

Current Perspectives in Nitrogen Fixation

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The isolation¹ of an iron-molybdenum cofactor from the dinitrogenase of *Azotobacter vinelandii* represented a significant development in the identification and description of redox components of this and other such proteins. This cofactor appears to be extracted, by *N*-methylformamide, as a discrete, low molecular weight species which contains iron, molybdenum and acid-labile sulphide in the approximate ratios 7:1:6.¹⁻⁴ Several spectroscopic studies have been reported; these not only reveal different facets of this cofactor but also establish that it is extruded reasonably intact. A distinctive manifestation of this cofactor is the e.p.r. spectrum, obtained in the presence of thiophenol and sodium dithionite, with *g* values of ca. 4.6, 3.3, and 2.2, consistent with existence of an $S = 3/2$ electronic spin level.⁴ E.p.r. spectroscopy allows the oxidation levels attainable by the cofactor to be monitored; oxidation of the $S = 3/2$ state produces an e.p.r. silent state and then an e.p.r. active state with an apparent *g* value of 4.38.³ All of the iron atoms of the cofactor at its $S = 3/2$ level exhibit the same ⁵⁷Fe Mössbauer chemical shift and quadrupole splitting; at 90 K these values are 0.37 and 0.75 mm s⁻¹, respectively. The EXAFS associated with the molybdenum *K* edge of two dinitrogenases and the iron-molybdenum cofactor is consistent with the molybdenum being adjacent to three or four sulphur atoms at a distance of ca. 2.35 Å, two or three iron atoms at a distance of ca. 2.72 Å, with perhaps one or two sulphur atoms ca. 2.49 Å away.⁵

The above results, together with the successful synthesis and characterisation of the analogues of the active sites of the iron-sulphur proteins,⁶ have stimulated many investigations of the formation and properties of iron-molybdenum-sulphur clusters. The compounds reported so far may be divided into two groups: (i) systems that contain (or probably contain) a tetrathiomolybdate group attached as a bidentate ligand to one or two iron atoms,⁷ (ii) systems that contain two {Fe₃MoS₄} cubane-like clusters linked across their molybdenum centres by: (a) three thiolate groups, [Fe₆Mo₂S₈(SR)₉]³⁻ or [Fe₆Mo₂S₈(SR)₃X₆]³⁻ (X = Cl or Br), (b) one sulphide and two thiolate groups, [Fe₆Mo₂S₉(SR)₈]³⁻, (c) three methoxide groups, [Fe₆Mo₂S₈(OMe)₃(SPh)₆]³⁻, or (d) a (μ-SR)₃Fe(μ-SR)₃ arrangement, [Fe₇Mo₂S₈(SR)₁₂]^{3-,4-}. The Mo...Mo separation for these latter complexes varies with the bridging groups as (a) 3.66(1) (b) 3.2(1) (c) 3.142(3), and (d) (3-) 6.638(2), (4-) 6.924(1) Å.⁸⁻¹⁰ The majority of the synthetic routes to the complexes (i) have involved [MoS₄]²⁻ reacting with a compound containing iron(II), whereas the complexes (ii) have been isolated subsequent to reactions between FeCl₃, NaSR, and [MoS₄]²⁻ (or [MoO_xS_{4-x}]²⁻) in MeOH or EtOH; also [(PhS)₂FeMoS₄]²⁻ reacts with FeCl₃ and NaSPh in MeOH to form [Fe₆Mo₂S₈(SPh)₉]³⁻. However, the products possible from given reactants, particularly if thiol groups are included, can vary with the stoichiometric ratios of the reactants, the solvent, the extent of the reaction, the cations present and/or the crystallisation conditions. For example, variation in the FeCl₃: NaSPh: [MoS₄]²⁻ ratio from 1:4:3.3 to 1:2.4:3.3, for reaction in MeOH, results in the major product changing from [Fe₆Mo₂S₈(SPh)₉]³⁻ to [Fe₆Mo₂S₈(OMe)₃(SPh)₆]³⁻. The structure of this latter anion is illustrated in Figure 1 to show two different views of the constituent cubane-like clusters.

The spectroscopic, redox, and structural properties of the complexes (i) do not compare favourably with the corresponding properties of the iron-molybdenum cofactor of the dinitrogenases. In particular, the Mo-S distances of ca. 2.15 and 2.26 Å^{7,10} are significantly shorter than those obtained from the EXAFS studies⁵ and the u.v./vis spectra of these complexes contain distinctive features in the 300-600 nm region, in contrast to the bland profile exhibited by the cofactor.¹ However, [Fe(MoS₄)₂]³⁻, in frozen MeCN solution ≤ 77 K, has an e.p.r. spectrum consistent with an $S = 3/2$ spin state with, at 14 K, apparent *g* values of ca. 5.2, 2.7, and 1.8.¹¹ The e.p.r. spectrum for powdered [NET₄]₃[Fe(MoS₄)₂], whilst retaining the principal features of a glass spectrum, shows a more complex profile, perhaps indicative of more than one type of anion in the unit cell and/or different relaxation processes for the solid and solution species.

