Characterization of a Variant Iron Protein of Nitrogenase That Is Impaired in Its Ability to Adopt the MgATP-induced Conformational Change*

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An Azotobacter vinelandii nitrogenase iron protein mutant has been created which contains an alanine to glycine substitution at amino acid 157. The strain expressing this mutant Fe protein is able to grow under nitrogen-fixing conditions. This contrasts with an A. vinelandii strain described previously which is unable to grow under nitrogen-fixing conditions and which expresses an Fe protein variant that has an alanine to serine mutation at position 157. The A157S Fe protein was unable to support substrate reduction by nitrogenase because of an inability to undergo a required MgATP-induced conformational change. Although the A157G strain grows at 55% of the rate of the wild-type strain, purified A157G Fe protein is only able to support substrate reduction in in vitro assays at a rate that is approximately 20% of the rate supported by the wild-type Fe protein. Electron paramagnetic resonance, circular dichroism spectroscopies, and enzymatic activity data indicate that the A157G Fe protein adopts the correct conformation upon the binding of MgATP. However, kinetic studies using chelation show that this protein undergoes the conformational change more slowly than the wild-type protein. Thus, this mutant has lower activity because of an impaired ability to undergo this conformational change. Comparison of two available x-ray crystal structures of the native Fe protein alone and complexed with the MoFe protein has provided us with a model to explain the change in activity in alanine 157 mutants. Steric interactions with the side chain of residue 157 influence the protein's ability to undergo the initial MgATP-induced conformational change. In the case of the A157G mutant, however, once the correct conformation is attained, the protein can participate in all subsequent reactions including complex formation, electron transfer, and MgATP hydrolysis. Thus, the role of alanine 157 is to stabilize the proper initial conformation upon MgATP binding.

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binding of two molecules of MgATP causes a large conformational
change that affects a single [4Fe-4S] cluster. Although the structure of the Fe protein with MgATP bound has not been
reported, there is substantial evidence from x-ray scattering experiments (20), the structure of an Fe protein-ADP-AlF4-
MoFe protein complex (10), and mutagenesis experiments (21–
25) that the conformational change involves interactions be-
tween the two subunits of the Fe protein.

Our earlier mutagenesis experiments have identified a highly conserved region of the Fe protein around Ala-157 which
is located at the subunit interface and appears to be critical for the MgATP-induced conformational change (26). This region
is not present in other nucleotide-binding proteins or in any other protein whose sequence is in the data base. X-ray crystallo-
graphic analysis of the Fe protein alone (9) and in the Fe protein-ADP-AlF4- MoFe protein complex (10) reveals that this
region is part of helix 5 located at the subunit interface which extends from residue 151 to 176. This helix moves substantially
when the free Fe protein structure is compared with the struc-
ture of the Fe protein in the complex. Our previous studies have shown that mutation of Ala-157 to Ser results in a protein
that can still bind MgATP normally but is unable to undergo the MgATP-induced conformational change. To understand
how the mutation of this residue can prevent the MgATP-in-
duced conformational change, we have begun to mutate this and neighboring conserved residues. The mutation of Ala-157
to residues larger than Ala leads to completely inactive protein. Here we report the construction and characterization of an
A157G Fe protein that is active.

**EXPERIMENTAL PROCEDURES**

Unless otherwise noted, all chemicals and reagents were obtained
from Fisher Scientific, Baxter Scientific, or Sigma.

**Construction and Expression of the Variant Azotobacter vinelandii Strain**—A fragment of the *A. vinelandii* chromosome containing the entire *nifH* and *nifD* genes as well as part of the *nifK* gene was cloned into the bacteriophage M13mp18. Site-directed mutagenesis was performed using the Mutagen mutagenesis kit, version 2, from Bio-Rad. The oligonucleotide used for mutagenesis was purchased from Inte-
gen DNA Technologies, Coralville, IA. The oligonucleotide was 29 bases long and was complementary to the region surrounding the Ala-
157 codon. The oligonucleotide was degenerate at the Ala-157 codon,
allowing the production of several Fe protein mutants at this position.

