Isolation and Characterization of an Acetylene-resistant Nitrogenase

(Received for publication, September 18, 1999, and in revised form, December 21, 1999)

Jason Christiansen‡, Valerie L. Cash‡, Lance C. Seefeldt§¶, and Dennis R. Dean§¶

From the ‡Department of Biochemistry, Virginia Tech, Blacksburg, Virginia 24061-0346 and §Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84322

A genetic strategy was developed for the isolation of a mutant strain of Azotobacter vinelandii that exhibits in vivo nitrogenase activity resistant to inhibition by acetylene. Examination of the kinetic features of the altered nitrogenase MoFe protein produced by this strain, which has serine substituted for the α-subunit Gly-Gly residue, is consistent with other studies that indicate the MoFe protein normally contains at least two acetylene-binding/reduction sites. The first of these is a high affinity site and is the one primarily accessed during typical acetylene reduction assays. Results of the present work indicate that this acetylene binding/reduction site is not directly relevant to the mechanism of nitrogen reduction because it can be eliminated or severely altered without significantly affecting nitrogen reduction. Elimination of this site also results in the manifestation of a low affinity acetylene-binding site to which both acetylene and nitrogen are able to bind with approximately the same affinity. In contrast to the normal enzyme, nitrogen and acetylene binding to the altered MoFe protein are mutually competitive. The location of the α-Ser-Gly substitution is interpreted to indicate that the 4Fe-4S face of the FeMo cofactor capped by the α-subunit Val residue is the most likely region within FeMo cofactor to which acetylene binds with high affinity.

Nitrogenase is composed of two component proteins designated the Fe protein and the MoFe protein. It catalyzes the MgATP-dependent reduction of nitrogen (N2), protons, and a variety of triply bonded substrates, including acetylene (1, 2). During catalysis, the Fe protein delivers electrons one at a time to the MoFe protein in a process that requires hydrolysis of two MgATP for each electron transfer event (3). The available evidence indicates that electrons are initially delivered from a [4Fe-4S] cluster located within the Fe protein to an [8Fe-7S] “P cluster” (4) contained within the MoFe protein. Intramolecular electron transfer then occurs from the P cluster to a [7Fe-9S-Mo-homocitrate] cluster called FeMo cofactor (5). Because FeMo cofactor has been identified as providing the substrate reduction site (6), its structure and reactivity has received considerable attention (7–10). Of particular interest to our laboratories is the contribution of the FeMo cofactor polypeptide to the nitrogenase activity resistant to inhibition by acetylene. Examination of the kinetic features of the altered nitrogenase MoFe protein produced by this strain, which has serine substituted for the α-subunit Gly-Gly residue, is consistent with other studies that indicate the MoFe protein normally contains at least two acetylene-binding/reduction sites. The first of these is a high affinity site and is the one primarily accessed during typical acetylene reduction assays. Results of the present work indicate that this acetylene binding/reduction site is not directly relevant to the mechanism of nitrogen reduction because it can be eliminated or severely altered without significantly affecting nitrogen reduction. Elimination of this site also results in the manifestation of a low affinity acetylene-binding site to which both acetylene and nitrogen are able to bind with approximately the same affinity. In contrast to the normal enzyme, nitrogen and acetylene binding to the altered MoFe protein are mutually competitive. The location of the α-Ser-Gly substitution is interpreted to indicate that the 4Fe-4S face of the FeMo cofactor capped by the α-subunit Val residue is the most likely region within FeMo cofactor to which acetylene binds with high affinity.

