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Current status of structure function relationships of vanadium nitrogenase

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Contents

Abstract

V-nitrogenase is both genetically and biochemically similar to the more intensively studied Mo-nitrogenase. The VFe protein contains P cluster redox centres and a catalytic FeVco centre, in which V is in polynuclear cluster with Fe, S and homocitrate with a chemical environment similar to Mo in MoFe proteins. Current preparations of VFe proteins are a mixture of functional and inactive species, hindering mechanistic studies. A rationale for their separation based on the formation of putative transition-state analogues is outlined.

Keywords: Mo-nitrogenase; Vanadium-nitrogenase; FeMoco; FeVco; Nitrogen fixation

1. Introduction

Nitrogenase, the microbial enzyme responsible for biological nitrogen fixation, the reduction of N_2 to NH_3, is responsible for the cycling of some 10^8 ton of N per year from the atmosphere to the soil, thereby making it available to higher organisms. Since the pioneering study of the metal requirement for N_2-dependent growth of the soil bacterium *Azotobacter vinelandii* by the microbial physiologist Bortels in the period 1930–1936, Mo had been accepted to have an essential role in nitrogen fixation (see Ref. [1]). Other early studies also showed that V was effective in supporting growth, but these findings were generally ignored. Some 50 years later V was shown unequivocally to have a role in biological nitrogen fixation, when V-containing nitrogenases were isolated from mutants of two species of Azotobacter unable to synthesise Mo-nitrogenase [2,3]. Although all nitrogen-fixing organisms have a Mo-nitrogenase, subsequent genetic and physiological studies showed that V-nitrogenases are widely distributed but were only synthesised when Mo was a limiting nutrient.

Mo-nitrogenase is a two component metallo-enzyme system that couples the hydrolysis of MgATP to the reduction of dinitrogen in the reaction:

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$$N_2 + 8H^+ + 8e + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16Pi$$

Mo-containing nitrogenases are made up of a molybdenum and iron containing protein (MoFe protein) and an Fe protein which functions as a specific MgATP-dependent electron donor to the MoFe protein [4,5]. The X-ray crystal structures of both individual proteins and the putative ADP-AlF_4 transition state complex of the two proteins of *A. vinelandii* have been determined [5,6]. The Fe protein is a γ_2 dimer which has a single [4Fe4S] centre ligated at the subunit interface and two nucleotide-binding sites, one on each subunit. The structures of the MoFe proteins have revealed $\alpha_2\beta_2$ subunit structure in which each dimeric αβ subunit pair binds a P cluster (a Fe_8–S_7 cluster) positioned at the subunit interface and a FeMo-cofactor centre (Fe_7S_9Mo-homocitrate) within the α subunit. This information, together with comparative spectroscopic data enabled a more meaningful interpretation of data obtained for VFe proteins, for which the structure has yet to be determined.

In this contribution, the molecular enzymology of Mo-nitrogenase (with a focus on the MoFe protein) are reviewed and compared with comparable data for the VFe protein. In general, review references are used for the earlier work on Mo-nitrogenase.

Molecular genetics played an essential part in putting V-nitrogenase research on a firm basis. It enabled the existence of V-nitrogenase to be established unequivocally, and identified likely common steps in the biosynthesis of the cofactor centres of Mo- and V-nitrogenase. My intention is to indicate that the V- and Mo-nitrogenases of *Azotobacter*, share a great deal of properties in common, allowing the structure of the V-containing cofactor of the VFe proteins to be predicted with some certainty. However, subsequent progress in characterizing V-nitrogenases and their reactivity has increasingly been hampered by the heterogeneity of the preparations of VFe proteins currently available, both with respect to their spectroscopic properties, and in their metal contents and enzymatic activities. These difficulties are discussed, and a potential method for removing partially-processed or damaged species of the VFe protein is presented.

