Elucidation of a MgATP Signal Transduction Pathway in the Nitrogenase Iron Protein: Formation of a Conformation Resembling the MgATP-Bound State by Protein Engineering†

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ABSTRACT: The present work defines one MgATP signal transduction pathway in the nitrogenase iron (Fe) protein. Deletion of an amino acid (Leu 127) by site-directed mutagenesis in the protein chain between Asp 125, located in the ATP binding site, and Cys 132, a ligand to the [4Fe−4S] cluster, resulted in protein conformational changes resembling the MgATP-bound state in the absence of any bound nucleotides. Specifically, ^{1}H nuclear magnetic resonance, electron paramagnetic resonance, and circular dichroism spectroscopic properties, along with Fe chelation assays, suggested that deletion of Leu 127 in the Fe protein resulted in changes in the electronic properties of the [4Fe−4S] cluster similar to those normally observed upon MgATP binding to the wild-type Fe protein. Deletion of Leu 127 of the Fe protein lowered the redox potential of the [4Fe−4S] cluster by 112 mV compared to the wild-type Fe protein (−412 mV compared to −294 mV). A nearly identical lowering of the redox potential by 120 mV occurs in the wild-type Fe protein upon binding MgATP (−294 mV compared to −420 mV). The L127Δ Fe protein did not contain bound nucleotides which could account for the observed conformational changes. The present results support a model in which the protein chain from Asp 125 to Cys 132 acts as one pathway for MgATP signal transduction and suggests a mechanism for this transduction to the [4Fe−4S] cluster. The L127Δ Fe protein was found to still bind 2 MgATP or 2 MgADP molecules/Fe protein. Unlike the wild-type Fe protein, the L127Δ Fe protein bound 2 ADP molecules/Fe protein in the absence of Mg^{2+}. Finally, the L127Δ Fe protein was found to bind to the MoFe protein, although the complex did not catalyze MgATP hydrolysis or substrate reduction. In concurrence with previous models, homologies between the Asp 125 to Cys 132 transduction pathway in Fe protein and the switch II region of the broad class of GTPase signal transduction proteins (G-proteins) are discussed.

The hydrolysis of MgATP is an absolute requirement for all substrate reduction reactions catalyzed by the enzyme nitrogenase. While a requirement for MgATP in nitrogenase reactions has been known since 1964 (Mortenson, 1964), the exact mechanism of coupling MgATP hydrolysis to electron transfer and substrate reduction remains largely undefined. The binding of MgATP to the iron (Fe) protein component of nitrogenase results in protein conformational changes that can be monitored by changes in the electronic and structural properties of its single [4Fe−4S] cluster (Mortenson et al., 1993). These protein conformational changes are a prerequisite to Fe protein docking to the other nitrogenase component, the molybdenum−iron (MoFe) protein. The MoFe protein contains the site of substrate reduction, a unique molybdenum−iron−sulfur−homocitrate containing cofactor (FeMoco) (Kim & Rees, 1992b). The Fe protein−MoFe protein complex catalyzes the hydrolysis of a minimum of 2 MgATP molecules bound to the Fe protein, which is coupled to the transfer of a single electron from the Fe protein [4Fe−4S] cluster to FeMoco in the MoFe protein (probably through the MoFe protein [8Fe−8S] cluster) (Howard & Rees, 1994; Peters et al., 1995). The oxidized Fe protein, with 2 molecules of MgADP bound, is released from the MoFe protein and another reduced Fe protein, with two bound MgATP molecules, binds to the partially reduced MoFe protein for a second round of MgATP hydrolysis and electron transfer (Hageman & Burris, 1978). This cycle is repeated until sufficient electrons have been transferred to the MoFe protein to reduce the bound substrate.

Thus, MgATP binding to the Fe protein and hydrolysis by the Fe protein−MoFe protein complex is an integral part of the nitrogenase mechanism. A detailed understanding of this mechanism requires an understanding of the mechanism of MgATP- or MgADP-induced protein conformational changes in the nitrogenase Fe protein and the role these conformational changes play in controlling electron transfer. The nitrogenase Fe protein is a homodimeric protein of 64 000 Da with a single [4Fe−4S] cluster bridged between its subunits (Howard et al., 1989; Georgiadis et al., 1992). Analysis of the position of a bound ADP molecule in the X-ray structure of the Fe protein from *Azotobacter vinelandii* (Georgiadis et al., 1992), along with the properties of a series of site-specifically altered Fe proteins (Seefeldt et al., 1992;

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[1] Abbreviations: Fe protein, iron protein of nitrogenase; MoFe protein, molybdenum−iron protein of nitrogenase; L127Δ, iron protein of nitrogenase with Leu 127 deleted; IDS, indigodisulfonate; Tris, tris(hydroxymethyl)aminomethane; MOPS, 3-(*N*-morpholino)propanesulfonic acid; Tricine, *N*-[tris(hydroxymethyl)methyl]glycine); BPS, 4,7-diphenyl-1,10-bathophenanthrolinedisulfonic acid; EPR, electron paramagnetic resonance; ^{1}H NMR, proton nuclear magnetic resonance; CD, circular dichroism.

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Wolle et al., 1992a; Seefeldt & Mortenson, 1993; Ryle et al., 1995; Lanzilotta et al., 1995b), has established the position of the nucleotide binding sites, one on each subunit, some 19 Å away from the [4Fe−4S] cluster. Upon binding either MgATP or MgADP, the Fe protein undergoes protein conformational changes which affect the properties of its [4Fe−4S] cluster (Mortenson et al., 1993). These changes in the [4Fe−4S] cluster must be communicated through protein conformational changes initiated at the nucleotide binding sites. One significant change induced by either MgADP or MgATP binding to the Fe protein is the lowering of the redox potential of the [4Fe−4S] cluster from −290 mV to −420 mV (Zumft et al., 1974; Watt et al., 1986). The lower redox potential of the [4Fe−4S] cluster appears to correlate with a form of the Fe protein capable of electron transfer to the MoFe protein. A variety of spectroscopic techniques including EPR (Zumft et al., 1972), NMR (Meyer et al., 1988; Lanzilotta et al., 1995a), CD (Stephens et al., 1979; Ryle et al., 1996), and Mössbauer (Lindahl et al., 1987b) spectroscopies have also been employed to monitor changes in the properties of the [4Fe−4S] cluster upon Fe protein binding nucleotides. Significantly, both CD (Ryle et al., 1996) and NMR (Lanzilotta et al., 1995a) studies have shown that MgATP or MgADP binding to the Fe protein result in different changes in the cluster, suggesting that these different conformations may be important in the mechanism of electron transfer. Another important observation comes from Fe K-edge X-ray absorption studies which have revealed that nucleotide binding to the Fe protein does not change the Fe−Fe or Fe−S bond distances of the [4Fe−4S] cluster significantly, suggesting that the changes in the properties of the [4Fe−4S] cluster must be the result of changes in the protein environment near the cluster (Lindahl et al., 1987a; Ryle et al., 1996).

