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Editorial

Perspectives

Papers

Hydrogen photoproduction by use of photosynthetic organisms and biomimetic systems

Suleyman I. Allakhverdiev,*[a,b,c,d] Vladimir D. Kreslavski,[a] Velmurugan Thavasi,[b] Sergei K. Zharmukhamedov,[a] Vyacheslav V. Klimov,[a] Toshi Nagata,[c] Hiroshi Nishihara[d] and Seeram Ramakrishna[b,e,f]

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Hydrogen can be important clean fuel for future. Among different technologies for hydrogen production, oxygenic natural and artificial photosyntheses using direct photochemistry in synthetic complexes have a great potential to produce hydrogen, since both use clean and cheap sources: water and solar energy. Artificial photosynthesis is one way to produce hydrogen from water using sunlight by employing biomimetic complexes. However, splitting of water into protons and oxygen is energetically demanding and chemically difficult. In oxygenic photosynthetic microorganisms such as algae and cyanobacteria, water is split into electrons and protons, which during primary photosynthetic process are redirected by photosynthetic electron transport chain, and ferredoxin, to the hydrogen-producing enzymes hydrogenase or nitrogenase. By these enzymes, e^- and H^+ recombine and form gaseous hydrogen. Biohydrogen activity of hydrogenase can be very high but it is extremely sensitive to photosynthetic O_2. In contrast, nitrogenase is insensitive to O_2, but has lower activity. At the moment, the efficiency of biohydrogen production is low. However, theoretical expectations suggest that the rates of photon conversion efficiency for H_2 bioproduction can be high enough (>10%). Our review examines the main pathways of H_2 photoproduction by using of photosynthetic organisms and biomimetic photosynthetic systems.

Introduction

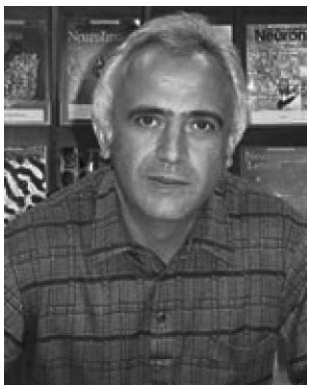
Solar energy is the most abundant and accessible renewable energy source available for future sustainable production of fuel and, finally, electricity. For effective use of solar energy it is important to develop more cost-effective systems with improved ability to convert solar energy into chemical energy conserved in fuel, such as H_2. Hydrogen is, likely, one of the most promising clean fuels for the future.[1] The combustion of the evolved H_2 yields only H_2O and thereby completes the clean energy cycle. A variety of process technologies have been employed for H_2 production, including splitting of water by water-electrolysis, photoelectrolysis and photo-biological production. However, for all H_2 production processes there is a need for significant improvement in efficiencies, reduced capital costs, and enhanced reliability and operating flexibility.[2] For instance, photo-electrolysis is at an early stage of development, and material cost and many practical issues have to be solved for application. Photo-biological H_2 production may be one of the alternatives to chemical and electrochemical technologies. Photosynthesis is a base for all biological solar-driven methods of H_2 production. Therefore, these approaches examine a link between photosynthetic efficiency, photosynthetic products and H_2 production.

Photosynthesis is based on conversion of solar energy into chemical energy by a series of electron transfer steps (Fig. 1).[3–5] Photosynthesis can be divided into oxygenic (O_2 producing) and anoxygenic photosynthesis.[5,6,7] Oxygenic organisms (higher plants, algae and cyanobacteria) use solar energy to extract electrons and protons from water mainly for the CO_2 assimilation cycle, and to produce oxygen (Fig. 1).[4,5,8] Anoxygenic photosynthesis occurs in simpler organisms such as green sulfur and purple non-sulfur bacteria. This review focuses only on oxygenic organisms such as algae and cyanobacteria that are able to split water and evolve H_2.

All oxygenic organisms extract electrons and protons from water and use them to reduce $NADP^+$ and plastoquinone for use as energy sources for metabolism such as the Calvin cycle (CO_2 fixation) and other pathways. However, oxygenic phototrophs such as cyanobacteria and microalgae can transiently produce H_2 under anaerobic conditions *via* proton reduction, catalyzed by a hydrogenase (or nitrogenase) in competition with other intracellular processes. In this case the electrons and protons, ultimately produced by water oxidation, are redirected at the level of ferredoxin/NADPH into hydrogenase.