After mutagenesis, bacteriophage containing mutated *nifH* genes were
selected through DNA sequencing using the Sequenase II sequencing kit
with some slight modifications. Between 0.5 and 1.0 kg of cell paste
was thawed in 1.4 volumes of 0.05 M Tris-HCl, pH 8.0, and degassed
thoroughly. Sodium dithionite was added to give a final concentration
of 2 mM, and the cells were then broken by passing them through a Gaulin
cell homogenizer two or three times at 6,000 p.s.i. to make a crude extract. The crude extract was then made 10 μg/ml with bovine
serum albumin (BSA). Purification of the Fe protein was performed by
electrophoresis through a 2.5% acrylamide gel in Tris-glycin buffer
and DNase, degassed for an additional 2 hr, loaded onto a 2.0 ml column of ADP-Sepharose 4B (Calbiochem Schuchardt) and then eluted
with 0.05 M Tris-HCl, pH 8.0, 0.1 M NaCl. The Fe protein-containing fraction was then eluted onto a 1.5 × 10-cm Q-Sepharose (Amersham
Pharmacia Biotech) column and eluted with a linear 0.1–0.5 M NaCl gradient.
The eluate was then loaded onto a 1.5 × 10 cm DEAE-
cellulose column and concentrated by elution in the reverse direction using 0.5 M NaCl. The purified Fe protein was analyzed by polyacyrly-
amide gel electrophoresis, and its concentration was determined using the
Biuret method (29).

**Spectroscopy**—For spectroscopic experiments, all samples were made
in a Vacuum Atmospheres dry box under argon. For EPR, the samples were 2 mM in sodium dithionite. The spectra were recorded on a Bruker ESP 300 EZ spectrophotometer equipped with an Oxford Instruments liquid helium flow cryostat. The samples were degassed by evacuation of the [4Fe-4S] cluster was oxidized by one of two methods. In one case, a degassed solution of 20 mM indigo disulfonate (MCB, Norwood, OH) was added to the protein until a blue color remained. The protein was incubated in the dye longer than 15 min and was then separated from the dye by passage over a 1 × 10-cm Sephadex G-25 (Amersham Pharmacia Biotech) column. The other method consists of oxidizing the protein by passage over a specially prepared column as described pre-
viously (30). The column (1 × 10 cm) consists of, from top to bottom, 5
5 cm of indigo disulfonate dye bound to AG-1-X8 (Bio-Rad) and 5 cm of P6DG (Bio-Rad). The protein was loaded onto the top of the column and was allowed to incubate longer than 15 min. It was then eluted with
0.05 M Tris-HCl, pH 8.0. The CD spectra were obtained using a Jasco
J720 spectropolarimeter. Ultraviolet/visible spectra were recorded on a
Heckard Packard 8452/A diode array spectrophotometer.

**Activity Assays**—Enzyme activity was determined by measuring both
hydrogen evolution under an argon atmosphere and ethylene produc-
tion under an atmosphere of 10% acetone and 90% argon. Assays were performed by incubating the Fe and MoFe proteins together at 30 °C in the presence of a reaction mixture and 20 mM sodium dithionite for 2 hr. Reaction mixtures contained 250 mM MES,1 pH 7.4, 100 mM ATP, 250 mM MgCl2, 300 mM creatine phosphate, and 500 units/ml creatine phosphokinase. 0.6 ml of the
reaction mix and 0.3 ml of 250 mM MES, pH 7.4, were degassed in calibrated vials. 0.1 ml of a 200 mM sodium dithionite solution was then added. The ratio of Fe protein to MoFe protein used was 0.5:1, and the total protein concentration was 0.5 mg. The reaction was initiated by the addition of the Fe protein. After incubation, the reaction was killed by the addition of 100 μl of 30% trichloroacetic acid. The amount of product evolved was determined on a Varian 3700 gas chromograph using either a flame ionization detector (ethylene production) or a thermal conductivity detector (hydrogen evolution). The crude extract ethylene production assay was carried out as above except that the reaction was initiated by the addition of 0.1 ml of either wild-type or A157G crude extract to a calibrated vial containing degassed reaction mixture, water, and 20 mM sodium dithionite. The total protein concentration used in the crude extract activity assay was determined using the Biuret method (29) and was found to be 45.2 and 36.6 mg/ml for the wild-type and A157G crude extracts, respectively. The ATP/2e
ratio was determined by performing a standard hydrogen evolution assay in the absence of creatine phosphate and creatine phosphokinase and then determining the amount of ADP generated over the course of the assay. The assays were incubated for increasing amounts of time. Times were selected such that the rate of hydrogen evolution was linear with respect to time
so as to avoid complications resulting from inhibition of the assay by the
formation of ADP. ADP concentrations were determined using a high
performance liquid chromatography method (24, 31). An aliquot of the
liquid from the killed activity assay was centrifuged to remove precipitated
protein. The nucleotide concentration of a portion of this was measured by loading it onto a Supelco LC-18-T C18 reversed phase

