Nitrogen Fixation by Mo Clusters

The Role of Mo Atoms in Nitrogen Fixation: Balancing Substrate Reduction and Dihydrogen Production[**]

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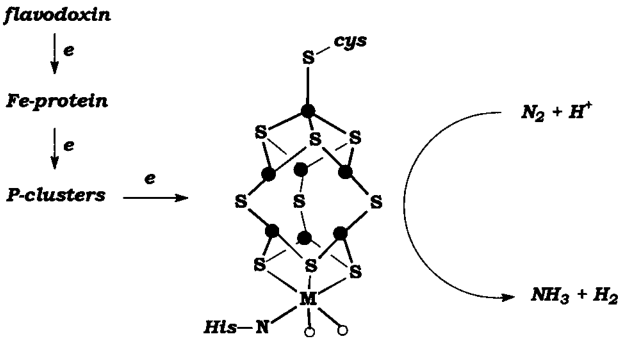
Nitrogenases are metalloenzymes that catalyze the conversion of dinitrogen into ammonia by coupled electron- and proton-transfer reactions.[1,2] Three types of nitrogenases have been characterized[3] and are distinguished by their metal content: molybdenum nitrogenase contains Mo and Fe atoms; vanadium nitrogenase contains V and Fe atoms; and iron-only nitrogenase contains Fe centers and no other transition-metal atoms. The difference in metal-atom content is associated with the Fe–S-based clusters called cofactors, which are the active sites of these enzymes.[4,5] In molybdenum nitrogenase the FeMo cofactor comprises one molybdenum, seven iron, and nine sulfur atoms (Figure 1). The structure of FeMo cofactor has been established by X-ray analysis,[2] but the structures of the other nitrogenases have yet to be determined. Nevertheless, the close homology between the enzymes[3] strongly indicates that the FeV and FeFe cofactors are structurally analogous to the FeMo cofactor, with V or Fe atoms in place of the Mo atom, respectively. Thus, Mo atoms are not essential for nitrogen fixation, but studies on these three types of nitrogenases have shown that the Mo nitrogenase is the most efficient.[3,6]

The different cofactors impart distinct reactivity characteristics to the nitrogenases. Most notably there are subtle differences in the product specificities formed with ethyne, and dinitrogen.[3] In the absence of substrates all nitrogenases reduce protons to dihydrogen, but in the presence of dinitrogen the electron flux is divided between reduction of dinitrogen (to form ammonia) and protons (to form dihydrogen). Since the formation and evolution of every dihydrogen molecule consumes two electrons (and hence four ATP molecules), dihydrogen production wastes energy.[2–4] The molybdenum nitrogenase is the most efficient, but it still produces at least one molecule of dihydrogen for every dinitrogen molecule converted into ammonia. The vanadium and iron-only nitrogenases are markedly less efficient (Figure 1). There is still much debate about the role of the Mo atoms in the cofactor. Herein, we present work that indicates that, irrespective of where dinitrogen binds to the cofactor, the Mo atoms affect the reactivity of the cofactor in a way that facilitates efficient nitrogen fixation. The Mo atoms slow the protonation of the cluster, which, in turn, suppresses dihydrogen production.



LIMITING STOICHIOMETRIES

M = Mo: $N_2 + 8H^+ + 8e \longrightarrow 2NH_3 + H_2$

M = V: $N_2 + 12H^+ + 12e \longrightarrow 2NH_3 + 3H_2$

M = Fe: $N_2 + 21H^+ + 21e \longrightarrow 2NH_3 + 7.5H_2$

Figure 1. Electron-transfer pathway for the nitrogenases, which culminates in the reduction of dinitrogen to ammonia, and protons to dihydrogen. The limiting stoichiometries of the three types of nitrogenase are shown.

The cuboidal clusters $[Fe_4S_4X_4]^{2-}$ and $[\{MoFe_3S_4X_3\}_2(\mu\text{-}SR)_3]^{3-}$ (X = Cl or RS; R = Ph or Et) are structurally simpler than cofactors. Although they contain many of the structural features of cofactors, the coordination environments of the central Fe atoms in cofactors have yet to be observed in synthetic systems.[7] Herein, we report the rates of proton transfer to both types of synthetic cluster (X = Cl) and show how the rates are affected by: 1) prior binding of the nucleophile to the cluster, and 2) the composition of the cluster core (i.e. $\{Fe_4S_4\}^{2+}$ or $\{MoFe_3S_4\}^{3+}$ ions).