One attractive way to harvest solar energy is to adopt the concept of natural photosynthesis to build artificial systems for H_2 bioproduction. Artificial photosynthesis employs synthetic complexes as photosensitizers (Pn) to harvest solar energy and utilize the energy to produce hydrogen from water.[9] This is an

[a]*Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Moscow Region 142290, Russia. E-mail: Suleyman@ibbp.psn.ru*
[b]*Nanoscience and Nanotechnology Initiative, National University of Singapore, 2 Engineering Drive 3, Singapore, 117576, Singapore*
[c]*Research Center for Molecular Scale Nanoscience, Institute for Molecular Science, 5-1 Higashiyama, Myodaiji, Okazaki 444-8787, Japan*
[d]*Department of Chemistry, School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan*
[e]*Department of Mechanical Engineering, National University of Singapore, 9 Engineering Drive 1, Singapore, 117576, Singapore*
[f]*Division of Bioengineering, National University of Singapore, 9 Engineering Drive 1, Singapore, 117576, Singapore*



Suleyman I. Allakhverdiev

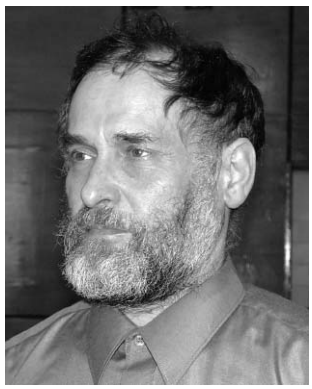
Suleyman I. Allakhverdiev is the Chief Research Scientist at the Institute of Basic Biological Problems, RAS, Pushchino, Russia. He received a Dr.Sci. degree in Photochemistry, Photobiology, and Plant Physiology (2002, Moscow), and Ph.D. in Physics and Mathematics (1984, Pushchino). He graduated from Azerbaijan State University, Department of Physics (Baku). He has been guest-editor and is a member of the Editorial Board of several international journals. His research interests include the structure and function of photosystem II, water oxidizing complexes, artificial photosynthesis, hydrogen photoproduction, and catalytic conversion of solar energy. He has been cited ca. *3000 times. E-mail: suleyman@issp.serpukhov.su*

Velmurugan Thavasi

Dr Velmurugan Thavasi is a Research Fellow at the NUS Nanoscience and Nanotechnology Initiative. He obtained an M.Eng. in chemical engineering and Ph.D. in chemistry from National University of Singapore. His research interests include materials synthesis and interface engineering for energy and electronic devices. E-mail: velnanotech@gmail.com

Hiroshi Nishihara

Hiroshi Nishihara received a D.Sc. degree in 1982 from The University of Tokyo. He was appointed research associate of Keio University in 1982, and promoted to associate professor in 1992. Since 1996, he has been a professor of The University of Tokyo. He was also a visiting research associate at the University of North Carolina at Chapel Hill (1987–1989), and a researcher at PRESTO, JRDC (1992–1996). His research focuses on the creation of new electro- and photo-functional materials, the invention of unidirectional electron transfer systems utilizing molecular layer interfaces, and the combination of the interfaces with biomolecules. E-mail: nisihara@chem.s.u-tokyo.ac.jp



Vladimir D. Kreslavski

Vladimir D. Kreslavski is a senior researcher and head of group in the laboratory of ecology and physiology of phototrophic organisms at Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia. His field of interests includes: molecular mechanisms of plant stress resistance and acclimation of photosynthetic apparatus, as well as the pathways of photosynthetic improvement. E-mail: vkreslav@mail.ru

Toshi Nagata

Toshi Nagata, born in 1964, received his Doctor of Science degree from Kyoto University in 1992. After working as a research associate in Kyoto University and a visiting scientist in Colorado State University, he joined Institute for Molecular Science as an associate professor in 1998. His research interests are synthetic organic chemistry and coordination chemistry, aiming at artificial photosynthesis. E-mail: toshi-n@ims.ac.jp

Seeram Ramakrishna

Prof. Seeram Ramakrishna is currently the Vice-President (Research Strategy) of the National University of Singapore (NUS). He received a Ph.D. from the University of Cambridge. He holds positions on many advisory boards, including companies, universities and journals. His research interests include the development of biomaterials, and materials for renewable energy based devices. The ISI web of knowledge currently ranks him at 95 out of 3463 most cited materials scientists in the world. Email: seeram@nus.edu.sg

