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The use of inorganic model complexes in understanding the nitrogenase system

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Abstract: The enzyme nitrogenase contains an Mo-Fe-S cluster (FeMoco), which has a unique extended FeS-bridged structure and appears to be the centre at which the reduction of N_2 and related molecules occurs. The possible mode of action of FeMoco is discussed in relation to its behaviour when isolated from the protein and to the behaviour of model complexes which are able to mimic various aspects of the nitrogenase function.

Nitrogenase and FeMoco

The nitrogenase enzyme contains two proteins, the Fe protein and the MoFe protein, which carry out the reduction of N_2 to NH_3 as shown in equation 1 (1).

$$N_2 + 8H^+ + 8e^- + 16\,ATP \rightarrow 2NH_3 + H_2 + 16\,ADP + 16Pi \quad \text{-----1}$$

A number of other unsaturated molecules can also be reduced *e.g.* CH_3NC (to CH_4 and NH_3) (2), cyanide ion (to CH_4 and NH_3) (3) and C_2H_2 (to C_2H_4) (4). In the absence of any other substrate, protons are reduced to H_2.

The two component proteins of nitrogenase have been characterised by X-ray crystallography for the organism *Azotobacter vinelandii*. In particular, the larger FeMo protein contains a cluster unit, the iron-molybdenum cofactor, FeMoco, which is considered to be the active site at which dinitrogen and other substrates are reduced. Its X-ray structure at 2.2Å resolution (1), is shown in Fig.1. FeMoco can be extracted from the FeMo protein only in small quantity and this limitation, together with its extreme sensitivity to dioxygen, has so far precluded crystallisation. Normally, FeMoco, extracted in the so-called semi-reduced form, is examined using its characteristic S = 3/2 electron paramagnetic resonance (EPR) and X-ray

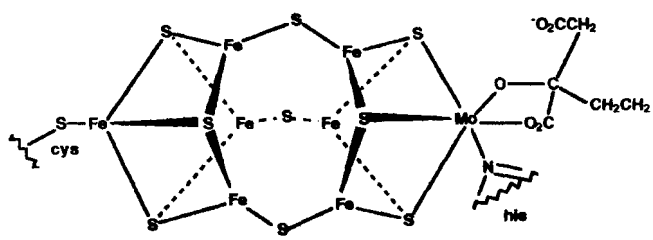


Fig. 1 X-ray structure of FeMoco

absorption (XAS) spectroscopies (5). Bond distances within FeMoco deduced from XAS investigation were used in deriving the crystallographic structure. Moreover, structural parameters from such compounds as **1** (6) were used in the formulation of the crystallographic model of FeMoco (1).

Isolated FeMoco contains no amino acids, thus the cysteine-Fe and histidine-Mo bonds to the protein (Fig. 1) are severed in the extraction process and replaced by solvent such as N-methyl formamide, or possibly its anion. FeMoco when isolated is anionic, with a probable 2- charge and is possibly dimeric or oligomeric (7). It reacts with thiols and selenols. A combination of EPR titration data and Fe- and Se-XAS studies have shown that this interaction is of one thiolate or selenate at an Fe, presumed to be the Fe originally bound to cysteine (8)(9).

Cyanide binds to two sites on FeMoco, one being Mo and the other is presumed to be Fe. When cyanide and thiolate are in competition for the FeMoco sites, it appears that thiolate displaces cyanide from Fe but not from Mo (10)(11). MeNC also interacts with FeMoco but di- and tri-thiols disrupt the cluster structure, giving as yet uncharacterised products (12)(13).

Electrochemical studies have shown that semi-reduced FeMoco can lose or gain an electron, but as yet no interaction with N_2 has been observed at any oxidation level (5-12).

The X-ray structural model of FeMoco has therefore not given a clear lead as to the site of binding and reduction of N_2 and other substrates of nitrogenase. The site could be Mo or Fe, possibly with proton transfer from sulfur, or perhaps in the case of substrates such as alkynes, direct interaction with bridging sulfur could occur, as occurs between alkynes and sulfur ligands bridging Mo atoms *e.g.* in the complex $[\{Mo(C_5H_5)_2\}_2(\mu\text{-}S_2)_2]$ (14).

