J|A|C|S
COMMUNICATIONS

Quantum Refinement of [FeFe] Hydrogenase Indicates a Dithiomethylamine Ligand

Ulf Ryde,*,[†] Claudio Greco,[‡] and Luca De Gioia[‡]

Theoretical Chemistry, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden, and Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 1, 20126 Milan, Italy

Received October 29, 2009; E-mail: Ulf.Ryde@teokem.lu.se

Hydrogenases play an important role in the metabolism of many microorganisms by performing reversible oxidation of hydrogen: $H_2 \rightleftarrows 2H^+ + 2e^-$.[1] Three classes are known, depending on the metal content of the active site: [NiFe], [FeFe], and [Fe] hydrogenases. The [FeFe] hydrogenases have been extensively studied using experimental and theoretical methods.[1–3] Crystal structures are available for [FeFe] hydrogenases from two bacteria.[4,5] The active site consists of a ferredoxin-like [4Fe4S] cluster connected via a Cys residue to a binuclear Fe_2 cluster, where each Fe ion has one CO and one CN^- ligand. In addition, there are three bridging groups, another CO, and a dithiolate ligand (Figure 1). The Fe ion distal to the [4Fe4S] cluster (Fe_d) has a vacant coordination site that is believed to be the reactive center. In some oxidized structures, a water molecule is found in this site.

The nature of the dithiolate ligand has been much discussed. It was originally tentatively assigned as $CH_2(CH_2S^-)_2$.[4a,b] However, several groups have suggested that $NH(CH_2S^-)_2$ is a more likely interpretation[2,4c,6] because the amine group could act as a proton acceptor during the catalytic cycle.[3] Recently, a new crystal structure was presented together with density functional theory (DFT) calculations indicating that the dithiolate ligand is actually $O(CH_2S^-)_2$.[5] The authors compared six ligands $X(CH_2S^-)_2$, where $X = CH_2$, NH, NH_2^+, S^-, SH, or O, and showed that a 200 atom model of the active site with $O(CH_2S^-)_2$ had the lowest relaxation energy when optimized in vacuum starting from the crystal structure.

Unfortunately, such an approach strongly depends on small errors in the crystal structure and the theoretical method, as is illustrated by the large relaxation energies involved (>200 kJ/mol). A more satisfactory approach is to include the DFT calculations in the crystallographic refinement itself, so each structure becomes an ideal compromise between DFT and crystallography. This is possible with the quantum refinement approach developed in our group,[7] in which the molecular mechanics force field normally used to supplement the raw crystallographic data is replaced by more accurate DFT calculations for the active site of the protein. In this way, the structure can be improved and the nature of the atoms (e.g., the protonation state of metal ligands) can be determined.[8] In this work, we used this approach to decide which form of the dithiolate ligand best fits the raw crystallographic data.

The calculations were based on the 1.39 Å structure of [FeFe] hydrogenase from *Clostridium pasteurianum* (PDB entry 3c8y).[5] We used the quantum-mechanical (QM) system $Fe_2(CO)_3(CN)_2(H_2O)(SCH_2XCH_2S)(CH_3S)_4(Fe_4S_4)(CH_3SH)$, wherein $X = CH_2$, O, NH_2^+, or NH with the H atom pointing either up (NH_{up}) or down (NH_{down}) relative to the Fe cluster (Figure 1). This model includes the catalytic Fe_2 cluster, the connected [4Fe4S] cluster, and a model of Cys299, which may accept or donate a hydrogen bond to the X group. In keeping with the previous combined crystallographic and DFT work,[5] we studied the cluster in the H_{ox} ($Fe^{II}Fe^{I}$; $Fe^{II}{}_2Fe^{III}{}_2$) state, which gives a net charge of −3 for each of the X groups except NH_2^+ (−2). We employed the BP86[9] DFT method and the def2-SV(P) basis set,[10] using the broken-symmetry approach.[11] The models had a surplus of one unpaired electron. Two pairs of antiferromagnetically coupled high-spin Fe ions were considered for the [4Fe4S] subcluster along with two low-spin Fe ions in the binuclear subsite, both with significant spin, but most on Fe_d.[3] Test calculations with other models, functionals, and basis sets gave similar results [see the Supporting Information (SI)]. The calculation speed was increased using the resolution-of-identity approximation.[12]

Figure 1. Quantum-refined structures of the states with $X = NH_{up}$ (left) and NH_{down} (right).

Table 1. Results of the Quantum Refinement Calculations[a]

X	Fe−O	R_{free}	residue *R*	ΔE_{QM1}	Δr
CH_2	2.21	0.141833	0.480	144	0.59
NH_2^+	2.46	**0.141727**	**0.406**	163	**0.40**
NH_{down}	2.40	0.141806	0.418	**142**	0.45
NH_{up}	2.15	0.141809	0.462	154	0.61
O	2.12	0.141844	0.472	**142**	0.66
crystal	2.38	0.142551	0.419		

[a] Fe−O distance and Δr in Å; ΔE_{QM1} in kJ/mol.

