Periplasmic Nitrate Reductase as a Model Molybdenum Enzyme: Mechanism, Oxygen Atom Transfer, and Substrate Promiscuity

Partha Basu,

Indiana University Indianapolis

Abstract.

Nitrate reduction is the first step in transforming nitrogen from its most oxidized state into biologically accessible forms and is therefore a key process in the global nitrogen cycle. Periplasmic nitrate reductase (NapA) is a mononuclear molybdenum enzyme that catalyzes the reduction of nitrate to nitrite and serves as an important model system for understanding oxygen atom transfer (OAT) chemistry in biology. While the structures of NapA and related enzymes have been known for many years, several aspects of their catalytic mechanisms remain subjects of active investigation.

This presentation will discuss recent studies of recombinant NapA from *Campylobacter jejuni* that combine structural, spectroscopic, biochemical, isotopic-labeling, and kinetic approaches. These studies provide new insights into the nature of the molybdenum center and its role in catalysis. The results are consistent with a terminal oxo ligand in the catalytically relevant Mo(VI) state and support a mechanism involving oxygen atom transfer during nitrate reduction. Isotope-labeling experiments, phosphine oxidation reactions, and kinetic isotope effect measurements offer a framework for understanding the elementary steps involved in catalysis.

The presentation will also describe the ability of NapA to catalyze reactions beyond nitrate reduction, including nitrite oxidation and the transformation of several alternative oxyanion substrates. Together, these observations highlight the reversible nature of oxygen atom transfer at the molybdenum center and provide insight into the factors that influence substrate recognition and reactivity. More broadly, the work illustrates how studies of NapA can inform our understanding of the chemistry and functional diversity of mononuclear molybdenum enzymes.