Kinetic and Electrochemical Properties of Cytochrome c Nitrite Reductase (ccNiR) Variants

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Abstract
Cytochrome c Nitrite Reductase (ccNiR, or NrfA) is a periplasmic, decahelix homodimeric enzyme that catalyzes the six-electron reduction of nitrite to ammonia (ammonification). Under physiological conditions ccNiR catalyzes the process without release of intermediates. However, in vitro we have found it possible to trap putative intermediates, or to release partially reduced nitrogen species such as nitric oxide, by controlling the electrochemical potential at which reduction takes place. Such experiments provide valuable insights regarding ccNiR-catalyzed ammonification of nitrite. After development of the ccNiR active site mutants R103Q and H257Q, we found very different reactivities with nitrite and partially reduced nitrogen species. We conclude H257Q is a key active site residue when nitrite is the substrate, possibly donating a proton to help cleave the N-O bond. The effects of R103Q are less pronounced. Interestingly, the H257 mutation doesn’t affect the catalytic turnover of hydroxylamine to ammonia nearly as profoundly as it does the nitrite reduction step. This result shows us how ccNiR tune the active site and do the conversion by using proton coupled reaction.

Methods and Materials
Construction of Site-Directed Mutants

Enzyme Expression and Purification

Results
Steady State Kinetics H257Q, R103Q and Nitrite

Steady State Kinetics WT, H257Q, R103Q ccNiR: Michaelis Menten (MM) Plot

Kinetic Values of WT and Mutant ccNiR

Conclusion
The mutations of H257 and R103 do not fully block the nitrite to hydroxylamine and hydroxylamine to ammonia conversions, and low Km values for ccNiR when nitrite is the substrate indicate that it continues to bind the protein with high affinity. The higher catalytic number for conversion nitrite to ammonia by R103Q compared to H257Q indicates the first proton which is used to cleave he N-O bond provided by the histidine. However, opposite result shown when conversion of hydroxylamine to ammonia indicate that arginine provided proton to facilitated the reaction. Spectroptotentiometry experiment show the variant histidine mutant ccNiR follow one electron reduction which also indicate the possible intermediate can be trapped to draw the mechanism.

Reference
1. Hydrogated Binding NirAase Tube Protein-Coupled. A. Andrew Pacheco, Biochemistry, 2014

Acknowledgments
Funding