

Intermediate complexes in chemical and biological nitrogen fixation

A.E. Shilov

Institute of Chemical Physics, 142432 Chernogolovka, Russia

Abstract

Mechanism of dinitrogen reduction in protic media is discussed in the light of results obtained for specially prepared binuclear complexes MNNM with bridging dinitrogen capable to be protonated and reduced. The conclusion is made that such complexes with d^2 or d^3 electronic configuration of M together with additional electron donor are likely intermediates in catalytic dinitrogen reduction by comparatively weak reducing agents. Model systems are compared with the active center of dinitrogen fixing enzyme, nitrogenase.

1. INTRODUCTION

Until the middle of the sixties a great gap existed between biological nitrogen fixation by bacteria and the chemistry of dinitrogen known at that time. N_2 was considered extremely chemically inert, unable to react with any substance at room temperature except metallic lithium. Therefore chemistry of biological nitrogen fixation long remained mysterious, particularly because until 1960 the process was observed only in living cells of nitrogen fixing bacteria.

Since then the situation has changed drastically. Nitrogen fixing enzyme, nitrogenase, was isolated capable to catalyze N_2 reduction to ammonia in vitro and the mechanism of enzymatic dinitrogen reduction becomes more and more understood. At the same time first chemical reactions of N_2 in solutions of transition metal complexes were discovered and since then the chemistry emerged of low-temperature dinitrogen reactions. Almost simultaneously first dinitrogen complexes with transition metal compounds were discovered and thus new branch of coordination chemistry, that of N_2 complexes was opened.

From the very beginning it was clear that the three fields of research: chemistry of enzymatic nitrogen fixation, low-temperature N_2 reduction in the presence of transition metal complexes and chemistry of N_2 complexes with transition metal compounds, are closely related. Nitrogenase contains transition metals, iron and molybdenum, and catalyzes N_2 reduction to ammonia. Chemical reactions of N_2 with transition metal complexes are mainly reductions and N_2 complexes should be among intermediates in enzymatic and purely chemical dinitrogen reduction.

However first results obtained with stable dinitrogen complexes were disappointing: N_2 ligand turned out to remain chemically inert. First dinitrogen reactions in solution were found to proceed under the action of very strong reducing agents and produced usually complex nitrides, whereas in the enzymatic process intermediate dinitrogen complex is producing ammonia evidently with participation of solvent protons.

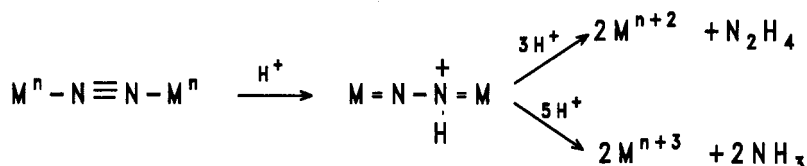
Only after long search dinitrogen complexes were discovered with N_2 capable to further reduction to hydrazine and ammonia. The reactions of N_2 in protic media were also discovered forming hydrazine and

ammonia with participation of solvent protons, i.e. similar from this point of view with the enzymatic reaction.

These reaction are slowly filling the gap between the chemistry of biological nitrogen fixation and that of N_2 reactions in model systems. Now we can try to answer the questions which were previously very difficult to chemists. What complexes should be formed to activate dinitrogen to further reactions? What are the requirements for optimum catalyst, i.e. a metal complex capable to perform the catalytic cycle with comparatively weak reducing agent at low temperatures and pressures? What could be said about intermediate complexes in enzymatic dinitrogen reduction? Are we really close to the enzymatic process in our model chemical reactions? These are the problems I shall try to deal with in my lecture.

2. POSSIBLE MECHANISMS OF DINITROGEN REDUCTION BY COMPARATIVELY WEAK REDUCING AGENTS (ref. 1)

Chemical inertness of dinitrogen is reflected in its characteristics. Triple bond dissociation energy is very high (225 kcal/mol), ionization potential is 15.6 eV, electron affinity has a large negative value. Dinitrogen is a very weak base virtually unable to be protonated even by strongest acids. Highest occupied MO of N_2 , $3\sigma_g$ is strongly bonding, while lowest unoccupied MO, $1\pi_g$, corresponds to high energy. At the same time considering dinitrogen properties one may find its weak points. Bond dissociation energy is very unevenly distributed between NN bonds. The first breaking bond is by far the strongest one among the three and corresponds to more than half of the triple bond energy. This is reflected also in redox potentials of electron transfer to dinitrogen: one- or two-electron reductions require very strong reducing agents with high negative values of redox potentials whereas four- and particularly six-electron reductions require much weaker, rather mild reducing agents. These considerations led us already long ago to a mechanism of dinitrogen reduction with participation of comparatively weak reducing agents through intermediate binuclear complexes:



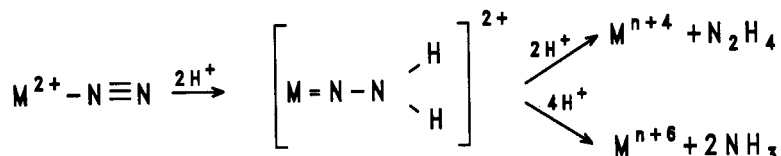
Here and after M is transition metal compound.

