J|A|C|S COMMUNICATIONS

Published on Web 06/10/2010

A Cyclodextrin Host/Guest Approach to a Hydrogenase Active Site Biomimetic Cavity

Michael L. Singleton, Joseph H. Reibenspies, and Marcetta Y. Darensbourg*

Department of Chemistry, Texas A & M University, College Station, Texas 77845

Received May 3, 2010; E-mail: marcetta@mail.chem.tamu.edu

The naturally engineered pockets that bind the catalytic centers of metalloenzymes impart stability to unusual molecular structures that are poised to facilitate molecular transformations. This is readily seen in the protein environment that surrounds the active site of diiron hydrogenase, [FeFe]-H_2ase, Figure 1, an enzyme that produces H_2 at an efficiency comparable to that of the noble metal platinum in fuel cells.[1−3]

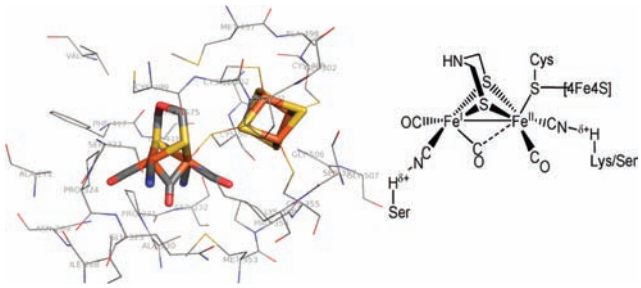


Figure 1. Structure of the hydrogen producing H-cluster of [FeFe]-H_2ase in the protein environment[4] (left) and as a Chemdraw figure (right) showing selected first and second coordination sphere interactions.

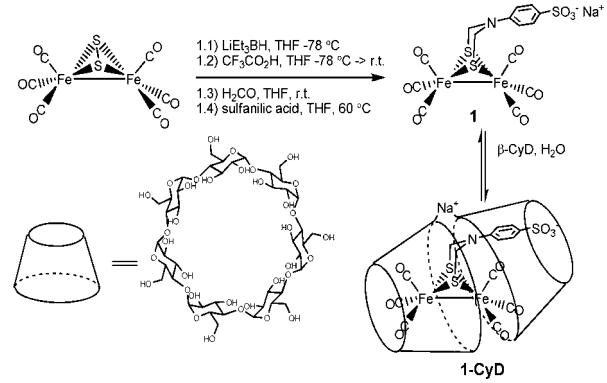
While strong donation into the [FeFe] unit by the CN^- and thiolate ligands contributes to the reactivity of the active site, a number of secondary interactions[4] stabilize a unique geometry in the organoiron motif that allows formation of a reactive terminal hydride.[5] In addition to an appropriately sized, largely hydrophobic cavity, dipole/hydrogen bonding interactions between nearby peptide residues and the cyanides and semibridging carbonyl result in the conservation of an open site in both the Fe^IFe^{II} and Fe^IFe^I states.[6] This important feature has been obtained in mixed valent Fe^IFe^{II} synthetic analogues of the oxidized enzyme active site; however, reduction back to Fe^IFe^I results in rearrangement to the all terminal CO conformation and loss of the open site.[7]

Thus a major goal for modeling the active site should be to identify and evaluate supramolecular constructs that might enforce constraints mimicing the natural binding cavity of the [FeFe]-H_2ase active site. While this has been explored both computationally and synthetically,[8−10] inclusion of a diiron model system inside of a discrete supramolecular host has not yet been reported.

Cyclodextrins (CyD) have properties long recognized as potential scaffolds for biomimetics.[11] Their hydrophobic cavities have in fact been demonstrated to serve as hosts for organometallics,[12] and the hydrophilic hydroxyl rims provide hydrogen bonding sites. In this report we describe an approach to the inclusion of a small molecule model of the [FeFe]-H_2ase active site, (μ-$SCH_2NH(C_6H_4SO_3^-)CH_2S)[Fe^I(CO)_3]_2$ (**1**), within β-CyD and the X-ray crystal structure of the sodium salt of the **1**·2 β-CyD·28 H_2O clathrate.

