Physical Chemistry

Unique peculiarities of the structure of the Fe—Mo cofactor of nitrogenase favorable for multicenter coordination of a nitrogen molecule

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The probable coordination mode of a nitrogen molecule on the Fe—Mo cofactor of nitrogenase has been theoretically considered, taking into account both the well-known data on the structure of the Fe—Mo cofactor and the substrate selectivity of nitrogenase and the results of semiempirical calculations of the electronic structures of the cofactor and its complexes with molecular nitrogen. The distances between the Fe atoms in the cofactor are favorable for different multicenter coordination modes of a nitrogen molecule: above the Fe_4 face along its diagonal, through this face, and inside the Fe_6 prism perpendicularly to its axes. It is important that the nitrogen atoms are open for protonation in all coordination modes. The first mode is disadvantageous due to steric hindrances. Of the other variants, the latter is the most favorable both energetically and from the viewpoint of weakening of the N—N bond.

Key words: nitrogenase, Fe—Mo cofactor, coordination of molecular nitrogen, quantum-chemical modeling.

Recent studies of the structures of Fe and Mo—Fe proteins of nitrogenase[1,2] make it possible to approach a deeper insight into the mechanism of the enzymatic nitrogen fixation.[3] As it is known, the Mo—Fe protein consists of two α- and two β-subunits, and, according to the published data,[2] the key groups are arranged in the following way: the Fe—Mo cofactor is inside the α-subunit, and the P-cluster pair is located at the boundary of the α- and β-subunits. Since there are no permanent channels between the protein surface and cofactor, it can be assumed that substrates are transported to the active center and the products are transferred in the opposite direction due to the appropriate fluctuations of the protein structure. The mechanism of the electron transfer is studied less, and the arrangement of ATPase centers and the mode of complex formation of Fe and Mo—Fe proteins are not quite clear. Of many yet unsolved problems of the mechanism of enzymatic nitrogen fixation, in this work we confine ourselves to the consideration of the modes of coordination of substrates on the Fe—Mo cofactor and to the electronic structures of the cofactor and its complexes with molecular nitrogen.

Fe—Mo cofactor

The core of the $MoFe_7S_9$-cofactor, (Cys-275)$Fe(\mu_3\text{-}S)_3Fe_3(\mu\text{-}S)_3Fe_3(\mu_3\text{-}S)_3Mo$(His-442)(HCit), is "attached" to the protein chain *via* the S atom of cysteine Cys-275 and the N atom of histidine His-442, and the molecule

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of homocitrate (HCit) is coordinated bidentately *via* the hydroxyl and carboxyl groups at C-2 with the molybdenum atom. The structure of the metal framework of Fe_7Mo is a two-capped triangular prism. The terminal iron and molybdenum atoms have tetrahedral and octahedral environments, respectively. The coordination numbers of six iron atoms forming the triangular prism are very unusual. Formally, all these numbers are equal to three; however, because of very short Fe—Fe distances (on the average, 2.51 Å* along the edges of the prism), an increase in the coordination numbers can be expected due to the formation of three Fe—Fe bonds.

The cluster of this unusual structure has no analogs among numerous synthetic Fe—S and Mo—Fe—S clusters[4]; however, mononuclear complexes with three-coordinate iron are known.[5] The $MoFe_7S_9$ cluster of the cofactor is unusually strong, as it can be isolated undergraded from nitrogenase under acidic conditions that bring about decomposition of ordinary Mo—Fe—S and Fe—S clusters. All this indicates that despite a relatively high exposedness of the Fe atoms with the coordination numbers of 3 (they are only embedded by 0.30 Å, on the average, with respect to the plane of three coordinated S atoms), they are rather inert. This can be explained by the highly compact structure of the cofactor and sufficiently high strength of the short Fe—Fe bonds, so that it can be decomposed only by a simultaneous attack at several centers, which, of course, is unlikely.

The Fe_3Mo tetrahedron is more compact than the Fe_4 tetrahedron. The average Mo—Fe distance (2.92 Å) is shorter than the average distance from the apical Fe atom to the Fe atoms in the base of the prism (2.96 Å), despite the greater radius of Mo. The Fe—Fe distances in the base of the prism from the side of the Mo atom are also shortened relative to the Fe—Fe distances in the opposite base: 2.77 and 2.93 Å (on the average). This corresponds to the pattern observed in Fe—Mo—S clusters. For example, in the doubled cluster $[MoFe_3(\mu_3\text{-}S)_4(SEt)_3(Pr_2cat)]_2^{4-}$ ("cat" is the catecholate dianion), the Mo—Fe and Fe—Fe distances are 2.73 and 2.71 Å,[6] respectively, while in the $[Fe_4(SCH_2Ph)_4(\mu_3\text{-}S)_4]^{2-}$ cluster the Fe—Fe distances vary from 2.732 to 2.766 Å,[7] and in the Fe_4S_4 cluster of bacterial ferredoxin the average Fe—Fe distances are[8] 2.85 Å.

