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Structure and function studies of recombinant clusterin

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Structure and Function Studies of Recombinant Clusterin

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DECLARATION

This thesis is submitted in accordance with the regulations of the University of Wollongong in fulfilling the requirements for the degree Master of Biotechnology - Research. It does not incorporate any material previously published or written by any person except where due reference is made in the text. The experimental work described in this thesis is original work and has not been previously submitted for a degree or diploma at any university.

Yvonne Teresa González Cendales

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ABSTRACT

Clusterin is a ubiquitous secreted glycoprotein that acts as an extracellular chaperone. It effectively inhibits stress-induced protein aggregation by an ATP independent mechanism, binding to partially unfolded proteins and forming soluble high molecular weight complexes with them (Wilson and Easterbrook-Smith, 2000). A model proposed by Yerbury *et al.* (2005) suggested that if clusterin mediates the clearance and degradation of misfolded extracellular proteins *in vivo* and this action is anti-inflammatory, a recombinant form of clusterin might be therapeutically useful to clear protein debris from sites of disease and inflammation and in this way suppress pathology. The expression of recombinant mammalian proteins is often performed using mammalian or insect cells because some post-translational processes (glycosylation, phosphorylation, oligomerization, specific protein cleavage and disulfide bond formation) are not performed by bacterial cells (Sambrook *et al*., 1989). However, since Stewart *et al*. (2007) showed that clusterin retains its chaperone function when deglycosylated and that deglycosylation did not induce major structural changes in the molecule, the possibility of producing chaperone-active human clusterin in bacteria became apparent. This thesis describes an attempt to produce a chaperone-active recombinant clusterin expressed in bacteria to provide a large scale supply of protein useful in therapeutic applications. In addition, two different commercially available recombinant clusterin proteins were compared in structure and chaperone function with human serum clusterin to provide additional insights into any functional limitations of recombinantly expressed clusterin proteins and to supply the information to the manufacturer.

Firstly, the expression of clusterin - MBP fusion protein was attempted in *E. coli* TBI but full length recombinant clusterin - MBP fusion protein was only detected as a minor species; a large part of the product was cleaved from MBP during expression and most of the MBP-clusterin fusion protein was degraded with time. Thus, because it was not possible to obtain a source of stable clusterin - MBP fusion protein, the expression of clusterin – 6xHis tag fusion protein was attempted.

Gateway® cloning technology was used for cloning and expressing clusterin – 6xHis tag fusion protein in *E. coli*. After expression, the protein was extracted and purified under denaturing conditions. The purified protein was subsequently refolded in 50 mM NaH_2PO_4, 300 mM NaCl, 1 mM DTT, 10% glycerol, 0.1% azide, alkylated with 50 mM IAA and dialyzed prior to developing functional and structural analyses. After expression in *E. coli* TBI, clusterin – 6xHis Tag fusion protein was detected along with disulfide-bonded high molecular weight aggregates and putative truncated products. While structural studies by CD revealed that the

predicted secondary structure content of clusterin – 6xHis tag fusion protein and plasma-derived clusterin were similar, functional analysis revealed that the former had little if any chaperone activity (its limited effects on the heat-induced precipitation of fibrinogen were not dose-dependent).

Structural (SEC, CD) and functional analyses (chaperone assays and IgG binding test) of Flag-Tagged full-length recombinant human clusterin (expressed in human embryonic kidney cells) and His-Tagged partial-length recombinant rat clusterin (expressed in *E. coli,* not post translationally processed, thus not cleaved into α and β chains and not glycosilated) (both commercial proteins from BioVendor- Laboratory Medicine, Inc.) indicated that the recombinant proteins tested had (i) an increased proportion of high molecular weight species when compared with plasma-derived clusterin, (ii) predicted secondary structure content similar to that of plasma-derived clusterin, (iii) chaperone activity but were less effective than plasma-derived clusterin (recombinant human 28%, recombinant rat 48% less effective) and (iv) were able to bind IgG, the IgG binding activity was dose-dependent and similar to that of plasma-derived clusterin. Furthermore, the results obtained for recombinant rat clusterin suggested that the expression of chaperone active clusterin in bacteria is possible and that cleavage into α and β chains and the C-terminus of the beta chain (residues 22-145) are not required for chaperone or IgG binding activity. Further studies will be required to optimize production of a chaperone active form of human clusterin in bacteria.

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ABBREVIATIONS

Bovine serum albumin	BSA
Citrate synthase	CS
Dithiothreitol	DTT
Iodoacetamide	IAA
Heat-denatured casein	HDC
Immunoglobulin G	IgG
Phosphate buffered saline	PBS
Tris EDTA	TE
Maltose Binding Protein	MBP
Lauria Bertani	LB
Isopropylthio-β-galactoside	IPTG
Tris Acetate EDTA	TAE
PBS/0.1% sodium azide	PBS/Az
Sodium dodecylsulphate polyacrylamide gel electrophoresis	SDS-PAGE
Size Exclusion Chromatography	SEC
Circular dichroism spectroscopy	CD
Millidegrees	mdeg
mean residue ellipticity	θ_{MRE}

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