In consecutive protonation of dinitrogen in the complex hydrazine can be formed avoiding thermodynamically unfavourable diazene N_2H_2 , or even ammonia can be formed directly avoiding hydrazine. This mechanism can be considered four- or six- electron reduction of dinitrogen. Indeed four electrons of two M are used already in the intermediate complex MNNM in forming the MN bonds and the electrons are shifted even more to the bridging dinitrogen under the action of protons.

More traditional terminology is often used and the intermediates like MNHNHM are regarded as coordinated diazene stabilized by two M, but it should be kept in mind that "stabilized" N_2H_2 may be in fact very close to a hydrazine derivative M-NH-NH-M.

Proceeding from consideration of molecular orbitals of the intermediate complex $M-N \equiv N - M$ an optimum electronic configuration may be derived which, at given redox potential, corresponds to maximum weakening of MN bonding and forming of maximum negative charge on N atoms in the binuclear complex.

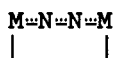
M with d^2 or d^3 electronic configuration have been proposed to be most suitable for such a role (see ref. 1). It should be taken into account that M must be comparatively strong reducing agent capable, however, to be reasonably stable in water or other protic solvents. It is clear therefore that such a combination is not too widespread in chemistry and special search for a suitable catalyst is needed. At the increase of the number of d electrons at M to more than four bridging binuclear MNNM complex is no longer a good candidate for an intermediate in dinitrogen reduction since higher orbitals occupied by additional electrons will be those stabilizing NN bond. At the same time for $d^5 - d^8$ electronic configuration there is another mechanism to reduce end-on coordinated dinitrogen in a mononuclear complex $M-N\equiv N$ with participation of an acceptor, e.g. acid:



From general considerations it follows that dinitrogen reduction via binuclear MNNM complexes requires less strong reducing agents for regeneration of initial (reduced) state of M than via mononuclear M-NN complexes and thus binuclear complexes seem to be preferable for catalytic reduction.

Two additional factors should be mentioned influencing strongly catalytic activity of M.

1. It is essential for a binuclear complex to have a common ligand binding both metal atoms, dinitrogen closing the cycle at coordination:



Several orders of magnitude for the reaction rate may be gained in this case because of less entropy losses.

2. For both kinds of complexes it is very important to have an additional electron source in close vicinity to the reaction center, increasing the electron capacity of the catalyst. This source, e.g. a cluster of low-valent metal ions, will increase negative charge on nitrogen atoms and help to protonate and further reduce dinitrogen.

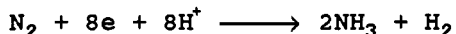
Therefore the effective catalyst must be an organized molecular system and this limits even more the choice of possible catalysts.

In the case of additional electron source close to the reaction center the advantages of binuclear active center forming MNNM complex in catalytic N_2 reduction as compared with end-on M-NN complexes are not so obvious.

3. CHEMISTRY OF NITROGENASE REACTIONS (ref. 2)

Nitrogenase isolated from different nitrogen fixing bacteria consist of two proteins: a smaller Fe protein (ca. 60 kD) and a larger MoFe protein (ca. 230 kD). In living bacteria ferredoxine acts as an electron donor, in vitro it can be replaced by dithionite $S_2O_4^{2-}$. Electrons are transferred from the reducing agent to Fe protein and then to MoFe protein, the latter presumably containing the actual site of dinitrogen activation and reduction. Fe protein contains a single Fe_4S_4 cluster, whereas MoFe protein contains two so called $FeMo$ cofactors (see below) and four special Fe_4S_4 clusters (P-clusters).

MgATP takes part in the process, being hydrolyzed in coupled reaction with electron transfer. Minimum number of MgATP molecules hydrolyzed per one electron transferred seems to be two. The mechanism of this coupling is not yet clear but it may be thought that the energy of ATP hydrolysis is used to shift the redox potential of MoFe protein to more negative values. Without dinitrogen H_2 is evolved in reaction with solvent protons and even at saturating dinitrogen pressures dihydrogen evolution proceeds in parallel coupled process with dinitrogen reduction and the stoichiometry of the whole reaction may be written schematically as follows:



Other substrates besides dinitrogen can be reduced in the presence of nitrogenase including acetylene, N_2O , CH_3NC , cyclopropene.

It was discovered recently (see ref. 3) that besides molybdenum containing nitrogenase another nitrogenase exists with vanadium replacing molybdenum. This nitrogenase is produced in *Azotobacter* strains which were lacking the DNA encoding molybdenum nitrogenase. In vanadium nitrogenase proteins similar to those present in molybdenum nitrogenase form a complex of VFe protein containing two vanadium atoms with Fe protein containing Fe_4S_4 cluster. Despite many similarities between two nitrogenases there are also some differences. E.g. in the case of vanadium nitrogenase some ethane is produced from acetylene besides ethylene which is the only product of C_2H_2 reduction by molybdenum nitrogenase. Hydrazine was found to be a by-product of dinitrogen reduction with vanadium nitrogenase (ref. 4), whereas ammonia is the only product of reduction by molybdenum nitrogenase. There have been reports about the third nitrogenase apparently based only on iron since no other transition metals are present in the enzyme. However when isolated this nitrogenase is very inactive towards dinitrogen and therefore it is not yet quite certain that it is iron only nitrogenase.