A significant question then is, how is MgATP or MgADP binding to the Fe protein transduced a distance of 19 Å to the [4Fe−4S] cluster? One possible pathway within the Fe protein for communication from the nucleotide binding sites to the [4Fe−4S] cluster has been suggested (Howard & Rees, 1994) from analysis of the X-ray structure of the Fe protein (Georgiadis et al., 1992) and from analysis of several site-specifically altered Fe proteins (Wolle et al., 1992a; Lanzilotta et al., 1995b). The protein chain from Asp 125 to Cys 132 offers the shortest pathway from the nucleotide binding site to the cluster. Asp 125 is known to be located in the nucleotide binding site, probably functioning in the interaction with the Mg^{2+} associated with the nucleotide (Wolle et al., 1992a). Cys 132 provides two of the four protein ligands to the [4Fe−4S] cluster (Howard et al., 1989). Recent studies of Fe proteins in which Asp 125 (Wolle et al., 1992a) and Asp 129 (Lanzilotta et al., 1995b) were changed to the amino acid Glu suggested a possible function of this protein domain in the nucleotide-induced conformational changes communicated to the [4Fe−4S] cluster.

Thus, it seemed possible that movement in the protein domain from Asp 125 to Cys 132 could be one pathway for nucleotide-induced changes in the environment of the [4Fe−4S] cluster (Howard & Rees, 1994). In order to test this model, two changes in this domain of the Fe protein were engineered by means of site-directed mutagenesis, namely, the deletion of a Leu at position 127 (L127Δ) and the insertion of a Leu between Val 126 and Leu 127. The Leu insertion-modified Fe protein proved unstable and could not be isolated. In contrast, the L127Δ Fe protein could be purified to homogeneity. Analysis of several properties of the L127Δ Fe protein revealed that shortening the amino acid chain by one amino acid resulted in protein conformational changes resembling those normally only induced by MgATP binding. Thus, by means of protein engineering, a MgATP-bound conformation has been created in the Fe protein in the absence of MgATP. The results presented in this work are discussed in the context of a possible mechanism for other nucleotide switch proteins.

MATERIALS AND METHODS

Site-Directed Mutagenesis, Expression, and Purification of Fe Proteins. Site-directed mutagenesis of the *A. vinelandii* nitrogenase Fe protein gene, *nifH*, was carried out as previously described (Jacobson et al., 1989; Seefeldt & Mortenson, 1993). Fe proteins were purified in 50 mM Tris buffer, pH 8.0, under anaerobic conditions in the presence of 2 mM dithionite (Seefeldt & Mortenson, 1993). All buffers used in the purification of the L127Δ Fe protein included 20% glycerol as a stabilizing agent. Prior to freezing, glycerol concentrations were reduced to less than 5% by dilution with glycerol-free buffer. Protein concentrations were determined by a modified biuret method using bovine serum albumin as the standard (Chromy et al., 1974). All proteins were purified to homogeneity as determined by analysis on SDS gels stained with Coomassie blue (Hathaway et al., 1979). All protein manipulations were carried out under an argon or nitrogen atmosphere.

Activity Assays and MgATP Hydrolysis. Acetylene and proton reduction rates were determined as previously described (Seefeldt & Ensign, 1994) except that the buffer was 100 mM MOPS, pH 7.0. MgATP hydrolysis rates were determined in assay solution without the MgATP-regenerating system (Seefeldt & Mortenson, 1993). MgADP formed during the assay was quantified by the HPLC method previously described (Seefeldt, 1994).

MgATP-Dependent Chelation of Fe^{2+} from Wild-Type and L127Δ Altered Fe Proteins. The MgATP-dependent chelation of Fe^{2+} from Fe proteins was followed continuously by the formation of the Fe^{2+}−4,7-diphenyl-1,10-bathophenanthrolinedisulfonic acid (BPS) complex, which was monitored spectrophotometrically at 534 nm using an absorption coefficient of 22 140 $M^{-1}\cdot cm^{-1}$ (Ljones & Burris, 1978). To a 1-cm path length, 2-mL cuvette fitted with a butyl rubber stopper was added BPS to a final concentration of 500 μM in 50 mM Tris buffer, pH 8.0. This solution was deoxygenated by bubbling with argon for 6 min, followed by the addition of dithionite to a final concentration of 2 mM from a 100 mM stock solution and nucleotides to a final concentration of 1 mM. In all cases, reactions were initiated by the addition of the Fe protein. All assays which included MgATP contained a MgATP-regenerating system (Seefeldt & Mortenson, 1993).

To determine the potential for Fe protein binding to MoFe protein, the effects of the addition of MoFe protein on the rate of Fe^{2+} chelation from the Fe protein in the BPS chelation assay were determined (Seefeldt, 1994). In these assays, 6 mM creatine phosphate and 0.125 mg/mL creatine phosphokinase were included to prevent any MgADP formation. The indicated quantity of MoFe protein was added to the assay vials prior to the addition of the Fe protein.

Nucleotide Binding to Wild-Type and L127Δ Altered Fe Proteins. The binding of nucleotides to wild-type and L127Δ Fe proteins was determined by the equilibrium column technique previously described (Ryle et al., 1995). A Sephadex G-25 column (0.5 × 15 cm) was equilibrated with 50 mM Tris buffer, pH 8.0, containing 2 mM dithionite and 1 mM MgATP, MgADP, ADP, or ATP. The column was developed following the addition of 4 mg of Fe protein. A single protein-containing fraction was collected and split into two equal aliquots. One aliquot was analyzed for protein concentration using the modified biuret method (Chromy et al., 1974), and the second for nucleotide concentration by the HPLC method previously described (Seefeldt & Mortenson, 1993). The molar ratio of MgATP, MgADP, ADP, and ATP bound to the Fe protein was determined.

^{1}H NMR Spectra of the Isotropically Shifted Protons for Wild-Type and L127Δ Altered Fe Proteins. Fe protein samples for ^{1}H NMR were prepared as previously described (Lanzilotta et al., 1995a). All spectra were recorded at 305 K in anaerobic glass NMR tubes fitted with Teflon stoppers (Wilmad, Buena, NJ). Fe protein samples were exchanged into Chelex-100-treated Tris-buffered D_2O solution (70 mg of Tris-HCl and 20 mg of Tris base in 10 mL of D_2O with 4 mM dithionite) by passage through a Sephadex G-25 column (1.0 × 15 cm). The Fe protein samples were concentrated to between 1 and 2 mM in a Centricon-30 concentrator (Amicon Division, Beverly, MA). Pulse conditions were as described previously (Lanzilotta et al., 1995a).

Circular Dichroism Spectra of Wild-Type and L127Δ Altered Fe Proteins. The circular dichroism spectrum of IDS-oxidized wild-type or L127Δ altered Fe proteins was recorded as previously described (Ryle et al., 1996). A 20-mg sample of Fe protein was desalted by passage down a Sephadex G-25 column (0.5 × 10 cm) equilibrated with 100 mM Tris buffer, pH 8.0. The Fe protein sample was then oxidized by the addition of 20 μL of 20 mM indigodisulfonate (IDS). The oxidized Fe protein was separated from IDS using a Dowex-1 column, equilibrated with anaerobic 100 mM Tris buffer, pH 8.0. The oxidized Fe protein was diluted to 4 mL and split into two equal fractions, and each fraction was transferred into a sealed 1-cm path length quartz cuvette under a nitrogen atmosphere. Nucleotides were added to a final concentration of 1 mM from anaerobic stock solutions. All spectra were recorded on an Aviv 62DS spectropolarimeter and were baseline-subtracted. The concentration of oxidized Fe protein was determined using an absorption coefficient of 13.3 $mM^{-1}\cdot cm^{-1}$ at 400 nm (Lanzilotta et al., 1995b).