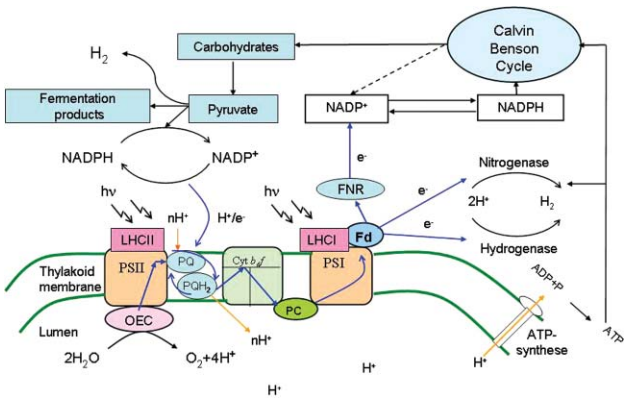


Fig. 1 Scheme of solar-powered H_2 production during oxygenic photosynthesis and subsequent formation of carbohydrates in cyanobacteria and microalgae. The oxygenic "light reactions" of photosynthesis are driven by the solar energy captured by the light-harvesting complexes of photosystem I (PSI) and photosystem II (PSII) or phycobilisoms. Electrons derived from H_2O by oxygen-evolving complex (OEC) of PSII are passed along the photosynthetic electron transport chain *via* plastoquinone (PQ), the cytochrome b_6f complex (Cyt b_6f), plastocyanin (PC), PSI, and ferredoxin (Fd), then by ferredoxin-$NADP^+$ oxidoreductase to $NADP^+$ with final production of NADPH. H^+ is released into the thylakoid lumen by PSII and the PQ/PQH_2 cycle and used for ATP production *via* ATP synthase. The ATP and NADPH generated during primary photosynthetic processes are consumed for CO_2 fixation in the Calvin-Benson cycle, which produces sugars and ultimately starch. Under anaerobic conditions, H_2ase (nitrogenase) can accept electrons from reduced Fd molecules and use them to reduce protons to H_2. Certain algae under anaerobic conditions can use starch as a source of H^+ and e^- for H_2 production (*via* NADPH, PQ, cyt b_6f, and PSI) using the H_2ase. In cyanobacteria the H^+ and e^- derived from H_2O can be converted to H_2 *via* a nitrogenase or fermentation. Here, carbohydrate stores such as starch can be converted to sugars and then pyruvate by glycolysis, before producing H_2 and organic acids (*e.g.*, formate, acetate, and butyrate). During fermentation process carbohydrate stores such as starch can be converted to sugars and subsequently pyruvate *via* glycolysis, before producing H_2 and organic acids (*e.g.*, formate, acetate, and butyrate). Adapted from ref. 3, 7 and 8.

emerging field and the hydrogen generation efficiency of man-made molecular systems is not high enough at the moment, but encouraging for researchers.

There are some problems in this field. One or several sensitizers are required for an artificial photosynthetic cell (Fig. 2). Excited Pn donates electrons to the reductive site of the artificial photosynthetic system and extracts electrons from oxidative site. A catalyst that operates at the very high redox potential is needed for the water-oxidation reaction, performs a four-electron reaction so as to maximize the energy efficiency, prevent the production of reactive intermediates such as hydroxyl radicals, and mediate proton-coupled redox reactions (Fig. 2).

To develop active and stable catalysts for water oxidation, it is important to use a multinuclear structure, which can accumulate and delocalize four oxidizing equivalents. Di- and tetranuclear manganese complexes as well as mono-, di-, and trinuclear ruthenium complexes have been reported as molecular catalysts capable of evolving O_2 from water.[10–14] The presence of oxygen at the catalytic site for hydrogen production can inactivate or decrease the performance of many known catalysts. Therefore,

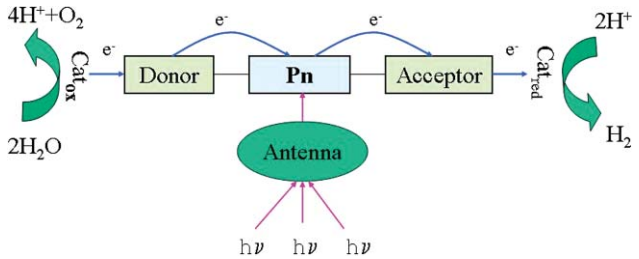


Fig. 2 Scheme of artificial photosystem, illustrating the photochemical splitting of water into H_2 and O_2. The following blocks are required for a functioning photosystem: photosynthesizer, composed of several molecules (Pn), electron transfer donor (Donor), electron-transfer acceptor (Acceptor), catalyst for chemical H_2 reduction (cat_{red}), catalyst for chemical water oxidation (cat_{ox}) and light-harvesting molecular construct (Antenna). Adapted from ref. 9.

the biomimetic system has to be developed to spatially separate the catalytic centers for production of hydrogen and oxygen.