Nitrogenase is composed of two component proteins designated the Fe protein and the MoFe protein. It catalyzes the MgATP-dependent reduction of nitrogen (N2), protons, and a variety of triply bonded substrates, including acetylene (1, 2). During catalysis, the Fe protein delivers electrons one at a time to the MoFe protein in a process that requires hydrolysis of two MgATP for each electron transfer event (3). The available evidence indicates that electrons are initially delivered from a [4Fe-4S] cluster located within the Fe protein to an [8Fe-7S] “P cluster” (4) contained within the MoFe protein. Intramolecular electron transfer then occurs from the P cluster to a [7Fe-9S-Mo-homocitrate] cluster called FeMo cofactor (5). Because FeMo cofactor has been identified as providing the substrate reduction site (6), its structure and reactivity has received considerable attention (7–10). Of particular interest to our laboratories is the contribution of the FeMo cofactor polypeptide environment toward its ability to bind and effect the reduction of various substrates. In previous work we gained insight about the functioning of nitrogenase by characterizing an altered Azotobacter vinelandii MoFe protein for which the α-subunit His residue was substituted by glutamine (11). This altered MoFe protein does not significantly reduce nitrogen (11) but is still able to reduce acetylene and does so with essentially unaltered kinetic parameters. Even though nitrogen cannot serve as an effective substrate for the α-Glu MoFe protein, it remains a potent inhibitor of both acetylene and proton reduction. The major conclusion from this previous work was that nitrogen and acetylene compete for occupancy of a common or shared site. This interpretation is complicated, however, by other observations. First, in the case of the normal MoFe protein, acetylene is a noncompetitive inhibitor of nitrogen reduction, whereas nitrogen is a competitive inhibitor of acetylene reduction (12–14). Second, kinetic and spectroscopic evidence obtained using the unaltered MoFe protein (15, 16) as well as a MoFe protein altered by amino acid substitution (17) indicates that there are two acetylene-binding sites located within the MoFe protein. In light of these observations we became interested in determining if it is possible to obtain a MoFe protein that is altered in its ability to reduce acetylene but remains capable of normal nitrogen reduction.

EXPERIMENTAL PROCEDURES

Isolation of Acetylene-resistant Strains and DNA Biochemistry—Strains of A. vinelandii used in this study were grown under diazotrophic (nitrogen-fixing) conditions using a modified Burk medium previously described (18). Spontaneous acetylene-resistant mutants were isolated from strain DJ939. This strain has the MoFe protein β-subunit tyrosine residue 98 substituted by histidine. The construction of this strain and the biochemical characterization of the altered MoFe protein it produces were previously described (19). Acetylene-resistant strains were isolated by plating approximately 107 cells of DJ939 on Burk medium Petri plates and then incubating these plates in gas-tight jars that included 0.025 atm of acetylene in air. Acetylene-resistant colonies that appeared after about 10 days were picked and streaked on fresh Burk’s medium Petri plates and again incubated under 0.025 atm of acetylene in air. In this way, five different independently isolated acetylene-resistant strains were obtained. To determine whether or not the acetylene-resistant phenotype for a particular strain was the result of a mutation within the nifD gene (the nifD gene encodes the MoFe protein α-subunit), the genomic segment corresponding to the nifD gene of each strain was inserted into the pUC119 cloning vector. Isolated DNA from each strain was then used in attempts to transform DJ939 to the acetylene-resistant phenotype. Of the five strains, two of them (DJ1250 and DJ1252) were found to carry a mutation within the nifD gene. Sub-segments of the nifD gene from each of these two clones were then used to determine that a specific region within nifD endows the acetylene-resistant phenotype. In both cases this segment was found to include the region encoding residues 37 to 214. DNA sequence analysis revealed that both strains carried a single nucleotide change so that the α-subunit residue-69 codon (GGC) was substituted by a serine codon (AGC).

To determine whether or not the α-Ser substitution can suppress the acetylene-sensitive phenotype in other mutant strains, plasmid pDB1096 was used as the donor DNA for transformation experiments using either strain DJ266 or DJ1036 as the recipient. In these transformation experiments, selection was for the acetylene resistance phe-
The Fe protein Arg 100 residue is substituted by Leu 100, and strain \textit{A. vinelandii} have been previously described (23–25). None of the recombinant plas- 
moids and strategies used for strain constructions in the present work 
were recorded on a Bruker ESP300E spectrometer equipped with a 
equation of the form, 

$$v = -\frac{[S]}{V_{max}/K_{m} + [S]}$$  

(Eq. 1) 

where \(v\) is the specific activity of the substrate being examined, \([S]\) is the concentra-
tion of substrate, and \(V_{max}\) is the theoretical maximum specific activity, which was also treated as a variable parameter during fitting.

Electron Paramagnetic Resonance (EPR) Spectroscopy—EPR spec-
tra were recorded on a Bruker ESP300E spectrometer equipped with a 
dual mode cavity and an Oxford ESR 900 liquid helium cryostat. Per-
pendicular mode spectra were recorded at \(-12\) K with a microwave 
power of 2.01 mW, microwave frequency of 9.65 GHz, modulation am-
plitude of 12.63 G, modulation frequency of 100 kHz, and a time con-
stant and conversion time of 20.48 ms.

