

Modeling a Central Ligand in the Nitrogenase FeMo Cofactor

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Nitrogenase catalyzes one of the most important reactions in biology, the conversion of nitrogen to ammonia (NH_3) and H_2 under ambient conditions.^{1–4} Structurally related systems are the Fe-only hydrogenases which catalyze the formation of H_2 .⁵ The most common form of nitrogenase consists of two metalloproteins, the Fe protein and the MoFe protein. The Fe protein contains a ferredoxin (4Fe-4S), and the MoFe protein contains two unique metal clusters, the P-cluster and the FeMo cofactor (FeMoco).² Most likely, the FeMoco is the active site, where N_2 binds and is reduced. The crystal structure of the FeMoco was first solved in 1992 by Kim and Rees, and, since then, the structures of all parts of nitrogenase have been determined with resolutions up to 1.6 Å.⁶ Very recently, a new crystal structure of the FeMo protein with a resolution of 1.16 Å has been determined by Einsle et al.⁷ This structure reveals that there is a ligand in the center of the FeMoco. The structure determined by Einsle et al. is shown in Figure 1a. They argue that the possible candidates for the atom type are nitrogen, oxygen, or carbon, but they cannot say which. In the present paper, we investigate the structural and energetic consequences of a central ligand in the FeMoco by means of density functional calculations. We conclude that nitrogen is the most likely candidate.

All calculations are carried out with the program *dacapo*,⁸ which uses a plane-wave expansion of the Kohn–Sham wave functions and the spin-dependent RPBE functional. For N_2 adsorption on Fe surfaces, where there are experiments with which to compare, this functional describes the adsorption energies and activation energies very well.⁹ We model the FeMoco by truncating the ligands, that is, substituting Cys by SH, His by NH_3 , and Homocitrate by two OH groups. The employed model is shown in Figure 1b. All atoms are relaxed. Our model has the same magnetic configuration as the one used in previous theoretical analyses of the reactions on the active site of the enzyme.^{10,11} It is clear that our model is very simple and does not take into account the surrounding protein structure. However, we base our conclusions on trends in energies and structures from one central ligand to the next, and they should be more accurate than the absolute results for each central ligand.

To investigate a central ligand in the FeMoco, one has to assign this ligand a meaningful oxidation state. In the case of nitrogen, this would be nitride (N^{3-}). This would require the transfer of three electrons to the cluster and a concomitant transfer of three protons. We model the coupled electron–proton transfers by introducing hydrogen atoms to the cluster. They are found to be most stable when binding to the bridging sulfur atoms, as it was suggested by Rod and Nørskov.¹⁰ Thus, we model the central ligand N by adding an N atom in the middle of the cluster and by adding three H atoms to the bridging sulfurs, which is shown in Figure 1c. In an analogous way, we model the O ligand, whose most sensible oxidation state is O^{2-} , by adding two H atoms to the bridging sulfurs, as shown in Figure 1d. The case of C as a central ligand is less straightforward, as C can have different oxidation states. We have chosen to model

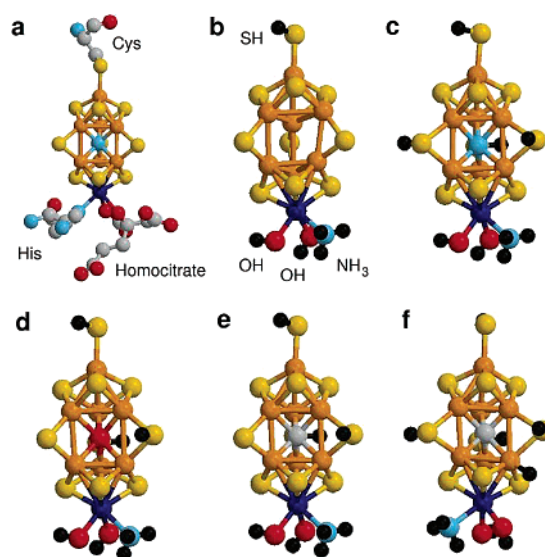


Figure 1. The nitrogenase FeMo cofactor from the recent crystal structure 1MIN⁷ (a) and the calculated model systems (b–f). The color scheme is dark blue (molybdenum), orange (iron), yellow (sulfur), red (oxygen), light blue (nitrogen), gray (carbon), black (hydrogen). The structures b–f are calculated energy minima. Note that structures a and f are rotated 120° clockwise relative to the other structures. In structure a, Einsle et al. assigned the central ligand to be nitrogen, hence the N color code. The figure was prepared using MolScript and Raster3D.¹²

Table 1. The Calculated Energy Differences and Magnetic Moments for the Structures in Figure 1b–f

structure in Figure 1	central ligand	no. of H atoms	molecule for comparison	ΔE [kJ/mol]	magnetic moment [μ_B]
c	N	3	NH_3	–56	3/2
d	O	2	H_2O	–38	5/2
e	C	2	$\text{CH}_4 - \text{H}_2$	61	3/2
f	C	4	CH_4	113	3/2

this situation with both two and four hydrogen atoms added, as shown in Figure 1e and f, respectively. In Figure 1f, we assume that the fourth H atom adsorbs end-on on one of the Fe atoms, as suggested by Rod and Nørskov.¹⁰

The energies of the structures in Figure 1b–f have been calculated, and the results are summarized in Table 1. To predict the stability of the FeMoco with a central ligand, we compare the energies of the clusters in Figure 1c–f to the energy of the FeMoco without a central ligand and an appropriate molecule in the gas phase. For N as a central ligand, it is meaningful to use NH_3 for comparison, as this is the main product. In the case of O, we use H_2O , as water is present around the cluster. Again, the case of C is less straightforward. In the case of two added H atoms, we use the energy difference between CH_4 and H_2 , and in the case of four H atoms added, we use CH_4 . Although CH_4 is not a product, it is