Several values of the weight factor between the QM and crystallographic energy functions (w_A) were tested (see the SI) and gave similar results. Therefore, only results for $w_A = 1$ (which gave the lowest values for the R_{free} factor) are presented in Table 1. All five X groups gave very similar Fe−Fe (2.54 Å) and Fe−ligand distances. Only the rather weakly bound H_2O ligand showed differences larger than 0.02 Å. In the NH_2^+ and NH_{down} structures, the X group forms a rather strong hydrogen bond to the water ligand (1.59 and 1.74 Å, respectively). Therefore, the Fe−O bond is essentially broken (2.46 and 2.40 Å), and the two hydrogen atoms of water point toward the CN and CO ligands of Fe_d (see Figure 1).

On the other hand, in the NH_{up} and O structures, the water ligand forms a hydrogen bond to the X group via one of its hydrogen atoms (1.57 and 1.65 Å), thereby strengthening the Fe−O bond

---

[†] Lund University.
[‡] University of Milano-Bicocca.

10.1021/ja909194f

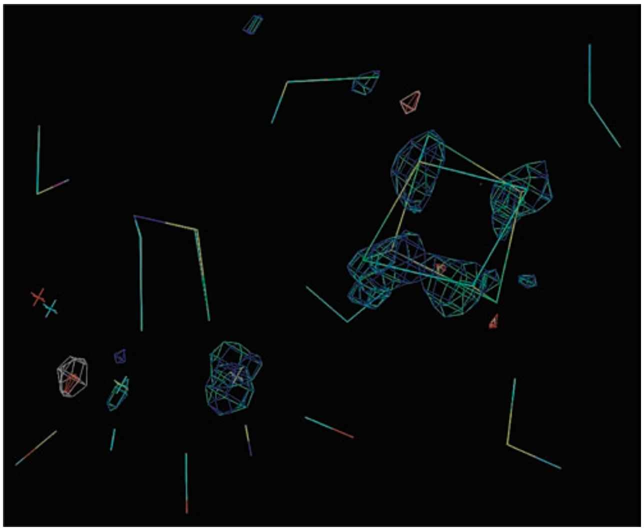

Figure 2. Electron density difference maps ($f_o - f_c$) for the states with X = NH_2^+ (atomic colors; red and blue densities) and O (cyan atoms; white and green densities). The densities (4.0 σ) are shown in red or white (negative) and blue or green (positive). The larger white volume at the lower left is noteworthy.

(2.15 and 2.12 Å). The CH_2 structure is intermediate because the CH_2 group can form only a weak H−O interaction (1.95 Å), giving an intermediate Fe−O bond (2.21 Å). In the original crystal structure, the Fe−O distance is 2.38 Å.

In the calculations with X = CH_2, NH_2^+, and NH_{up}, the SH group of Cys299 accepts a hydrogen bond from the X group, with H−S distances of 2.93, 2.97, and 2.93 Å, respectively (Figure 1a). For the two other systems, the SH group of Cys299 instead donates a hydrogen bond to X with H−N/O distances of 2.11 Å (Figure 1b).

Because of these small differences in the geometry, there are only small differences in the crystallographic *R* factors. However, both the R_{free} and *R* factors are lowest for the NH_2^+ structure. Likewise, the residue (real-space) *R* factor is lowest for this structure (Table 1), indicating that the NH_2^+ structure fits the experimental electron density better than the other structures. This is also confirmed by the electron density maps, which show smaller differences for NH_2^+ than for the other ligands (Figure 2).

However, comparison of the quantum-refined structures with the same structures optimized in a vacuum (which shows the preferred structure of the active site) shows that the strain energy ΔE_{QM1} (i.e., the difference in the QM energies of the quantum system optimized in vacuum and in the crystal) is lowest for the NH_{up} and O structures (142 kJ/mol) and slightly higher for the NH_2^+ structure (166 kJ/mol) (Table 1). In contrast, comparison of the sums of the differences in the Fe−ligand bond distances for the structures optimized in vacuum and in the crystal (Δr in Table 1) shows that the NH_2^+ structure has the lowest deviation (0.40 Å). The latter two comparisons are complicated by the fact that the water ligand dissociates in vacuum for all of the complexes. Therefore, we performed the vacuum calculations with the Fe−O distance fixed to the value observed in the quantum refinements. We also deleted the Cys299 model because it otherwise may have moved to form hydrogen bonds with other atoms in the structure. The results also depended somewhat on the QM model used, although the structures with N atoms always gave the best results (see the SI). Thus, it is clear that the quality estimates indicate that a N atom is more favorable in the dithiolate ligand than either a C or an O atom.