Catalysts such as Co[15,16] and molecules mimicking hydrogenase structure and possessing hydrogenase activity[17–19] are more favorable for hydrogen production.

In any case, whether the devices mimicking photosynthesis are composed of natural biomolecules or organic or inorganic molecules, the architecture and spatial arrangements at multiple length scales should play a crucial role.[5]

Natural systems

Oxygenic organisms (cyanobacteria and microalgae)

Photosynthesis involves a sequential chain of reactions that include light absorption, charge separation, water splitting, electron transport, reducing $NADP^+$ and creation of a proton gradient. Several main complexes are involved in the process of oxygenic photosynthesis: Photosystem II (PSII), including water oxidation complex, Photosystem I (PSI), cytochrome b_6/f and ATP-synthase complexes (Fig. 1). Components of the photosystems and electron transport from water to $NADP^+$ are described in detail in a number of papers.[4,8,14]

Electrons are transferred from PSII through PSI to ferredoxin (Fd). Normally, Fd shuttles electrons to an enzyme ferredoxin-NADP-reductase that reduces $NADP^+$ to NADPH, an important source of electrons needed to convert CO_2 to carbohydrates in the Calvin-Benson cycle. Here, protons (H^+) outside the thylakoid are carried to the inner thylakoid space and exert a proton gradient across the thylakoid membrane. Under anaerobic conditions, hydrogenase (nitrogenase) can accept electrons from reduced Fd molecules and use them to reduce protons to molecular hydrogen (H_2). In this case, photosynthetic reducing power can partition between at least two pathways: CO_2 reduction and H_2 production. CO_2 reduction requires an ATP pathway for using reductant from water, whereas H_2 production does not use ATP, which is the desired pathway for renewable energy production.

Under normal conditions, the competition for the electron donor favors the CO_2 fixation pathway. However, in the absence or limitation of CO_2, the favorable pathway is the H_2 production, which is down-regulated due to non-dissipation ΔpH caused by the lack of ATP utilization.

There are two major research challenges related to the conversion of protons and electrons by light energy into H_2. 1: The level of solar light intensities that can be efficiently utilized to drive photosynthesis should be optimized for microorganisms. 2: For H_2ases, there are kinetic limitations on electron transport to the hydrogenase under H_2-producing conditions. Nitrogenase uses ATP during the production of H_2, therefore the efficiency of nitrogenase system is lower than using the hydrogenase.

Enzymes for biohydrogen production

Terrestrial plants are not capable of photoproduction of H_2. On the contrary, most of the microalgae and cyanobacteria are able to produce hydrogen.[8,20–22]

Cyanobacteria use two major types of enzymes: nitrogenases that produce H_2 contaminant with N_2 fixation, and [NiFe]-hydrogenases.[7,8] Nitrogenase is not known to be present in any eukaryote, including the microalgae, whereas H_2ases are widespread and synthesized in many of the microalgae and cyanobacteria.[22–24]

Among microalgae, many unicellular green algae have the highest rate of H_2 photoproduction. Green algal H_2ases that belong to the class of [FeFe]-hydrogenases are involved in much higher specific activities than ones of cyanobacterial [NiFe]-hydrogenases.[25]

In green algal cells, H_2 production reaction is catalyzed by the [FeFe]-hydrogenase enzyme, and as shown in reaction equation: $2H^+ + 2Fd^- \rightarrow H_2 + 2Fd$. Ferredoxin, being the natural electron donor, transports the electrons to the algal [FeFe]-hydrogenase as well as to the nitrogenase (Fig. 1).

Electrons and protons are initially extracted from water ($2H_2O \rightarrow 4H^+ + 4e^- + O_2$) by oxygenic photosynthesis. Here, the hydrogen-producing enzymes act as a H^+/e^- release valve by recombining H^+ (from the medium) and e^- (from the reduced ferredoxin) to produce H_2. The metal clusters of hydrogenases are unique in having CO_2 and CN ligands, but they are sensitive to O_2 and CO. [NiFe]-hydrogenases and [FeFe]-hydrogenases can be inactivated by these inhibitors especially in the latter case, the inactivation by O_2 is irreversible.[22] The stoichiometric release of one O_2 and two molecules of H_2 is possible only under the conditions of real anaerobiosis. This is required for the transcription of the hydrogenase gene and supporting hydrogenase activity.[6] However, very little is known at the moment about regulation of [FeFe]-hydrogenase gene transcription and maturation.[22] Such issues as well as structure and function of the enzymes [FeNi] and [FeFe]-hydrogenases and nitrogenase are need to be examined.

