Rational Design of Galactose Oxidase Mimetic Proteins

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Over the last 15 years, a new class of enzymes containing covalently crosslinked amino acid cofactors has emerged. In galactose oxidase (GO), new bond forming reactions result in the formation of a tyrosine-cysteine(YC) crosslinked cofactor that, along with copper, carries out two-electron redox chemistry. Using a geometry based bioinformatics approach, and the YC crosslink as a model, we were able to predict amino acid mutations in 500 structurally resolved proteins that could potentially lead to crosslink formation. In order to explore the chemical criteria for formation of the YC crosslink, five of the predicted sites in rat intestinal fatty acid binding protein (IFABP) were selected for experimental analysis and the DNA constructs for these sites were generated using recombinant DNA methods. Two oxidative methods, photooxidation by d^6 polypyridal complexes and peroxidase chemistry, are being used in order to model oxidative stress conditions. We have seen initial evidence for YC crosslink formation through MALDI analysis of proteolytic digests. This data is further supported by evaluating the loss of free thiols in the oxidized samples using 5',5-dithiobis(2-nitro-benzoic acid). Further analysis of crosslink formation will use NMR and LC/MS. These results will be discussed.