2. Mo-nitrogenase

The description of MoFe proteins given here is not comprehensive, having been selected for the purpose of comparison with the more restricted range of data available for VFe proteins. MoFe proteins (see Refs. [4,5,7,8]) are a highly conserved protein family and the α and β subunits encoded by *nifD* and *nifK* genes, show a high degree of sequence conservation among nitrogen-fixing organisms. The proteins are $\alpha_2\beta_2$ tetramers with a $M_r \sim 240\,000$, that in preparations with highest activity contain ca. 2 Mo, and 30–34 Fe atoms and 30 acid-labile sulfur atoms. Mössbauer spectroscopy indicated that the Fe atoms being organised into two types of cluster, the P clusters and paramagnetic centres with an $S = 3/2$ spin system. The centre giving rise to the EPR signal is an Fe- and Mo-containing cofactor centre (FeMoco), which under denaturing conditions can be extracted into *N*-methyl formamide (NMF), in a form capable of activating the inactive apo-MoFe protein isolated from mutant strains unable to synthesise FeMoco. Reconstitution experiments of this type showed that FeMoco isolated from MoFe protein of a *nifV* mutant unable to synthesise homocitrate, transferred the phenotype of forming an acetylene-reductase activity but with low activity with N_2 as a substrate. This pattern of activity of the reactivated MoFe protein, provides the strongest evidence that FeMoco forms the active site of nitrogenase [9]

Extensive spectroscopic studies (particularly EXAFS) of both MoFe proteins and isolated FeMoco provided information as to the environment of the Mo. The problem of the chemical structure of FeMoco was resolved by the determination of the structures of several MoFe proteins by X-ray crystallography (see Refs. [5,7,10]). These structures show that FeMoco, with the composition Fe_7S_9Mo-homocitrate, is anchored to the protein by two amino acid residues. In the MoFe protein of *Klebsiella pneumoniae* these are the α-Cys273 residue providing a thiolate ligand to the Fe atom at one end of the cofactor, and the α-His440 residue where the imidazole $^{\delta}$N atom is coordinated to the Mo atom. Structurally FeMoco is made up of a 4Fe–3S cluster and a1Mo–3Fe–3S cluster linked by three bridging sulfides with (*R*)-homocitrate coordinated to the Mo atom by its 2-hydroxy and 2-carboxy groups (Fig. 1). The Mo atom is six-coordinate and six of the Fe atoms are in an unusual distorted trigonal geometry.

The biosynthesis of FeMoco is complex, and the detail is not well understood at the biochemical level [11]. A number of *nif* genes have been shown to have roles in this process, and are also required for the

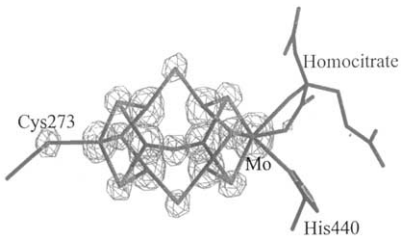

Fig. 1. The FeMoco centre of *K. pneumoniae* MoFe protein at 1.6 Å resolution contoured at 6 σ. At this level only electron density associated with the heavier atoms, Fe, Mo, S is visible [10].

formation of an active V-nitrogenase providing strong presumptive evidence for the presence of similar types cofactor in this system. *NifH* encoding the Fe protein of Mo-nitrogenase is also involved in both the synthesis of FeMoco and in its insertion into apo-MoFe protein. *NifB* is involved in the formation of NifB-co, a low molecular weight material containing Fe and acid-labile sulfide, which can be solubilized from cell extracts by detergents, and *nifV* as a homocitrate synthase, catalyzing the condensation of α-oxoglutarate with acetylCoA to form homocitrate, a ligand to Mo in FeMoco. *Nif EN* are also essential for FeMoco biosynthesis, and the products of these genes, which show homology with *nifDK* have a suggested role as a template on which FeMoco is synthesised. A generalized scheme for FeMoco biosynthesis is shown in Fig. 2.

The remainder of the Fe in the MoFe proteins is associated with P clusters, which are Fe/S clusters capable of undergoing multi-electron redox processes and exhibit unusual spectroscopic properties. Characteristic spectroscopic features of these clusters are the unusually steep magnetization curves of the low-temperature M.C.D. transitions in dye-oxidized proteins, and a range of E.P.R. signals exhibited by oxidized MoFe proteins when poised at redox potentials in the range −500 to +100 mV (see Ref. [4]).

The P clusters are located at the α/β subunit interface, ligated by two cysteine residues from both subunits. In the dithionite-reduced protein they are made up of two [4Fe4S] clusters linked by bridging cysteine thiolate groups. On oxidation a change in structure occurs and a single sulfur atom is shared by the cubanes [10,12]. The bridged double cubane structure of the P clusters, and the structural variant of a shared sulfur atom, readily accounts for their unusual spectral properties, and their ability to undergo multi-electron redox processes.

The P clusters and the FeMoco centres are separated by some 19 Å, and the two FeMoco centres 70 Å apart, distances supporting the view that each αβ subunit pairs function independently during catalysis. The consensus view is that the FeMoco centre is the site of substrate binding and activation and that the P clusters function in electron transfer from the Fe protein to this catalytic centre. The P clusters become transiently oxidized during enzyme turnover under N_2 providing an experimental basis for their long-suggested role as electron capacitors [13]. The amino acid residues ligating these redox centres (Fig. 3) are invariant in all known MoFe protein sequences.

