7.7: Multisite Redox Enzymes (Part 3)

Nitrogenases

Nitrogen fixation is a key reaction of the biological nitrogen cycle.[233] Fixed nitrogen, in which N is in molecules other than N_2, is frequently the limiting factor in plant growth.[234] Since natural systems often cannot provide enough fixed nitrogen for agriculture or animal husbandry, industrial processes have been developed to "fix nitrogen" chemically. The major process in use, often referred to as ammonia synthesis, is the Haber-Bosch process, in which N_2 and H_2 are reacted at temperatures between 300-500°C and pressures of more than 300 atm, using catalysts (usually) based on metallic iron. 235 Hundreds of massive chemical plants are located throughout the world, some producing more than 1,000 tons of NH_3/day. In contrast, in the biological process, N_2 is reduced locally as needed at room temperature and ~0.8 atm by the enzyme system called nitrogenase (variously pronounced with the accent on its first or second syllable).

1. The Scope of Biological Nitrogen Fixation

Biological nitrogen fixation occurs naturally only in certain prokaryotic organisms (sometimes called diazotrophs). Although the majority of bacterial species are not nitrogen fixers, the process of nitrogen fixation has been confirmed in at least some members of many important phylogenetic groups. Nitrogen fixation occurs in strict anaerobes such as *Clostridium pasteurianum*, in strict aerobes such as *Azotobacter vinelandii*, and in facultative aerobes such as *Klebsiella pneumoniae*. Much of the established biochemistry of N_2 reduction has been gleaned from studies of these three species. However, nitrogen fixation has a far broader range, occurring in archaebacterial methanogens such as *Methanobacillus omelianskii*, which produce methane, and eubacterial methanotrophs such as *Methylococcus capsulatus*, which oxidize methane. Photosynthetic organisms ranging from the purple bacterium *Rhodobacter capsulatus* (formerly *Rhodopseudomonas capsulata*) to the cyanobacterium (blue-green alga) *Anabaena cylindrica* fix nitrogen. Nitrogen fixation occurs mostly in mesophilic bacteria (existing between 15 and 40 °C), but has been found in the thermophilic archaebacterial methanogen *Methanococcus thermolithotrophicus* at 64 °C.

Many organisms fix N_2 in nature only symbiotically. Here the most studied systems are species of *Rhizobium* and *Bradyrhizobium*, which fix nitrogen in the red root nodules of leguminous plants such as soybeans, peas, alfalfa, and peanuts. The red color inside the nodules is due to leghemoglobin, a plant O_2-binding protein analogous to animal myoglobins and hemoglobins (Chapter 4). Other symbioses include that of blue-green algae such as *Anabaena azollae* with *Azola* (a water fern); actinomycetes such as *Frankia* with trees such as alder; and *Citrobacter freundii*, living in the anaerobic hind gut of termites. The distribution of nitrogenase clearly points to its adaptability as a metabolic option for species occupying widespread ecological niches.

The absence of nitrogen fixation in eukaryotes therefore seems somewhat puzzling. There would appear to be no fundamental limitation to the existence of nitrogen fixation in higher organisms. Indeed, *nif* genes have been transferred to yeast, where they work effectively under anaerobic conditions. Furthermore, the problem of the simultaneous presence of nitrogen fixation and aerobiosis has been solved effectively by aerobic bacteria such as *Azotobacter*, *Gleocapsa*, and *Anabaena*. Indeed, the lack of a fundamental limitation has encouraged researchers to propose the construction of nonsymbiotic nitrogen-fixing plants (whose niche to date is limited to the grant proposal).

Due to the mild conditions under which it occurs, the biological nitrogen-fixation process may seem inherently simpler than the industrial one. However, it is not; the biological process displays a complexity[235,235a] that belies the simplicity of the chemical conversion of $N_2 \rightarrow 2NH_3$. Genetic analysis reveals that *at least* twenty genes are required for nitrogen fixation in the bacterium *Klebsiella pneumoniae*.[236,237] These *nif* genes (illustrated in Figure 7.25) specify proteins that are involved in regulation (*nif* A and L), pyruvate oxidation/flavin reduction (*nif* J), electron transfer (*nif* F for flavodoxin), the subunits of the structural proteins of the nitrogenase (*nif* H, D, K), Fe-S cluster assembly (*nif* M) and biosynthesis of the iron-molybdenum cofactor, FeMoco (*nif* N, B, E, Q, V, H).[238] The last two functions specify proteins that are responsible for the incorporation of unusual transition-metal sulfide clusters into the nitrogenase proteins. These clusters have allowed nitrogenase to be studied by biophysical and bioinorganic chemists to establish aspects of its structure and mechanism of action.

