Bioengineering Approaches for Hydrogen Production

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Hydrogen with high energy content is considered to be a promising alternative clean energy source. Biohydrogen production through microbes provides a renewable and immense hydrogen supply by utilizing raw materials such as inexhaustible natural sunlight, water, and even organic waste, which is supposed to solve the two problems of “energy supply and environment protection” at the same time. Hydrogenases and nitrogenases are two classes of key enzymes involved in biohydrogen production and can be applied under different biological conditions. Both the research on enzymatic catalytic mechanisms and the innovations of enzymatic techniques are important and necessary for the application of biohydrogen production.

biohydrogen production hydrogenases nitrogenases

1. Introduction

A deep understanding of the hydrogen metabolism and regulation is necessary to conduct genetic engineering for enhancing hydrogen production. Hydrogenases are widespread in microbes and they are involved in various metabolic pathways such as methane formation pathway, nitrogen fixation with nitrogenase-hydrogenase co-regulation, remediation of toxic heavy metals, and the virulence of pathogenic bacteria and parasites [1]. Different from the hydrogenases involved in many different metabolic pathways, nitrogenases are responsible for converting dinitrogen into ammonia in biological nitrogen fixation, providing a nitrogen source for microorganisms [2].

Extensive research on molecular mechanisms and physiological functions of hydrogenases and nitrogenases has paved new ways for enzyme engineering to improve biohydrogen production efficiency. In this section, six different bioengineering strategies will be discussed.

2. Improvement of O_2 Tolerance

O_2 is an important regulating factor in anaerobic hydrogen production. Both nitrogenases and hydrogenases are sensitive to O_2. Nitrogenases must be manipulated under anaerobic conditions and their mechanisms of O_2 inactivation are possibly oxidative damage of metalloclusters [3]. A total of 5–10% of cells in many filamentous cyanobacteria are specially differentiated cells which are called heterocysts, and they can isolate nitrogenases and provide a microaerobic environment for hydrogen production from protons [4].

Hydrogenases have different O_2 sensitivities and need to either be protected from or tolerate the presence of O_2 during photosynthetic hydrogen production.

A few O_2-tolerant hydrogenases exist in nature. Natural O_2-tolerant [FeFe] hydrogenase was found in *Clostridium bjerinckii* SM10 (CbA5H) [5]. Three different O_2-tolerant [NiFe] hydrogenases from *Ralstonia eutropha* were found but they have lower enzyme activity than O_2-sensitive hydrogenases [6]. Other O_2-tolerant [NiFe] hydrogenases were also found in *Aquifex aeolicus*, *Escherichia coli*, and *Desulfovibrio fructosovorans* [7][8]. [NiFe] hydrogenase *Ko*Hyd3 purified from *Klebsiella oxytoca* HP1 displayed remarkable O_2 tolerance and exhibited substantial hydrogen evolving activity under 10–20% O_2 in the gas phase [9].

Studies on the O_2-tolerant membrane-bound hydrogenase showed that O_2 resistance originates from its unusual redox properties and kinetic behavior. The proximal iron–sulfur cluster located in [NiFe] hydrogenases contains an unusual [4Fe–3S]–6Cys cluster with two more cysteine residues compared to the standard [4Fe–4S]–4Cys cluster, which can transmit two electrons and may be responsible for regulating unusual redox potentials [10]. The mechanism of O_2 tolerance is complex and inter-domain electron transfer between the distal clusters is proposed to be one way of increasing the O_2 tolerance of [NiFe] hydrogenase [11]. In O_2-tolerant [FeFe] hydrogenase, the O_2 sensitivity of the H-cluster is strongly influenced by the protein environment and can be reversibly converted from the active state into the inactive state [5].