The level of O_2 is crucial for algal [FeFe]-hydrogenases. H_2 production is often limited mainly because of the extreme sensitivity of H_2ases to molecular oxygen.

Pathways for H_2 production

There are several hydrogenase-dependent pathways available for H_2 production in cyanobacteria and algae.[7,8] The first pathway is the photo-dependent H_2, in which the electron transport occurs *via* two photosystems from water to Fd (Fig. 1). H^+ that is released from lumen and e^- from reduced ferredoxin are used for H_2 production by hydrogenase (H_2ase). This is an efficient pathway for cyanobacteria, but inefficient for green algae. However, under conditions of low activity of PSII, for instance, upon sulfur deprivation, which significantly eliminates O_2, the rate of H_2 photoproduction can be significant.[8,26]

The second pathway for H_2 production is photo-fermentative, which effectively occurs in two temporal stages. During the first stage, the photosynthetic processes produce carbohydrates, providing mitochondrial respiration and cell growth. During the second stage, under anaerobic conditions, H_2ase expression is induced, and NADPH pumps electrons from stored reductants to the PQ pool.

PSI accepts e^- and H^+ delivered to the PQ pool, which is fully reduced under anaerobic conditions by enzymatic oxidation of intracellular reductants derived from fermentation. Mitochondrial oxidative phosphorylation is largely inhibited. The temporal separation of H_2 and O_2 flows is crucial for increasing the efficiency of this pathway.

Thus, anaerobic conditions force some H_2 producers to reroute the energy stored in carbohydrates to chloroplast H_2ase, likely using a NADPH–PQ electron transfer mechanism, which presumably facilitates ATP production *via* photophosphorylation. The two stage pathway seems to be the most effective for H_2 bioproduction.[8]

The third pathway, similar to the second, produces H_2 from water but uses nitrogenase of cyanobacteria. Here, electrons and protons are delivered from photosynthesis. However, this pathway requires the largest numbers of photons, failing results in lower efficiency, in comparison with other pathways and hence makes it economically impractical.

Oxygen sensitivity of hydrogenases

Like nitrogenases, the majority of H_2ases are also very sensitive to O_2.[22,27] It is an important issue, and pathways for suppressing O_2 production and improving H_2 production yield were discussed in several recent reviews.[7,8,28,29] Due to the fact that H_2ase is hypersensitive to oxygen and is located in the chloroplast, where PSII releases O_2, H_2 production rate is usually low. Therefore it is important to decrease the O_2 concentration. Natural mechanisms that could be used for this are: the enhancement of respiration and chemical reduction of O_2 by PSI, and reversible inactivation of O_2 evolution in PSII.[8]

One of the approaches to decline the rate of oxygenic photosynthesis is sulfur deprivation, which is described in detail in earlier reviews (see ref. 22 and 27).

One of the most effective pathways for generation of H_2 is indirect biophotolysis that intends to eliminate the oxygen sensitivity of the H_2ases by separating H_2-producing reactions from the oxygen evolving ones.[24,26,30]

Reduced antenna size and increased PQ pool

The efficiency of light utilization is one of the important factors that determine the H_2 photoproduction yield. Enhanced H_2 production may be achieved by engineering the antenna size to suppress fluorescence and heat dissipation that causes a reduction in efficiency.[8,31] Genes that regulate the Chl antenna size in the model green alga *Chlamydomonas reinhardtii* were identified and characterized.[29] Analysis of the *tla1* and *tlaX* mutants with decreased Chl antenna size in comparison with the wild type

demonstrated higher yields of photosynthesis in microalgae with a truncated Chl antenna size.

The increase of PQ pool capacity and strong proton buffer capacity can also be considered for improving light utilization, since it is able to accelerate electron transport to PSI, slow down the back reactions in the PSII, and oxidase reducing equivalents stored during CO_2 fixation. Besides, down regulation of competing pathways can redirect the fluxes of electrons *via* PSI and Fd into H_2ase (Fig. 1).

