Effect of Water on Nitrogen Fixation

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Several possible models for the nitrogen fixation are tested in order to make the nature of the nitrogen fixation clear. Among the various schemes, the intermediate water structure scheme in the dinitrogen reduction to an ammonia was mainly investigated by inserting the water molecules around the chosen substrate, and the process of the dinitrogen reduction can be explained well on the basis of our schemes. The water molecules in the nitrogen fixation are not merely an inert or static environment but have an important role as in other biological systems. In our calculations, the model using $4H_3O^+$ ($4H_2O+4H^+$) where these water molecules constitute a six-membered ring between the substrates shows the most reasonable results. This six-membered ring being composed of the water molecules with the nitrogen atom upon the Fe atom of our substrate is slightly puckered to minimize the energy and shows a significant process, and it can explain the nitrogen fixation successfully. In these processes, $2H_3O^+$ ($2H_3O+2H^+$) and N_2 are consumed to convert the dinitrogen into the diammonia together with producing the oxygen-related compounds. These oxygen-related compounds being a direct oxygen molecule or the peroxide molecules make it possible to interpret a lot of experimental evidences and to explain the processes of the nitrogen reduction successfully.

Introduction

Nitrogen is an essential element for constituting the cell of the plants or animals. However, there are severe limitations for the plants and animals to utilize the elemental nitrogen even though there are vast amount of the elemental nitrogens in the gaseous form. Some primitive micro-organisms - particular bacteria or blue green algae - have the enzymic capability to convert the elemental nitrogen into a combined form, namely ammonia. Since the term 'nitrogen fixation' was applied to these processes and the enzyme has such ability was called nitrogenase, a lot of scientific and engineering researches have been done on this field because of it importances in human life.

The nitrogenase enzyme complex is mainly consisted of two types of proteins; the Fe-protein[1,2] being composed of the α_2 dimeric sub-unit structure contains 4Fe and $4S^{-2}$ g·atom/mol, and these proteins are known to transfer the electrons that are supplied by an external reductant, with concomitant hydrolysis of ATP; and the Fe-Mo-protein[3-5] being known to have an $\alpha_2\beta_2$ subunit structure with the molecular weight in the ranges of 200000-240000 catalyzes the reduction of a large variety of the substrates when coupled with the electron-carrying Fe protein in the presence of Mg·ATP and an electron source. Four 4Fe-4S clusters being the main component of the Fe-protein are considered to be present in the Mo-Fe-protein molecule. Some respects in enzymological and physical evidence, it is believed that the Fe-Mo-cofactor of a cluster of the composition of $MeFe_{6-8}S_{8-9}$ is the catalytic site for the reduction of dinitrogen to an ammonia but the Fe/Mo ratio is still open to question. Since the bacterial nitrogenase was first extracted in an active form[6] and the well-characterized dinitrogen complex was isolated[7], there have been a great outpouring of theoreticals[8-12] and experimentals[13-17] in order to identify the nature of the nitrogen fixation.

Especially by the chemist and biochemist, many plausible mechanisms for the reduction of all nitrogenase substrates were formulated and they were used for the explanation of the observed results on the basis of the suggested models. Bulen and Newton[18,19] suggested some specific model in the respect of the relaltionship of HD formation to N_2 reduction and H_2 formation, and Stiefel[20] proposed his shceme for N_2 reduction, H_2 evolution, and C_2H_2 reduction by the nitrogenase, and precisely explained the observed 1 : 1 stoichiometry of N_2 fixation and H_2 evolution. Futhermore, Schrauzer[21] schematically provided the mechanism of the biological nitrogen fixation with combining the available evidence from the enzymological studies and the model experiments, and Thorneley and Lowe[22] developed the kinetic models for the nitrogen fixation and simulated the mechanism of the substrate reduction by the nitrogenase under the various conditions. One of the earliest observations made during the studies on the nitrogen fixation was that all of the nitrogen-fixing organisms contained the hydrogenase, many workers[23,24] tried to explain the role of hydrogenase, because N_2 is the only one that is inhibited by H_2[25,26]. It is also known that CO inhibits the hole processes of the nitrogen fixation by combining with the Fe-substrate[27,28].

The nature and the mechanism of the nitrogen fixation are still obscure only with being suggested the possible schemes as above. Now we try to identify the mechanism of the nitrogen reduction to an ammonia on the basis of the intermediate water structure model by introducing the water molecules to the nitrogen fixation system, where the water molecules have been investigated and known as doing the important roles in the biological systems[29-32] because the living organisms are mainly composed of the water and regulated by the water. With the same reason we can easily guess that the nitrogen fixation can also occur in coexistance with the water and the water molecules might be acting an important role as the intermediate in our processes.

