A look into the “H-Cluster” of FeFe-Hydrogenase through spectroscopic and synthetic methods

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Introduction

Dihydrogen, which is the simplest molecule, has become very important lately as an energy carrier to our current drive to rid our fixation on fossil fuels. While our attempts at a catalyst that makes the simple, but yet energetically difficult to synthesize, molecule have led to ineffectual or harmful catalysts, which contain “rare-earth” metals. Currently, our main source of dihydrogen is from reformation of fossil fuels. Nature, alternatively, has done this process with abundant iron or iron-nickel enzymes called hydrogenases. The hydrogenases probably developed at the early stages of life on our planet due to the hydrogen-rich atmosphere. There are three distinct classes of hydrogenases: [NiFe], [FeFe] and [Fe] hydrogenases. While all three distinct classes are important to life on our planet, but this paper will only be discussing the FeFe-hydrogenases.

Since 1998, the structure of one of the FeFe-hydrogenase (Clostridium pasteurianum (CpI)) has been known. At that time, five iron-sulfur clusters were found, a 2Fe2S cluster and three 4Fe4S clusters with one very unique 4Fe4S with an attached 2Fe cluster attached. The unique 6Fe cluster, which has been named the “H-cluster”, is catalytically active site of the enzyme. While, all of the clusters are important for electron donation to the catalytically active site, this paper will discuss the importance of the “H-cluster” and various synthetic models based off of the active site. The original cluster and the synthetic models will be analyzed through their spectroscopic characteristics.

“The H-Cluster”

There are aspects of the catalytically active “H-cluster” still under debate, including what the “bridge-head” group is in the 2Fe cluster. The current theories about the makeup of the active sites are shown in the figures 1 and 2. The “bridge-head” group has been thought to be one of two groups, a propane dithiol (PDT) or a di-thiomethyl-amine (DTN). While both groups are possible due to the ambiguity in the crystal structure, the DTN has become the “leading” group due to its ability to proton accept and donate. The iron atoms at the active 2Fe cluster have been found to be low-spin iron metal atoms due to S2-, CO and CN- ligands. It is remarkable that CO and CN- are bound to the protein structure to the relative “metal toxicity” of those ligands.

Finding the oxidation states of the 2Fe cluster’s iron atoms was found to more difficult than expected due to the unlikely behavior of the iron...
The oxidized inactive form ($H_{\text{ox-air}}$), although, was not difficult because the H-cluster was found to be EPR inactive. This means that the two iron atoms have to be diamagnetic and in the low spin $d^6$ or $+2$ valence state. There is also a chance that the cluster has iron atoms in the low spin $d^5$ or $+3$ valence state. The irons would then have to be antiferrically coupled to each other. While, both are EPR silent, the leading theory is that the iron atoms are in the $+2$ valence state due to the lack of magnetic coupling to the 4Fe4S cluster.

A one electron reduction of the inactive site yields what the literature refers to as the transient state ($H_{\text{trans}}$). The reduction occurs supposedly at the 4Fe4S cluster. The reasoning that it is theorized that the reduction occurs at the 4Fe4S cluster is that the EPR active signal is consistent with that of other reduced 4Fe4S clusters. This also helps stabilize the belief that the 4Fe4S cluster is used for electron trafficking, so that the 2Fe cluster can move electrons back and forth when “needed”. Upon further lowering the redox potential, the EPR signal shifts to something that does not resemble a 4Fe4S cluster. The shift suggests that at least one of the iron atoms in the 2Fe cluster ($H_{\text{ox}}$) changes its oxidation state. The signal shows that the proximal iron atom to the 4Fe4S cluster is only weakly magnetic coupled to the 4Fe4S cluster. This means that the proximal iron is diamagnetic and stays in the $+2$ low spin oxidation state. Since the 2Fe cluster is EPR active, the distal low spin iron atom must be paramagnetic, meaning that it must be in the $+1$ or $+3$ oxidation state. While the Mössbauer data initially theorized that the distal iron atom must be in the more abundant $+3$ oxidation state, FTIR studies have confirmed the rare $+1$ oxidation state. This also is intuitive due to the fact that the cluster has been reduced and the iron atoms can only go from the $+2$ oxidation state to the $+1$ or lower oxidation states. Upon further reduction both iron atoms in the 2Fe cluster ($H_{\text{red}}$) go to the $+1$ oxidation state. This cluster is a $d^7$ with one unpaired electron on each iron atom, but the EPR signal is silent. This means that the two iron atoms are antiferromagnetically coupled to each so that the spins are in opposite directions and the spin state is 0. (See figure 3 for complete analysis of the oxidation-reduction scheme, but note that $H_{\text{red}}$ is actually Fe(II) and $H_{\text{ox-air}}$ and $H_{\text{trans}}$ are Fe(I) states [also red means EPR active and blue is EPR inactive].) Also important to mention is what happens to the active H-cluster when excess CO gas is introduced to the site, the H-cluster ($H_{\text{ox-CO}}$) becomes inactive again. The molecule is again EPR active and no oxidation state changes are made. Why the structure becomes inactive is discussed in the following paragraph.

