JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by ISTANBUL TEKNIK UNIV

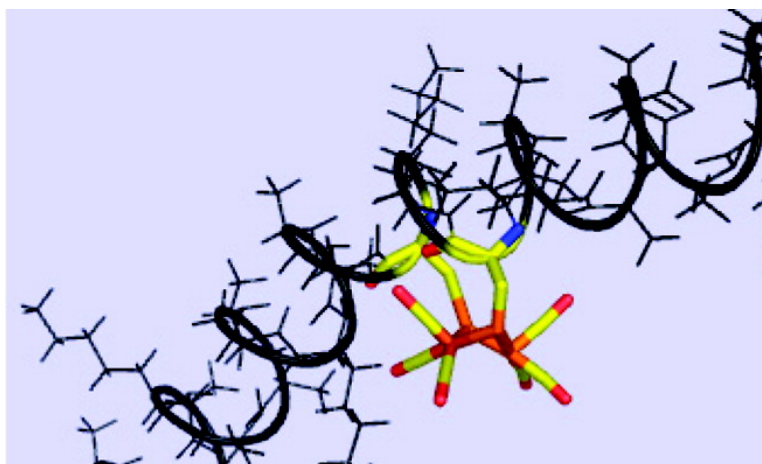
Communication

Synthetic Hydrogenases: Incorporation of an Iron Carbonyl Thiolate into a Designed Peptide

Anne K. Jones, Bruce R. Lichtenstein, Arnab Dutta, Gwyneth Gordon, and P. Leslie Dutton

J. Am. Chem. Soc., **2007**, 129 (48), 14844-14845 • DOI: 10.1021/ja075116a

Downloaded from http://pubs.acs.org on February 9, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Published on Web 11/13/2007

Synthetic Hydrogenases: Incorporation of an Iron Carbonyl Thiolate into a Designed Peptide

Anne K. Jones,*,§,† Bruce R. Lichtenstein,§ Arnab Dutta,† Gwyneth Gordon,‡ and P. Leslie Dutton§

Department of Biochemistry and Biophysics, Johnson Research Foundation, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, and Department of Chemistry and Biochemistry and School of Earth and Space Exploration, Arizona State University, Tempe, Arizona 85287

Received July 10, 2007; E-mail: jonesak@asu.edu

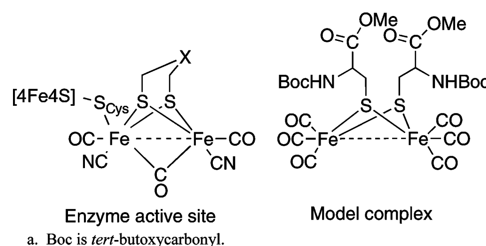
Hydrogen is exploited as a fuel by a diverse array of organisms.[1] Hydrogenases, enzymes for the reversible oxidation of hydrogen into protons and electrons, utilize base metals, either Fe or Ni and Fe, to catalyze this important reaction. Understanding these enzymes is essential not only for the development of hydrogen-based biofuel cells but also for providing mechanistic insights into a broad array of natural oxidoreductases. However, complexity in hydrogenase structure and their catalytic cofactors means that their direct study has left many fundamental catalytic and biosynthetic questions unanswered.[2] Considerable help addressing these questions has come from inorganic chemists who have sought to create mimics of the hydrogen-activating site of hydrogenases.[3,4] Their small organometallic models have proven invaluable in mimicking the catalytic cluster and reproducing hydrogenase activity. However, contributions to mechanism and activity that in nature are derived from burial of the catalytic cluster in a heterogeneous site in combination with second coordination sphere contacts are necessarily absent in these models. De novo designed proteins, maquettes, can be constructed in a size scale intermediate between enzymes and organometallic model compounds and promise many of the advantages of both regimes. Maquettes have been designed that bind a number of natural and unnatural metallocofactors, and they have proven an invaluable tool for the study of complex oxidoreductases.[5−8] In this paper, we have designed a cysteine-containing peptide that reacts with $Fe_3(CO)_{12}$ to assemble a maquette containing a diiron cluster related to the [FeFe] hydrogenase active site.

The hydrogen-activating cluster (H-cluster) of the [FeFe] hydrogenases is depicted in Chart 1. It consists of a [4Fe4S] cluster bridged via cysteine to an unusual [2Fe2S] cluster.[9] A variety of organometallic diiron dithiolate model complexes have been synthesized that reproduce some of the spectroscopic and structural features of the H-cluster.[10−17] Inspired by the direct reaction of $Fe_3(CO)_{12}$ with the Boc-protected methyl ester of cysteine to produce the model compound shown in Chart 1,[12] we opted to utilize cysteinyl sulfur as the anchor for a diiron site within a designed peptide.

