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The consequences of an interstitial N atom in the FeMo cofactor of nitrogenase

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The atom-centred FeMo cofactor of nitrogenase most likely contains N, resting at the $[NMoFe_7]^{18+}$ redox level, inserted from N_2, and subsequently restricting the modes of binding of substrate to the NFe_6 core.

The nitrogenase enzyme that effects the remarkable transformation of N_2 to NH_3 under ambient conditions was shown in 1992[1] and subsequently[2] to contain an unprecedented iron–molybdenum–sulfide cofactor (FeMoco), with the composition [(homocitrate)$MoFe_7S_9$]. This cluster, where substrates are bound and reduced following sequential electron transfer from cognate Fe_4S_4 proteins,[3,4] is bonded to its polypeptide surrounds through just two residues, cysteine and histidine. An unusual trigonal prism of six under-coordinated Fe atoms in FeMoco drew attention to the open Fe_4 faces of FeMoco as the probable atomic sites for the binding of substrates and inhibitors, and this has been supported by experiment[5] and theory.[6] But now, using high quality X-ray diffraction data, Rees *et al.*[7] have shown that this Fe_6 trigonal prism is centrally occupied by a single atom, probably N, although the X-ray data do not exclude C or O.

This new surprise (Fig. 1) raises many additional chemical questions.[7,8] What is the electronic structure of this centred cofactor, in relation to the observed spectroscopic properties and the redox states of the cofactor? How might the N atom be inserted, or included in the biosynthesis of the cofactor? While central-C is chemically unlikely, what about central-O, or -F? How does central-N affect the dynamic properties of FeMoco, and its binding of substrates and inhibitors? Is central-N part of the catalytic cycle? I report here density functional (DF) calculations[9] of the complete FeMoco cofactor including the ligating components of homocitrate and bound cysteine and histidine, addressing these questions. The empty cluster is [(OCH_2COO)(imidazole)$MoS_9Fe_7(SCH_3)$] **1**, and the model calculated is $[(\mu_6\text{-X})\cdot\mathbf{1}]^z$, illustrated in Fig. 1, plus substrates.

The most probable identity of the central atom is nitrogen. The theoretically optimized geometry for $[(\mu_6\text{-N})\cdot\mathbf{1}]^{-1}$ has a symmetrical NFe_6 core, with N–Fe distances (1.98 to 2.02 Å) and Fe–Fe distances (2.55 to 2.70, mean 2.63 Å) indistinguishable from those observed (N–Fe 1.95 to 2.07 Å; Fe–Fe 2.58 to 2.67, mean 2.62 Å).[7] In contrast, $[(\mu_6\text{-O})\cdot\mathbf{1}]^{-1}$ optimises to an enlarged off-centre unsymmetrical geometry, with O–Fe distances ranging 1.92 to 2.36, and Fe–Fe distances ranging 2.69 to 2.93 Å, inconsistent with the crystal structure.[10]

What is the electronic structure of $[(\mu_6\text{-N})\cdot\mathbf{1}]^z$ at possible redox levels? Fig. 2 shows six levels, identified by the charge z and the $[NFe_7Mo]$ charge, plotted according to energies (eV) of the highest occupied molecular orbitals (HOMO), which indirectly calibrate the redox state of the cluster (see below). Relative energies (kcal mol^{-1}) the three lowest-energy spin states for each redox level are provided (not to scale): the spin state is relevant because it provides a connection to the observed S = 3/2 resting state of the enzyme. Two of the redox levels (z = +1, −1) have S = 3/2 ground states, while z = −3 has the S = 3/2 state only 5 kcal mol^{-1} above the ground state. However, the influences of the protein environment[11] are larger than these differences, and could change the relative ordering of