Immobilization of microbial cultures

The reported rates of H_2 production by sulfur-deprived cultures are still far below the maximum potential rate of H_2 photoproduction for an algal system[32] mainly due to the partial inactivation of photosynthetic water oxidation.[33]

On the another hand, the immobilized cyanobacteria produce H_2 at much higher volumetric rates than suspension cultures.[34] The improved and longer-term H_2 photoproduction by immobilized green alga cells was successfully demonstrated.[28,30,33,35] It was shown that sulfur-deprived cultures of *C. reinhardtii* cells can be immobilized by inexpensive matrices and sulfur-deprivation stress can be successfully applied to immobilized algal cells.

Moreover, both natural cultures and future ideal artificial photobiodevices for H_2 photoproduction should be based on two reactions: photosynthetic water splitting to O_2 and H^+ on the donor side and the H^+ reduction on the acceptor side of PSII, using only this photosystem alone. Nevertheless in the case of this approach a few problems exist: separating H_2 gas from other contaminants, first of all O_2, inhibiting the catalytic site of water oxidation system by molecular hydrogen, *etc.* Thus, it is important to separate the processes of O_2 evolution and H_2 photoproduction. The immobilization approach can solve the problem of compartmentalization.

The use of mimics of water oxidation systems

Another approach to overcome partial inactivation of photosynthetic water oxidation systems leading to low efficiency and instability of H_2 photoproduction is the use of mimics of the natural Mn-cluster. It is well known that the water oxidation complex is composed of a special tetra manganese cluster with a composition of $Mn_4Ca_1Cl_x$,[36] which is very important in photosynthetic oxygen evolution. However, the oxygen evolving center of PSII is not suitable for engineering application such as H_2 photoproduction due to its limited stability.

It is believed that performing a directed molecular design and broad synthesis of different artificial metal–organic complexes with different ligand spheres and matrices that mimic natural the Mn-cluster of PSII might avoid the problems associated with low H_2 photoproduction rates and scale-up of bioreactors. Such systems would have more versatility, and might split water with sun light and produce hydrogen and oxygen, with a high efficiency and long term stability.

Many synthetic Mn complexes with different ligands have been synthesized and examined with various degrees of restoration of the original function of PSII, including oxygen evolution in Mn-depleted PSII complexes,[10,37–43] and some of them even produced hydrogen peroxide.[13]

If protons from a water oxidation complex can be captured and reduced to H_2 in the above reconstructed photosynthetic systems, it could provide an interesting approach for future. Then next approach could be combination of an artificial Mn-containing water oxidation complex with an H_2ase system stabilized by inexpensive matrices.

Enhanced resistance to environmental stress conditions

To increase productivity, the algal cells must be maintained in a healthy, active state during H_2 production for a longer period of time. The tolerance of cell cultures to environmental stresses such as photoinhibition, salt stress and high temperatures is necessary for sustainable photosynthesis and, hence, H_2 production.[7,8] The efficiency of the recovery of PSII, from damage induced by high light or environmental stress is one of the key factors in photosynthetic resistance.[44,45]

The use of mutants

An alternative approach for improving H_2 production in photosynthetic organisms is the systematic genetic screening for mutants with an increased ability for effective production of H_2. Genetic engineering has shown significant promise for increasing H_2 production both in algae and cyanobacteria.[6,22,24,29]

Molecular engineering that makes the algal H_2ase enzyme insensitive to the presence of O_2 was suggested.[22,23] Besides, replacing the algal hydrogenase with a strongly oxygen tolerant, or at least reversibly inactivated, bacterial enzyme may be possible.[46]

It is difficult to judge which organisms are the most promising systems for H_2 production. The [NiFe]-hydrogenases of cyanobacteria have the advantage compared to Fe-hydrogenases of algae and strict anaerobes: they have much higher tolerance to O_2 and resistant to various unfavorable environments.[47] On the other hand, algal H_2ases can reach very high specific activities that are much higher than those of cyanobacterial hydrogenases but they are very oxygen sensitive.[22]

Therefore it is important to develop strategies for reducing the O_2 sensitivity. For example, it is possible to engineer an algal [FeFe]-hydrogenase resistant to O_2 inactivation or introduce a gene encoding for a [NiFe]-hydrogenase with increased resistance into photosynthetic cyanobacterial cells.

