

Influence of His-195 on the nitrogenase FeMo-cofactor activity

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ABSTRACT

Mo-nitrogenase is the enzyme responsible for the conversion of atmospheric nitrogen to ammonia. Its active site is the iron-molybdenum cofactor, FeMo-co, which is composed by two incomplete cubes, Fe_4S_3 and MoFe_3S_3 , connected through three bridging S. N_2 adsorption is expected to take place on the FeMo-co, and to be catalytically converted to two NH_3 molecules through the successive addition of protons and electrons. Mutant studies have evidenced that the activity of the nitrogenase FeMo-co is significantly influenced by the environment. In particular the mutation of HIS-195 to glutamine inhibits its ability to reduce nitrogen, though leaving intact its capability to adsorb N_2 . In this work we investigated theoretically the adsorption of N_2 and of one hydrogen atom in presence of HIS-195. We performed our simulations with both C and N as central atoms, as it has been recently shown experimentally that N might not be the FeMo-co interstitial atom and C is a reasonable candidate. We report energetic results for four different N_2 adsorption mechanisms. In particular we identify a favorable adsorption mechanism characterized by a partial opening of the FeMo-co which is here proposed for the first time.

Keywords: Biochemistry, Nitrogenase, Enzyme, Quantum Chemistry, Simulation.

1 INTRODUCTION

The enzyme is composed by two metallo-proteins, the iron protein, that has the function of transferring electrons, and the molybdenum iron protein, on which the nitrogen fixation takes place. The enzyme active site is the iron-molybdenum cofactor, FeMo-co, which can be considered as composed of two incomplete cubes, $\text{Fe}_4(\mu_3\text{-S})_3$ and $\text{MoFe}_3(\mu_3\text{-S})_3$, connected through three bridging $\mu_2\text{-S}$. The structure of the FeMo-co has been the subject of extensive experimental¹⁻³ and theoretical investigations,⁴⁻⁸ which showed that an interstitial atom is present at the center of the MoFe-co. The theoretical attribution of the central atom identity to Nitrogen^{4,9} has been recently questioned on the basis of experimental studies.³ N_2 adsorption is expected to take place on the FeMo-co, and to be catalytically converted to two NH_3 molecules through the successive addition of protons and electrons. Mutant studies have evidenced that the activity of the nitrogenase MoFe-co is significantly influenced by the environment. In particular the mutation of

α HIS-195 to glutamine and α GLN-191 to lysine inhibits the ability of the MoFe-co to reduce nitrogen, though living intact its capability to adsorb N_2 . While GLN-191 is relatively far from the candidates N_2 adsorption sites of the FeMo-co, HIS-195 is positioned in proximity of a bridging $\mu_2\text{-S}$, with which it can interact to form an N-H-S bond. This suggests that N_2 adsorption is likely to take place in proximity of a $\mu_2\text{-S}$, and that one or both of the two Fe atoms to which it is connected are directly involved. It has been proposed that the action of HIS-195 is either that of assisting directly the N_2 adsorption, or to play an active role in transposing of adsorbed hydrogen atoms from the FeMo-co and N_2 .

2 METHOD

In this work we investigated theoretically the adsorption of N_2 and of the first of the hydrogen atoms necessary for the Thornely-Lowe scheme, in presence of HIS-195. Simulations were performed with density functional theory using the BLYP functionals. The Stuttgart-Dresden effective core potential basis sets were used for the Fe and Mo atoms while for all the other atoms we adopted the Dunning-Huzinaga double ζ basis set. All simulations were performed with the Gaussian 03 computational suite.¹⁰ The molecular model used for the simulations is shown in Fig. 1. It has been obtained truncating the protein structure and replacing CYS-275 by SH and homocitrate with OCH_2CO_2 .