EPR. EPR spectra of the dithionite-reduced Fe protein were recorded at 12.7 K, 0.5 mW microwave power, 5 G modulation amplitude, 100 KHz modulation frequency, and a microwave frequency of 9.50 GHz, as previously described (Seefeldt et al., 1992). Where indicated, MgATP was added as a 10-fold molar excess over protein. Spectra were recorded with either a Bruker ER300E spectrometer with an Air Products liquid helium cryostat or a Bruker ESP300E spectrometer with an Oxford liquid helium cryostat.

Electrochemical Redox Titrations. Electrochemical redox titrations were performed essentially as previously described (Dutton, 1978; Morgan et al., 1986). Iron protein (40 mg) was desalted to remove excess dithionite by passage through a Sephadex G-25 column equilibrated with 100 mM Tricine buffer, pH 8.0. The dithionite-free Fe protein was oxidized by the addition of a minimum volume of 20 mM IDS solution. Reduced and oxidized IDS were removed from the Fe protein sample by passage through a 3-mL Dowex-1 column equilibrated with 100 mM Tricine buffer, pH 8.0. The oxidized Fe protein was diluted to a final concentration between 10 and 15 mg/mL in a volume of 2−3 mL with a 100 mM Tricine buffer solution (pH 8.0) containing 500 mM NaCl, 100 μM methyl viologen, 100 μM benzyl viologen, and 100 μM flavin mononucleotide. When nucleotides were present, the solution also contained either 2 mM MgADP or 2 mM MgATP. Tricine buffer was used to minimize pH changes upon freezing of the samples for EPR (Williams-Smith et al., 1977). Redox potentiometry was carried out in a stirred cell in an argon-filled glovebox essentially as previously described (Morgan et al., 1986) except that the working electrode was a gold wire and the reference electrode was Ag/AgCl. The reference electrode was calibrated against a saturated calomel electrode. Sodium dithionite was used as the reductant and IDS as the oxidant. Aliquots (220 μL) were removed at defined potentials and frozen in calibrated EPR tubes immediately in liquid nitrogen. The fraction of reduced Fe protein was determined by measuring the peak to peak height between the $g = 2.05$ and $g = 1.91$ EPR signals for wild-type Fe protein both in the absence of nucleotide and in the presence of MgADP. For both the wild-type Fe protein in the presence of MgATP and the L127Δ Fe protein without added nucleotides, the fraction of reduced Fe protein was determined by measuring the peak to peak height between the $g = 2.03$ and the $g = 1.88$ EPR signals. EPR spectra were recorded at 15 K with 6.36 mW microwave power. All potentials are reported with respect to the normal hydrogen electrode (NHE). Midpoint potentials were determined from least-squares fits to the Nernst equation using the program Igor Pro (Wavemetrics, Lake Oswego, OR).

Binding of the L127Δ Fe Protein to the MoFe Protein. To test the possibility that the L127Δ Fe protein could bind to the MoFe protein, a competitive binding assay was employed (Lanzilotta et al., 1995b). Each assay vial contained 253 μg (1.0 nmol) of wild-type MoFe protein and various amounts of the L127Δ Fe protein (0−3.4 nmol). Each reaction was initiated by the addition of 134 μg (2.1 nmol) of wild-type Fe protein.

RESULTS

Site-Directed Mutagenesis. Figure 1 illustrates a proposed signal transduction pathway in *A. vinelandii* nitrogenase Fe protein located between Asp 125 in the nucleotide phosphate binding site and Cys 132, one ligand to the [4Fe−4S] cluster (Howard & Rees, 1994). The intervening protein chain is composed of the amino acid sequence

$$D^{125}V^{126}L^{127}G^{128}D^{129}V^{130}V^{131}C^{132}$$

In order to test the possibility that this chain might function as a nucleotide signal transduction switch in the Fe protein, two altered Fe proteins were created using site-directed mutagenesis of the Fe protein gene, *nifH*. The first mutation was an in-frame deletion of the Leu at position 127 (L127Δ), which shortened the peptide chain by one amino acid. The second mutation was an in-frame insertion mutation where the amino acid Leu was inserted between the amino acids Val 126 and Leu 127 (Leu126Ins), which lengthened the

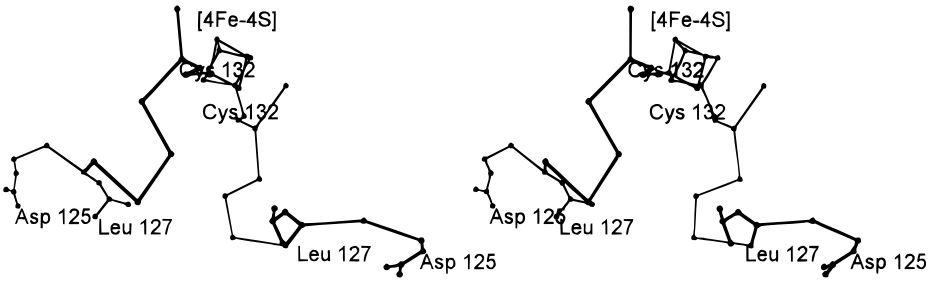

FIGURE 1: Stereoscopic view of a possible signal transduction pathway between the nucleotide binding site and the [4Fe−4S] cluster of *A. vinelandii* nitrogenase Fe protein. Molecular models were generated using the program Molecular Images (U.S. Science, San Diego, CA) from the X-ray coordinates of the *A. vinelandii* Fe protein (Georgiadis et al., 1992). The model shows the α-carbon backbone between Asp 125 and Cys 132 for both subunits. The side chains of Asp125, Leu127, and Cys132, as well as the [4Fe−4S] cluster (cubane structure), are also shown.

peptide chain between Asp 125 and Cys 132 by one amino acid. The site for these alterations was chosen to minimize the effects on Asp 125 and Asp 129, both of which have recently been shown to be essential to nucleotide interactions (Wolle et al., 1992a; Lanzilotta et al., 1995b) and Cys 132, a ligand to the cluster (Howard et al., 1989). Each of the altered proteins was expressed in *A. vinelandii* cells in place of the wild-type Fe protein using a chromosomal replacement strategy previously employed for numerous altered Fe proteins (Seefeldt & Mortenson, 1993). Cells expressing either the L127Δ or Leu126Ins Fe proteins were unable to grow under nitrogen-fixing conditions, suggesting that these amino acid changes had resulted in inactive Fe proteins. The expression of both proteins was confirmed by analysis of whole cell extracts on SDS gels, where the proteins were observed to migrate at their normal molecular mass, suggesting that full-length proteins were produced. Attempts to purify the Leu126Ins Fe protein were unsuccessful, which suggested that the alteration created an unstable Fe protein. In contrast, the L127Δ Fe protein could be purified to homogeneity in quantities comparable to wild-type Fe protein.

Unlike the wild-type Fe protein, however, the L127Δ Fe protein was unable to support acetylene or proton reduction or MgATP hydrolysis when combined with the wild-type MoFe protein. In the sections that follow, data are presented revealing that deletion of Leu127 in the Fe protein results in a protein conformation which mimics that normally found only upon MgATP binding.

