Characterization of an Altered MoFe Protein of Nitrogenase from a ΔnifV strain of
Azotobacter vinelandii

by

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**ABSTRACT**

The site of substrate binding and reduction for the nitrogenase complex is located on the iron molybdenum cofactor (FeMo-co) which is contained within the α-subunit of the molybdenum iron protein. FeMo co consists of a metal sulfur core composed of an FeS cluster bridged by three inorganic sulfides to a MoFeS cluster. An organic acid, homocitrate, is coordinated to the Mo atom through its 2-carboxy and 2-hydroxy groups. Homocitrate is formed by the condensation of acetyl-CoA and α-ketoglutarate, which is catalyzed by a homocitrate synthase encoded by *nifV*. By deleting the *nifV* gene from *Azotobacter vinelandii* we were able to study the role of homocitrate in nitrogenase catalysis. A poly-histidine tail was incorporated into the C-termini of the α-subunit permitting isolation of the homocitrateless MoFe protein by using metal affinity chromatography. We have found that the addition of a poly-histidine tag does not alter the catalytic behavior of the native enzyme. In NifV strains of *Klebsiella pneumoniae*, citrate has been found to replace homocitrate as the organic constituent of FeMo-co. We have found no evidence this is so in *A. vinelandii*. Gas chromatography mass spectrophotometry studies indicate little or no organic acids are associated with FeMo-co. We examined the catalytic properties of the NifV MoFe protein. In the mutant, H₂ evolution is inhibited by the addition of CO, unlike in the wild type. We have found that the NifV MoFe protein from *A. vinelandii* is able to catalyze the reduction of acetylene to both ethylene and ethane.
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