### RESULTS

Rationale and Design of Experiments for Isolation of Acet-
ylene-resistant Strains—Our initial goal was to determine the 
possibility of isolating a mutant strain of \textit{A. vinelandii} that is 
 altered in its ability to reduce acetylene without a concomitant 
impairment in its ability to reduce nitrogen. Because acetylene 
is known to be a specific inhibitor of nitrogen fixation, a 
straightforward genetic approach to address this issue would 
be to isolate mutant strains exhibiting \textit{in vivo} acetylene resis-
tance when cultured under diazotrophic growth conditions. Al-
though acetylene has been known for many years to specifically 
inhibit \textit{A. vinelandii} diazotrophic growth (1), the explosive 
levels of acetylene required for a clean genetic selection has 
denied the use of this direct approach. We therefore developed 
an indirect approach that relied on the flux-dependence of 
nitrogenase substrate discrimination as described below.

The rate at which electrons are delivered to the nitrogenase 
substrate reduction site is usually referred to as flux and can be 
controlled \textit{in vitro} by adjusting the relative ratio of Fe protein 
to the MoFe protein in the assay mixture. High flux conditions 
are obtained by using a high Fe protein/MoFe protein ratio. In 
their analysis of the \textit{in vitro} catalytic properties of nitrogenase, 
Davis et al. (31) show that under low flux conditions, acetylene 
is a more effective inhibitor of nitrogen reduction than under 
high flux conditions. The explanation offered for this phenom-
enon is that a more reduced state of the enzyme might be 
required for nitrogen binding than for acetylene binding.

In the case of acetylene reduction measured in typical assays, this 
suggestion has been strongly supported by a series of detailed 
kinetic analyses described by Burgess and Lowe (9) and Thor-
neley and Lowe (32). We therefore reasoned that strains that 
exist low flux under \textit{in vivo} conditions as a result of an amino 
acid substitution in one of the nitrogenase component proteins 
might exhibit diazotrophic growth that is hypersensitive to 
acetylene. This prediction proved to be correct because many of 
the mutant strains from our collection having amino acid sub-
stitutions within either the Fe protein or the MoFe protein and 
that otherwise exhibit nearly normal diazotrophic growth were 
found to be unable to grow in the presence of 0.025 atm of 
acetylene. For example, certain mutants that produce nitrogen-
ases that are only modestly impaired in their capacity for 
intercomponent electron transfer (20) or intramolecular electron 
transfer between the P cluster and FeMo cofactor (19) or 
are slightly altered in their FeMo cofactor environments (21, 
33) all exhibit diazotrophic growth that is highly sensitive to 
acetylene (Fig. 1 and Table I). In contrast, there is little or no 
effect on diazotrophic growth when the wild type strain is 
cultured in the presence of 0.025 atm of acetylene (Fig. 1 and 
Table I).

A mutant strain that produces a MoFe protein having histi-
dine substituted for the MoFe protein \(\beta\)-subunit Tyr 288 residue 
(19) and that exhibits diazotrophic growth that is hypersensit-
tive to acetylene was used as the parental strain for the selec-
tion of a resistant mutant strain resistant to acetylene (Fig. 1 and 
Table II). Two of five independently isolated acetylene-resis-
tant strains retain the original \(\beta\)-H288 substitution and also 
carry a second substitution within the MoFe protein \(\alpha\)-subunit 
as determined by genetic mapping experiments. DNA sequence 
analysis of the \(nifD\) gene isolated from these two mutants 
revealed that both of them encode a MoFe protein \(\alpha\)-subunit 
having the Gly 307 residue substituted by serine. The other three 
acetylene-resistant mutants were true revertants.

\footnote{The abbreviation used is: EPR, electron paramagnetic resonance.}
Acetylene-resistant Nitrogenase

FIG. 1. Acetylene-resistant and acetylene-sensitive phenotype of A. vinelandii strains. The figure shows the diazotrophic growth properties of various strains when cultured under air (left panel) or under air plus 0.025 atm of acetylene (right panel). The strains streaked on the plates are wild type (A), DJ1250 (α-Ser^69^-β-His^46^) (B), DJ939 (β-His^46^) (C), and DJ1252 (α-Ser^69^-β-His^46^) (D). Strains DJ1250 and DJ1252 are independently isolated versions of the same strain.