An understanding of the equilibria between the biological solutes and the surrounding water is essential in the study of the reactions of biopolymers. Water is not merely an inert or static environment but sometimes it can form the particular structures acting a significant effect on the enzymic activity. With such ideas, we take the nitrogenase substrate con-

taining the one iron atom as $Fe(NH_3)_4Cl$[33-35] and $4H_3O^+$ capturing the dinitrogen, where the water molecules are composing a six-membered ring with the nitrogen atom upon the iron atom of the substrate. This ring structure made by the captured water molecules between the iron and nitrogen atom shows special processes during the optimization as producing the ammonia and the oxygen-related compounds. The oxygen-related compounds are the direct oxygen molecule or the peroxide molecules. There are a lot of experimental evidences that the nitrogenase enzyme is regulated by the leghemoglobin[36-38] and oxygen[39,40]. So we can explain such experimental evidences including H_2 evolution and H_2 inhibition on the basis of our intermediate water structure and in the respects of equilibria, and it is discussed more precisely in later. In the next section, we described briefly the computational method that we used in these calculations and its shortcomings. On the basis of our new shcemes for the nitrogen fixation with the intermdeiated water sturcture constructed by introducing the water molecules between the substrates, we can explain the various experimental results of the nitrogen fixation, and our calculated results are discussed and compared with the other works in later.

Computational Methods

The calculations that we performed on these systems were of the intermediate neglet of differential overlap (INDO) type[41-44] which has been successfully applied to the biological systems[45,46]. Self-consistent field (SCF) calculations were applied by using a generalized close-shell or open-shell operator described elsewhere[47] to the model of the nitrogenase substrate taken as $Fe(NH_3)_4Cl$ capturing the dinitrogen that Boca and *et al.*[33-35] investigated in the CNDO/2 formalism. The SCF calculations with a configuration interaction (CI) using a Rumer diagram technique[48-50] were done on our metal-dinitrogen complex. In the CI, only the single excitations were included to be consistent with the parametrization of this model Hamiltonian.

Results and Discussion

To explain the structure of an active site in the nitrogenase enzyme and to search the mechanism of the dinitrogen reduction into an ammonia are one of the most significant problems in the theoretical researches for the nitrogen fixation. According to the physicochemical properties of nitrogenase, there have been suggested a lot of models about the active site in nitrogenase[51-53]. Up to now, the Fe-protein containing the Fe-Me-protein is belived to be an active site in nitrogenase. By taking the active site of nitrogenase as $Fe(NH_3)_4Cl$ that was discussed by Boca *et al.*[33-35], we focused our research on how to make the mechanism of the nitrogen fixation clear and on how to explain the arrising phenomena in the biological organisms[18-21]. At the beginning of our work, we investigated two different types of the iron-dinitrogen complex in their orientation such as $Cl(NH_3)_4Fe\text{-}N\text{-}N$ (end-on bonding) and $Cl(NH_3)_4Fe\begin{matrix}N\\|\\N\end{matrix}$ (side-on bonding) in order to find out the correct pathway of the dinitrogen when it is approaching to the iron atom of the chosen model. The model of side-on bonding is hard to accept as the reasonable approach for producting an ammonia because of its seperation during the optimization process as $Cl(NH_3)_4Fe+N_2$, thus we take the end-on bonding type as the more reasonable substrate in performing the further processes of nitrogen fixation.

Table 1. Structural Parameters of $Fe(NH_3)_4Cl(N_2)$

	Distance (Å)	
	INDO/CI	R. Boca *et al.*[33]
Fe-Cl	2.53	2.31
Fe-NH_3	2.13	1.90
Fe-N_2	2.12	1.90
N-N	1.15	1.15

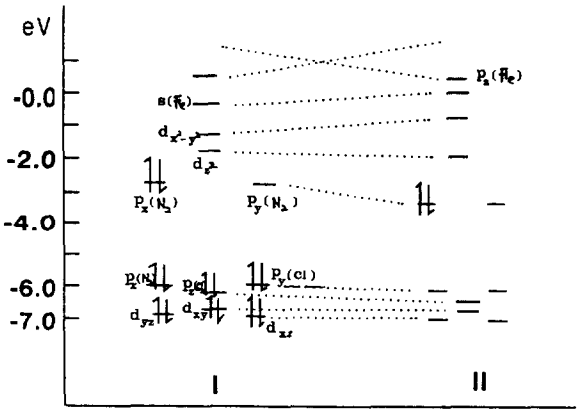


Figure 1. Electronic diagram of $Fe(NH_3)_4Cl(N_2)$. I: Obtained after INDO/CI optimization on RHF. II: Obtained after SCF/CI by using the structural data in ref. 33.