Limiting the diffusion of O_2 to the active site is an alternative approach for O_2 tolerance enhancement. Since the active site is located inside the [NiFe] hydrogenases and connected to the protein surface through a hydrophobic channel, the diffusion of O_2 to the active site can be limited by reducing the gas channel size at the cavity interface with the active site. Two conserved hydrophobic residues (Val and Leu) are located at the end of the hydrophobic channel in O_2-sensitive [NiFe] hydrogenases and they are replaced by larger Ile and Phe in O_2-tolerant [NiFe] hydrogenases [12]. [FeFe] hydrogenase CpI was engineered in combination with cell-free mutant screening for improving O_2 tolerance, and M4 mutant $CpI^{N160DI197VA280VN289D}$ showed higher O_2 tolerance than the wild-type CpI. After exposure to 1% O_2 for 5 min, the wild-type CpI retained only 23% activity while the M4 mutant retained 62% [13]. Using rational mutant libraries of *Clostridium* [FeFe] hydrogenase, $CpI^{T356V/S357T}$ was identified to be the most O_2-tolerant variant and has an equivalent aerobic hydrogen production rate in the presence of 5% O_2 [14]. Enzyme engineering was shown to be a feasible tool for upscaling biohydrogen production, and meanwhile, the cellular context is also considered to be of great importance [15].

3. Immobilization Technology

Hydrogenase has a high catalytic conversion rate and a low overpotential under mild conditions and has potential applications in replacing Pt as an electrocatalyst to develop hydrogen biofuel cells [16]. Immobilization technology can help hydrogenase to be reusable, maintain its stability and catalytic activity on the electrode surface, and improve electron-transfer efficiency [17].

The design of novel nanostructured electrodes for enzyme fixing can facilitate direct electron transfer between enzymes and solid carriers, thereby alleviating the demand for enzymes as electronic media and making biotechnological applications such as biofuel cells and biosensors simpler [16]. Covalent immobilization of [NiFe] hydrogenases onto SAM-modified gold surfaces makes enzymatic electrodes relatively stable, the rate of electron transfer increased, and redox mediators were not required [18].

Nanomaterials can be used to promote electron transfer efficiency. The study on the immobilization of Fd-HydA1 on black TiO_2 nanotubes (bTNTs) found that direct electron transfer happened between black TiO_2 and Fd-HydA1 [19]. The effect of molecular weight on the catalytic and electrochemical properties of hydrogenase was investigated by fixing truncated enzymes ($Pf_{\alpha\delta}$ and Pf_{α}, containing the subunits αδ and the α subunit only, respectively) derived from a four-subunit (αβγδ) [NiFe] hydrogenase *Pf*SHI to multiwalled carbon nanotubes (MWCNTs), and results showed that $Pf_{\alpha\delta}$ with a shortened distance between the electrode and enzymes exhibited a higher electron transfer rate than *Pf*SHI [20].

4. Modification of Nitrogenase Substrate Selectivity

The electron flux through nitrogenases is largely independent of substrates being reduced, implying all nitrogenase substrates including N_2 (for BNF), acetylene (to produce ethylene), and protons (for BHP) can compete for the same pool of electrons effectively [21].

Because hydrogen production by nitrogenases is independent of N_2 reduction, the replacement of N_2 by Ar can enhance the electron flux to proton reduction and keep away from N_2 reduction. This method is effective to produce only H_2, but the higher operational cost may follow [22].

Mutagenesis provides an alternative approach to overcome the N_2 competition. The catalytic FeMo cofactor has been identified to provide substrate reduction sites, so a lot of research works had focused on its structure, its reactivity, and the development of genetic strategies for altering the substrate selectivity of dinitrogenase. Nonpolar or bulky residues such as Val, Phe, and Trp have functions in substrate access, substrate binding, and FeMo cofactor positioning. All of them constitute an important regulated network for enzymatic function, providing target sites in engineering [23].

