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Artificial hydrogenase: biomimetic approaches controlling active molecular catalysts

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Hydrogenase catalyses reversible transformation of H_2 to H^+ using an active site which includes an iron or nickel atom. Synthetic model complexes and molecular catalysts inspired by nature have unveiled the structural and functional basis of the active site with remarkable accuracy and this has led to the discovery of active synthetic catalysts. To further improve the activity of such molecular catalysts, both the first and outer coordination spheres should be well-organized and harmonized for an efficient shuttling of H^+, electrons, and H_2. This article reviews recent advances in the design and catalytic properties of artificial enzymes that mimic the hydrogenase active site and the outer coordination sphere in combination with a peptide or protein scaffold.

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Introduction

Molecular hydrogen (H_2) produced from water is an ideal feedstock alternative to fossil fuels. Because of the potential use of H_2 as an energy source, extensive efforts have been devoted to development of an efficient catalyst for oxidation of or production of H_2. The natural enzyme known as hydrogenase (H_2ase) catalyses the reduction of H^+ and also catalyzes the opposite reaction, oxidation of H_2 to yield H^+. Both reactions occur under mild conditions [1,2]. The active site of [FeFe]-H_2ase, an H-cluster, is formed by a diiron core with an unusual bridging azadithiolate ligand and diatomic ligands such as CO and CN^- (Figure 1). The coordination arrangement of the active site contributes to the structural stability and the reactivity of the H-cluster [3–5]. Efforts to artificially replicate the H-cluster have led to successful design of molecular catalysts for H_2 production and H_2 oxidation [6–8,9••,10••,11••]. Since the molecular catalysts require electrons to produce H_2 from H^+, a single- or multi-component photochemical system where electrons are provided in a cascade to the molecular catalysts has been constructed using a visible light-driven photosensitizer [12,13]. To further improve the catalytic performance of this system, its outer coordination sphere should be tailored to promote efficient shuttling of electrons, H^+, or H_2. Efforts to develop artificial metalloenzymes with an embedded non-natural active site within a protein scaffold have proven that there is potential to improve specificity, selectivity, and efficiency of catalysis [14–21]. This short review summarizes recent advances in development of an artificial H_2ase on the basis of a peptide or protein framework. Hybrid H_2ases in other combinations, such as dendrimers, sugars and nanomaterials will not be [22–26] discussed.

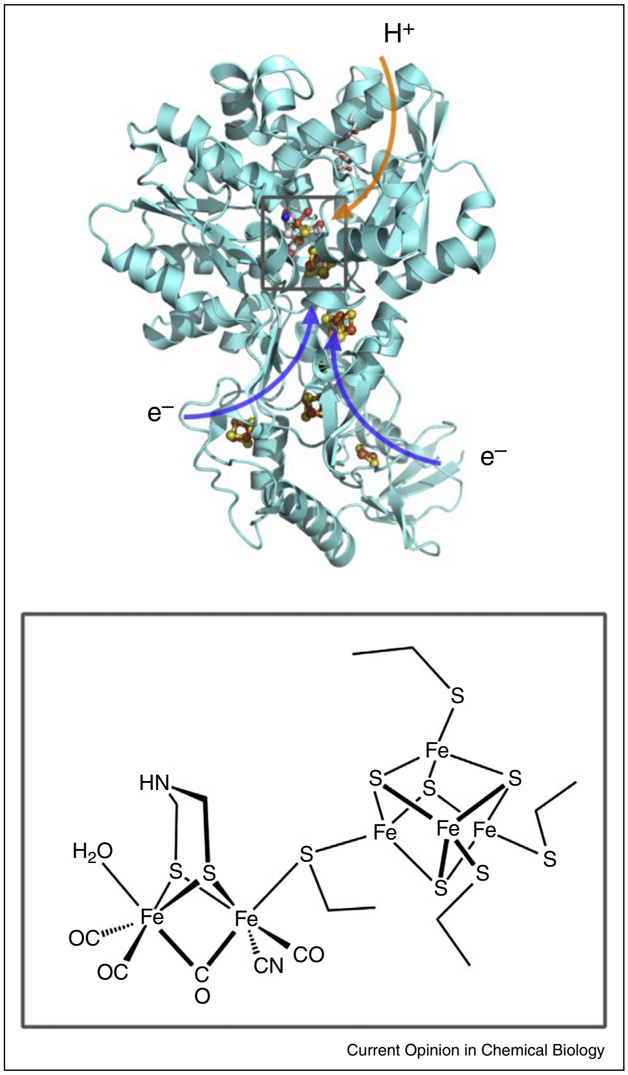
FeFe and NiFe active site models

Jones *et al.* were the first to report a synthetic H_2ase mimicking the dithiolate bridging ligands of H_2ase using a designed peptide. The diiron entity was incorporated by two cysteines located on the same face of the α-helix at the *i*, and the *i* + 3 positions. The 36-residue peptide was treated with $Fe_3(CO)_{12}$, generating a $[Fe_2(\mu\text{-}S(Cys))(CO)_6]$ core datively anchored to the surface of the α-helical peptide (Figure 2a, **1**), which serves as a first generation of [FeFe]-hydrogenase maquette [27].

