Approaches to developing biological H_2-photoproducing organisms and processes

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Abstract

The development of efficient biological systems for the direct photoproduction of H_2 gas from water faces several challenges, the more serious of which is the sensitivity of the H_2-evolving enzymes (hydrogenases) to O_2, an obligatory by-product of photosynthesis. This high sensitivity is common to both FeFe and NiFe hydrogenases, and is caused by O_2 binding to their respective metallocatalytic sites. This overview describes approaches to (i) molecular engineering of algal FeFe-hydrogenase to prevent O_2 access to its catalytic site; (ii) transform a cyanobacterium with an O_2-tolerant bacterial NiFe hydrogenase or (c) partially inactivate algal O_2-evolution activity to create physiologically anaerobiosis and induce hydrogenase expression.

Introduction

Biological H_2 production linked to photosynthetic water oxidation is a promising technology that may play a major role in the future of renewable energy [1,2]. Photosynthetic microorganisms, such as green algae and cyanobacteria, have the potential to store efficiently the energy of incident sunlight as high-energy H_2 molecules, with a maximum theoretical efficiency of approx. 13%. To achieve sustained H_2 production, however, photosynthetic organisms need to be able to produce H_2 gas directly from water at maximum photosynthetic efficiency. This presents a major challenge due to the high sensitivity of hydrogenases to O_2 inactivation [3]. Three approaches are being pursued at the NREL (National Renewable Energy Laboratory) to address this issue. In this overview, we describe these approaches and discuss recent advances in each.

Engineering an O_2-tolerant Fe-hydrogenase

In the green alga *Chlamydomonas reinhardtii*, H_2 photoproduction is catalysed by one or two Fe-hydrogenases [4,5]. Both enzymes are transcriptionally and post-translationally regulated by O_2 concentration and by other still unidentified metabolic factors [3,6]. These observations agree with the fact that Fe-hydrogenases are more sensitive to O_2 than their NiFe counterparts [7]. In the literature, one finds two main determinants of hydrogenase O_2 sensitivity: the structure of the catalytic site [8] and the accessibility of the catalytic site to O_2 gas [9]. In our work, we have focused on the latter determinant, and initiated detailed studies of gas diffusion through Fe-hydrogenases to identify the most probable pathways for O_2 and H_2 gases. Our molecular dynamics simulations are based on the crystal structure of the highly homologous *Clostridium pasteurianum* CpI Fe-hydrogenase, due to the lack of a solved structure for the algal enzymes. Preliminary experiments have identified a few potential individual pathways for O_2 gas diffusion, but multiple pathways for H_2 diffusion (see Cohen et al. in this colloquium [9a]). These results are in contrast with the observation that there is a single H_2 channel that links the catalytic site to the surface of the enzyme, as proposed for the *Desulfovibrio desulfuricans* Fe-hydrogenase by probing its X-ray structure with a 0.75 Å (1 Å = 0.1 nm) probe [9]. The difference between the two observations may be due to the dynamic nature of our simulations, as opposed to the static assumptions of [9]. Nevertheless, our results underscore the possibility of selectively affecting O_2 diffusion into the enzyme without disrupting H_2 gas diffusion from the catalytic site. Further *in silico* and *in vitro* experiments are being developed to test this hypothesis, taking advantage of a recently developed system for expression of Fe-hydrogenases in *Escherichia coli*.

Construction of a recombinant cyanobacterium expressing an O_2-tolerant NiFe-hydrogenase

In contrast with green algae, cyanobacteria are photosynthetic microbes that contain NiFe-hydrogenases. Until recently, it was not clear whether photosynthetic reductants could be directly utilized for hydrogenase-catalysed H_2 production in these microbes [10]. However, Cournac et al. [11] recently demonstrated that a *Synechocystis* strain, deficient in the type I NADPH-dehydrogenase complex was able to produce a significant amount of H_2 in the light. This observation opens up the door to explore cyanobacteria as an additional potential system for H_2 photoproduction from water.

Key words: biohydrogen, cyanobacteria, green algae, hydrogenase, O_2 inactivation, sulphur deprivation.

