Engineering Human Copper-Zinc Superoxide Dismutase for therapeutic application

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Copper-zinc superoxide dismutases (CuZnSOD) are found in both eukaryotes and in prokaryotes. Although the overall protein fold for these enzymes isolated from the two classes is similar they differ in their quaternary assemblies. Eukaryotic CuZnSOD are predominantly found as dimers whereas the prokaryotic enzymes are monomeric. Subcellular localisation is also markedly different for the two classes. Eukaryotic CuZnSOD is invariably found in the cytosol of the cell while prokaryotic CuZnSOD is found in the periplasmic space. The latter finding is relevant to the known requirement for a disulphide bond in the monomer which can readily form in the periplasm but is less likely to form in the reducing conditions of the prokaryotic cytosol.

We are investigating the interesting differences between human and bacterial CuZnSODs. We have constructed a chimeric form of the protein to see if some properties of the bacterial enzyme may be acquired by the human construct. Some properties may give the constructed protein potential as a human therapeutic agent. We will express wild-type human and our chimeric CuZnSODs after sub-cloning into a very efficient expression vector. A novel strain of *Escherichia coli* genetically engineered to promote disulphide bond formation will be the host for expression studies. The enzymes will then be purified to very high degree in order to study the physicochemical properties further. This will include measurements of molecular mass (both primary and quaternary), structural integrity, enzymatic activity and efficiency and three dimensional structure of the chimeric form may be determined. Techniques which will be utilised to carry out this project include PCR (polymerase chain reaction), transformation, cell culture and harvesting, affinity column chromatography, polyacrylamide gel electrophoresis, size exclusion chromatography, circular dichroism spectrometry, spectrophotometry (including stopped-flow) and protein crystallisation.