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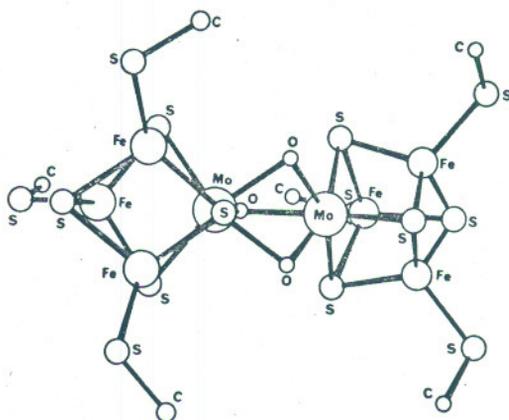


Figure 1. Representation of the structure of $[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{OMe})_3(\text{SPh})_6]^{3-}$, the phenyl rings and the methoxide carbons have been omitted for clarity.

The structures of the $\{\text{Fe}_3\text{MoS}_4\}$ cubane-like clusters, with Mo-S_μ and $\text{Mo}\cdots\text{Fe}$ distances *ca.* 2.35 and 2.73 Å, respectively, have a remarkably close correspondence to those values obtained from the EXAFS studies.⁵ ^{57}Fe Mossbauer studies of $[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SR})_9]^{3-}$ complexes have shown¹² that at 77 K the average isomer shift and quadruple splitting is *ca.* 0.41 and 1.20 mm s^{-1} , respectively. This former value is close to that of $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ complexes and thus implies that the average oxidation state of the iron atoms in $[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SR})_9]^{3-}$ complexes is near 2.5. The average magnetic moment versus temperature (in the range 300-1.8 K) or applied field (from 0.125 to 2.0 T) have been obtained for $[\text{NBu}_4]_3[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SPh})_9]$ and $[\text{NET}_4]_3[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{OMe})_3(\text{SPh})_6]$. The latter data have been interpreted (Figure 2) in terms of antiferromagnetic spin coupling between $\text{Fe}^{\text{II}} + 2\text{Fe}^{\text{III}}$ centres within the separate

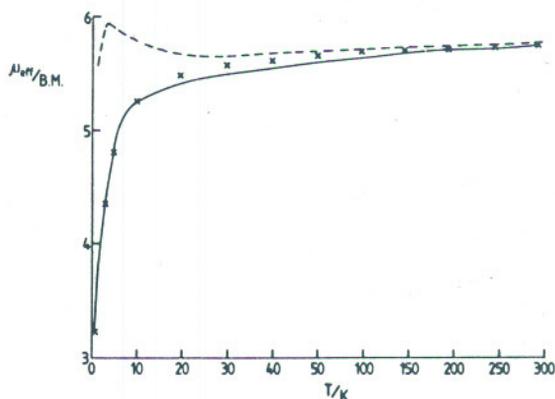


Figure 2. Variation of μ_{eff} with T for $[\text{NET}_4]_3[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{OMe})_3(\text{SPh})_6]$ (—) and $[\text{NBu}_4]_3[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SPh})_9]$ (---) and a theoretical model (X) assuming no intercluster coupling. (Petrouleas, V., Collison, D., Christou, G., Mabbs, F.E., and Garner, C.D., submitted for publication).

$\{\text{Fe}_3\text{MoS}_4\}$ cubane-like clusters with no intercluster coupling.¹⁰ The profile observed for the former complex is consistent with a similar magnetic model but involving a small amount of intercluster coupling. This conclusion that the bulk of the unpaired electron spin density is concentrated on the iron atoms is consistent with the ^1H n.m.r. spectra of $[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SR})_9]^{3-}$ complexes,^{9,13} since the isotropic shifts for the protons of the bridging ligands are small and much less than those for the protons of the corresponding terminal ligands. $[\text{Fe}_6\text{Mo}_2\text{S}_9(\text{SET})_8]^{3-}$ exhibits a rich e.p.r. spectrum; at 4.2 K in DMF the apparent g values are ~ 10 , 4.0, 1.9, and 1.2.⁹ In the solid state $[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SR})_9]^{3-}$ and $[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{OMe})_3(\text{SPh})_6]^{3-}$ (Figure 3) are also e.p.r. active; the intensity of the signals are remarkably temperature dependent and show significant differences from the powder to a frozen solution (*cf.* $[\text{Fe}(\text{MoS}_4)_2]^{3-}$). Earlier we reported¹⁴ that the reduction of

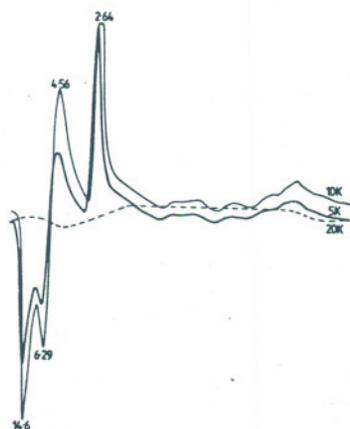


Figure 3. E.p.r. spectra of powdered crystalline $[\text{NET}_4]_3[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{OMe})_3(\text{SPh})_6]$.

