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Density Functional Calculations on the Binding of Dinitrogen to the FeFe Cofactor in Fe-Only Nitrogenase: FeFeco(μ_6-N_2) as Intermediate in Nitrogen Fixation

Zexing Cao,*,† Zhaohui Zhou,† Huilin Wan,† Qianer Zhang,† and Walter Thiel‡

Department of Chemistry, State Key Laboratory for Physical Chemistry of Solid Surfaces,
Center for Theoretical Chemistry, Xiamen University, Xiamen 361005, China, and
Max-Planck-Institut für Kohlenforschung, Mülheim an der Ruhr, Germany

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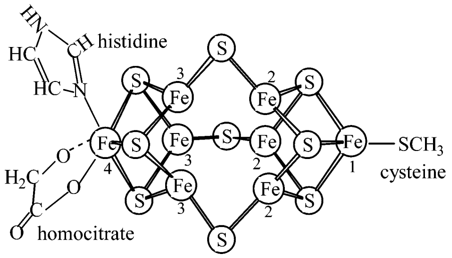
The geometries and stabilities of the FeFe cofactor at different oxidation states and its complexes with N_2 have been determined by density functional calculations. These calculations support an EPR-inactive resting state of the FeFe cofactor with four Fe^{2+} and four Fe^{3+} sites ($4Fe^{2+}4Fe^{3+}$). FeFeco(μ_6-N_2) with a central dinitrogen ligand is predicted to be the most stable complex of the FeFe cofactor with N_2. It is easily formed by penetration of N_2 into the trigonal Fe_6 prism of the FeFe cofactor with an approximate barrier of 4 kcal mol^{-1}. The present DFT results suggest that an FeFeco(μ_6-N_2) entity is a plausible intermediate in dinitrogen fixation by nitrogenase. CO is calculated to bind even more strongly than N_2 to the FeFe cofactor so that CO may inhibit the reduction of nitrogen by Fe-only nitrogenase.

The enzymatic activity of the molybdenum-bearing nitrogenase, the most extensively characterized of nitrogenases,[1−3] centers on the transfer of electrons from the P cluster to the FeMo cofactor of the FeMo protein. The FeMo cofactor is the active site of dinitrogen (N_2) binding and reduction. Nitrogenases containing iron and vanadium have been found in bacteria;[3,4] both molybdenum and vanadium are absent in Fe-only nitrogenase. The enzymatic activity of V-nitrogenase and Fe-nitrogenase is lower than that of Mo-nitrogenase; however, as such nitrogenases display high rates of hydrogen formation by proton reduction, they are of potential use in biotechnology.[4] Biochemical, EPR,[3] EXAFS, and Mössbauer[4] studies have shown that the FeFe cofactor (FeFeco) of Fe-nitrogenase and the FeMo cofactor (FeMoco) of Mo-nitrogenase are isostructural, the only important difference being the replacement of the molybdenum by an iron atom (Scheme 1). In the FeFeco part of Fe-only nitrogenase,[4] the Fe−S distance is 2.29 Å and the Fe−Fe distance is 2.63 Å; the nonbonded Fe−Fe distance in the Fe_6 prism is 3.68 Å. Spectroscopic data for the interaction of N_2 with nitrogenases are not available.[5] Theoretical calculations on N_2 binding in complexes that resemble FeMoco have been performed at several levels of theory ranging from the semiempirical extended Hückel approach,[6] CNDO,[7] and INDO[8] to density functional theory (DFT).[9−12]

Scheme 1. The $[Fe_8S_9(SCH_3)(imidazole)(O_2CCH_2O)]^-$ Anion Calculated as a Model for the FeFe Cofactor in Fe-Only Nitrogenase



The present model for the FeFe cofactor is the $[Fe_8S_9(SCH_3)(imidazole)(O_2CCH_2O)]^-$ anion **a** (Scheme 1), which possesses four Fe^{2+} and four Fe^{3+} sites ($4Fe^{2+}4Fe^{3+}$) in accordance with recent EXAFS and Mössbauer spectroscopic measurements of the Fe-only nitrogenase from *Rhodobacter capsulatus*.[4] DFT calculations were performed with the DMol3 (Accelrys) program,[13] which determines variational

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* Author to whom correspondence should be addressed. E-mail: zxcao@xmu.edu.cn.