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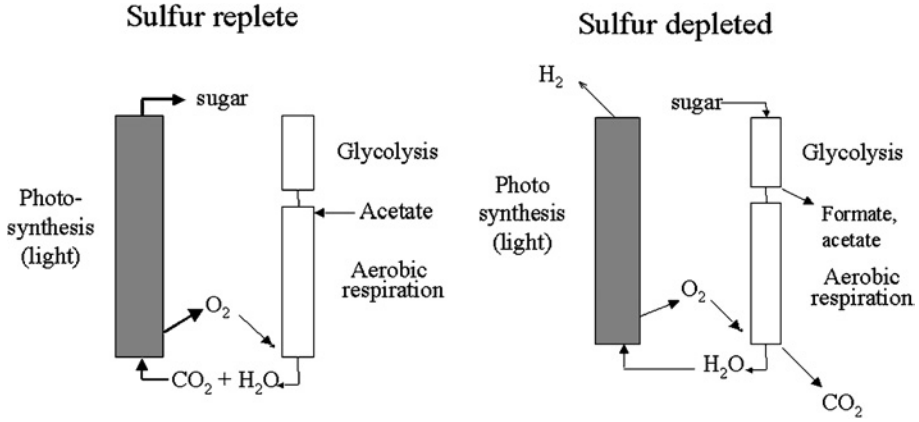
Figure 1 | Physiological effects of sulphur deprivation



Research conducted at NREL has uncovered an O_2-tolerant NiFe hydrogenase from the purple non-sulphur photosynthetic bacterium, *Rubrivivax gelatinosus* CBS. The *in vivo* half-life of this hydrogenase is 21 h in air, and 6 h in air when the protein is partially purified [12]. Genes encoding the CBS hydrogenase and its accessory proteins were sequenced recently. Work has been initiated to transfer and express the O_2-tolerant hydrogenase genes from CBS to *Synechocystis* so that the resultant recombinant organism can evolve H_2 continuously during normal oxygenic photosynthesis.

Green algal H_2 production following partial inactivation of photosynthetic O_2-evolution activity

A third approach to sustain algal H_2 photoproduction consists of depriving an algal culture of sulphate, required to provide proteins with sulphurylated amino acid residues. The protein most affected by sulphur deprivation in green algae is D1, the components of the water-oxidizing Photosystem II that displays the fastest rate of turnover. As the cultures move from sulphur-replete to sulphur-deprived medium, D1 turnover decreases, causing an overall reduction in photosynthesis [13]. Indeed, we have demonstrated that, within 24 h, the photosynthetic capacity of the cultures decreases below that of respiratory O_2 consumption. As shown in Figure 1, as a result, the culture becomes anaerobic, CO_2 fixation is interrupted and the accumulated starch is degraded. Depending on the rate of starch degradation, the cultures can either (i) oxidize all starch to CO_2, thereby consuming the residual photosynthetically evolved O_2, or (ii) resort to partial anaerobic metabolism as well, excreting formate and acetate into the medium [14].

The H_2-production phase is temporary, due to the eventual effect of sulphur deprivation on all other cellular functions. We have demonstrated that the cultures can cycle between a sulphur-replete, photosynthetic O_2 evolution mode and the sulphur-depleted, H_2-photoproduction mode for at least three times without significant loss of activity [15]. However, preliminary economic analyses at NREL attributed significant costs to the cycling of cultures, which led us to develop a continuous, chemostat-based system for algal H_2-production [16]. This latter development resulted in a decrease in the cost of H_2 production by a factor of three. Finally, current developments include attempts to immobilize sulphur-deprived cells on to glass fibres at high cell density, which has resulted in increased stability and increased volumetric rates of H_2 production.

Conclusion

Although still under investigation, biological H_2 photoproduction represents a potentially revolutionary technology to harvest solar energy and store it in an easily transportable chemical form. The O_2 sensitivity of the hydrogenase enzyme is, however, only one of the many obstacles to the development of a truly commercial system. Major developments in algal physiology and genetics, electron transport, light-harvesting optimization and photobioreactor engineering will also be required to achieve efficient coupling of photosynthetically generated reductants with H_2 gas production.

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