# Zinc Binding Studies on Yeast, Bovine and Human SOD1 Reveal Differences Between the Apo Structures

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Copper-zinc superoxide dismutases (SOD1s) are 32 kDa homodimeric proteins that bind one copper and one zinc ion per monomer. Yeast SOD1 has been shown previously to stand apart from bovine and human SOD1s in that it has a "phantom subunit", a monomer that cannot be remetallated *in vitro* under conditions where bovine and human SOD1s dimers can be fully reconstituted. Further investigations into the binding of zinc to these three proteins reveal additional dissimilarities in the thermodynamics of binding zinc to the polypeptides as well as effects on the overall structure upon metal binding. H/D exchange and protein modification assays on bovine and human SOD1 demonstrate asymmetry in the structural effects of zinc binding, where the first zinc has a much more profound effect on the dimeric structure than the binding of the second ion. Yeast SOD1 shows a much more severe "one zinc effect" where the second monomer does not bind zinc. Isothermal titration calorimetry (ITC) experiments were used to determine the thermodynamic parameters of zinc binding to the apo proteins. Significant differences in the binding constants, enthalpies and entropies of zinc binding were found, suggesting that zinc binding to all three SOD1 proteins is more complex than zinc binding to a preformed empty binding site. Indeed, ITC experiments on bovine SOD1 carried out over a wide range of temperatures show a large, negative change in heat capacity indicating a change in solvent accessible surface area. These results suggest that zinc binding induces a structural rearrangement. A plot of observed enthalpies versus temperature observed by ITC experiments on the yeast SOD1, however, shows significant curvature across temperatures well below its melting point. These data suggest an even more complicated metal binding linked protein folding event than seen for bovine SOD1. Implications for metal binding processes *in vivo* are discussed.