DEPC Modification of the CuA Protein from *Thermus thermophilus*

**Abstract:** The CuA protein is the soluble portion of subunit II of Cytochrome c Oxidase. It contains a dinuclear copper center that contains two bridging cysteines, and is ligated by two histidines, a methionine, and a carbonyl in the peptide backbone of a glutamine residue. Diethyl pyrocarbonate (DEPC) is a common chemical modifier that reacts with deprotonated histidines, lysines, the N-terminus and tyrosines. DEPC was used to probe if one or both of the ligating histidines would react with DEPC. UV-Visible and Visible Circular Dichroism were used to monitor the reaction. At least one ligating histidine becomes modified by DEPC and use of fewer equivalents of DEPC show that the histidine is highly reactive, requiring around 5 equivalents. Additionally, electrochemical techniques have been developed that allow the determination of the reduction potential of the modified protein. The reduction potential increases by nearly 70 mV upon modification. These results all point to the role of the ligating histidines in modulating the redox properties of this cluster. Mononuclear proteins, including azurin and Sco, were also tested and these proteins do not display ligating histidines reactive toward DEPC. Thus, DEPC reactivity is not a property of all ligating histidines.