3. V-nitrogenase

V-nitrogenase is comprised of a Fe protein and a VFe protein, encoded by structural genes homologous to, but distinct from, those of Mo-nitrogenase [14,15]. Thus despite biochemical similarity, the vanadium system does not arise from the simple substitution of V for Mo in the MoFe protein. Biochemical studies of V-nitrogenases are restricted to the proteins isolated from

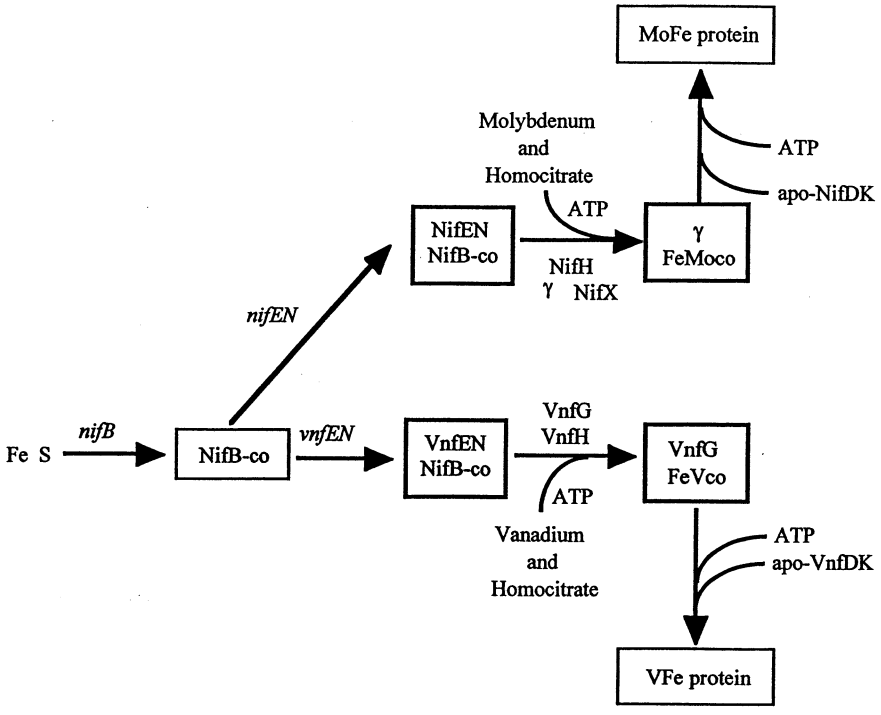

Fig. 2. A model for FeMoco and FeVco biosynthesis and insertion into MoFe and VFe protein.

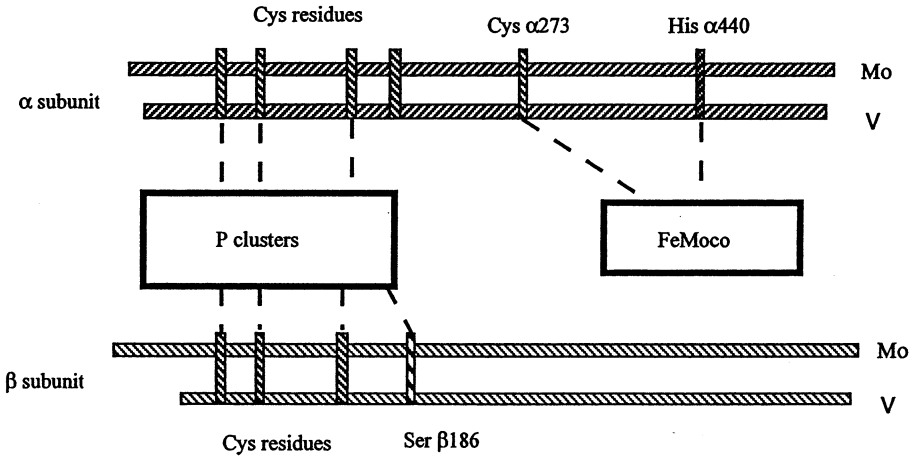

Fig. 3. Amino acids conserved in all MoFe protein sequences and involved in binding P clusters and FeMoco are also conserved in VFe proteins.

