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, $O_2^{\cdot-}$, these being the initial reduction products of oxygen, by catalysing their dismutation into O_2 and H_2O_2.[1] 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. C_{IPP} 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

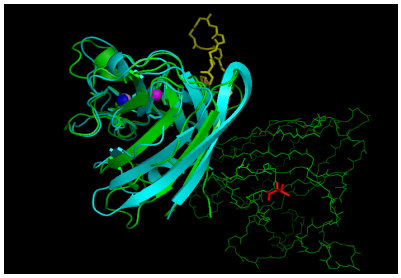
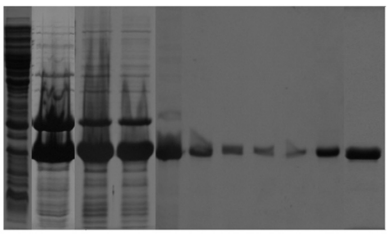
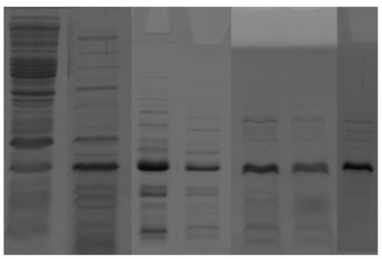


Fig. 1: Human CuZn SOD dimer (depicted in green) superimposed on the *E. coli* CuZn SOD monomer (depicted in cyan). The extra loop shown in yellow was introduced, by site directed mutagenesis, into the human dimeric protein at the site shown in red.



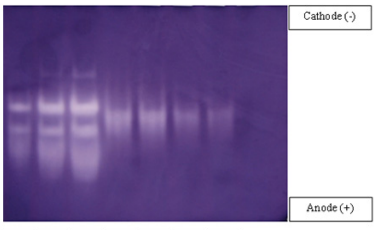
Lane number	1	2	3	4	5	6	7	8	9	10	11
Protein Loaded	Lysate	Heat Denatured at 75°C	T.W.	S.W	5 mM	10 mM	20 mM	30 mM	40 mM	50 mM	Gel Filtration
			Metal Chelation Affinity Chromatography		Ion Exchange Chromatography at different KP molarities						
% Purity	3.14	36.14	36.68	48.10	70	100					100
Protein concentration (mg/mL)	148	186	125	100	5.38	4.70					2.91

Fig. 2: SDS PAGE analysis of L-1 SOD purification process, using IEC at different KP molarities.



Lane number	1	2	3	4	5	6	7
Protein loaded	Lysate	Heat Denatured at 75°C	T.W.	S.W.	Wash 1	Wash 2	Gel Filtration
			Metal Chelation Affinity Chromatography		Ion Exchange Chromatography at 50 mM KP		
% Purity	5.95	12.21	20.63	21.95	53.00	54.47	90.27
Protein concentration (mg/mL)	139	125	112	90	6.37	6.42	0.55

Fig. 3: SDS PAGE analysis of L-Loopy SOD purification process, using IEC at 50 mM KP pH 7.8.



Lane number	1	2	3	4	5	6	7

Fig. 4: 8% Native PAGE gel showing that both L-1 SOD and L-Loopy SOD retained their activity following their purification processes.

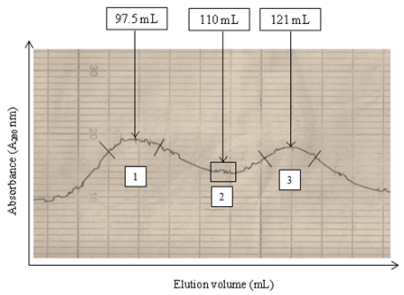


Fig. 5: Gel filtration chromatogram depicting the elution volume of L-Loopy SOD protein.

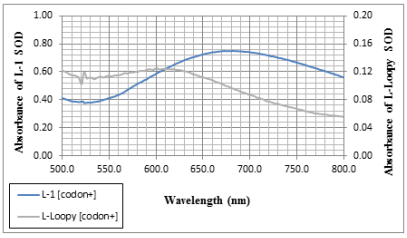


Fig. 6: Curve showing the absorbance values of L-1 SOD and L-Loopy SOD on the y axis against the wavelength values on the x axis.

Conclusions

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 human dimeric CuZn SOD, altered its active site. However, this mutation had no effect on the activity of this protein, as depicted by Native PAGE gels. The molecular weights of L-1 SOD and L-Loopy SOD were determined to be 32,027 Da and 34,995 Da, respectively. However, L-Loopy SOD also eluted at a volume which corresponded to the molecular weight of the monomeric configuration, and the formation of an equilibrium between the monomeric and the dimeric form is subject to further research studies.

References

1. Parker, M. W.; Schinina, M. E.; Bossa, F.; Bannister, J. V. Chemical Aspects of the Structure, Function and Evolution of Superoxide Dismutases. *Inorganica Chimica Acta*. **1984,** 91, 307-317.
2. El Shafey, H. M.; Bahashwan, S. A.; Alghaithy, A. A.; Ghanem, S. Microbial superoxide dismutase enzyme as therapeutic agent and future gene therapy. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*. **2010,** 435-440.