TABLE I
Acetylene-resistant phenotypes of A. vinelandii strains

| Strain   | Substitution(s) | 0.025 atm C_2H_2 |
|----------|-----------------|------------------|
| DJ       | None            | Sensitive        |
| DJ939    | β-His^46^-γ-His | Sensitive        |
| DJ867    | α-190Ser^-γ-Gly | Sensitive        |
| DJ776    | α-191Glu^-γ-Pro | Sensitive        |
| DJ841    | α-192Ser^-γ-Aux | Sensitive        |
| DJ987    | α-359Arg^-γ-Lys | Sensitive        |
| DJ1036   | α-381Pro^-γ-Leu | Sensitive        |
| DJ266^a  | γ-100Arg^-γ-Leu | Resistant        |
| DJ1270   | β-His^46^-γ-His | Resistant        |
| DJ1289   | α-381Pro^-γ-Leu | Resistant        |
| DJ1257^a | γ-100Arg^-γ-Leu | Resistant        |

^a γ refers to the subunits of the Fe protein homodimer.

Nature of the Acetylene Resistance Phenotype Elicited by the α-Ser^69^ Substitution—Acetylene resistance resulting from the α-Ser^69^ substitution could be explained by either a compensation in the electron-flux defect caused by the original β-His^46^ substitution or by alteration in the accessibility of an acetylene-binding site such that acetylene is no longer an effective physiologic inhibitor of nitrogen reduction. These possibilities were distinguished by showing that the α-Ser^69^ substitution can effectively suppress the acetylene-sensitive phenotype when placed in combination with a variety of other individual substitutions, each of which lowers flux in a different way (Table I). The most striking example of this phenomenon is illustrated by the observation that the α-Ser^69^ substitution within the MoFe protein effectively suppresses the acetylene-sensitive phenotype of a mutant strain that has leucine substituted for the Fe protein Arg^100^ residue. The Fe protein Arg^100^ residue is located at the component protein-docking interface (34, 35), and substitutions at this position have been shown to affect intercomponent electron transfer (20, 36). The general ability of the α-Ser^69^ substitution to suppress the acetylene-sensitive phenotype is best explained by a structural barrier that prevents acetylene from effectively binding to the active site of the altered protein.

Characterization of the α-Ser^69^ MoFe Protein—To assess the catalytic consequences of the α-Ser^69^ substitution without interference by the effects of any other amino acid substitution, a strain was constructed that produces an altered MoFe protein for which α-Ser^69^ is the only substitution. A comparison of the catalytic properties of the isolated α-Ser^69^ MoFe protein and the wild type MoFe protein are summarized in Fig. 2 and Table II. The acetylene reduction behavior is graphically illustrated in the Lineweaver-Burk plot shown in Fig. 2, where the wild type MoFe protein and the α-Ser^69^ MoFe protein data exhibit very different slopes yet approach the same y intercept, indicative of a large change in K_m accompanied by almost no change in the V_max between the two proteins. The inset for Fig. 2 shows the actual enzyme saturation data with the accompanying fit to the hyperbolic form of the Michaelis-Menten equation (as described under “Experimental Procedures”). The resulting values for K_m and V_max derived from these fits are given in Table II. These results show that the α-Ser^69^ MoFe protein exhibits a K_m of ~0.14 ± 0.01 atm, an approximately 20-fold increase from the apparent K_m observed for acetylene binding to the wild type MoFe protein. Table II also illustrates that, in similar experiments, the α-Ser^69^ MoFe protein reduces nitrogen with kinetic parameters almost identical to what is observed for wild type MoFe protein.

Also shown in Table II are the interactions among some of the substrates and inhibitors of nitrogenase catalytic activity. Acetylene inhibition of nitrogen reduction catalyzed by the α-Ser^69^ MoFe protein is changed from a noncompetitive inhibition pattern to a competitive pattern. The α-Ser^69^ MoFe exhibits proton reduction specificity activity and nitrogen reduction specific activity very similar to that of the unaltered MoFe protein. Also, like the wild type MoFe protein, the nitrogen reduction activity of the α-Ser^69^ MoFe protein is inhibited by hydrogen. Finally, the α-Ser^69^ MoFe protein exhibits an S = 3/2 EPR spectrum that is identical to the wild type in both line-shape and intensity.