A. vinelandii ($Av1^V$) [2] and *A. chroococcum* ($Ac1^V$) [16]. Following their isolation in 1985, rapid progress was made in defining the biochemical properties, building on the extensive body of experience and structural spectroscopic and genetic data available for Mo-nitrogenase (see Ref. [17]). Much of the work focused on the types of redox centres that the VFe proteins contain, and to investigate how the presence of V changes the catalytic properties compared with the more extensively studied Mo-nitrogenase. The types of redox centres present in VFe proteins have been investigated by E.P.R., [16,18,19] M.C.D., [20] Mössbauer [21] and X-ray absorption spectroscopies [22,23]. As summarized below, the spectroscopic data for VFe proteins are fully consistent with the presence of redox centres very similar to those of MoFe proteins. An indication of the similarity of these systems is the ability of the Fe and VFe proteins to form fully functional hybrid nitrogenases with components of the Mo-nitrogenase [16]. V-nitrogenase of *A. chroococcum* catalyses the reaction:

$$N_2 + 12e^- + 12H^+ + 40MgATP \rightarrow 2NH_3 + 3H_2 + 40MgADP + 40P_I$$

By comparison with Mo-nitrogenase the enzyme evolves more H_2 and consumes more ATP during the reduction of N_2, the reasons and possible significance of these differences are currently unclear.

4. Molecular genetics of V-nitrogenase

The structural genes have been cloned and sequenced from *A. vinelandii*, [15] *A. chroococcum* [14] and the Cyanobacterium *Anabaena variabilis* [24]. The genes of the α and β subunits of VFe protein (*vnf DK*) are separated by a gene (*vnf G*) in *A. vinelandii* and *A. chroococcum* that encodes a third small (δ) subunit of the VFe protein in these organisms. The δ subunit has no counterpart in the MoFe proteins, but is essential for V-nitrogenase function [25], and has a suggested role in the incorporation of the cofactor centre into VFe proteins (see Ref. [11]). Comparison of the derived amino acid sequences of *vnf DK* genes with *nif DK* of Azotobacter shows the α subunits to be 32% and the β subunits 35% identical in sequence. Of particular significance is the presence of residues that the X-ray structure of MoFe proteins show to ligate P clusters and FeMoco ([5,7,10], see Fig. 3) leading to the expectation that similar redox centres would be present in VFe proteins.

5. Enzymology of V-nitrogenase

The VFe proteins $Av1^V$ and $Ac1^V$ generally have very similar properties [3,16]. They differ from the MoFe proteins in their subunit structure, since an additional δ subunit is present, resulting optimally in a $\alpha_2\beta_2\delta_2$ hexamereric structure of $M_r \sim 250\,000$. As discussed below, the activities, metal contents and subunit stoichiometry of current preparations of VFe proteins are variable. They contain in preparations with highest activity, 0.7–2 V atoms, and 9–19 Fe atoms and 20 acid-labile sulfur atoms. Although these metal contents are lower than are currently found in a typical preparation of MoFe protein, this difference is probably not significant, since in the case of MoFe proteins comparable values were common, but have increased with time.

5.1. Evidence for P clusters in VFe proteins

Low temperature (4.2 K) Mössbauer spectra of reduced ^{57}Fe-substituted $Av1^V$ are complex (as expected for a system with a large number of Fe atoms) [21]. The spectra show components with relative intensities, isomer shifts and quadrupole coupling constants very

similar to those of MoFe proteins. One spectral component had magnetic hyperfine structure attributed to a paramagnetic species assigned to the $S = 3/2$ spin species of a presumptive FeVco, discussed below. A second feature was comprised of three quadrupole doublets and assigned to P clusters in a diamagnetic state.

Additional evidence is provided from low-temperature M.C.D. spectroscopy [20] that monitors magnetically induced dichroism of the Fe–S charge-transfer band of paramagnetic Fe/S centres. A unique feature of the M.C.D. characteristics of oxidized P clusters is their unusually steep magnetization curves when compared with data for other FeS containing proteins. Oxidation of $Av1^{v}$ resulted in very steep magnetization curves being obtained, very similar to those of oxidized MoFe protein, indicating that the paramagnetic chromophores present in these oxidized proteins have very similar magnetic properties.