Conformational State of the L127Δ Fe Protein. Given the possibility that deletion of Leu 127 in the Fe protein might have an effect on the nucleotide-mediated conformational status of the [4Fe−4S] cluster, it was essential to probe the state of the [4Fe−4S] cluster. One assay that has been used to monitor the conformation of the [4Fe−4S] cluster of the Fe protein is Fe^{2+} chelation from the cluster by iron-specific chelators such as α,α′-dipyridyl (Walker & Mortenson, 1974) and bathophenanthroline (Ljones & Burris, 1978). In the absence of bound MgATP, these chelators are unable to remove any Fe^{2+} from the Fe protein over long incubation times. However, upon the addition of MgATP, a time-dependent chelation of Fe^{2+} from the cluster occurs which can be continuously monitored spectrophotometrically as the formation of the colored chelator-Fe^{2+} complex. Figure 2 (panel A) illustrates the time-dependent chelation of Fe^{2+} from both wild-type and L127Δ Fe proteins by bathophenanthrolinedisulfonate (BPS). In the absence of added MgATP, no Fe^{2+} was chelated from the wild-type Fe protein (trace 7) over the 300-s assay. MgATP addition stimulated the chelation of Fe^{2+} from the wild-type Fe protein (trace 6) with an apparent first-order rate constant of 0.016 s^{-1}. In contrast to the wild-type Fe protein, the L127Δ Fe protein

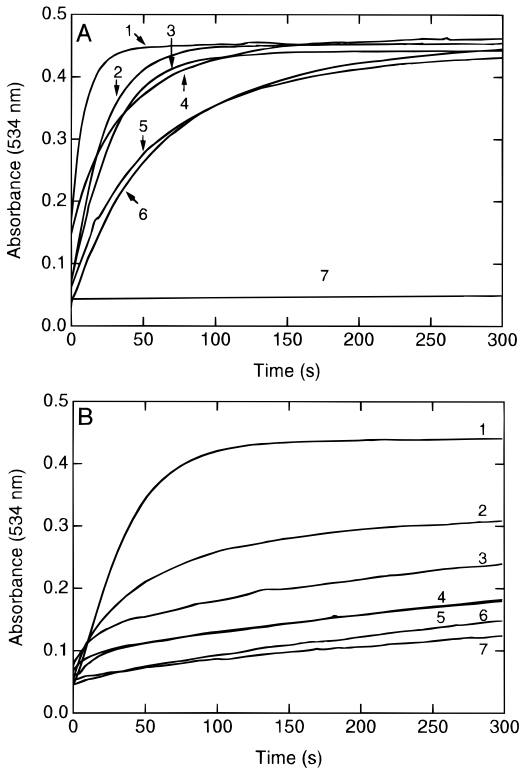

FIGURE 2: Time course for the MgATP-dependent chelation of Fe^{2+} from Fe proteins by bathophenanthrolinedisulfonate (BPS). Reaction conditions were as described in Materials and Methods. The absorbance at 534 nm of the Fe^{2+}−BPS complex versus time was recorded. Prior to addition of the Fe protein, nucleotides were added, where noted, to a final concentration of 1.0 mM. Each reaction was initiated by the addition of 0.4 mg (6.25 μmol) of Fe protein. Panel A: The rate of Fe^{2+} chelation from the L127Δ Fe protein in the presence of ADP (trace 1), ATP (trace 2), no nucleotide added (trace 3), MgATP (trace 4), or MgADP (trace 5) is presented. The rate of Fe^{2+} chelation for wild-type Fe protein in the presence of 1 mM MgATP (trace 6), or in the absence of nucleotides (trace 7) is also presented. Panel B: Protection by the MoFe protein was examined by the addition of 1.29 mg (5.3 μmol) of wild-type MoFe protein to each assay cuvette prior to Fe protein addition. The time-dependent chelation of Fe^{2+} from L127Δ Fe protein with ADP (trace 2), L127Δ Fe protein with ATP (trace 3), L127Δ Fe protein without nucleotides (trace 4), wild-type Fe protein with MgATP (trace 5), L127Δ Fe protein with MgATP (trace 6), or L127Δ Fe protein with MgADP (trace 7) is presented. For comparison, the time-dependent chelation from L127Δ Fe protein, in the absence of nucleotides, without MoFe protein addition, is shown (trace 1).

showed Fe^{2+} chelation by BPS in the absence of added MgATP (trace 3). Interestingly, the rate of chelation from the L127Δ Fe protein in the absence of added MgATP was somewhat faster (0.032 s^{-1}) than the rate observed for the wild-type Fe protein in the presence of saturating MgATP. The presence of ATP (trace 2) (0.040 s^{-1}) or MgATP (trace 4) (0.025 s^{-1}) in the chelation reaction of the L127Δ Fe protein resulted in changes in the rate of Fe^{2+} chelation.

MgADP is normally a potent inhibitor of MgATP-stimulated Fe^{2+} chelation from the wild-type Fe protein. Addition of MgADP to the chelation reaction with L127Δ Fe protein (trace 5) had only a slight inhibitory effect, with a measured apparent first-order rate constant of 0.017 s^{-1}, which was nearly identical to that observed for the wild-type Fe protein with MgATP (trace 6). Interestingly, ADP in the absence of Mg^{2+} was found to significantly accelerate the rate of Fe^{2+} chelation from the L127Δ Fe protein (trace 1) (0.088 s^{-1}). ADP in the absence of Mg^{2+} normally does not bind to the wild-type Fe protein. Thus, the stimulation in the rate of chelation observed for the L127Δ Fe protein suggests that ADP can bind to the altered protein.

The chelation reactions described above were repeated with the chelator α,α′-dipyridyl and qualitatively the same results were obtained.

In all cases described above, the L127Δ Fe protein showed the same total Fe released as the wild-type Fe protein in the presence of MgATP (3.1 iron/Fe protein). This suggests that the altered Fe protein (L127Δ) contains a full complement of Fe and that all of the Fe can be chelated from the cluster. In separate total Fe determination experiments, the L127Δ Fe protein was confirmed to contain 3.2 iron/Fe protein compared to 3.4 iron/wild-type Fe protein (Seefeldt et al., 1992).

The observation that the L127Δ Fe protein showed a time-dependent chelation by BPS in the absence of added MgATP suggested that the protein was in a conformation similar to that which results from the addition of MgATP. It was possible that this was the result of tightly bound nucleotides which might have copurified with the L127Δ Fe protein. To test for such a bound nucleotide, the L127Δ Fe protein was analyzed for bound nucleotides following acid denaturation (Seefeldt & Mortenson, 1993). No bound nucleotides (ATP, ADP, AMP, GTP, or GTP) above the detection limits of 0.01 nucleotide bound/L127Δ Fe protein could be detected. These results suggest that the L127Δ Fe protein is in a conformation similar to the MgATP-bound state in the absence of any bound nucleotides.