The incorporation of charged functional groups into the guest molecules of cyclodextrin host/guest systems has been reported to provide a degree of stability to the inclusion complex.[13] For the diiron guest in our system, an aryl sulfonate group was incorporated into the S-to-S linker, as shown in Scheme 1. On addition of β-CyD to an aqueous slurry of $\mathbf{Na^+1}$, the latter completely dissolved. ESI mass spectral data indicated a peak bundle at $m/z = 1671$ representative of a 1:1 adduct in this solution. Infrared spectral monitoring also found a shift in the ν(CO) bands of **1** to higher wave numbers, as the β-CyD ratio was increased, Figure S1.

Scheme 1. Syntheses of Complex **1** and **1-CyD**



Crystals of X-ray diffraction quality were obtained by carefully layering a concentrated aqueous solution of **1-CyD** with a dilute aqueous solution of $[Ph_3P{=}N{=}PPh_3]^+Cl^-$. While the latter was necessary for crystal growth, the red needles that resulted on standing for two weeks were of the Na^+ salt only.[14a] The structure of **1-CyD** shows two β-CyD molecules serve as hosts to one [FeFe]-model complex guest, Figure 2. As anticipated, the aryl group of **1** is entirely encapsulated and the sulfonate projects through the primary hydroxyl rim of the β-CyD. The apical CO ligand of the $Fe(CO)_3$ unit on the same side is also enclosed within the cyclodextrin cavity while the basal CO's are close to the secondary hydroxyl rim. The $Fe(CO)_3$ group distal to the aryl sulfonate is encapsulated by the second β-CyD. The two β-CyD units interact with one another through hydrogen bonds that form between the secondary hydroxyl groups on the rim and also through ion−dipole interactions with the Na^+ counterion. This Na^+ links the two β-CyD's together and also interacts with neighboring units in the extended two-dimensional crystalline array, Figure 2. A total of 28 water molecules per Na^+ **1**·2 β-CyD unit are also in the crystal lattice, with four located near, or closing off, the end of the cyclodextrin nanocapsule opposite the sulfonate, Figure S5. No water molecules are located in the inner part of the cyclodextrin construct. The hydrophobic character of the 2 β-CyD cavity is emphasized by comparison of the electrostatic potential plots of **1-CyD** and that of the FeFe-H_2ase active site cavity which includes an ∼22 Å amino acid sphere, Figure S9.

10.1021/ja103774j

While the Fe−Fe bond distance of the included complex is not significantly altered from that of the free complex, 2.502(2) vs 2.499(1) Å, respectively, there are some minor structural differences between the structures of **1**[14b] and of **1** as found in **1-CyD**. Most important is a ca. 20° increase in the torsion angle between the apical CO groups of the $Fe(CO)_3$ subunits in **1** vs **1-CyD** indicating destabilization of the more eclipsed conformation.

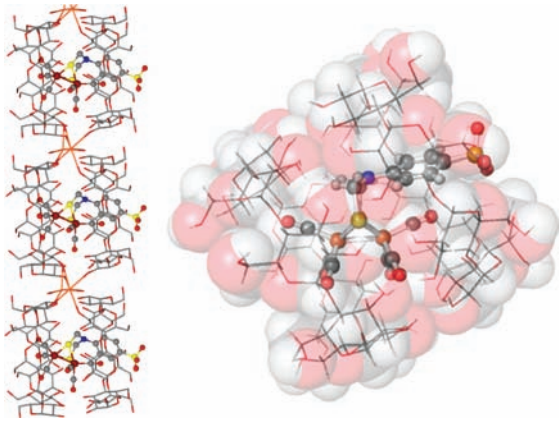


Figure 2. Extended structure (left) and unit cell (right) of **1 -CyD**. Protons and water molecules have been removed for clarity.

