The Biosynthesis and Function of Nitrogenase Metalloclusters

by Patricia C. Dos Santos

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APPROVED

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D. R. Dean, chairman

_____ _____
D.R. Bevan T.J. Larson

_____ _____
S. Luckhart S. Kim

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By Patricia C. Dos Santos

Dennis R. Dean, Chairman
Biochemistry Department

(ABSTRACT)

Nitrogenase catalyzes the biological reduction of N_2 to ammonia (nitrogen fixation). The metalloclusters associated with the nitrogenase components include the [4Fe-4S] cluster of the Fe protein, and the P-cluster [8Fe7S] and FeMo-cofactor [7Fe-9S-Mo-X-homocitrate], both contained within the MoFe protein. These metal-complexes play a vital role in enzyme activity during electron transport and substrate reduction. It is known that the FeMo-cofactor provides the site of substrate reduction, but the exact site of substrate binding remains a topic of intense debate. Some models for the substrate binding location favor the molybdenum atom, while other models favor one or more iron atoms within FeMo-cofactor. We have shown that the α-70 residue of the MoFe protein plays a significant role in defining substrate access to the active site: α-70 approaches one 4Fe-4S face of the FeMo-cofactor. Substitutions at this position alter enzyme specificity for reduction of alternative alkyne substrates. These altered MoFe proteins and alternative alkyne substrates, such as propargyl alcohol, were used to trap an intermediate during substrate reduction. Further studies involving the effect of pH on substrate reduction of these altered MoFe proteins pinpointed the location of the bound substrate-derived intermediate on the FeMo-cofactor to a specific Fe atom, designated Fe6.

In addition to understanding how substrates are bound and reduced at the active site, understanding how these clusters are biologically assembled is a second point of interest. Inactivation of NifU or NifS has been shown to affect the activity of both nitrogenase components. NifS is a cysteine desulfurase that provides the sulfur for cluster formation and NifU serves as a molecular scaffold during [Fe-S] cluster assembly. Genetic and biochemical experiments involving amino acid substitutions within the N-terminal and C-terminal domains of NifU indicate that both domains can separately

participate in nitrogenase-specific [Fe-S] cluster formation. Furthermore, the NifU and NifS protein appear to have specialized functions in the maturation of metalloclusters of nitrogenase and cannot functionally replace the *isc* [Fe-S] cluster system used for the maturation of other [Fe-S] proteins. These results indicate that, in certain cases, [Fe-S] cluster biosynthetic machineries have evolved to perform only specialized functions.

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ABBREVIATIONS

[Fe-S] cluster, Iron sulfur cluster

ADP, adenosine 5’ diphosphate

Allyl-NH_2, allyl amine

Allyl-OH, allyl alcohol

ATP, adenosine 5’ triphosphate

B-media, Burk media without nitrogen source

BN-media, Burk media with nitrogen source (ammonium acetate or urea)

CO, carbon monoxide

DFT calculations, density functional theory calculation

DTT, dithiothreitol

EDTA, Ethylene diamine tetra-acetic acid

ENDOR, electron-nuclear double resonance

EPR, electron paramagnetic resonance

Fe protein, iron protein.

FeMo-cofactor, iron-molybdenum cofactor

G, Gauss

Hz, Hertz

K, Kelvin

K_m, Michaelis Menten constant

MES, 4-morpholineethanesulfonic acid

MgADP, magnesium adenosine 5’ diphosphate

MgATP, magnesium adenosine 5’ triphosphate

MoFe protein, molybdenum-iron protein

MOPS, 4-morpholinepropanesulfonic acid

mW, milli Watts

nif, nitrogen fixation gene

P cluster, [8Fe-7S] cluster of molybdenum iron protein

PAGE, Polyacrylamide gel electrophoresis

P_i, inorganic phosphate

PLP, pyridoxal 5’ phosphate

Propargyl-NH_2, propargyl amine

Propargyl-OH, propargyl alcohol

S, Electron paramagnetic spin state

SDS, Sodium dodecyl sulfate

TAPS, 3-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}-1-propanesulfonic acid

Tris-HCl, Tris(hydroxymethyl) aminomethane hydrochloride

V_{max}, maximum velocity

WT, wild type