It is evident that the $MoFe_3$- and Fe_4-subsystems, if considered separately, possess different redox properties. In addition, both clusters with tetrahedral metalloframeworks have high electron capacities. Therefore, one can expect the existence of the intracluster electron transfer between the subsystems resulting in different effective charges of the $MoFe_3$ and Fe_4 clusters. At the total null charge, their charges will be evidently opposite, which will result in the mutual attraction of the $MoFe_3$- and Fe_4-clusters and, as a consequence, in the shortening of the intercluster Fe—Fe distances.

By contrast, when the system is charged, the signs of the effective charges on the clusters can become identical, hence, the attraction will be changed to repulsion. Thus, based only on purely Coulomb interactions (electron orbital effects will be analyzed below), one can expect that in a certain state the cofactor will possess the most compact structure, while oxidation and reduction of the system will result in its weakening. This structural rearrangement is favorable for the coordination of both the substrates and the products of their reduction.

Cofactor charge

The analysis of the charge of the cofactor will be based on the principle of electroneutrality, *i.e.*, the impossibility of creating high local noncompensated charges in a protein. The study of the structural data published[2] shows that two residues Arg 96 and Arg 359 as well as His 195 capable of carrying positive charges are located in close proximity to the cofactor (Fig. 1). If

* It should be noted for comparison that the Fe—Fe distance in metallic α-Fe is 2.48 Å.

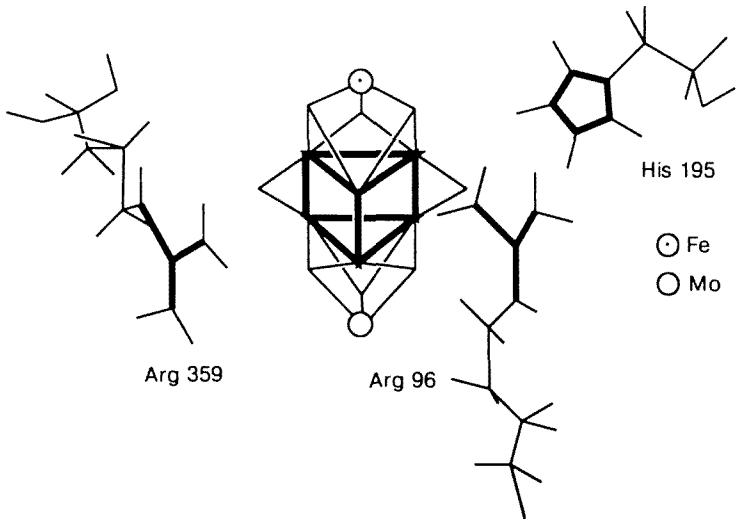

Fig. 1. General view of the core of the Fe—Mo cofactor surrounded by amino acid residues that can carry positive charges.

it is assumed that both the cofactor itself and two free carboxyl groups of homocitrate can act as counterions, the overall negative charge of the cofactor along with homocitrate can vary from 0 to −3 with the whole system being electroneutral. Taking into account the possibility of two forms for carboxyl groups (charged and noncharged), we can conclude that the inherent charge of the cofactor can vary from +2 to −3.

The extreme cases corresponding to two uncharged arginine residues and both nondissociated groups of the homocitrate ligand should be considered unlikely. Taking into account the known data that the valent state of Mo^{IV} is the most probable and it does not change in the reduction of the cofactor,[9] we have the following degrees of oxidation.

