

## Activation and protonation of dinitrogen at the FeMo cofactor of nitrogenase

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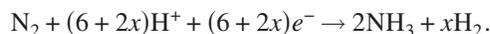
The protonation of N<sub>2</sub> bound to the active center of nitrogenase has been investigated using state-of-the-art density-functional theory calculations. Dinitrogen in the bridging mode is activated by forming two bonds to Fe sites, which results in a reduction of the energy for the first hydrogen transfer by 123 kJ/mol. The axial binding mode with open sulfur bridge is less reactive by 30 kJ/mol and the energetic ordering of the axial and bridged binding modes is reversed in favor of the bridging dinitrogen during the first protonation. Protonation of the central ligand is thermodynamically favorable but kinetically hindered. If the central ligand is protonated, the proton is transferred to dinitrogen following the second protonation. Protonation of dinitrogen at the Mo site does not lead to low-energy intermediates. © 2005 American Institute of Physics.

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### I. INTRODUCTION

Although dinitrogen is the main part of the atmosphere, it is, in its molecular form, inaccessible to biological organisms. Its inactivity is caused by the triple bond—one of the strongest covalent bonds in nature. While high pressure and high temperature are required to convert N<sub>2</sub> to NH<sub>3</sub> in the industrial Haber-Bosch process, biological nitrogen fixation achieves the same goal at ambient conditions. For this purpose nature employs the enzyme nitrogenase, which is one of the most complex bioinorganic catalysts in nature.

Nitrogenase converts N<sub>2</sub> to biologically accessible ammonia.<sup>1–4</sup> During the reaction, nonstoichiometric<sup>5</sup> amounts of hydrogen are produced.



Values for  $x$  range from  $<1$  (Ref. 6) upwards and there is an ongoing discussion about the question whether hydrogen is produced in stoichiometric amounts.<sup>7</sup> In the gas phase, the first protonation is by far the most difficult step in the reaction. The catalyst has to find a way to dramatically reduce this barrier.

The active center of the enzyme, shown in Fig. 1, is the FeMo cofactor, MoFe<sub>7</sub>S<sub>9</sub>N homocitrate. The FeMo cofactor is linked to the protein via two amino acid residues. The question arises why nature employs such a large multicenter cluster.

The reaction mechanism is being studied for over 40 years, but the atomistic mechanism of substrate conversion at the FeMo cofactor still remains an open issue. In a previous paper, we presented the most salient features of the reaction cycle as it emerged from our simulations.<sup>8</sup> In the

present paper, we present the results of state-of-the-art first-principles calculations of the most difficult step in the reaction pathway, the first protonation of N<sub>2</sub> bound to the FeMo cofactor.

Nitrogenase consists of two proteins: (1) the molybdenum-iron protein, which holds the FeMo cofactor (FeMoco) and (2) the iron protein, which hydrolyzes MgATP and uses the energy obtained to provide the molybdenum-iron protein with electrons.

The structures of the two proteins were resolved already in 1992.<sup>9–11</sup> Since then increasingly more refined crystallographic data became available<sup>12–14</sup> until, in 2002, a central ligand of the FeMoco, shown in Fig. 1 was found,<sup>15</sup> which was undetected in previous studies. While crystallographic studies determined the central ligand to be either a C, N, or O atom, theoretical studies<sup>16–19</sup> provide strong support for it to be a nitrogen atom. It turns out that the central ligand (N<sub>x</sub>) plays a critical role in the mechanism.<sup>8</sup>

Kinetic studies of the mechanism of biological nitrogen fixation<sup>20–23</sup> indicate that the rate-limiting step of the reaction is the dissociation of the two proteins. In each of these association-dissociation cycles one electron is transferred to

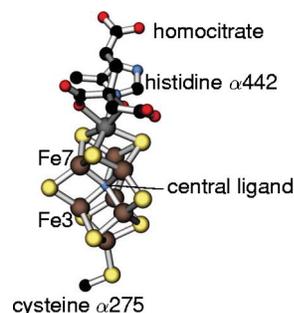


FIG. 1. (Color) The FeMo cofactor of nitrogenase with its homocitrate ligand and the two residues linking the cofactor to the protein: histidine and cysteine.

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FeMoco. The so-called Thorneley-Lowe scheme provides insight into the first reduction steps, stating that  $N_2$  binds after three or four electrons have been transferred to the MoFe protein. Theoretical models<sup>24,25</sup> indicate that geometrical changes of the backbone of the Fe-protein are responsible for using the energy from MgATP to transfer electrons to the MoFe protein.

Since the appearance of the first crystal structures, nitrogenase has been subject to numerous theoretical investigations.<sup>26–52</sup> Unfortunately, the central ligand was unknown at that time, so that the conclusions have to be reconsidered. However, several more recent theoretical studies<sup>8,16–19,53,54</sup> have been performed taking the central ligand into account.

A critical parameter for theoretical calculations is the oxidation state of the cofactor. By comparing theoretical results<sup>17,18,54</sup> with experimental observations, a consensus has been reached that the cofactor in the resting state is charge neutral, i.e.,  $[MoFe_7S_9N]^0$ .

