

Artificial models of iron hydrogenases for their potential use in the generation of molecular hydrogen: a mini-review

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Abstract- The mimicking of natural processes and the development of alternative methods to obtain clean fuels such as H₂, have been of great interest in recent years. In nature, the hydrogenase enzymes at some bacteria and algae are responsible of the fermentation and biophotolysis processes. This class of enzymes performs the reduction of hydronium ions to molecular hydrogen. Due to the composition of its active site, the hydrogenase enzymes are classified as: [Fe-Ni], [Fe-Fe] and [Fe] -hydrogenases. The study of its active centers has allowed inspiring model compounds that promote the advance in the development of efficient catalysts for the generation of hydrogen. In this review, we present some complexes that have been studied as models to imitate the active centers of the hydrogenases. Also, we analyze some electronic properties that they present. The most commonly used methods to mimic hydrogenases are structural, spectral or functional. Some of the common metals for this purpose are iron, nickel or cobalt, among others. In order to obtain an accurate model of a hydrogenase enzyme, several properties of the metals and ligands used for the generation of a metallic complex should be considered. These might promote some effects that could interfere with the electronic transfer and the subsequent redox process in the generation of molecular hydrogen.

Index Terms- Catalytic center, metal complex, hydrogenases, hydrogen production.

I. INTRODUCTION

Fuels have been essential elements in our civilization, it is estimated that by 2080 molecular hydrogen could be the main source of energy [1]. Currently, hydrogen is considered an alternative fuel, with potential that could decrease environmental damage by reducing the consumption of fossil fuels [2, 3]. In order to generate an environmentally and economically sustainable society, molecular hydrogen must be obtained by environmentally friendly methods and green technologies should be developed, for example the water splitting using sunlight[4]. In 1912 Giacomo Ciamician manifested the necessity to abandon the use of fossil fuels and theorized the generation of new technologies based on photochemical devices, with the potential to mimic the natural systems such photosynthesis [5]. This process is considered the most important reaction in biosphere, the advances in synthetic chemistry and the understanding of natural processes might allow the mimicking of biological processes and the possible generation of clean fuels such as H₂, using water as a raw material[6]. In nature, hydrogenase enzymes carry out the reduction reactions of hydrogen ions to generate molecular hydrogen. These enzymes inspire the design of catalysts for this reduction process [7]. In order to contribute to the understanding of the function, structure and promote the imitation of hydrogenase enzymes, several compounds with an analogous structure to the active center of this enzyme have been synthesized[8]. However currently, the search continues for compounds that can work as catalysts in the generation of molecular hydrogen[6]. A common strategy involves the modification of ligands to bind two metals and allow the synthesis of binuclear complexes [9]. In this review, we show some complexes that have been synthesized as models to mimic hydrogenases. Some of its advantages and disadvantages are analyzed, as well as its properties in the electron transfer reactions.

II. HYDROGENASES IN THE HYDROGEN GENERATION

A. Hydrogen production

Molecular hydrogen has a great potential for energy storage: the energy stored per mole of molecular hydrogen at 298K is $\Delta G^\circ = 237.2$ KJ/mol, considering its small mass, the capacity of hydrogen to store energy per gram is of 119000 J/g. When this value is compared to fossil fuels which provide 40,000 J/g, the energy value of molecular hydrogen is considered excessively high [10]. Also, it has the advantage of generating clean combustion, because when combined with oxygen, it produces heat, electricity and only water vapor as a by-product. This characteristic is considered one of the great attributes as an alternative energy source, but only if it is obtained by sustainable methods[11]. A slight disadvantage of hydrogen is its detonation limit in air, which is 4%, while that of butane is 1.86%, so the use of hydrogen may not be more dangerous than using natural gas[10].