DISCUSSION

An explanation of the effect of the α-Ser^69^ substitution on the ability of the altered MoFe protein to reduce acetylene can be considered in the context of previous work. First, Davis et al. (15) report evidence for the presence of high affinity and low affinity acetylene-binding sites on the Clostridium pasteurianum nitrogenase enzyme. The K_m they report for the low affinity acetylene-binding site (0.23 ± 0.01 atm) is similar to the one reported here for the α-Ser^69^ MoFe protein (0.14 ± 0.01 atm). Second, EPR studies by Lowe et al. (16) were interpreted to indicate the presence of two binding sites for acetylene on the MoFe protein from Klebsiella pneumoniae (16). Finally, we previously reported that substitution of the α-Arg^-γ^- residue by histidine elicits CO-induced cooperativity of acetylene binding to the altered MoFe protein (17). Thus, there is clear evidence that at least two acetylene binding sites are located within the MoFe protein. In the case of the α-Ser^69^ MoFe protein, the high affinity acetylene-binding site appears to have been eliminated or severely altered without significantly altering the capacity of the altered enzyme to reduce nitrogen. Also, the remaining low affinity acetylene binding site in the α-Ser^69^ MoFe protein does not appear to be significantly altered, maintaining a K_m value similar to the value previously reported by Davis et al. (15) and a V_max that is only slightly less than the value derived for wild-type MoFe protein (Table II).

Elimination of the high affinity acetylene-binding site by the α-Ser^69^ substitution can be considered in light of the FeMo cofactor structure and its polypeptide environment (Fig. 3a). This structure has a central prismatic waist of six Fe atoms, each of which is ligated to three sulfides in a nearly trigonal planar geometry. These six Fe atoms are arranged such that three pairs of Fe atoms are shared at the intersection of three geometrically identical 4Fe-4S faces within the FeMo cofactor (Fig. 3b). The α-Gly^-γ^- residue is located immediately adjacent to the α-Val^-γ^- residue that caps one of the 4Fe-4S faces of FeMo cofactor. These two residues are located near the end of a short helix that is positioned between the P cluster and FeMo cofactor. We propose that the high affinity acetylene-binding site is provided by the 4Fe-4S face capped by α-Val^-γ^- and that the α-Ser^69^ substitution results in movement of α-Val^-γ^- so that the high affinity acetylene-binding site is no longer accessible. An
paring the kinetic behavior of the site. If the 4Fe-4S face of FeMo cofactor that is capped by this site is hereafter referred to only as the "nitrogen binding" site and the nitrogen-binding site are the same. For simplicity, the 3/2 EPR signal of the 5 $^{39}$S has been noted and because there is no apparent perturbation in the kinetic parameters, nitrogen is bound to the active site. A simple working model that explains this situation is that the nitrogen binding site cannot be bound and reduced at the high affinity site when nitrogen with normal affinity but does not significantly reduce nitrogen. Yet, nitrogen remains an effective competitive inhibitor of acetylene reduction. Thus, acetylene cannot be bound and reduced at the high affinity site when nitrogen is bound to the active site. A simple working model that explains this situation is that the nitrogen binding site overlaps with the high affinity acetylene-binding site, both of which are located within the same 4Fe-4S face of FeMo cofactor. For this reason and because there is no apparent perturbation in the partial pressure of acetylene (in argon) used for the assay. The inset shows the raw saturation curve data for the reduction of acetylene that was fit to a hyperbolic function as described under "Experimental Procedures." The resulting Michaelis constants ($K_m$) are shown in Table II.