Looking at the IR spectra of the various oxidation states can elucidate much about ligand orientation and bonding (see figure 5 for complete IR data about the various H-cluster states.) The CN- and CO vibrations are optimal due to the fact that the stretching frequencies occur in a characteristic region that is free from
protein vibrations. The region can not only tell what ligands are attached, it can, since the IR stretching is so sensitive to metal-ligand charge density, tell what possible oxidation states of the iron atoms are. The initial FTIR study of the H-cluster of oxidized inactive (H_{ox-air}) *Defulsovibrio vulgarism*, a FeFe hydrogenase, in 1998, even before the crystal structure was known, (shown in figure 4) shows 5 distinct peaks of interest; 2106, 2087, 2008, 1847 cm\(^{-1}\). The first two peaks, 2106 and 2087 cm\(^{-1}\), are terminal CN\(^{-}\) ligands. The fact that there are two peaks signifies that each iron has its own unique CN\(^{-}\) ligand with a unique stretching due to distinctive sigma bonding. The next two peaks are terminal CO ligands again with two peaks signifying that each iron has its own CO ligand with distinct sigma and also pi* bonding. Finally, the 1847 cm\(^{-1}\) confirms that there is a bridging (µ) CO ligand. The initial Albracht et al. FTIR study also believed that the stretching could be due to an NO ligand, but upon further investigation into the crystal structure it has been confirm to be a CO ligand.

So upon the first reduction of H_{ox-air} to H_{trans}, the ligands are affected in bonding and anti-bonding strength alone since the new stretching bands are 2100, 2075, 1983, 1977, 1836 cm\(^{-1}\). The first two bands (2100 and 2075 cm\(^{-1}\)) are CN\(^{-}\) bands (the CN\(^{-}\) bands from here on will be neglected for simplicity). The next two stretches are again the CO ligands and the last stretch is the µ-CO ligand. The bands all go downward in frequency due to the excess electron density on the 4Fe4S cluster which will push the density onto the 2Fe cluster. The 2Fe cluster will then in turn donate more electron density into the pi* orbital of the CO ligands and lower the stretching frequency. After further reducing the cluster, the H_{trans} goes to the H_{ox} cluster. The stretching frequencies for the CO ligands in the state are 1965, 1940 and 1802 cm\(^{-1}\). This reduction pushes even more electron density onto the iron atoms, which will further add electron density in to the pi* orbitals. After the reduction of H_{ox} to H_{red}, a significant IR spectra is observed of the CO ligands of 1965, 1916, and 1894 cm\(^{-1}\). This is significant because the last stretching frequency, 1894 cm\(^{-1}\) shows that the µ-CO has been lost and has been moved to a terminal CO ligand. The µ-CO ligand has been found to be moved to the distal iron site from X-ray crystallographic studies. The reason that the µ-CO is lost in the H_{red} structure is from probable H\(_2\) bonding or release. The discussion of the possible mechanism is beyond the scope of this paper, but the fact that the µ-CO can become terminal and then go back to being µ-CO is one of the very probable reasons for the facilitation of such an efficient reaction of dihydrogen.

Also looking at the IR spectrum of the H_{ox-CO} cluster can possibly clarify why the cluster becomes inactive. The IR has four distinct CO bands 2016, 1921, 1963, 1810 cm\(^{-1}\). There is still the µ-CO band at 1810 cm\(^{-1}\) but there is now three distinct CO bands signifying that there is an extra CO which would have to bond in the open position where the water normally rests. This does not allow dihydrogen to come or be release because the molecules can not come in as close to the cluster.
“Synthetic Models”

Due to the importance of the dihydrogen to the future of our energy economy, synthetic models have been developed to mimic the activity of the H-cluster. Many models of 2Fe2S clusters have tried to imitate the activity with a variety of ligand systems (phosphines, borides, etc.), but the goal of this paper will be to discuss two models with ligand systems reminiscent of the original cluster.