A large number of the structural studies of the transition metal-dinitrogen complexes has been made[54-56], but the bonding nature is still not clear and it shows a large variety of the bonding distance in the metal-nitrogen or nitrogen-nitrogen. In our calculations, it shows 2.12 Å for the Fe-N bond distance and 1.15 Å for the N-N bond distance, and the structural parameters of $Fe(NH_3)_4Cl(N_2)$ that we have taken as the substrate of the nitrogen fixation are summarized in Table 1. In general, when there are few electrons in the metal-dinitrogen complex, the highest energy level of the N-N bonding orbitals is unoccupied and the N-N bond distance is considerably longer[54-56]. In the molecular orbital eigenvalues for $Fe(NH_3)_4Cl(N_2)$, it shows that an activation to the dinitrogen orbital is depending on the geometric or electronic factors as depicted in Figure 1. The highest occupied molecular orbital (HOMO) and the lowest unoccupied molekcular orbital (LUMO) of our model become degenerate P_x and P_y orbital of the chlorine or dinitrogen. The HOMO being the degenerate P_x and P_y orbital of the chlorine is mixed with d_{xz} or d_{yz} orbital of the Fe atom according to its geometry. Due to the above facts, it shows that the electrons in the chlorine atom are easily excited to the upper states and the chlorine atom can get the positive charge by loosing the electrons during the processes of optimization.

Table 2. Müllikan Population Analysis of Fe $(NH_3)_4Cl(N_2)$

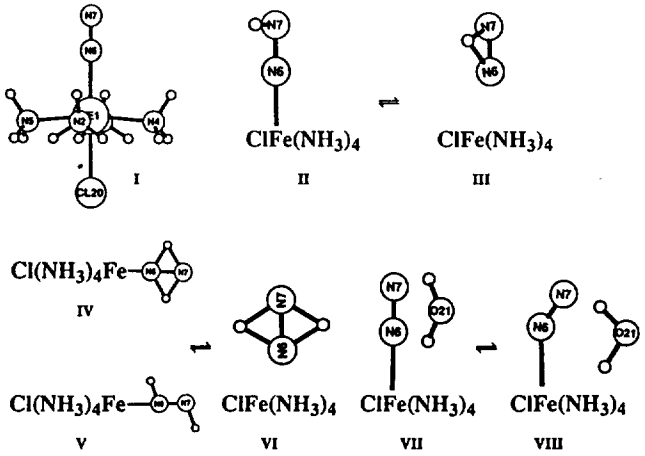
		INDO/CI[a]	SCF/CI[b]
F	d_{z2}	0.255	0.391
	d_{x2-y2}	0.303	0.447
	d_{xy}	1.996	1.981
	d_{xz}	1.977	1.918
	Net d	6.508	6.686
	$4s$	0.209	−0.027
	$4P_x=4P_y$	0.179	0.211
	$4P_z$	0.041	−0.106
N (in NH_3)	net charge	−0.362	−0.387
	π	1.152	1.147
N_1 (in N_2)	net charge	−0.044	−0.139
	π	1.117	1.128
N_2 (in N_2)	net charge	0.105	0.027
	π	1.053	1.039
Cl	net charge	−0.878	−0.954
	π	1.767	1.739

[a]Obtained after optimizing the structure. [b]Using the fixed structural data from ref. 39.

Such a phenomena causes to make the chlorine atom seperate from the substrate-it means that the original structure of the reduction substrate is changed or broken-unless the additional electrons are supplied from the external electron source such as Mg·ATP or flavodoxins[57]. Special shemes are prepared to illustrate the above fact and it is discussed in the later part of this chapter. The flexibility of the N-N bond length in the metal-dinitrogen complex might be caused by the fact that the electrons in the lower energy level are excited to the molecular orbital of nitrogen being the LUMO according to the energy difference between the HOMO and the LUMO. Thereby the N-N bond distance becomes longer or shorter depending on how much charges are exposed to the nitrogen, and we have considered such an effect in our calculation and discussed it later. The geometrical structure of $Fe(NH_3)_4Cl(N_2)$ is shown in structure I and the stereographic structure as depicted in structure I is replaced as $Cl(NH_3)_4Fe$-N-N for the simplified notation in all of our works, and its Müllikan populations at SCF level are reported in Table 2.

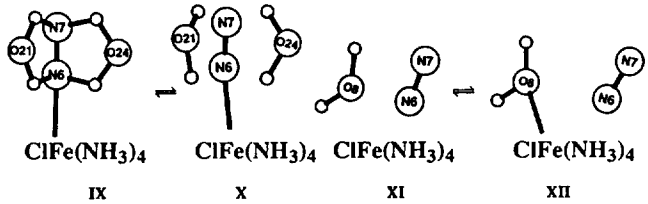
In the nitrogen fixation research, the word mechanism evokes a variety of the different images in paracticing the diverse disciplines. By the stoichiometric mechanism meaning the delineation of the elementary steps that occur during the optimization processes, we try to identify the mechanism of the dinitrogen reduction into an ammonia. Most of the suggested mechanisms for the nitrogen fixation being published elsewhere[18-22] were directly used to combine the dinitrogen with the hydrogen molecules or protons (H^+), even though the biological organisms are mainly composed of the water that has been doing the important roles on keeping the life. In such a respect, a lot of interest about the effects of water on the biological system is now increasing greatly and lots of the biological problems[29-32] are tried to solve by inserting the water molecules. Water is not merly an inert or static environment but is essential in the study of biopolymers.