† Xiamen University.

‡ Max-Planck-Institut.

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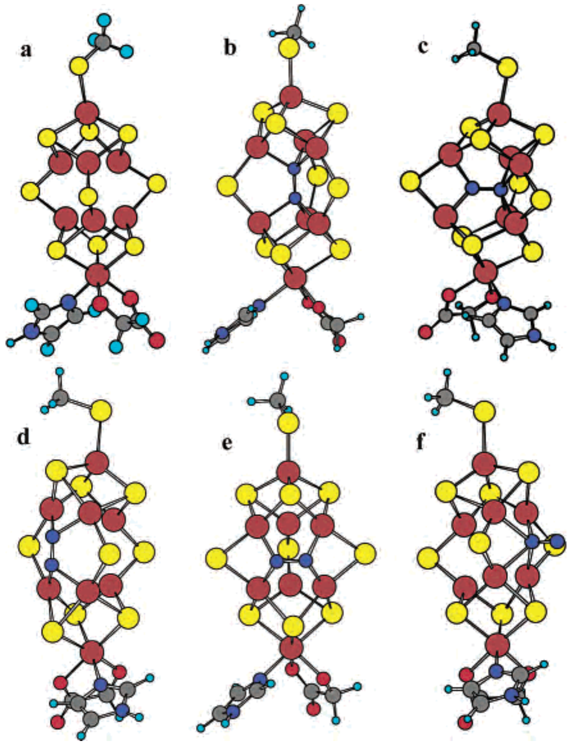

Figure 1. The structures of complexes of the FeFe cofactor (**a**) with N_2 bonded in inside-prism (**b**, **c**), side-on (**d**, **e**), and end-on (**f**) fashions. Atoms are identified by color: S, yellow; O, red; C, gray; Fe, brown; N, blue; H, green.

self-consistent solutions to the DFT equations, expressed in a numerical atomic orbital basis. The Perdew−Wang PW91 functional[14] and double numerical basis sets[13] in connection with effective core potentials for iron atoms[15] were employed in spin-unrestricted Kohn−Sham (UKS) computations. The spin contamination in these UKS calculations was generally found to be negligible. For example, the expectation values of $\langle S \rangle^2$ for the doublet and triplet states of the neutral and anionic FeFeco forms are 0.7593 and 2.0204, respectively. In the case of singlet states, the UKS treatment always started from an appropriately chosen initial spin coupling scheme for the eight Fe sites (consistent with the given oxidation levels, see below), but converged to the same results as separate closed-shell calculations (e.g., for **a**−**f** in Figure 1).

These calculations predict in **a** Fe−Fe bond lengths for Fe(2)−Fe(2) and Fe(3)−Fe(3) of 2.63 and 2.61 Å, respectively (Table 1). The averaged optimized Fe(2)−Fe(3) cross-cluster separation, i.e., the diagonal of the Fe_4 facet, is 3.68 Å. These calculated bond lengths agree very well with available experimental data.[4] It should be noted that the calculated Fe(3)−Fe(4) distance is 2.94 Å, larger than the Fe(1)−Fe(2) separation, and may correspond to the Fe−Fe distance of 2.92 Å from the EXAFS spectra,[4] even though the latter has been assigned to the P cluster. The singlet EPR-silent form of the FeFeco model is more stable than the triplet paramagnetic form by ca. 1 kcal mol^{-1}. Ionization of the FeFeco model anion requires 77.9 kcal mol^{-1}, giving rise to a doublet neutral form with five Fe^{3+} and three Fe^{2+} sites (oxidation level $5Fe^{3+}3Fe^{2+}$). The relatively high stability of the EPR-inactive $4Fe^{2+}4Fe^{3+}$ FeFeco model **a** suggests that it can serve as candidate for the resting state of FeFeco in Fe-only nitrogenase. It can accommodate an electron to yield the $3Fe^{3+}5Fe^{2+}$ oxidation state with an exothermicity of ca. 40 kcal mol^{-1}. The addition of another electron leads to a more reduced $2Fe^{3+}6Fe^{2+}$ cofactor structure which is less stable than the $4Fe^{2+}4Fe^{3+}$ FeFeco model by ca. 46 kcal mol^{-1}. The optimized $2Fe^{3+}6Fe^{2+}$ cofactor structure has a distorted Fe_8S_9 cage, whereas the other three less reduced FeFeco species have similar undistorted geometries (Table 1).

