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Molecular insights into nitrogenase FeMoco insertion – The role of His 274 and His 451 of MoFe protein α subunit

Aaron W. Fay [a], Yilin Hu [a], Benedikt Schmid [b], Markus W. Ribbe [a,*]

[a] *Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92697-3900, USA*
[b] *Department of Biotechnology, Friedrich-Alexander-University Erlangen-Nürnberg, 91052 Erlangen, Germany*

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This paper is dedicated to the memory of Professor Ed Stiefel

Abstract

The final step of FeMo cofactor (FeMoco) assembly involves the insertion of FeMoco into its binding site in the molybdenum–iron (MoFe) protein of nitrogenase. Here we examine the role of His α274 and His α451 of *Azotobacter vinelandii* MoFe protein in this process. Our results from combined metal, activity, EPR, stability and insertion analyses show that mutations of His α274 and/or His α451, two of the histidines that belong to a so-called His triad, to small uncharged Ala specifically reduce the accumulation of FeMoco in MoFe protein. This observation indicates that the enrichment of histidines at the His triad is important for FeMoco insertion and that the His triad potentially serves as an intermediate docking point for FeMoco through transitory ligand coordination and/or electrostatic interaction.

Keywords: Nitrogenase; Biosynthesis; FeMoco; MoFe protein

1. Introduction

Nitrogenase catalyzes the nucleotide-dependent reduction of the inert atmospheric dinitrogen to the bioavailable form of ammonia (for recent reviews see Refs. [1–5]). The molybdenum (Mo) nitrogenase of *Azotobacter vinelandii* is a two-component system, comprising the iron (Fe) protein and the molybdenum–iron (MoFe) protein. The Fe protein (also designated Av2) is a homodimer (encoded by *nifH*), with one ATP binding site per subunit and a single [4Fe–4S] cluster bridged between the subunits. The MoFe protein (also designated Av1) is an $\alpha_2\beta_2$-tetramer (encoded by *nifD* and *nifK*), containing two unique metal clusters per αβ subunit pair: the [8Fe–7S] P-cluster [6], which is bridged between α- and β-subunits and ligated to six Cys ligands; and the [Mo-7Fe-9S-X-homocitrate][1] FeMo cofactor (FeMoco) [7], which is located within the α-subunit and bound to the protein by Cys α275 at the terminal Fe and His α442 at the opposite Mo that is further coordinated by an endogenous homocitrate. Nitrogenase catalysis involves complex association/dissociation between the Fe protein and the MoFe protein, during which process electrons, concomitant with ATP hydrolysis by the Fe protein, are sequentially transferred from the [4Fe–4S] cluster in the Fe protein through the P-cluster to the FeMoco in the MoFe protein, where substrate reduction takes place.

The biosynthesis of nitrogenase component proteins and complex metal clusters are controlled by the nitrogen fixation (*nif*) genes [8]. The assembly of MoFe protein occurs in two steps. First, a P-cluster-replete, yet FeMoco-depleted form is synthesized in a process that involves the expression of *nifD* and *nifK*—the structural genes encoding the α- and β-subunits of the MoFe protein, and the formation of P-cluster—likely through fusion of its substructural, [4Fe–4S] cluster units at the target location (*in situ*) in the protein [8–10]. Then, the FeMoco—assembled outside of the MoFe protein (*ex situ*) in a process that involves the participation of *nifB*, *nifE*, *nifN*, *nifH*, *nifV* and *nifQ*, is

* Corresponding author. Tel.: +1 949 824 9509; fax: +1 949 824 8551.
E-mail address: mribbe@uci.edu (M.W. Ribbe).
[1] The identity of X is unknown but it is considered to be C, O or N [7].

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inserted into its binding site, resulting in the formation of an active, holo form of MoFe protein [8]. The *ex situ* assembly of FeMoco is presumably initiated with the formation of an Fe/S core on NifB, followed by the relocation and rearrangement of the core on the NifEN complex [11–14]. Recently, we identified a Mo-free, NifEN-bound FeMoco precursor that closely resembled the core structure of the mature FeMoco [11,12]. We also showed that, coupled to the hydrolysis of ATP, the Fe protein inserted Mo and homocitrate into the precursor while it was still bound to NifEN, resulting in a fully complemented cluster that could be inserted into the MoFe protein through direct protein–protein interaction [13,14]. These recent developments on *ex situ* FeMoco biogenesis not only clarify certain aspects of the cluster assembly in nitrogenase, they may also provide useful insights into the biosynthetic strategy of complex metal clusters in other systems [15–17].