Charge of cofactor	Degree of oxidation	State of the nearest environment
+1	Mo^{IV} $3Fe^{II}$ $4Fe^{III}$	($HCit^{2-}$, Arg^+ or $HCit^-$)
0	Mo^{IV} $4Fe^{II}$ $3Fe^{III}$	($HCit^{2-}$, 2 Arg^+ or $HCit^-$, Arg^+)
−1	Mo^{IV} $5Fe^{II}$ $2Fe^{III}$	($HCit^{2-}$, 2 Arg^+, His^+ or $HCit^-$, 2 Arg^+)
−2	Mo^{IV} $6Fe^{II}$ Fe^{III}	($HCit^-$, 2 Arg^+ His^+)

Since the resting state of the cofactor has[10] the spin $S = 3/2$, the cofactor should be considered noncharged, taking into account the existence of both oxidized and reduced forms of the cofactor.[10] The Fe^{II} : Fe^{III} ratio seems acceptable, if the known redox potentials of the Fe—S and Mo—Fe—S clusters are taken into account. In this case, the overall charge −1 is the most probable, *i.e.*, one free carboxyl group is dissociated, which corresponds to the known data on pK of dicarboxylic acids (if dissociation constants of coordinated homocitrate and dicarboxylic acid are taken to be equal).

It is likely that the ability of the homocitrate ligand to exist in different charge states is important for the matched transfer of electrons and protons to the cofactor during the catalytic cycle. As follows from the data on the changes in the activity of nitrogenase on chemical modification[11] of the homocitrate ligand, the homocitrate ligand itself possesses the optimum structural organization to perform this function.

Coordination of the N_2 molecule

Several modes for the coordination of the N_2 molecule with the cofactor can be suggested, especially if a possibility of substitution of several ligands is assumed. Many of these modes have been analyzed theoretically.[12] In this work, formulating the mode for the coordination of molecular nitrogen, we will proceed from the known regularities of the reduction of various substrates with nitrogenase rather than from the comparative analysis of numerous types of coordination (due to the difficulty of absolute comparison in semiempirical approaches).

As is known, nitrogenase readily reduces acetylene and does not reduce ethylene. It is quite reasonable to assume that the coordination modes of nitrogen and acetylene molecules are identical since they are isoelectronic. Then, accepting the assumption that the reason for the basic difference in the activities of acetylene and ethylene is a coordination mode of acetylene, which cannot be realized for ethylene, we can draw the following conclusion. If the structure of the cofactor is not changed, the only form of the external coordination of nitrogen and acetylene acceptable from the viewpoint of geometry is realized when they are arranged above the tetragonal face of the prism along its diagonal (Fig. 2, **A**). It turns out that this coordination is impossible for ethylene because H...S contacts are so short (~1.00—1.50 Å) that they are even shorter than the S—H bonds.

A reasonable deformation of this structure can hardly provide the disappearance of these contacts. This coordination mode will be analyzed below. It has not been considered previously,[12] and of all coordination modes for nitrogen discussed so far, only one mode of the type of the roof above the edge of the Fe_6 prism instead of the μ-S atom is also possible for acetylene. However, coordination of this type is also possible for ethylene. Therefore, according to the criterion accepted, we consider the coordination of nitrogen to the edge to be substantially less probable, despite the fact that it turned out to be the most preferable of all the modes considered.[12] In fact, if it is accepted that the coordination of nitrogen instead of the bridge S atom is possible, it should also be possible for ethylene as a more active ligand, because the existence of any substantial electron forbiddance factors can hardly be expected.

The intriguing possibility of multicenter coordination of molecular nitrogen inside the Sm_2Li_4 cluster has been recently shown,[13] which virtually results in a cleavage of the N—N bond. Based on these data, we can consider two more types of the multicenter coordination of the nitrogen molecule on the cofactor: perpendicularly to the Fe_4 face upon its "half-embedding" inside the Fe_6 prism (Fig. 2, **B**) and under the Fe_2 edge (Fig. 2, **C**). This results in the appearance of 10 and 8 Fe—N bonds, respectively, against 6 bonds for the coordination above the face. Of the multicentered coordination modes, only the second mode with embedding into the Fe—Mo cofactor is possible for acetylene and also for its derivatives with the terminal triple bond. However, as regards the coordination of the nitrogen molecule itself, the difference between these structures is not so considerable and consists in variable extent of immersion inside the cofactor.