Earlier work has shown that for all synthetic Fe–S-based clusters, protonation of the cluster core (presumably at the S atoms) is a common reaction.[8–10] Recently,[11,12] we studied the substitution reactions of $[Fe_4S_4Cl_4]^{2-}$ clusters with PhS^- ions to form $[Fe_4S_4(SPh)_4]^{2-}$ clusters, in the presence of $[H_2N(CH_2)_3CH_2]^+$ ions, in MeCN. Because the chloro ligands in the $[Fe_4S_4Cl_4]^{2-}$ cluster are labile, and the $[H_2N(CH_2)_3CH_2]^+$ ion is a weak acid ($pK_a = 21.5$ in MeCN), the protonation of the cluster core is the rate-limiting step in the reaction.[11] Stopped-flow spectrophotometric studies of

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the reactions between the $[\{MoFe_3S_4Cl_3\}_2(\mu\text{-}SR)_3]^{3-}$ clusters (R = Et or Ph) and PhS^- ions in the presence of $[H_2N\overline{(CH_2)_3C}H_2]^+$ ions show that these reactions have kinetic behavior analogous to that of the reaction between the $[Fe_4S_4Cl_4]^{2-}$ cluster and PhS^- ions. The reaction of the $[\{MoFe_3S_4Cl_3\}_2(\mu\text{-}SEt)_3]^{3-}$ cluster exhibits a first-order dependence on the concentration of the PhS^- ions (Figure 2) and a nonlinear dependence on the concentration of acid as described by Equation (1).

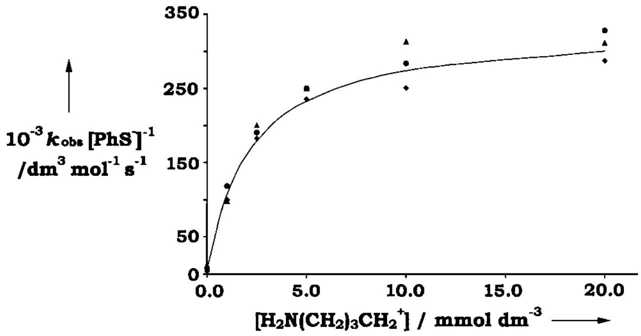

Figure 2. Kinetic data for the reaction of the $[\{MoFe_3S_4Cl_3\}_2(\mu\text{-}SEt)_3]^{3-}$ cluster with PhS^- ions in the presence of $[H_2N\overline{(CH_2)_3C}H_2]^+$ ions in (MeCN, 25.0 °C). The data points correspond to: ■ $[PhS^-] = 0.50$; ▲ $[PhS^-] = 1.25$; ◆ $[PhS^-] = 2.50$ mmol dm^{-3}. The curve is that defined by Equation (1) and the corresponding values are shown in Table 1.

$$\frac{-\mathrm{d}[\text{cluster}]}{\mathrm{d}t} = \frac{k_1^{\text{PhS}}(k_2^{\text{PhS}} + k_5^{\text{PhS}}\,[\mathrm{H_2N\overline{(CH_2)_3C}H_2^+}])\,[\mathrm{PhS^-}]\,[\text{cluster}]}{k_{-1}^{\text{PhS}} + k_2^{\text{PhS}} + k_5^{\text{PhS}}[\mathrm{H_2N\overline{(CH_2)_3C}H_2^+}]} \tag{1}$$

This rate law is consistent with the mechanism of path a in Figure 3, in which the initial binding of the PhS^- ion to the cluster is followed by the protonation or dissociation of a chloride ion. A comparison of the rate constants (Table 1), which were obtained from an analysis of the data,[13] indicates the following features: 1) The $\{MoFe_3S_4\}^{3+}$ clusters have a higher affinity than the $\{Fe_4S_4\}^{2+}$ clusters for binding PhS^- ($K_1^{PhS} = k_1^{PhS}/k_{-1}^{PhS}$). Such behavior is entirely general and has been observed before for a variety of molecules and ions (halides, PhS^- ions, CO, and N_2O).[14] The increased affinity is the result of a combination of an increase in the rate of binding (k_1^{PhS}), and a decrease in the rate of dissociation (k_{-1}^{PhS}). 2) When the PhS^- ion is bound, the rate of protonation of the $\{MoFe_3S_4\}^{3+}$ cluster is little different to that of the $\{Fe_4S_4\}^{2+}$ cluster, and corresponds to the maximum rate of protonation of Fe–S-based clusters ($k \sim 10^6\,dm^3\,mol^{-1}\,s^{-1}$).[11] It seems likely that the binding of thiolate to the cluster increases the basicity of the cluster, and the presence of the bound thiolate is the major factor influencing the rate of proton transfer.[15]