To consider further likely mechanisms for the binding and reduction of N_2 and other substrates on nitrogenase, we turn to the established chemistry of these ligands at metal centres, noting that at least in azotobacter, two alternative nitrogenases exist; in the first molybdenum is replaced by vanadium and in the second it appears to have been replaced by iron (15).

Reduction of nitrogenase substrates at metal centres

Dinitrogen can be bound and reduced to NH_3 at Mo, V or Fe phosphine-ligated centres, *e.g.*$\{M(diphosphine)_2\}$ (M = Mo, V or Fe) (16-20). The most thoroughly studied metal is Mo and using this chemistry an electrochemical cycle has been developed for the production of

ammonia (21). Alternative substrates such as isocyanides and alkynes are bound and reduced at the Mo centres to give similar products to those from nitrogenase (22). These centres also bind H_2, which can be displaced by N_2; this might relate to the reduction of protons by nitrogenase and to the release of H_2 when N_2 is bound and reduced (21)(22). A similar chemistry is developing for the related V and Fe systems (17)(18)

The above chemistry has primarily involved mononuclear metal sites, but dinitrogen has a well documented chemistry in the bridging mode (23). Although reduction of bridging dinitrogen usually gives hydrazine, ammonia can be produced *e.g.* from acid treatment of $[V\{(C_6H_4CH_2NMe_2)_2(C_5H_5N)\}_2(\mu\text{-}N_2)]$ (24).

Thus on this basis, Fe and Mo in FeMoco (or Fe and V or Fe only in the alternative nitrogenases) are equal candidates for the substrate binding centre. However, because the metals in nitrogenase have a sulfur-ligand environment, efforts have been made to prepare complexes of dinitrogen or its derivatives such as hydrazine and diazene, and of other nitrogenase substrates, at metal centres where the coligands have sulfur-donor atoms. The most successful system in terms of binding N_2 is the macrocyclic thioether complex *trans*-$[Mo(N_2)_2(Me_8[16]aneS_4)]$ (25). Its dinitrogen ligands can be reduced, but the yields of ammonia are low and the greater susceptibility of the thioether ligand to degradation make an electrochemically-driven cycle for ammonia unlikely at present (26)(27).

As yet no analogues of *trans*-$[Mo(N_2)_2(Me_8[16]aneS_4)]$ in which V or Fe replace Mo have been obtained.

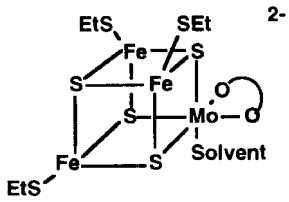
Cluster complexes and reactions of hydrazines at metal centres

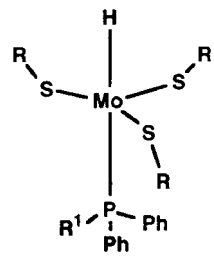
The ligation of Mo in FeMoco is mimicked closely in the cluster complexes such as **1** where the coordination environment of the Mo can be S_3O_2N [*e.g* S_3 from μ-sulfides, O_2 from catecholate or citrate, N from MeCN (28)(29)or imidazole (30)]. As noted earlier, structural parameters from such compounds were used in the formulation of the crystallographic model of FeMoco.

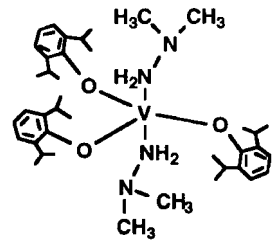
Although complexes **1** do not react with N_2, cluster anions such as $[MoFe_3S_4Cl_3(C_6Cl_4O_2)(MeCN)]^{2-}$ catalyse the disproportionaton and reduction of hydrazine. The site of binding of N_2H_4 appears to be Mo, since $PhNHNH_2$ has been shown to bind at Mo in **1** (29). These observations have relevance to the possible function of nitrogenase in that bound hydrazine, and derivatives of it such as N_2H_2, NNH_2^{2-}, or NNH_3^{2-} are likely intermediates in the reduction of N_2, whether at a mononuclear or a multinuclear site.