Multicenter coordination of nitrogen inside the cofactor has also been considered.[12] However, in this mode the nitrogen molecule was arranged completely inside the Fe_6 prism along its approximate symmetry axis of the third order, which required a substantial divergence of the triangular bases of Fe_3. This model leads to other difficulties in the interpretation of the catalytic cycle of

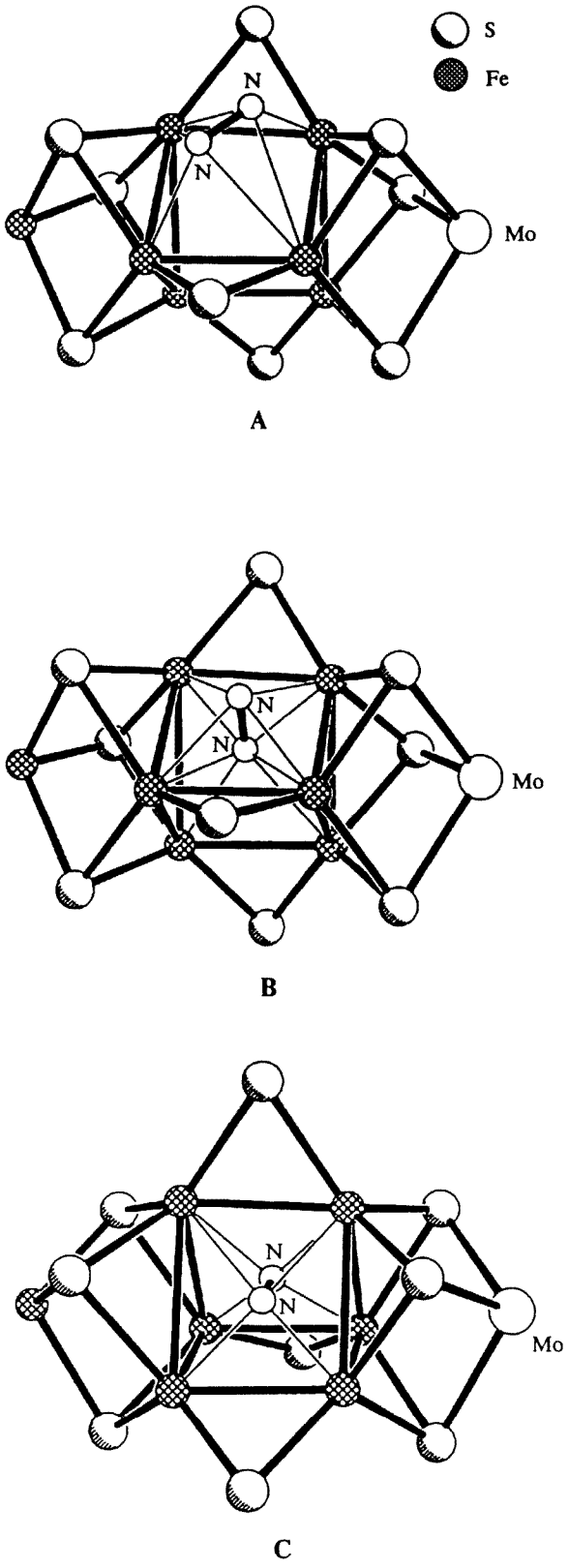

Fig 2. Variants of the multicenter coordination of the nitrogen molecule on the Fe—Mo cofactor.

the nitrogen reduction due both to the inaccessibility of the "sealed" nitrogen molecule for protonation and the necessity of the reverse assembling of the compact structure of the cofactor after the stage of the formation of ammonia. It is also difficult to imagine that other substrates of nitrogenase (this coordination mode is inaccessible for the molecules more than diatomic) react in a similar way; therefore, to explain the known data on the mutual effect of nitrogenase substrates upon their joint reduction we should use additional postulates.

The modes for the considered coordination of the nitrogen molecule do not create hindrances for its protonation and at the same time they are also probable in principle for other substrates, including alkynes with the terminal triple bond. The only exception is diazirine, for which only the external coordination of the N=N bond is possible. It will be clear from further discussion that the internal coordination of nitrogen has several advantages over the external coordination, which qualitatively explains an insignificant ability of nitrogenase to reduce diazirine, despite its high energy capacity and weakening of the N=N bond.

It can be assumed from general considerations that the coordination modes of charged and neutral substrates are least equivalent from the viewpoint of the perturbation created by them in the enzyme. Therefore, we cannot accept the results,[14] according to which the cyanide ion is coordinated by the Mo atom in the isolated Fe—Mo cofactor, as a basis for the analogy with the coordination of the nitrogen molecule.