Table 2. Comparison of Selected Calculated and Reported Experimental Parameters (Å and deg)^a

bond ^b	exp. FeMoco ^c (a)	FeMoco (b)	FeMoco + N + 3H (c)	FeMoco + O + 2H (d)	FeMoco + C + 2H (e)	FeMoco + C + 4H (f)
Fe'–X	1.98 ± 0.05		2.07 ± 0.10	2.16 ± 0.06	1.98 ± 0.12	2.00 ± 0.13
Fe''–X	2.02 ± 0.04		1.98 ± 0.01	2.13 ± 0.01	1.96 ± 0.02	2.00 ± 0.14
∠Fe'–X–Fe''	80.9 ± 2.5		83.5 ± 1.2	83.6 ± 2.5	82.3 ± 2.3	83.6 ± 2.5
Fe–Fe'	2.67 ± 0.01	2.65 ± 0.03	2.69 ± 0.01	2.70 ± 0.02	2.66 ± 0.02	2.68 ± 0.02
Fe'–Fe'	2.65 ± 0.01	2.74 ± 0.18	2.59 ± 0.02	2.82 ± 0.12	2.57 ± 0.02	2.61 ± 0.05
Fe'–Fe''	2.59 ± 0.02	2.72 ± 0.05	2.70 ± 0.09	2.86 ± 0.05	2.60 ± 0.02	2.67 ± 0.08
Fe''–Fe''	2.61 ± 0.02	2.66 ± 0.09	2.65 ± 0.06	2.70 ± 0.12	2.57 ± 0.02	2.65 ± 0.07
Fe''–Mo	2.69 ± 0.02	2.82 ± 0.08	2.76 ± 0.03	2.75 ± 0.03	2.74 ± 0.06	2.72 ± 0.03
Fe'–Fe''	3.70 ± 0.01	3.78 ± 0.61	3.76 ± 0.07	3.98 ± 0.10	3.66 ± 0.11	3.71 ± 0.23
diagonal ^d						

^a Average and standard deviation determined over the same bond types. ^b Fe denotes the upper Fe atom, Fe' denotes the Fe atoms in the triangle adjacent to the Fe atom, and Fe'' denotes the Fe atoms in the triangle adjacent to the Mo atom. X denotes the central atom (X = N, O, or C). ^c Taken from the crystal structure 1M1N and averaged over the four copies of the FeMo cofactor. ^d Averaged over the three face diagonals.

the simplest hydrocarbon and thus a meaningful energy level in an organic environment. The binding energy ΔE has been determined from the calculated total energies with the expression

$$\Delta E = E_{\text{tot}}(\text{FeMoX}) - [E_{\text{tot}}(\text{FeMo}) + E_{\text{tot}}(\text{X})] \quad (1)$$

where FeMoX is the FeMoco with a central ligand (Figure 1c–f), FeMo is the FeMoco without a central ligand (Figure 1b), and X is the molecule used for comparison.

From the binding energies in Table 1, one can see that only the central ligands N and O lead to a stable cluster structure, as the binding energies are negative. Both with two and with four adsorbed hydrogen atoms, C as a central ligand does not lead to a stable structure. This suggests that C likely can be ruled out as a central ligand. However, with both N and O as the central ligand, the FeMoco is stable with respect to without a central ligand and NH₃ or H₂O, respectively. However, from the fact that the binding energy ΔE for N is somewhat lower than that for O (18 kJ/mol), one cannot decide unambiguously which ligand is present. Therefore, we turn to the geometry of the structures, where we have chosen to focus on the Fe–ligand and Fe–Fe distances. In Table 2, averages of bond distances and angles are listed. Concerning the Fe–ligand distances, N and C are consistent with the experimental value, whereas the Fe–O distance is approximately 0.1 Å too large. The same applies to the Fe–Fe distances, especially the bond lengths within and between the two triangles, and shows that the cluster is expanded as compared to the experimental structure. Thus, from the bond distances we can conclude that the values for N and C are consistent with the experimental values, but we can render O as a rather improbable central ligand. Together with the energetics, this renders nitrogen as the most probable central ligand, which agrees with the suggestion of Einsle et al.

Thermodynamic considerations show that if O was the central ligand and if it was produced by dissociating water, the fraction of FeMoco present with a central ligand would be extremely low, due to the entropy loss of the water. This also supports the proposal that the central ligand is nitrogen. To describe the dynamics of the insertion, one would need the transition state and the barrier energy for, for example, the dissociation of NH₃, insertion of the N into the FeMoco, and adsorption of the H atoms on the bridging S atoms. The central nitrogen must be formed during turnover, and it is therefore also interesting to compare its stability to, for example,

N₂ and H₂ in the gas phase, see Table 1. It is clear that there is a thermodynamic driving force for this reaction to take place.

It remains to be seen whether the possible nitrogen atom in the center of the cluster is an active intermediate for the reaction, an inactive spectator, or perhaps a product formed after turnover is ceased (for instance, from adsorbed NH₃ which cannot be removed). More experiments and calculations are needed to solve this problem.

In summary, we have shown that N is the only central ligand which leads both to an energetically stable structure and to a geometry resembling the one found experimentally. Thus, we conclude that the central ligand is most likely nitrogen.

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Supporting Information Available: Technical details of the DFT calculations and coordinates of the minimized structures (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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