At first, we reviewed the mechanism without using the water molecules and tried the nitrogen fixation but we couldn't accept such schemes represented by structures II to VI as the process of the nitrogen fixation because the dinitrogen was seperated from the substrate as soon as the proton is approaching to the dinitrogen as shown in structures III and VI. At this point, we thought more strongly that



the water molecules are necessary to make to proton (H^+) enable to attatch toward the nitrogen. The water molecules are inserted around the dinitrogen with changing the position and angle between the water molecules as shown in structures VII and IX, but still we couldn't get the desirable process any more.

Thus we tested the energy difference and the energy gradient between the dinitrogen and the water molecule when it attatches to $Fe(NH_3)_4Cl$, and also tested the competition between them as shown in structures XI and XII. When the N_2 or H_2O is moved to the 0.01 Å longer and the shorter from the reference geometry, the energy difference with the reference structure in the eV unit is 0.1108 and −0.1151 or 0.0865 and −0.3732, respectively. Here we hitted upon an idea that the water molecule is more easily attatched to the Fe-substrate than the dinitrogen and the water molecule is a primary molecule reacting with the Fe site of the Fe-complex, because the nitrogenase is always coexisted with the water in the living organism before the dinitrogen come close to the nitrogenase. A lot of possible models was constructed on the basis of above concept and they are depicted in structures XIII to XVI, but the dinitrogen still doesn't make a bond directly with any structure from XIII to XVI.



The results shown in structures XIII to XVI make us think the fact that nitrogen fixation can't arrise only using the water molecules in such a scheme but can achieve in acompany with the proton and water such as H_2O+H^+ (H_3O^+). On the basis of the above appearance, we tried to solve the

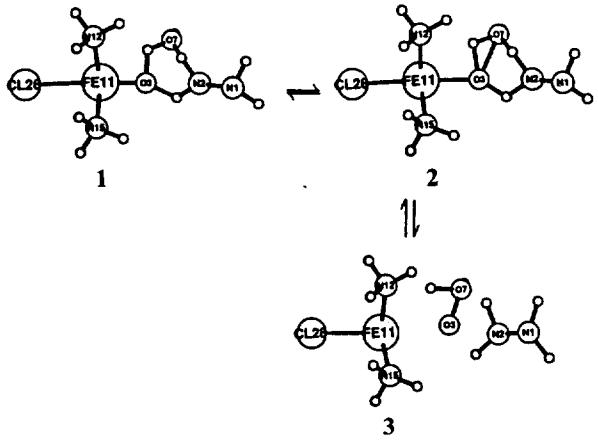


Figure 2. Processes of INDO optimization with using $2H_3O^+$. Structure 1 is the starting model and others are the intermediate structure obtained during the optimization.

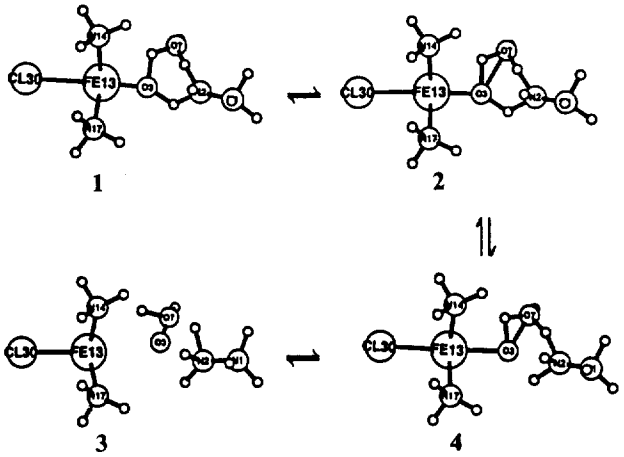
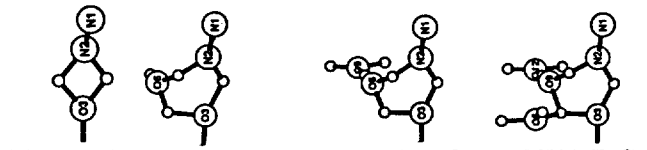


Figure 3. Processes of INDO optimization with using $2H_3O^+ + 2H^+$. Structure 1 is the starting model and others are the intermediate structure obtained during the optimization.



$(NH_3)_4FeCl$ $ClFe(NH_3)_4$ $(NH_3)_4FeCl$ $(NH_3)_4FeCl$

XIII XIV XV XVI

problems by using the water molecules and protons together as illustrated in Figures 2 and 3 and their charge densities are shown in Tables 3 and 4.

