Expressing βF99H & βF99H/βS188A Variants of Nitrogenase in Azotobacter vinelandii

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Introduction & Abstract
Nitrogen is essential for the biosynthesis of nucleic acids and proteins. Although N₂ is the most abundant gas in Earth’s atmosphere, organisms are unable to access this Nitrogen until it is fixed into ammonia (NH₃) or other reduced forms. There is only one enzyme known to catalyze the N₂ fixation process: Nitrogenase, a metalloenzyme produced by diazotrophs. In order for Nitrogenase’s active site, FeMo-cofactor (FeMoCo), to be activated, a series of electron transferences must relay electrons to FeMoCo, which is located in MoFe protein (MoFeP). Surprisingly, evidence suggests that the 8Fe-7S P-cluster, also found in MoFeP, relays electrons to FeMoCo prior to being reduced by the 4Fe-4S cluster in Fe protein (FeP). However, despite this, the P-cluster cannot perform this “deficit spending” of electrons in the absence of FeP, suggesting that conformational gating may play a role in Nitrogenase’s electron transport chain. O-based amino acid residues that can ligate the P-cluster upon its oxidation have been identified. Across many WT versions of Nitrogenase, Serine acts as a hard, O-based ligand that is important in order to ligate the P-cluster as a part of Nitrogenase’s electron transport pathway.

Method for Making Mutants
1.) PCR
2.) Transformation into E. coli
3.) Miniprep
4.) Sequencing
5.) Fe starve A. v.
6.) Transformation into A. v. cells
7.) Colony PCR
8.) Sequencing
9.) Expression

Results & Predicted Structure of the Variants
We successfully induced mutations that code for P99H and P99H/S188A variants of Mo Nitrogenase in the nifH gene. We are currently in the process of transforming these mutations into Azotobacter vinelandii, which will express the two variant enzymes. Pictured below are the predicted structures of the P-cluster & surrounding amino acid residues in P99H & P99H/S188A. For reference, the structure of the corresponding region in wild-type Mo Nitrogenase is also included.

Future Directions & Experiments
- Purification of βF99H and βF99H/βS188A
- Crystal structures of βF99H and βF99H/βS188A
- Assays on βF99H and βF99H/βS188A
  - ATP hydrolysis assay
  - H₂ production assay
  - NH₃ production assay
- Once the ATP, H₂, and NH₃ assays have been completed, we will have the data necessary to determine the relationship between ATP hydrolysis and N₂ fixation in the βF99H & βF99H/βS188A variants. This will help elucidate the role of O-based amino acids that ligate the P-cluster as a part of Nitrogenase’s unconventional electron transport pathway.

References

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