Evidence for inclusion complex formation in solution comes from the ^{1}H NMR studies in D_2O. As the mole fraction of β-CyD is increased, there is a significant upfield shift in the aromatic protons of **1**, with a concomitant downfield shift for the methylene protons in the S to S linker. Similarly, ^{13}C NMR spectra show a 1.0 ppm shift in the CO resonance at 206.9 ppm. For the cyclodextrin, the ^{1}H NMR spectra exhibit only minor shifts with variation in [**1**] ($\Delta\delta$ of $\pm$0.02) for most of the ^{1}H resonances. However the C3 and C5 protons shift by 0.10 and 0.19 ppm respectively. The large change for these intracavity protons is reported to be indicative of inclusion within the β-CyD cavity.[15] Averaged Job plots based on these changes have maxima near 0.5 indicating a 1:1 β-CyD/[**1**] ratio in solution, Figure S15.[16] However the accuracy of Job plots is highly dependent on the nature of the binding constant of the first and second β-CyD units, making this assignment tentative.

While the changes in the chemical shift provide good evidence that the inclusion complex is forming in solution, at no point were separate signals observed for the free and encapsulated **1** indicating that the rate of exchange in and out of the cyclodextrin cavity is faster than can be observed by NMR. Even upon cooling to 5 °C in the presence of 10 equiv of cyclodextrin only a single broad signal in the ^{13}C NMR spectrum is observed for all six CO ligands. These results are consistent with not only the fact that the complex is exchanging rapidly in and out of the cyclodextrin but also, and likely due to this exchange, that the cyclodextrin architecture does not prevent the well studied intramolecular dynamic processes observed in similar diiron systems.[17] Despite the apparent instability of **1-CyD**, the formation of the inclusion complex still produces interesting changes in the electrochemical properties of **1**.

Similar to most (μ-SRS)$[Fe(CO)_3]_2$ models, **1** acts as an electrocatalyst for H_2 production from weak acid. In CH_3CN solution, **1** has very similar redox properties to (μ-$S(CH_2)_3S)[Fe(CO)_3]_2$,[18] showing a response (increase in current) to added increments of HOAc at a more negative potential than the initial Fe^IFe^I/Fe^IFe^0 reduction. In 10 mM aqueous NaCl solution the electrochemical behavior is quite different. For free **1** the response is observed at the first reductive feature, the $Fe^IFe^I \rightarrow Fe^IFe^0$ couple at −1.2 V vs the Ag/AgCl (sat'd KCl) couple. In the presence of cyclodextrin however, the Fe^IFe^I/Fe^IFe^0 reduction is shifted ∼80 mV more negative and two reductive events are observed upon addition of HOAc. The first, based on control experiments without catalyst, is attributed to the reduction of HOAc by glassy carbon while the second event, assigned to proton reduction by **1-CyD**, occurs more negative (−1.4 V) reflecting the hydrophobic environment of the cavity, Figures S21 and S22.

In summary, we have used β-CyD to provide a first generation artificial protein environment for a small molecule model of the [FeFe]-hydrogenase enzyme active site. Inclusion in the cyclodextrin not only produces structural distortions in the diiron motif, as observed in the X-ray structure of **1-CyD**, but also affects change in the redox and electrocatalytic properties of **1**. Through synthetic modification of the host and guest components of the system, a model with intermolecular interactions that facilitate H^+ reduction or H_2 oxidation could be realized.

Acknowledgment. We thank Prof. Gyula Vigh for many helpful discussions. We also acknowledge financial support from the National Science Foundation (CHE-0910679 to M.Y.D., and CHE-0541587) and the R. A. Welch Foundation (A-0924).