There has actually been a synthetic model of the H-cluster that has been known for about 70 years before the original H-cluster was seen through X-ray crystallography. This model, which is full carbonyl 2Fe2S cluster \([\text{Fe}_{2} (\mu-SR)_{2} (\text{CO})_{6}]\), was the starting point for three distinct groups after the crystal structure was known. The groups, which all independently, synthesized a 2CN\(^{-}\) system that replaced two of the CO ligands of the cluster (see figure 6).\(^6\)\(^{10-11}\) The CN\(^{-}\) addition was confirmed through FTIR with important bands at 2078, 2029, 1962, 1880, 1867 cm\(^{-1}\).\(^11\) The first two bands again are the CN\(^{-}\) bands, and the last four are terminal CO ligands. This molecule is EPR silent and the oxidation state of the iron atoms are assumed to be in the +1 state due to antiferrocoupling.\(^8\) Even upon CN\(^{-}\) addition, the oxidation state of the iron atoms does not change to do the use of counter-ions. This early model, which resembles only a 2Fe2S cluster whereas the H-cluster is a 2Fe3S cluster due to the attached cysteine residue, was expanded to a 2Fe3S system through the “bridge-head”. The synthesis of the cluster \([\text{Fe}_{2} (\mu-(\text{SCH}_{2})_{2}-\text{MeCHCHSMMe}_{2})_{2} (\text{CO})_{6}]\), which was done by Pickett et al., was shown to be closely resemble the H-cluster if the “bridge-head” is the PDT group (see figure 7, note that the proposed PDT 2Fe3S carbonyl cluster is molecule 7). When the 2Fe3S cluster is reacted through cyanation of the carbonyl cluster, eventually a synthetic model that very closely mimics the H-cluster is found even with a bridging CO ligand. This structure (seen as molecule 9 in figure 7) which is only a stable intermediate can be confirmed through FTIR.\(^12\) The structure which has CN\(^{-}\) bands at 2083 and 2077 cm\(^{-1}\) and terminal CO bands at 1957, 1919, and 1878 cm\(^{-1}\).\(^12\) The \(\mu\)-CO band occurs at 1780 cm\(^{-1}\).\(^12\) Recalling the H-clusters IR bands, there was \(\mu\)-CO band at 1802 cm\(^{-1}\) meaning that the \(\mu\)-CO in the synthetic 2Fe3S is receiving more \(\pi^{*}\) donation from the iron atoms. The rationale for the lower \(\mu\)-CO is shown in the EPR data. Since the EPR data shows a diamagnetic signal, the oxidation states are assumed to be the same as in the 2Fe2S carbonyl cluster which is +1. Recalling the \(\text{H}_{\text{ox}}\) cluster which

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has Fe(I) and Fe(II) atoms, the synthesized 2Fe3S cluster has both iron atoms in the +1 oxidation state. Since two Fe(I) will have more electron density than a Fe(I) and a Fe(II), the synthetic model will have more electron density to donate to the π* CO orbital. This interaction will lower the μ-CO band.

The other important synthetic model that will be discussed is the 2Fe cluster that models the DTN version of the H-cluster. The model which was synthesized by Rauchfuss et al. is shown in figure 8. The compound was confirmed via FTIR and showed no N-Fe bonding interaction. The IR spectra showed CN- bands at 2075 and 2032 cm\(^{-1}\) and CO bands at 1963, 1922, 1884, 1875 cm\(^{-1}\), indicating that there is no μ-CO ligand.

Conclusion

Spectroscopic tools can be utilized to analyze cluster characteristics. Tools, such as FTIR and EPR, can be used to find oxidation states, ligand motifs, and ligand bonding such as difference between terminal CO ligands and a bridging CO ligand. While it was not discussed in this paper these techniques along with computational tools can also be used to elucidate the possible mechanism of dihydrogen bonding and release.\(^8,\text{14}\) These tools have given potential aspects of the H-cluster to be utilized in order to synthesize models that will duplicate the reactivity of the important cluster.

Using the two synthetic models along with models with exotic non CO or CN- ligands could be beneficial to finding a catalyst that will make dihydrogen efficiently. This system would be ideal for our environment and energy economy as it utilizes iron metal which is very abundant and “earth friendly” to make and clean up. While currently there is no iron-based synthetic model that can match the efficiency of the H-cluster, there is hope with the current research drive to make an effective system.
References: