Involvement of the P Cluster in Intramolecular Electron Transfer within the Nitrogenase MoFe Protein*

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Nitrogenase is the catalytic component of biological nitrogen fixation, and it is comprised of two component proteins called the Fe protein and MoFe protein. The Fe protein contains a single Fe₄S₄ cluster, and the MoFe protein contains two metallocluster types called the P cluster (Fe₃S₄) and FeMo-cofactor (Fe₇S₉Mo-homocitrate). During turnover, electrons are delivered one at a time from the Fe protein to the MoFe protein in a reaction coupled to component-protein association-dissociation and MgATP hydrolysis. Under conditions of optimum activity, the rate of component-protein dissociation is rate-limiting. The Fe protein’s Fe₄S₄ cluster is the redox entity responsible for intermolecular electron delivery to the MoFe protein, and FeMo-cofactor provides the substrate reduction site. In contrast, the role of the P cluster in catalysis is not well understood although it is believed to be involved in accumulating electrons delivered from the Fe protein and brokering their intramolecular delivery to the substrate reduction site. A nitrogenase component-protein docking model, which is based on the crystallographic structures of the component proteins and which pairs the 2-fold symmetric surface of the Fe protein with the exposed surface of the MoFe protein’s pseudosymmetric αβ interface, is now available. During component-protein interaction, this model places the P cluster between the Fe protein’s Fe₄S₄ cluster and FeMo-cofactor, which implies that the P cluster is involved in mediating intramolecular electron transfer between the clusters. In the present study, evidence supporting this idea was obtained by demonstrating that it is possible to alter the rate of substrate reduction by perturbing the polypeptide environment of the P cluster and FeMo-cofactor without necessarily disrupting the metallocluster polypeptide environments or altering component-protein interaction.

The MgATP-dependent reduction of nitrogen gas to yield ammonia has a minimal stoichiometry usually indicated as follows.

\[ \text{N}_2 + 8\text{e}^- + 8\text{H}^+ + 16\text{MgATP} \rightarrow 2\text{NH}_3 + 8\text{H}_2 + 16\text{MgADP} + 16\text{P}_i \]

**Reaction 1**

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and FeMo-cofactor, that could participate in electron transfer between the two clusters (Fig. 2). The Tyr<sup>b</sup> residue located on one of these helices and is situated in a direct line between the two clusters. This residue is also one of a group of hydrophobic residues that provide the polypeptide environment of the P cluster without being in contact with it. Furthermore, Tyr<sup>b</sup> approaches the terminal carboxyl of the homocitrate moiety of FeMo-cofactor and may indirectly interact with it by hydrogen bonding through water.\(^1\) The homologous residue within the \(\alpha\)-subunit, \(\alpha\)-Tyr<sup>91</sup>, is also found on a helix located between the clusters but its side chain is directed away rather than toward the FeMo-cofactor (Fig. 2). To assess the role of the P cluster and the possible participation of Tyr<sup>b</sup> in intramolecular electron transfer, this residue was substituted by Phe, Leu, and His and the catalytic, kinetic, and spectroscopic properties of the resulting altered MoFe proteins were examined. Thus, the experimental rationale was to ask whether or not it is possible to perturb intramolecular electron transfer without disrupting the polypeptide environments of the metalloclusters or altering component protein interaction. In a parallel set of experiments, the \(\alpha\)-subunit residue, Tyr<sup>91</sup>, was also substituted by Phe, Leu, and His, and the catalytic and kinetic properties of the altered His<sup>91</sup> MoFe protein were examined as well. These latter experiments were intended to serve as a control, with the substitutions at this position considered less likely to have an effect on intramolecular electron transfer.

\(^1\) J. T. Bolin, personal communication.
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Construction and diazotrophic growth of A. vinelandii mutant strains

| Strain | Oligonucleotide* | Plasmid | Substitution | Doubling time |
|--------|------------------|---------|--------------|---------------|
| Wild type | TGGGCCGACGACTCGCCGCGCCGG | pDB880 | None | 3.0 h |
| DJ 1038 | TGGGCCGACGACTCGCCGCGCCGG | pDB880 | α-His98 | 3.5 h |
| DJ 933 | TGGGCCGACGACTCGCCGCGCCGG | pDB894 | α-Leu94 | 3.5 h |
| DJ 933 | TGGGCCGACGACTCGCCGCGCCGG | pDB894 | α-Leu98 | 3.5 h |
| DJ 933 | TGGGCCGACGACTCGCCGCGCCGG | pDB797 | β-His98 | 6.0 h |
| DJ 1028 | TGGGCCGACGACTCGCCGCGCCGG | pDB860 | β-Leu98 | 4.0 h |
| DJ 982 | TGGGCCGACGACTCGCCGCGCCGG | pDB796 | β-Phe98 | 4.0 h |

*Oligonucleotide refers to the synthetic DNAs used in site-directed mutagenesis experiments. Nucleotides differing from the wild type sequence are underlined.

RESULTS

Diazotrophic Growth of Mutant Strains and Activities of the Purified, Altered MoFe Proteins—All six mutant strains constructed by substituting either Tyr91, Tyr98, or Phe91 by Phe, Leu, and His, were capable of diazotrophic growth (Table I). The mutant strains, which produce altered MoFe proteins having either the β-Phe98 or the β-Leu98 substitutions, grew diazotrophically almost as well as wild type (4- to 3-h doubling time), while the strain with the His91 substitution grew slowest with a 6-h doubling time. All three mutant strains with substitutions at the Tyr98 position exhibited, at most, only a small increase in diazotrophic doubling time.

MoFe proteins were isolated and purified in parallel from all three strains with substitutions at Tyr98, from the His91 strain, and from wild type. The isolated His91 MoFe protein exhibited kinetic and catalytic properties nearly identical to the wild-type MoFe protein, confirming that this substitution has no discernible effect on electron transfer from Fe protein to substrate. As expected from the good growth rates of their parent strains, the altered Phe98 and Leu98 MoFe proteins exhibited catalytic activities for H2 evolution, C2H2 reduction, N2 fixation, and concomitant MgATP hydrolysis comparable with those of the wild type. In contrast to the other altered MoFe proteins, the His98 MoFe protein showed a significant decrease in the maximum specific activity for substrate reduction, while the overall rate of MgATP hydrolysis was maintained near the wild type rate (Table II). In other words, the reaction catalyzed by the altered His98 MoFe protein exhibits MgATP hydrolysis that is partially uncoupled from electron transfer. For all of the altered MoFe proteins, the effect of 10% CO was to divert all electron flux to H2 evolution, which was insensitive to the presence of CO2, as seen for wild type. No methane was observed from catalyzed C2H2 reduction.

A number of experiments were performed on the His98 protein.

2 The abbreviations used are: EPR, electron paramagnetic resonance; MCD, magnetic circular dichroism.
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MoFe protein to determine whether or not its lower specific activity is due to an alteration in intramolecular electron delivery. The effect of varying electron flux was investigated to probe if its ability to deliver electrons to the substrate had been compromised. Component-protein ratio titrations performed in parallel for both wild-type and His\textsuperscript{98} MoFe proteins revealed that their specific activities maximized at different Fe protein:MoFe protein ratios (Fig. 3). For the wild-type MoFe protein, a maximum specific activity of 2250 nmol of H\textsubscript{2} formed/min/mg of MoFe protein was achieved at an Fe protein:MoFe protein molar ratio of greater than 10:1 under conditions of proton reduction. Under the same conditions, a maximum specific activity of 1100 nmol of H\textsubscript{2} formed/min/mg of MoFe protein was observed for the His\textsuperscript{98} MoFe protein, and this value was achieved at the lower Fe protein:MoFe protein molar ratio of approximately 5:1 (Fig. 3). This effect was also apparent in analogous titrations performed under conditions of acetylene reduction, where the activity of the His\textsuperscript{98} MoFe protein maximizes at about 600 nmol of C\textsubscript{2}H\textsubscript{4} produced/min/mg of MoFe protein at the same 5:1 molar ratio, while the wild type reaches the expected maximum activity of 2100 nmol C\textsubscript{2}H\textsubscript{4} formed/min/mg of MoFe protein at a molar ratio of greater than 20:1. The \( K_m \) values for C\textsubscript{2}H\textsubscript{4} reduction were also determined for the wild-type and His\textsuperscript{98} MoFe proteins and found to be comparable at 0.0055 and 0.0040 atm, respectively. These values are in the range previously reported for wild type (Dilworth, 1966; Scholhorn and Burris, 1967; Kim et al., 1995).

The lowered maximum C\textsubscript{2}H\textsubscript{4} reduction activity for the His\textsuperscript{98} MoFe protein, when compared with its maximum H\textsuperscript{+} reduction activity, is not compensated for by increased H\textsuperscript{+} reduction under 10% C\textsubscript{2}H\textsubscript{2} to maintain a constant electron flux (Table I). Thus, the His\textsuperscript{98} MoFe protein appears to suffer greater inhibition of electron flux under a 10% C\textsubscript{2}H\textsubscript{2} atmosphere than under either 100% N\textsubscript{2} or 100% argon atmospheres. The inhibition of electron flux during C\textsubscript{2}H\textsubscript{2} reduction catalyzed by the His\textsuperscript{98} MoFe protein was partially relieved by carbon monoxide, bringing it to approximately the same level observed under 100% N\textsubscript{2} or 100 argon atmospheres.

Stopped-flow Spectrophotometry—The possibility that the reduced activity and uncoupled MgATP hydrolysis exhibited by the His\textsuperscript{98} MoFe protein is due to the altered MoFe protein being unable to easily transfer an electron from the P cluster to FeMo-cofactor, such that intramolecular electron transfer is rate-limiting, was investigated using two different types of continuous stopped-flow spectrophotometric experiments. In the first series of experiments, the turnover rate for catalysis, i.e. the dissociation rate of the Fe protein-MoFe protein complex involving either the wild-type MoFe protein or the His\textsuperscript{98} MoFe protein, was determined indirectly by measurement of the rate of nitrogenase-dependent oxidation of flavodoxin from its hydroquinone form to the semiquinone (Fig. 4). Wild-type MoFe protein-dependent flavodoxin oxidation was found to exhibit a single linear function having a calculated turnover rate of 6.3 s\textsuperscript{-1}. This value, which is calculated from the rate of absorbance change, the \( \Delta_{\text{absorb}} \) is 5.7 m\textsuperscript{-1} cm\textsuperscript{-1} for the hydroquinone form of flavodoxin (Klugkist et al., 1986), and the Mo content of the isolated MoFe protein is in excellent agreement with the value of 6.4 s\textsuperscript{-1} previously reported for the dissociation rate of the Klebsiella pneumoniae nitrogenase component.

MoFe protein

| Addition | Product | 10% C\textsubscript{2}H\textsubscript{2}, 90% argon | 100% argon | 100% N\textsubscript{2} |
|----------|---------|-----------------------------------------------|------------|-------------|
| Wild-type (\( \beta \)-Type\textsuperscript{98}) | None | H\textsubscript{2} | 290 | 2331 | 566 |
| | | C\textsubscript{2}H\textsubscript{2} | 2196 | 0 | 0 |
| | | NH\textsubscript{3} | 0 | 0 | 930 |
| | | Total 2e\textsuperscript{b} | 2486 | 2331 | 1961 |
| | | ATP/2e\textsuperscript{c} | 5.5 | 5.4 | 5.4 |
| | CO\textsuperscript{d} | H\textsubscript{2} | 2460 | 2442 | 1930 |
| | | C\textsubscript{2}H\textsubscript{2} | 0 | 0 | 0 |
| | | NH\textsubscript{3} | 0 | 0 | 0 |
| | | Total 2e\textsuperscript{e} | 2460 | 2442 | 1930 |
| | | ATP/2e\textsuperscript{e} | 5.6 | 5.3 | 5.1 |
| | CO | H\textsubscript{2} | 61 | 1142 | 362 |
| | | C\textsubscript{2}H\textsubscript{2} | 703 | 0 | 0 |
| | | NH\textsubscript{3} | 0 | 0 | 456 |
| | | Total 2e\textsuperscript{e} | 764 | 1142 | 1046 |
| | | ATP/2e\textsuperscript{e} | 11.3 | 8.1 | 7.8 |
| \( \beta \)-His\textsuperscript{98} | None | H\textsubscript{2} | 1335 | 1197 | 1275 |
| | | C\textsubscript{2}H\textsubscript{2} | 0 | 0 | 0 |
| | | NH\textsubscript{3} | 0 | 0 | 0 |
| | | Total 2e\textsuperscript{e} | 1335 | 1197 | 1275 |
| | | ATP/2e\textsuperscript{e} | 6.5 | 7.7 | 5.4 |

\( ^{a} \) Specific activity is expressed in nmol of product formed/min/mg of purified MoFe protein under the atmosphere indicated and the conditions specified under "Experimental Procedures."