It is notable that the QM energies of the NH_{down} and NH_{up} states are directly comparable (they contain the same atoms). The energies indicate that NH_{down} is the more stable conformation by 0−19 kJ/mol.

Consequently, we conclude that the quantum refinement calculations do not support the suggestion that the dithiolate ligand should be $O(CH_2S^-)_2$. On the contrary, the quality criteria in Table 1 indicate that central atom instead is nitrogen. These results are in accordance with recent EPR measurements as well as studies of the role of the HydG maturation factor.[13] The reason that the present results differ from the previous DFT study,[5] which was based on the same crystal structure, is that we used structures representing an optimum compromise between DFT and crystallography and directly measured how well the structures fit the raw crystallographic data (not the crystal coordinates, which are the result of an involved process of model building and refinement involving a molecular mechanics potential) in the form of crystallographic *R* factors.

Acknowledgment. This investigation was supported by grants from the Swedish Research Council and by computer resources of Lunarc at Lund University.

Supporting Information Available: Detailed description of the method, optimized Fe−ligand distances and quality criteria with three different QM systems, and results of calculations with the B3LYP method or larger basis sets. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Adams, M. W. W. *Biochim. Biophys. Acta* **1990**, *1020*, 115. (b) Vignalis, P. M.; Billoue, B.; Meyer, J. *FEMS Microbiol. Rev.* **2001**, *25*, 455.

(2) (a) Lubitz, W.; Reijerse, E.; van Gastel, M. *Chem. Rev.* **2007**, *107*, 4331. (b) Tard, C.; Pickett, C. J. *Chem. Rev.* **2009**, *109*, 2245.

(3) (a) Siegbahn, P. E. M.; Hall, M. B. *Chem. Rev.* **2007**, *107*, 4414. (b) Greco, C.; Bruschi, M.; De Gioia, L.; Ryde, U. *Inorg. Chem.* **2007**, *46*, 5911.

(4) (a) Peters, J. W.; Lanzilotta, W. N.; Lemon, B. J.; Seefeldt, L. C. *Science* **1998**, *282*, 1853. (b) Nicolet, Y.; Piras, C.; Legrand, P.; Hatchikian, C. E.; Fontecilla-Camps, J. C. *Structure* **1999**, *7*, 13. (c) Nicolet, Y.; de Lacy, A. L.; Venede, X.; Fernandez, V. M.; Hatchikaian, E. C.; Fontecilla-Camps, J. C. *J. Am. Chem. Soc.* **2001**, *123*, 1596. (d) Nicolet, Y.; Lemon, B. J.; Fontecilla-Camps, J. C.; Peers, J. W. *Trends Biochem. Sci.* **2000**, *25*, 138.

(5) Pandey, A. S.; Harris, T. V.; Giles, L. J.; Peters, J. W.; Szilagyi, R. K. *J. Am. Chem. Soc.* **2008**, *130*, 4533.

(6) (a) Nicolet, Y.; Cavazza, C.; Fontecilla-Camps, J. C. *J. Inorg. Biochem.* **2002**, *91*, 1. (b) Barton, B. E.; Olsen, M. T.; Rauchfuss, T. B. *J. Am. Chem. Soc.* **2008**, *130*, 16834.

(7) (a) Ryde, U.; Olsen, L.; Nilsson, K. *J. Comput. Chem.* **2002**, *23*, 1058. (b) Ryde, U. *Dalton Trans.* **2007**, 607.

(8) (a) Ryde, U.; Nilsson, K. *J. Am. Chem. Soc.* **2003**, *125*, 14232. (b) Nilsson, K.; Ryde, U. *J. Inorg. Biochem.* **2004**, *98*, 1539.

(9) (a) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098. (b) Perdew, J. P. *Phys. Rev. B* **1986**, *33*, 8822.

(10) Weigend, F.; Ahlrichs, R. *Phys. Chem. Chem. Phys.* **2005**, *7*, 3297.

(11) Noodleman, L.; Peng, C. Y.; Case, D. A.; Mouesca, J. M. *Coord. Chem. Rev.* **1995**, *144*, 199.

(12) (a) Eichkorn, K.; Treutler, O.; Öhm, H.; Häser, M.; Ahlrichs, R. *Chem. Phys. Lett.* **1995**, *240*, 283. (b) Eichkorn, K.; Weigend, F.; Treutler, O.; Ahlrichs, R. *Theor. Chem. Acc.* **1997**, *97*, 119.

(13) (a) Silakov, A.; Wenk, B.; Reijerse, E.; Lubitz, W. *Phys. Chem. Chem. Phys.* **2009**, *11*, 6592. (b) Pilet, E.; Nicolet, C.; Mathevon, C.; Douki, T.; Fontecilla-Camps, J. C.; Fontecave, M. *FEBS Lett.* **2009**, *583*, 506.

JA909194F