The mechanisms for nitrogen fixation proposed up to now may be divided in two main groups: (1) conversion at Mo and (2) conversion at Fe.

- The Mo atom has been the target of numerous theoretical<sup>42,45,46,55,56</sup> studies. A strong indication in favor of Mo comes from experiment: an Mo-based model complex<sup>57</sup> has been found to catalytically reduce  $N_2$ . However, there are also active nitrogenases<sup>3</sup> with Mo replaced by V or Fe, which indicates that Mo is not essential.
- Numerous proposals have been made for the reduction involving the Fe atoms. (1) The direct way for  $N_2$  to bind at Fe atoms is head-on binding.<sup>30,31,38–40,58</sup> In this mechanism  $N_2$  binds to one Fe atom of the central cluster, where it is protonated until one and then the second ammonia dissociate. The intermediates of the complete cycle have been identified, albeit still without the central ligand by Rod and co-workers.<sup>38–40</sup> Recently, Hinemann and Nørskov<sup>58</sup> extended this proposal to the cofactor with central ligand. The theoretical predictions are, however, at variance with this mechanism because the calculations show that  $N_2$  binding is highly endothermic.<sup>58</sup> (2) Sellmann and co-workers<sup>59–62</sup> suggested an opening of the cage in analogy to smaller Fe complexes. In his model, two octahedrally coordinated low-spin Fe atoms positioned in close proximity bind dinitrogen between them, where it is reduced. The mechanism has been lined out up to the first four protonations. (3) Our own recent calculations<sup>8,54</sup> support the view of cage opening even though, in our model, the Fe atoms are in a high-spin tetrahedral coordination, which points towards a quite different chemistry than expected for low-spin octahedral Fe atoms. The opening and closing of an SH bridge at the cofactor is critical for the activation of  $N_2$  and for the dissociation of the second ammonia. A critical role is attributed to the central ligand, which enables required bond rearrangements. In a similar approach, we were able to explain many experimental findings in the conversions of acetylene by

nitrogenase.<sup>63</sup> (4) Another proposal<sup>19</sup> suggests the opening of a sulfur bridge upon coordination of water to an Fe atom, complete protonation of the central ligand, and dissociation of ammonia. Then  $N_2$  inserts into the central cavity of the cofactor, where one nitrogen atom is fully protonated and dissociated, which closes the catalytic cycle. This intriguing proposal seems to be in conflict with isotope exchange electron spin echo envelope modulation/electron-nuclear double resonance (ESEEM/ENDOR) experiments<sup>64</sup> that exclude an exchange of a central nitrogen ligand.

## II. CALCULATIONAL DETAILS

We considered the complete FeMo cofactor with truncated ligands as in the previous study.<sup>54</sup> The histidine was replaced by imidazole, the homocitrate ligand by glycolate and the cysteine, bound to the terminal iron atom by an SH group.

We performed density-functional theory<sup>65,66</sup> (DFT) calculations based on the projector-augmented wave<sup>67,68</sup> (PAW) method. The gradient-corrected PBE (Ref. 69) functional was used for exchange and correlation. The PAW method is a frozen-core all-electron method. Like other plane-wave-based methods, the PAW method leads to the occurrence of artificial periodic images of the structures. This effect was avoided by explicit subtraction of the electrostatic interaction between them.<sup>70</sup> Wave-function overlap was avoided by choosing the unit cell large enough to keep a distance of more than 6 Å between atoms belonging to different periodic images. We used a plane-wave cutoff of 30 Ry for the auxiliary wave functions of the PAW method. The following shells were treated in the frozen-core approximation: Fe [Ne], Mo [Ar3d<sup>10</sup>], S [Ne], O [He], N [He], and C [He]. The following sets of projector functions were employed, Fe  $2s2p2d$ , Mo  $2s2p2d$ , S  $2s2p2d$ , O  $2s2p1d$ , N  $2s2p1d$ , C  $2s2p1d$ , and H  $2s1p$ , which provides the number of projector functions per angular momentum magnetic and spin quantum numbers  $m$ ,  $s$  in each main angular momentum channel  $\ell$ .

Atomic structures were optimized by damped Car-Parrinello<sup>71</sup> molecular dynamics with all degrees of freedom relaxed. The convergence was tested by monitoring if the kinetic temperature remains below 5 K during a simulation of 0.05 ps (200 time steps). During that simulation, no friction was applied to the atomic motion and the friction on the wave-function dynamics was chosen sufficiently low to avoid a noticeable effect on the atomic motion.