\( ^{b} \) Total 2e\textsuperscript{e} represents the electron pairs derived from all products in a particular experiment to allow direct comparisons among substrates. 1 nmol of C\textsubscript{2}H\textsubscript{4} or H\textsubscript{2} is equivalent to 1 nmol of 2e; and 1 nmol of NH\textsubscript{3} is equivalent to 1.5 nmol of 2e.  

\( ^{c} \) ATP/2e\textsuperscript{e} represents nmol of MgATP hydrolyzed per electron pair transferred to substrate.

\( ^{d} \) Approximately 10% carbon monoxide (CO) was added, which is sufficient for complete inhibition of C\textsubscript{2}H\textsubscript{2} or N\textsubscript{2} reduction activities.
proteins determined from steady-state data (Thorneley and Lowe, 1983). For the His$^{98\text{ab}}$ MoFe protein, the apparent rate of component-protein dissociation was calculated as 6.0 s$^{-1}$ but only for approximately the first 300 ms. After this brief initial time period, the apparent rate of component protein dissociation in the His$^{98\text{ab}}$ MoFe protein-catalyzed reaction was slowed approximately 2-fold to a linear rate of 2.5 s$^{-1}$. From the known amounts of all reactants in the continuous stopped-flow experiments, the number of electrons delivered to the MoFe protein prior to the observed lowering in turnover rate was calculated as 2.4 indicating that the P cluster is able to accumulate at least two electrons prior to the intramolecular electron delivery event.

In the second set of stopped-flow experiments, the rates of both primary and secondary intramolecular electron transfers to both the wild-type and His$^{98\text{ab}}$ MoFe proteins were measured, as were the absorbance changes that occur at longer times after the initial electron transfers (after 150 ms). The primary and secondary electron transfer rates to both the wild-type and His$^{98\text{ab}}$ MoFe proteins were found to be identical at 158 s$^{-1}$ (Fig. 5; data for determination of the secondary electron transfer rate are not shown) and within the range previously reported (Thorneley, 1975; Fisher et al., 1991). In contrast to the wild-type MoFe protein, the reaction involving the His$^{98\text{ab}}$ MoFe protein exhibits a gradual decrease in optical absorbance after about 150 ms in the pre-steady state experiment (Fig. 5). Further, this decrease in absorbance occurs exponentially with a rate constant of 2.7 s$^{-1}$, which is reminiscent of the protein-component complex dissociation rate of 2.5 s$^{-1}$ that is measured for the latter part of this biphasic process. Thus, the absorbance decrease may reflect reduction of the oxidized Fe protein as it dissociates from the complex.

**Fig. 5.** Electron transfer from the Fe protein to the wild type and His$^{98\text{ab}}$ MoFe protein and subsequent absorbance changes occurring after primary electron transfer. The top panel is a comparison of stopped-flow spectrophotometry traces of His$^{98\text{ab}}$ MoFe protein- (a) and wild-type MoFe protein-dependent Fe protein oxidation (b). The traces are an enlargement of the first 0.03 s shown in the lower trace. The lower panel is an expanded trace (0.8 s) and shows the absorbance changes that occur after primary electron transfer. Trace c was obtained with wild-type MoFe protein and is typical of that reported previously (Lowe et al., 1993), whereas trace d with the His$^{98\text{ab}}$ MoFe protein-dependent reaction shows a single exponential absorbance decrease ($k_{abs} = 2.5$ s$^{-1}$) after primary electron transfer.