1 The abbreviation used is: TES, N-tris(hydroxymethyl)methyl-2- aminothanesulfonic acid.
column and eluting with 100 mM potassium phosphate buffer, pH 6.0. Nucleotides were detected using a Waters 486 tunable absorbance detector at 259 nm. An extinction coefficient of 15,400 M⁻¹ cm⁻¹ was used to determine the concentration of nucleotide.

Chelation Assay—The chelation assay followed published methods (21, 32). Chelation was performed by degassing a solution of 50 mM Tris-HCl, pH 8.0, and 6.25 mM a,a'-dipyridyl in a stopped quartz cuvette. Fe protein was added to a concentration of 0.8 mg/ml and the spectrophotometer blanked. The chelation reaction was started by the addition of MgATP to a final concentration of 5 mM ATP and 10 mM MgCl₂. The progress of the reaction was followed by monitoring the absorbance of the solution at 520 nm. The ability of the Fe protein to interact with the wild-type MoFe protein was assessed by performing the chelation assay in the presence of MoFe protein (1.29 mg/ml) (25, 32). In this case, a regenerating system was used to prevent the accumulation of ADP. The regenerating system used consisted of 6 mM creatine phosphate and 0.125 mg/ml creatine phosphokinase.

RESULTS AND DISCUSSION

Growth of an A. vinelandii Strain Expressing A157G Fe Protein—When A. vinelandii is grown under nitrogen-fixing conditions its growth rate is limited by the availability of fixed nitrogen and is therefore controlled by the activity of the enzyme nitrogenase. In a previous study we established that the A157S Fe protein variant could bind MgATP but could not undergo the MgATP-induced conformational change (26). Because that Fe protein was inactive, expression of the protein in its native background in A. vinelandii led to a strain that was unable to grow under nitrogen-fixing conditions. This Nif phenotype was also observed when other residues larger than Ala were substituted for Ala-157. However, a strain expressing an A157G variant could grow under nitrogen-fixing conditions at 55% of the wild-type rate. The A157G variant strain has a much longer doubling time, 5.6 h, compared with 3.1 h for the wild-type strain. Based on purification profiles, SDS-polyacrylamide gel electrophoresis of crude extracts, and purification yields, the A157G Fe protein is present in approximately wild-type levels, thus we do not believe that the longer doubling time is caused by lower amounts of A157G Fe protein in vivo.