Our interpretation of the reciprocity in the competitive inhibition of nitrogen and acetylene reduction exhibited by the $\alpha$-Ser$^{69}$ MoFe protein is that the low affinity acetylene-binding site and the nitrogen-binding site are the same. For simplicity, this site is hereafter referred to only as the "nitrogen binding" site. If the 4Fe-4S face of FeMo cofactor that is capped by $\alpha$-Val$^{70}$ provides the high affinity acetylene-binding site, where is the nitrogen binding site? Our results do not provide a definitive answer to this question. Nevertheless, based on the results described here and results previously obtained with an altered MoFe protein having the $\alpha$-His$^{195}$ MoFe protein substituted by glutamine, we argue that this site is probably provided by the same 4Fe-4S face that is capped by $\alpha$-Val$^{70}$. Inspection of the FeMo cofactor polypeptide environment shows that the $\epsilon$ imidazole nitrogen of the $\alpha$-His$^{195}$ residue is hydrogen-bonded to a central sulfide that is a member of the 4Fe-4S face that is capped by $\alpha$-Val$^{70}$ ( Ref. 37 and Fig. 3). Substitution of $\alpha$-His$^{195}$ by glutamine results in an altered MoFe protein that can bind nitrogen with normal affinity but does not significantly reduce it (11). Nevertheless, the $\alpha$-Gln$^{195}$ MoFe protein is unaltered in its ability to either bind or reduce acetylene, so the high affinity acetylene-binding/reduction site must remain intact. An important observation is that the $\alpha$-Gln$^{195}$ MoFe protein cannot significantly reduce nitrogen, yet nitrogen remains an effective competitive inhibitor of acetylene reduction. Thus, acetylene cannot be bound and reduced at the high affinity site when nitrogen is bound to the active site. A simple working model that explains this situation is that the nitrogen binding site overlaps with the high affinity acetylene-binding site, both of which are located within the same 4Fe-4S face of FeMo cofactor.

---

**TABLE II**

Kinetic parameters for wild type and $\alpha$-69$^{Ser}$ MoFe proteins

| Parameter                  | Wild type MoFe protein | $\alpha$-69$^{Ser}$ MoFe protein |
|----------------------------|------------------------|----------------------------------|
| Acetylene reduction        |                        |                                  |
| $K_m$                      | 0.007 ± 0.001 atm      | 0.14 ± 0.01 atm                  |
| $V_{max}$                  | 2000 ± 100 nmol/min/mg | 1800 ± 50 nmol/min/mg            |
| Inhibition by $N_2$        | Yes (competitive)      | Yes (competitive)                |
| Inhibition by $H_2$        | No                     | No                               |
| Inhibition by CO$^-$       | Yes                    | Yes                              |
| Nitrogen reduction         |                        |                                  |
| $K_m$                      | 0.08 ± 0.02 atm        | 0.09 ± 0.04 atm                  |
| $V_{max}$                  | 1120 ± 50 nmol/min/mg  | 810 ± 150 nmol/min/mg            |
| Inhibition by $C_2H_2$     | Yes (noncompetitive)   | Yes (competitive)                |
| Inhibition by $H_2$        | Yes                    | Yes                              |
| Inhibition by CO$^-$       | Yes                    | Yes                              |
| Proton reduction           |                        |                                  |
| Specific Activity$^d$       | 2100 ± 10 nmol/min/mg  | 2000 ± 50 nmol/min/mg            |
| Inhibition by $N_2$        | Yes                    | Yes                              |
| Inhibition by $C_2H_2$     | Yes                    | Yes                              |
| Inhibition by CO$^-$       | No                     | No                               |

$^a$ Determined using a 36:1 molar ratio of Fe protein to MoFe protein.

$^b$ Determined using a 4:1 molar ratio of Fe protein to MoFe protein.

$^c$ Determined using a 36:1 molar ratio of Fe protein to MoFe protein under 0.10 atm of CO gas.

$^d$ Determined using a 36:1 molar ratio of Fe protein to MoFe protein under a 100% Ar atmosphere.