Low-molecular iron-molybdenum cofactor was isolated from MoFe protein (see ref. 5). It contains one molybdenum atom, 6-8 iron atoms, 8-9 sulfur atoms and also a molecule of organic substrate homocitrate ((R)-2-hydroxy-1,2,4-butanetricarboxylic acid). According to the information obtained by various physical methods FeMo cofactor is a cluster with a spin $S = 3/2$. Molybdenum is believed to be in the state of Mo^{IV} . Evidently activation and reduction of dinitrogen proceeds with participation of two cofactors in MoFe protein. The exact mechanism of their action is not yet clear and will be discussed later. Here it may be mentioned only that the composition of iron-molybdenum protein is in agreement with the conclusions made earlier about an optimum catalyst for dinitrogen reduction with metal complexes: two cofactors could form a complex with bridging dinitrogen by means of two molybdenums or two vanadiums with d^2 or d^3 electronic configuration. Other mechanisms could be suggested as well with N_2 bound end-on to Mo or to Fe or with N_2 bridging between Mo and Fe, and these latter mechanisms must be considered of course if the two cofactors function separately. For any mechanism iron-sulfur clusters situated near the reaction center may function as electron reservoirs.

4. DINITROGEN REDUCTION IN THE PRESENCE OF TRANSITION METAL COMPLEXES (ref. 1)

Dinitrogen reduction in solution was observed for the first time in a pioneering work published by Vol'pin and Shur in 1964 (ref. 6). Dinitrogen was shown to be reduced by strong reducing agents, such as $RMgX$, $LiAlH_4$, LiR , AlR_3 , $Mg+MgI_2$ in the presence of compounds of various transition metals (Ti, V, Cr, Fe, Mo etc.). Naturally the reaction is possible only in aprotic media. Generally complex nitrides of transition metals are produced, forming ammonia when decomposed by an acid.

Dinitrogen reduction in protic media was observed for the first time in our work in 1970 (see ref. 1). Since then we have found several systems capable to reduce dinitrogen in water or methanol. They are summarized in Table 1. The systems are based on smaller number of transition metals than for reduction in aprotic media. They include titanium(II), vanadium(II), molybdenum(III), niobium(III), and tantalum(III). Among them molybdenum(III) requires the presence of a stronger reducing agent: Ti^{III} , Cr^{II} or Ta^{III} hydroxides, sodium amalgam or mercury cathode (for electrochemical reduction). Compounds of the other metals mentioned can both activate and reduce N_2 . It is seen that all of them have d^2 (for Ti^{II} , Nb^{III} , Ta^{III}) or d^3 (for V^{II} , Mo^{III}) electronic configurations i.e. those optimum for dinitrogen reduction via MNNM complex. Chromium(II) in its hydroxide is apparently also somewhat active with d^4 electronic configuration. It is significant that two metals forming most active systems, molybdenum and vanadium, function in two nitrogenases.

In all cases only polynuclear metal complexes are active towards dinitrogen in protic media. They include hydroxides, soluble polynuclear vanadium and molybdenum complexes.

TABLE 1. Systems reducing dinitrogen in protic media^a.

M	Reducing agent	Products	Yield ^b	Conditions ^c t°C
Ti^{II}	Na(Hg)	N_2H_4 , NH_3	0.01	20
Mo^{III}	$Ti(OH)_3$	N_2H_4 , NH_3	1	60
	$Ti(OH)_3+Mg(OH)_2$ (without Mo)	N_2H_4 , NH_3	170 0.005	110 180
	$Cr(OH)_2$ (without Mo)	N_2H_4 , NH_3	0.80 0.015	90 90
	Na(Hg)+ Mg^{2+}	N_2H_4	2.5	20
	Na(Hg)+ Mg^{2+} +PC ^d + R_3P	N_2H_4	10,000	20
	Cathode+ $Ti(OH)_3$	N_2H_4 , NH_3	0.5	20
	V^{II}	$V(OH)_2+Mg(OH)_2$	N_2H_4 , NH_3	0.65
NH_3			0.35	20, water, pH 12
V^{II} +catechol		NH_3	0.75	20, pH 10.5
Nb^{III}	$Nb(OH)_3$	N_2H_4 , NH_3	0.09	35
Ta^{III}	$Ta(OH)_3$	N_2H_4	0.02	35

^aFor references see ref. 1; Ti^{II} see ref. 7.

^bMoles per mol Mo or Ti for systems with Mo and Ti; current yield for electrochemical reduction; for other systems according to the stoichiometry $4Red + 4H^+ + N_2 \rightarrow N_2H_4 + 4Ox$.

^cMedium: aq. methanol unless otherwise stated; pressure: ca. 100atm N_2 .

^dPC : phosphatidylcholine.

