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HYDROGENASE ACTIVE SITES: A NEW PARADIGM FOR NATURAL PRODUCT-INSPIRED SYNTHESIS BASED ON ORGANOMETALLIC CHEMISTRY

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The scientific career of F.G.A. Stone witnessed the birth of transition metal organic chemistry and contributed greatly through an amazing period of development. The maturation and acceptance of this new field depended on the accumulated syntheses and careful characterization of compounds that spanned the transition metal series, setting "in Stone" the precedents, principles, and tenets of structure and bonding of species containing a metal-carbon bond.[1] These well-described observations from Stone's laboratory permitted predictions that guided applications to homogeneous catalysis from the organic chemist's bench to the industrial plant processes. This new organometallic chemistry ultimately changed our lives. Nevertheless, there was "old" organometallic chemistry, well developed in nature from billions of years of evolution. It was just waiting to be discovered when the chemistry in our Schlenk flasks, signals from our spectra, and structures from our crystal tubes were sufficiently understood.

***Keywords*: hydrogenase, organoiron, small molecule models, synthetic analogues**

***Abbreviations*: eas, enzyme active site; H_2ase, hydrogenase**

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That organometallics would be found in nature was only a matter of time with proper detective attention. Still, even the discovery[2] of the Co-C bond in vitamin B_{12} did not sufficiently inspire chemists to search hard enough for *what had to be* in those microorganisms whose livelihood depended on C-C coupling reactions or H_2 uptake/H_2 production processes. We were thus pleasantly surprised when in 1994 evidence from FT IR spectroscopy and biochemical labeling confirmed endogeneous CO and CN^- ligands were bound to the metal(s) in the active site of nickel-iron hydrogenase, [NiFe]-H_2ase.[3,4] These data, coupled with X-ray crystallography[5,6] were convincing that nature had long ago utilized diatomic ligand for the maintenance of iron(II) in low spin configuration for heterolytic hydrogen splitting or for hydrogen production from protons and electrons:

$$H_2 = H^- + H^+$$

$$H_2 = 2\ H^+ + 2\ e^-$$

On many levels, this discovery was paradigm changing. From a personal view of the author, the ability to connect, via vibrational spectroscopy (Figure 1),[7,8] synthetic analogues of the active site to the myriad redox levels of [NiFe]-H_2ase (previously characterized by EPR spectroscopy),[9] permitted entry to a research direction and stimulating application of organoiron chemistry which has, over the past 15 years, grown into its own branch of bioorganometallic chemistry.

There are three main classes of hydrogenase enzymes, formed within some 100 or more microorganisms.[10] They are classified according to their metal content. The [NiFe]-H_2ase and [FeFe]-H_2ase have bimetallic active sites (Figure 2), typically with a complement of iron sulfur clusters aligned within the protein so as to serve as efficient electron shuttle routes connecting the deeply buried active sites to their redox partners that are docked into the periphery of the H_2ase proteins.[5,10,11] A third hydrogenase has no need for an electron access route as its active center, a single iron(II) is redox invariant.[12] This mono-iron hydrogenase, [Fe]-H_2ase (also known as Hmd or the $\underline{H_2}$-forming $\underline{m}$ethylene tetrahydromethanopterin $\underline{d}$ehydrogenase) is known to promote heterolytic H_2 cleavage, ridding organisms of excess H_2 by extracting a hydride and placing it on the methenyl carbon of a strategically placed carbocation substrate, Figure 3. The coordination sphere shown in Figure 3 is a result of tour de force protein X-ray crystallography and biochemical

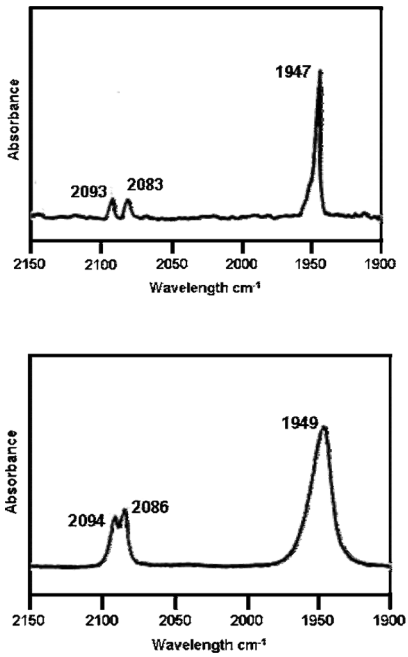

Figure 1. The infrared spectra in the diatomic region of (top) the "as-isolated" [NiFe]-H_2-ase, and (bottom) a small organoiron model of the $Fe^{II}(CN)_2CO$ motif.[8]