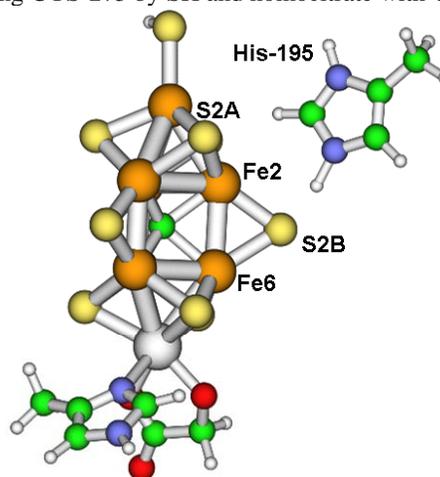


Figure 1. Site model

Only the imidazole ring of HIS-195 and HIS-442 is considered, while a CH₃ group is adopted to truncate the connection with the protein. The protonated form of HIS-195 was used in all the simulations, as it gave the results, in term of structural configuration, in best agreement with experimental data. The C atoms of the CH₃ groups and the S atom of the Cys residues are held fixed in the X-Ray positions of the 1M1N pdb structure during all optimizations. Given the uncertainty about the identity of the FeMo-co interstitial atom, two different sets of simulations, with N and C as central atoms, were performed. It seems in fact reasonable that, if N is not the central atom, than the second best candidate, on the basis of previous computational studies and electronic properties, is C. The total molecular charge and spin used in all the simulations were those of minimum energy. Interestingly the energy of the system as a function of the charge has a minimum at -2, of which -2.71 (N central atom) and -2.75 (C central atom) localized on the FeMo-co. This is in agreement with previous computational studies,⁸ which predicted that, if the interaction of the FeMo-co with its protein environment is considered, the FeMo-co charge should be -3.

3 RESULTS AND DISCUSSION

An ample conformational search was performed with the aim of identifying possible interaction structures of HIS-195 with the FeMo-co. It has in fact been proposed that the adsorption of N₂ on the nitrogenase active site might lead to a significant structural change of the FeMo-co itself.⁶ Our calculations showed that a possible interaction mechanism for HIS-195 with the FeMo-co is to exchange a H atom with a μ₂-S and bind the vicinal Fe atom of the Fe₄(μ₃-S)₃ cluster through a N-Fe bond (Fig. 2).

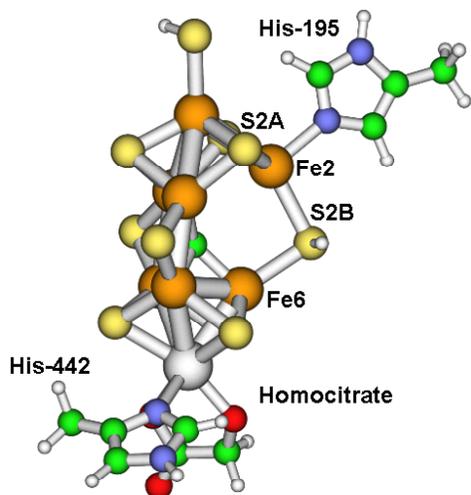


Figure 2. Open site structure.

This structure, which we will refer to as open, is slightly unstable with respect to the closed structure of Fig. 1 by

about 1.2 kcal/mol if N is the central atom and by 7.7 kcal/mol if it is C, which is consistent with the fact that the observed structure is in the closed form. However the transition from closed to open structure might take place either upon the adsorption of hydrogen atoms, or in concomitance with the N₂ adsorption, provided that the open structure is capable of interacting more efficiently with the adsorbing molecules. Two different FeMo-co structures are thus possible for the adsorption of N₂, the closed (Fig. 1) and open (Fig. 2) structures.