Transition states were determined by applying a one-dimensional constraint on the atomic positions. In the present application, bond-length, angle, and torsion constraints were used. To get a first upper bound for the barrier, the specific constraint was varied within 1000 molecular-dynamics (MD) steps. If this upper bound is less than 20 kJ/mol, the barrier will be easily overcome and it has not been calculated more accurately. In case of a higher estimate, the bond length was fixed to discrete values around the transition state to maximize the energy while all unconstrained degrees of freedom

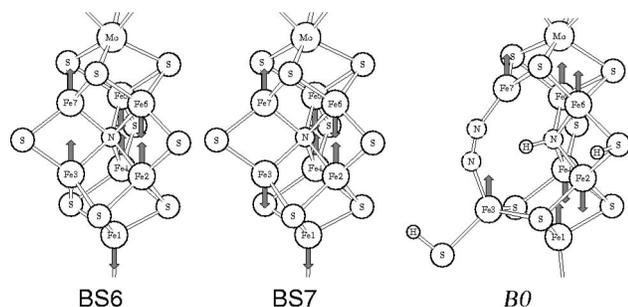


FIG. 2. The two relevant spin ordering BS6 and BS7 as well as the spin ordering of B0. BS6 and BS7 differ by a spin flip of Fe3. BS7 is the same spin ordering as obtained for the cofactor in the resting state *M*. It is characterized by ferromagnetically coupled Fe–Fe and Fe–Mo pairs, which are antiferromagnetically aligned relative to each other (Ref. 54). The spin ordering only defines the directions of the site spins, not their absolute value.

were allowed to relax to minimize the energy. Proof that this approach, when converged, determines exactly the first-order transition states is given elsewhere.<sup>72</sup>

The reaction rates  $\Gamma$  can be estimated using  $\Gamma = \Gamma_0 e^{-E_A/(k_B T)}$  from the calculated activation energy  $E_A$  and a typical vibrational frequency  $\Gamma_0 = 3 \times 10^{13} \text{ s}^{-1}$  corresponding to about  $1000 \text{ cm}^{-1}$ .

The FeMoco exhibits seven high-spin iron atoms antiferromagnetically coupled to each other. Many different spin configurations may easily lead to metastable states in conventional collinear spin-polarized calculations. Therefore, we used a noncollinear description of the spin density for our calculations. In a noncollinear description each one-electron wave function is a two-component spinor wave function.<sup>73–76</sup> This method not only correctly describes real noncollinear spin states, which occur within the reaction mechanism, but also avoids artificial barriers between different spin configurations occurring in collinear calculations. Our resulting spin distribution is therefore independent of the random starting conditions. Such a dependence is a common problem of conventional (collinear) spin-polarized calculations for this system. We found that the spin ordering depends on subtle changes in the atomic structure. It also changes between different states of the reaction mechanism. The spin orderings encountered in our calculations are given in Fig. 2, where we follow the notation introduced by Lovell *et al.*<sup>48</sup> Noncollinear spin arrangements have been found, in this study, only for energetically unfavorable states, which is why we do not specify them further.

The spin quantum number  $S$  is specified alongside with the corresponding structures in Figs. 3 and 4. A spin with  $S=1$ , for example, corresponds to a triplet. Noncollinear states are indicated by a spin quantum number that differs from half-integer values. This corresponds, in analogy with the collinear expression, to the absolute value of the integrated spin density divided by  $\hbar$ .

During the reaction, protons and electrons are transferred to the cofactor and the substrate. In this work we made the assumption that electrons and protons are transferred in a correlated way, i.e., that one proton is transferred with each electron transfer. This assumption implies one of the two scenarios: either a reduction of the cofactor increases the proton affinity such that a proton transfer is induced. Or, if

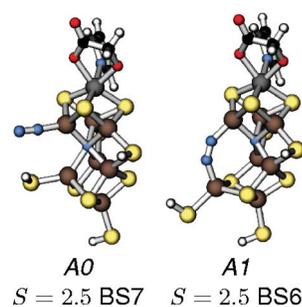


FIG. 3. (Color)  $\text{N}_2$  binding modes, their spin states, and their spin ordering (BS state) obtained from DFT.

the proton transfer precedes the electron transfer, it implies that the electron affinity is sufficiently enhanced by the positive charge next to the cofactor to induce an electron transfer to the cofactor. This is the main assumption in our work besides the accuracy of the density functionals and the neglect of the protein environment. This assumption has been shown to be valid for the cofactor before the binding of the substrate.<sup>54</sup>

The energies of protons and electrons, which are consumed during the reaction, affect the overall reaction energy. Thus we need to define a value that reflects their energies in the environment. For protons, the relevant environment is the proton transfer channel, while for electrons it is expected to be the *P*-cluster. The exact energies cannot be determined by theory alone.