Purification and Activity Measurements—The Fe protein of nitrogenase has at least three functions: electron transfer to the MoFe protein (11); the initial biosynthesis of FeMo cofactor (33–35); and the insertion of preformed FeMo cofactor into an inactive, MoFe cofactor-deficient MoFe protein (36). Because the MoFe cofactor center of the MoFe protein has a characteristic EPR signal (37), the ability of an Fe protein to function in FeMo cofactor biosynthesis and insertion can be readily tested by EPR. Analysis of extracts from the strains expressing the A157G Fe protein (Fig. 1) clearly show the EPR signal that arises from protein-bound FeMo cofactor, leading to the conclusion that the like the previously characterized A157S Fe protein (26), the A157G Fe protein does function normally in FeMo cofactor biosynthesis and insertion. The slower than wild-type growth rate of the A157G strain therefore results from some defect in the electron transfer function of the protein. To identify the defect, the A157G Fe protein was purified to homogeneity using a modification of the original anaerobic purification procedure (28) described under “Experimental Procedures.” The A157G Fe protein behaved normally throughout the purification process. As shown in Table I, the protein was then assayed for catalytic activity as described under “Experimental Procedures.”

The activity of the purified protein was approximately 20% of the wild-type level for both hydrogen evolution and ethylene production. We note that this is lower than expected based on our observed growth rate.

Nucleotide-induced Conformational Change—A critically important step in the overall nitrogenase mechanism is the change in the conformation of the Fe protein which occurs upon MgATP binding (11). As shown in Fig. 2 one of the most striking effects of MgATP binding by the Fe protein is the change in the reactivity of the [4Fe-4S]-cluster with iron chelators. In the absence of MgATP the [4Fe-4S] cluster is not accessible to attack by a,a'-dipyridyl (32). When MgATP binds, the chelator is able to remove all of the Fe from the Fe protein rapidly (38). Fig. 2 shows that the same chelation reaction occurs for the A157G Fe protein but that the initial rate of Fe chelation is much slower. As expected (32), neither wild-type nor A157G Fe protein was chelated in the presence of MgADP. Although chelation of iron from the Fe protein by a,a'-dipyridyl has been shown to be biphasic (38), a pseudofirst-order rate can be fitted to the initial portion of the data. The wild-type observed rate of Fe chelation was calculated to be 8.5 × 10⁻³ s⁻¹, whereas that of the A157G Fe protein was found to be 1.5 × 10⁻³ s⁻¹. This chelation rate of only 17% the wild-type rate corresponds well with the unexpectedly low activity we see in our in vitro activity assays. Thus, either the mutant undergoes the conformational change more slowly than the wild-type protein, or the final conformation is different. To distinguish these possibilities spectroscopic methods were employed.

As shown in Fig. 3, the EPR spectrum of the reduced [4Fe-4S]⁺ cluster in the Fe protein exhibits an S = ½ signal with a rhombic line shape (37). The addition of MgATP results in a change in the shape of the S = ½ signal from rhombic to axial (39). The S = ½ signal exhibited by the A157G Fe protein is qualitatively and quantitatively indistinguishable from the

![Fig. 1. EPR spectra of reduced (A) wild-type and (B) A157G crude extracts. The spectra were measured at a microwave power and frequency of 50 mW and 9.43 GHz, respectively. The receiver gain was 5 × 10⁵, modulation frequency was 100 kHz, modulation amplitude was 5.131 G, and the temperature was 10 K. The total protein concentration of the wild-type crude extract was 68.7 mg/ml, and that of the A157G crude extract was 57.2 mg/ml. Both extracts contained 2 mM sodium dithionite.](http://www.jbc.org/)

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signal exhibited by the wild-type Fe protein both in the presence and absence of MgATP. Thus, by this criterion, the A157G Fe protein appears to adopt the same final conformation as the wild-type protein.

A second method that has been used to study the MgATP-induced conformational change is visible region CD spectroscopy which, in general, is a useful way to monitor the environment of [Fe-S] clusters in proteins. Studies of Fe proteins from three organisms have shown that the CD is measurable in the oxidized [4Fe-4S] oxidation state and is very sensitive to the addition of nucleotides (11, 40–42). Fig. 4 shows that, as was the case with the EPR, the shapes of the CD spectra for the wild-type and A157G Fe proteins are the same before and after the addition of MgATP. The binding of MgADP also causes a change in the conformation of the Fe protein which is quite distinct from that induced by MgATP. The addition of MgADP does not allow chelation of the Fe atoms of the [4Fe-4S] cluster and does not cause substantial change in the EPR spectra (11). However, the addition of MgADP has been observed to cause a large change in the CD spectra of the oxidized protein (41, 42). As shown in Fig. 4, the changes in shape observed in the spectra of the wild-type and the A157G Fe proteins upon addition of MgADP are indistinguishable from each other.