Currently, the hydrogen production is carried out by methods of hydrocarbon reforming by steam or coal gasification. Both methods use fossil fuels, under high conditions of temperature and pressure, which promotes the generation of greenhouse gases [4]. There are some green technologies for the production of hydrogen, such as: the electrolysis of water by photovoltaic systems [9], the photolysis of water through the use of microorganisms [4], some other pioneering technologies that are still in development [12-14] and the artificial photosynthesis [9, 11]. The use of photovoltaic systems is initially very expensive, due to the cost of the equipment used [3]. The production of hydrogen through bacterial photosynthesis has some important barriers, such as inactivation by oxygen and light capture, among other [15]. Also, the microorganisms that have been studied, show a production of hydrogen in a very low rate [4]. On the other hand, artificial photosynthesis is considered one of the most promising alternatives for the production of hydrogen from water. Artificial photosynthetic systems must carry out key processes, such as photo-excitation and oxidation, which allows an electron to be generated and with this, a subsequent reduction of chemical species [8]. To achieve a design of artificial systems producing hydrogen, attention should be focused on chemical compounds that mimic the hydrogenase enzymes present in nature and consider the structure of metal clusters at the active site, where the generation of hydrogen takes place [15].

B. Hydrogenases

The molecular hydrogen production in microorganisms is linked to the activities of electron transport of its biogeochemical cycle [16]. Hydrogenase enzymes are present in microorganisms that incorporate hydrogen into their metabolisms, such as sulfate-reducing photosynthetic bacteria and some eukaryotic algae [7, 17]. Hydrogenase enzymes have diverse functions, mainly catalyze the conversion of hydrogen ions and electrons to molecular hydrogen [7], according to reaction (1):



The hydrogenase enzymes are classified according to the structure of its active center into three types: iron-iron hydrogenases or [FeFe]hydrogenases, nickel-iron hydrogenases or [NiFe]hydrogenases and iron hydrogenases or [Fe]hydrogenases [8]. The functions performed by the hydrogenase enzymes, depends on its site in cell where they are present, so many bacteria have two or more different hydrogenase enzymes. Also several bacteria have a single type of enzyme [NiFe]hydrogenases or [Fe]hydrogenases [7]. Most of the hydrogenases can catalyze the reaction (1) in vitro in any direction [7, 8, 17]. [FeFe]hydrogenases and [NiFe]hydrogenases perform as redox catalysts, while [Fe]hydrogenases catalyze the reversible heterolytic cleavage of H_2 [8] according to reaction (2):



In recent years, using studies of X-ray crystallography, spectroscopy and chemical modeling, different structures of hydrogenase enzymes have been characterized. This has allowed distinguishing the chemical structure in the three types of hydrogenases. Despite the spectral differences, the metal centers of the hydrogenase enzymes have structural and chemical analogies [7].

The three enzymes classes contain H_2 activating sites, around one iron unit coordinated with a carbonyl (CO) and sulfur atoms. The CO and cyanide (CN⁻) are structural elements of each active center and is the unique feature of the hydrogenase enzymes [8]. An important aspect which has generated great controversy, is the oxidation state of the metal atoms in the active center, the results in several studies are contradictory [8]. Some artificial models of hydrogenase enzymes that have been synthesized, show that their structure can be manipulated with a degree of predictability. Also several of its physical properties such as solubility, basicity and redox potential [18]. In addition, the development of several feasible method to generate the catalytic splitting of H_2O for the production of H_2 on a large scale have been proposed [8].

III. HYDROGENASE ARTIFICIAL MODELS

Several studies have reported diverse synthetic models to mimic the hydrogenase enzymes. Some of these compounds are based on the structural or spectral mimicking of the active site of the enzymes. For this purpose, some metal ions such as ruthenium, osmium, platinum, cobalt, iron and nickel have been used. Nevertheless iron and nickel are present in the active center of the enzymes, therefore they are the best candidates for participate in mimicking the structure of the active site [6].

A. [NiFe]hydrogenase artificial models

A crystallographic structure of the [NiFe]hydrogenase was obtained by isolating the enzyme from a strain of one reducing-sulfate bacterium: *Desulfovibrio vulgaris Miyazaki*. Its study showed that the metallic center NiFe, allows the stabilization of iron atom at the redox state +2 [19]. During the catalytic cycle, the nickel atom is the site where the reaction begins which changes for several oxidation states [20] which allows to the iron atom remain at oxidation state of +2 [8]. The studies carried out on these enzymes have been a key point for the understanding of structural and functional properties of the active center. Some of the electronic transfers in the metallic center can be reflected in the spectrophotometric studies of hydrogenase enzymes such the signal of maximum absorbance at 360 nm of hydrogenases isolated from a strain of *M. marburgensis* [21]. The electronic structure of the enzyme is related to its catalytic activity, this encourage the purpose to mimic the structure of the active-center of [NiFe]hydrogenases.