Quantum-chemical modeling

For the quantum-chemical calculations, the cluster core of the $MoFe_7S_9$ cofactor was reduced to the idealized symmetry C_{3v} with the following distances (in Å): $Fe—Fe_a$ = 2.96, $Fe_a—Fe_a$ = 2.93, $Fe_b—Fe_a$ = 2.51, $Fe_b—Fe_b$ = 2.77, $Fe_b—Mo$ = 2.92, $Fe—S_a$ = $Fe_a—S_a$ = 2.35, $Fe_b—S_b$ = 2.31, $Mo—S_b$ = 2.46, and $Fe_a—S$ = $Fe_b—S$ = 2.27 obtained by averaging equivalent pairs of the distances for both cofactors in the Mo—Fe protein[2] (the Fe atoms and the μ_3-S atoms connected with them in the bases of the Fe_6 prism and closest to the apical Fe and Mo atoms are designated by indices a and b, respectively). In addition, to simplify the system, the external ligands (coordinated groups attached to the protein and the homocitrate molecule) are substituted by the H^- ligands placed regularly at the distances of 1.8 and 2.0 Å from the Fe and Mo atoms, respectively.

The calculations were performed by the extended Hückel method, modified so as to take into account intraatomic interelectronic interactions[15] with the same parametrization as in the previous quantum-chemical study of the cluster model of the cofactor of nitrogenase.[15] It is significant that the account of the Hund energy results in the electron decoupling within the

Table 1. Results of the calculation of the model of the $(H)_3MoFe_3S_9Fe_3Fe(H)$ cofactor

Atom or bond	Configuration* (state) 77/43 (ox)	77/44 (rest)	77/45 (red)	60/60 (ox)	61/60 (rest)	61/61 (red)
	Charges on atoms and groups					
Mo	1.13	1.00	0.99	2.75	2.62	2.49
Fe	0.50	0.48	0.48	−0.24	−0.42	−0.58
Fe_a	0.47	0.43	0.27	−0.28	−0.31	−0.35
Fe_b	0.53	0.48	0.37	0.44	0.30	0.16
S	−0.51	−0.54	−0.55	−0.35	−0.37	−0.39
$MoFeS_3$	2.02	1.65	1.21	3.92	3.32	2.72
$FeFeS_3$	1.14	0.99	0.47	−0.84	−1.18	−1.51
	Bond populations					
Fe_a—Fe	−0.01	−0.01	−0.01	0.05	0.04	0.04
Fe_b—Mo	0.02	0.02	0.02	0.07	0.08	0.09
Fe_a—Fe_b	0.17	0.17	0.20	0.23	0.23	0.23
S—Fe_a	0.51	0.51	0.51	0.58	0.58	0.58
S—Fe_b	0.50	0.50	0.50	0.61	0.60	0.60

* In designations of the configuration a/b, the numbers a and b are equal to the upper boundaries of occupation for α- and β-electrons, respectively.

band of the d-type levels and the formation of local high-spin states of Fe and Mo atoms. This conclusion corresponds to the ENDOR [16] and NGR data[17] and is ultimately caused by the relation of the gain in energy upon the electron decoupling on the centers and the width of the band of d-levels. The quantitative estimations show that in the resting state the energy minimum is achieved for the decoupling of approximately 31 electrons. Two electrons populate the lower orbital doublet of the d-states of the Mo atom occupying the upper part of the band of d-levels, and the local spins of all d-atoms of the cofactor come to correspond to the weak ligand field: $S = 1$ for Mo^{IV}, $S = 2$ for Fe^{II}, and $S = 5/2$ for Fe^{III}.

The result of the two-step occupation is that the Mo atom gains the correct electronic configuration d^2, and the charge distribution becomes more realistic (see Table 1). The one-step occupation used previously[12] results in the nonphysical electron density distribution over the atoms and, hence, prevents correct consideration of the problem of the activation of the nitrogen molecule. As can be seen from Table 2, when electrons are distributed over levels without the consideration of the Hund energy, there are noticeable differences in the weakening of the N—N bond and the electron density transfer to the nitrogen atom. The populations of the N—N bond obtained with the chosen models of the nitrogen coordination on the cofactor (1.29 to 1.36 at rest state) are considerably lower than the value of 1.47 determined[12] for the optimum coordination of the nitrogen molecule at the one-step occupation of the band of d-states of the cofactor.