Several valine substitutions in the α subunit of dinitrogenase have been intensively studied. The α-70Val site is predicted to have effects on the access of substrates to the active site. The substitution of V70A allows larger substrates, and V70I is just the opposite with the ability to block the access of acetylene and N_2 to the active site except for protons [24][25]. Both hydrophilic and hydrophobic channels were supposed to be accessible for substrates to reach the buried active site. The α-71Val site conserved in Mo-nitrogenases is predicted to be in the hydrophobic channel and α-75Val is directly near the active site. Both α-71 and α-75 sites affect substrate specificity and modification of α-70Val, α-71Val, and α-75Val can result in higher hydrogen production [26][27].

5. Enzyme Compartmentalization

Bacterial microcompartments (BMCs) are found in a broad range of bacteria. They are self-assembled and functional analogs of eukaryotic organelles. A BMC is composed of an outer selectively permeable protein shell and an enzymatic core which performs a specific metabolic process and contributes to the functional diversity of BMCs [28]. BMCs can serve as physical barriers to protect cargo enzymes inside and provide a natural microenvironment for enhancing catalytic performance. BMCs have many potential applications for providing functional compartmentalization within cells to synthesize non-native metabolites or to deliver medical molecules [29].

Carboxysomes are anabolic BMCs and are found in all cyanobacteria and some chemoautotrophic bacteria. They have a proteinaceous icosahedral outer shell of roughly 800 to 1400 angstroms in diameter and they house enzymes involved in carbon fixation [30]. Carboxysome is prospective to be engineered for constructing nanoreactors. Carboxysome protein-encoding genes were expressed and self-assembled into robust carboxysome shells in *E. coli*. The empty shells were proved to have the capacity of encapsulating catalytic [FeFe] hydrogenases and functional partners together to create nanoreactors for hydrogen production and the O_2 tolerance of the enzymes was improved at the same time [31].

6. Metabolic Engineering

Metabolic engineering provides a very promising strategy to improve hydrogen yield by redirecting biochemical pathways. Genetic engineering approaches have been used in both natural hydrogen-producing strains such as green microalgae and cyanobacteria and model organisms such as *E. coli* to favor hydrogen production. Genetically modified strains with a higher ability to generate biohydrogen have been successfully constructed [32] [33].

7. Artificial Hydrogenases

Studies on molecular structures and catalytic mechanisms of diverse enzymes involved in biohydrogen production inspired researchers to develop novel catalysts as artificial hydrogenases and construct more stable and efficient catalytic systems for hydrogen gas generation.

The metal center substitution was used to prepare the first artificial hydrogenase nickel-substituted rubredoxin (NiRd) with a structure of a mononuclear Ni ion coordinated by four cysteine residues, and the artificial hydrogenase showed a capability of catalyzing hydrogen evolution [34]. In heme-binding proteins, the native cofactor iron protoporphyrin IX was replaced by cobaltous protoporphyrin IX (CoP) and the replacement resulted in modest catalysts for proton reduction to produce hydrogen. In addition, the CoP–myoglobin system showed strong O_2-tolerant catalytic behavior [35]. Cobaloxime catalysts and electron transfer proteins with light-harvesting properties, such as ferredoxins and apo-flavodoxins, can efficiently self-assemble and provide the photocatalytic ability for proton reduction.

Many other efforts have been made to design various cofactors potentially to be integrated into [FeFe] hydrogenases. Both iron atoms were replaced with the non-native metallic element ruthenium and this [RuRu] analog of [FeFe] hydrogenase has the advantage to trap the key hydride intermediate state which is transient for [FeFe] hydrogenase. The stability of ruthenium hydrides can provide deep insight into the [FeFe] hydrogenase catalytic mechanism. In the analog, the catalysis reaction cannot proceed because the ruthenium atoms in the hydride intermediate state are redox-inactive [36]. In another study, the element nitrogen located at the bridgehead of the bridging dithiolate was substituted with its homolog phosphorous. Three new phosphorous-based [FeFe] hydrogenase mimics were synthesized by reacting $(HSCH_2)_2P(O)R$ (R = Me, OEt, OPh) with $Fe_3(CO)_{12}$ and showed that the phosphorous could be reduced which may potentially improve catalytic activity regarding hydrogen evolution reaction [37]. The diiron carbonyl compounds with aromatic dithiolate bridges were also used to mimic the catalytic site of [FeFe] hydrogenase. They are robust and readily reducible, and aromatic dithiolate bridges are helpful for catalytic intermediate stabilization, molecular engineering, assembly with functional materials, and so on. These mimics exhibited relatively positive potentials as effective catalysts for electro- or photochemical hydrogen production [38].