Given the effects of the addition of MgATP, ATP, MgADP, or ADP on the chelation rates from the L127Δ Fe protein, it was important to determine if these nucleotides could bind to the protein and to determine the binding stoichiometry. This was determined directly by an equilibrium column binding technique previously described (Ryle et al., 1995). Both the wild-type and L127Δ Fe proteins bound approximately 2 molecules of MgATP or MgADP/Fe protein (Table 1). Previous studies have shown that the presence of a divalent metal (e.g. Mg^{2+}) associated with ATP or ADP is absolutely required for nucleotide binding to the wild-type Fe protein (Tso & Burris, 1973). As expected from this observation and the lack of an effect of ATP addition on the chelation rate for the L127Δ Fe protein, ATP in the absence of added Mg^{2+} did not bind to either the wild-type or the L127Δ Fe proteins (Table 1). In contrast, ADP in the absence of Mg^{2+} was found to bind to the L127Δ Fe protein with a stoichiometry of 2 ADP/Fe protein. This contrasts with the observation that ADP, in the absence of Mg^{2+}, has no affinity for the wild-type Fe protein. The binding of ADP to the L127Δ Fe protein does, however, fit with the observed increase in the rate of chelation in the BPS assay for the L127Δ Fe protein upon ADP addition (Figure 2A, trace 1).

Table 1: Number of Nucleotides Bound to Wild-Type and L127ΔFe Proteins at Saturating Nucleotide Concentrations[a]

	nucleotides bound (μmol/μmol of Fe protein)			
Fe protein	ATP	MgATP	ADP	MgADP
wild-type	0	2.06	0	1.98
L127Δ	0	2.05	2.15	1.97

[a] The number of nucleotides bound to reduced wild-type and L127Δ Fe proteins, at nucleotide concentrations of 1 mM, were determined as described in Materials and Methods.

Earlier studies have shown that the inclusion of the MoFe protein in Fe chelation assays results in a decreased rate of Fe^{2+} chelation from the Fe protein (Walker & Mortenson, 1974; Seefeldt, 1994). This is attributed to MoFe protein binding to the Fe protein and protecting the [4Fe−4S] cluster from access by the chelator. Figure 2 (panel B) illustrates the effects of added MoFe protein on the rates and extents of Fe^{2+} chelation from wild-type and L127Δ Fe proteins. Consistent with previous observations (Seefeldt, 1994), the MgATP-stimulated chelation of Fe^{2+} from the wild-type Fe protein was significantly reduced by the addition of MoFe protein (trace 5). When the MoFe protein was included in the chelation assay for the L127Δ Fe protein with ADP, ATP, MgADP, or MgATP, a decreased rate of chelation was observed in all cases, indicating that under the conditions of the assay the L127Δ Fe protein could still bind to the MoFe protein. Surprisingly, the L127Δ Fe protein−BPS chelation rate in the absence of any added nucleotides was decreased by the inclusion of the MoFe protein. A control protein, such as bovine serum albumin, had no inhibitory effect on the rate of Fe chelation from the L127Δ Fe protein. The rate of chelation for the L127Δ Fe protein with MoFe protein was nearly identical to that observed for the wild-type Fe protein with MgATP and MoFe protein. This result reveals that the L127Δ Fe protein binds with significant affinity to the MoFe protein in the absence of added nucleotides. This suggests not only that has shortening the amino acid chain between Asp 125 and Cys 132 caused changes in the [4Fe−4S] cluster environment consistent with the MgATP-bound state but also that this conformation is sufficient to allow binding to the MoFe protein.

Conformational State of the L127Δ Fe Protein Monitored by ^{1}H NMR, CD and EPR. Given the observation from the chelation assay that the L127Δ Fe protein might be in an MgATP-like conformation in the absence of MgATP, we investigated the properties of the [4Fe−4S] cluster of the L127Δ Fe protein by three different techniques which are sensitive to nucleotide-induced conformational changes in the cluster, namely, ^{1}H NMR, CD and EPR spectroscopies.

The downfield portion of the ^{1}H NMR spectrum of Fe protein is a sensitive monitor of changes in the properties of the [4Fe−4S] cluster. The α-CH and β-CH_2 protons of the cysteinyl ligands to the [4Fe−4S] cluster are shifted out of the diamagnetic envelope, providing a fingerprint of the

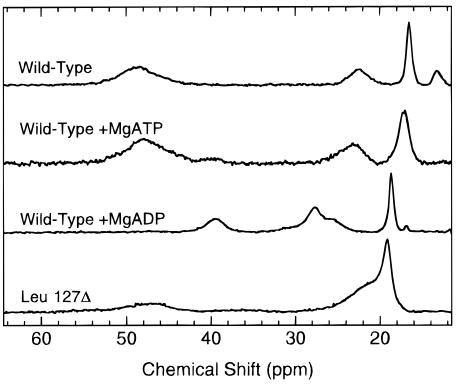

FIGURE 3: Downfield portion of the ^{1}H NMR spectra of wild-type and L127Δ Fe proteins. Reduced Fe protein samples were prepared as described in Materials and Methods. The downfield portions of the 400-MHz ^{1}H NMR spectra, at 305 K, for wild-type Fe protein (1.5 mM) in the absence of nucleotides (wild-type), with MgATP (wild-type + MgATP), or with MgADP (wild-type + MgADP) are presented. The spectrum for the L127Δ Fe protein in the absence of nucleotides is also presented. Where noted, nucleotides were added to a 5-fold molar excess over protein from stock solutions.

cluster environment. Recently, each of the four isotropically shifted proton resonances observed for the wild-type Fe protein have been assigned to α-CH and β-CH_2 cysteinyl ligand protons (Lanzilotta et al., 1995a). The chemical shift of each of these proton resonances is sensitive to the binding of MgATP or MgADP, with the binding of either nucleotide resulting in different chemical shifts for each of the four resonances (Figure 3). Figure 3 compares the downfield portion of the ^{1}H NMR spectrum of wild-type Fe protein with that recorded for the L127Δ Fe protein. As can be seen, the L127Δ Fe protein, in the absence of any added nucleotides, showed a downfield ^{1}H NMR spectrum which was somewhat different from that observed for the wild-type Fe protein in the absence of nucleotides. A comparison of the L127Δ Fe protein spectrum with that for the wild-type Fe protein in the presence of either MgATP or MgADP reveals similarities to MgATP- and MgADP-bound states. The two most downfield-shifted resonances observed for the L127Δ Fe protein appeared to be shifted similarly to the corresponding resonances in the MgATP-bound state of the wild-type Fe protein. The sharp signal in the L127Δ Fe protein spectrum at 19 ppm was shifted similarly to the corresponding resonance in the wild-type Fe protein with MgADP bound. The addition of either MgATP or MgADP to the L127Δ Fe protein resulted in no detectable changes in the downfield proton NMR resonances.

Circular Dichroism Spectroscopy. The visible-region CD spectrum of the oxidized Fe protein provides a sensitive way to detect changes in the environment of the [4Fe−4S] cluster (Stephens et al., 1979; Ryle et al., 1996). MgATP or MgADP binding to the Fe protein results in distinct changes in the CD spectrum, providing a useful way to monitor the conformational state of the Fe protein (Figure 4, panel A). The CD spectrum of the L127Δ Fe protein in the absence of nucleotides is shown in Figure 4 (panel B). This spectrum was strikingly similar to the CD spectrum of wild-type Fe protein with MgATP bound, although the total ellipticity of the L127Δ Fe protein was slightly lower. These results suggest that the L127Δ Fe protein is in a MgATP-bound like conformation in the absence of nucleotides. The addition of MgATP, MgADP, ATP, or ADP to the L127Δ Fe protein resulted in negligible changes to the CD spectrum.