**DISCUSSION**

Evidence that the P cluster is the primary acceptor of electrons from the Fe protein and that it subsequently brokers the intramolecular delivery of electrons to the FeMo-cofactor can be considered in the context of the proposed structural models for the nitrogenase component proteins from A. vinelandii (Georgiadis et al., 1992; Kim and Rees, 1992). A docking model that is based on the structures of the individual component proteins (Kim and Rees, 1992; Howard, 1993) and that takes into account amino acid substitution studies (Wolle et al., 1992) and chemical cross-linking experiments has been proposed (Willing et al., 1989; Willing and Howard, 1990). This model (Fig. 1) pairs the 2-fold symmetric surface of the Fe protein homodimer with the exposed surface of a MoFe-protein pseudo-symmetric αβ-unit interface. In this arrangement, the Fe protein's Fe$_4$S$_4$ cluster is positioned in the closest possible proximity to the MoFe protein's P cluster, which then lies between the Fe protein's Fe$_4$S$_4$ cluster and FeMo-cofactor. Evidence supporting the docking model has come from biochemical and kinetic analyses of altered component proteins having one or more amino acid substitutions located within the respective
In the present work, the possibility that substitutions for Tyr$^{198}$ might alter intramolecular electron transfer was investigated because this residue is located on a helix, which spans the P cluster and FeMo-cofactor, and yet does not directly contact either the P cluster or FeMo-cofactor (Fig. 2). Thus, the primary objective was to determine whether or not it is possible to alter intramolecular electron transfer between these prosthetic groups without disrupting either of their respective polypeptide environments. Studies of the effects of substitutions at Tyr$^{198}$ were carried out in parallel with identical amino acid substitutions at the corresponding residue in the α-subunit, Tyr$^{193}$, which served as an internal control because the side chain of this residue is directed away from rather than across the direct line from the P cluster to the FeMo-cofactor. Substitutions at Tyr$^{193}$ were, therefore, considered much less likely to affect intramolecular electron transfer. Of the six mutant strains resulting from the substitution of either Tyr$^{193}$ or Tyr$^{198}$ by Phe, Leu, or His, only the His$^{198}$ substituted strain showed a significant increase in diazotrophic growth-doubling time, which correlated with the His$^{198}$ FeMo protein being the only one to exhibit significantly reduced maximal specific activity for $N_2$ fixation, $H_2$ evolution, and $C_2H_4$ reduction. These results were consistent with our hypothesis that the Tyr$^{193}$ residue would have no role in intramolecular electron transfer.

The decreased steady-state maximum activity observed for the His$^{198}$ FeMo protein is best explained as arising from an alteration in electron transfer capability that occurs after intramolecular electron transfer between the Fe protein and the MoFe protein because the primary and secondary rates of intramolecular electron transfer were found to be identical for both the altered His$^{198}$ MoFe protein and the wild-type MoFe protein (Fig. 5). Moreover, the component-protein titration experiments can be explained by a model where, under high flux conditions (i.e. high Fe protein:MoFe protein ratios), substrate reduction catalyzed by the His$^{198}$ MoFe protein becomes limited by intramolecular electron transfer rather than complex dissociation. In other words, the amount of Fe protein required to achieve maximum His$^{198}$ specific activity is lowered in the titration experiments shown in Fig. 3 because the maximum flux through the system is limited as a consequence of a defect in intramolecular electron transfer per se.

This conclusion is supported by MCD spectrophotometric analysis of the thionine-oxidized state of the altered His$^{198}$ FeMo protein, which was unchanged when compared with the wild type, indicating no apparent changes in the P cluster structure or its electronic environment. Similarly, no perturbation of FeMo-cofactor’s S = 3/2 EPR spectra was observed for any of the altered dithionite-reduced FeMo proteins when compared to the wild-type spectrum. Also, none of the altered MoFe proteins having substitutions at the Tyr$^{198}$ position exhibited any of the characteristic substrate reduction changes associated with perturbation of the FeMo-cofactor’s polypeptide environment (Scott et al., 1990, 1992; Kim et al., 1995; Table II). These results, together with comparable $K_m$ values for acetylene reduction for both the wild-type and His$^{198}$ MoFe protein, indicate that the lowered maximum specific activity for the His$^{198}$ MoFe protein under conditions of high flux is unlikely to occur as a result of an alteration in the substrate reduction site. However, the unusually low electron flux, plus the increased uncoupling of MgATP hydrolysis (see below) observed only under 10% $C_2H_4$ with the His$^{198}$ MoFe protein, both of which are relieved by CO, suggests that different substrates may be served by different or multiple electron-transfer pathways.