As a consequence of our assumption that the reduction and protonation occur in a correlated manner, only the sum  $\mu_H$  of the energies of protons and electrons is relevant for the relative energies of the intermediates. A range of possible values can be derived by comparing experimental x-ray and extended x-ray-absorption fine structure (EXAFS) data with our calculated geometries: we found indirect evidence for the

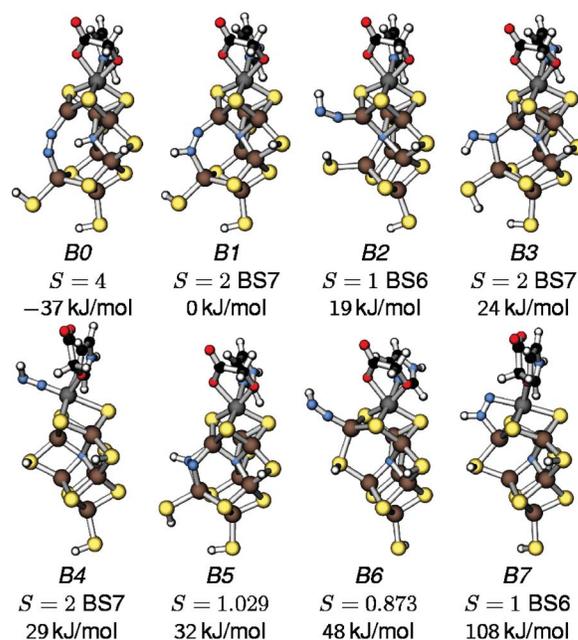


FIG. 4. (Color) Different binding modes of the  $\text{N}_2\text{H}$  adduct at the FeMoco. The spin state and the spin ordering (BS state) are given, as well as the energies, which are given relative to B1.

cofactor being unprotonated in the resting state, while being protonated in the reduced state.<sup>54</sup> Therefore,  $\mu_H$  is sufficiently high to drive protonation, that is,  $\mu_H > E(MH) - E(M)$ . On the other hand, no protonation occurs under the same conditions, but in the absence of MgATP. Thus the chemical potential in the absence of MgATP, denoted by  $\mu'_H$ , must be sufficiently low not to drive protonation, that is,  $\mu'_H < E(MH) - E(M)$ . As two MgATP are hydrolyzed in each electron transfer, the difference between the chemical potentials with and without MgATP is smaller than twice the energy of hydrolysis of MgATP, that is,  $\mu_H - \mu'_H < 64.4$  kJ/mol.<sup>77</sup> It is smaller because a fraction of the energy supplied by MgATP will be dissipated. Therefore, we assume the lower bound for  $\mu_H$ , that is,  $\mu_H = E(MH) - E(M)$ , in our calculations. This is the most conservative assumption possible. A less conservative value would make the reactions including protonation more exothermic.

Previous studies<sup>19,38–40,58</sup> made a different choice for  $\mu_H$ , namely,  $\mu_H = \frac{1}{2}E(H_2)$ . This would be the appropriate choice if the protons and electrons would be obtained from gaseous hydrogen. While the production of gaseous hydrogen  $2H^+ + 2e^- \rightarrow H_2$  is energetically neutral with this choice, this reaction is exothermic by 71 kJ/mol when using our choice of  $\mu_H = \frac{1}{2}E(H_2) + 35$  kJ/mol. Thus our reaction energies can be translated to the values for  $H_2$  reference by adding 35 kJ/mol per added hydrogen atom. We additionally list the reaction energies with  $H_2$  as the reference energy in parentheses after the values we obtain with our  $\mu_H$  for all those energies were influenced by the choice of  $\mu_H$ .

In our work we evaluate not only the energetics of the intermediates, but also the barriers for the transitions. To estimate the barriers for protonation, we need, however, to specify a proton donor, which models the proton channel. We used the ammonium ion to mimic the proton donor. Note, however, that this choice only affects the barriers but not the relative energies of the intermediates. Our finding that the barriers for protonations are small and will therefore be overcome easily indicates that the barriers for protonation are not relevant for the overall picture.

The notation for the structures is chosen as follows. The complexes of dinitrogen with the cofactors are given in letters in alphabetic order according to the number of proton transfers and in numerals for their energetic order. A numeral 0 denotes the ground state for the selected composition.

### III. RESULTS

The most difficult step of the reaction from  $N_2$  to  $NH_3$  is the first protonation of dinitrogen. In the reaction in the gas phase we find that the reaction step  $N_2 + H^+ + e^- \rightarrow N_2H$  is endothermic with 164 kJ/mol using the choice for  $\mu_H$  described under calculational details (and 199 kJ/mol with  $H_2$  as reference). The main goal for nitrogenase must be to lower this barrier. At the FeMo cofactor, the reaction energy is dramatically reduced to only 41 kJ/mol (76 kJ/mol). Nevertheless, in the catalyzed reaction this remains the most endothermic step.

We explored eight different isomers of the  $N_2H$  adduct bound to the  $MH_2$  state of the cofactor.  $MH_2$  represents the

resting state ( $M$ ) of the cofactor reduced by two electrons and protonated at two of the sulfur bridges. The isomers are shown in Fig. 4 and will be discussed in the following.

#### A. Protonation of the central ligand

Nitrogen in the bridged binding mode A1 opens the structure of the cofactor and leaves space for the coordination at the central ligand. Protonation of the central ligand leads to the most stable isomer with one protonated nitrogen, namely  $B0$ , which is lower in energy by 37 kJ/mol than the second most stable configuration, the protonated dinitrogen bridge  $B1$ .