Several research groups have synthesized and characterized multiple compounds with structural analogies such as the bonding distances and geometric arrangement analogous to the metallic center of the enzyme[22] or synthesizing compounds with metal centers analogous to this enzyme, which incorporate an iron and a nickel atom. Also, it has been determined its activity in the molecular hydrogen generation, this has allowed increasing the understand of the mechanism of this enzyme[23]. The ligand is an essential constituent for the synthesis of these compounds, in several studies have reported that a substituted 2,2'-bipyridyl could provide the necessary characteristics for catalyzing the molecular-hydrogen generation reaction, by promoting structural and spectroscopic properties analogous to hydrogenase enzyme[23]. Some other compounds are used as ligands: 1,3,5,7-tetraphenyl-1,5-diaza-3,7-diphosphacyclooctane, substituted with aromatic rings or mercaptoimidazole. These compounds have been reported as models of the active-center of the enzyme, also the generation of molecular hydrogen has been catalyzed when a nickel complex has been coordinated with this ligand by phosphorus atoms, which induces a stability to the complex[24]. An additional complex studied is nickel(II)-bis(2-mercapto-1-methylimidazole)borate, which shows structural characteristics similar to [NiFe]hydrogenase with interesting redox properties [25]. In another study, residues of cysteine and phenylalanine coordinated to the metal center were used, the aromatic rings of the ligands promote a electronic transfer which catalyzes the reaction of reduction [26]. Finally, a binuclear nickel compound was studied, $[\text{Ni}_2(\text{L})(\text{MeCN})_2]^{3+}$ (L^{2-} macrocycle incorporating imine N and thiolate S donors) with a Ni(III) and Ni(II) centers, linked by a two thiolates bridge, also a nickel atom is coordinated with two acetonitrile molecules [27]. While several studies focused on the synthesis of diverse compounds, other studies focused on the electronic characterization of the active center or its model compounds. Several spectroscopic techniques such as UV-Vis and Raman spectroscopy were used to characterize two model complexes with a similar structure to the active-center of hydrogenase enzyme. One of these compounds with an iron atom bonded to a thiolate bridge shows peaks of visible spectrum at 400, 500 and 590 nm. A different compound has a hydrogen atom that performs as a bridge, shows absorption signals at 290, 350, 450 and 575 nm. The peaks were assigned to a charge transfer from nickel to the phosphine and thiolate ligands, while the other assignment corresponds to the transfer between iron and nickel atoms. Another signal that was found corresponds to a charge transfer from the thiolate ligand to the iron center [28]. In a different study, some nickel complexes with trimethylphosphine, phenylselenolate or ethanethiolate, were spectrally characterized. If phenylselenolate is coordinated, absorption bands at 590 and 620 nm are showed. On the other hand if a chloride ion is coordinated its signals changed to 954 and 990 nm, with ethanethiolate were observed bands of 577 and 920 nm [29]. Other exotic studies evaluated the effect of the ligand trimethylphosphine which has an electron donor-acceptor capacity [30]. Also, have been synthesized compounds with a Ni-Fe metal-center attached to a ferrocene complex [31]. There is great controversy about the redox inactivity of iron in the proposed mechanism of [NiFe]hydrogenases enzymes. In order to promote the redox activity that nickel has in the enzyme, some studies have synthesized complexes with a metallic center that contains only nickel[8].