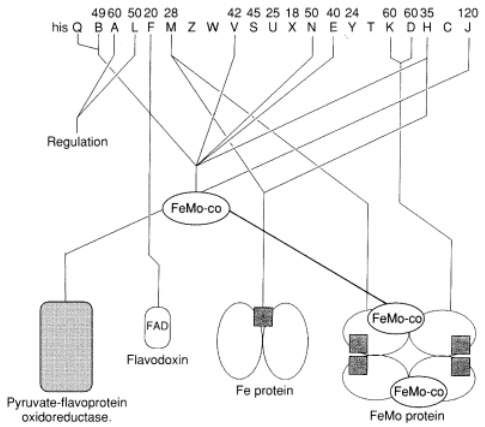

Figure 7.25 - Nif genes required for nitrogen fixation as arranged in Klebsiella pneumoniae, and their respective gene products. The numbers on the top are the molecular weights in kilodaltons of the respective gene products.

We will first discuss the N_2 molecule and focus on its reduction products, which are the presumed intermediates or final product of nitrogen fixation. We then present what has been called[239,240] the "Dominant Hypothesis" for the composition, organization, and function of molybdenum-based nitrogenases. Until 1980, it was thought that molybdenum was essential for nitrogen fixation. However, work starting in 1980 led finally in 1986 to the confirmation of vanadiumbased nitrogen fixation. The newly discovered vanadium-based nitrogenases differ in reactivity from the Mo-based enzyme in having "alternative" substrate specificity. The distinct reaction properties of the different nitrogenases point to the importance of the study of alternative substrate reactions in probing the mechanism of nitrogen fixation.

2. Dinitrogen: The Molecule and Its Reduced Intermediates

The N_2 molecule has a triple bond with energy 225 kcal/mole, a ν(N≡N) stretch of 2331 cm^{-1}, and an N≡N distance of 1.098 Å. The stable isotopes of nitrogen are ^{14}N(I = 1) with natural abundance of 99.64 percent and ^{15}N(I = $\frac{1}{3}$) with an abundance of 0.36 percent.

The challenge to which nitrogenase rises is to break and reduce at a reasonable rate the extremely strong N≡N triple bond. The kinetic inertness of N_2 is highlighted by the fact that carrying out reactions "under nitrogen" is considered equivalent to doing the chemistry in an inert atmosphere. Despite this kinetic inertness, thermodynamically the reduction of N_2 by H_2 is a favorable process,

$$N_2 + 3H_2 \rightarrow 2NH_3 \qquad \Delta G^o = -3.97 kcal/mole \tag{7.10}$$

and at pH = 7 the reaction

$$N_2 + 8H^+ + 6e^- \rightarrow 2NH_4^+ \qquad E^{o\,\prime} = -280 mV \tag{7.11}$$

has an E°' value that makes it easily accessible to biological reductants such as the low-potential ferredoxins discussed earlier in this chapter.

What, then, is the cause of the kinetic inertness of the N_2 molecule? The thermodynamically favorable reduction of N_2 to $2NH_3$ is a six-electron process. Unless a concerted $6e^-$, $6H^+$ process can be effected, intermediates between N_2 and NH_3 must be formed. However, all the intermediates on the pathway between N_2 and NH_3 are higher in energy than either the reactants or the products. The EO' values for the formation of N_2H_2 (= diimine, diazene, diamide) or N_2H_4 (hydrazine) are estimated[241] as

$$N_2 + 2H^+ + 2e^- \rightarrow N_2H_2 \qquad E^{o\,\prime} \sim -1000 to -1500 mV \tag{7.12}$$

$$\$\$N_2 + 5H^+ + 4e^- \rightarrow N_2H_5^+ \qquad E^{o\,\prime} = -695 mV \tag{7.13}$$

Clearly, these potentials are sufficiently negative that the normal biological reductants cannot effect the reaction. The difficulty of reaching these intermediates is indicated in Figure 7.26.