The products are hydrazine and (or) ammonia. Generally hydrazine is not the precursor of ammonia: they are formed in parallel. Prevalence of N_2H_4 or NH_3 depends mainly on the system reducing ability. For relatively strong reducing agents hydrazine is usually the main product, whereas weaker reductants produce ammonia. If the reducing agent is not sufficiently strong to produce hydrazine the system is apparently waiting for additional two electrons to cleave N-N bond and to form thermodynamically more favourable ammonia.

Dihydrogen is evolved in all the systems in parallel with dinitrogen reduction. This parallel reaction is partly responsible for low yields of N_2 reduction products in some of the systems.

Other substrates of nitrogenase are also reduced in these systems but requirements for their reduction are not so severe as for dinitrogen. Thus acetylene can be reduced by hydroxide of molybdenum(III) to ethylene and ethane without a stronger reducing agent, and several hydroxides which are inactive towards dinitrogen without molybdenum(III) can reduce acetylene.

5. CATALYTIC DINITROGEN REDUCTION IN THE PRESENCE OF MOLYBDENUM COMPLEXES

As it was mentioned molybdenum(III) requires an additional reducing agent to reduce dinitrogen to hydrazine and ammonia. This property opens the way for catalytic dinitrogen reduction, molybdenum compounds playing the role of the catalyst. Indeed we have found two kinds of systems which are catalytic with respect to molybdenum(III). The first involves titanium(III)-magnesium(II) mixed hydroxide and becomes catalytic at elevated temperatures (see Table 1). The second, based on sodium amalgam, is catalytic already at room temperature and atmospheric pressure. The system may be developed and becomes particularly active when molybdenum compound is mixed with magnesium salt before reduction to molybdenum(III) and the process is performed in the presence of organic phosphines and certain surface-active materials, e. g. phospholipid, lecithin (phosphatidylcholine). The molybdenum complex is incorporated in the thin film of the phospholipid covering the amalgam surface. Dinitrogen and solvent (methanol and water) molecules diffuse through the film to the complex situated near the amalgam surface which is the electron donor of the system. The activity of this highly organized and very effective catalytic system is close to that of nitrogenase, though making the comparison one has to have in mind that the artificial system requires much stronger reducing agent. We have isolated several complexes of molybdenum(V) and (VI) which are the precursors of the catalytic complexes and become catalysts when reduced to the state of molybdenum(III). The structures of the two complexes found by X-ray analysis are presented in Fig. 1 and 2. They contain two and eight molybdenum atoms each and are stabilized by magnesium bridges.

According to the results obtained by gel chromatography the sizes of the complexes remain the same when they are reduced to molybdenum(III) state. Therefore apparently they contain the same number of molybdenum atoms though exact structures of the molybdenum(III) complexes remain to be determined. Kinetic experiments have shown that the larger is the number of molybdenum atoms in the complexes the higher is their catalytic activity. The structures confirm the essential role of polynuclear complexes as catalysts for dinitrogen reduction. Activating role of phosphines is probably due to their basic properties. They increase the reducing ability of molybdenum(III) entering its coordination sphere. The exact way of dinitrogen coordination remains unclear. N_2 may form a bridge between two molybdenums replacing oxygen or there could be an end-on coordination and subsequent protonation to hydrazido complex $Mo=N-NH_2$, electrons being provided from another molybdenum in the complex.

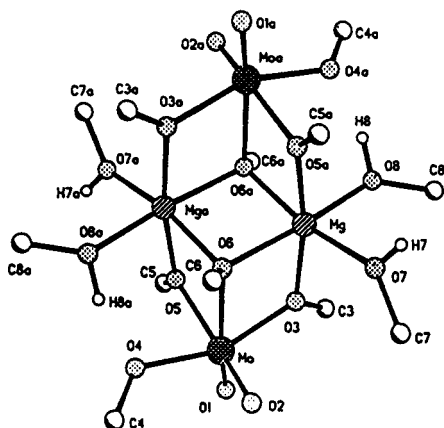


Fig. 1. The structure of the complex $\text{Mo}_2\text{Mg}_2\text{O}_4(\text{OCH}_3)_8(\text{CH}_3\text{OH})_4$

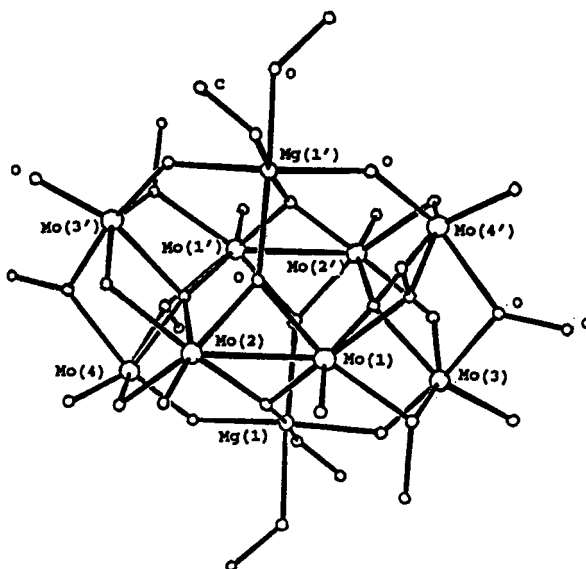


Fig. 2. The structure of dianion $[\text{Mo}_8\text{Mg}_2\text{O}_{22}(\text{OCH}_3)_8(\text{CH}_3\text{OH})_4]^{2-}$

6. REDUCTION OF DINITROGEN IN COMPLEXES WITH TRANSITION METALS

The first dinitrogen complex, $[\text{Ru}(\text{NH}_3)_5\text{N}_2]^{2+}$, identified by Allen and Senoff (ref. 9), as well as many similar mononuclear complexes prepared soon afterwards (ref. 10) turned out to be unable to activate coordinated dinitrogen to further reduction. Dinitrogen in binuclear complexes like $[(\text{NH}_3)_5\text{RuN}_2\text{Ru}(\text{NH}_3)_5]^{4+}$ also remain inert.