There is only limited space for a donor to access the central ligand in A1. Correspondingly, we find a large barrier for protonation of 79 kJ/mol from  $NH_4^+$ , which was used as the model for the proton donor. This barrier corresponds to a reaction rate of the order of  $0.1 - 1$  s<sup>-1</sup>, which is substantially lower than the electron transfer rate. Furthermore, as discussed below, A1, the common starting configuration for both  $B0$  and  $B1$  can be protonated with a negligible barrier at dinitrogen leading to  $B1$ . Since the pathways to  $B0$  and  $B1$  are in direct competition, protonation of the central ligand is kinetically hindered. Therefore, we consider a reaction path via protonation of the central ligand unlikely.

In  $B0$ , one iron atom is in a planar threefold coordination shell, which appears to be an unusual configuration. To explore if this structure is an artifact of our structural model, we investigated if this iron atom could form a complex bond to a nearby water molecule, which would restore a tetrahedral coordination of the Fe atom. However, in our calculations including an additional water molecule, no significant complex bonds between the three-coordinate Fe atom and a water are formed: for  $B0$  an Fe–O distance of 2.7 Å was calculated, which is substantially larger than typical complex bonds to Fe.

One may ask if the solvent effects affect the barrier to access  $B0$ . We find that the interaction between an additional  $H_2O$  molecule and the triangular Fe atom in the initial state A1 and in the transition state is even weaker than in the final state  $B0$ , expressed in even longer Fe–O distances. From the absence of strong interactions we conclude that the large barrier is not appreciably influenced by the solvent.

A slow rate of formation does not rule out that the cofactor is accidentally trapped in this low-energy intermediate. Therefore, we investigated the subsequent steps starting from  $B0$ . We find that after one reduction the next proton attaches to the bridged dinitrogen, which is similar to the second most stable intermediate discussed below. However, upon protonation of dinitrogen, the proton at the central cluster is destabilized and is transferred to the unprotonated nitrogen atom of dinitrogen. The reaction energy for the internal proton transfer from the central ligand to the protonated dinitrogen is -69 kJ/mol. The reaction has a barrier of 20 kJ/mol. Thus even if the central ligand is protonated, the reaction mechanism quickly leads back to what we consider the most likely pathway. Intermediate structures of this side branch of the reaction cycle are shown in Fig. 5.

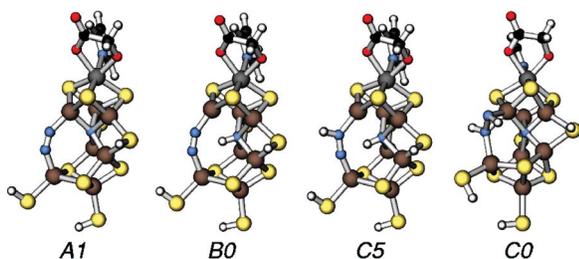


FIG. 5. (Color) Intermediates for the protonation of the central ligand.

## B. Protonation of bridged dinitrogen

The second most stable isomer with one protonated nitrogen is the bridged binding mode *B1* shown in Fig. 4.

As shown previously,<sup>54</sup> dinitrogen can dock at the FeMo cofactor *MH*<sub>2</sub> in two configurations with similar energies. They are shown in Fig. 3. The axial binding mode, denoted by *A0*, is slightly more stable than the bridged one, denoted by *A1*.

One of the most interesting questions of biological nitrogen fixation is how dinitrogen is activated for the first protonation. Therefore, we investigated the two binding modes in some detail.

The most relevant geometric parameters and the N–N stretching vibration frequencies of dinitrogen bound to the cofactor are given in Table I and compared to the experimental data obtained from a model complex with a N<sub>2</sub> bridging two low-coordinated iron atoms.<sup>78</sup> The elongation of the N–N bond as well as the reduction of the vibration frequency with respect to gaseous N<sub>2</sub> are indications of the activation of the triple bond for the following protonation. The elongation of the dinitrogen bond and the reduction of the stretch frequency in *A1* compares well with the model complex, while *A0* appears to be much less activated.

The activation of *A1* can be traced back to occupied N<sub>2</sub>– $\pi^*$  orbitals as shown in Fig. 6. These orbitals can be seen as an antisymmetric combination of the Fe–N bonds. Interestingly they are only dominant in the minority spin direction of the two neighboring Fe sites Fe3 and Fe7. The origin is that the interaction with the unoccupied *d* states split the  $\pi^*$  orbital in a bonding and an antibonding orbital. The bonding orbital, still having  $\pi^*$  character but containing the Fe–N bonding contribution, becomes occupied and is located about

TABLE I. Bond distances (Å), angles (°), and vibration numbers (cm<sup>-1</sup>) of the N<sub>2</sub> binding modes and free N<sub>2</sub>. They are compared to experimental data on N<sub>2</sub> and a model complex.