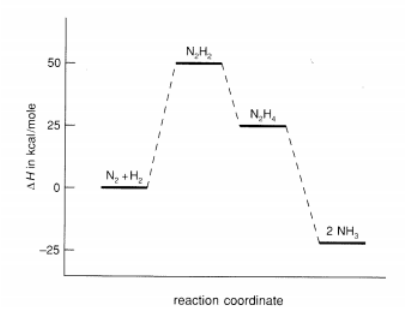

Figure 7.26 - Energetics of N_2, NH_3, and some potential intermediates along the reaction pathway for their interconversion.

Several factors may allow this barrier to be overcome. First, the six-electron reduction might be carried out in a concerted or near-concerted manner to avoid the intermediates completely. Alternatively, the intermediates could be compIexed at metal centers to stabilize them to a greater extent than either the reactants or products. Finally, the formation reaction for the unfavorable intermediate could be coupled with ATP hydrolysis or with the evolution of dihydrogen, each a favorable process, so that the overall process is favorable. Which of the above strategies is used by nitrogenase is unknown, but it seems likely that some combination of the last two of these is used to effect the difficult reduction of N_2 to NH_3. To probe the possibilities, a variety of complexes of N_2, diazenes, and hydrazines has been prepared and chemically characterized, and these are discussed toward the end of this section.

3. The Dominant Hypothesis for Molybdenum Nitrogenase[239,240,242,243]

The action of the Mo-nitrogenase enzyme involves the functioning of two separately isolatable component proteins, as sketched in Figure 7.27A. The larger of the two proteins, sometimes incorrectly[244] designated[245] dinitrogenase has, in the past, been called molybdoferredoxin, azofermo, or component I. More often this protein is called the MoFe or FeMo protein ([MoFe] or [FeMo]). The smaller protein, formerly called azoferredoxin or component II, is sometimes incorrectly[244] referred to[245] as dinitrogenase reductase.* This protein is properly called the Fe protein or [Fe]. A useful nomenclature for discussions of kinetics and comparative biochemistry designates the FeMo protein as *Xyl*, where X and y are the first letters of the first and second name of the bacterial source, respectively. For example, *Cpl* is the FeMo protein from *Clostridium pasteurianum*. Similarly, for the Fe protein the designation *Xy2* is given; for example, the Fe protein of *Azotobacter vinelandii* is called *Av2*. This system will be used where appropriate to distinguish the protein source. Properties of representative Mo nitrogenases are given in Table 7.6.

Table 7.6: Properties of some representative nitrogenases.

Organism	Component	MW	Metal Content	Reference
Azotobacter vinelandii	[MoFe] [Fe]	234,000 64,000	2Mo, 34-38Fe, 26-28S 3.4Fe, 2.8S	366, 367
Azotobacter chrococcum	[MoFe] [Fe]	227,000 65,400	2Mo, 22Fe, 20S 4Fe, 3.9S	366, 368
Clostridium pasteurianum	[MoFe] [Fe]	221,800 55,000	2Mo, 24Fe, 24S 4Fe, 4S	366, 369
Klebsiella pneumoniae	[MoFe] [Fe]	229,000 66,800	2Mo, 20Fe, 20S	366
Anabaena cylindrica	[MoFe] [Fe]	223,000 60,000	2Mo, 20Fe, 20S	370
Rhodospirillum rubrum	[MoFe] [Fe]	215,000 60,000	2Mo, 25-30Fe, 19-22S	371

The schematic diagram in Figure 7.27 shows some of the compositional and functional relationships of the nitrogenase proteins. The iron protein contains two identical subunits of MW ~ 30 kDa. The subunits are products of the *nif* H gene.[246] A single Fe_4S_4 center is present in the protein and appears to be bound between the subunits.[246a] A recent x-ray structure[246b] of the iron protein confirms this picture. As shown in Figure 7.27A, the single Fe_4S_4 center is located at one end of the molecule, in the only region of

significant contact between the two subunits. *In vivo*, the Fe protein is reducible by ftavodoxin or ferredoxin. *In vitro*, artificial reductants such as dithionite or viologens are generally used. The single Fe_4S_4 center undergoes a single one-electron redox process, wherein the reduced form is EPR-active and the oxidized form is diamagnetic. As such, this center resembles four-iron-cluster-containing ferredoxins. Its redox potential is dependent on the ATP or ADP level in the solution. For example, *Cp2* (the Fe protein from *Clostridium pasteurianum*) shows E°' = -294 mV in the absence and -400 mV in the presence of MgATP.[247] Two equivalents of MgATP and MgADP each bind to [Fe].