Binding to the MoFe Protein—Taken together, the above data provide strong evidence that the A157G and wild-type proteins begin in the same conformation, that upon addition of MgATP it takes much longer for the A157G protein to adopt the correct conformation, but that the final conformation obtained for the two proteins in the presence of a large excess of MgATP appears to be the same. The next step in a normal nitrogenase reaction is for the reduced Fe protein with MgATP bound to bind to the MoFe protein. One method that has been used to examine the binding of the Fe protein to the MoFe protein involves the addition of MoFe protein to a chelation experiment of the type shown in Fig. 2. When the MoFe protein is complexed with the Fe protein the [2Fe-2S] cluster because CD spectroscopy of chelated A157G Fe protein does not show the expected signal characteristic of a [2Fe-2S] cluster.
protection it from exogenous chelator (32, 43). Fig. 5 shows that, unlike the situation for the wild-type Fe protein, the MoFe protein is not able to protect the A157G Fe protein [4Fe-4S] cluster from chelation.

In a previous study, we showed that the A157S protein that failed to undergo the MgATP-induced conformational change also could not compete with the wild-type Fe protein in an activity assay. That study (26) combined with other data (21, 25, 41) show that the MgATP-induced conformational change is a prerequisite for productive binding of the Fe protein to the MoFe protein. The A157G protein must be able to bind productively to the MoFe protein at least some of the time because it has catalytic activity (Table I), which again supports the conclusion that the final conformation of the mutant protein is the same as that of the wild-type protein. However, if the A157G protein takes a relatively long time to adopt that conformation, then at any point in time only a relatively small percentage of the molecules might be in the required conformation to bind productively to the MoFe protein, a phenomenon that could explain the lack of protection from chelation (Fig. 5) and the low activity (Table I).

MgATP Hydrolysis Is Coupled to Electron Transfer—In the next step in nitrogenase turnover, the Fe protein transfers one electron to the MoFe protein, and two molecules of MgATP are hydrolyzed to two molecules of MgADP and inorganic phosphate (11). Because the Fe protein does not hydrolyze MgATP by itself, complex formation must cause a second conformational change in the Fe protein which brings the catalytic residues in the Fe protein into the correct position to hydrolyze MgATP (6, 10). The stoichiometry of MgATP hydrolyzed to electrons transferred is only slightly larger for the A157G mutant than it is for the wild-type protein. The values are 5.9 ± 0.2 and 3.8 ± 0.3 MgATP/two electrons for A157G and wild-type protein, respectively. Thus, once the A157G Fe protein has bound to the MoFe protein, it can adopt the conformation required for MgATP hydrolysis.

The rate-limiting step in the Fe protein cycle is dissociation of the Fe protein-MgADP complex from the MoFe protein (17). The chelation protection assay shown in Fig. 5 shows that, unlike an Fe protein variant with a deletion at Leu-127 (25, 44), the A157G protein does not have a problem with dissociation. The CD data shown in Fig. 4 further show that the conformation of the oxidized A157G Fe protein-MgADP complex is likely to be the same as that of the wild-type complex.

A Model for the Role of Residue 157—The A157S mutant could not undergo the initial MgATP-induced conformational change and was also blocked for all subsequent steps in the reaction. Using the active A157G mutant, it is now possible to examine the role of this residue in subsequent reactions. Taken together, the above data indicate that upon MgATP binding, the A157G mutant is slow to adopt the conformation required for productive binding to the MoFe protein, but once that conformation is obtained it can undergo all subsequent reactions. Thus, residue 157 is critical only for the initial conformational change. Using the structure of the Fe protein in the absence of nucleotide and the structure of the Fe protein-MgADP-AlF4 2−-MoFe protein complex we can address two questions: How does mutation of Ala-157 to Ser prevent the initial conformational change? Why does putting a Gly in that position increase the time required to get into the correct conformation?