The rate of proton transfer to the clusters prior to the binding of the nucleophile (i.e. path b of Figure 3) can be measured in the substitution reactions of the clusters with Br^- ions in the presence of $[H_2N\overline{(CH_2)_3C}H_2]^+$ ions. Bromide is a poor nucleophile for $[Fe_4S_4Cl_4]^{2-}$ and $[\{MoFe_3S_4Cl_3\}_2(\mu\text{-}SR)_3]^{3-}$ clusters, consequently proton transfer from the $[H_2N\overline{(CH_2)_3C}H_2]^+$ acid is faster than the binding of the bromide ion.[18] Hence, by measuring the kinetics of the reaction of the clusters with Br^- ions in the presence of $[H_2N\overline{(CH_2)_3C}H_2]^+$ ions, the rate constants for the protonation of the clusters before the binding of the nucleophile can be determined. At low concentrations of acid the reaction with

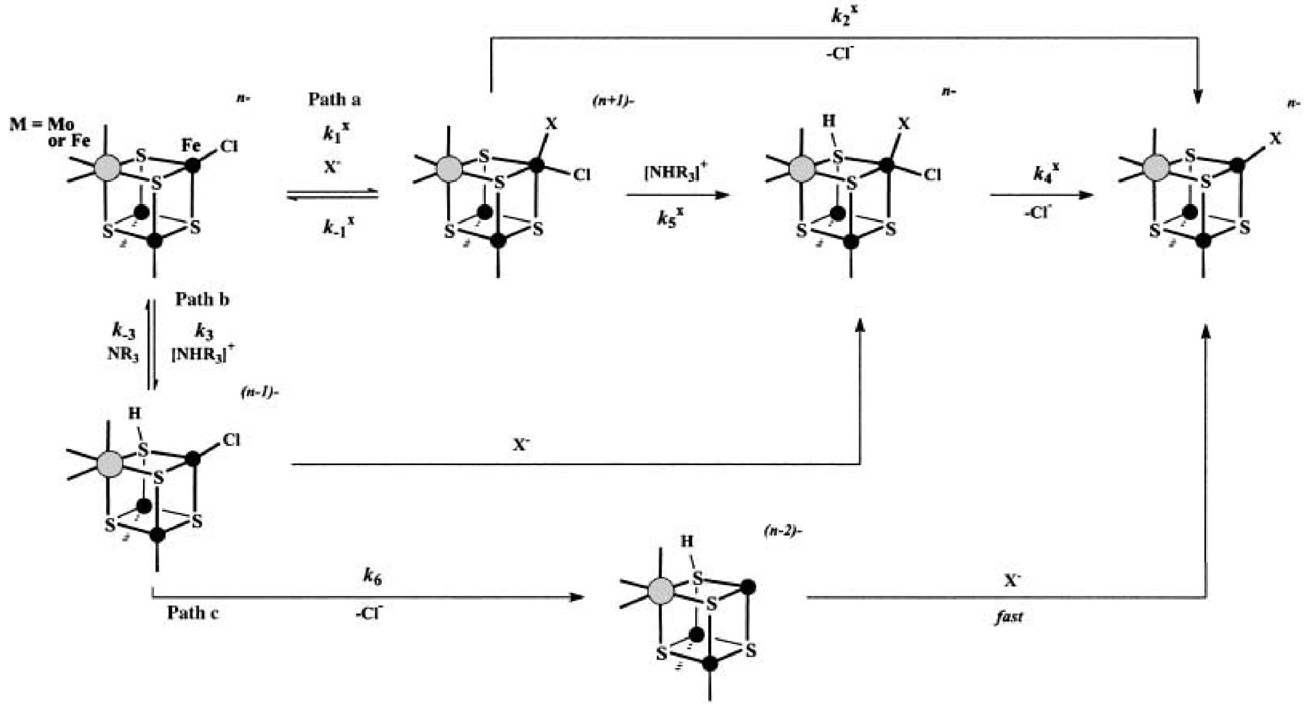

Figure 3. Summary of the pathways for the substitution reactions of synthetic Fe–S clusters in the presence of acids, where $[NHR_3]^+ = [H_2N\overline{(CH_2)_3C}H_2]^+$ ion. Path C is not discussed herein but is included for completeness since this is the acid-catalyzed dissociative pathway observed in the reactions of many clusters ($X^- = PhS^-$, Ph^-).[8,9,13]