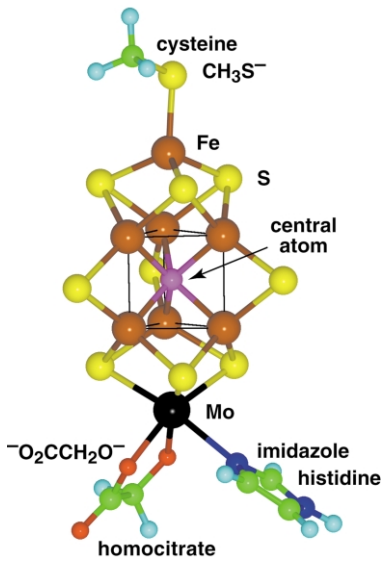

Fig. 1 The FeMo cofactor (homocitrate)MoS_9Fe_7, bonded to protein by histidine coordinated at Mo and cysteine coordinated at Fe, and the model $(\mu_6\text{-X})(OCH_2COO)$(imidazole)$MoS_9Fe_7(SCH_3)$ used in the calculations: C green; O red; N blue; H cyan; S yellow; Fe brown; Mo black.

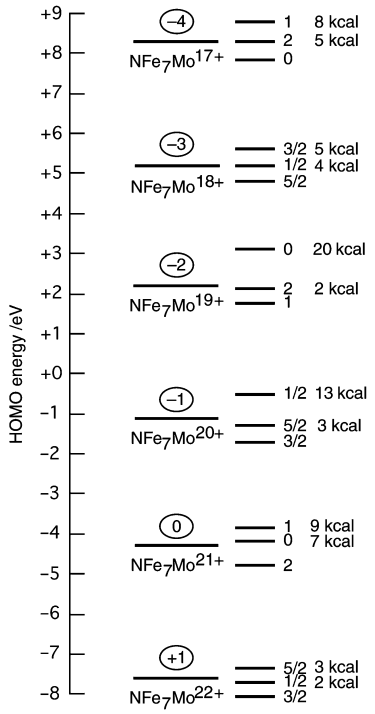

Fig. 2 Schematic representation of six redox levels of $[(\mu_6\text{-N})\cdot\mathbf{1}]^z$, identified by overall charge z (encircled) and charge on NFe_7Mo, plotted according to the calculated energy of the highest occupied molecular orbital (HOMO). For each redox level the relative energies (kcal mol^{-1}) of the three lowest spin states S are marked.

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the energies of these spin states. So, S = 3/2 is a feasible ground spin state for any of the redox levels $z = +1, -1, -3$.

Identification of the resting redox level of the N-centered FeMo cofactor can be approached also through the correlation of the electrochemical potential for oxidation with the energy of the HOMO. Basing this correlation on related clusters containing Fe, S, Mo, and thiolate ligation, all calculated with the same DF procedure, and incorporating potentials for clusters in proteins wherever possible, it is estimated that the redox level $z = -3$ would correspond to a mid-point potential for oxidation of −100 (±200) mV (*vs* NHE).[12] Since the observed potential for FeMoco in various proteins ranges 0 to −180 mV,[4] it is concluded that $z = -3$ is the probable resting redox level for an N-centred FeMoco. This corresponds to $[NFe_7Mo]^{18+}$, which could be formalised as $(N^{3-})(Mo^{4+})(Fe^{3+})_3(Fe^{2+})_4$, consistent with experimental data for Mo[13] and Fe.[14]

There is a feasible mechanism for insertion of N into empty FeMoco. Calculations have already shown that when N_2 binds to an Fe_4S_4 face of empty FeMoco cluster in the 'oblique arrow' conformation,[15] the distal N atom of N_2 is positioned such that three surrounding S atoms can function to protonate it, and with concomitant electronation NH_3 is generated. At the same time the proximal N of N_2 is drawn into the Fe_4 face, and further energy minimisation without protonation causes it to move to the centre of the Fe_6 trigonal prism (Fig. 3). It has been suggested that μ_6-N as nitride could be an intermediate in the catalytic cycle, to be protonated to NH_3. This has an impossibly large energy barrier. While a single atom can pass through an expanded Fe_4 face of FeMoco, a protonated atom cannot.

