IMPLEMENTING JAGUAR 4.0 IN THE UNDERGRADUATE LABORATORY: A COMPUTATIONAL INVESTIGATION OF THE NICKEL-IRON HYDROGENASE

Crystal Cunningham, Arlen Viste, and Gary Earl
Department of Chemistry
Augustana College
Sioux Falls, SD 57197

ABSTRACT

The family of metalloproteins called hydrogenase catalyze the reaction $H_2 \leftrightarrow 2H^+ + 2e^-$. The active site of the enzyme contains two metal atoms, iron and nickel. Several iron-sulfur clusters surround the active site and participate in electron transfer. The reaction mechanism of this enzyme has been extensively studied both experimentally and theoretically. Two detailed theoretical analyses (Shuqiang 1999, Pavlov 1998) prompted the current investigation. Density Functional Theory (DFT) methods were used to investigate the active site and iron-sulfur clusters in various redox, protonated, and spin states. The DFT results were in good agreement with experimental values of bond lengths and bond angles. The calculations were performed using Jaguar 4.0 and gOpenMol was used to view plots of the electron density, electrostatic potential, and molecular orbitals. (Schrödinger 2000, Laaksonen 2000) This paper suggests a smooth convergence of structures of this type in the Jaguar software system. Another result of this effort proved Jaguar 4.0 to be efficient in the undergraduate laboratory for the computational exploration of heavy metal atoms.

Keywords
Hydrogenase, metalloproteins, Jaguar, DFT

INTRODUCTION

The driving force for ATP formation in sulfur reducing microorganisms is the oxidation of $H_2$

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

The reaction results in a pH gradient that gives rise to ATP via ATPase (Pavlov, 1998). There are three metalloprotein and one novel organic hydrogenase (Thauer, 1996) that catalyze the oxidation reaction. Both the Fe-only and Ni-Fe enzymes complex with an iron-sulfur thiocubane structure (Td symmetry).
that assists in electron transfer. The Fe-S clusters provide electron transfer pathways to and from the active site, the H-cluster. Molecular hydrogen binds to the Fe atom in the H-cluster. This initial binding begins a complicated series of redox reactions which eventually yield the two electrons and protons formed from $\text{H}_2$.

X-ray diffraction has provided the structures of both the Fe-only (Peters, 1998) and Ni-Fe hydrogenase (Amara, 1999). The proteins were isolated from the bacterium *Desulfovibrio desulfuricans* a sulfate reducing bacterium. The resolved crystal structures show that the Ni-Fe H-cluster contains bridging sulfur ligands between the Fe and Ni. In addition to the two bridging sulfur atoms, Ni has two cysteinethiolate ligands. The infrared studies show three diatomic ligands located on the Fe atom (Pavlov, 1998): one CN and two CO molecules. When the sixth ligand, molecular hydrogen, binds the Fe atom takes an octahedral geometry. The thiocubane and the H-cluster from the *D. gigas* are depicted in Figures 1 and 2.

EPR data gives evidence of several accessible states of the enzyme (1). Detailed computational studies by Pavlov et al (Pavlov, 1998) and Suquiang Niu et al (Niu, 1999) propose mechanistic schemes for this enzyme based on the EPR, NMR and X-Ray data. Suit-
able species for the experimental results were proposed by Shuquiang Niu et al. This work prompted the current investigation to employ Jaguar 4.0 (Schrödinger, 2000) and gOpenMol (Laaksonen, 2000) computational software in the undergraduate laboratory to better understand the complexity of enzyme catalysis through the use of computational modeling.

The use of an accurate \textit{ab initio} approach to study this system is very challenging considering the number of heavy metal atoms in the enzyme. The thiocubane structure and the H-cluster are considered to be too large of systems to obtain realistic results using \textit{ab initio} methods. On the other hand, various density functional theory (DFT) methods overcome this many body problem. The accuracy of the DFT method weighs heavily on which method is employed. This research used a Local Density Approximation (LDA) to study the thiocubane structure and a Non-Local Density hybrid to study the H-cluster of the Ni-Fe hydrogenase. Several studies have shown that DFT methods have the capacity to treat these larger biochemical systems with the accuracy of \textit{ab initio} calculations (Siegbahn, 2000).

\section*{COMPUTATIONAL METHODS}

The Jaguar 4.0 Computational program was used for all calculations. The calculations were carried out under SuSE 6.4 Linux on a PC.

\subsection*{Thiocubane Optimization}

DFT calculations used a SCF local density approximation Slater VWN, with the LACVP** basis set. This basis set gives an effective core potential (ECP) for the iron and provides a 6-31G** basis set for all other electrons. The HF initial guess was that of Ligand Field Theory. The SCF level shift was set to 2 using the GVB-DIIS convergence scheme with an ultrafine accuracy level. An \texttt{atomic} file was added to the Jaguar input in order to complete the calculation. In geometry optimization, bond angles were frozen to maintain the cubic structure but the bond lengths were allowed to optimize.

