# Copper in the Transduction of $NO_x$-derived Signals: Mechanisms and Biological Implications

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The biological chemistry of nitrogen oxides ($NO_x$) has been under intense investigation since the discovery that nitric oxide (NO) influences key biological functions including vasodilation and neuronal signaling. The NO signal is transduced in part *via* the posttranslational S-nitrosation of cysteine residues. Low-molecular-weight S-nitrosothiols, such as S-nitrosoglutathione (GSNO), are speculated to S-nitrosate protein thiols *in vivo*. We have found that trace copper, added as either $CuSO_4$ or Cu,Zn-superoxide dismutase (CuZnSOD), is required as a redox catalyst in NO transfer between GSNO and the physiologically abundant proteins, hemoglobin and human brain calbindin D28k (HCalB). CuZnSOD is a more efficient and selective catalyst than $CuSO_4$, and mechanisms of copper-catalyzed protein S-nitrosation by low-molecular-weight species will be discussed with emphasis on the proposed role of NO in sensing anoxia.

One-electron reduction of NO gives nitroxyl (HNO), which also is biologically active. HNO is a versatile modifier of protein thiols as will be shown using ESI mass spectra of protein incubates with the HNO donor, Angeli's salt (*AS*) ($HN_2O_3^- \rightarrow HNO + NO_2^-$). Control $NaNO_2$ incubates confirmed that HNO is the reactive species at physiological pH, and addition of EDTA demonstrated that HNO-mediated protein derivatization is not likely metal catalyzed. Prior blocking with the thiol-specific reagent, *N*-ethylmaleimide, revealed that protein thiols are the main targets of HNO and four distinct derivatives were detected. Thiols in HCalB and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were converted to sulfinamides ($RSONH_2$), and intramolecular sulfinamide crosslinking of Cys34 to neighboring Lys and/or Arg residues occurred in bovine serum albumin (BSA). HNO also induced S-cysteinylation of HCalB in the presence of free cysteine, and intramolecular disulfide (Cys149-Cys153) formation in GADPH. The previously reported orthogonal bioactivity of HNO and NO donors will be discussed in light of the results presented here.