The E.P.R. spectra of the VFe proteins as isolated are more complex than spectra of MoFe proteins, since several paramagnetic species are present. Some of these features have been assigned to partially damaged centres [26]. $Av1^{v}$ shows a broad, poorly resolved E.P.R. signal with g values at 5.8 and 5.4 which has been assigned to transitions from the ground state of a spin $S = 3/2$ Kramers doublet which integrate to 0.89 spins per V atom, assignable to a putative cofactor centre [3]. The spectrum of $Ac1^{v}$ is more complex and is difficult to analyse, since the g values at 5.6, 4.3 and 3.77 appear to arise from a mixture of $S = 3/2$ species, presumably associated with different environments of the cofactor centre [13]. In both cases this signal is some 10-fold less intense that that of the MoFe proteins. Both proteins also exhibit an axial E.P.R. signal with g values of 2.04 and 1.93 assigned to an $S = 1/2$ ground state spin system, which integrates to ca. 0.2 spin mol^{-1}. In the case of $Ac1^{v}$ the intensity of this signal is low in samples of high activity and has been assigned to a FeS centre present in an inactive de-vanado species [27]. However, this signal in $Av1^{v}$ remains at a constant ratio to the $S = 3/2$ signal on further purification, and it is thought to be associated with active protein [28].

In oxidative redox titrations VFe proteins exhibit E.P.R. signals characteristic of one and two electron-oxidized P clusters. The detection of a E.P.R. signal with $g = 12$ arising from the two-electron oxidized state, most easily detected in the parallel mode, is a unique and characteristic spectroscopic fingerprint for P clusters [28,29].

In summary, E.P.R. studies of the VFe proteins in the dithionite-reduced state provide additional evidence for the presence of a FeVcofactor centre and in the oxidized proteins, for their containing P clusters.

5.2. Evidence for FeVco centres in VFe protein

In addition to the sequence and spectroscopic data discussed above there is a body of compelling evidence for V being in a cofactor centre with a chemical environment similar to that of Mo in FeMoco. Firstly, two of the genes involved in FeMoco biosynthesis are also essential for V-nitrogenase activity. The involvement *nifB* and *nifV* in the formation of active V-nitrogenase [30–34] together with the presence of a reiteration of *nifEN*-like genes in *A. vinelandii* [35] and *A. chroococcum* [36], provides very strong genetic evidence for the presence a V cofactor analogous to FeMoco, and one that has homocitrate as a component. The available data suggest that the branch-point in the biosynthesis of both cofactors occurs after the formation of a NifBco–*vnf*EN complex (see Fig. 2).

Secondly, the cofactor centre can be extracted from VFe protein using methods developed for the extraction of FeMoco from MoFe proteins [35]. Extracted cofactor contained V, Fe and acid-labile sulfide in the ratio ca. 1:6:5, comparable to those of FeMoco with V replacing Mo. The E.P.R. spectra of extracted FeVco exhibited weak signals characteristic of a spin $S = 3$/system. FeVco can bind FeMoco binding-site in MoFe protein, since it reactivates apo-MoFe protein for H_2 evolution, C_2H_2 reduction but not N_2 reduction, indicating that specific interactions of the cofactor centre with the polypeptide, required for the reduction of N_2 were missing [35]. Additional evidence for the presence of cofactor binding sites on VFe proteins is provided by the ability of isolated FeMoco to bind and activate apo-$Av1^{v}$ [36].

In the absence of a crystal structure of a VFe protein, the detail of the environment of V has been provided from EXAFS studies of $Ac1^{v}$ [23] and $Av1^{v}$ [22]. These studies were the first biological application of V K-edge X-ray absorption spectroscopy, and indicated the V atom was in a similar environment to V in the model compound $[Me_4N][VFe_3S_4Cl_3(DMF)_3]$. The data for $Av1^{v}$ and $Ac1^{v}$ are very similar, and the EXAFS region for both proteins can be simulated by a three component fit with Fe, S, and O as nearest neighbours to the V atom. These assignments and the distances of the V atom from the scattering atoms are very similar to those for Mo in the FeMoco centre of MoFe proteins, (Table 1). As discussed above, both homocitrate and the corresponding His421 residue are implicated in V-nitrogenase function, allowing assignment of the light atoms in the first co-ordination shell of the V.