On the other hand, the mechanism of the final step of FeMoco assembly—namely, how FeMoco is inserted into its target binding site in the MoFe protein, remains largely unknown. Much of the current understanding in this regard comes from the crystallographic studies of a Δ*nifB* MoFe protein from a *nifB*-deletion strain of *A. vinelandii* [18]. In agreement with the genetically-based theory that *nifB* is the starting point of FeMoco biosynthesis, the Δ*nifB* MoFe protein is FeMoco-deficient, yet it contains normal P-clusters [18]. More importantly, the Δ*nifB* MoFe protein can be readily reconstituted, *in vitro*, into an active holo protein by addition of isolated FeMoco, suggesting that it represents a physiologically relevant snapshot of MoFe protein right before the final insertion of FeMoco [18]. In contrast to the wild-type MoFe protein, the Δ*nifB* MoFe protein contains a positively charged funnel that could allow the entry and navigation of the negatively charged FeMoco toward its target location [18]. Such a FeMoco insertion funnel is created as a result of major structural rearrangement of the αIII domain of the Δ*nifB* MoFe protein, which involves the significant repositioning of a number of residues. Some of these residues belong to a stretch of polypeptide ranging from α353 to α364 which, upon repositioning by distances up to 20 Å, forms a loop at the entrance of the funnel. Among them are the positively charged Arg α359 and Arg α361, as well as the His α362, which could serve as the first contact point for FeMoco during the insertion process. Moving into the funnel, the C_α of His α442 is relocated by a distance of approximately 5 Å and joins His α274 and His α451 to form a striking His triad (Fig. 1), which could help guide the negatively charged FeMoco to the destined location. The movement of His α442 also leads to a switch in position of this residue with Trp α444, which could help secure FeMoco at its final location. Additionally, Lys α426 is shifted by roughly 5 Å, which could serve as another anchoring point for FeMoco during insertion. These observations have led to the proposed mechanism of FeMoco insertion, which involves the initial docking of FeMoco at the entrance loop of the

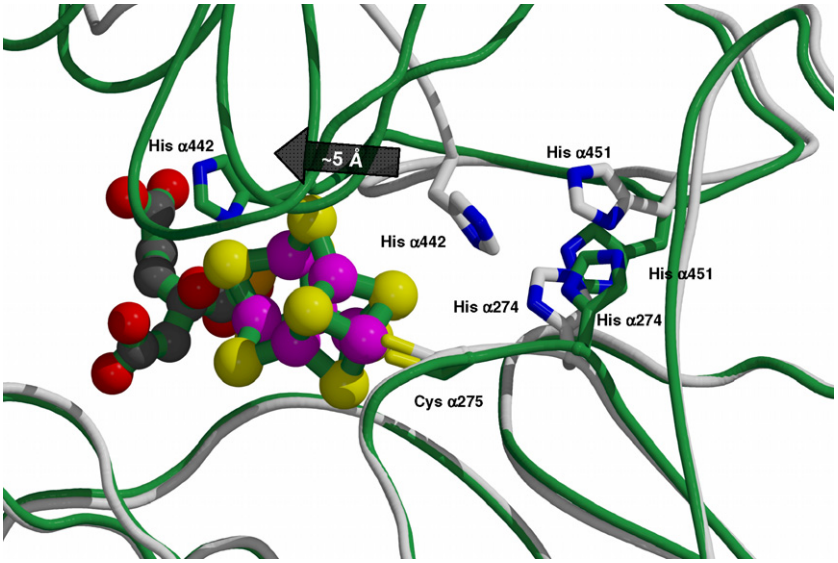

Fig. 1. Overlaid protein environment of Av1$^{\text{wild-type}}$ and Av1$^{\Delta nifB}$ in the vicinity of the FeMoco binding site. Parts of the C_α backbones of Av1$^{\text{wild-type}}$ and Av1$^{\Delta nifB}$ are shown and colored as follows: Av1$^{\text{wild-type}}$ in green and Av1$^{\Delta nifB}$ in light grey. FeMoco is shown in ball-and-stick presentation and colored as follows: oxygen in red, carbon in dark grey, molybdenum in orange, sulfur in yellow, iron in magenta. Note that, from this angle, only a small portion of the molybdenum atom is visible, while the central atom X (of unknown origin) is invisible. The side chains of His α274, His α451, His α442 and Cysα275 are shown in line presentation and colored as follows: His α274, His α451, His α442 of Av1$^{\text{wild-type}}$ in green and blue, His α274, His α451, His α442 of Av1$^{\Delta nifB}$ in light grey and blue, Cys α275 of Av1$^{\text{wild-type}}$ in green and yellow, and Cys α275 of Av1$^{\Delta nifB}$ in light grey and yellow. His α442 and Cys α275 are the two protein ligands that are covalently attached to FeMoco in Av1$^{\text{wild-type}}$. In Av1$^{\Delta nifB}$, the position of Cys α275 remains largely unchanged, whereas His α442 is re-positioned by a distance of approximately 5 Å and joins His α451 and His α274 in the formation of a striking His triad, which presumably serves to guide the FeMoco to its final location. The distances between the His triad residues, measured by the distances between their respective C2 atoms of the imidazole rings (the carbon atom between the two ring nitrogen atoms) are: (i) 3.62 Å between His α451 and His α274; (ii) 3.60 Å between His α274 and His α442; and (iii) 3.66 Å between His α442 and His α451. Additionally, His α442 can potentially form a hydrogen bond with the nearby Asp α234.