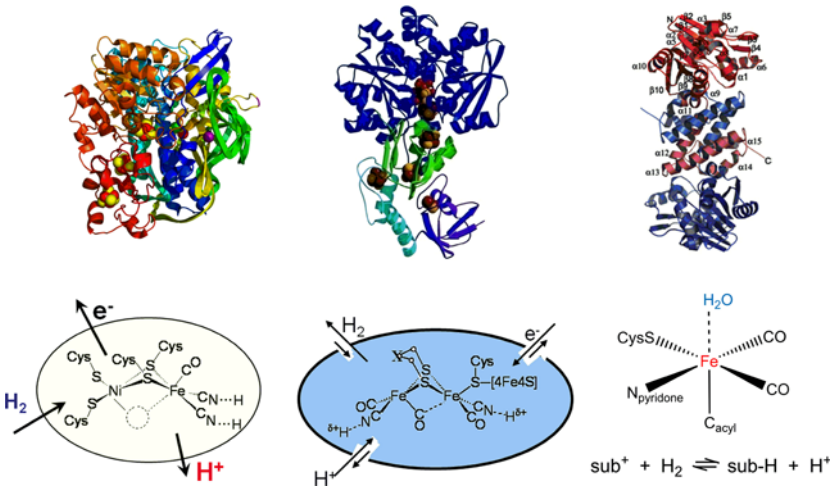

Figure 2. Structures of the three hydrogenase enzymes with blow-ups of their active sites: [NiFe-H_2ase,[5] [FeFe]-H_2ase,[11] and the [Fe]-H_2ase.[12] (Figure appears in color online.)

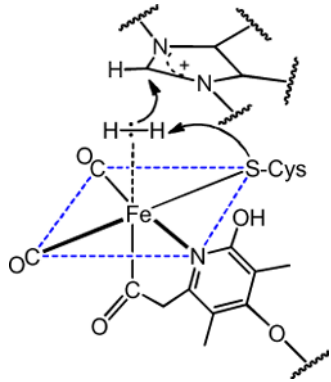

Figure 3. The currently accepted active site of the [Fe]-H_2ase and a suggestion for its mechanism of action.[12b] (Figure appears in color online.)

manipulation of proteins. The accepted active site structure is that of a penta-coordinate iron, surrounded by two cis carbonyls, an acyl carbon, a pyridone nitrogen in a large unique cofactor seemingly designed to be positioned as a ligand via covalent interactions with the protein. A cysteinyl sulfur is the sole covalent link of the iron to the protein.

The cartoon shown in Figure 3 is a working hypothesis of an iron sufficiently electrophilic to attract H_2, with the hydride-accepting substrate ideally positioned 3 Å away, and a nearby base (either the cysteinyl sulfur or the pyridone hydroxyl) available to dispense of the proton.[12] As of this writing, there is still no structure of the holo, intact [Fe]-H_2ase enzyme, but rather the coordination complex shown in Figure 3 is a composite from different isolates and versions of manipulated protein and cofactors.[12] With this relatively clear picture of the active site, synthetic chemists have tackled small molecule models of this site, making considerable progress recently.[13–15]

Our initial approach was to search for ligation settings that stabilize penta-coordinate iron, reproducing compositional and structural features of the [Fe]-H_2ase in as much as possible. To introduce a nitrogen and sulfur as donors, we selected the NS bidentate ligand that had, in the laboratories of Professor Wen Feng Liaw, Taiwan, permitted access to a cyanocarbonylate, $(O\text{-}S(NH)C_6H_4) - Fe(CO)_2(CN)^-$.[16] Of course the good π-donor character of the ortho-amidothiophenylate, or NS^{2-} ligand is responsible for stabilization of iron in the 2+ oxidation state. However, this ligand epitomizes non-innocence in electron distribution

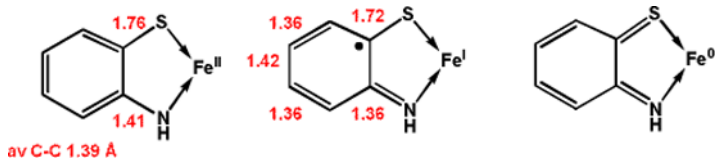

Figure 4. Representation of three redox levels of the o-amidophenylthiolate ligand bound to iron: Non-innocent behavior.[17] (Figure appears in color online.)

properties, with the possibilities of metal-ligand redox state interplay as shown in Figure 4.[17] Thus a series of $(NS)Fe(CO)_2PR_3$ complexes was prepared and examined for the structural and spectral indicators of electron delocalization (Figure 5). The ν(CO)IR patterns were consistent with cis carbonyls at 90° angles (Figure 5).[18] The ν(CO) positions varied as expected for the electron-donating ability of the series of phosphines. Analysis of X-ray crystallographic results found distorted square pyramids while Mössbauer spectroscopy suggested the iron is more reduced than in similar compounds not containing the non-innocent NS ligand.[18] Consistent with this π-electron shielding of the "Fe^{II}", the uptake of exogeneous CO was observed only after protonation, which occurred at nitrogen (Figure 6). This redirecting of electron density from the Fe(NS) π system into the new N-H σ bond increases the electrophilicity of Fe^{II} sufficiently for the binding of a sixth