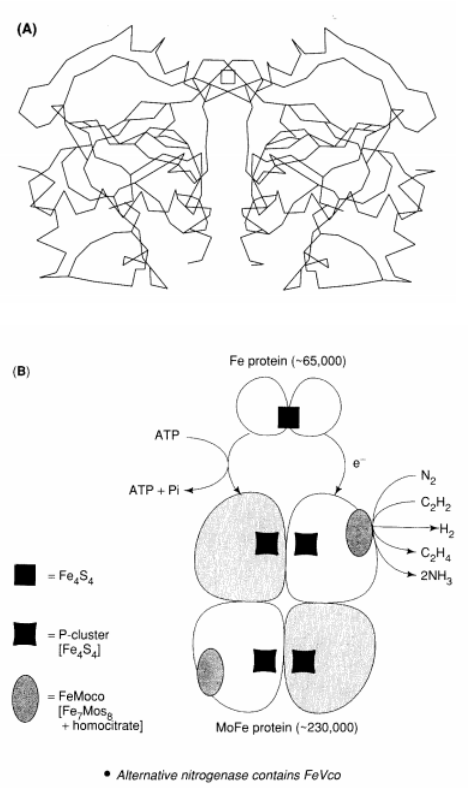

Figure 7.27 - (A) Preliminary x-ray crystal structure of the Azotobacter vinelandii nitrogenase Fe protein.[246b] (B) Schematic of the nitrogenase proteins illustrating their composition and mode of action.

Until recently there was a major mystery over the number of Fe_4S_4 centers in [Fe] as deduced by EPR quantitation of the Fe_4S_4 centers compared to the number derived analytically or by extrusion experiments.[239,248] However, it has now[9,200,249,250] been clearly established that the single Fe_4S_4 center can exist in this protein in two spin states, $S = \frac{1}{2}$ and $S = \frac{3}{2}$ Only that part of the EPR signal corresponding to the $S = \frac{1}{2}$ form, with its g values near 2, was considered in earlier spin quantitations. When the $S = \frac{3}{2}$ center, with g values between 4 and 6, is included, the EPR spin integration shows one paramagnetic site per Fe_4S_4 unit. Model systems[121,122] and theoretical studies[123,124,251] strongly support the ability of Fe_4S_4 to exist in various spin states. During enzyme turnover, the single Fe_4S_4 of the Fe-protein center transfers electrons to the FeMo protein in one-electron steps. There is no evidence for any difference in redox behavior between the $S = \frac{1}{2}$ and $S = \frac{3}{2}$ states of the protein.[200]

The Fe protein binds two molecules of MgATP.[252] The recent structure[246b,378] suggests that a cleft between the two subunits may serve as the ATP binding site. As the enzyme system turns over, a minimum of two molecules of MgATP are hydrolyzed to MgADP and phosphate in conjunction with the transfer of each electron to the FeMo protein.[253] The ATP/2e$^-$ ratio is generally accepted to have a minimum value of 4. Higher numbers represent decreased efficiency, often attributed to "futile cycling," where back electron transfer from [FeMo] to [Fe] raises the effective ratio.[248,253] Except for an as-yet-unconfirmed report of reduction by thermalized electrons produced by pulse radiolysis,[254] there is no evidence that the FeMo protein can be reduced to a catalytically active form without the Fe protein present.