As stated above, Ala-157 is part of helix α5, which includes residues 151–176. Fig. 6 shows that this helix changes orientation in the complex structure as opposed to the noncomplex...
A comparison of these two structures in this region shows that the formation of the correct orientation is important for two reasons. First, the structure of helix $a_5$ changes near its COOH-terminal end in the complex structure (Fig. 6). A bend occurs in the helix near the complex interface around Gly-167, and a 2.8 Å salt bridge is formed between the backbone oxygen of Asn-173 and the e amino group of MoFe $b$ subunit residue Lys-171 (10). Changes in the protein which alter these observed movements of helix $a_5$ would therefore affect the ability of the Fe protein to bind to the MoFe protein. Because of the lack of symmetry observed in the Fe protein-MoFe protein complex, only Asn-173 from one of the Fe protein subunits (monomer 2) interacts with the MoFe protein. In this discussion, monomer 2 refers to polypeptide chain E, and monomer 1 refers to polypeptide F in the complex crystal structure (10). When necessary, the individual subunits of the noncomplexed form of the protein are referred to as monomer A or B, which correspond, respectively, to polypeptide chains A and B in the noncomplex crystal structure PDB file (9).

Second, the NH2-terminal region of helix $a_5$ is in close proximity to the trigger region composed of residues 9–16 (Fig. 7). Although the two regions do not contact each other directly, examination of both crystal structures indicates that these two regions are very close. In the complex structure, the $\beta$ methyl group of Ala-157 is 4.1 Å away from the $\beta$ methylene of Lys-10 and 3.8 Å away from the $\delta$ methyl group of Ile-13 (Table II). Additionally, Glu-154 comes within 3.4 Å of the Ala-157 side chain, and Ser-152 comes within 3.3 Å. Thus, in the complex crystal structure, the amino acid packing in this region is such that there is a pocket formed in which there is no room for a larger residue at position 157. Another observation from the original crystal structure is that the distances between amino acids vary when comparing this pocket in both subunits. The distances between the Ala-157 side chain and residues 10, 13, 152, and 154 are as much as 1.0 Å longer in monomer B than those in monomer A, making the pocket formed by the residues around Ala-157 slightly larger in monomer B (Table II). This variation between the two subunits is not seen in the complex crystal structure. In the complex structure, this pocket around Ala-157 has almost identical dimensions in both subunits, suggesting that the structure and interactions seen in the complex crystal structure are more critical than those seen in the MgATP-free crystal structure.

It is thought that the binding of MgATP triggers a conformational change by breaking a salt bridge between Lys-15 and Asp-125 (22, 23). Replacement of Ala-157 with a larger residue would introduce the problem of steric clashes between it and residues Ile-13, Lys-10, Ser-152, and Glu-154. The availability of the complex crystal structure has greatly assisted us in our understanding of the importance of specific interactions in this region. Lys-10 is seen to interact across the subunit interface with the $\beta$ phosphate of the ADP molecule and has been proposed to be involved in the stabilization of the leaving group during ATP hydrolysis (10). To avoid steric clashes with these

