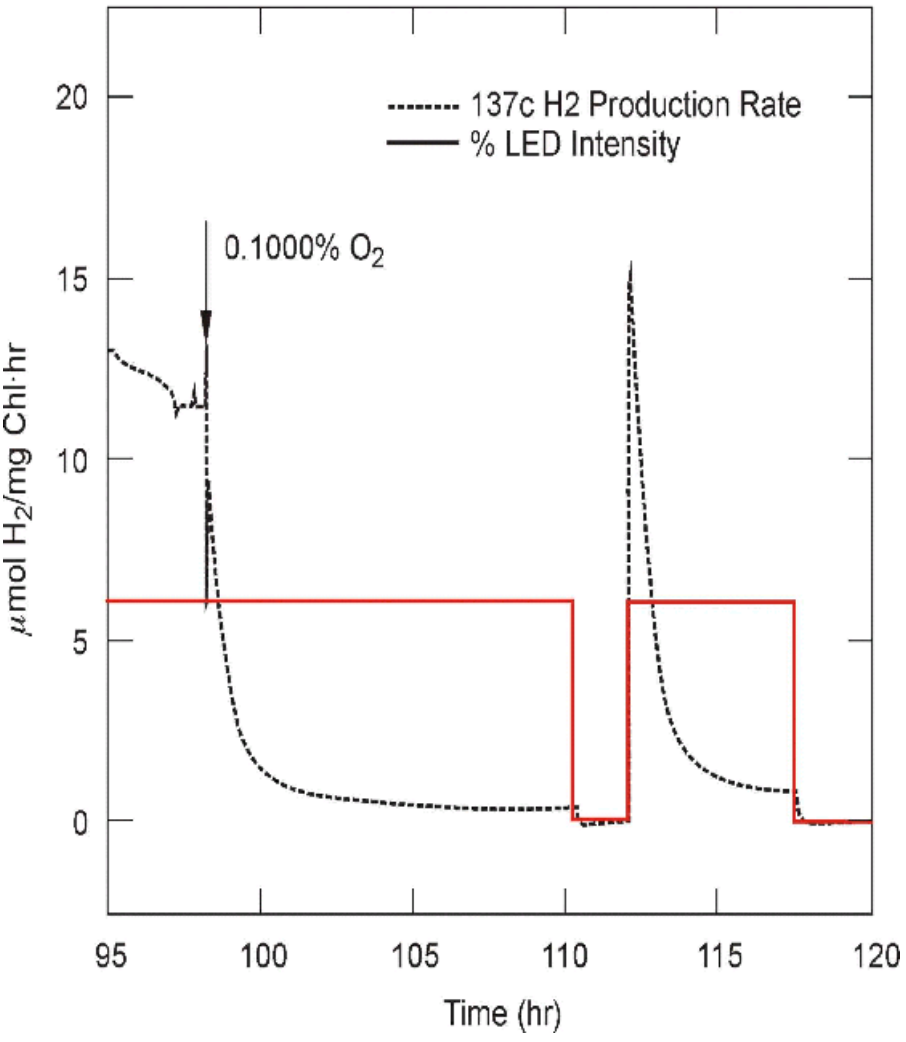

Figure 2. Observation of a new oxygen sensitivity to algal H_2 production in *Chlamydomonas reinhardtii.*

This observation clearly

indicated that hydrogenase activity was not the limiting factor for photoevolution of H_2 at this O_2 level. There must be an alternative electron transport pathway that takes the photogenerated electrons away from ferredoxin to O_2. The observed reduction of H_2 production after the introduction of 0.1000% O_2 can be explained by this alternative pathway that competes for electrons with the hydrogenase-catalyzed H_2 production pathway. This is an important discovery since it really redefines the meaning of "oxygen tolerance" in algal H_2 production. Our future work will focus on mapping this alternative electron transport pathway and on developing a technique to control this pathway to enhance the production of H_2.

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References

1. Greenbaum, E. and J. W. Lee 1998. "Photosynthetic hydrogen and oxygen production by green algae." In *BioHydrogen*, Edited by O. R. Zaborsky et al., pp. 235–241. Plenum Press, New York.

2. Ghirardi, M. L., R. K. Togasaki, and M. Seibert 1997. "Oxygen sensitivity of algal H_2-production" *Applied Biochemistry and Biotechnology*, 63-65:141-151.

3. Lee, J.W., S. L. Blankinship, and E. Greenbaum 1995. "Temperature effect on production of hydrogen and oxygen by Chlamydomonas cold strain CCMP1619 and wild-type 137c" *Applied Biochemistry and Biotechnology*, 51/52:379-385.