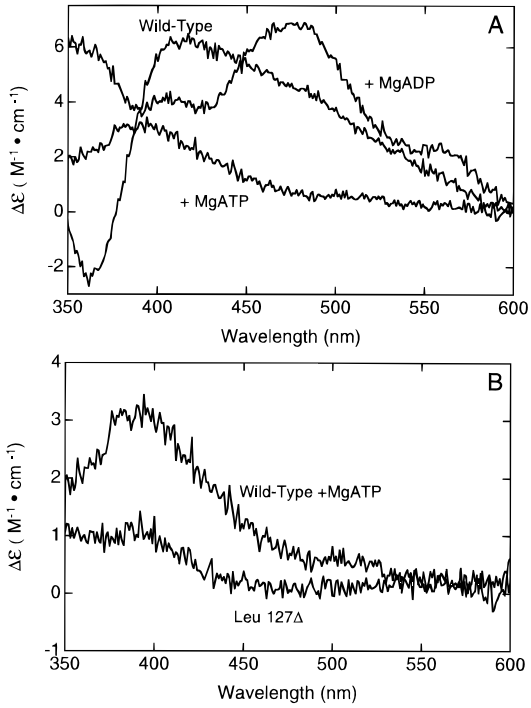

FIGURE 4: Circular dichroism spectra of wild-type and L127Δ Fe proteins in the presence or absence of nucleotides. The visible region circular dichroism (CD) spectra of oxidized wild-type and L127Δ Fe proteins were recorded as described in Materials and Methods. Panel A: CD spectra of the wild-type Fe protein in the absence (wild-type) or in the presence of MgADP (+MgADP) or MgATP (+MgATP). Panel B: CD spectra of L127Δ Fe protein in the absence of nucleotides. For comparison, the CD spectrum for wild-type Fe protein with MgATP bound (wild-type + MgATP) is shown. All spectra were baseline-subtracted.

EPR. EPR has been used extensively to monitor both the oxidation state and the electronic environment of the [4Fe−4S] cluster in the Fe protein (Zumft et al., 1973; Lindahl et al., 1987b). In the absence of nucleotides, the reduced Fe protein exhibits a rhombic EPR spectrum centered at $g = 1.93$ (Figure 5). The addition of MgATP, but not MgADP, results in a change in the EPR line shape from rhombic to axial (Figure 5). This change in line shape is diagnostic for the MgATP-induced conformational changes in the environment of the [4Fe−4S] cluster. The L127Δ Fe protein, in the absence of added nucleotides, revealed an axial EPR spectrum similar to that observed for the wild-type Fe protein observed only in the presence of MgATP (Figure 5). The addition of MgATP, ATP, ADP or MgADP to the L127Δ Fe protein did not result in any significant change in the EPR spectrum. The $g = 1.87$ EPR signal of the L127Δ Fe protein was somewhat broader than the corresponding $g = 1.89$ signal in the MgATP-bound state of the wild-type Fe protein.

Redox Potential. MgATP binding to the wild-type Fe protein results in approximately a 120-mV lowering of the redox potential of the [4Fe−4S] cluster from −300 to −420 mV (Figure 6). MgADP binding to the wild-type Fe protein results in approximately a 160-mV lowering of the redox potential to −440 mV (Figure 6). These changes in the redox potential are a significant manifestation of the nucleotide-induced conformational changes in the environment of the [4Fe−4S] cluster. The redox potential of the L127Δ Fe protein was found to be −412 mV in the absence of added nucleotides, which was very similar to the redox potential of the wild-type Fe protein in the presence of MgATP (Figure

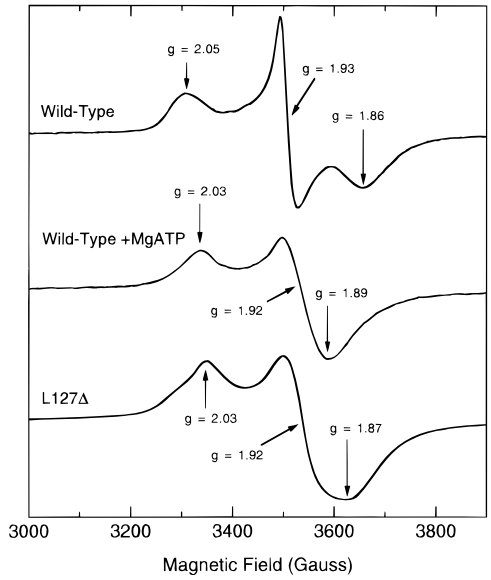

FIGURE 5: EPR spectra of wild-type and L127Δ Fe proteins in the presence or absence of nucleotides. Wild-type (30 mg·mL^{-1}) and L127Δ (85 mg·mL^{-1}) Fe proteins were prepared, and spectra were recorded, as described in Materials and Methods. Samples were maintained in the reduced state by the presence of 2 mM dithionite. Spectra were recorded at 12 K, 9.50 GHz, and 0.5 mW microwave power. Where noted, nucleotides were added as a 10-fold molar excess over proteins prior to freezing.

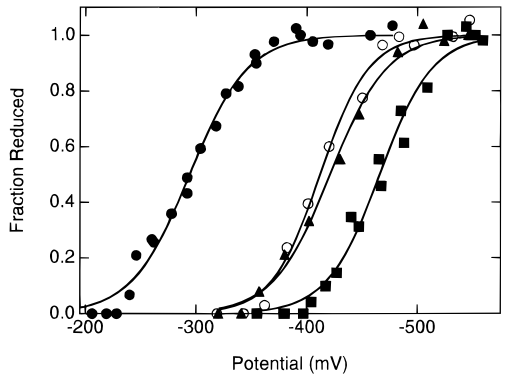

FIGURE 6: Redox titrations of wild-type and L127Δ Fe proteins. Electrochemical redox titrations using EPR were performed as outlined in Materials and Methods. Results for wild-type Fe protein (●) (E_m = −294 mV), wild-type Fe protein with MgATP (▲) (E_m = −420 mV), wild-type Fe protein with MgADP (■) (E_m = −460 mV), and L127Δ Fe protein (○) (E_m = −412 mV) are shown. Each Fe protein sample (10−15 mg·mL^{-1}) was prepared in 50 mM Tricine buffer, pH 8.0, with 0.25 M NaCl, 50 μM methyl viologen, 50 μM benzyl viologen, and 50 μM flavin mononucleotide. EPR spectra were recorded at 15 K, 6.36 mW microwave power, and at a frequency of 9.64 GHz. The peak to peak height of the EPR signal was plotted against the measured potential. Midpoint potentials (E_m) were calculated from fitting the data to the Nernst equation and are reported relative to the normal hydrogen electrode (NHE).

6). Thus, deletion of Leu at position 127 of the Fe protein has resulted in a 112-mV lowering in the redox potential of the Fe protein compared to the unaltered Fe protein.

