

# Visualization of small molecules bound to copper nitrite reductase

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The process of denitrification involves the sequential reduction of nitrate and nitrite to dinitrogen via the following pathway:



The nitrite reductase from *A. faecalis* (NiR) is a green 110 kDa homotrimer with each monomer containing one type I and one type II copper sites. The type I Cu is the site of electron transfer from pseudoazurin. Electrons are then donated internally to the type II Cu site, where  $\text{NO}_2^-$  is reduced to NO. In the presence of chemical reductants and nitrite, NiR is able to further reduce NO to  $\text{N}_2\text{O}$ .

To visualize the binding mode of nitrite, nitric oxide and nitrous oxide, crystals structures of NiR in complex with these molecules were solved to 1.5 Å resolution or better. Nitrite binds to the Cu (II) primarily by one of its oxygens; the second oxygen and the nitrogen atoms are situated ~2.3 Å away from the cofactor, thus positioning nitrite almost face-on with respect to the metal. The close proximity of these non-coordinating atoms would facilitate the production of nitric oxide since only small conformational changes are necessary at the active site. To visualize the binding mode of the product, NiR crystals were reduced with ascorbate, exposed to NO saturated solution and frozen in a glove box. Refinement of this structure revealed a molecule of NO bound at the active site Cu in side-on coordination, where the N and O atoms are equidistant from the metal. During the NiR-catalyzed reduction of  $\text{NO}_2^-$  to NO,  $\text{N}_2\text{O}$  is produced if NO is not removed from the reaction vessel. Under these conditions, a Cu-NO intermediate is proposed to accumulate and to react with  $\text{NO}_2^-$  or with NO derived from  $\text{NO}_2^-$  to yield  $\text{N}_2\text{O}$ . To examine the ability of NiR to bind to nitrous oxide, crystals were soaked in a saturated  $\text{N}_2\text{O}$  solution. In contrast to nitrite and similar to nitric oxide, nitrous oxide bound to the copper via two of its atoms. Asp98 is a catalytically important residue that provides a proton during catalysis. The carboxylate of Asp98 forms a hydrogen bond to both bound substrate and product. In the  $\text{N}_2\text{O}$  bound NiR, this H-bond is not present and Asp98 has undergone a conformational change.