Table 1. Selected Calculated Average Bond Lengths (Å)[a]

FeFeco	R_{12}/R_{34}	R_{22}/R_{33}	R_{23}/R'_{23}	Fe−S
		DFT		
$2Fe^{3+}6Fe^{2+}$	2.70/3.11	2.60/2.60	2.59/3.61	2.19−2.40
$3Fe^{3+}5Fe^{2+}$	2.67/2.93	2.62/2.62	2.58/3.67	2.18−2.39
$4Fe^{3+}4Fe^{2+}$	2.67/2.94	2.63/2.61	2.58/3.68	2.18−2.40
$5Fe^{3+}3Fe^{2+}$	2.67/2.97	2.63/2.63	2.58/3.68	2.18−2.40
		EXAFS[b]		
		2.63/2.63	/3.68	2.29

[a] R_{ij} denotes the distance between the Fe(i) and Fe(j) atoms; the numbering refers to Scheme 1; R' indicates the Fe−Fe cross-cluster distance in the Fe_6 trigonal prism. [b] Reference 4.

It should be mentioned that although complex **a** is a fairly complete model of FeFeco, the description of the oxidation and reduction of the cluster does not take into account the surrounding protein structure and water, and calculated numerical values may bear little relation to the actual experimental values. The relative stability and geometry of FeFeco at different oxidation states may be altered by the influence of the protein environment as shown in previous theoretical studies on P450 enzymes.[16] The $2Fe^{3+}6Fe^{2+}$ oxidation state for FeFeco has been studied by recent DFT calculations,[17] which provide Mössbauer isomer shifts in agreement with experimental values.

Among the complexes of N_2 bonded to FeFeco in inside-prism, side-on, and end-on fashions, the complex FeFeco-(μ_6-N_2), **b**, which has the N_2 molecule in the center of FeFeco, is the most stable form (Figure 1 and Table 2). In the Fe_6 prism formed by the three Fe(2) and three Fe(3) atoms (Scheme 1) the Fe(2)−Fe(3) edge is much longer in **b** (3.45 Å) than in FeFeco **a** (2.58 Å), whereas other Fe−Fe separations increase only slightly, by less than 0.1 Å. Although the Fe_6 prism stretches significantly as N_2 enters into the FeFeco cage, the complex **b** is still more stable than the separated components by ca. 29 kcal mol^{-1}. Such high stability for FeFeco(μ_6-N_2) shows that the FeFeco cage is fairly flexible.

The second most stable complex is another inside-prism form **c** with a binding energy of ca. 20 kcal mol^{-1}. As in the case of **b**, the trigonal prism in **c** expands strikingly in

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Table 2. Calculated Binding Energies (kcal mol^{-1}), Selected FeFe Separations, NN Bond Lengths (Å), and Mulliken Charge Populations on Dinitrogen in Complexes of the FeFe Cofactor with N_2

	b	**c**	**d**	**e**	**f**
$E_b{}^a$	29.5	19.7	10.9	12.9	4.2
$R_{22}{}^b$	2.786	3.310	2.600	2.800	2.570
R_{23}	3.450	2.720	3.387	2.700	2.680
R_{NN}	1.290	1.290	1.245	1.232	1.197
q_{NN}	−0.027	−0.103	−0.022	−0.085	−0.223

[a] Binding energy: $E_b = [E_{tot}(\text{FeFeco}) + E_{tot}(N_2)] - E_{tot}(\text{FeFeco}(N_2))$. [b] R_{ij} denotes the distance between the Fe(i) and Fe(j) atoms; the numbering refers to Scheme 1, where only Fe atoms in the Fe_4 facet bonding N_2 are considered.

comparison with **a** (Tables 1 and 2). The side-on complexes **d** and **e** of N_2 coordinated to one of the Fe_4 facets of the cofactor have comparable stability (ca. 11−13 kcal mol^{-1}). The end-on complex **f** of N_2 attached to the Fe_4 facet is only weakly bound by ca. 4 kcal mol^{-1}. In the complexes **b** and **c**, the NN bond length of 1.29 Å is intermediate between that of a single N−N bond and a double N═N bond, showing that dinitrogen is significantly activated by FeFeco.