Effective nitrogenase catalysis requires the coupled hydrolysis of about four MgATP for each pair of electrons transferred to substrate. However, MgATP hydrolysis can become partially uncoupled from electron transfer under certain conditions, such as extremely low flux (Ljones and Burris, 1972; Hageman and Burris, 1978), high or low pH (Jeng et al., 1970; Imam and Eady, 1980), and high or low temperature (Watt et al., 1975; Watt and Burns, 1977). Certain amino acid substitutions that alter either component-protein interaction (Wolle et al., 1992; Seefeldt, 1994) or the substrate reduction site (Kim et al., 1995) have also been shown to uncouple MgATP hydrolysis from electron transfer. Such uncoupling of MgATP hydrolysis from substrate reduction can be explained either by the back donation of an electron from the MoFe protein to an oxidized Fe protein, called futile cycling (Orme-Johnson and Davis, 1977) or by the occurrence of MgATP hydrolysis upon component-protein interaction without electron transfer (Thorley et al., 1991). A reasonable explanation for the uncoupled MgATP hydrolysis in reactions catalyzed by the His$^{198}$ MoFe protein (Table II) is that, as a consequence of disturbing the intramolecular electron transfer pathway, the capacity for the P cluster to accept electrons becomes saturated (after one or more rounds of component-protein interaction and intramolecular electron transfer), and any further component-protein interactions result in MgATP hydrolysis but not necessarily a net electron transfer from the Fe protein to the MoFe protein.

This explanation is consistent with the biphasic nature of the apparent component-protein dissociation rate. Here electron transfer to the P cluster of the His$^{198}$ MoFe protein occurs at an initial rate comparable with the wild type only until its capacity for storing electrons is reached, at which time the apparent component-protein dissociation rate becomes substantially lowered due to a defect in intramolecular electron transfer. Moreover, the gradual absorbance decrease observed after 150 ms for the His$^{198}$ MoFe protein contrasts dramatically with the absorbance increase observed with the wild-type MoFe protein. Thus, according to the model of Lowe et al. (1993), it appears that P cluster oxidation within the His$^{198}$ MoFe protein occurs only at a relatively slow rate and is masked by the reduction of the oxidized Fe protein as it dissociates from the complex, consistent with the hypothesis that the altered MoFe protein is unable to achieve normal intramolecular electron transfer. Further, the apparent ability of the P cluster to accumulate at least two electrons is consistent with the proposed role of the P cluster as an electron storage unit and the proposed corner-to-corner disulfide link between the P cluster subfragments (Rees et al., 1993). Although the present results provide no insight as to whether the P cluster donates single electrons or electron pairs (or both) during substrate reduction, they do provide some credence to the possibility that a two-electron transfer from the P cluster to the substrate reduction site could occur during turnover.

Finally, the helix containing Tyr$^{198}$ has been suggested as one possible electron transfer pathway from the P cluster to FeMo-cofactor (Kim and Rees, 1992), in particular the portion from the P cluster-ligating Cys$^{195}$ to Tyr$^{198}$ and then through a hydrogen bond to homocitrate, which ligates the molybdenum atom of FeMo-cofactor. However, both the Phe$^{198}$ and Leu$^{198}$ MoFe proteins, each of which would be incapable of hydrogen bonding to homocitrate, exhibit rates of substrate reduction similar to wild type. Thus, this pathway could be viewed as unattractive and the simple interpretation invoked that neither the hydroxyl group nor the aromatic feature of the Tyr$^{198}$ residue is critical for productive intramolecular electron transfer. In this context, however, it should be noted that intramo-

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3 M. K. Johnson, personal communication.
molecular electron transfer rates measured for certain other proteins (reviewed by Farid et al. (1993)) are orders of magnitude faster than the rate reported for nitrogenase component-protein dissociation (Thorneley and Lowe, 1983), the rate-limiting step in nitrogenase catalysis. Consequently, a dramatic decrease in the rate of intramolecular electron transfer might be necessary to become manifested as a lower rate of enzyme turnover. Thus, because the rate of intramolecular electron transfer within the MoFe protein cannot be directly measured, it remains premature to conclude that neither the hydroxyl group nor the aromatic nature of the Tyr988 residue is involved in intramolecular electron transfer. Moreover, the significant changes in the rates of MgATP hydrolysis and catalyzed substrate reduction exhibited by the His978 MoFe protein, resulting from an introduced structural perturbation, suggest that this helix could provide a significant electron transfer pathway.

Similar studies to those described above on the residues constituting the other prosthetic group-spanning helices should provide insight into how electrons are both accommodated within and intramolecularly transferred among the redox-active moieties of the MoFe protein. Such information will be necessary to describe the complete mechanism of biological nitrogen fixation and could impact generally on our understanding of electron transfer processes in complex biological systems.

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