The thiolate-hydride complexes $[MoH(SR)_3(PR^1Ph_2)]$ (R = $C_6H_2Me_3$-2,4,6 or $C_6H_2Pr^i_3$-2,4,6; R^1 = Me or Et) **2** (31), also catalyse disproportionation and reduction of hydrazine. The adducts $[MoH(SC_6H_2Pr^i_3\text{-}2,4,6)_3(PMePh_2)(L)]$ (L = N_2H_4 or NH_3) have been isolated from this reaction, indicating that it occurs at a single Mo (32). Although binding of N_2 at this type of site has not yet been demonstrated, isolation of such a compound may be a matter of time, since the complex $[Re(SC_6H_2Pr^i_3\text{-}2,4,6)_3(N_2)(PPh_3)]$ is known (33).

Similar patterns of reaction are emerging for vanadium. Hyrazine adducts such as $[VCl_3(Me_2NNH_2)_2]$ (34), $[V(OC_6H_2Pr^i_3\text{-}2,6)_3(Me_2NNH_2)_2]$ **3** (35), and hydrazide complexes such as $[VCl_2(NNMePh)(NH_2NMePh)]Cl$ (36) and $[NH_2Me_2][\{V(SC_6H_2Pr^i_3\text{-}2,4,6)_3\}_2(\mu\text{-}NNMe_2)_2]$ (35), have been obtained.







1; for O-O and solvent see text **2**; R = aromatic, R^1 = Me or Et **3**

Diazene has been stabilised between two iron atoms in the complex $[\{Fe(N_HS_4)\}_2(\mu\text{-}NHNH)]$ [N_HS_4 = 2,2'-bis(2-mercaptophenylthio)diethylamine] (37).

Conclusions

Although the necessity of binding N_2 to a metal to allow its reduction has been established, at the present time any of the metals present in FeMoco and its analogues could be the candidate for such binding, with some caveats.

For example, if Mo is the binding site in FeMoco, since it is 6-coordinated, it must either lose a ligand in order to bind substrate, or increase its coordination number. Although cyanide ion appears to bind in place of histidine at Mo in isolated FeMoco (10)(11), if 6-coordinate Mo is retained during substrate binding in the intact protein, it seems likely that rather than displacement of histidine, partial displacement of the chelating homocitrate ligand should occur, perhaps as as a result of a protonation of ligating oxygen (38). Although an increase of coordination number of Mo in FeMoco cannot be ruled out and seven-coordination of Mo with sulfur-donor ligands is known, [*e.g.* in the cluster complex $[MoI(SPh)(CO)_3\{Fe_4(C_5H_5)_4S_2(\mu\text{-}S_2)_2\}]$ (39)] as yet no compounds of this type have been prepared with N_2 as a ligand.

It could be that the apparently unsaturated trigonal Fe atoms at the relatively open centre of FeMoco provide the site for N_2 reduction. It has been suggested that N_2 could occupy the central cavity in FeMoco in the active state of the enzyme (1), although it is difficult to see how this site could accommodate the larger, partially reduced states of N_2. Theoretical studies currently favour binding of N_2 between two irons, either on an edge of the central cavity or across a face (40)(41).

As yet there are no iron complexes of N_2 from which one can draw support for these proposals. The only compounds which approach the low coordination number and geometry of the central trigonal Fe atoms are the thiolato-complexes formulated as $[\{Fe(SAr)\}_2(\mu\text{-}SAr)_2]$ (SAr = *e.g.*, S $C_6H_2Bu^t_3$-2,4,6) (42). To date the complex $[\{Fe(N_HS_4)\}_2(\mu\text{-}NHNH)]$ is the closest model of possible intermediates in the reduction of N_2 at sulfur-ligated Fe.

Further study of metal complexes of N_2 and other substrates, particularly where the metal environments are close to those seen in FeMoco, is clearly necessary before definitive mechanisms can be established for the function of nitrogenase at the atomic level.

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