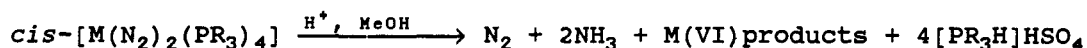
The first dinitrogen complex found to be intermediate in dinitrogen reduction was observed in ether solution in interaction of N_2 with the products of the reaction of $(\text{C}_5\text{H}_5)_2\text{TiCl}_2$ with *i*-PrMgCl (ref. 1a). The complex is very unstable (its heat of formation is only ca. 5 kcal/mol) and was observed only at low temperatures. Nevertheless it was isolated and happened to be a binuclear complex $[(\text{C}_5\text{H}_5)_2\text{Ti}^{\text{III}}\text{R}]_2(\mu\text{-N}_2)$. In excess of the reducing agent in solution the complex is further reduced to a product which forms hydrazine at protonation. The structure of the similar but more stable complex with aryl group at titanium was shown to be linear with respect to the fragment TiNNTi (ref. 11).

Intermediate complexes in dinitrogen reductions in protic media are all unstable and difficult to be isolated. From kinetic results obtained for dinitrogen reduction by mixed titanium(III)-magnesium(II) hydroxide in the presence of molybdenum(III) complex it follows that the enthalpy of the N_2 complex formation is -7 kcal/mol (ref. 12). The value of the complex formation entropy (-17 e.u.) indicates that coordination of dinitrogen is likely to be accompanied by the loss of two water molecules, therefore MoN bonds in the complex are probably much stronger than formation enthalpy would indicate.

Some of the results obtained definitely support the mechanism with polynuclear transition state and bridging dinitrogen between two transition metal atoms as the activated form of N_2 . E.g. kinetics of dinitrogen reduction by vanadium(II) catechol complex (second order with respect to V^{II} and first order with respect to N_2 concentrations) show that two V^{II} complexes are involved in reaction with N_2 (ref. 13). Each vanadium complex according to the EPR spectrum contains three V^{II} atoms. Therefore six-nuclear vanadium(II)-dinitrogen complex was concluded to be the transition state in N_2 reduction. Moreover the EPR spectrum indicates that three vanadiums in the complex form an open triangle which could then form a cycle with bridging dinitrogen.

Results obtained with heterogeneous hydroxide systems as well as with molybdenum complexes catalyzing dinitrogen reduction by sodium amalgam also suggest that polynuclear systems are involved as the intermediates.

However no direct data have been obtained for the structure(s) of intermediate dinitrogen complexes in protic media. Meanwhile J. Chatt and his colleagues in Brighton found that mononuclear molybdenum and tungsten dinitrogen complexes are able to undergo N_2 reduction at protonation (ref. 14). Thus the complexes *cis*- $[M(N_2)_2(PMe_2Ph)_4]$ or *trans*- $[M(N_2)_2(PPh_2Me)_4]$ ($M = Mo$ or W), on treatment with sulfuric acid in methanol, give high yields of ammonia (with a little hydrazine in some cases).



The yields of ammonia according to this equation is essentially quantitative for $M = W$, but ca. 0.7 mol per metal atom for $M = Mo$.

Some intermediate complexes have been also demonstrated, such as hydrazido complexes $[MX_2(NNH_2)(PMe_2Ph)_3]$ and diazenido complexes, e.g. $[trans-MX(N_2H)(dppe)_2]$. ($X = F, Cl, Br$ or I ; $dppe = Ph_2PCH_2CH_2PPh_2$).

These reactions were extensively studied in several laboratories (see ref. 15) and invoked much interest among those involved in the work on nitrogen fixation. Apparently since then the mechanism of N_2 reduction via mononuclear complexes have been widely accepted as a general one and was spread on the biological dinitrogen reduction, though actually they are not the intermediates in catalytic or stoichiometric dinitrogen reduction in protic media: they are first synthesized in aprotic conditions and then are protonated by an acid producing ammonia or hydrazine.