	Theory		Experiment		
	<i>A0</i>	<i>A1</i>	N <sub>2</sub>	N <sub>2</sub>	Model Complex <sup>a</sup>
<i>d</i> (N–N)	1.146	1.173	1.105	1.098	1.182(5)
<i>d</i> (N–Fe7)	1.777	1.827			1.770(5)
<i>d</i> (N–Fe3)		1.834			1.779(5)
<i>d</i> (SH–Fe3)	2.298	2.349			
∠(N–N–Fe7)	176	147			180
∠(N–N–Fe3)		147			180
$\bar{\nu}$ (N–N)	1979	1792	2339	2331	1778

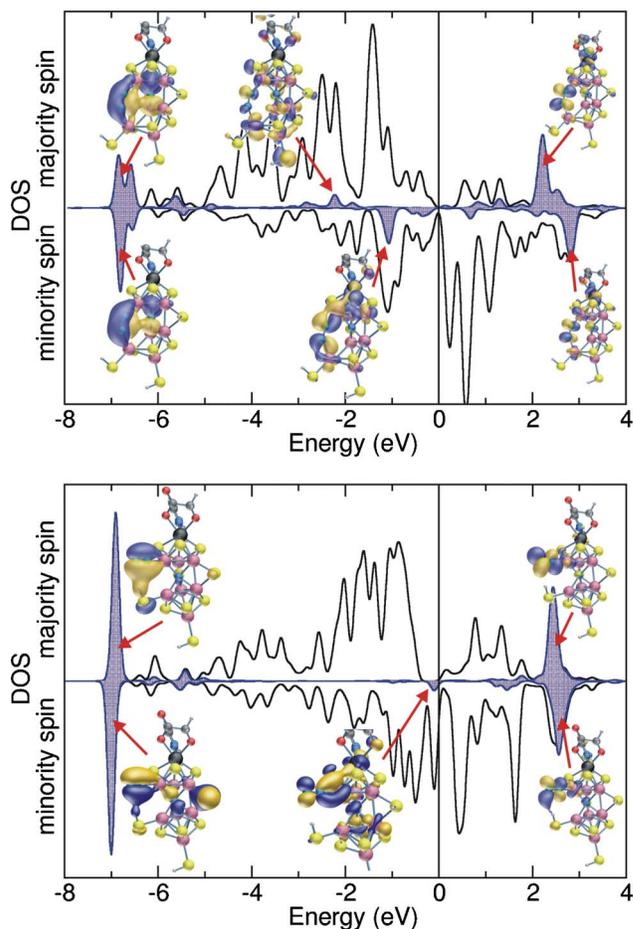
<sup>a</sup>Reference 78.

FIG. 6. (Color) Density of states of the bridged (*A1*, top) and the axial (*A0*, bottom) binding modes illustrating N<sub>2</sub> activation. The full line indicates the density of states projected onto the *d* orbitals of the Fe atoms directly bound to N<sub>2</sub>. The shaded curve is the density of states projected onto the  $\pi$  and  $\pi^*$  orbitals of dinitrogen. The insets show the relevant wave functions. Note that both states have occupied  $\pi^*$  orbitals, which is more dominant and lower in energy in the bridged binding mode *A1*.

1 eV below the highest occupied molecular orbital (HOMO), while the antibonding orbital, having the Fe–N antibonding contribution, remains unoccupied. The former, bonding orbital is the relevant frontier orbital for the protonation.

The energy to add a hydrogen atom to dinitrogen in the bridged binding mode *A1* and to obtain *B1* is 41 kJ/mol (76 kJ/mol), substantially less than the 164 kJ/mol (199 kJ/mol) of the gas-phase reaction. *B1* is energetically slightly more favorable by 8 kJ/mol than protonating the other nitrogen atom of N<sub>2</sub>.

The protonation of the reduced complex from an ammonium, which mimics the proton donor in our calculations, proceeds with a negligible barrier of only 4 kJ/mol and is exothermic by 63 kJ/mol. Note, however, that the calculated protonation energies taken individually are not as reliable as the reaction energy, as the former depend on the choice of NH<sub>4</sub><sup>+</sup> as the proton donor. Nevertheless, this calculation indicates that protonation of the reduced complex at dinitrogen proceeds without difficulty and much more readily than protonation at the central ligand.

Interestingly, the spin ordering of the cofactor, as obtained from DFT, changed from BS6 in *A1* to BS7 in *B1*

during the reduction and protonation. (See Fig. 2 for a definition of the spin labels.) This corresponds to a spin flip of the iron atom Fe3, to which N<sub>2</sub> as well as the SH group are bound. In comparison, the FeMo cofactor in the resting state has the same spin arrangement BS7 as B1. After two protonations and reductions, BS7 of the resting state is transformed to a noncollinear spin distribution, which changes to BS7 during dinitrogen binding in the axial mode A0 and to BS6 during the conversion to the bridged binding mode A1. These transitions show the importance of allowing spin flips and noncollinear spins when simulating the reaction. Previous calculations<sup>38–40,58</sup> assumed a fixed spin ordering during the entire reaction, which may cause errors in the energy profile.