The H-cluster of all [FeFe] hydrogenases consists of a cubane-like [4Fe–4S] cluster and a [2Fe–2S] cluster with additional CO and CN ligands and bridged by an azadithiolate (ADT) [37][39]. As the homogeneity and simplicity of the [2Fe–2S] sub-cluster and crystal structures of [FeFe] hydrogenases were determined, many synthetic cofactors were developed to replace the active site of [FeFe] hydrogenase and biohybrid systems were also investigated by using synthetic diiron carbonyl moieties and non-hydrogenase protein matrixes [40]. The synthetic $[(\mu\text{-}S_2)Fe_2(CO)_6]$ motif can mimic the [2Fe] subsite of [FeFe] hydrogenases and provide evident photo-induced hydrogen production under photocatalytic conditions, which is valuable for designing noble metal-free catalysts for electrochemical hydrogen production. Due to the poor water solubility of $[(\mu\text{-}S_2)Fe_2(CO)_6]$, a bioactivated [FeFe] hydrogenase mimic with two pyrene moieties was further prepared and integrated on multi-walled carbon nanotube (MWNT)-based electrodes through π-interactions to provide remarkable stability and activity in electrocatalytic hydrogen production under aqueous conditions [41]. The active site mimics also have potential applications for hydrogen fuel cells with more economical materials. Model compounds can be introduced into an environment to help them maintain catalytic effectiveness and stability. Successful examples include a covalent attachment to polymer backbones and oligopeptide chains, encapsulated in peptide hydrogels and micelles [42][43][44].

The “iron–sulfur world” theory proposes the FeS/H_2S pair as the origin of life and a potential ancestor of [FeFe] hydrogenase [45][46][47]. Compartmentalization is considered to form an autocatalytic inorganic metabolic system to fulfill the requirements of life development. A membrane-bound [FeFe] hydrogenase model was prepared, and this vesicular system can exhibit catalytic action under particular conditions. This [FeFe] hydrogenase compartmentalization system can be applied as a minimal cell model or a nanoreactor to generate hydrogen [47].

Diiron complexes as mimic [FeFe] hydrogenase have been successfully immobilized on a metal–organic framework (MOF) or mesoporous silica (MS). Periodic mesoporous organosilica (PMO) with thiol groups (SH-PMO) was also developed for artificial [FeFe] hydrogenase anchoring for turnover number (TON) improvement [48].

Tremendous research works have been conducted to develop synthetic systems to mimic natural enzyme reactivity and even obtain enhanced stability and more remarkable catalytic activities. Undoubtedly, challenges to developing economical hydrogen production systems remain, and the efforts of understanding enzymes and their applications will continue.