Another possibility for the adsorption of N₂, is to strongly modify the FeMo-co structure and interact with more than one Fe or Mo atoms contemporarily, which would thus compensate the energy required to modify the original cluster, as originally proposed by some authors. The interaction with vicinal aminoacids, among which HIS-195, is sometimes invoked to support this proposal. To account for all these possibilities, we investigated the N₂ adsorption for four different configurations. The first is the adsorption of N₂ on the FeMo-co without HIS-195, and in particular on one of the Fe atoms of the Fe₄(μ₃-S)₃ cluster, that we found, as other authors⁹, to interact more strongly with N₂ than the Fe atoms of the MoFe₃(μ₃-S)₃ cluster and is better positioned, from a sterical point of view, to justify a significant contribution of HIS-195. The second and the third are the closed and open structures of Figs. 1 and 2. The candidate Fe atom is the same as that of the first conformation. The fourth is the results of an ample conformational search started from the structures proposed by Blochl et al. and performed under the hypothesis that HIS-195 must be somehow involved and that N₂ must interact with more than 1 Fe atom. We refer to this structure as 'bridged'. The minimum energy structures so determined are sketched in Fig. 3-6, while the computed adsorption energies are reported in Table 1 (In Figure 7 is reported the Global process of formation of adsorbed N₂H specie adopting the open structure of the FeMo-co).

Table 1. N₂ and H adsorption energies (kcal/mol) and energy required to transfer a H atom from a Fe site to adsorbed N₂ for C and N central atoms.

	Centra l Atom	FeMo His195 Closed	FeMo His195 Open	FeMo His195 Bridge	
N ₂ ads	N	-5.6	4.0	-5.4	0.8
H ads on Fe6	N	-25.7	-23.2	-20.6	-38.3
FeH → N ₂ H	N	-6.3	-14.5	-2.1	1.0
N ₂ H ads	N	-37.6	-33.7	-28.1	-36.3
N ₂ ads	C	-12.2	4.9	-10.6	3.0
H ads on Fe6	C	-30.8	-36.3	-24.8	-30.4
FeH → N ₂ H	C	-2.0	-7.5	-10.3	-12.5
N ₂ H ads	C	-41.1	-38.9	-45.7	-40.0

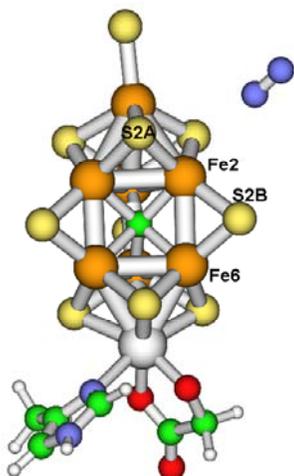


Figure 3. Structure of the FeMo-co without HIS-195 with N₂ adsorbed.

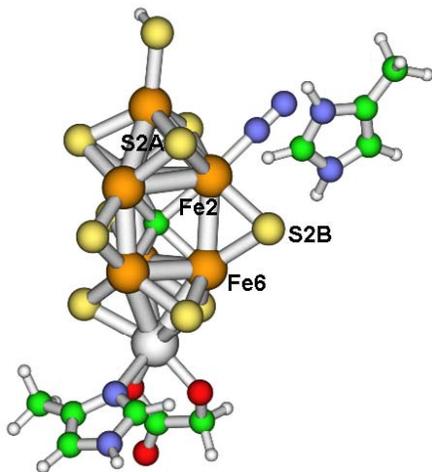


Figure 4. Closed structure of the FeMo-co with N₂ adsorbed.

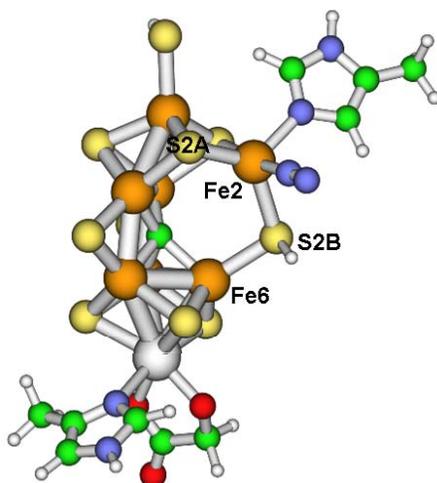


Figure 5. Open structure of the FeMo-co with N₂ adsorbed.