---

**Fig. 2.** Lineweaver-Burk plot comparing the kinetic behavior of the $\alpha$-Ser$^{69}$ MoFe protein (filled circles) and the wild type MoFe protein (filled squares) for the reduction of acetylene. The plot shows the reciprocal of the partial pressure of acetylene (in atm) used for the assay. The inset shows the raw saturation curve data for the reduction of acetylene that was fit to a hyperbolic function as described under "Experimental Procedures." The resulting Michaelis constants ($K_m$) are shown in Table II.
tor. Second, the P cluster is known to undergo a redox-dependent structural rearrangement that could be coupled to electron or proton transfer to the FeMo cofactor (4, 38). The short helix that spans from the P cluster to the FeMo cofactor is an ideal candidate for providing communication between the two cluster types. For example, slight movement of α-Val70 residue as a consequence of events occurring at the P cluster could play an important role in the correct positioning of substrate for reduction (Fig. 3c). From this perspective it is interesting that the amino acid sequence between and including α-Cys62 to α-Val70 is among the most highly conserved primary sequences among all known MoFe proteins. The conservation in primary sequence of this segment also extends to the corresponding region from the alternative nitrogenases (39). However, in this context it is interesting to note that the residue within the VFe protein corresponding to α-Gly69 from the MoFe protein is substituted by a leucine. The VFe protein also exhibits a Kₘ for acetylene binding that is approximately 10 times higher than for the MoFe protein (40). Arguments made for a possible functional role for the α-Val70 residue in nitrogen catalysis can also be made for the α-Arg66 residue. The α-Arg66 residue is hydrogen-bonded to the same FeMo cofactor 4Fe-4S face capped by the α-Val70 (Fig. 3c). This residue is also connected by a short helix to the α-Cys66 residue, which provides a bridging ligand to the two subcluster fragments of the P cluster. Whether or not either or both of these residues directly participate in catalysis, the 4Fe-4S face they approach is distinguished from the two other 4Fe-4S faces in its potential for communication with the P cluster. Third, of the three 4Fe-4S faces provided by FeMo cofactor, the richest source of potential proton donors necessary for substrate reduction is found in proximity to the 4Fe-4S face capped by α-Val70 (Fig. 3, a and c). Such potential proton donors include α-His135, α-Arg66, and a pool of water molecules organized around the α-carboxylate group of homocitrato.

In summary, substitution of the MoFe protein α-Gly69 residue by serine results in elimination of a high affinity acetylene-binding site located within the MoFe protein. This substitution also eliminates the nonreciprocity in the mutual inhibition patterns of acetylene and nitrogen reduction exhibited by the wild type protein. The location of the α-Ser69 substitution leading to the acetylene-resistant phenotype indicates that the 4Fe-4S face capped by α-Val70 is a likely candidate to provide the high affinity acetylene-binding site. We believe that the genetic approach described here should also prove useful for the evaluation of the binding of other nitrogenase substrates and inhibitors.