Fe K-edge EXAFS spectra of $Av1^{v}$ [37], $Ac1^{v}$ [38]and the extracted FeVco of $Ac1^{v}$ [38] are dominated by Fe–S and Fe–Fe interactions similar to those of FeMoco (Table 1). Based on these data and the chemical structure of FeMoco determined from the crystal structures of MoFe proteins, the proposed structure of

Table 1
Comparison of the environment of Fe and V in the cofactor centre of VFe proteins and Fe and Mo in the cofactor centre of MoFe proteins determined from EXAFS measurements

	Type of interaction	*N*	*R* (Å)
MoFe protein of *A. vinelandii*	Mo···O(N)	2	2.12
	Mo···S	4.5	2.37
	Mo···Fe	3.5	2.67
	Fe···Fe	1.4	3.78
FeMoco	Mo···O(N)	2	2.09
	Mo···S	3	2.36
	Mo···Fe	3.5	2.68
	Fe···Fe	1.3	3.68
$Av1^{v}$	V···O(N)	2–3	2.15
	V···S	3–4	2.33
	V···Fe	3 ± 1	2.76
	Fe···Fe	1	3.76
$Ac1^{v}$	V···O(N)	3 ± 1	2.15
	V···S	3 ± 1	2.31
	V···Fe	3 ± 1	2.75
FeVco	Fe···S	3	2.24
	Fe···V	1	3.69
	Fe···Fe	2	2.65
	Fe···Fe	1	3.69

Data for $Av1^{v}$ [23,37], $Ac1^{v}$ [22], and isolated FeVco [38]. Data for MoFe protein and isolated FeMoco [37].

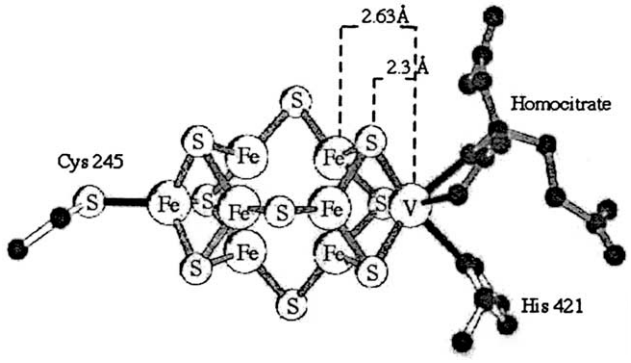

Fig. 4. A proposed structure for the FeVco centre of VFe proteins.

FeVco is shown in Fig. 4. Using the sequence numbering for $Ac1^{V}$ the putative amino acids which link FeVco to the protein are His 241 and Cys 254. As is observed with Mo-nitrogenase little change is seen in V EXAFS data on oxidation or reduction of the cofactor centre [39].

6. Problems associated with current preparations of V-nitrogenase

The initial rapid progress of the 1980s in this area has not been maintained, and it has become clear that apparently homogenous preparations of VFe proteins are in fact mixtures. In the case of $Av1^{v}$ the stoicheometry of the α and β subunits has been shown to be variable, and species corresponding to subunit composition $\alpha\beta_2$ and $\alpha_2\beta_2$ have been separated by chromatography on Q sepharose and characterised [40]. The variability in subunit ratio suggests that subunit interactions are weaker than those of MoFe proteins which invariably purify as a $\alpha_2\beta_2$ species. Such variation in subunit composition has not been detected with $Ac1^{v}$ even in preparations ranging in specific activity from 200 to 1800 [16]. The metal and sulfide contents of the $\alpha\beta_2$ species of $Av1^{v}$ are consistent with it containing one FeVco centre, one P cluster and a [4Fe4S] centre. The $S = 1/2$ EPR signal in the protein as isolated has been assigned to the [4Fe4S] cluster. Based on a vanadium content of 2 V per tetramer, the $\alpha_2\beta_2$ species of $Av1^{v}$ with a V:Fe:S ratio of 2:30:34 has a full complement of redox centres. On oxidation a complex pattern of EPR signals is seen, that in the case of the $\alpha\beta_2$ species has provided a model for redox-mediated conformational changes triggering electron transfer from the P centres to the FeVco centre [28].

This variability in the composition preparations of VFe proteins currently available has hindered mechanistic studies of V-nitrogenase and undoubtedly contributed to the difficulty in obtaining crystals for structural studies.

7. Transition state complexes as a tool for selective separation of species of VFe proteins

During the past few years stable but inactive complexes of nitrogenase components have been isolated. These have provided structural information about the interaction of the Fe and MoFe proteins and also provided the first evidence for long-range conformational changes occurring in the MoFe protein. Both 2:1 and 1:1 complexes of Fe:MoFe protein have been isolated. Of particular relevance in the present context, was the finding that MoFe protein lacking a full occupancy of P clusters and FeMoco centre reacted at a faster rate to form complexes [41]. V-nitrogenase components form similar complexes (Eady unpublished work), thus providing a potential approach to the specific separation of the mixed forms of VFe protein described above.

