Expression, Purification and Characterisation of Human Copper-Zinc Superoxide Dismutase Protein and Human-Escherichia coli Copper-Zinc Superoxide Dismutase Chimeric Protein

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Introduction

Superoxide dismutases (SODs) represent the first line of defense to counter oxidative stress caused by superoxide radicals, $O_2^-$, these being the initial reduction products of oxygen, by catalysing their dismutation into $O_2$ and $H_2O_2$. The latter is further decomposed by peroxidases and catalases. CuZn SOD has been considered as being almost exclusively a dimeric eukaryotic enzyme. However, CuZn SODs adopting the monomeric configuration have recently been isolated from E. coli. Since the monomeric bacteriocuprein activity was determined to be comparable to that of the dimeric protein, this showed that the subunit interaction does not play a role in the protein’s activity.[2] Hence, the human/ E. coli CuZn SOD chimeric protein (L-Loopy SOD), has been genetically engineered, in an attempt to produce a stable, active, monomeric human CuZn SOD, for therapeutic applications. The human wild-type CuZn SOD has also been mutated to express this dimeric protein (L-1 SOD) in E. coli cytoplasm.

Methodology

E. coli CodonPlus® cells, containing either L-1 or L-Loopy SOD, were used to overexpress these proteins. Following the extraction of both soluble L-1 and L-Loopy SODs, their activity was determined, followed by the modeling of their 3-D structures. Czion purification schemes were attempted on both proteins, with L-1 SOD acting as the control. Heat denaturation experiments, followed by metal chelation affinity chromatography, isolated the target proteins. Furthermore, the bulk of impurities and background E. coli proteins were eliminated by ion exchange chromatography, carried out at different KP molarities and different pHs. Gel filtration acted as the final polishing step. Protein characterisation was also performed so as to determine molecular weights, and hence whether L-Loopy SOD was adopting the monomeric or dimeric configuration. Peak wavelengths measured in the visible region of the electromagnetic spectrum were determined so as to indicate whether L-Loopy SOD active site was altered.

Results

A high degree of purity for both proteins was achieved by developing efficient, multi-step purification schemes. Ion exchange chromatography at different pHs resulted in the precipitation of L-Loopy SOD, hence concluding that this chimeric protein is more pH sensitive than L-1 SOD. 2.5 mL of 100% pure L-1 SOD protein (2.91 mg/mL) and 2 mL of 90.3% pure L-Loopy SOD protein (0.55 mg/mL) were achieved. The low absorbance value and the shift in the maximum absorbance to a lower wavelength observed in the absorption spectrum of L-Loopy SOD indicated that the introduction of the bacterial amino acid loop into the protein (2.91 mg/mL) and 2 mL of 90.3% pure L-Loopy SOD protein (0.55 mg/mL) were achieved. The low absorbance value and the shift in the maximum absorbance to a lower wavelength observed in the absorption spectrum of L-Loopy SOD indicated that the introduction of the bacterial amino acid loop into the protein (2.91 mg/mL) and 2 mL of 90.3% pure L-Loopy SOD protein (0.55 mg/mL) were achieved. The low absorbance value and the shift in the maximum absorbance to a lower wavelength observed in the absorption spectrum of L-Loopy SOD indicated that the introduction of the bacterial amino acid loop into the protein (2.91 mg/mL) and 2 mL of 90.3% pure L-Loopy SOD protein (0.55 mg/mL) were achieved. The low absorbance value and the shift in the maximum absorbance to a lower wavelength observed in the absorption spectrum of L-Loopy SOD indicated that the introduction of the bacterial amino acid loop into the protein (2.91 mg/mL) and 2 mL of 90.3% pure L-Loopy SOD protein (0.55 mg/mL) were achieved. The low absorbance value and the shift in the maximum absorbance to a lower wavelength observed in the absorption spectrum of L-Loopy SOD indicated that the introduction of the bacterial amino acid loop into the protein (2.91 mg/mL) and 2 mL of 90.3% pure L-Loopy SOD protein (0.55 mg/mL) were achieved. The low absorbance value and the shift in the maximum absorbance to a lower wavelength observed in the absorption spectrum of L-Loopy SOD indicated that the introduction of the bacterial amino acid loop into the protein (2.91 mg/mL) and 2 mL of 90.3% pure L-Loopy SOD protein (0.55 mg/mL) were achieved.

Conclusions

Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology

References