The other NN bond lengths listed in Table 2 indicate that dinitrogen activation also occurs in complexes **d**−**f**, but to a lesser extent. According to the Mulliken charge populations in Table 2, there is only a net charge of −0.027 on dinitrogen in complex **b**, which suggests that the dinitrogen activation in FeFeco(μ_6-N_2) involves a donation−back-donation bonding interaction between N_2 and the two planar Fe_3 moieties of the Fe_6 prism.

An exploration of the potential energy surface (PES) shows that the most stable complex **b** is easily accessible. A PES scan along a path from **d** to **b** by a series of constrained optimizations reveals a barrier of only ca. 4 kcal mol^{-1} for penetration of N_2 into the Fe_6 prism (through the Fe_4 facet). The low barrier implies that the entry of N_2 into the trigonal prismatic Fe_6 unit is a facile process. It thus seems that there are only weak Fe−Fe interactions in the prism, which can be easily replaced by multiple Fe−N interactions upon association of N_2 to FeFeco.

Carbon monoxide is an inhibitor for the reduction of dinitrogen by nitrogenase. Interactions of carbon monoxide with the FeMo cofactor in Mo nitrogenases have been studied by EPR, ENDOR, and IR spectroscopy.[18−21] For comparison, the binding of CO to FeFeco was investigated by DFT calculations. Figure 2 displays two selected optimized structures **g** and **h**. Coordination of CO inside the Fe_6 prism (**g**) yields FeFeco(μ_6-CO) with an exothermicity of ca. 37 kcal mol^{-1}. Association of CO to one Fe_4 facet in side-on fashion (**h**) is also exothermic, by ca. 23 kcal mol^{-1}. The CO bond lengths in **g** and **h** are 1.300 and 1.251 Å, respectively, showing significant CO activation in both cases. Since the computed complexation energies of CO are even slightly higher than the corresponding values for N_2 (see above), CO should act as an inhibitor of the reduction of dinitrogen by Fe-only nitrogenase.

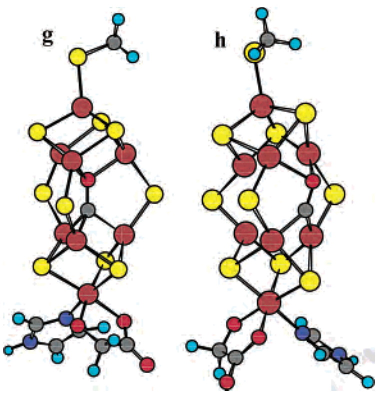

Figure 2. The structures of complexes of the FeFe cofactor with CO bonded in inside-prism (**g**) and side-on (**h**) fashions. Atoms are identified by color: S, yellow; O, red; C, gray; Fe, brown; N, blue; H, green.

In the case of the FeMo protein, FeMoco(μ_6-N_2) was postulated as a possible binding mode of dinitrogen in the cavity of the FeMo cofactor some time ago,[22] and upon optimizing a complex of the FeMoco model with dinitrogen inside the prism, we indeed find that FeMoco(μ_6-N_2) is quite similar to FeFeco(μ_6-N_2) both in geometry and in stability: N_2 coordinates inside the FeMoco model with an exothermicity of ca. 29 kcal mol^{-1}. However, a recent high-resolution (1.16 Å) crystal structure of the FeMo protein[23] has revealed a light atom (C, N, or O) in the center of the FeMoco cage. Chemical arguments[23] and theoretical calculations[24−26] indicate that this central ligand is most likely a nitrogen atom which, according to ENDOR and ESEEM measurements,[27] does not exchange during catalysis. It is clear that the presence of such an interstitial atom will block the cavity and will most probably prevent the formation of FeMoco(μ_6-N_2). It remains to be seen whether the isostructural FeFe cofactor in the Fe-only nitrogenase also contains a central ligand atom that has not been detected so far. If so, the most stable inside-prism complexes **b** and **c** should not be accessible, and the side-on complexes **d** and **e** might then become mechanistically more relevant (see Figure 1). Further work is in progress to explore these possibilities.

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