The processes of H_2 photoproduction based on using cyanobacteria and other cell cultures demonstrated relatively low conversion efficiencies.[2] Besides, H_2 production can be improved by the mutants with reduced antenna size for decreasing heat losses and fluorescence[29,31] and effective redirecting of H^+ and electron fluxes to their corresponding H_2-producing enzymes (Fig. 1). The theory predicts that solar light to H_2 photon conversion efficiency of 10% can be reached.[8]

Role of photosystems in H_2 photoproduction

Besides activity of PSI, at least some activity of PSII is required to sustain the H_2 photoproduction. This is in line with recent observations on the use of inhibitors.[48] This last study indicated that the vast majority of the electrons driving H_2 production originates from water oxidation. The effect of progressive impairment of PSII photochemical activity in sulfur-deprived *C. reinhardtii* D1-R323 also demonstrated the progressive decrease in O_2 evolution and

activity and loss of photochemical activity of PSII.[49] The mutants exhibited a lower H_2 yield compared to the wild type.

An interesting problem is the direct evolution of H_2 by PSII. Earlier studies have demonstrated that H_2 can be produced from PSII under certain conditions, both in mutants lacking PSI[50] and preparations of PSII.[51] The wild type and mutants, lacking PSII, of green alga of *Chlamydomonas reinhardtii* produced H_2 with high efficiency, but a mutant lacking PSI demonstrated low efficiency in H_2 production.[50] Conversely, subchloroplast preparations enriched in PSII in the presence of an electron donor TMPD exhibited higher H_2 evolution rates (up to 30 nmol per mg Chl per h) than preparations enriched in PSI under the same conditions.[51] It is interesting that H_2 photoproduction was stimulated 10-fold after removal of manganese (by tris-treatment) from PSII and this reaction was suppressed by DCMU (5 μM), dinoseb (10 μM), atrazine (10 μM), *o*-phenanthroline (10 μM) or CO (0.4%).[51] The data on the suppression of H_2 evolution by well-known inhibitors of PSII (DCMU, dinoseb, atrazine) proved that the H_2 photoproduction is sensitized by the reaction center of PSII. Moreover, it has been shown that the mid-point redox-potential of the intermediate electron acceptor of PSII, pheophytin (Pheo), is –0.61 V.[52] Theoretically, this potential is sufficient to allow PSII to photoreduce electron acceptors with redox-potential of *ca.* –0.4 V (ferredoxin, $NADP^+$, methylviologen, benzylviologen, NO_2^-, NO_3^-, SO_4^{2-}, *etc.*) typical for PSI, and photoreduction of H_2 (–0.42 V).[53]

These results demonstrate that theoretically, isolated PSII can produce H_2 under sun light. However, the detailed characterization and application of this unique approach of H_2 photoproduction by PSII should be a subject of research in near future.

Artificial systems

Catalysts for H_2 production: overview

The concept of artificial photosynthesis for H_2 production is illustrated in Fig. 2. Due to the constraint of space, we will focus on the most important topic: the catalysts for H_2 production.[54]

The classical, and still the most efficient, catalyst for H_2 production is Pt. It has almost all the requisites of catalysts for the hydrogen economy: low overpotential, high reaction rate, good electron capacity and conductivity, and high chemical and mechanical stability. As such, it is the ideal catalyst for laboratory research. However, when it comes to industrial applications, its high cost and limited quantity are serious shortcomings. Much researches is under way to find reasonable alternatives to Pt metal catalysts.

The known H_2 catalysts can be roughly classified into three categories: those based on precious metals (Pt, Rh, Ir), on common metals (Co, Ni, Fe), and those related to (and/or inspired by) the natural enzyme H_2ase. These classifications are by no means rigorous, and there are significant overlaps between them. Nevertheless, such classification will be beneficial for us to understand the current status of this fast-growing research area.

H_2 catalysts with precious metals

Rhodium(III) polypyridine complexes, initially used as electron carriers for H_2 production (together with Pt metal catalysts), were later found to be capable of generating hydrogen without Pt.[55] Rhodium porphyrins are also known to catalyze electrochemical generation of H_2.[56] Iridium(III) complexes have also been used for photoproduction of H_2.[57]

Recently, covalent assembly of a rhodium center and a ruthenium photosensitizer gave rise to better performance of rhodium-based photoproduction of H_2.[58] Actually, the first example of such a "combined" photocatalyst was based on Pt for hydrogen evolution and Ru as photosensitizer.[59]

Although these catalysts are quite interesting from the viewpoint of coordination chemistry, they share with Pt catalysts the problem of high cost and limited availability (both Rh and Ir are even less abundant than Pt in the Earth's crust). It is not likely that these catalysts will become of practical importance, unless some compounds with extremely high activity are discovered.