Binding of the L127Δ Fe Protein to the MoFe Protein. Two earlier observations made it interesting to determine the affinity of binding of the L127Δ Fe protein to the MoFe protein. First, the observation that the MoFe protein inhibited Fe^{2+} chelation from the L127Δ Fe protein suggested that the L127Δ Fe protein could still bind to the MoFe protein with high affinity. Second, the L127Δ appears to be in a conformation which mimics the wild-type Fe protein with

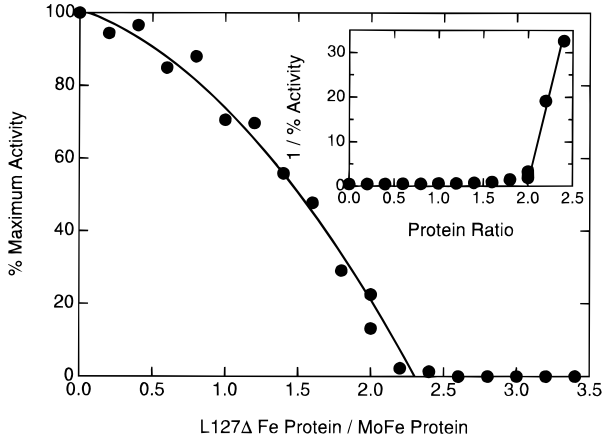

FIGURE 7: Inhibition of wild-type nitrogenase acetylene reduction activity by L127Δ Fe protein. Acetylene reduction assays were performed as described under Materials and Methods. A constant amount, 253 μg (1.05 nmol), of wild-type MoFe protein and increasing amounts of L127Δ Fe protein (0−3.4 nmol) were added to each assay vial. Each reaction was initiated by the addition of 134 μg (2.1 nmol) of wild-type Fe protein. The percentage of the maximum acetylene reduction activity (nanomoles of C_2H_2 formed per minute) was plotted against the ratio of L217Δ Fe protein to wild-type MoFe protein. The maximum activity of the wild-type Fe protein was 2176 nmol of C_2H_2·min^{-1}·mg^{-1}. Inset: A Dixon plot showing the inverse of the percent maximum activity plotted against the protein ratio (nanomoles of L127Δ Fe protein per nanomol of wild-type MoFe protein).

MgATP bound. Earlier studies have suggested at least three stages to Fe protein docking to the MoFe protein (Willing & Howard, 1990; Seefeldt, 1994) and that the MgATP-bound conformation of the Fe protein is required for the last two stages of this docking. Thus it was of interest to determine if the L127Δ Fe protein could still bind to the MoFe protein. To better quantify the interactions of the L127Δ Fe protein with the MoFe protein, we employed a competitive binding assay (Lanzilotta et al., 1995b). Since the L127Δ Fe protein was unable to support substrate reduction when combined with the MoFe protein, it was possible to include increasing concentrations of L127Δ Fe protein in a normal wild-type Fe protein−MoFe protein assay to determine the effects on the substrate reduction rates. The assumption was that if the L127Δ Fe protein could bind to the MoFe protein to form an inactive complex, then a decrease in the substrate reduction rate catalyzed by the wild-type Fe protein−MoFe protein complex would be observed. If the L127Δ Fe protein did not bind to the MoFe protein, then increasing L127Δ Fe protein concentrations in a normal wild-type Fe protein−MoFe protein assay would have no effect on the substrate reduction rates. Figure 7 illustrates the effect of increasing L127Δ Fe protein concentrations on the acetylene reduction activity catalyzed by the wild-type Fe protein−MoFe protein complex, demonstrating that the L127Δ Fe protein inhibits this assay and thus binds to the MoFe protein. It is interesting to note that at a molar ratio of 2 L127Δ Fe proteins: 1 MoFe protein, the reaction was fully inhibited. Each MoFe protein has 2 Fe protein binding sites (Kim & Rees, 1992a), so a molar ratio of 2 Fe proteins:1 MoFe proteins fits with the model for the number of binding sites. What was surprising was the observation that at the ratio of 2 L127Δ Fe proteins:1 MoFe protein (with 2 wild-type Fe proteins present), the activity was fully inhibited. This suggested that the affinity of the L127Δ Fe protein for binding to the MoFe protein was much higher than was the

affinity of wild-type Fe protein for the MoFe protein. This was further illustrated by plotting the inverse of percent maximum activity against the concentration of L127Δ Fe protein added (Figure 7, inset). The resulting Dixon plot is typical for tight-binding inhibitors, suggesting that the L127Δ Fe protein forms a tight complex with the MoFe protein with a measured stoichiometry of approximately 2 L127Δ Fe proteins:1 MoFe protein.

DISCUSSION

Elucidating the mechanism of long-range MgATP signal transduction to the [4Fe−4S] cluster in the nitrogenase Fe protein is critical to a detailed understanding of the function of MgATP in reactions catalyzed by nitrogenase. The results of the present study support an earlier hypothesis (Howard & Rees, 1994) that the protein domain from Asp 125 to Cys 132 serves as one pathway for signal transduction. Removing one amino acid in the protein chain between Asp 125 (at the ATP binding site) and Cys 132 (a [4Fe−4S] cluster ligand) resulted in protein conformational changes detected by the properties of the [4Fe−4S] cluster that were strikingly similar to those normally induced only by MgATP binding. This suggests that the protein chain from Asp 125 to Cys 132, and more specifically movement in this chain, is central to the mechanism of MgATP signal transduction to the environment of the [4Fe−4S] cluster.

MgATP Conformational Changes. A model for MgATP binding and conformational changes in the nitrogenase Fe protein can be developed in light of the current results. Several recent reports on site-specifically altered Fe proteins (Seefeldt et al., 1992; Wolle et al., 1992a; Seefeldt & Mortenson, 1993; Ryle et al., 1995; Lanzilotta et al., 1995b), along with analysis of the X-ray structure of the Fe protein (Georgiadis et al., 1992), have begun to define the amino acids in the Fe protein involved in MgATP binding and hydrolysis. Lys 15, Ser 16, Asp 125, and Asp 129 have all been implicated in some aspect of nucleotide binding or hydrolysis. Ser 16 appears to coordinate the Mg^{2+} associated with the bound ATP (Seefeldt & Mortenson, 1993), while Asp 129 (Lanzilotta et al., 1995b) has been suggested to bind a water molecule associated with the bound ATP (possibly activating the water for hydrolysis of the γ-phosphate). Lys 15 and Asp 125 appear to form a salt bridge in the absence of bound MgATP (Georgiadis et al., 1992). From site-specifically altered Fe proteins, Lys 15 (Ryle et al., 1995) has been suggested to bind to the phosphate portion of the bound nucleotide and Asp 125 (Wolle et al., 1992a) with the associated Mg^{2+}. Upon Fe protein binding MgATP, the salt bridge between Lys 15 and Asp 125 is expected to be broken, with each residue moving into position to bind either the phosphate or Mg^{2+}. Breaking the salt bridge between Asp 125 and Lys 15 might be expected to result in movement in adjoining amino acids. This idea can be coupled to the results presented in this work suggesting that movement in the Asp 125−Cys 132 protein chain is essential to the MgATP-induced changes in the [4Fe−4S] cluster. Since shortening of the Asp 125−Cys 132 chain by deletion of Leu 127 induced a MgATP-like conformational change, this would suggest that movement of Asp 125 to accommodate the binding of MgATP results in a "pull" on the protein chain from Asp 125 to Cys 132. Consistent with this model, recent small-angle X-ray scattering experiments on the Fe protein have suggested that MgATP binding results in an approximately 2-Å decrease in the radius of gyration (Chen et al., 1994). This suggests that MgATP binding results in a tightening of the overall structure of the Fe protein, consistent with a model in which MgATP binding results in a movement of the peptide chain from Asp 125 to Cys 132.