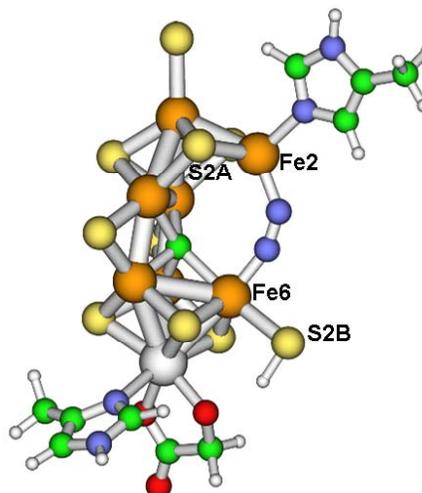


Figure 6. Bridged structure of the FeMo-co with N₂ adsorbed.

The calculated adsorption energies indicate that HIS-195 can significantly influence the adsorption process. In particular the comparison between N₂ adsorption on the FeMo-co with and without HIS-195 show that, in the absence of a major conformational change, such as the transition from closed to open site, the adsorption energy is positive. The N₂ adsorption energy for the open site conformation reported in Table 1 is calculated with reference to Fig.1a structure. Thus it does not comprise the energy necessary to reach the open site conformation (+1.2 and 7.7 kcal/mol for N and C as central atoms), despite of which the process is however overall exothermic. The nature of the central atom can influence the adsorption process. In particular FeMo-co clusters with C as central atom are more reactive towards N₂ than those with N, probably because of the lower extent of interaction of C with Fe than N. This is confirmed by the fact that the N₂ adsorption in the bridge conformation is highly unstable if N is the central atom, while the bond energy increases significantly, though not becoming negative, with N as central atom.

As the reduction of N₂ to NH₃ requires the adsorption of several H atoms, we determined the adsorption energy of H on one of the Fe atoms of the MoFe₃(μ₃-S)₃ cluster, and used it as a reference value to determine the energy required to transfer the adsorbed H to N₂ to form N₂H. The Fe2 atom of the MoFe₃(μ₃-S)₃ cluster is in fact likely to be one of the entry sites for H on the FeMo-co cluster¹¹, and a favorable energetic profile for transferring it from Fe2 to N₂ is required to let the N₂ reduction process proceed. H adsorption energies were computed with reference to gas phase molecular hydrogen. H transposition is significantly exothermic for almost all the examined configurations, with the notable exception of the bridge site with central N, which allows to rule out this conformation as a possible intermediate of the N₂ reduction mechanism. It is interesting to observe that the nature of the central atom can

influence significantly hydrogen adsorption energies, and in particular the protonation of S2B in the open form greatly decreases the Fe-H bond energy. It is also interesting to observe that some preliminary studies of the stable conformations of two adsorption H atoms revealed that the open site conformation can adsorb both of them in a pseudo-octahedral structure on Fe2, which is very similar to one recently proposed to explain experimental observations.² The energy of this structure is among those of lowest energy we identified through an ample conformational search.

The main results of this study can be summarized as follows. The closed site conformation might be a candidate for the N₂ adsorption, provided that a sufficient amount of H is already adsorbed on the FeMo-co site and that the hydrogen transposition kinetics to form N₂H is sufficiently fast, as the energy gained through this reaction is sufficient to energetically stabilize adsorbed N₂. In this case the N₂ reduction would appear as dynamic process in which it is the high flux of H on the FeMo-co to create the feasible conditions for the reaction to proceed. The role of HIS-195

in this case would thus be that of transferring protons of N₂. A similar consideration holds for the bridge site with central C, though, given the large conformational change necessary to reach the N₂ adsorbed structure, it seems reasonable that this pathway might be kinetically limited. An interesting possibility for N₂ adsorption is that represented by the transition from closed to open site. This conformation is in fact only slightly unstable with respect to the closed site and the energy released by N₂ adsorption would make the structural change energetically accessible. A PES scan of the reaction of transition from closed to open site structure showed that this conformational change does not require overcoming any energetic barrier. In particular, if C is the central atom, the transposition of H to S2B and migration of HIS-195 towards Fe2 is helped by the formation of an intermediate structure, similar to Fig1b but with the FeMo-co in the 'closed' form, which has a similar energy to that in which HIS-195 is protonated (Fig. 1). Such structure would rapidly lead to the formation of the 1e structure in presence of N₂.

