A Copper Nitrosyl in Nitrite Reductase

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Nitrite reductase (NiR) is an enzyme that reduces nitrite to nitric oxide as part of the global nitrogen cycle. Two distinct families of NiRs are found in nature. One family employs two types of copper cofactors whereas the other family uses a pair of heme (cd_1) cofactors. The reaction in the well-characterized heme enzyme is initiated by N-coordinated nitrite binding to the ferrous d_1 heme. Protonation yields a ferric nitrosyl which dissociates before electron transfer from the *c* heme.

In Cu containing NiRs, electron transfer from external donors is mediated by a type 1 Cu site and nitrite reduction occurs at a type 2 Cu site. Evidence for a copper nitrosyl intermediate in Cu containing NiRs suggests a parallel mechanism to the cd_1 NiRs may operate in these enzymes as well. To gain insight into the mechanism of Cu NiR, crystal structures to beyond 1.4 Å resolution were obtained of the Cu(II) nitrite complex and of the Cu(I) nitric oxide complex. Unexpectedly, nitrite and nitric oxide are bound in an almost face-on and side-on coordination to the copper. In contrast, the inhibitor azide binds end-on to the type 2 copper. Also, acetate and nitrate coordinate through both oxygens (bidentate), whereas nitrite is coordinated by a single oxygen that forms an H-bond to the active site aspartate, an interaction that is likely to be essential for efficient catalysis. Crystal structure of active site mutants show that the aspartate along with a nearby non-coordinating histidine are found to control binding of nitrite to the type 2 Cu and to largely define the chemical reactivity of NiR. The crystallographic work supports an alternative mechanism where nitrite binds to Cu(II) and the reaction is initiated by electron transfer from the type 1 site.

Type 2 copper sites are found in a variety of versatile oxidoreductases that mediate reactions involving oxygen or nitrogen oxides and are found throughout all branches of life. Interestingly, NiR is able to reduce oxygen to hydrogen peroxide in vitro, eventually leading to enzyme inactivation. The NiR type 2 copper site shares a similar coordination sphere to those of superoxide dismutase and amine oxidases suggesting the possibility of common mechanistic features.