More recently a new branch of dinitrogen complexes with N_2 capable to further reduction appeared in literature. The complexes are binuclear and contain dinitrogen as a bridging ligand (ref. 16). Some of them are presented in Table 2. Besides complexes of titanium(II) and zirconium(II) known earlier they include complexes of vanadium(II), niobium(III), tantalum(III), molybdenum(IV) and tungsten(IV). All of them have electronic configurations d^2 or d^3 . According to the data on NN and MN bond lengths and NN vibration frequencies dinitrogen molecule in these binuclear complexes is considerably more activated than in end-on mononuclear ones. For some of the complexes the structure $M=N-N=M$ seems to be more appropriate than the structure with NN triple bond $M-N \equiv N-M$. They illustrate very well four-electron mechanism of dinitrogen reduction discussed earlier. The results with protonation of bridging dinitrogen confirm the conclusion that dinitrogen ligand is strongly activated. Niobium and tantalum complexes form hydrazine quantitatively when protonated by HCl. Vanadium, molybdenum and tungsten complexes produce mainly ammonia, the yields being smaller than quantitative: part of coordinated dinitrogen is evolved as free N_2 . These differences in their behaviour may be reasonably explained in similar way to N_2 reduction in different systems in protic media by different reducing properties of the metals in the complexes. Nb^{III} and Ta^{III} are sufficiently strong two-electron reductants to form hydrazine. In the case of the other complexes they are unable to easily produce hydrazine and have to receive two more electrons to break NN bond and to produce ammonia. These additional electrons are provided by other dinitrogen complexes present in the solution and this results in their oxidation with free dinitrogen evolution. If the protonation is performed in the presence of an added reducing agent (zinc or sodium amalgam) the yields of ammonia are considerably increased (Table 2), since additional electrons for ammonia formation are provided by the reductants and therefore less dinitrogen is lost from the complexes.

TABLE 2. Protonation^a of bridging dinitrogen complexes.

Complex	N-N(Å)	Products (yields %) ^b	Ref.
[Zr(C ₅ Me ₅) ₂ (N ₂) ₂] ₂ (μ-N ₂)	1.182(5)	N ₂ (67), N ₂ H ₄ (33)	17
[V(o-Me ₂ NC ₆ H ₄) ₂ PY] ₂ (μ-N ₂)	1.228	NH ₃ (33), V ^{III}	18, 19
[Nb(S ₂ CNEt ₂) ₃] ₂ (μ-N ₂)	1.25(2)	N ₂ H ₄ (100)	20
[Ta(S ₂ CNEt ₂) ₃] ₂ (μ-N ₂)		N ₂ H ₄ (100)	20
[Mo(C ₅ Me ₅) ₂ Me ₃] ₂ (μ-N ₂)	1.236	NH ₃ (16)	21
with Zn(Hg)		NH ₃ (32-36)	
[Mo(C ₅ Me ₅) ₂ Me ₃](μ-N ₂) - -[W(C ₅ Me ₄ Et)Me ₃]	1.235	NH ₃ (16)	21
with Zn(Hg)		NH ₃ (83-93)	
[W(C ₅ Me ₅)Me ₃](μ-N ₂)		NH ₃ (17)	21
with Na(Hg)		NH ₃ (38)	

^a HCl data. In the presence of Zn(Hg) or Na(Hg) lutidine-HCl or lutidine-HOSO₂CF₃ were used.

^b Based on total N₂ content.

The reduction of bridging dinitrogen at protonation in specially prepared binuclear complexes may be considered as a model of the second stage of dinitrogen reduction in protic media (described above), the first stage being complex formation.

In this connection it is of interest to compare metals (and their oxidation states) which are found to activate dinitrogen to reduction in protic media (a) with those able to form bridging binuclear MNNM complexes with N₂ undergoing reduction at protonation (b):

- a) Ti^{II} V^{II} Cr^{II} Nb^{III} Mo^{III} Ta^{III}
 b) Ti^{II} V^{II} Zr^{II} Nb^{III} Mo^{IV} Ta^{III} W^{IV}

Of course it may be foreseen that other metal complexes will be found in future for both dinitrogen activation and reduction in protic media and the synthesis of binuclear MNNM complexes with N₂ reducible at protonation. Nevertheless the resemblance of the both groups is striking and hardly accidental: metals of IV-VI groups with d² or d³ electronic configuration turn out to be able to reduce N₂ in protic media and form appropriate complexes for further dinitrogen reduction.

Similarly to N₂ reduction in protic media the choice between hydrazine and ammonia in products of protonation of MNNM complexes is determined mainly by M reducing ability: N₂H₄ is formed in the case of sufficiently strong two-electron reductants, while less strong reducing

agents produce ammonia as the final product. In this case sometimes the yields can be increased by addition of the same reducing agent (e.g. sodium amalgam) which is electron donor for catalytic N_2 reduction in protic media.

All these similarities strongly support the view that similar (although less stable) complexes are formed intermediately in N_2 reduction in protic media, where they were so far difficult to observe. It should be reminded that these results are also in good agreement with theoretical considerations presented above. As it was mentioned earlier the instability of the complexes in protic media (formed in all cases in the presence of water) does not mean that dinitrogen is less activated: dinitrogen replaces water which is a good ligand and heat of complex formation corresponds to the difference between two M-N and two M-OH₂ bond energies.

Why the elements of IV-VI groups are preferable to activate N_2 in d^2 or d^3 electronic configuration? Apparently it is because these configurations for the groups of smaller numbers correspond to too strong reducing agents unable to exist in protic media, while for the groups of higher numbers d^2 and d^3 electronic configuration would mean too weak reductant (e.g. Mn^{IV} or Fe^{VI}) unable to form complexes and to activate N_2 to further reduction.