Table 1: Summary of rate constants for the elementary reactions of $[Fe_4S_4Cl_4]^{2-}$ and $[\{MoFe_3S_4Cl_3\}_2(\mu\text{-}SR)_3]^{3-}$ clusters with PhS^- or Br^- ions in the Presence of $[H_2N(CH_2)_3CH_2]^+$ in MeCN at 25.0°C

Cluster	Nucleophile	$10^{-5}\,k_1^{PhS}$ [$dm^3\,mol^{-1}\,s^{-1}$]	$10^{-5}\,k_{-1}^{PhS}$ [s^{-1}]	k_2^{PhS}	$10^{-6}k_5^{PhS}$ [$dm^3\,mol^{-1}\,s^{-1}$]	$10^{-2}\,k_3$ [$dm^3\,mol^{-1}\,s^{-1}$]	k_6 [s^{-1}]
$[Fe_4S_4Cl_4]^{2-}$	PhS^-	1.4 ± 0.03	2.20 ± 0.24	300 ± 20	1.80 ± 0.27		
	Br^-					240 ± 40[a]	156 ± 12
$[\{MoFe_3S_4Cl_3\}_2(\mu\text{-}SEt)_3]^{3-}$	PhS^-	3.3 ± 0.3	1.3 ± 0.04	260 ± 15	6.0 ± 0.4		
	Br^-					2.5 ± 0.4[b]	
$[\{MoFe_3S_4Cl_3\}_2(\mu\text{-}SPh)_3]^{3-}$	PhS^-	3.8 ± 0.4	1.3 ± 0.1	230 ± 20	1.6 ± 0.3		
	Br^-					5.0 ± 0.8	

[a] $\Delta H^{\neq} = 0.45 \pm 0.2$ kcal mol^{-1}; $\Delta S^{\neq} = -47 \pm 5$ cal deg^{-1} mol^{-1}. [b] $\Delta H^{\neq} = 0.0 \pm 0.2$ kcal mol^{-1}; $\Delta S^{\neq} = -65 \pm 27$ cal deg^{-1} mol^{-1}.

the $[Fe_4S_4Cl_4]^{2-}$ clusters exhibits a first-order dependence on the concentration of $[H_2N(CH_2)_3CH_2]^+$ ions, and is independent of the concentration of Br^- ions (Figure 4). The rate constants are summarized in Table 1. At high concentrations of the acid, the protonation of the $[Fe_4S_4Cl_4]^{2-}$ cluster is sufficiently fast that a unimolecular reaction becomes rate-limiting. It seems likely that this observation corresponds to the rate-limiting dissociation of the chloro group (k_4).

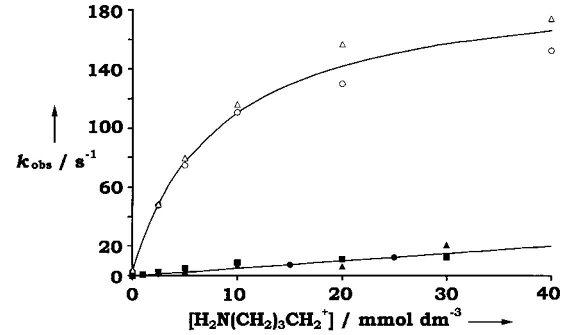

Figure 4. Kinetic data for the reactions of $[Fe_4S_4Cl_4]^{2-}$ (curve) and $[\{MoFe_3S_4Cl_3\}_2(\mu\text{-}SEt)_3]^{3-}$ (line) clusters with Br^- ions in the presence of $[H_2N(CH_2)_3CH_2]^+$ ions (MeCN, 25.0°C). The data points correspond to the $[Fe_4S_4Cl_4]^{2-}$ cluster: ○ $[Br^-] = 5.00$; $[Br^-] = 5.00$ mmol dm^{-3} in the presence of $[D_2N(CH_2)_3CH_2]^+$ (△) and $[\{MoFe_3S_4Cl_3\}_2(\mu\text{-}SPh)_3]^{3-}$: ■ $[Br^-] = 2.50$; ▲ $[Br^-] = 5.0$; ● $[Br^-] = 10.0$ mmol dm^{-3}. The curve and linear fits to the data are those defined by the rate constants shown in the Table 1.