For the first generation of our hydrogenase maquettes, we sought a peptide sequence that would present the two cysteine side chains necessary for formation of the diiron complex on the same face of an α-helix. Short, unusually stable, alanine-rich peptides have been described by Marqusee and co-workers.[18] These sequences, with the addition of two cysteine residues placed at positions i and $i+3$, formed the basis for our hydrogenase maquette, SynHyd1. Its sequence is NH_2-AAKAAAAKAACAKCAAAKAAAAKAAAA-KAAAAKAAW-$CONH_2$.

§ University of Pennsylvania.
† Department of Chemistry and Biochemistry, Arizona State University.
‡ School of Earth and Space Exploration, Arizona State University.

Chart 1. [FeFe] Hydrogenase Active Site and a Model Compound Utilizing a Cysteine Ligand



a. Boc is *tert*-butoxycarbonyl.

SynHyd1 was synthesized via solid-phase peptide synthesis and purified using reversed phase HPLC. Circular dichroism demonstrated that the peptide had a well-defined secondary α-helical structure. Purified peptide was then reacted with $Fe_3(CO)_{12}$, and the reaction mixture was repurified via HPLC to obtain peptide with optical absorbance maxima at 280 and 335 nm.[19] Reaction of $Fe_3(CO)_{12}$ under the same conditions either in the absence of peptide or in the presence of a peptide in which the cysteines were exchanged for alanines did not lead to any species with an absorbance maximum at 335 nm, indicating that the cysteine residues are essential for the reaction. Furthermore, the molecular weights of SynHyd1 and Fe_2-SynHyd1 were determined via mass spectrometry to be 3193 and 3473, respectively. This difference of 280 is consistent with incorporation of a $Fe_2(CO)_6$ cluster and demonstrates that Fe_2-SynHyd1 is a monomeric peptide.

Figure 1A shows the circular dichroism (CD) spectrum of Fe_2-SynHyd1, indicating that the secondary structure of the Fe-containing peptide remained α-helical. Cline and co-workers have demonstrated in peptides related to apo-SynHyd1 that the exact placement of cysteine ligands within the helix does not diminish As(III) binding, but the secondary structure of the resulting As peptide is dramatically influenced by ligand position.[20] Experiments are currently underway to investigate the effects of the relative placement of the two cysteine ligands on both the synthesis of the diiron cluster and the secondary structure of the resulting Fe-containing peptides.

Figure 1B shows the UV−vis spectrum of Fe_2-SynHyd1. Two features characteristic of (μ-SRS)$[Fe(CO)_3]_2$ complexes are observed: an intense absorption with a local maximum at 335 nm (ϵ = 10 000 M^{-1} cm^{-1}) and a weaker, broader absorbance centered at 460 nm (ϵ = 1040 M^{-1} cm^{-1}). Both the positions and extinction coefficients of these bands are very similar to those observed for the comparable FeFe hydrogenase model compound μ-$(S(CH_2)_3S)$-$Fe_2(CO)_6$. The characteristic absorbance of the tryptophan residue is also seen in the 280 nm region. The CO and CN^- ligands of hydrogenases and their model compounds absorb in the IR region 1800−2200 cm^{-1}, a region devoid of other peptide absorbances. Thus, the presence and chemical environments of these ligands can

10.1021/ja075116a CCC: $37.00 © 2007 American Chemical Society

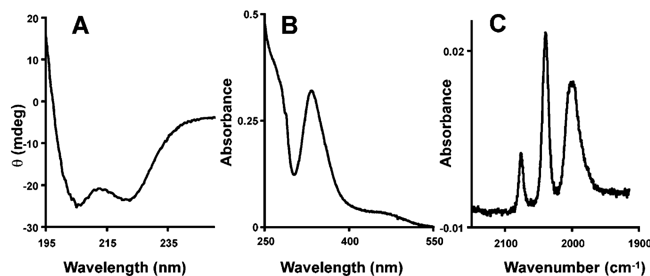


Figure 1. Characterization of Fe_2-SynHyd1 via (A) CD (peptide concentration 7.8 μM in 10 mM phosphate buffer at pH 8), (B) UV−vis spectroscopy (Fe cluster concentration of 32 μM in water), and (C) FTIR spectroscopy (0.49 mM peptide in water).

be conveniently monitored with FTIR spectroscopy. Figure 1C shows the FTIR spectrum of Fe_2-SynHyd1 in this region. Three clear bands attributable to the CO ligands are observed at 2076, 2040, and 2000 cm^{-1}. Both the positions and shapes of these bands are very similar to those observed for μ-$(S(CH_2)_3S)Fe_2(CO)_6$ at 2072, 2033, and 1993 cm^{-1}, confirming that the CO ligands are present in the peptide-coordinated cluster and that the electronic structure of the cluster is not substantially different from that of inorganic complexes.[21]

In this paper, we have demonstrated the synthesis of a $(\mu\text{-SR})_2Fe_2(CO)_6$ complex coordinated to a simple, α-helical peptide via two cysteine residues. This peptide serves as a first generation [FeFe] hydrogenase maquette and opens the chemical door for the creation of more sophisticated peptides containing second coordination sphere residues designed to modulate the properties of the diiron site. We have also created the first model system for studying the reactions of dithiolato diiron carbonyl compounds in de novo designed peptides. Details regarding the biosynthetic pathway of FeFe hydrogenases remain scarce,[2] and hydrogenase maquettes will no doubt serve as convenient systems for testing biosynthetic hypotheses. Finally, through redesign, hydrogenase maquettes offer a promising opportunity to develop robust, artificial, water-soluble, base-metal containing, cheap hydrogen production catalysts for use in environmentally friendly alternative energy production strategies.