From this point of view the existence of the third nitrogenase based on iron as the only transition metal, if confirmed, would mean that an end-on FeNN complex is probably the intermediate in the reaction. There is a report indicating that N_2 can be reduced in protic media in the presence of iron complexes (ref. 22) and there are some examples of dinitrogen Fe^0 complexes (d^8 electronic configuration) which reduce N_2 at protonation (ref. 23, 24)

7. ABOUT THE NATURE OF NITROGENASE ACTIVE CENTER

Oxidation state of molybdenum in nitrogenase is believed to be Mo^{IV} and it is definitely not lower than Mo^{III} . For vanadium nitrogenase V^{II} or V^{III} is involved in the active center. Thus in both cases the electronic configuration is d^2 or d^3 , i.e. optimum for binuclear intermediate complex MNNM. In the light of the knowledge that two molybdenums or two vanadiums are present in nitrogenase molecule it was natural to conclude that they form a binuclear intermediate with bridging dinitrogen. Therefore two cofactors must form a united system, four Fe_4S_4 clusters being also involved in the system to increase its electron capacity. In this case we would have an optimum catalytic system capable to reduce N_2 by a comparatively weak reducing agent.

Long ago I came to this conclusion (which was supported by our results in model dinitrogen reducing systems) and suggested this mechanism in my articles (e.g. see ref. 1b). This hypothesis was by no means generally accepted by others engaged in the work on nitrogen fixation. On the contrary it was almost universally concluded that two FeMo cofactors are far apart from each other and function separately. As it was mentioned above this was probably connected initially with the finding by Chatt et al of mononuclear (though zero-valent) molybdenum and tungsten complexes capable to reduce dinitrogen at protonation. Later some other indirect evidence was presented to support this mechanism. E.g. kinetic data were interpreted to conclude that two cofactors reduce dinitrogen separately (ref. 25). Obviously in fact kinetic results cannot distinguish between the two alternative mechanisms if both cofactors are always included in one protein molecule: the only apparent difference will be that the rate constant calculated for two cofactors acting together will be twice as large as for two cofactors functioning separately. The final conclusion can be made on the base of X-ray analysis of molybdenum iron protein. The first results on the X-ray analysis of a crystal of MoFe protein isolated from *Azotobacter vinelandii*, published in our paper in 1986

(ref. 26), gave the opportunity only to find the spots of high electron density. According to these results the authors of ref. 26 came to the conclusion that all clusters of the protein are close to each other and form united system with intense electron exchange and maximum electron capacity. Based on the relative sizes of the peaks it was proposed that the two FeMo cofactors are adjacent with the distance between the edges of the two cofactors being ca. 7Å. No places for molybdenum atoms have been found but it was suggested on the base of the above considerations that this picture was consistent with the intermediate MoNNMo complex provided two molybdenums were situated on the edges. Recently, however, another report was published (ref. 27) claiming also on the base of X-ray data, but for MoFe protein from *Clostridium pasteurianum* that the distance between the two cofactors is ca. 70Å, and therefore naturally they function separately. It is unlikely that nitrogenases of different organisms are so different from each other and thus the results demonstrate the existing controversy. To solve it the full X-ray structure of MoFe protein and of the whole nitrogenase complex has to be established. However some independent data seem to contradict to the results of the ref. 27. MoFe protein is known to consists of four subunits (2α and 2β). Electron microscopic studies (ref. 28) show that they form a distorted tetrahedron. Results of the X-ray analysis obtained in ref. 26 are in agreement with this structure. Electron microscopy results show that Fe protein has two-fold symmetry axis and is bound to two identical subunits of MoFe protein which also has two-fold symmetry. Full X-ray structure of Fe protein was resolved recently (ref. 29) and revealed that Fe_4S_4 cluster is placed between the two subunits of the protein which has two-fold symmetry in agreement with the earlier results of electron microscopy. Therefore Fe protein which is the electron donor for MoFe protein contains the Fe_4S_4 cluster evidently close to the boundaries between two subunits of MoFe protein. The dinitrogen activating center of the latter must be placed not far from the Fe_4S_4 cluster of Fe protein. For the structure proposed in ref. 27 the distance between the Fe_4S_4 cluster and each of the two cofactors should be evidently more than 35Å which is definitely too long for effective electron transfer. For the structure published in ref. 26 the distance between Fe_4S_4 cluster and the reaction center of MoFe protein is close to 10Å.

Of course the full structure of MoFe protein is needed to make a more definite decision, but even after the structure is known the structure of the intermediate complex with dinitrogen may remain not quite certain. In principle the nature may use a mechanism of dinitrogen activation still unknown in chemistry. Conclusions based on analogies only are always somewhat doubtful.

Whatever is the intermediate complex, high electron capacity is definitely needed to dinitrogen enzymatic reduction, since otherwise there would have been no necessity to arrange so many iron and sulfur atoms close to the reaction center in the molecule of the enzyme.

It is also certain that the mechanism of dinitrogen reduction via MNNM intermediate proposed many years ago is valid at least for a number of purely chemical dinitrogen fixing systems in solution.