B. [FeFe]hydrogenase artificial models

The [FeFe]hydrogenases enzymes have their active center conformed by a metallic cluster with two iron atoms [8], with a three-dimensional structure of square pyramid shaped, where both iron atoms are linked by a dithiolate bridge [32]. In addition to the metal cluster, the hydrogenases have an additional ferredoxin clusters: [4Fe-4S] and [2Fe-2S], which during the catalytic cycle performing as electron transfer centers [33]. Some studies about the mechanism of H_2 formation that occurs in [FeFe]hydrogenases have proposed that the first step for catalysis is the transfer of a first electron to the iron center. Subsequently, a second transfer carries out the electron to the ferredoxin center that is farthest from the di-iron center, so that the ferredoxin centers can store the second electron. Finally, the stored electrons are used for the reduction of hydrogen ions and generate molecular hydrogen [34-36]. During all steps of catalytic cycle, one of the two iron atoms of the metal center maintains its oxidation state of +2, while the other iron atom is constantly changing its oxidation state [37]. Other compounds promote a similar structure to the active-center of the enzyme using cyanide as a ligand to replace carbonyls [38] and pyridine which can generate molecular hydrogen [39]. Also, other compounds with structural characteristics different from the enzyme have been studied, using ligands such as dimethyl-azadithiolate [40], trimethylphosphine [41, 42]. It was found that due to the electrons delocalization a change in the oxidation states of the metal-centers occurs. Also, a model was synthesized with 3,6-dichlorobenzene-1,2-dithiolate and used in conjunction with a ruthenium photosensitizer, showing activity in the hydrogen generation [43]. A few complexes has been synthesized with more than two iron atoms, binding a di-iron compound with a [4Fe4S] cluster, which can be an aid to improve the understanding of the chemical processes that occur in the natural process [44, 45]. In another study, several tetra-nuclear iron compounds were synthesized with benzene-1,2,3,4-tetra-thiolato [46]. Spectral studies of the enzyme and its artificial models are a key for the understanding of the electronic structure and the catalytic mechanism for the molecular hydrogen generation. Some of the electronic transitions occurring in the enzyme active-center have been studied by UV-Vis methods. A [FeFe]hydrogenase enzyme was purified from *DesulfoVibrio desulfuricans*, showing absorption bands at 280 and 390 nm [45]. Some models that have been studied show absorption bands between 260 and 420 nm analogous to the enzyme. Using benzylamine and chitosan a compound was synthesized. It was determined that chitosan promotes a stabilization of the molecules linked to the metal center and increases the electrons transfer for the generation of H_2 . In addition, by spectrophotometric techniques were observed signals at 336 nm [44]. In another work, a compound was synthesized with the azadithiolate cofactor showing UV absorption bands at 330 nm, this system is able to use light to photocatalyze the hydrogen formation [47].

In another study, a compound was synthesized from three ether chains linked. In this compound were observed absorption bands at 350 nm and emission bands at 670 nm [48]. Also, a di-iron cluster linked to tetra-phenyl-porphyrin was synthesized, which shows a maximum absorption at 419 nm and emission at 655 and 719 nm. It is believed that these signals are due to the electrons transfer from the porphyrin to the di-iron metal-center [49]. The 7-mercapto-4-methylcoumarin was also used as ligand, in which showed absorption bands at 288 and 338 nm and emission at 370 nm. This spectral analysis indicated a photoinduced electron transfer of 7-mercapto-4-methylcoumarinligand to metal center [50]. Also, a dinuclear iron center linked with the photosensitizer *bis*-2,2'-bipyridyl-2-phenylpyridyl-iridium(I), is able of carrying out all process for the molecular hydrogen generation from water. This compound shows absorption bands between 260-320 nm attributed to $\pi-\pi^*$ transitions located in 2,2'-bipyridyl and 2-phenylpyridyl, other signals at 300-400 nm were assigned to metal to ligand charge transfer transitions [51].