\subsection*{Ni-Fe H-Cluster Optimization}

The Ni-Fe hydrogenase (Fig. 2) was optimized with a net molecular charge of -2 and in the triplet state. The same LACVP** basis set used for the thiocubane optimizations was used for these optimizations. The specific DFT theory used was the Becke three-parameter hybrid exchange functional and the Lee-Yang-Parr correlations functional (B3LYP). This optimization also required the use of an \texttt{atomic} section. This species has both Fe and Ni in the +2 oxidation state and possesses triplet multiplicity. Two other species were also optimized. The first species was a bridged Ni-H-Fe complex. This bridging structure contained Fe\textsuperscript{2+} and Ni\textsuperscript{3+}, the molecular charge was -1 and a doublet multiplicity was designated. The bridging hydrogen was assigned a -1 charge and the terminal sulfur was protonated.
Transition state search

An attempt was made to find a possible transition state for movement of H from a bridging position Fe-H-Ni to and S-H position. The quadratic synchronous transit (QST) search was used first since both product and reactant input are known. The second method employed was the linear synchronous transit (LST) search. For this search the most recent structure and its corresponding Hessian provided by QST were used as the proposed transition state. The optimizer can now search along the linear path to the product.

RESULTS AND DISCUSSION

The thiocubane structure Fe₄S₄ structure is shown in Figure 1. In the optimization, bond angles were left fixed but bond lengths were varied. In comparison with crystallographic distances of Mak for Fe₄S₄, our calculated Fe-S distance is 2.10 vs 2.22 Å, calculated S-S 3.54 vs 3.49 Å, but calculated Fe-Fe 3.30 vs 2.67 Å. (Mak, 1992). Calculated Fe-S in [Fe₄S₄]⁺ and [Fe₄S₄]⁻ were 2.86 and 2.85 Å.

The Ni-Fe hydrogenase (Fig. 2) has both Fe and Ni in the +2 oxidation state and possesses triplet multiplicity. Two other species were also optimized. The first species was a bridged Ni-H-Fe complex. This bridging structure contained Fe²⁺ and Ni³⁺, the molecular charge was -1 and a doublet multiplicity was designated. The bridging hydrogen was assigned a -1 charge and the terminal sulfur was protonated. The third species contained Fe²⁺ and Ni²⁺. The molecular charge was also -1 in the doublet state. This species had both terminal sulfurs protonated. These species were proposed by Shuquiang Niu et al as intermediate structures of active site species. The two latter species, Ni-Fe(II,III) and Ni-Fe(II,II) are shown in Figures 3 and 4 respectively. Figure 5 shows the electron density surface of the hydride bridged structure (of Fig. 3), colored with the electrostatic potential.

The transition state search did not fully succeed. However Figure 6 shows the closest approximation reached in this study.

The final distance between the transferring hydride ion and the S3 atom is 2.32 angstroms. This short distance places the hydride ion in a bridging position between the S1 and S3 atoms.

The LST search gave rise to a structure in which the CO ligand on the Fe atom became bent toward the Ni atom. This was similar to the investigation by Pavlov et al in which the CN- ligand bent toward the Ni atom. (Pavlov, 1998).

CONCLUSION

This work concludes that the thiocubane structure and its redox states can be successfully investigated using DFT methods in a time efficient manner appropriate for the undergraduate laboratory.
Figure 3. Hydride bridged Fe-Ni-S4 cluster.

Figure 4. Fe-Ni-S4 cluster with both H on S.

Figure 5. Hydride bridged cluster (from Fig. 3) with electrostatic potential colored on the electron density.

Figure 6. Transition state search.
Plots of the electrostatic potential and electron densities of the H-Cluster show it to be a spin delocalized system. DFT methods give insight into the structure and reactivity patterns of the H-Cluster of the hydrogenase enzyme and can be a useful tool to study enzyme structure and mechanisms. This work optimized two thermodynamically stable structures in the H₂ cleavage cycle. A partial transition state between these two structures suggests that the hydride ion is transferred to a bridging sulfur atom. During this transfer the Ni-S bond breaks and bending of the CO ligand occurs.

One of the reasons for the difficulty in locating the transition state is that the structure labeled as product was optimized in a way that placed the hydride ion on the back-side of the sulfur. It may be possible to rotate the hydrogen on the S₃ atom and use this structure as the product target and then make a transition to the structure in Figure 4. Another option to consider is that the hydride transfer might not be a single-step concerted process but might instead occur by a two step process. Further investigations should shed light on the location of the transition state and further use of Jaguar in the laboratory.

LITERATURE CITED

ACKNOWLEDGMENTS

We thank Augustana College and its Department of Chemistry for software and hardware support. Thanks also to Dr. Nola Borman of the Augustana College Department of Biology for pointing out supporting literature involving microorganism metabolism.