How then could movement in the Asp 125−Cys 132 protein chain result in changes in the properties of the [4Fe−4S] cluster? X-ray absorption spectroscopy studies suggest that the Fe−Fe and Fe−S bond distances of the [4Fe−4S] cluster do not change significantly upon Fe protein binding MgATP (Lindahl et al., 1987a; Ryle et al., 1996). Thus, the observed changes in the electronic properties of the [4Fe−4S] cluster seen in the CD, EPR, and NMR spectra, along with the decrease in the midpoint potential, must be mediated by changes in the protein environment of the cluster. Several models have been developed to explain how the protein environment in Fe−S cluster-containing proteins can control the properties of Fe−S clusters (Jensen et al., 1994; Warshel et al., 1994). The proximity of charged or hydrophobic amino acids near the Fe−S cluster, along with backbone hydrogen bonding to the cysteinyl ligands, appears to modulate the properties of the cluster. It would seem reasonable to assume that the movement induced in the Asp 125−Cys 132 protein chain in the Fe protein upon binding MgATP changes the arrangement of amino acids near the cluster. This hypothesis is supported by proton NMR studies which reveal changes in the orientation of the protons of the cysteinyl ligands (including Cys 132) relative to the cluster upon binding MgATP (Meyer et al., 1988; Lanzilotta et al., 1995a). The NMR results also suggest that the changes around the cluster upon the protein binding MgATP are subtle, not requiring major protein rearrangements (Lanzilotta et al., 1995a). The details of the protein changes near the cluster are not clear, but it is noteworthy that two of the three amino acids known to be within van der Waals contact of the cluster (Phe 135 and Val 130) are connected to the Asp 125−Cys 132 chain (Georgiadis et al., 1992). Thus, movement in the Asp 125−Cys 132 chain might be expected to result in changes in the positions of Val 130 and Phe 135, which might control the properties of the cluster, either directly or by controlling water access to the cluster.

MgADP Conformational Changes. MgADP binding to the Fe protein is known to result in different spectroscopic properties of the [4Fe−4S] cluster compared to the native or MgATP-bound Fe protein (Meyer et al., 1988; Lanzilotta et al., 1995a; Ryle et al., 1996). This is contrasted with the observation that both MgATP and MgADP binding result in a significant lowering of the redox potential of the [4Fe−4S] cluster (Zumft et al., 1973; Watt et al., 1980). These results suggest that MgADP and MgATP binding to the Fe protein result in some common and some different changes in the protein environment of the cluster. Clearly, the presence of the γ-phosphate on ATP (or the position of the Mg^{2+} bound between the β- and γ-phosphates) must account for the differences between the MgADP- and MgATP-bound states. Shortening the protein chain from Asp 125 to Cys 132 by deletion of Leu 127 resulted in Fe protein with MgATP-bound-like character, suggesting that this change mimics the MgATP-induced changes. How then are MgADP-induced conformational changes communicated to the [4Fe−4S] cluster? Two possibilities seem likely. One is that the Asp 125−Cys 132 protein chain also communicates the MgADP-induced changes, with slight differences in the

movement of the chain accounting for the different properties of the cluster. A second possibility is that a second signal transduction pathway exists in the Fe protein, which may be involved in MgADP signal transduction (Howard & Rees, 1994).

An interesting observation of the present work is that the L127Δ Fe protein binds to the MoFe protein, both in the presence and in the absence of MgATP. It appears that the MoFe protein docking sites on the Fe protein include several regions near the [4Fe−4S] cluster (Lowery et al., 1989; Willing & Howard, 1990; Wolle et al., 1992b; Peters et al., 1994; Seefeldt, 1994). Thus the observed need for the MgATP-bound conformation in the Fe protein for docking to the MoFe protein suggests conformational changes induced by MgATP binding near the [4Fe−4S] cluster. The observation in the present study that the L127Δ Fe protein docks to the MoFe protein in the absence of MgATP suggests that the conformational change necessary for the later stages of component docking (Seefeldt, 1994) have been induced by the deletion of Leu 127. This again implicates the Asp 125−Cys 132 protein chain in signaling the conformational changes necessary for MoFe protein docking. It is possible that other protein conformational changes in the Fe protein may also account for the docking signal.

Other Nucleotide Binding Proteins. A series of recent studies have all pointed toward many common features between the nitrogenase Fe protein and the large class of GTPase signal transduction proteins (G-proteins) (Wolle et al., 1992a; Howard & Rees, 1994). In both cases, nucleotide binding and hydrolysis appears to provide a kinetic mechanism for protein-induced conformational changes essential to the function of the proteins. Both groups of proteins contain highly conserved Walker A and B protein sequences, which are involved in nucleotide binding and both have similar overall protein folds. Of particular interest to the present study is the striking homologies between Asp 125 and the adjoining protein chain to Cys 132 to an equivalent Asp residue in the G-proteins and the adjoining protein chain (Howard & Rees, 1994). High-resolution X-ray structures now exist for four different G-proteins with or without bound nucleotides, including Ras p21 (Milburn et al., 1990; Schlichting et al., 1990), EF-Tu (Jurnak, 1985; Kjeldgaard & Nyborg, 1992), G_t (Lambright et al., 1994), and $G_{i\alpha 1}$ (Mixon et al., 1995). Common among each of these G-proteins is the presence of an Asp residue at the phosphate-binding site and a signal transduction pathway originating from this Asp residue. The specific changes induced in the protein chain associated with the Asp residue are different for each of the G-proteins, but in all cases the changes in this protein chain appear to be essential to the signal transduction. The protein chain originating from the Asp residue has been designated as switch II for several of these proteins (Milburn et al., 1990). The similar position of Asp 125 of the nitrogenase Fe protein and the adjoining chain suggests that the protein chain from Asp 125 to Cys 132 may share a common function in signal transduction with switch II in the G-proteins. In the case of the Fe protein, the function of the Asp 125−Cys 132 chain is to transduce MgATP binding and hydrolysis to the [4Fe−4S] cluster for electron transfer and docking to the MoFe protein. Given the strong similarities between the Fe protein and the G-proteins, we suggest that the Asp 125−Cys 132 protein chain in Fe protein be designated as "switch II" in keeping with the nomenclature adopted for the homologous region in the G-proteins. Work to identify other signal transduction pathways in the Fe protein is underway.

In summary, the present work has defined a MgATP signal transduction pathway in the nitrogenase Fe protein which includes the protein chain from Asp 125 to Cys 132 (designated switch II). Deletion of an amino acid residue in this protein chain (Leu 127) resulted in an Fe protein with MgATP-bound character in the absence of MgATP, suggesting that shortening of switch II of the Fe protein can mimic the MgATP-bound protein conformation. It will be interesting to determine the X-ray structure of the L127Δ Fe protein to better define the exact conformational changes (specifically around the [4Fe−4S] cluster) associated with MgATP signal transduction.

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