Supporting Information Available: Full structure files; complete description of experiments; cyclic voltammograms and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Nicolet, Y.; Lemon, B. J.; Fontecilla-Camps, J. C.; Peters, J. W. *Trends Biochem. Sci.* **2000**, *25*, 138–143.
(2) Fontecilla-Camps, J. C.; Volbeda, A.; Cavazza, C.; Nicolet, Y. *Chem. Rev.* **2007**, *107* (10), 4273–4303.
(3) Hambourger, M.; Gervaldo, M.; Svedruzic, D.; King, P. W.; Gust, D.; Ghirardi, M.; Moore, A. L.; Moore, T. A. *J. Am. Chem. Soc.* **2008**, *130*, 2015–2022.
(4) Pandey, A. S.; Harris, T. V.; Giles, L. J.; Peters, J. W.; Szilagyi, R. K. *J. Am. Chem. Soc.* **2008**, *130*, 4533–4540.
(5) (a) Barton, B. E.; Rauchfuss, T. B. *Inorg. Chem.* **2008**, *47* (7), 2261–2263. (b) Jablonskyte, A.; Wright, J. A.; Pickett, C. J. *Dalton Trans.* **2010**, *39* (12), 3026–3034.
(6) Nicolet, Y.; de Lacey, A. L.; Vernède, X.; Fernandez, V. M.; Hatchikian, E. C.; Fontecilla-Camps, J. C. *J. Am. Chem. Soc.* **2001**, *123*, 1596–1601.
(7) (a) Liu, T.; Darensbourg, M. Y. *J. Am. Chem. Soc.* **2007**, *129*, 7008–7009. (b) Justice, A. K.; Rauchfuss, T. B.; Wilson, S. R. *Angew. Chem., Int. Ed.* **2007**, *46*, 6152–6154. (c) Singleton, M. L.; Bhuvanesh, N.; Reibenspies, J. H.; Darensbourg, M. Y. *Angew. Chem., Int. Ed.* **2008**, *47*, 9492–9495. (d) Justice, A. K.; De Gioia, L.; Nilges, M. J.; Rauchfuss, T. B.; Wilson, S. R.; Zampella, G. *Inorg. Chem.* **2008**, *47* (16), 7405–7414.
(8) Dy, E. S.; Kasai, H.; Redshaw, C.; Pickett, C. J. *Surf. Interface Anal.* **2008**, *40*, 1092–1097.
(9) Jones, A. K.; Lichtenstein, B. R.; Dutta, A.; Gordon, G.; Dutton, P. L. *J. Am. Chem. Soc.* **2007**, *129*, 14844–14845.
(10) Song, L.-C.; Liu, X.-F.; Ming, J.-B.; Ge, J.-H.; Xie, Z.-J.; Hu, Q.-M. *Organometallics.* **2010**, *29*, 610–617.
(11) Breslow, R.; Dong, S. D. *Chem. Rev.* **1998**, *98*, 1997–2011.
(12) (a) Harwani, S.; Telford, J. R. *Chemtracts* **2005**, *18* (8), 437–448. (b) Hapiot, F.; Tilloy, S.; Monflier, E. *Chem. Rev.* **2006**, *106*, 767–778.
(13) Kano, K. *Colloid Polym. Sci.* **2008**, *286*, 79–84.
(14) (a) Crystal data for **1-CyD**: $C_{98}H_{204}Fe_2NNaO_{107}S_3$, M = 3339.49, triclinic, $P1$ (No. 1), a = 15.346(4), b = 15.422(4), c = 17.595(4) Å, α = 113.517(3), β = 98.507(3), γ = 103.154(3)°, V = 3582.9(15) Å^3, Z = 1, D_c = 1.548 g/cm3, F_{000} = 1766, Final GooF = 0.999, $R1$ = 0.0832, $wR2$ = 0.1797. (b) Crystal data for **TEA$^+$1**: $C_{22}H_{24}Fe_2N_2O_9S_3$, M = 668.31, monoclinic, $P2(1)/c$, a = 22.1279(2), b = 7.6853(5), c = 16.7425(10) Å, α = 90, β = 99.752(2), γ = 90°, V = 2806.1(2) Å^3, Z = 4, D_c = 1.582 g/cm3, F_{000} = 1368, Final GooF = 1.064, $R1$ = 0.0408 $wR2$ = 0.0888.
(15) Schneider, H.-J.; Hacket, F.; Rüdiger, V.; Ikeda, H. *Chem. Rev.* **1998**, *98*, 1755–1785.
(16) Connors, K. *Binding Constants. The measurement of Molecular Complex Stability*; Wiley: New York, 1987.
(17) Lyon, E. J.; Georgakaki, I. P.; Reibenspies, J. H.; Darensbourg, M. Y. *J. Am. Chem. Soc.* **2001**, *123*, 3268–3278.
(18) Felton, G. A. N.; Mebi, C. A.; Petro, B. J.; Vannucci, A. K.; Evans, D. H.; Glass, R. S.; Lichtenberger, D. L. *J. Organomet. Chem.* **2009**, *694* (17), 2681–2699.

JA103774J