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Investigations into the biochemical basis for the formation of the barrier in the aging human lens

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Investigations into the Biochemical Basis for the Formation of the Barrier in the Aging Human Lens

A thesis submitted in fulfilment of the requirements for the degree of

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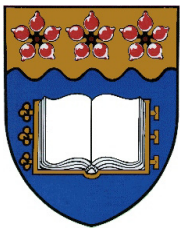

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Declaration

The work described in this thesis does not contain any material which has been accepted for the award of any other degree or diploma in this or any other University and to the best of my knowledge and belief contains no material previously published by any other person, except where due reference has been acknowledged.

M.G. Friedrich

April, 2009

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Publications

Sections of the work described in this thesis have been reported in the following publications:

1. Heys, K., M. Friedrich, and R.J.W. Truscott, *Presbyopia and heat: changes associated with aging of the human lens suggest a functional role for the small heat shock protein, alpha-crystallin, in maintaining lens flexibility.* Aging Cell, 2007. **6**(6): p. 807-815.

2. Heys, K.R., M.G. Friedrich, and R.J.W. Truscott, *Free and Bound Water in Normal and Cataractous Human Lenses.* Invest. Ophthalmol. Vis. Sci., 2008. **49**: p. 1991-1997.

3. Friedrich M.G. and R.J.W. Truscott, *Membrane association of proteins in the aging human lens: profound changes take place in the 5th decade of life*. Invest. Ophthalmol. Vis. Sci. (Accepted manuscript with revisions).

Abstract

The aging lens is characterised by a variety of physical changes, such as stiffening of the lens core and the formation of the barrier to diffusion. The lens may be unique in that proteins formed prior to birth are present for the lifetime of the individual. As we age increasing amounts of crystallins becoming more insoluble. It is assumed that changes in protein integrity are caused by the post-translational modifications of lens crystallins over time decreasing their solubility and resulting in aggregation.

In this thesis, protein solubility was examined in four regions of the human lens (outer, barrier, inner and core) and was found to depend on age and region of the lens. The barrier region displayed a gradual decrease of water soluble protein (WSP). The core and inner regions differed from the barrier with the majority of soluble protein decreasing between the age of 40 and 50. By age 50 *ca.* 50% of protein in the core was insoluble and increasing amounts of protein appeared to be associated with membranes.

In order to examine the associations of protein with membranes further samples were examined by sucrose density gradient centrifugation. Distinct protein density patterns were observed in the barrier and outer, and inner and core regions. With age progressively more protein was found to sediment at higher densities. Mass spectrometry was used to examine membrane lipids in each protein interface in the core. Remarkably by the age of 50 the majority of core lens lipids were associated with high density protein bands. The barrier region was different, with most aggregation not associated with lens membranes.

HPLC demonstrated that, prior to the huge protein and membrane density changes in the lens core, high molecular weight (HMW) protein increased until age 30 and then decreased. This loss of HMW was accompanied by a near total loss of α-crystallin by the age of 40. These results are consistent with α-crystallin acting as a molecular chaperone. It was only when α-crystallin was lost and HMW protein had decreased that changes in protein and membrane density were observed. In the barrier region α-crystallin was found to be present even in relatively old lenses, suggesting that α-crystallin may hinder the interaction of lens membranes with crystallins.

To understand the molecular basis for changes in protein density, the highest

density bands of lens protein in a sucrose gradient were analysed by iTRAQ^{TM}. Preliminary experiments showed substantial increases in αB, β-crystallins and γ-crystallins at the interface between 70 and 80% sucrose (SG1). Oddly αA crystallin did not change with age which may be because of an initial high amount of this protein in the most dense band by age 50. At the interface between 60 and 70% sucrose (SG2) there was a slight decrease of both αA and αB crystallin. Interestingly, cytoskeletal proteins were found in both SG1 and SG2 further indicating the presence of membranes at these high density interfaces. The SG2 interface from the barrier, similar to SG1 from the core, displayed substantial increases in β-crystallins and γ-crystallins.

An important finding from this thesis was that thermal denaturation of lens crystallins could lead to similar density changes to those observed in the aging human lens. These age-related changes could be mimicked simply by heating young intact human lenses at 50 ºC. Indeed, these findings may provide a biochemical reason for the formation of the lens barrier at middle age. Large scale binding of denatured proteins to lens membranes after middle age may cause occlusion of integral membrane pores such as aquaporin and connexons.

The human lens increases in stiffness with age and has been associated with presbyopia. The loss of α-crystallin coincided with its incorporation into HMW and insoluble protein at a time when large increases in lens stiffness occurred. Incubation of porcine lenses at 50 ºC mimicked (reproduced) these changes and suggests that α-crystallin through acting as a molecular chaperone may help maintain lens flexibility. These results also suggest that presbyopia may be the result of loss of α-crystallin in the lens centre as a result of thermal denaturation of lens crystallins.

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Abbreviations

1D	one-dimensional
2D	two-dimensional
APAF	Australian Proteome Analysis Facility
AQP0	aquaporin 0
BHSM	betaine-homocysteine S-methyltransferase
BHT	butylated hydroxytoluene
α –crystallin	alpha-crystallin
β –crystallin	beta-crystallin
γ –crystallin	gamma-crystallin
CR	carbonyl reductase
DMA	Dynamic Mechanical Analysis
DTT	1,5-dithiolthreitol
EDTA	ethylenediaminetetraacetic
EGTA	ethylene glycol tetraacetic acid
ESI-MS	electrospray-ionisation mass spectrometry
FA	formic acid
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GSH	reduced glutathione
HCl	hydrochloric acid
HMW	high molecular weight
HPLC	high performance liquid chromatography
MALDI	matrix-assisted laser desorption ionisation
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MW	molecular weight
m/z	mass-to-charge ratio
NaCl	sodium chloride
NaOH	sodium hydroxide
NH_4HCO_3	ammonium Bicarbonate
PMF	peptide mass fingerprinting

PTMs	post-translational modifications
RD	retinal dehydrogenase
SD	sorbitol dehydrogenase
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate polyethylene glycol
TGA	thermo-gravimetric analysis
TOF	time of flight
Tris-HCl	tris(hydroxymethyl)aminomethane hydrochloride
WSP	water soluble protein
USP	urea soluble protein