### C. Protonation of axial dinitrogen

If N<sub>2</sub> is axially bound as in A0 during the first protonation, the lowest-energy protonation site is the terminal nitrogen atom. The resulting structure is B2, shown in Fig. 4. The protonation does not induce major structural changes. The energy of B2 is 19 kJ/mol higher than the energy of B1, i.e., the bridged mode. Nevertheless, even though the energy for protonation of the reduced complex from an ammonium is, compared to the one of the bridged mode, 30 kJ/mol smaller, the reaction is still exothermic with 33 kJ/mol and proceeds with a negligible barrier of less than 10 kJ/mol.

Also in this case, the spin ordering changed during the reduction and protonation from BS7 in A0 to BS6 in B2.

### D. Other binding modes

Only 5 kJ/mol above the energy of B2, we find the complex B3 bridging two Fe atoms with a single nitrogen atom. This structural principle is found again later in the reaction cycle.<sup>8</sup> However, like the axial binding mode, it lies substantially, that is, by 24 kJ/mol, higher in energy than the bridged mode B1.

The protonated dinitrogen bound to molybdenum, i.e., B4, lies 29 kJ/mol above B1. Together with B7, it will be discussed in Sec. III E, where we discuss the potential role of molybdenum.

As in B5, dinitrogen can also bridge the two Fe atoms with its axis perpendicular to the Fe–Fe direction, so that both nitrogen atoms are connected to both Fe atoms. A similar binding mode has recently been found experimentally for nitrogen bridging two zirconium centers.<sup>79</sup> The energy of this structure, i.e., B5, lies 32 kJ/mol above that of B1.

In the relevant intermediate B6 of the mechanism proposed by Hinnemann and Nørskov,<sup>58</sup> dinitrogen binds axially to one Fe atom like our structure B2. In contrast to B2, however, the sulfur bridge is still intact and the bond between this Fe atom and the central ligand is broken. This structure is 48 kJ/mol above B1 and it is 29 kJ/mol less stable than the axial binding mode B2 discussed previously.

### E. Molybdenum as coordination site

Numerous theoretical<sup>42–46,56</sup> and experimental<sup>57,80</sup> studies have been performed to investigate the Mo atom as a possible adsorption site. For this reason, we discuss nitrogen

coordination to molybdenum here in some detail. As shown previously,<sup>54</sup> the molybdenum atom is, according to our simulations, not a favorable adsorption site for nitrogen: nitrogen bound to molybdenum is higher in energy by 50 kJ/mol than the one bound to the iron atoms. Nevertheless we investigate the most favorable pathway for the first protonation via dinitrogen bound to the Mo site.

As shown previously, nitrogen adsorption at pentacoordinate Mo is endothermic by 30 kJ/mol.<sup>54</sup> This indicates that, even if the coordination site is vacant, dinitrogen binds to Mo only for fleetingly short periods of time. If protonation proceeds sufficiently easy so that the proton is transferred during these short periods, dinitrogen may be stabilized bound to Mo. However, protonation leading to B4, shown in Fig. 4, is energetically unfavorable. B4 is 29 kJ/mol higher in energy than B1, with N<sub>2</sub>H bound to Fe atoms.

Durrant<sup>45</sup> proposed a transition of the protonated dinitrogen B4 into a bridging position between Mo and Fe as in B7 shown in Fig. 4. We find B7 to be 79 kJ/mol higher in energy than B4.

## IV. DISCUSSION

The following picture emerges from our calculations: dinitrogen exists in two competing docking modes at the cofactor, the axial mode A0 and the bridged mode A1.<sup>54</sup> They are separated by a large barrier of 66 kJ/mol, which, however, is still sufficiently small to be overcome.<sup>54</sup> Before the reduction and protonation take place, the axially bound dinitrogen is even slightly more stable by 6 kJ/mol than the bridged configuration.<sup>54</sup> We assume that axial and bridged modes are in equilibrium until the proton is transferred. However, the electron transfer and the protonation reverse the energetic order between them so that the axially bound dinitrogen B2 ends up 19 kJ/mol higher in energy than the bridged dinitrogen B1.

In the bridged binding mode A1, N<sub>2</sub> is activated for accepting a proton by forming bonds to the Fe sites. As a result the  $\pi^*$  orbitals are occupied, which implies that the triple bond is effectively broken. The occupied  $\pi^*$  orbital exposes two nucleophilic lobes to which a proton can bind.

These occupied  $\pi^*$  orbitals are binding combinations of the dinitrogen  $\pi^*$  orbital with the *d* orbitals of the Fe sites. They are only occupied in the minority spin direction of the Fe atoms binding to dinitrogen. This is because only the high-lying *d* orbitals of the minority spin direction can lead to a stabilization of the  $\pi^*$  orbital; hybridization with the low-lying Fe-*d* states of the majority spin direction would shift the  $\pi^*$  orbitals further up, while the corresponding bonding orbital is mostly of Fe character. Note here that both Fe sites binding to dinitrogen have the same spin orientation in A1.