REFERENCES

1. Burris, R. H. (1979) in A Treatise on Dinitrogen Fixation, Sections I and II: Inorganic and Physical Chemistry and Biochemistry (Hardy, B. W. F., Bottemelly, F., and Burns, R. C., eds) pp. 569–604, John Wiley & Sons, Inc., New York
2. Yates, M. G. (1991) in Biological Nitrogen Fixation (Stacey, G., Burris, R. H., and Evans, H. J., eds) pp. 665–733, Chapman & Hall, New York
3. Seefeldt, L. C., and Dean, D. R. (1997) Acc. Chem. Res. 30, 260–266
4. Peters, W. P., Stowell, M. H. B., Solis, M. S., Finnegan, M. G., Johnson, M. K., and Rees, D. C. (1997) Biochemistry 36, 1181–1197
5. Burgess, B. K. (1990) Chem. Rev. 90, 1377–1406
6. Dean, D. R., Bolin, J. T., and Zheng, L. (1993) J. Bacteriol. 175, 6737–6744
7. Dance, I. (1996) J. Biol. Inorg. Chem. 1, 581–586
8. Howard, J. B., and Rees, D. C. (1996) Chem. Rev. 96, 2965–2982
9. Burgess, B. K., and Lowe, D. J. (1996) Chem. Rev. 95, 2983–3011
10. Peters, J. W., Fisher, K., and Dean, D. R. (1995) Annu. Rev. Microbiol. 49, 335–386
11. Kim, C.-H., Newton, W. E., and Dean, D. R. (1995) Biochemistry 34, 2798–2808
12. Dilworth, M. J. (1966) Biochim. Biophys. Acta 125, 285–294
13. Rivers-Otn, J. H., and Burris, R. H. (1975) J. Bacteriol. 122, 537–545
14. Hwang, J. C., Chen, C. H., and Burris, R. H. (1979) Biochim. Biophys. Acta 292, 256–270
15. Davis, L. C., Henzi, M. T., Burris, R. H., and Orme-Johnson, W. H. (1979) Biochemistry 18, 4860–4869
16. Lowe, D. J., Eady, R. R., and Thorneley, R. N. F. (1978) Biochem. J. 173, 277–290.
17. Shen, J., Dean, D. R., and Newton, W. E. (1997) Biochemistry 36, 4884–4894.
18. Strandberg, G. W., and Wilson, P. W. (1968) Can. J. Microbiol. 14, 25–31.
19. Peters, J. W., Fisher, K., Newton, W. E., and Dean, D. R. (1995) J. Biol. Chem. 270, 27007–27013.
20. Welle, D., Kim, C., Dean, D., and Howard, J. B. (1992) J. Biol. Chem. 267, 3667–3673.
21. Lee, H. I., Thrasher, K. S., Dean, D. R., Newton, W. E., and Hoffman, B. M. (1998) Biochemistry 37, 13370–13378.
22. Christiansen, J., Goodwin, P. J., Lanzilotta, W. N., Seefeldt, L. C., and Dean, D. R. (1998) Biochemistry 37, 12611–12623.
23. Page, W. J., and von Tigerstrom, M. (1979) J. Bacteriol. 139, 1058–1061.
24. Jacobson, M. R., Cash, V. L., Weiss, M. C., Laird, N. F., Newton, W. E., and Dean, D. R. (1989) Mol. Gen. Genet. 219, 49–57.
25. Robinson, A. C., Burgess, B. K., and Dean, D. R. (1986) J. Bacteriol. 166, 180–186.
26. Chromy, V., Fischer, J., and Kulhanek, V. (1974) Clin. Chem. 20, 1362–1363.
27. Laemmli, U. K. (1970) Nature 227, 680–685.
28. Peters, J. W., Fisher, K., and Dean, D. R. (1994) J. Biol. Chem. 269, 28076–28083.
29. Burgess, B. K., Jacobs, D. B., and Stiefel, E. I. (1980) Biochim. Biophys. Acta 614, 196–209.
30. Dilworth, M. J., Eldridge, M. E., and Eady, R. R. (1992) Anal. Biochem. 207, 6–10.
31. Davis, L. C., Shah, V. K., and Brill, W. J. (1975) Biochim. Biophys. Acta 403, 67–78.
32. Thorneley, R. N. F., and Lowe, D. J. (1985) in Molybdenum Enzymes (Spira, T. G., ed) pp. 221–284, John Wiley & Sons, Inc., New York.
33. Newton, W. E., and Dean, D. R. (1993) in Molybdenum Enzymes, Cofactors, and Model Systems (Stiefel, E. I., Coucouvanis, D., and Newton, W. E., eds) pp. 216–30, American Chemical Society, Washington, D. C.
34. Howard, J. B. (1993) in Molybdenum Enzymes, Cofactors, and Model Systems (Stiefel, E. I., Coucouvanis, D., and Newton, W. E., eds) pp. 271–289, American Chemical Society, Washington, D. C.
35. Schindelin, H., Kisker, C., Schlessman, J. L., Howard, J. B., and Rees, D. C. (1997) Nature 392, 370–376.
36. Lowery, R. G., Chang, C. L., Davis, L. C., McKenna, M. C., Stephens, P. J., and Ludden, P. W. (1989) Biochemistry 28, 1206–1212.
37. Kim, J., and Rees, D. C. (1992) Nature 360, 553–560.
38. Lanzilotta, W. N., Christiansen, J., Dean, D. R., and Seefeldt, L. C. (1998) Biochemistry 37, 11376–11384.
39. Joerger, R. D., Loveless, T. M., Pau, R. N., Mitchenall, L. A., Simon, B. H., and Bishop, P. E. (1996) J. Biol. Chem. 172, 3400–3408.
40. Eady, R. R. (1996) Chem. Rev. 96, 3013–3030.
41. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950.
42. Bacon, D. J., and Anderson, W. F. (1988) J. Mol. Graphics 6, 219–220.
43. Merritt, E. A., and Murphy, M. E. P. (1994) Acta Crystallogr. Sec. D 50, 869–873.
Isolation and Characterization of an Acetylene-resistant Nitrogenase
Jason Christiansen, Valerie L. Cash, Lance C. Seefeldt and Dennis R. Dean

J. Biol. Chem. 2000, 275:11459-11464.
doi: 10.1074/jbc.275.15.11459

Access the most updated version of this article at http://www.jbc.org/content/275/15/11459

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 37 references, 10 of which can be accessed free at http://www.jbc.org/content/275/15/11459.full.html#ref-list-1