Even though [Fe] must be present for catalysis to take place, the Dominant Hypothesis[239] designates [FeMo] as the protein immediately responsible for substrate reduction, and genetic/biochemical evidence supports this view. The FeMo protein contains an ($\alpha_2\beta_2$ subunit structure, where α and β are coded by the *nif* D and *nif* K genes,[15,229] respectively. The overall molecular weight of about 230 kDa reflects the 50- to 60-kDa MW of each of its four subunits. In addition to protein, a total of 30 Fe, 2 Mo, and 30

S^{2-}, all presumed to be in the form of transition-metal sulfide clusters, add relatively little to the molecular weight, but are presumed to be major parts of the active centers of the protein. Figure 7.27, which is highly schematic, displays the cluster types in accord with the Dominant Hypothesis.

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* The nomenclature proposal[245] that [FeMo] be designated as dinitrogenase and [Fe] as dinitrogenase reductase, although sometimes used in the literature, is incorrect or, at best, premature.[244] The suggested nomenclature implies that both [FeMo] and [Fe] are enzymes. However, neither protein can function catalytically in the absence of the other. [FeMo] will not reduce N_2 or C_2H_2 or evolve H_2 in the absence of [Fe]. The iron protein will not hydrolyze MgATP in the absence of [FeMo]. Nitrogen fixation requires the simultaneous presence of both proteins. Although mechanistic considerations[255] point to [FeMo] as the substrate binding and reducing protein, and [Fe] as the ATP binding locus, catalytic reactions characteristic of this enzyme system have never been consumated by one protein in the absence of the other (but see later for the uptake of H_2). In this chapter, we use the [FeMo] and [Fe] designations in accord with most workers in the field.

4. Protein Purity and Active Sites

It has been almost 20 years since the first relatively pure preparations of nitrogenase became available. Indeed, homogeneous preparations are a *sine qua non* for progress in our understanding of the chemical nature and reactivity of any active site. In metalloproteins, there are two levels of homogeneity. The first involves purity with respect to the protein/subunit composition. This type of purity is achieved by conventional protein-purification techniques, and can be monitored by gel electrophoresis under native and denaturing conditions. In the language of polymer science, the macromolecular portion of the protein can be said to be monodisperse, corresponding to a single molecular weight for the polypeptide chain(s). However, even if the protein is homogeneous by this criterion, it may be inactive or only partially active because it does not have a full complement of active metal sites. The metal sites may be empty, filled with the wrong metals, or otherwise imperfect. Often the apo or inactive enzyme has chromatographic, electrophoretic, and centrifugal behavior very much like that of the holo protein, and therefore copurifies with it. Therefore, purification to electrophoretic homogeneity is only the first step. It is then necessary to ensure the chemical homogeneity of the active site. Very often activity is the major criterion for the approach toward such purity; i.e., the most homogeneous preparations are usually those in which the activity is highest. Several studies done on preparations that lacked active-site homogeneity were, as a result, not meaningful.

The two types of centers present in the nitrogenase FeMo protein are designated P clusters and FeMoco (or M) centers. Both types of centers display unique spectroscopic properties, but only FeMoco continues to display most of those properties when it is extracted from the protein.

5. FeMoco

The presence of the FeMo cofactor within the FeMo protein of nitrogenase, i.e., the M center, is revealed through spectroscopic and redox studies.[239] In the resting state of [FeMo], as isolated in the presence of dithionite, the M center has a distinct S = $\frac{3}{2}$ EPR signal, which is discussed below (see Figure 7.28).

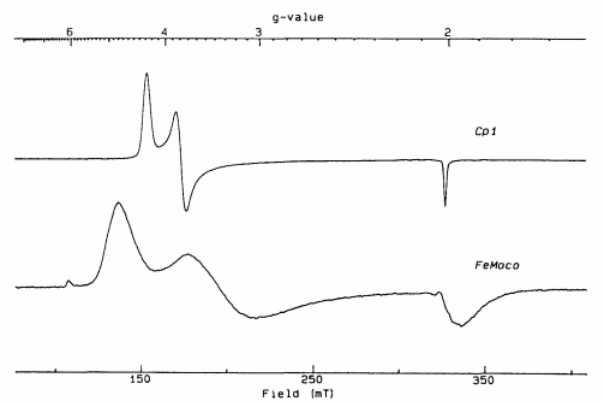


Figure 7.28: EPR spectra: (A) the S = $\frac{3}{2}$ M center in Clostridium pasteurianum nitrogenase FeMo protein; and (B) the FeMoco extracted into NMF from the protein. (Spectra courtesy of R. Bare and G. N. George.)