References

1. Vignais, P.M.; Billoud, B. Occurrence, classification, and biological function of hydrogenases: An overview. Chem. Rev. 2007, 107, 4206–4272.
2. Sickerman, N.S.; Hu, Y.; Ribbe, M.W. Nitrogenases. In Metalloproteins: Methods and Protocols; Hu, Y., Ed.; Springer: New York, NY, USA, 2019; pp. 3–24.
3. Einsle, O.; Rees, D.C. Structural enzymology of nitrogenase enzymes. Chem. Rev. 2020, 120, 4969–5004.
4. Golden, J.W.; Yoon, H.S. Heterocyst development in Anabaena. Curr. Opin. Microbiol. 2003, 6, 557–563.
5. Morra, S.; Arizzi, M.; Valetti, F.; Gilardi, G. Oxygen stability in the new -hydrogenase from Clostridium beijerinckii SM10 (CbA5H). Biochemistry 2016, 55, 5897–5900.
6. Burgdorf, T.; Lenz, O.; Buhrke, T.; van der Linden, E.; Jones, A.K.; Albracht, S.P.J.; Friedrich, B. -hydrogenases of Ralstonia eutropha H16: Modular enzymes for oxygen-tolerant biological hydrogen oxidation. J. Mol. Microbiol. Biotechnol. 2005, 10, 181–196.
7. Lukey, M.J.; Parkin, A.; Roessler, M.M.; Murphy, B.J.; Harmer, J.; Palmer, T.; Sargent, F.; Armstrong, F.A. How Escherichia coli is equipped to oxidize hydrogen under different redox conditions. J. Biol. Chem. 2010, 285, 3928–3938.
8. Topin, J.; Diharce, J.; Fiorucci, S.; Antonczak, S.; Golebiowski, J. O2 migration rates in hydrogenases. A joint approach combining free-energy calculations and kinetic modeling. J. Phys. Chem. B 2014, 118, 676–681.
9. Wu, X.; Liang, Y.; Li, Q.; Zhou, J.; Long, M. Characterization and cloning of oxygen-tolerant hydrogenase from Klebsiella oxytoca HP1. Res. Microbiol. 2011, 162, 330–336.
10. Liebgott, P.P.; Dementin, S.; Leger, C.; Rousset, M. Towards engineering O2-tolerance in hydrogenases. Energy Environ. Sci. 2011, 4, 33–41.
11. Wulff, P.; Thomas, C.; Sargent, F.; Armstrong, F.A. How the oxygen tolerance of a -hydrogenase depends on quaternary structure. J. Biol. Inorg. Chem. 2016, 21, 121–134.
12. Volbeda, A.; Montet, Y.; Vernede, X.; Hatchikian, E.C.; Fontecilla-Camps, J.C. High-resolution crystallographic analysis of Desulfovibrio fructiosovorans hydrogenase. Int. J. Hydrog. Energy

2002, 27, 1449–1461.