The kinetics of the reactions of $[\{MoFe_3S_4Cl_3\}_2(\mu\text{-}SR)_3]^{3-}$ clusters with Br^- ions in the presence of $[H_2N(CH_2)_3CH_2]^+$ ions are similar to those of the $[Fe_4S_4Cl_4]^{2-}$ cluster with Br^- ions; the rate exhibits a first-order dependence on the acid concentration and is independent of the concentration of Br^- ions (Figure 4). A comparison of the k_3 values shows that: 1) proton-transfer to Fe–S clusters is appreciably slower before a nucleophile is bound, and 2) proton transfer to $[\{MoFe_3S_4Cl_3\}_2(\mu\text{-}SR)_3]^{3-}$ clusters is approximately 50–100 times slower than proton transfer to the $[Fe_4S_4Cl_4]^{2-}$ cluster.

Why the Mo atoms within Fe–S clusters lower the rate of proton transfer could be simplistically attributed to the Mo atoms exerting a general electron-withdrawing effect from the cluster, and this same effect could lead to the increased affinity of the cluster for binding substrates. However, earlier studies[9] established that the pK_a values of protonated Fe–S clusters fall in the narrow range, $18.9 > pK_a > 17.9$ (in MeCN), irrespective of the nature of the terminal ligands, the topology, the nuclearity, the coordination of the S atom (μ_2-S or μ_3-S), or the composition of the core; specifically, for the clusters studied here, $[Fe_4S_4Cl_4]^{2-}$ $pK_a = 18.8$, and $[\{MoFe_3S_4Cl_3\}_2(\mu\text{-}SEt)_3]^{3-}$ $pK_a = 18.6$. The fact that the presence of the Mo atom has little effect on the basicity of the cluster is evidence against there being a general electron-withdrawing effect exerted by the Mo atom. Previously, we showed that the rates of protonation of Fe–S clusters are affected by bond-length changes of the ligands during proton transfer.[11,12] It may be that bond-length changes are energetically more demanding in Mo–Fe–S clusters.

Proton transfer, coupled with electron transfer, is central to the action of cofactors in nitrogenases: to transform dinitrogen into ammonia, and to act as a prequel to dihydrogen production by coupling hydrogen atoms, either on the sulfur atom, or after the hydrogen atoms have migrated from the sulfur atom to a metal center. The presence of the Mo atom in synthetic Fe–S-based clusters makes proton transfer to the clusters slow, and consequently is advantageous to a site that fixes dinitrogen, especially in the binding of the substrate. The bonding between a transition-metal center and dinitrogen consists of nitrogen-to-metal σ-donation and metal-to-nitrogen π-donation.[19] Consequently, a binding site[20] that activates dinitrogen towards protonation (ammonia formation) must be electron rich and thus is inherently susceptible to being protonated.[21] Therefore, any nitrogen-fixing site must maximize the binding and protonation of dinitrogen, but minimize the protonation of the binding site. As noted above, the protonation of core sulfur atoms is a persistent characteristic of Fe–S clusters,[22] including the FeMo cofactor.[9,23] The protonation of sulfur in the FeMo cofactor would make bound dinitrogen labile. This effect could be annulled if protonation is followed by electron transfer. However, the reduction of protonated cofactor could also facilitate dihydrogen production. The simplest way to maximize dinitrogen binding to a site that is also capable of being protonated is to ensure that protonation of the binding site is slow. Proton transfer to all Fe–S cluster cores is slow (at least 10^4 times slower than the diffusioncontrolled limit) and, most importantly, Mo–Fe–S clusters protonate appreciably slower than Fe–S-only clusters. The presence of Mo in FeMo cofactor may facilitate nitrogen fixation in the following ways: 1) By slowing protonation of the active site and maximizing the opportunity for dinitrogen to bind. Irrespective of the intimate mechanism of dihydrogen production, when the protonation process is slowed the rate of dihydrogen production is suppressed. 2) Earlier work[14] has shown that Mo-containing Fe–S-based clusters have an increased affinity to bind substrates compared to Fe–S-only clusters. Our results indicate a previously unidentified possible role for Mo in nitrogenase: to make the enzyme a good nitrogenase but a poor hydrogenase.

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[15] Studies with solutions containing $[D_2\overline{N(CH_2)_3C}H_2]^+$ ions show that the protonation reaction does not exhibit a discernable isotope effect. This observation is in line with other protonation reactions of Fe–S clusters.[16] However, studies in the temperature range 5–40 °C shows that these reactions are associated with small $\Delta H^{\ddagger}$ values, which is consistent with proton transfer reactions,[17] and negative $\Delta S^{\ddagger}$ values (Table 1), which is consistent with an ordered transition state involving the acid and cluster.

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[21] Studies on simple mononuclear complexes such as *trans*-$[Mo(N_2)_2(Ph_2PCH_2CH_2PPh_2)_2]$ have shown that protonation of the binding site deactivates dinitrogen.[1]

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