Acknowledgment. Financial support for this research was provided by DOE DF-FG02-05ER46223 to P.L.D. and NIH GM073443 to A.K.J. This work was supported in part by the MRSEC program of the National Science Foundation under award #DMR05-20020. James Lear is thanked for performing analytical ultracentrifugation experiments. Prof. Ian Gould is thanked for the use of his fluorimeter.

Supporting Information Available: Experimental details, analytical HPLC traces, and mass spectrometry data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Cammack, R.; Frey, M.; Robson, R. *Hydrogen as a Fuel: Learning from Nature*; Taylor & Francis: London, 2001.
(2) Boeck, A.; King, P. W.; Blokesch, M.; Posewitz, M. C. Maturation of Hydrogenases. In *Advances in Microbial Physiology*; Academic Press Ltd.: London, 2006; Vol. 51, pp 1−71.
(3) Evans, D. J.; Pickett, C. J. *Chem. Soc. Rev.* **2003**, *32*, 268−275.
(4) Bouwman, E.; Reedijk, J. *Coord. Chem. Rev.* **2005**, *249*, 1551−1581.
(5) Pecoraro, V. L.; Farrer, B.; Matzepetakis, M.; Ghosh, D. *J. Inorg. Biochem.* **2003**, *96*, 30.
(6) Lombardi, A.; Summa, C. M.; Geremia, S.; Randaccio, L.; Pavone, V.; DeGrado, W. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6298−6305.
(7) Laplaza, C. E.; Holm, R. H. *J. Am. Chem. Soc*. **2001**, *123*, 10255−10264.
(8) Rabanal, F.; DeGrado, W. F.; Dutton, P. L. *J. Am. Chem. Soc*. **1996**, *118*, 473−474.
(9) Peters, J. W.; Lanzilotta, W. N.; Lemon, B. J.; Seefeldt, L. C. *Science* **1998**, *282*, 1853−1858.
(10) Tye, J. W.; Lee, J.; Wang, H.-W.; Mejia-Rodriguexz, R.; Reibenspies, J. H.; Hall, M. B.; Darensbourg, M. Y. *Inorg. Chem*. **2005**, *44*, 5550−5552.
(11) van der Vlugt, J. I.; Rauchfuss, T. B.; Whaley, C. M.; Wilson, S. R. *J. Am. Chem. Soc*. **2005**, *127*, 16012−16013.
(12) He, C.; Wang, M.; Zhang, X.; Wang, Z.; Chen, C.; Liu, J.; Akermark, B.; Sun, L. *Angew. Chem., Int. Ed.* **2004**, *43*, 3571−3574.
(13) Schwab, D. E.; Tard, D.; Brecht, E.; Peters, J. W.; Pickett, C. J.; Szilagyi, R. K. *Chem. Commun*. **2006**, 3696−3698.
(14) Liu, X.; Ibrahim, S. K.; Tard, C.; Pickett, C. J. *Coord. Chem. Rev*. **2005**, *249*, 1641−1652.
(15) Tard, C.; Liu, X. M.; K., I. S.; Bruschi, M.; De Gioia, L.; Davies, S. C.; Yang, X.; Wang, L. S.; Sawers, G.; Pickett, C. J. *Nature* **2005**, *433*, 610−613.
(16) Stanley, J. L.; Rauchfuss, T. B.; Wilson, S. R. *Organometallics* **2007**, *26*, 1907−1911.
(17) Justice, A. K.; Linck, R. C.; Rauchfuss, T. B. *Inorg. Chem*. **2006**, *45*, 2406−2412.
(18) Marqusee, S.; Robbins, V. H.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5286−5290.
(19) Lyon, E. J.; Georgakaki, I. P.; Reibenspies, J. H.; Darensbourg, M. Y. *J. Am. Chem. Soc*. **2001**, *123*, 3268−3278.
(20) Cline, D. J.; Thorpe, C.; Shneider, J. P. *J. Am. Chem. Soc*. **2003**, *125*, 2923−2929.
(21) Lyon, E. J.; Georgakaki, I. P.; Reibenspies, J. H.; Darensbourg, M. Y. *Angew. Chem., Int. Ed.* **1999**, *38*, 3178−3180.

JA075116A