13. Bingham, A.S.; Smith, P.R.; Swartz, J.R. Evolution of an hydrogenase with decreased oxygen sensitivity. Int. J. Hydrog. Energy 2012, 37, 2965–2976.
14. Koo, J.; Swartz, J.R. System analysis and improved hydrogenase O2 tolerance suggest feasibility for photosynthetic H2 production. Metab. Eng. 2018, 49, 21–27.
15. Elman, T.; Schweitzer, S.; Shahar, N.; Swartz, J.; Yacoby, I. Engineered clostridial -hydrogenase shows improved O2 tolerance in Chlamydomonas reinhardtii. Int. J. Hydrog. Energy 2020, 45, 30201–30210.
16. Rudiger, O.; Gutierrez-Sanchez, C.; Olea, D.; Pereira, I.A.C.; Velez, M.; Fernandez, V.M.; De Lacey, A.L. Enzymatic anodes for hydrogen fuel cells based on covalent attachment of Ni-Fe hydrogenases and direct electron transfer to SAM-modified gold electrodes. Electroanalysis 2010, 22, 776–783.
17. Liu, J.; Wu, W.J.; Fang, F.; Zorin, N.A.; Chen, M.; Qian, D.J. Immobilization of hydrogenase on carbon nanotube polyelectrolytes as heterogeneous catalysts for electrocatalytic interconversion of protons and hydrogen. J. Nanopart. Res. 2016, 18, 220.
18. Reddy, K.R.; Hassan, M.; Gomes, V.G. Hybrid nanostructures based on titanium dioxide for enhanced photocatalysis. Appl. Catal. A 2015, 489, 1–16.
19. Liu, X.; Risbakk, S.; Carvalho, P.A.; Yang, M.Y.; Backe, P.H.; Bjoras, M.; Norby, T.; Chatzitakis, A. Immobilization of FeFe-hydrogenase on black TiO2 nanotubes as biocathodes for the hydrogen evolution reaction. Electrochem. Commun. 2022, 135, 107221.
20. Wang, Y.M.; Song, Y.H.; Ma, C.L.; Xia, H.Q.; Wu, R.R.; Zhu, Z.G. Electrochemical characterization of a truncated hydrogenase from Pyrococcus furiosus. Electrochim. Acta 2021, 387, 138502.
21. Hageman, R.V.; Burris, R.H. Kinetic studies on electron transfer and interaction between nitrogenase components from Azotobacter vinelandii. Biochemistry 1978, 17, 4117–4124.
22. Benemann, J.R.; Weare, N.M. Hydrogen evolution by nitrogen-fixing Anabaena cylindrica cultures. Science 1974, 184, 174–175.
23. Stripp, S.T.; Duffus, B.R.; Fourmond, V.; Leger, C.; Leimkushler, S.; Hirota, S.; Hu, Y.L.; Jasniewski, A.; Ogata, H.; Ribbe, M.W. Second and outer coordination sphere effects in nitrogenase, hydrogenase, formate dehydrogenase, and CO dehydrogenase. Chem. Rev. 2022, 122, 11900–11973.
24. Mayer, S.M.; Niehaus, W.G.; Dean, D.R. Reduction of short chain alkynes by a nitrogenase α-70Ala-substituted MoFe protein. J. Chem. Soc. Dalton Trans. 2002, 4, 802–807.
25. Barney, B.M.; Igarashi, R.Y.; Dos Santos, P.C.; Dean, D.R.; Seefeldt, L.C. Substrate interaction at an iron-sulfur face of the FeMo-cofactor during nitrogenase catalysis. J. Biol. Chem. 2004, 279,

53621–53624.

26. Igarashi, R.Y.; Seefeldt, L.C. Nitrogen fixation: The mechanism of the Mo-dependent nitrogenase. Crit. Rev. Biochem. Mol. Biol. 2003, 38, 351–384.
27. Weyman, P.D.; Pratte, B.; Thiel, T. Hydrogen production in nitrogenase mutants in Anabaena variabilis. FEMS Microbiol. Lett. 2010, 304, 55–61.
28. Kerfeld, C.A.; Aussignargues, C.; Zarzycki, J.; Cai, F.; Sutter, M. Bacterial microcompartments. Nat. Rev. Microbiol. 2018, 16, 277–290.
29. Frank, S.; Lawrence, A.D.; Prentice, M.B.; Warren, M.J. Bacterial microcompartments moving into a synthetic biological world. J. Biotechnol. 2013, 163, 273–279.
30. Tanaka, S.; Kerfeld, C.A.; Sawaya, M.R.; Cai, F.; Heinhorst, S.; Cannon, G.C.; Yeates, T.O. Atomic-level models of the bacterial carboxysome shell. Science 2008, 319, 1083–1086.
31. Li, T.P.; Jiang, Q.Y.; Huang, J.F.; Aitchison, C.M.; Huang, F.; Yang, M.R.; Dykes, G.F.; He, H.L.; Wang, Q.; Sprick, R.S.; et al. Reprogramming bacterial protein organelles as a nanoreactor for hydrogen production. Nat. Commun. 2020, 11, 5448.
32. Khetkorn, W.; Baebprasert, W.; Lindblad, P.; Incharoensakdi, A. Redirecting the electron flow towards the nitrogenase and bidirectional Hox-hydrogenase by using specific inhibitors results in enhanced H2 production in the cyanobacterium Anabaena siamensis TISTR 8012. Bioresour. Technol. 2012, 118, 265–271.
33. Nyberg, M.; Heidorn, T.; Lindblad, P. Hydrogen production by the engineered cyanobacterial strain Nostoc PCC 7120 ΔhupW examined in a flat panel photobioreactor system. J. Biotechnol. 2015, 215, 35–43.
34. Slater, J.W.; Shafaat, H.S. Nickel-substituted rubredoxin as a minimal enzyme model for hydrogenase. J. Phys. Chem. Lett. 2015, 6, 3731–3736.
35. Esmieu, C.; Raleiras, P.; Berggren, G. From protein engineering to artificial enzymes—Biological and biomimetic approaches towards sustainable hydrogen production. Sustain. Energy Fuels 2018, 2, 724–750.
36. Sommer, C.; Richers, C.P.; Lubitz, W.; Rauchfuss, T.B.; Reijerse, E.J. A analogue of an -hydrogenase traps the key hydride intermediate of the catalytic cycle. Angew. Chem. Int. Ed. 2018, 57, 5429–5432.
37. Wittkamp, F.; Boydas, E.B.; Roemelt, M.; Apfel, U.P. New phosphorous-based -hydrogenase models. Catalysts 2020, 10, 522.
38. Gao, S.; Liu, Y.; Shao, Y.D.; Jiang, D.Y.; Duan, Q. Iron carbonyl compounds with aromatic dithiolate bridges as organometallic mimics of hydrogenases. Coord. Chem. Rev. 2020, 402, 213081.