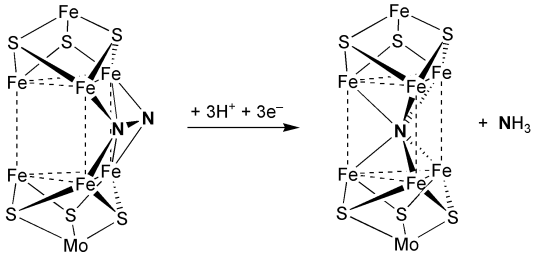

Fig. 3 The calculated conversion of N_2 bound in the oblique arrow conformation at an Fe_4 face of empty FeMoco, to $(\mu_6\text{-N})$·FeMoco + NH_3: not all atoms are shown.

One of the key attributes of empty FeMoco is substantial geometrical (and electronic) plasticity, due mainly to the undercoordination of the six Fe atoms and their linkage by only two-connected sulfur atoms.[15] The occurrence of a central atom bound strongly (at 2.0 Å) to all six Fe atoms imposes rigidity on $(\mu_6\text{-N})$·FeMoco: structures with Fe_6 twisted towards an octahedron, or skewed with N off-centre, revert to the observed symmetrical structure upon energy minimisation.

An atom at the centre of the Fe_6 prism has steric influence on substrate binding, because it is *ca* 0.7 Å inside an Fe_4 face, and too close to substrate atoms bound directly over the face. Therefore the N_2 binding modes **2**, **3**, and **4** (Fig. 4), possible for the empty cluster, are not feasible when it is filled. Two modes for N_2 binding to an Fe_2 edge, **5** and **6**, while not obstructed by the central N atom, are apparently not energy minima and transform to structure **7**. Structures **7**, **8** with terminal Fe–N–N binding are minima, as is **9** with Fe–η^2-N_2 edge binding. For **7**, **8** and **9** the N_2 binding can occur at Fe nearer to the Mo-histidine end or at Fe nearer to the Fe-cysteine end of $(\mu_6\text{-N})$·FeMoco.

It is concluded that (a) the most likely resting state for an atom-centered FeMo cluster in nitrogenase is $[(\mu_6\text{-N})Fe_7MoS_9$(homocitrate)(histidine)(cysteine)] at the $[NFe_7Mo]^{18+}$ redox level, (b) there is a mechanism of insertion of the central N atom, but not passage for protonated atoms, (c) the cluster is relatively inflexible, and (d) the cluster can bind N_2 at an Fe_2 edge or Fe atom, but not over an Fe_4 face.

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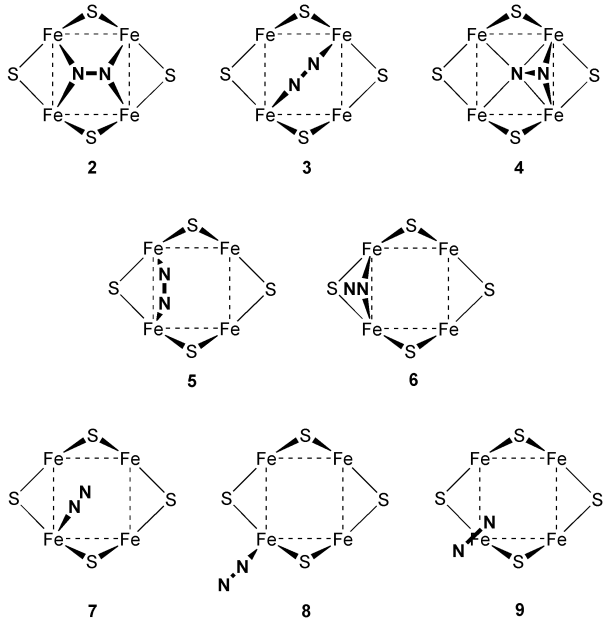

Fig. 4 Structures considered for the binding of N_2 to $(\mu_6\text{-N})$·FeMoco: the central N atom and atoms other than one Fe_4S_4 face of $(\mu_6\text{-N})$·FeMoco are omitted. Structures **2**–**4** incur serious conflict between N_2 and the central N, while **5**–**9** do not.

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