H_2 catalysts with common metals

Among the more common metals in the first-row transition elements, Fe, Co and Ni have shown promising results as H_2 catalysts. These are reasonable choices: Fe and Ni are known as active metals in H_2ases, and Co belongs to the same family in the periodic table as Rh and Ir, which are active metals for H_2 production as we saw above.

Like Rh complexes, cobalt complexes are often used as electron carriers, however they are also useful for production of H_2.[60] A BF_2-bridged diglyoxime Co complex produces H_2 electrochemically at potentials of –0.28 V *vs.* SCE, which is one of the most positive (*i.e.* the least energy-demanding) potentials reported for complex catalysts.[15] Covalent assembly of a ruthenium photosensitizer and a cobalt complex is also reported.[61]

Macrocyclic complexes of iron[62] and nickel[63] have been used for photochemical or electrocatalytic H_2 production. These metals are often related to the active center of H_2ase (see below), however a simple mononuclear Fe(I) complex can be active for H_2 production.[64]

The difficulty of handling coordination compounds of first-row transition metals lies in their high susceptibility towards ligand exchange, particularly in aqueous solutions. To utilize the inherent power of these elements, it is necessary to design the coordination environment carefully, thereby improving the stability and controlling the reactivity.

Hydrogenase and related synthetic compounds

Hydrogenase enzymes depend on the cooperation of two metal centers in their active sites (Fe_2 or Fe–Ni) to produce H_2. Recent progress in researches on the structures and function of the H_2ases has been provided by X-ray analysis, spectroscopic techniques, theoretical methods, and model studies.[65]

As for the synthetic model studies, much attention has been focused on the active sites of the Fe-only H_2ases, which feature a bimetallic iron center bridged with a dithiolate and have CO/CN auxiliary ligands.[66] On the other hand, the synthetic models of [NiFe]-hydrogenases are more difficult to prepare. By use of Ru in place of Fe (Ru is electronically similar to but more robust than Fe), a Ni–Ru complex with a bridging hydride ligand was successfully isolated.[17]

The Fe-only H_2ases catalyze the reduction of protons to H_2 with almost zero overpotential.[67] On the other hand, the synthetic dinuclear compounds still require large negative overpotentials (–0.4 to –1.4 V). There are theoretical studies to clarify the detailed mechanism of H_2ase function,[68] and zero overpotential of H_2ase is claimed to be reproducible by computation.[69] Such attempts will be helpful for the design of new model complexes with better performance.

Towards the future

Research of artificial biomimetic photosynthesis for photoproduction of H_2 is still in its infancy and has not yet reached to the stage where large-scale practical application is feasible. Nevertheless, there has been significant progress in various aspects in this area. Towards the future, there should be continuous efforts in the development of synthetic catalysts for H_2/O_2 production using water splitting, photochemical conversion machinery for controlled electron transfer, and light-harvesting units. Even more important is to integrate these components into functional assemblies, which is likely to be realized with the aid of profound understanding of the structural/functional features of biological systems.

Conclusions

Both the natural and biomimetic photosynthetic processes are efficient and cost-effective for water splitting, and H_2 production.

The actual photoproduction of hydrogen will have to be carried out in a sealed photobioreactor, and also requires careful reactor designs[70] for the substantial improvements of hydrogen production rates and yields. A prerequisite challenge is to improve current systems at the biochemical level so that they can generate hydrogen at a rate, and approach the 10% energy efficiency, which has been already surpassed in photoelectrical systems.[71,72]

Currently and in the near future, researchers could focus on increasing the O_2 tolerance of [FeFe]-hydrogenases and the use of immobilized microbial cultures to reach this target, as these methods are promising. Reduced antenna size and increased PQ pool and decreased PSI cyclic electron transport, as well as enhanced resistance to environmental stress conditions, should be considered for the improvement of photohydrogen production. These studies will guide further molecular engineering research improving the efficiency of hydrogen bioproduction.

Thus, research is needed to understand the diversity and capacity of natural hydrogen production systems and to optimize their utilization in H_2 production processes.

Abbreviations

AP	Artificial photosynthesis
DCMU	3-(3′,4′-Dichlorophenyl)-1,1-dimethylurea
Fd	Ferredoxin
H_2ase	Hydrogenase
PQ	Plastoquinone
PSII	Photosystem II
PSI	Photosystem I
TMPD	*N*,*N*,*N*′,*N*′-Tetramethyl-*p*-phenylenediamine

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