39. Land, H.; Senger, M.; Berggren, G.; Stripp, S.T. Current state of -hydrogenase research: Biodiversity and spectroscopic investigations. ACS Catal. 2020, 10, 7069–7086.
40. Wang, M.; Chen, L.; Li, X.Q.; Sun, L.C. Approaches to efficient molecular catalyst systems for photochemical H2 production using -hydrogenase active site mimics. Dalton Trans. 2011, 40, 12793–12800.
41. Zamader, A.; Reuillard, B.; Pecaut, J.; Billon, L.; Bousquet, A.; Berggren, G.; Artero, V. Non-covalent integration of a -hydrogenase mimic to multiwalled carbon nanotubes for electrocatalytic hydrogen evolution. Chem. Eur. J. 2022, 28, e202202260.
42. Quentel, F.; Passard, G.; Gloaguen, F. A binuclear iron-thiolate catalyst for electrochemical hydrogen production in aqueous micellar solution. Chem. Eur. J. 2012, 18, 13473–13479.
43. Wright, J.A.; Webster, L.; Jablonskyte, A.; Woi, P.M.; Ibrahim, S.K.; Pickett, C.J. Protonation of -hydrogenase sub-site analogues: Revealing mechanism using FTIR stopped-flow techniques. Faraday Discuss. 2011, 148, 359–371.
44. Frederix, P.; Kania, R.; Wright, J.A.; Lamprou, D.A.; Ulijn, R.V.; Pickett, C.J.; Hunt, N.T. Encapsulating -hydrogenase model compounds in peptide hydrogels dramatically modifies stability and photochemistry. Dalton Trans. 2012, 41, 13112–13119.
45. Wächtershäuser, G. Before enzymes and templates: Theory of surface metabolism. Microbiol. Rev. 1988, 52, 452–484.
46. Wächtershäuser, G. On the chemistry and evolution of the pioneer organism. Chem. Biodivers. 2007, 4, 584–602.
47. Menzel, K.; Apfel, U.P.; Wolter, N.; Ruger, R.; Alpermann, T.; Steiniger, F.; Gabel, D.; Forster, S.; Weigand, W.; Fahr, A. -Hydrogenase models assembled into vesicular structures. J. Liposome Res. 2014, 24, 59–68.
48. Himiyama, T.; Waki, M.; Esquivel, D.; Onoda, A.; Hayashi, T.; Van der Voort, P.; Inagaki, S. A heterogeneous hydrogen-evolution catalyst based on a mesoporous organosilica with a diiron catalytic center modelling -hydrogenase. ChemCatChem 2018, 10, 4908–4913.