A similar dative anchoring approach was applied to incorporate a photocatalytically active diiron core into a Cys-X-Y-Cys fragment of apo-cytochrome *c* (apoCyt) [28•]. ApoCyt was reacted with diiron nonacarbonyl ($Fe_2(CO)_9$) to form a $[Fe_2(\mu\text{-}S(Cys))(CO)_6]$ core (Figure 2b, **2**). H_2 was produced using [FeFe]-apoCyt in the presence of $[Ru(bpy)_3]^{2+}$ as a photosensitizer and ascorbate as a sacrificial reagent. Photocatalytic production of H_2 reached a plateau after two hours, yielding a turnover number (TON) of ~80 per [FeFe]-apoCyt with a maximum turnover frequency (TOF) of ~2.1 per minute. By contrast, a small model where a diiron cluster is formed with a heptapeptide fragment YKCAQCH was found to promote H_2 production with a TOF of 0.47 per minute. This indicates that the protein environment near the diiron core provides a favorable platform for efficient photocatalytic H_2 production.

A hydrogenase model core positioned close to a photosensitizing unit is expected to improve the catalytic activity. Our group further constructed an H_2ase model

Figure 1



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Structure of *Clostridium pasteurianum* [FeFe]-hydrogenase (PDB ID: 3C8Y) and the H-cluster active site.

containing both a datively anchored $[Fe_2(\mu\text{-}S(Cys))(CO)_6]$ cluster and a Ru complex in a peptide platform. The octadecapeptide YIGKACGNCHENFRDKEG (Pep18), a fragment of cytochrome c_{556} from *Rhodopseudomonas palustris*, was used as a platform for both metal complexes. The peptide was reacted with $Fe_2(CO)_9$ on the CXXCH sequence of Pep18, followed by a reaction with $[Ru(bpy)(tpy)(H_2O)](PF_6)_2$ (bpy = 2,2′-bipyridine, tpy = 2,2′:6′,2″-terpyridine) to form a photosensitizer-linked diiron carbonyl cluster within the peptide (Figure 2c, **3**) [29]. Photochemical H_2 production reached a plateau after two hours, yielding a TON of ~9 per [FeFe][Ru]-Pep18 with a maximum TOF of ~0.19 per minute calculated from the initial rate of H_2 production. By contrast, a reference system comprising a 1:1 mixture of $[Ru(bpy)(tpy)(im)]^{2+}$ (im = imidazole) and [FeFe]-Pep18(H10A) where the histidine residue is replaced with alanine did not promote H_2 production. The difference in the activities indicates that the histidine residue adjacent to the cysteine residue serves as a coordination site for the Ru photosensitizer. As a result, efficient electron transfer is found to take place from the photoexcited Ru moiety to the diiron cluster within the peptide matrix.

In efforts to design and engineer the outer coordination sphere of the H_2ase model catalyst, Jones *et al.* recognized that an artificial amino acid ligand tethering a diiron complex for incorporation into peptide frameworks would be promising. This group developed a general method for modification of a $[Fe_2(\mu\text{-}S)_2(CO)_6]$ core with a peptide via on-resin coupling in solid-phase peptide synthesis (Figure 2d, **4**). A propanedithiol unit was coupled with the ε-amine group of the lysine residue in an octapeptide (Ac-WASKLPSG) on the resin. The dithiolate ligand on the peptide was further used to coordinate the $Fe_2(CO)_6$ moiety [30]. The $[Fe_2(\mu\text{-}S)_2(CO)_6]$ core tethered to the peptide was shown to undergo ligand substitution reaction with PMe_3.

Ghirlanda *et al.* designed an [FeFe]-H_2ase mimic with a helical peptide scaffold including a non-natural amino acid bearing a 1,3-dithiol at an *i* position and a lysine at an *i* + 3 position (Figure 2d, **5**). The dithiol unit and the non-coordinating lysine residue were intended to mimic the dithiolate bridge and Lys358 in the vicinity of the H-cluster in *Clostridium pasteurianum* [FeFe]-H_2ase. The [FeFe]-H_2ase mimic using a helical peptide catalyzes H_2 production with a TON of 84 over 150 min in the presence of a 15-fold excess of $[Ru(bpy)_3]^{2+}$ as a photosensitizer and 50 mM ascorbate as a sacrificial electron donor [31]. This mimetic system demonstrated irreversible oxidation and reduction of a Fe^IFe^I/Fe^IFe^0 redox couple approximately at −1.1 V versus NHE, a value which depends on the pH value of the solution.

Our group has also prepared an [FeFe]-H_2ase model by introducing a $[Fe_2(\mu\text{-}S)_2(CO)_6]$ core with a tethered maleimide moiety into a cavity of aponitrobindin (apo-NB), a protein with a rigid β-barrel structure. This was accomplished by forming a covalent linkage between the maleimide moiety and a cysteine residue within the β-barrel cavity (Figure 2e, **6**) [32]. The β-barrel structure provides a remarkably rigid platform for supporting the construction of an artificial active site [33,34], because the protein matrix of the NB family, which transports an NO molecule in *Arabidopsis thaliana*, retains the β-barrel folding even in the apo-form after removal of the heme cofactor. Photochemical H_2 production experiments were performed with NB-**6** in the presence of excess ascorbate as a sacrificial donor and a photosensitizer. The photocatalytic production of H_2 reached a plateau after six