Finally, we introduce the process of the dinitrogen reduction into an ammonia with construction a dinitrogen-water-$Fe(NH_3)_4Cl$ complex as depicted in Figure 4, and their charge densities are reported in Table 5. Even though the process of producing the ammonia or the amine by using two water molecules as shown in Figures 2 to 5 are performed on RHF and UHF calculation there is a slight difference between them in preforming the process of the nitrogen fixation. But the schme that produces the ammonia in Figures 3 is less reasonable than the scheme in Figures 2 and 4, because

Table 3. Formal Charge Density of an Individual Atom Shown in Figure 2

	Formal charge		
	1[a]	2[a]	3[a]
Fe	0.918	0.910	0.678
N_1	−0.133	−0.088	−0.028
N_2	−0.248	−0.200	−0.089
N_{12}	−0.528	−0.538	−0.555
Cl	−0.289	−0.419	−0.498
O_3	−0.507	−0.371	−0.359
H_4	0.499	0.498	0.496
H_5	0.406	0.410	0.514
H_6	0.358	0.392	0.443
O_7	−0.519	−0.426	−0.298
H_8	0.534	0.480	0.418
H_9	0.422	0.456	0.443
H_{10}	0.344	0.359	0.403

[a] The numerical numbers mean the structure depicted in Figure 2, and the molecular energy (in *au*) of each stucture is −144.7112 for 1, −144.9932 for 2, and −145.4181 for 3.

Table 4. Formal Charge Density of an Individual Atom Shown in Figure 3

	Formal charge			
	1[a]	2[a]	3[a]	4[a]
Fe	0.817	0.816	0.874	0.876
N_1	−0.292	−0.268	−0.251	−0.248
N_2	−0.533	−0.442	−0.362	−0.266
N_{14}	−0.541	−0.543	−0.554	−0.596
Cl	0.756	0.726	0.637	0.172
O_3	−0.376	−0.361	−0.386	−0.211
H_4	0.487	0.484	0.463	0.461
H_5	0.346	0.361	0.483	0.538
H_6	0.425	0.422	0.424	0.464
O_7	−0.346	−0.313	−0.331	−0.026
H_8	0.503	0.446	0.437	0.362
H_9	0.508	0.515	0.516	0.418
H_{10}	0.383	0.379	0.376	0.406
H_{11}	0.417	0.411	0.402	0.424
H_{12}	0.356	0.325	0.279	0.308

[a] The numerical numbers mean the structure depicted in Figure 3, and the molecular energy (in *au*) of each structure is −144.5756 for 1, −144.5757 for 2, −144.8162 for 3, and −145.1753 for 4.

it couldn't keep the balance with the solvated water as $2H_3O^+ + 2H^+$ and the proton being not solvated with the water is not acceptable in the biological systems. Here, we can find out some meaningful facts that the water molecules are composed of a six-membered ring with the nitrogen atom upon the iron atom of the substrate and only these ring structure schemes give the significant results as depicted in Figures 2 and 4. This six-membered ring is slightly puc-

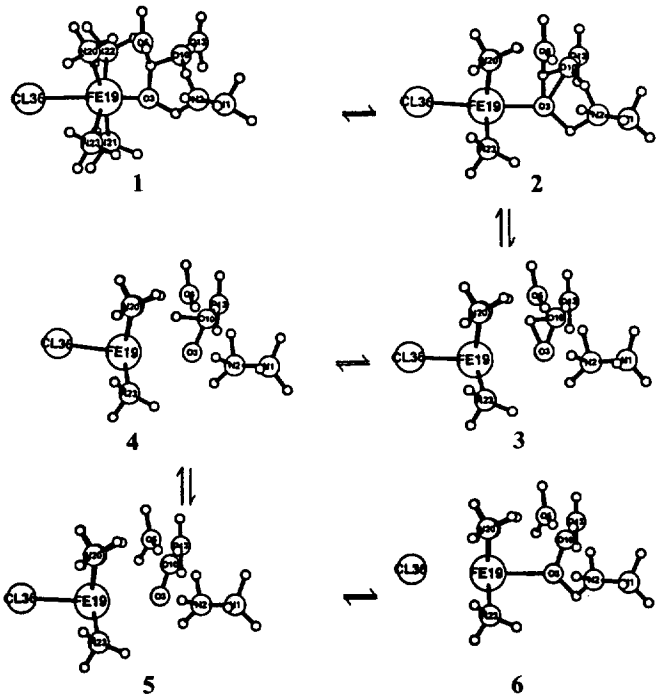


Figure 4. Proesses of INDO optimization with using $4H_3O^+$. Structure 1 is the starting model and others are the intermediate structure obtained during the optimization.