In the axial binding mode A0, the corresponding  $\pi^*$  orbital lies close to the Fermi level and its weight on dinitrogen is substantially smaller than in the corresponding orbital of the bridging mode. These differences between the bridged and axial binding modes are also reflected in the bond-length

expansion and the reduction of the stretch frequency of the dinitrogen bond, and suggest a smaller activation of the axial binding mode.

Nevertheless, dinitrogen is readily protonated both in the axial and in the bridged binding mode after the transfer of one electron. Compared to the gas phase, the reaction energy for the first protonation is dramatically reduced from 164 kJ/mol (199 kJ/mol) for the gas phase to 41 kJ/mol (76 kJ/mol) at the FeMo cofactor in the bridged mode.

Interestingly, the most stable site for protonation is the central ligand. The resulting isomer is more stable by 37 kJ/mol than the one with protonated dinitrogen. However, this isomer is not considered relevant because protonation of the central ligand directly competes with protonation of dinitrogen. While protonation of the central ligand requires a large barrier of 79 kJ/mol to be overcome, protonation of dinitrogen proceeds nearly barrierless. We attribute the large barrier to the breaking of the bond between the central ligand and one of the Fe atoms, which changes its tetrahedral coordination to a trigonal planar one.

Even if the central ligand is protonated, the second proton is added to dinitrogen and induces an internal proton transfer from the central ligand to dinitrogen.<sup>8</sup> Thus even if the first proton binds to the central ligand, this step neither poisons the catalyst nor does it lead to an entirely different branch of the reaction cycle. This side branch may potentially be relevant at small turnover frequencies.

The branch via protonation of the central ligand is reminiscent of the proposal by Huniar *et al.*,<sup>19</sup> who propose complete protonation of the central ligand and its cleavage as ammonia, before dinitrogen binds to the cofactor. In their study, the central cage is opened by a water molecule coordinating to one Fe atom. Additional calculations would be necessary to directly compare our results with their proposal. However, the latter seems to be in conflict with isotope exchange (ESEEM/ENDOR) experiments<sup>64</sup> that exclude an exchange of a central nitrogen ligand.

A mechanism via dinitrogen bound to the Mo site is inconsistent with our calculations. Binding to the Mo atom is substantially more endothermic than binding to the Fe sites.

Dinitrogen bridging two Fe atoms is part of the proposal by Sellmann and co-workers.<sup>59-62</sup> Our model differs from theirs, in that all Fe atoms remain tetrahedrally coordinated and in the high-spin state. We are not aware of any theoretical investigations of this system including the cofactor with its central ligand.

The presence of the central ligand is crucial for the reaction mechanism of nitrogen fixation at the FeMo cofactor, and may explain why the mechanism has remained elusive for a long time. The main feature of the central ligand is its ability to form a variable number of bonds to the six Fe atoms. The central ligand changes its coordination from sixfold to fivefold and fourfold. This allows other ligands such as nitrogen and sulfur to form and cleave bonds to the Fe atoms without deviations from the preferred tetrahedral coordination of the latter. Tetrahedral coordination seems to be a common structural principle to nearly all relevant intermediates of the reaction cycle. This is particularly apparent when dinitrogen binds:<sup>54</sup> while the Fe atom to which dinitro-

gen binds in the axial binding mode A0 maintains its coordination shell by giving up its sulfur bridge, the bridged binding mode A1 would result in an unfavorable fivefold coordination of one Fe atom, if the latter would not give up its bond to the central ligand.

Let us mention here some variants of the reaction steps discussed: our calculations do not allow to distinguish between Fe3 and Fe7 as potential docking sites of dinitrogen. We find that the axial binding mode A0 is more stable, that is, by 30 kJ/mol, on Fe7 than on Fe3. The bridged mode accessed via the axial binding at Fe7 is more stable by 14 kJ/mol than the other variant. We expect that these differences are sensitive to the environment of the cofactor: due to the large motion of the sulfur atom, the opening of the SH bridge may be strongly affected by the shape and the specific interactions of the cavity. Thus, no conclusive answer regarding the initial binding site can be given at this point.

The cofactor has an approximate threefold symmetry, which is broken by the ligands and the protein environment. As long as the protein environment is not taken into account, as in the present study, the energetics will proceed similar for all three equivalent orientations. Nevertheless, the position of the proton channel in the protein indicates that nitrogen fixation proceeds near the iron atoms Fe3 and Fe7. Furthermore the cavity in this region provides sufficient space to accommodate nitrogen bound to an Fe atom.<sup>54</sup>

## V. SUMMARY

We have studied the first protonation of dinitrogen at the cofactor on the basis of density-functional calculations. We made an effort to explore the phase space for the reaction without prejudice for one particular model of the mechanism. A large number of intermediates and the barriers between them have been explored and placed into perspective. While an unambiguous determination of this reaction step is not yet possible, three possible branches could be identified. One proceeds via dinitrogen in the bridged configuration, a second one proceeds via axially bound dinitrogen, and the third proceeds via protonation of the central ligand. The latter, however, is unlikely to play a role in the reaction cycle due to kinetic competition with direct protonation of the bridging dinitrogen. The activation of dinitrogen is discussed in detail.

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