### Table II

| Surrounding atom | Noncomplex monomer A | Noncomplex monomer B | Complex monomer 1 | Complex monomer 2 |
|------------------|----------------------|----------------------|------------------|------------------|
| Lys-10 $\beta$ CH$_3$ | 4.72 | 5.67 | 4.05 | 4.05 |
| Lys-10 e NH$_3$ | 4.61 | 5.65 | 6.67 | 6.69 |
| Ile-13 $\delta$ CH$_3$ | 4.60 | 4.57 | 3.80 | 3.79 |
| Ser-152 O | 3.55 | 3.74 | 3.72 | 3.68 |
| Ser-152 $\gamma$ O | 3.85 | 4.86 | 3.34 | 3.33 |
| Glu-154 O | 2.98 | 3.13 | 3.40 | 3.39 |
| Glu-154 e O | 3.71 | 4.32 | 4.28 | 4.29 |
important residues, one could envision two possibilities, one
being the movement or rearrangement of the trigger region and
the other being the movement or rearrangement of helix α5.
Interfering with the trigger region would very likely affect the
affinity of the protein for MgATP. However, the A157S Fe
protein was shown to bind MgATP in a manner comparable to
wild-type Fe protein (26). Presumably, the proper binding of
MgATP in the A157S mutant would still break the salt bridge
between Lys-15 and Asp-125. However, in the A157S Fe pro-
tein, the subsequent signal transduction to the [4Fe-4S] cluster
through the switch II sequence, which consists of residues
125–132 (23, 25), is somehow prohibited. Helix α5 does not
interact directly with the switch II sequence. The closest dis-
tance between these two regions in the complex structure is a
4.1 Å separation between the side chain of Ile-164 and the
oxygen of Gly-128. Thus, this region exerts its influence apart
from the signal transduction sequence and highlights the im-
portance of concerted interactions throughout the protein for
the formation of the proper conformation upon MgATP binding.
A second possibility is that of the movement or rearrangement
of helix α5. As stated above, in the complex this helix has
interactions at its COOH-terminal end with the MoFe protein.
At its NH2-terminal end, it contains residues that help to
stabilize the binding of nucleotide through intersubunit inter-
actions between Glu-154 and Arg-213 (10) (Fig. 8). Also, Met-
156 has close interactions both with residue Asp-43 at a dis-
tance of 3.4 Å, a residue on the other subunit which is about 3.5
Å from the AlF group in the complex structure and with the
ADP molecule, at a distance of 300 Å from the α phosphate
group. These interactions further suggest that the proper ori-
entation of this helix, both at its COOH terminus and espe-
cially at its NH2 terminus, is critical to attaining the confor-
mation observed in the complex structure. As indicated above,
in the complex structure, replacement of Ala-157 with Ser
would cause steric interference between this residue and resi-
dues in the trigger region, probably causing a reorganization
of the structure of the NH2-terminal portion of helix α5 which
would result in a failure of the protein to be able to adopt the
proper conformation, hence the inactivity seen in the A157S Fe
protein.

Replacement of Ala-157 with Gly would not cause this steric
interference. The helix would be free to move and would be able
to attain the correct orientation for the MgATP-induced con-
formational change and subsequent complex formation to oc-
cur. This is supported by our observations that A157G Fe
protein does undergo the conformational change. This portion
of the protein sequence is highly conserved across all Fe pro-
teins, suggesting that the structure observed at the NH2-ter-
minal loop region of helix α5 is very important to the proper
function of the protein. This is further supported by the “tight-
ening up" of this pocket when comparing the complex structure with the noncomplex structure as discussed above and illustrated in Table II. The removal of steric constraints imposed by the presence of the alanine side chain would affect this region by failure to force the proper conformation of the residues around Ala-157. Furthermore, the removal of this side chain would allow the formation of alternate conformations in this region. In the latter case, the proper orientation of helix α5 for the conformational change and subsequent MoFe protein-Fe protein complex formation would not be impossible but might be less likely given the increased opportunity for alternate conformations. This would explain the lowered activity observed in the A157G Fe protein. It is important to emphasize the fact that this residue appears to be critical only for the initial MgATP-induced conformational change. Its ability to support substrate reduction indicates that once the initial conformational change has occurred, the protein is able to proceed normally with MoFe protein binding, MgATP hydrolysis, electron transfer, and complex dissociation.

The results presented in this paper suggest that the role of Ala-157 lies in the stabilization of the proper conformation of helix α5 in the protein. The proper movement of helix α5 from its orientation in the noncomplex structure to that seen in the Fe protein-ADP-MoFe protein complex structure is essential for proper Fe protein activity. Specifically, it is important for the initial conformational change. Replacement of Ala-157 with other residues destabilizes the proper conformation of the NH₂-terminal end of helix α5 and has profound effects on the ability of the Fe protein to function normally.

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