Table 5. Formal Charge Density of an Individual Atom Shown in Figure 4

	Formal charge					
	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]
Fe	0.978	0.959	0.995	0.913	0.759	0.665
N_1	−0.312	−0.231	−0.253	−0.237	−0.233	−0.277
N_2	−0.538	−0.418	−0.328	−0.321	−0.332	−0.361
N_{20}	−0.569	−0.343	−0.572	−0.581	−0.581	−0.560
Cl	0.319	0.242	0.193	0.254	0.124	0.333
O_3	−0.610	−0.479	−0.359	−0.317	−0.271	−0.274
H_4	0.503	0.460	0.461	0.456	0.485	0.475
H_5	0.487	0.517	0.557	0.493	0.500	0.488
O_6	−0.518	−0.544	−0.536	−0.529	−0.506	−0.497
H_7	0.417	0.381	0.396	0.409	0.436	0.429
H_8	0.360	0.298	0.304	0.329	0.373	0.360
H_9	0.405	0.396	0.430	0.428	0.432	0.430
O_{10}	−0.409	−0.402	−0.302	−0.310	−0.226	−0.381
H_{11}	0.475	0.444	0.393	0.383	0.365	0.409
H_{12}	0.526	0.492	0.411	0.431	0.422	0.471
O_{13}	−0.446	−0.466	−0.459	−0.465	−0.428	−0.472
H_{14}	0.440	0.422	0.421	0.414	0.438	0.425
H_{15}	0.499	−0.482	0.475	0.460	0.487	0.472
H_{16}	0.355	0.346	0.374	0.370	0.365	0.364
H_{17}	0.398	0.376	0.396	0.385	0.384	0.382
H_{18}	0.336	0.227	0.252	0.254	0.262	0.275

[a] The numerical numbers mean the structure depicted in Figure 4, and the molecular energy (in *au*) of each structure is −180.6611 for 1, −180.9043 for 2, −181.2118 for 3, −181.4482 for 4, −181,5709 for 5, and −181.6573 for 6.

kered to minimize the energy and the water molecules shown in Figure 4 keep the balance with the proton (H^+) as $4H_2O+4H^+$, namely $4H_3O^+$. Such a phenomenon suggests the fact that the protons make a desirable bond with the dinitrogen only on the help of the water molecules, where many workers[29-32] begin to believe that the water molecules bound to the biological solutes mainly constitute a six-membered ring and gives a special environmental effect on that system.

Supposed that the nitrogen fixation were occurred in the same condition as shown in Figures 2 and 4, there are produced $2NH_3$ and the oxygen-related compounds being O_2 or H_2O_2. Fortunately, there are a lot of conclusive evidences[36-38,58,59] for the role of the oxygenated-leghemoglobin (LbO_2) as an oxygen carrier to the bacteroids. Bergersen *et al.*[36,58] added LbO_2 to the respiring bacteroid suspensions and observed a doubling of the O_2 uptake and twenty fold increase in the nitrogenase activity, and Wittenberg *et al.*[37,59,60] investigated the effect on the nitrogenase activity in the bacteroid suspensions of series of the O_2-carrying proteins and concluded that the stimulation of the nitrogenase activity was related to the kinetics of O_2-binding to the carrier. Thus the oxygen-related compounds shown in our schemes might be coorperated with the leghemoglobin and hydrogenase[23,24] being contained in all of the nitrogen-fixing organisms to produce LbO_2 or H_2 such as in the following reaction.

$$H_2O_2 \text{ (or } O_2) < \xrightarrow{Lb} > LbO_2 + H_2 \qquad (1)$$

Now, we can interpret the above experimental facts as the leghemoglobin stimulates the process of the nitrogen fixation by consuming the appeared oxygen on the assumption that the leghemoglobin reacts with the oxygen-related compounds produced in our calculations and devides these compounds into a carriable oxygen as the form of LbO_2 and the hydrogen molecule under the help of hydrogenase that was known to coorperate with nitrogenase. Based on the above equation and concepts, we can also solve the H_2 evolution and the observed 1 : 1 stoichiometry of N_2 fixation and H_2 evolution, and especially we can get the another solution about H_2 inhibition to nitrogen fixation[25,26] in the respect of the equilibria of Eq. (1) and in Eqs. (2) and 3.

$$Fe(NH_3)4Cl + N_2 + 4H_3O + (4H_2O + 4H^+)$$

$$< \xrightarrow{e^-} > Fe(NH_3)_4Cl + 2NH_3 + \begin{cases} H_2O_2 + 2H_2O \\ O_2 + 2H_3O^+ \end{cases} \qquad (2)$$

$$< \xrightarrow{Lb} > \begin{cases} Fe(NH_3)_4Cl + 2NH_3 + LbO_2 + 2H_2O + H_2 \\ Fe(NH_3)_4Cl + 2NH_3 + LbO_2 + 2H_2O^+ \end{cases} \qquad (3)$$

On the assumption of producting the NH_3 and the oxygen-related compound with the help of the leghemoglobin as in Eqs. (1), (2) and (3), therefore, the 3rd step in Figure 2, 4th steps in Figure 3 and the 3rd, 4th, and 5th steps in Figure 4 were re-optimized respectively with removing the $2NH_3$ and/or the oxygen-related compounds in order to check the any variance on the substrate, but there is no difference between the starting substrate and the final substrate. And we have tried the various models that was thought to be possible, almost the similar process and the pro-