$[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SPh})_9]^{3-}$ with ~ 2 equivalents of sodium acenaphthalenide in MeCN, followed by rapid freezing, produces a species which has apparent g values at *ca.* 5.1, 4.3 and 2.0; this species remains uncharacterised. The u.v./vis absorption spectra of $[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SR})_9]^{3-}$ complexes are nearer to the bland spectral profile of the iron-molybdenum cofactor than are those of complexes containing bidentate $[\text{MoS}_4]^{2-}$ groups. However, two shoulders are apparent on a smooth profile of increasing absorbance with decreasing wavelength; for $[\text{NBu}_4]_3[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SPh})_9]$ in DMSO, these occur at *ca.* 446 nm ($\epsilon_M = 3.5 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) and *ca.* 350 nm ($\epsilon_M = 5.0 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$). Consistent with assignments for $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ complexes,⁶ the lower energy feature is attributed to a thiolate sulphur to iron charge-transfer transition and, as expected, this is not apparent for $[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SR})_3\text{Cl}_6]^{3-}$ complexes. M.c.d. spectral studies¹⁰ of $[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SCH}_2\text{CH}_2\text{OH})_9]^{3-}$ indicate that the u.v./vis absorption profile envelopes a large number of spectral transitions and this technique should afford an excellent fingerprint of the cluster core type and its electronic state.

Extensive electrochemical studies of $[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SR})_9]^{3-}$ and related clusters^{9,10,12} have established that: (1) These complexes seem like two linked $\{\text{Fe}_4\text{S}_4\}$ clusters; where an $\{\text{Fe}_4\text{S}_4\}$ cluster exhibits a one-electron oxidation and two one-electron reductions,⁶ the cubane-like cluster dimers exhibit one *pair* of one-electron oxidations and two *pairs* of one-electron reductions. (2) The separation of the components of each of these pairs is *ca.* 200 mV and corresponds to a weak coupling across the bridge and this remains unchanged for an Mo \cdots Mo separation of 3.142(3) Å, in $[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{OMe})_3(\text{SPh})_6]^{3-}$, to *ca.* 3.66 Å in $[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SR})_9]^{3-}$ complexes; the pair of reductions reported⁹ for $[\text{Fe}_7\text{Mo}_2\text{S}_8(\text{SET})_9]^{4-}$ have a separation of *ca.* 100 mV and correspond more nearly to non-interaction between the individual cubes over the Mo \cdots Mo separation of *ca.* 6.92 Å. (3) The first reduction potential for a $[\text{Fe}_6\text{Mo}_2\text{S}_8\text{L}_9]^{3-}$ complex is very close to that for the corresponding $[\text{Fe}_4\text{S}_4\text{L}_4]^{2-}$ complex and shows a similar variation with the nature of the terminal ligands, whereas changes in the bridging region have little effect. (4) Cyclic voltammetric studies have established that, only the first two reductions of $[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SET})_9]^{3-}$ approximate to full

electrochemical reversibility at high scan rates (ca. 5,000 mV s⁻¹).^{10,12}

Therefore, substitution of one molybdenum for one iron atom in an {Fe₄S₄} cluster does not lead to a richer redox chemistry nor to a shift in the redox potential for the first reductions (comparing [Fe₆Mo₂S₈(SR)₉]³⁻ and [Fe₄S₄(SR)₄]²⁻ clusters). These observations imply that the molybdenum of the iron-molybdenum cofactor of the nitrogenases is neither incorporated to allow the cluster to accumulate more electrons for substrate reduction, nor to operate at a significantly different potential, from a similar aggregate based on a corresponding iron-sulphur cluster. Therefore, two possible functions seem to remain for the role of the molybdenum in the biological systems; either it is employed to allow the formation of an iron-molybdenum-sulphur cluster assembly not possible with iron and sulphur alone and/or it is used in the binding and activation of the substrate prior to reduction. Both of these possibilities remain undemonstrated by chemical studies, however, the observation that molybdenum is usually six co-ordinate, whereas iron is four co-ordinate, in a metal-sulphur cluster assemblies, is not inconsistent with these ideas.

Comparisons between molybdenum and tungsten would also appear to be informative concerning the possible role of molybdenum in the dinitrogenases. Although tungsten can substitute for molybdenum in the dinitrogenases, the resultant enzyme is apparently inactive.¹⁵ There are no structural differences between [Fe₆M₂S₈(SR)₉]³⁻ (M = Mo or W) cubane-like cluster dimers,^{9,16} nor is the electrochemical behaviour different in terms of the redox potentials and accessible redox levels. However, the cluster [Fe₆M₂S₈(SEt)₉]³⁻ is, for M = W particularly in its reduced state, sensitive to attack by oxygen donor solvents which results in the elimination of the normal electron transfer characteristics of the system, whereas the corresponding M = Mo cluster is immune to such attack. Thus, the functionality of molybdenum in the dinitrogenases could require the thiolate co-ordination exterior to the metal-sulphur cluster assembly, implied by the EXAFS⁵ studies; substitution of tungsten into this site would lead to a chemically less robust centre, with the W-SR linkages being sensitive to rupture by oxygen donor ligands.

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