When the enzyme is turning over the EPR signal essentially disappears, leaving an EPR-silent state in which the FeMoco site is super-reduced to what is presumed to be its catalytically active form. In addition, a third state in which the S = $\frac{3}{2}$ EPR signal

disappears is produced upon oxidation under non-turnover conditions. Thus the M center within the protein shows three states of oxidation, and these appear to have been reproduced in the FeMoco extracted from the protein:[255a]

$$FeMoco\ (oxidized) \xrightarrow{e^-} FeMoco\ (reduced) \xrightarrow{e^-} FeMoco\ (super-reduced) \tag{7.14}$$

The detailed characterization of the FeMoco site has involved parallel studies of the site within the protein and in its extracted form. The authentication of the extracted FeMoco involves the production and use of mutant organisms that make an inactive FeMo protein that contains all subunits and P clusters, but lacks the FeMoco sites.[172,256] A mutant of *Azotobacter vinelandii* called UW-45 (UW = University of Wisconsin) was first used to assay for isolated FeMoco.[257] Since several genes are involved in specifying FeMoco biosynthesis, mutants lacking these genes produce FeMo protein either lacking FeMoco or having a defective version of FeMoco. Mutants such as *Nif* B^- of *Klebsiella pneumoniae*[172] lack cofactor, and an inactive "apo" protein can be isolated from them.

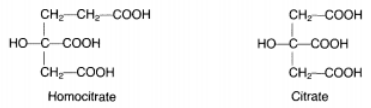
The breakthrough in this field[257] came in 1976, when FeMoco was extracted from [FeMo] into N-methylformamide[258] after the protein was acidified and then neutralized, The acidification removes most of the acid-labile P clusters, and partially denatures the protein. Reneutralization precipitates the protein (near its isoelectric point) and the precipitated denatured protein can then be extracted.

It has been shown that FeMoco can be extracted into many organic solvents,[10,257,259-259b] provided proper combinations of cations and anions[259a] are present in the solvent. The role of the cation is to balance the charge of the negatively charged cofactor. The role of the anion is to displace the cofactor from anion-exchange columns, such as DEAE cellulose or TEAE cellulose, to which the cofactor and/or its protein source had been adsorbed. The ability to dissolve cofactor in such solvents as CH_3CN, acetone, THF, and even benzene should facilitate attempts at further characterization and crystallization.[259,259a]

The biochemical authenticity of FeMoco has been assayed by its ability to activate the FeMo protein from the cofactor-less mutant organism.[258] The stoichiometry of the cofactor is $MoFe_{6\text{-}8}S_{7\text{-}10}$, with the variability likely due to sample inhomogeneity. The extracted cofactor resembles the M-center unit spectroscopically and structurally as shown in Table 7.7, The differences are presumed to result from differences in the peripheral ligands of the metal-sulfide center between the protein and the organic solvent.[260]

Strong evidence to support FeMoco as the site of substrate binding and reduction comes from the study of *nif* V mutants.[261-263] (The V designation is somewhat unfortunate, as *nif* V has nothing to do with vanadium.) The *Nif* V mutants do not fix nitrogen *in vivo*, and have altered substrate specificity *in vitro*. Dihydrogen evolution by isolated *nif* V nitrogenase is inhibited by CO, in contrast to the wild type, where H_2 evolution is insensitive to CO. FeMoco can be extracted from the *nif* V protein and used to reactivate the FeMoco-deficient mutants, such as *nif* B or UW-45. Remarkably, the reconstituted FeMo protein has CO-sensitive H_2 evolution, which is characteristic of *nif* V; i.e., the *nif* V phenotype is a property of FeMoco and not of the protein.[263] This result clearly implicates the FeMoco site as an important part of the substrate reactions of the nitrogenase enzyme complex.