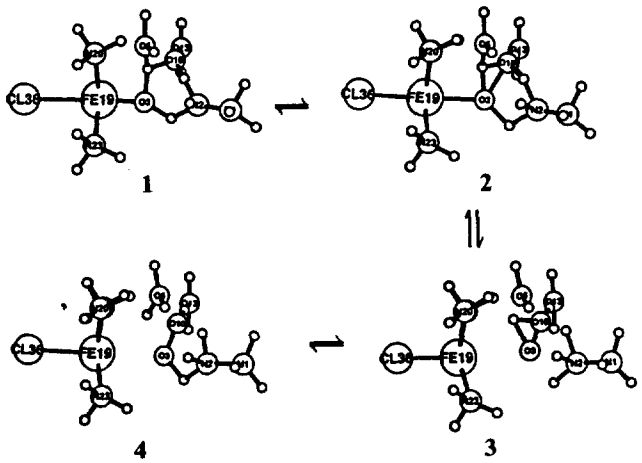


Figure 5. Processes of INDO optimization with the assignment of electrons in *d* orbitals of Fe atom and with the addition of two electrons. Structure 1 is the starting model and others are the intermediate structure obtained during the optimization.

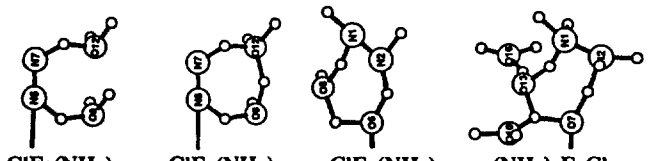
Table 6. Formal Charge Density of an Individual Atom Shown in Figure 5

	Formal charge		
	1[a]	2[a]	3[a]
Fe	0.922	0.966	0.998
N_1	−0.293	−0.214	−0.276
N_2	−0.628	−0.467	−0.363
N_{20}	−0.534	−0.542	−0.557
Cl	−0.605	−0.698	−0.736
O_3	−0.547	−0.456	−0.367
H_4	0.499	0.462	0.367
H_5	0.481	0.572	0.561
O_6	−0.532	−0.544	−0.541
H_7	0.352	0.342	0.357
H_8	0.294	0.286	0.302
H_9	0.342	0.347	0.369
O_{10}	−0.515	−0.494	−0.384
H_{11}	0.520	0.468	0.401
H_{12}	0.545	0.506	0.418
H_{13}	−0.487	−0.478	−0.477
H_{14}	0.388	0.391	0.381
H_{15}	−0.430	0.432	0.416
H_{16}	0.319	0.310	0.319
H_{17}	0.351	0.341	0.347
H_{18}	0.317	0.218	0.204

[a]The numerical numbers mean the structure depicted in Figure 5, and the molecular energy (in *au*) of each structure is −182.1903 for 1, −182.4788 for 2, and −183.1549 for 3.

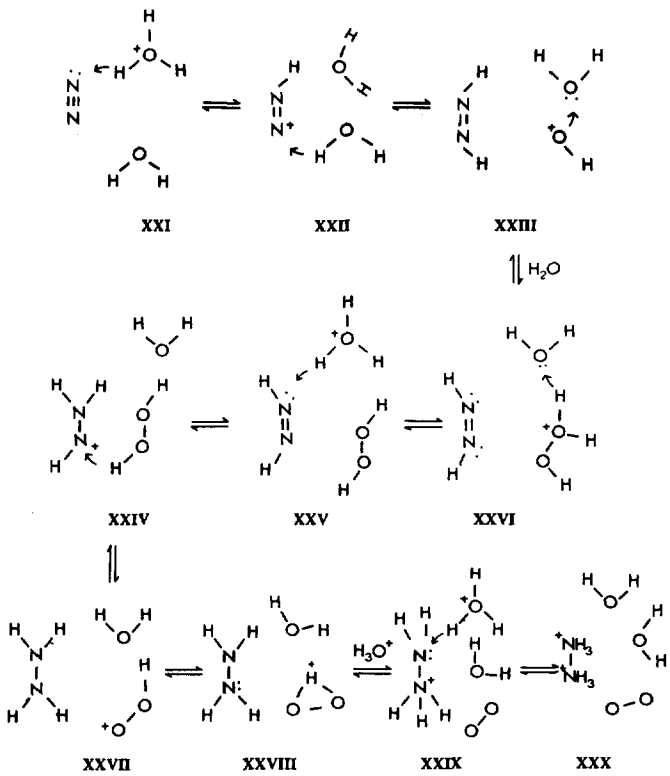
ducts are obtainable except some special case.

Almost similar results are obtained as shown in Figure 5, when we optimized the process even though the electrons of *d* orbitals in the Fe atom and/or *P* orbitals in the Cl atom of our starting substrate in Figure 4 were modified. The formal charge densities of each atom depicted in Figure 5 are reported in Table 6. Futhermore, by re-optimizing the 6th step in Figure 4 with adding two electrons, the chlorine atom being aparted from the Fe-substrate can make rebond with the Fe-substrate. This phenomena strongly recommends the facts the external electron sources are necessary to perform the nitrogen fixation as pointed out by many workers[57,61,62]. The water molecules were made the various ring structure of different styles as the structures from XVII to XX of the following schemes, however, none of them can create the reasonable process, because N_2 is seperated from the Fe-substrate as Fe-substrate + N_2 during the optimization.