REFERENCES

1. a) A.E. Shilov, in: A Treatise on Dinitrogen Fixation, pp. 31-108, R.W.F. Hardy (Ed.), Wiley, New York (1979). b) A.E. Shilov, in: New Trends in the Chemistry of Nitrogen Fixation, pp. 121-150, J. Chatt, L.M. da Câmara Pina and R.L. Richards (Eds), Academic Press, London (1980). c) A.E. Shilov, J. Mol. Catalysis **41**, 221-234 (1987).
2. B.K. Burgess, in: Advances in Nitrogen Fixation Research, pp. 103-114, C. Veeger and W.E. Newton (Eds), Nijhoff, Boston (1991).
3. R.N. Pau, Trends in Biochem. Sci. **4**, 183-186 (1989).
4. R.R. Eady and M.J. Dilworth, J. Inorg. Biochem. **43**, 477 (1991).
5. B.K. Burgess, Chem. Rev. **90**, 1377-1406 (1990).

6. M.E. Vol'pin and V.B. Shur, Dokl. Acad. Nauk SSSR **15**, 1102-1104 (1964)
7. G.V. Nikolaeva, N.T. Denisov, O.N. Efimov, S.I. Kulakovskaya and A.E. Shilov, Kinetika i Kataliz, in press.
8. M.Yu. Antipin, L.P. Didenko, L.M. Kachapina, A.E. Shilov, A.K. Shilova and Yu.T. Struchkov, J. Chem. Soc. Chem. Comm. 1467-1468 (1989). M.Yu. Antipin, A.E. Shilov, A.K. Shilova and Yu.T. Struchkov, Gazetta Chim. Ital., in press.
9. A.D. Allen and C.V. Senoff, Chem. Comm. 621-622 (1965)
10. F. Bottomley, in: A Treatise on Dinitrogen Fixation, pp. 109-167, R.W.F. Hardy (Ed), Wiley, New York (1979).
11. J.H. Teuben, in: New Trends in the Chemistry of Nitrogen Fixation, pp. 233-247, J. Chatt, L.M. da Câmara Pina and R.L. Richards (Eds), Academic Press (1980).
12. N.T. Denisov and N.I. Shuvalova, Reaction Kinet. Catal. Lett. **4**, 431-435 (1976).
13. N.P. Luneva, A.P. Moravsky and A.E. Shilov, Nouv. J. Chim. **6**, 245-252 (1982).
14. J. Chatt, J. Dilworth and R. Richards, Chem. Rev. **78**, 589-625 (1978).
15. M. Hidai, in: Molybdenum Enzymes, pp. 285-341, T.G. Spiro (Ed), Wiley, New York (1985).
16. R.A. Henderson, Transition Met. Chem. **15**, 330-336 (1990).
17. R.D. Sanner, D.M. Duggan, T.C. McKenzie, R.E. March and J.E. Bercaw, J. Amer. Chem. Soc. **98**, 8358-8365 (1976).
18. J. Edema, A. Meetsma and S. Gambarotta, J. Amer. Chem. Soc. **111**, 6878-6880 (1989).
19. G.J. Leigh, R. Prieto-Alcón J.R. Sanders, J. Chem. Soc. Chem. Comm. 921-922 (1991).
20. J.R. Dilworth, R.A. Henderson, A. Hills, D.L. Hughes, C. Macdonald, A.N. Stephens and D.R.M. Walton, J. Chem. Soc. Dalton Trans. 1077-1085 (1990).
21. R.R. Schrock, R.M. Kolodziej, A.H. Liu, W.M. Davis and M.G. Vale, J. Amer. Chem. Soc. **112**, 4338-4345 (1990).
22. N.T. Denisov, N.I. Shuvalova and A.E. Shilov, Kinetika i kataliz **32**, 1250-1253 (1991).
23. T.A. Bazhenova, I.N. Ivleva, L.M. Kachapina, A.K. Shilova, A.E. Shilov and B. Tchoubar, J. Organomet. Chem. **296**, 95-101 (1985).
24. G.J. Leigh, M. Jimenez-Tenerio, J. Amer. Chem. Soc. **113**, 5862-5863 (1991).
25. R.N.F. Thorneley and D.J. Lowe, Biochem. J. **224**, 887-894 (1984).
26. N.I. Sosfenov, V.I. Andrianov, A.A. Vagin, B.V. Strokopytov, B.K. Vainshtein, A.E. Shilov R.I. Gvozdev, G.I. Likhtenshtein, I.Z. Mitsova and I.S. Blazhchuk, Dokl. Acad. Nauk SSSR **291**, 1123-1127 (1986).
27. J.T. Bolin, N. Campobasso, S.W. Muchmore, W. Minor, L.E. Mortenson and T.V. Morgan, J. Inorg. Biochem. **43**, 477 (1991).
28. V.L. Tsuprun, I.Z. Mitsova, I.S. Blazhchuk, R.I. Gvozdev, E.V. Orlova and N.A. Kiselev, Eur. J. Biochem. **149**, 389-395 (1985).
29. A.S. Moffat, Science **253**, 1513 (1990).