Table 2. Calculated charges on atoms and populations of bonds in model nitrogen complexes $(H)_3MoFe_3S_9(N_2)Fe_3Fe(H)$

Atom or bond	State ox	rest*		red
		A		
Mo	0.58	0.56	(2.42)	0.53
Fe	0.36	0.33	(−0.50)	0.23
Fe_a	0.71	0.61	(−0.21)	0.59
	0.26	0.16	(−0.38)	0.09
	0.50	0.31	(−0.19)	0.26
Fe_b	0.55	0.43	(0.21)	0.20
	0.30	0.27	(0.03)	0.20
	−0.41	−0.43	(−0.44)	−0.43
S	−0.43	−0.44	(−0.45)	−0.45
	−0.53	−0.55	(−0.41)	−0.56
N′	0.01	−0.01	(0.25)	−0.05
N	0.11	0.10	(0.40)	0.08
$FeFeS_3$	1.33	0.87	(−0.94)	0.60
$MoFeS_3$	1.96	1.54	(2.62)	0.87
N—N′	1.34	1.34	(1.63)	1.35
Σ(N—$Fe_{a,b}$)	0.43	0.43	(0.43)	0.42
Σ(Fe_a—Fe_b)	0.51	0.57	(0.61)	0.58
		B		
Mo	0.83	0.83	(2.02)	0.82
Fe	0.50	0.48	(−0.65)	0.12
Fe_a	0.60	0.30	(−0.35)	0.13
	0.72	0.64	(0.37)	0.51
Fe_b	0.49	0.30	(−0.23)	0.25
	0.66	0.64	(0.43)	0.60
S	−0.59	−0.63	(−0.41)	−0.63
	−0.57	−0.59	(−0.39)	−0.60
N	0.12	0.11	(0.45)	0.10
N′	0.19	0.12	(0.09)	0.10
$FeFeS_3$	1.76	1.24	(−0.31)	0.44
$MoFeS_3$	1.73	1.44	(1.99)	1.28
N—N′	1.33	1.34	(1.29)	1.33
Σ(N—$Fe_{a,b}$)	1.29	1.27	(1.69)	1.26
Σ(Fe_a—Fe_b)	0.23	0.28	(0.39)	0.27
		C		
Mo	0.97	0.97	(1.92)	0.95
Fe	0.54	0.23	(−0.61)	0.21
Fe_a	0.66	0.56	(0.48)	0.52
	0.57	0.40	(0.05)	0.31
Fe_b	0.68	0.67	(0.54)	0.61
	0.56	0.50	(−0.02)	0.26
S	−0.60	−0.60	(−0.43)	−0.61
	−0.57	−0.58	(−0.48)	−0.62
N	0.23	0.22	(0.37)	0.18
$FeFeS_3$	1.51	0.74	(−0.05)	0.49
$MoFeS_3$	1.83	1.66	(1.73)	1.09
N—N	1.29	1.29	(1.25)	1.32
Σ(N—$Fe_{a,b}$)	1.98	1.99	(2.29)	1.91
Σ(Fe_a—Fe_b)	0.21	0.20	(0.43)	0.23

* The numbers in parentheses refer to the electronic configuration with the one-step occupation.

A correlation between the weakening of the N—N bond and the total population of the Fe—N bonds is observed for the multicenter coordination of nitrogen.

As is seen from Table 2, the Fe—N bonds strengthen at the expense of the weakening of the Fe—Fe bonds along the edges of the Fe_6 prism. This means the weakening of the cofactor structure upon the nitrogen fixation. For three types of coordination, the maximum weakening of the N—N bond is observed when the nitrogen molecule is located under the edge of Fe_2, although the differences are not great. The greatest differences are observed for the electron density transfer from the Fe—Fe bonds to the Fe—N bonds, and the coordination inside the Fe_6 prism is accompanied by the maximum total population of the N—Fe bonds, although the weakening of the Fe—Fe bonds does not differ strongly from that in the case of the coordination at the edge of the prism.

The formation of complex **C** is accompanied by the gain in energy (~10 kcal mol^{-1} at rest). For the two other types, **A** and **B**, the energy of complex formation is already negative, and its maximum change is observed for complex **A**, probably due to steric hindrances (the $N—S_{a,b}$ distances are shorter than the sum of the van der Waals radii). This ratio of the complex formation energies is mainly qualitative, because their absolute values depend on the structural relaxation of the system during the interaction of the substrate with the active center. For example, for complex **A** the extension of the cofactor and other appropriate changes in its structure can level out the role of steric hindrances and simultaneously enhance the Fe—N interactions.

Thus, this consideration shows that the highly organized coordination of the nitrogen molecule on the Fe—Mo cofactor of nitrogenase, *i.e.*, a good structural and orbital correspondence of the active center and the substrate, is possible.

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