Work on these complexes [42,43] stemmed from the realisation that the Fe proteins show a peptide folding pattern similar to other nucleotide-binding proteins such as the G family of proteins and myosin, where transient protein complexes couple nucleotide hydrolysis to signal and energy transduction processes. Aluminium fluoride has been extensively used to examine MgATP binding by gated proteins, its primary role is that of a phosphate

analogue. In the case of nitrogenase, MgATP hydrolysis requires the presence of both the Fe protein and the MoFe protein and several groups have exploited these similarities to form stable but inactive nitrogenase complexes. The parallels with myosin and G proteins, have led to the proposal that the ADP–AlF complex can be considered to be an analogue of the E-ADP·Pi species in which the AlF_4 mimics the trigonal bipyrimidal geometry of the terminal phosphate undergoing nucleophilic attack by a water molecule. Similarly the formation of a ADP–BeF_x complex of Kp-nitrogenase [44,45] is considered as a ATP-bound transition state analogue.

The crystal structure of the ADP–AlF-transition state complex of Av-nitrogenase shows two Fe proteins docked onto the pseudo-symmetric interfaces of the αβ subunit interfaces of the MoFe protein [6]. In the absence of excess AlF, complexes of this type slowly dissociate through an intermediate 1:1 complex to yield free active MoFe and Fe proteins that can be separated from any residual complex and isolated by gel permeation or ion exchange chromatography [46].

Small angle X-ray scattering experiments have shown that half-active MoFe protein containing 1 Mo per mol has an asymmetric shape with one half of the $\alpha_2\beta_2$ structure being more open than the other [42]. This species has been shown to react some 20-fold faster to form the inactive complex compared with MoFe protein with a full complement of metal centres [38]. This approach is being investigated as a potential method for the isolation of fully competent VFe protein.