C. [Fe]hydrogenase artificial models

Iron hydrogenases or [Fe]hydrogenases were discovered in 1990, in their structure, they have only iron atom in their active center [8]. Although [Fe]hydrogenases maintain analogies to [FeFe]- and [NiFe]hydrogenases, they are completely different. This was determined by mass spectroscopy and infrared spectroscopy studies [52]. In the iron hydrogenases, the generation of molecular hydrogen occurs in the active center which is an inverted site with a quadrangular pyramid structure, which allows the electron density to flow from the ferredoxin centers [32]. In 1972 a research group performed spectrophotometric studies on purified hydrogenases obtained from a strain of *Clostridium pasteurianum*, in this study were observed absorption bands at 340, 415 and 440 nm in oxidized-state and signals at 410 and 590 nm in reduced-state [53]. To evaluate the relationship between the enzyme spectrum and the active-center iron geometry were performed studies where the *A. Eutrophus* hydrogenase absorption spectrum was compared with other proteins that have iron components. It was found that the signals are similar to iron proteins without heme groups, showing maximum absorption at 380 and 420 nm, and emission at 526 nm [54]. Also, have been synthesized iron complexes with acyl, carbonyl and pyridine ligands, with structural analogies to those of enzyme in nature. Also, phosphine has been used as ligand, which induced a significant stability to the compound, in a greater proportion than N-donor ligands [55]. Song's group synthesized and characterized compounds similar to enzyme metal-center, linked to acylmethyl(hydroxymethyl)pyridine [56, 57]. They was observed that the geometry is similar to exists in the enzyme active-center [57]. Also, have been synthesized complexes with 2-pyridylamino-N,N-bis(2-methylene-4-methyl-6-tert-butylphenol) and 2-pyridylamino-N,N-bis(2-methylene-4-methoxyl-6-tert-butylphenol), which show absorption bands at 348 and 520 nm, these were attributed to a charge-transfer from ligands phenolate to iron(III) atom [58]. On the other hand, some studies with models that mimic the [Fe]hydrogenases, show the importance of the ligands linked to the metal center, which can induce link-up of the hydrogen ions to the metal center for its subsequent reduction [59]. In a similar study researches found that the use of π -acceptor ligands provide a strong interaction to the iron center [60]. Finally, it was observed that methyl thiolate compounds can be incorporated with iron and these which can improve the modeling of the active center of the enzyme [61].

D. Artificial models with other metal centers

To mimic the activity of hydrogenase enzymes in the generation of molecular hydrogen, additional to the use of iron and nickel, other metal ions such as ruthenium, rhodium, osmium and cobalt have been studied. The use of these transition metals have allowed to partially elucidate the mechanism of hydronium ion reduction to molecular hydrogen [17]. The first chemical model that mimics the hydrogenase enzyme reaction was a complex with ruthenium(III) reported by Harrod et al., in which multiple reactions are promoted with several changes in the ruthenium oxidation state, which bring to molecular hydrogen generation [62]. Other ruthenium complexes have been synthesized to obtain molecular hydrogen using 2-pyridyl, 2-N-methylimidazolyl, porphyrins and phosphines as ligands. It was observed that by using 2-pyridyl-2-N-methylimidazolyl is promoted the electrons transfer between metal centers [63]. With porphyrin, it was determined that the H₂ formation do not occur, possibly due to its rigid shape, which limits hydrogen binding to an only coordination site [64]. Also, has been analyzed the reactivity of two ruthenium complexes with 1,2-*bis*(dihydroxypropylphosphino)ethane and 1,2-*bis*(dihydroxymethylphosphino)ethane ligands [65]. A rhodium complex was synthesized with 1,2-*bis*[(2-mercapto-3,5-di-*tert*-butylphenylthio)ethane, which was studied to mimic the catalytic mechanism of hydrogenase enzymes, is considered the first compounds that promotes the understanding of the mechanism of these enzymes [66]. Also, have been synthesized osmium complexes with benzophenone-imine ligand, which has allowed studying the interaction of the metal atom with hydrogen atoms and its subsequent reduction to molecular hydrogen [67, 68]. On the other hand, the interaction between palladium and molecular hydrogen has been studied and it has been concluded that it can occurs from electronic delocalization and charge transfer in the complex. This allows to theorize the effect of metal complexes and hydrogen interactions and generation [69]. Since the 1980s, cobalt has been one of the most explored metal ions in the complexes formation that mimic the hydrogenases active center. Studies with this metal have focused in the hydronium reduction for the molecular hydrogen generation, some are able of generate molecular hydrogen with a low production rate. These systems show spectral characteristics similar to enzyme systems, with signals that appear between 230-350 nm and 420-470 nm.