Recently, a heat-stable factor called the V-factor has been discovered that restores the wild-type phenotype when added to *nif* V mutants during *in vitro* FeMoco assembly reactions.[264] The V-factor has been shown to be homocitrate (see Scheme 7.15) and ^{14}C labeling strongly suggests that homocitrate (or a part of it) is a component of the cofactor center. Interestingly, the far more metabolically common citrate appears to be present in the *nif* V mutant.[265a] Replacement of homocitrate by analogues that differ in structure or stereochemistry yields modified FeMoco sites that have altered substrate specificities.[265b] Thus, as is true for many cofactors (e.g., heme = porphyrin + iron; B_{12} = corrin + cobalt; F430 = corphin + nickel; Moco, the molybdenum cofactor = Mo +molybdopterin), both inorganic and organic components are present in FeMoco.



(7.15)

The biosynthesis of the cofactor and its insertion into [FeMo] apparently requires the presence of [Fe] and ATP in A. vinelandii.[266,266a] Whether this involves redox or conformational change in [FeMo] induced by [Fe] is unknown, but the fact that inactive versions of [Fe] are effective would seem to favor the nonredox mechanism. An attractive idea[266] is that [Fe]•MgATP binds to [FeMo], producing a state that is conformationally accessible for cofactor insertion.

Recently, site-directed mutagenesis studies[266b,c] have shown that cysteine residues are involved in binding FeMoco to the subunits of [FeMo]. Moreover, these studies again implicate FeMoco in the substrate-reducing site.

6. The P-clusters

Evidence has been presented[229] for the presence of four Fe_4S_4-like clusters (designated as P-clusters) in [FeMo]. The P-clusters are, however, by no means ordinary Fe_4S_4 clusters, and may not be Fe_4S_4 clusters at all. P-clusters are manifest[239,248] in electronic absorption and, especially, MCD and Mössbauer spectra of [FeMo]. These spectra are clearly not conventional; i.e., they are not like those found in ferredoxins and have not yet been seen in model compounds. In their oxidized forms, the P-clusters are high-spin, probably S = $t\frac{7}{2}$ according to EPR studies.[267] Mössbauer spectra reveal decidedly inequivalent Fe populations,[268,269] indicating that the putative Fe_4S_4 clusters are highly distorted or asymmetric. The four P-clusters do not appear to behave identically under many circumstances, and it is clear that they form at least two subsets. There is open disagreement over the redox behavior of these sets.[239,270,271] Furthermore, an additional Mössbauer signal sometimes designated as S may also be part of the P-cluster signal.[268]

Although spectroscopic studies of the P-clusters do not unequivocally reveal their structural nature, extrusion of these clusters from the protein leads to the clear identification of three or four Fe_4S_4 clusters.[248,272] As discussed previously, the extrusion technique has inherent uncertainties, because it may be accompanied by cluster rearrangement. Nevertheless, the experimental result does support the Dominant Hypothesis, which designates the P centers as highly unusual Fe_4S_4 clusters.* The P-clusters are thought to be involved in electron storage and transfer, and presumably provide a reservoir of low-potential electrons to be used by the M center (FeMoco) in substrate reduction. Attractive as it may seem, there is no direct evidence to support this notion.

Table 7.7 - Comparison of the FeMo protein and isolated FeMoco.[a]

a) Distance in Å with number of atoms in parentheses.
b) From 287; earlier study reported in 286.
c) Data from 373; earlier study reported in 372.
d) Data from 290.
e) Data from 291.
f) Data from 288, 289.

	FeMo protein (M center)	FeMoco (in NMF)
EPR		
g' values	4.27	4.8
	3.79	3.3
	2.01	2.0
EXAFS[a]		
Mo-S	2.36 (4)[b]	2.37 (3.1)[c]
Mo-Fe	2.69 (3)[b]	2.70 (2.6)[c]
Mo-O or N	2.18 (1)[b]	2.10 (3.1)[c]
Fe-S		2.25 (3.4)[d] 2.20 (3.0)[e]
Fe-Fe		2.66 (2.3)[d] 2.64 (2.2)[e] 3.68 (0.8)[e]
Fe-Mo		2.76 (0.4)[d] 2.70 (0.8)[e]
Fe-O or N		1.81 (1.2)[d]
XANES MoO_3S_3 fits best[f]		

* Recent x-ray crystallographic results show that, if Fe_4S_4 clusters are present, they are very close together in two pairs,[379,380] which may account for their unusual properties.