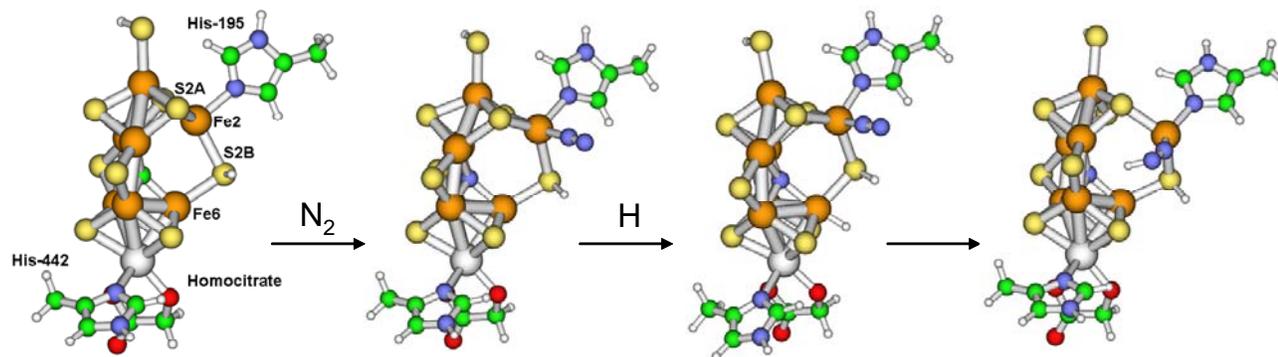


Figure 7. Global process of formation of adsorbed N₂H species adopting the open structure of the FeMo-co.

References

- (1) Einsle, O.; Tezcan, F. A.; Andrade, S. L. A.; Schmid, B.; Yoshida, M.; Howard, J. B.; Rees, D. C. *Science* **2002**, *297*, 1696-1700.
- (2) Igarashi, R. Y.; Laryukhin, M.; Dos Santos, P. C.; Lee, H. I.; Dean, D. R.; Seefeldt, L. C.; Hoffman, B. M. *J. Am. Chem. Soc.* **2005**, *127*, 6231-6241.
- (3) Yang, T. C.; Maeser, N. K.; Laryukhin, M.; Lee, H. I.; Dean, D. R.; Seefeldt, L. C.; Hoffman, B. M. *J. Am. Chem. Soc.* **2005**, *127*, 12804-12805.
- (4) Dance, I. *Chem. Comm.* **2003**, 324-325.
- (5) Hinnemann, B.; Norskov, J. K. *J. Am. Chem. Soc.* **2004**, *126*, 3920-3927.
- (6) Huniar, U.; Ahlrichs, R.; Coucouvanis, D. *J. Am. Chem. Soc.* **2004**, *126*, 2588-2601.
- (7) Kastner, J.; Hemmen, S.; Blochl, P. E. *J. Chem. Phys.* **2005**, *123*.
- (8) Lovell, T.; Li, J.; Liu, T. Q.; Case, D. A.; Noodleman, L. *J. Am. Chem. Soc.* **2001**, *123*, 12392-12410.

- (9) Hinnemann, B.; Norskov, J. K. *J. Am. Chem. Soc.* **2003**, *125*, 1466-1467.

(10) Gaussian03 Revision C. 01, see supplementary informations for full reference Wallingford CT, 2003.

- (11) Dance, I. *J. Am. Chem. Soc.* **2005**, *127*, 10925-10942.