Several ligands used in the cobalt complexes promote electronic transitions and wavelengths analogous to the nature systems, these ligands are: (difluoroboryl)dimethylglyoximato that shows absorption bands at 328 and 456 nm [70]; N,N-bis(2-pyridinylmethyl)-2,2'-bipyridine-6-methanamine, shows bands assign to $\pi-\pi^*$ transitions (247 y 300 nm), d-d transitions (420/470 nm) and other transitions at 337 nm [71]; 2,2'-bipyridyl [72], with absorption bands at 300 nm [73]; 2-*bis*(2-pyridyl)(methoxy)methyl-6-pyridylpyridine with absorption bands at 550 and 600 nm [74]; 2-acetylpyridine shows absorption bands due $\pi-\pi^*$ transitions (275 y 230 nm), d-d transitions (450 nm) and other electronic transfers at 310 nm [75]. Porphyrins are used also, showing absorption signals

at 407 and 526 nm [76]; using 1,1,1-*tris*(diphenylphosphinomethyl) [77], shows absorption bands at 680 nm [78]. By correlating the transitions with complexes stoichiometry can be an aid to know the electronic structure and the metal centers geometry [79]. Also, it is important to consider the properties of the ligand coordinated to the metal-center, for example, the ligand: 2-bis(2-pyridyl)(methoxy)methyl-6-pyridylpyridine can stabilize the complex in aqueous solutions [74]. On the other hand, ligands such diimines and pyridines are used due to their properties to stabilize the complex and generate a electronic delocalization[80]. This allows a high electron transfer to the ligands rings, promoting stability to the complexes, with metal ions that have d-orbitals, this is probably the result of the ability to form π -bonds by overlapping d full orbitals and π^* empty orbitals of ligand ring [79]. Although most complexes have been used individually, some studies report the simultaneous use of a photo-sensitizer with metal ions such iridium or ruthenium. One of the most prominent systems involves cobalt and ruthenium complexes with 2,2'-bipyridyl, in which it was determined that the activity molecular hydrogen generation increases when 2,2'-bipyridyl is substituted with methyl groups [72]. Several system uses the *tris*-2,2'-bipyridyl-ruthenium(II) complex as photosensitizer together cobalt(II)-dithiolene complex [81], shows an absorption band at 390 nm and also the photosensitizer characteristic bands. Finally a study use *tris*-(2,2'-bipyridyl)cobalt(II) complex like reducer agent and a gadolinium(III) as photosensitizer, which shows an comparable photocatalytic efficiency compared to $[\text{Ru}(\text{bipy})_3]^{2+}$ complex [82].

IV. CONCLUSION

Hydrogenase enzymes have been studied extensively. However, the development of an efficient catalyst for hydrogen generation, with structural analogies to enzymes, is at a very early stage. This can be attributed to the fact that the electron transfer in the reactions promoted by this enzyme has a high efficiency, promoting the proposals of very complex mechanisms. To design an artificial model of a hydrogenase enzyme, it is important to know the previous studies of artificial models, as well have access to structural and spectroscopic information of the enzyme. The study of the intramolecular structure of the enzyme hydrogenase might allow to know the electron transfer reactions during the redox cycles of metal atoms in the active center and contribute to future designs of artificial systems. On the other hand, different studies indicate that it is important to choose the metal and ligands used to obtain these complexes. The ligand might promote a single coordination site or several in the coordination sphere, this modify the geometry and stability of the complex. To generate a catalytic activity, artificial models require that the metal ions involved should generate complexes with an adequate coordination environment and promote high stability. These factors have limited the mimic the hydrogenases metallic center. The synthesis of metal complexes requires several steric and electronic properties of ligands, to reduce bond angles, distances and decrease the intramolecular tensions, which can affect the stability. This promote to focusing investigations on the synthesis of active functional model. Finally, several research groups have initiated the study of different metallic complexes, mono, di and multi-nuclear, with iron, nickel, cobalt, ruthenium and osmium, which have been reported to achieve the reduction of hydrogen ions to molecular hydrogen.

ACKNOWLEDGMENT

The authors would like to thank the Secretaría de Educación Pública (SEP) through the “Convocatoria 2017 de Fortalecimiento de Cuerpos Académicos” and Secretaría de Innovación, Ciencia y Educación Superior (SICES) through their program “Convocatoria de Investigadores Jóvenes 2016”, in the project “Obtención de Clústeres Manganese-Calcio para su Aplicación Potencial en Sistemas de Fotosíntesis Artificial”, for their economic support, is gratefully acknowledged.

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