Side-on Cu-Nitrosyl Coordination by Nitrite Reductase

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Nitric oxide (NO) is one of the smallest and simplest biologically active molecules. In mammals, NO is produced from arginine by isoforms of nitric oxide synthase, and it functions in signal transduction and as a cytoprotective or cytotoxic agent. In bacteria, NO is produced by nitrite reductase (NiR), a copper-containing enzyme, which is responsible for the reduction of nitrite to nitric oxide (NO) in the process of dissimilatory denitrification. Cu-containing NiRs are homotrimers with two distinct Cu sites per monomeric unit (*1*). The type 1 Cu site buried within each monomer relays electrons from external donors such as pseudoazurin to the type 2 Cu site where nitrite is reduced to NO.

Towards understanding the mechanism of NiR, the crystal structures of the substrate and product bound enzyme were determined (*2*). Beamline 7-1 at Stanford Synchrotron Radiation Laboratory (SSRL) played a significant role to determine these structures to the resolution required to define the mode of binding of the ligands to the active site copper. The structure of substrate-soaked NiR crystals to 1.4 Å resolution revealed an asymmetric *O*-coordination of nitrite to Cu. The bent *O*-coordination shows an almost face-on interaction of nitrite with the metal. The binding mode of the substrate places the N atom of nitrite in close proximity to catalytically important residues and the copper, thus facilitating in NO formation.

Due to their instability and sensitivity to oxygen, few Cu-nitrosyl complexes are well characterized. To obtain the product-bound NiR, crystals were reduced with ascorbate and exposed to NO in an anaerobic environment. The 1.3 Å resolution structure (Fig.1) revealed an unprecedented binding mode of NO to Cu. The side-on binding places the O and the N atom equidistantly from the metal. The Cu-nitrosyl of NiR is the first such complex in a biological system to be characterized with side-on coordination of a diatomic molecule. Comparison of the nitrite and NO complexes suggests a mechanism for NiR that resolves the paradox of how an *O*-coordinated nitrite can give rise to an *N*-coordinated nitrosyl during catalysis.

This mode of binding of NO to a copper expands the possibilities for NO interactions in other copper proteins such as superoxide dismutase and prions. Interaction of nitric oxide to Cu has been proposed in models of some neurodegenerative diseases. *In vitro* studies show that

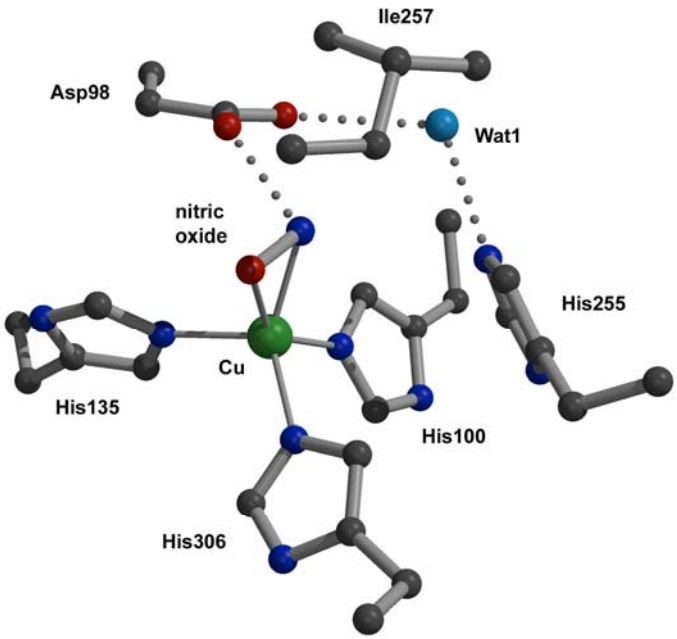

Figure 1. Overview of the essential features of the active site of NiR bound with nitric oxide. Metal-ligand bonds (solid), as well as H bonds and other electrostatic interactions (dashed), are shown as grey lines of the type indicated. Carbons (black), oxygens (red), nitrogens (blue), type 2 Cu (green), and waters (cyan) are colored as indicated.

the formation of a Cu-nitrosyl in variant forms of superoxide dismutase (SOD) yields highly reactive radicals that are implicated in some types of amyotrophic lateral sclerosis (*3*). Interestingly, the type 2 Cu sites of NiR and SOD share structural and functional features (*4, 5*). In reduced state, the SOD Cu is coordinated by three histidyl residues in an approximately tetrahedral arrangement, as is the type 2 Cu of NiR. A fourth histidine of SOD, which links the Cu and the Zn sites, superimposes with a catalytically important residue His255 of NiR. Furthermore, NiR from *A. xylosoxidans* is reported to have SOD activity (*5*). More recently, Cu binding to prion proteins was linked to the production of nitric oxide that autocatalyzes the removal of glypican 1-heparin sulfate side chains, a process associated with the formation of amyloid deposits (*6*). Thus, a better understanding of the binding and activation of NO by copper sites should provide insight into these destructive neural processes.

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