XVII XVIII XIX XX

Based on our calculations and various experimental evidences mentioned above, the following mechanisms that produce the amine, the ammonia, the peroxide form water, and the oxygen molecule from the peroxide during the optimization are strongly recomendable. These precise steps are not



appeared in an individual phase of the optimization. It was omitted one or two steps in the above schemes according to the starting conditions but the trends of the hole process to make the dinitrogen reduce to the ammonia are almost similar and these schemes are implied under the optimization. At the above schemes, $2H_3O^+$ are comsumed to produce the desired products and the external electron sources are necessary because the electrons are deficient in the hole mechanism. Three types of the N-N bond distance, 1.15

Å, 1.28 Å, and 1.41 Å for corresponding respectively to the triple bond, double bond, and single bond type as mentioned earlier[54-56] were applied to the same starting substrate used in Figure 4 in order to find out the effect of N-N bond distance on the process of optimization. The triple bond type is exactly same as done in Figure 4 and the other two types also produced the $2NH_3$ and the oxygen-related-compounds but not exactly same as in Figure 4.

Conclusions

We have considered the case of the dinitrogen reduction to an ammonia, being recently increased the importance and the usefullness in the biological and industrial respects. Using the INDO method that has been applied well to the biological systems, we set up the process of the dinitrogen reduction from the initial substrate and nitrogen molecule captured in the intermediate water molecules. The water molecules constructing an active six-membered ring in our system are our key point in this work and it make us accept its importance as in the general biological systems, and this six-membered ring mainly composed by the nitrogenase substrate and two water molecules gives a best meaningfull process among the several investigated models as discussed.

It consumes $2H_3O^+$ ($2H_2O+2H^+$) together with the nitrogen molecule, and then produces $2NH_3$ and the oxygen molecule in some process as $4H_3O^+ + N_3 \Leftrightarrow (NH_3\text{-}NH_3)^{2+} + 2H_3O^+ + O_2$. In the other process-most of processes using the intermediate water structure-it shows the reaction as $4H_3O^+ + N_2 + (e^-\ \text{source}) \Leftrightarrow H_2O_2 + 2NH_3 + 2H_2O$. Supposed that this hydrogen peroxide were devided into the oxygen molecules and the hydrogen molecule with the help from the other enzymes, we can make an explanation of the hole nitrogen reduction processes together with the hydrogen evolution as $H_2O_2 + Lb \Leftrightarrow LbO_2 + H_2$. Here we can easily image that the leghemoglobin might be an enzyme mentioned above, and this leghemoglobin located in the nodules must be coorperated with the nitrogenase and hydrogen evolution as shown in the experimentals.

Our schemes being based on the intermediate water structure and being different from the others that have been published untill now are sufficiently recommendable for the explanation of the nitrogen fixation including the dinitrogen reduction into an ammonia, H_2 evolution, H_2 inhibition, CO inhibition, and the regulation by the leghemoglobin and O_2 in the respect of equilibria shown as $Fe(NH_3)_4 + N_2 + 4H_3O^+ + Lb + e^-\ \text{source} \Leftrightarrow Fe(NH_3)_4Cl + 2NH_3 + LbO_2 + H_2 + 2H_2O$.

Almost the same processes are obtained in spite of varying the starting condition in our work by assigning the electrons in the P or *d* orbitals of the Cl or Fe atom and re-optimizing with adding the electrons within some special step. Especially the Fe-substrate was recovered to the original substrate by re-optimizing with adding the electrons despite of its variation, and the Fe-substrate are also clearly restored to the original state by continuing the optimization process with removing the products of our aim, namely NH_3 and the oxygen-related compounds. Thus the nitrogen fixation could be circulated by constructing the proper structure on this recovered substrate.

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Thermal Conversion Between Diastereomeric 1,4-Dipolar Cycloadducts

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Thermal conversion between diastereomers formed *via* 1,4-dipolar cycloaddition was identified by ^{1}H-NMR spectroscopic study depending on temperature and reaction time. Hereupon the formed product by kinetic control was converted to the thermodynamically controlled product. Various diastereomeric 1,4-dipolar cycloadducts were synthesized by the reacting of 5,6-dihydro-3-phenyl-7-[*N*-phenyl(carbamoyl)]imidazo[2,1-*b*]thiazolium-betaine with a series of *para*-substituted phenacyl bromides and the substituent effects were investigated.

Introduction

N-Bridged thiazolium-betaines show the strong nucleopilicity and basicity based on the presence of the carbanion. These betaines, which are highly reactive organic compounds, can be used for the synthesis of complicated heterocyclic compounds *via* ring transformation and 1,4-dipolar cycloaddition reactions.