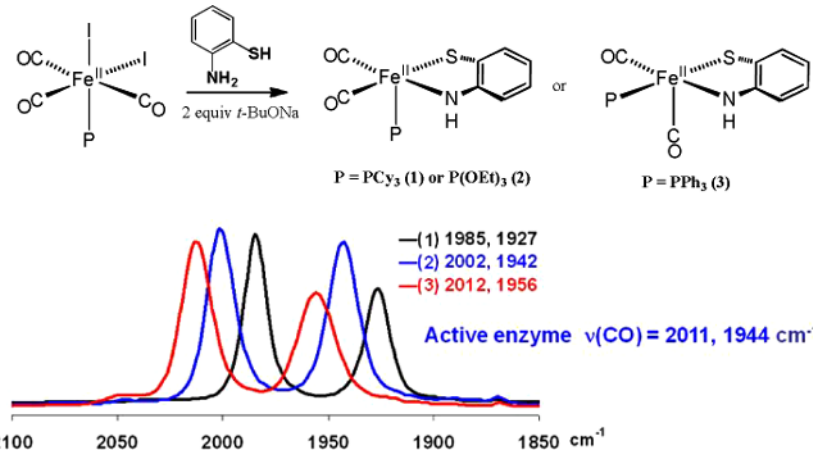

Figure 5. Synthetic approach to rudimentary models of the active site of [Fe]-H_2ase and ν(CO) IR data.[18] (Figure appears in color online.)

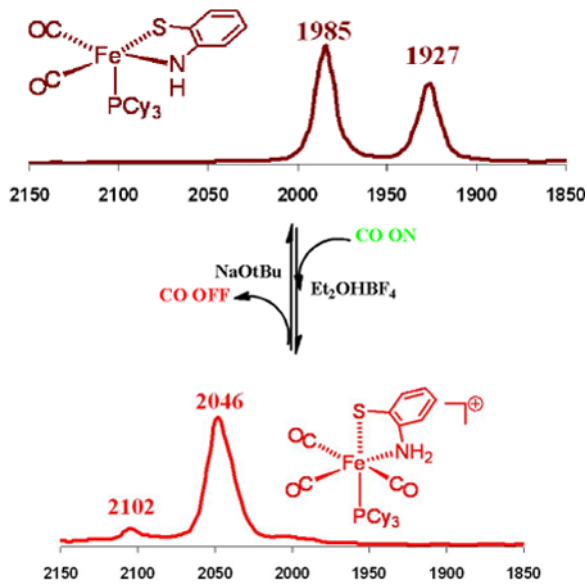

Figure 6. Protonation of complex 1 in the presence of CO gas and changes in ν(CO) IR spectra indicating reversible CO uptake.[18] (Figure appears in color online.)

ligand, achieving the favored 18-electron configuration so well established in organometallic chemistry. Such a plasticity of electron distribution that gives rise to tuning of potential binding sites is the "stuff" of catalysis, particularly within proteins where specific H-bonding sites can achieve various levels of such tuning.

We continue to stalk more faithful models of the hydrogenase active sites. We learn principles from one that apply to the other. They hold in common the design of nature to use by 1st, 2nd, and 3rd coordination sphere manipulations to render the earth abundant metals, iron and nickel, as catalysts for reactions that are performed in fuel cells that now use the resource-limited platinum. In this regard, we have recently imbedded a small molecule model of the [FeFe]-H_2ase active site within a cyclodextrin as a biomimetic of the second coordination sphere surrounding the active site.[19] A unique X-ray crystal structure is shown in Figure 7. This construct engenders water solubility on a hydrophobic moiety, a needed advance for utilizing molecular electrocatalysts as potential replacements for platinum. We continue to strive for the secrets of nature's success.

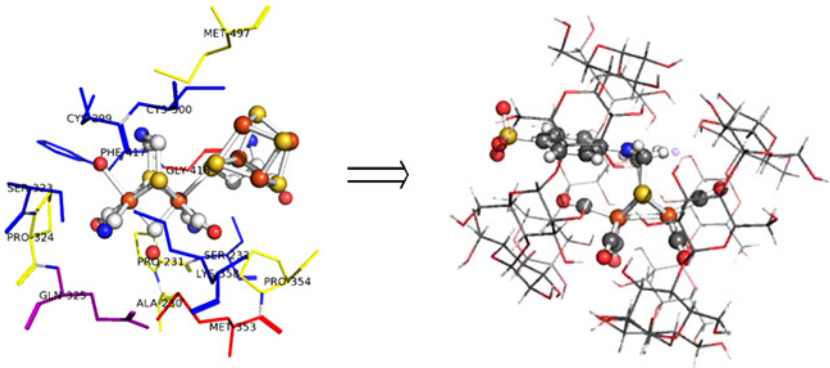

Figure 7. The active site of [FeFe]-H_2ase and 2nd coordination sphere protein residues. Right: Molecular structure of a simple diiron carbonyl model complex included within two cyclodextrins.[19] (Figure appears in color online.)

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