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References

[1] V.K. Shah, R.A. Ugalde, J. Imperial, W.J. Brill, Ann. Rev. Biochem. 53 (1984) 231.
[2] R.L. Robson, R.R. Eady, T.H. Richardson, R.W. Miller, M. Hawkins, J.R. Postgate, Nature (London) 322 (1986) 388.
[3] B.J. Hales, E.E. Case, J.E. Morningstar, M.F. Dzeda, L.A. Mauterer, Biochemistry 25 (1986) 7251.
[4] B.K. Burgess, D.J. Lowe, Chem. Rev. 96 (1996) 2983.
[5] J.B. Howard, D.C. Rees, Chem. Rev. 96 (1996) 2965.
[6] H. Schindelin, C. Kisker, J.L. Schlessman, J.B. Howard, D.C. Rees, Nature (London) 387 (1997) 370.
[7] J.B. Howard, D.C. Rees, in: E.W. Triplett (Ed.), Prokaryotic Nitrogen Fixation: A Model System for Analysis of a Biological Process (Chapter 4), Horizon Scientific Press, Wymondham, UL, 2000, pp. 43–53.
[8] J. Christiansen, D.R. Dean, L.C. Seefeldt, Annu. Rev. Plant Mol. Biol. 52 (2001) 269.
[9] T.R. Hawkes, B.E. Smith, Biochem. J. 209 (1984) 43.
[10] S.M. Mayer, D.M. Lawson, C.A. Gormal, S.M. Roe, B.E. Smith, J. Mol. Biol. 292 (1999) 871.
[11] P. Rangaraj, C. Rüttimann-Johnson, V.K. Shah, P.W. Ludden, in: E.W. Triplett (Ed.), Prokaryotic Nitrogen Fixation: A Model System for Analysis of a Biological Process (Chapter 5), Horizon Scientific Press, Wymondham, UL, 2000, pp. 55–79.
[12] J.W. Peters, M.H.B. Stowell, S.M. Soltis, M.G. Finnegan, M.K. Johnson, D.C. Rees, Biochemistry 6 (1997) 1181.
[13] D.J. Lowe, K. Fisher, R.N.F. Thorneley, Biochem. J. 292 (1993) 93.
[14] R.L. Robson, P.R. Woodley, R.N. Pau, R.R. Eady, EMBO J. 8 (1989) 1217.
[15] R.D. Joerger, T.M. Loveless, L.A. Mitchenal, B.H. Simon, P.E. Bishop, J. Bacteriol. 172 (1990) 3400.
[16] R.R. Eady, R.L. Robson, T.H. Richardson, R.W. Miller, M. Hawkins, Biochem. J. 244 (1987) 197.
[17] R.R. Eady, Chem. Rev. 96 (1996) 3013.
[18] B.J. Hales, A.E. True, B.M. Hoffman, J. Am. Chem. Soc. 111 (1989) 8519.
[19] J.E. Morningstar, B.J. Hales, J. Am. Chem. Soc. 109 (1987) 6854.
[20] J.E. Morningstar, M.K. Johnson, E.E. Case, B.J. Hales, Biochemistry 26 (1987) 1795.
[21] N. Ravi, V. Moore, S.G. Lloyd, B.J. Hales, B.H. Huynh, J. Biol. Chem. 269 (1994) 20902.
[22] J.M. Arber, B.E. Dobson, R.R. Eady, P. Stevens, S.S. Hasnain, C.D. Garner, B.E. Smith, Nature (London) 325 (1987) 372.
[23] G.N. George, C.L. Coyle, B.J. Hales, S.P. Cramer, J. Am. Chem. Soc. 110 (1988) 4057.
[24] T. Thiel, J. Bacteriol. 175 (1993) 6276.
[25] S.I. Waugh, D.M. Paulsen, P.V. Mylona, R.H. Maynard, P. Premakumar, P.E. Bishop, J. Bacteriol. 177 (1985) 1505.
[26] C.Z. Blanchard, R.C. Tittsworth, B.J. Hales, Biochemistry 35 (1996) 472.
[27] R.R. Eady, R.N. Pau, D.J. Lowe, F.J. Luque, in: P.M. Gresshoff, L.E. Roth, G. Stacey, W.E. Newton (Eds.), Nitrogen Fixation: Objectives and Achievements, Chapman and Hall, London, 1990, p. 125.
[28] R.C. Tittsworth, B.J. Hales, Biochemistry 35 (1995) 479.
[29] A.J. Perik, D.J. Lowe, M.E. Eldrige, S. Marritt, J. Farrah, A.J. Thompson, R.R. Eady, in: R. Palacios, W.E. Newton (Eds.), New Horizons in Nitrogen Fixation, Kluwer Academic, Dordrecht, 1993, p. 149.
[30] R.D. Joerger, R. Premakumar, P.E. Bishop, J. Bacteriol. 168 (1986) 673.
[31] C. Kennedy, D.R. Dean, Mol. Gen. Genet. 231 (1992) 494.
[32] K. Schuderkopf, S. Hennecke, U. Liese, M. Kutsch, W. Klipp, Mol. Microbiol. 8 (1993) 637.
[33] E.M. Lyons, T. Thiel, J. Bacteriol. 177 (1995) 1570.
[34] R.D. Joerger, M.R. Jacobson, R. Premakumar, E.D. Wolfinger, P.E. Bishop, J. Bacteriol. 171 (1989) 1075.
[35] (a) D.J. Evans, R. Jones, P.R. Woodley, R.L. Robson, J. Gen. Microbiol. 134 (1998) 931;
(b) B.E. Smith, R.R. Eady, D.J. Lowe, C. Gormal, Biochem. J. 250 (1988) 299.
[36] V.G. Moore, R.C. Tittsworth, B.J. Hales, J. Am. Chem. Soc. 116 (1994) 12101.
[37] J. Chen, J. Christiansen, R.C. Tittsworth, B.J. Hales, G.N. George, D. Coucouvanis, S.P. Cramer, J. Am. Chem. Soc. 258 (1993) 299.
[38] I. Harvey, J.M. Arber, R.R. Eady, B.E. Smith, C.D. Garner, S.S. Hasnain, Biochem. J. 266 (1990) 929.
[39] M. Arber, B.R. Dobson, R.R. Eady, S.S. Hasnain, C.D. Garner, T. Matsushita, M. Nomura, B.E. Smith, Biochem. J. 258 (1988) 733.

[40] C.Z. Blanchard, B.J. Hales, Biochemistry 36 (1996) 472.
[41] F.K. Yousafzai, R.R. Eady, Biochem. J. 339 (1999) 511.
[42] M.G. Duyvis, H. Wassink, H. Haaker, FEBS Lett. 380 (1996) 233.
[43] K.A. Renner, J.B. Howard, Biochemistry 35 (1996) 5353.
[44] T.A. Clarke, F.K. Yousafzai, R.R. Eady, Biochemistry 31 (1999) 9906.
[45] R.W. Miller, R.R. Eady, S.A. Fairhurst, C.A. Gormal, B.E. Smith, Eur. J. Biochem. 268 (2001) 809.
[46] J.G. Grossmann, S.S. Hasnain, F.K. Faridoon, R.R. Eady, J. Biol. Chem. 276 (2001) 6582.