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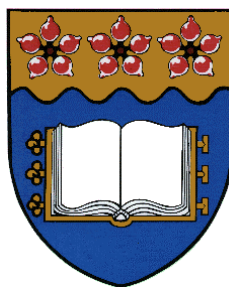
3-Hydroxykynurenine in the Human Lens

A thesis submitted in partial fulfillment of the
requirements for the award of the degree

Doctor of Philosophy

From

University of Wollongong



by

Anastasia Korlimbinis BMedChem(Hons)

Department of Chemistry

February 2006

Certification

I, Anastasia Korlimbinis, declare that this thesis, submitted in partial fulfillment of the requirements for the award of Doctor of Philosophy, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Anastasia Korlimbinis

February 2006

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Publications

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

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Abbreviations

ACN	acetonitrile
AGE	advanced glycation end
AHBDG	4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid <i>O</i> - β -D-diglucoside
AHBG	4-(2-amino-3-hydroxyphenyl)-4-oxobutanoic acid <i>O</i> - β -D-glucoside
ARN	age-related nuclear
Boc	butyloxycarbonyl
CLP	calf lens protein
Cys	cysteine
Da	dalton
DCI	deuterium chloride
D ₂ O	deuterium oxide
DTND	5,5'-dithio-bis(2-nitrobenzoic acid)
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
Em	emission
ESI-MS	electrospray ionisation mass spectrometry
Ex	excitation
Guanidine HCl	guanidine hydrochloride
GSH	glutathione (reduced)
His	histidine
H ₂ O ₂	hydrogen peroxide
HRP	horseradish peroxidase
Kyn	kynurenine
Kyn-GSH	kynurenine-glutathione
Lys	lysine
Met	methionine
M _{ox}	methionine sulfoxide
MS/MS	tandem mass spectrometry
Msr	methionine sulfoxide reductase

MW	molecular weight
NanoESI-MS	nanoelectrospray ionisation mass spectrometry
Na ₂ CO ₃	sodium carbonate
NaHCO ₃	sodium bicarbonate
NaN ₃	sodium azide
NMR	nuclear magnetic resonance
3OHKyn	3-hydroxykynurenine
3OHKyn-GSH	3-hydroxykynurenine-glutathione
3OHKynG	3-hydroxykynurenine <i>O</i> -β-D-glucoside
3OHKynG-GSH	3-hydroxykynurenine <i>O</i> -β-D-glucoside-glutathione
OPD	<i>o</i> -phenylenediamine
PDA	photodiode array
PMSF	phenylmethylsulfonyl fluoride
PSH	protein sulfhydryl
PTM	post-translational modification
RP-HPLC	reversed phase high performance liquid chromatography
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SH	sulfhydryl
TFA	trifluoroacetic acid
Tris-HCl	tris(hydroxymethyl)aminomethane hydrochloride
Trp	tryptophan
W _{ox}	oxidised tryptophan

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Abstract

The human lens contains three kynurenine UV filters, 3-hydroxykynurenine *O*- β -D-glucoside (3OHKynG), kynurenine (Kyn) and 3-hydroxykynurenine (3OHKyn), and it absorbs UV light in the 300-400 nm region due to their presence. UV filters may also prevent UV-induced photodamage to the retina and lens. After middle age, the UV filters, 3OHKynG and Kyn become bound progressively to proteins in the centre of our lenses. This feature is, in part, responsible for normal age-dependant human lens colouration.

To provide proof that 3OHKyn is bound to normal human lenses, model studies were undertaken. Cysteine (Cys), histidine (His) and lysine (Lys) residues in lens proteins had been previously shown to bind to UV filters *in vivo*, therefore adducts of these amino acids and 3OHKyn were synthesised and characterised by mass spectrometry, fluorescence, UV-visible and NMR spectroscopy in Chapter 2. The stability properties of each of the 3OHKyn amino acid adducts were also determined, with incubations performed at pH 4.0 and pH 7.2. 3OHKyn-*t*-Boc-His was identified as the most stable of the three adducts. 3OHKyn-*t*-Boc-Lys and 3OHKyn-Cys both decomposed at pH 7.2 forming numerous oxidation products. The stability of each adduct to acid hydrolysis was also examined.

In Chapter 3, calf lens protein was incubated with 3OHKyn, and acid hydrolysis showed that Cys was the primary site of modification when the incubation was undertaken at pH 7.2. However, when the incubation was undertaken at a higher pH (for example, pH 9.5), 3OHKyn readily modified Cys, His and Lys residues. Previously acid hydrolysis of human lens protein had identified Kyn attachment to the proteins. However, acid hydrolysis was not an appropriate method for detecting 3OHKyn attached to human lens proteins because 3OHKynG is also bound to human lens proteins. Therefore, a new assay was developed, and it was found that 3OHKyn does indeed bind to human lens proteins in an age-dependant manner. The assay also provides data for 3OHKynG and Kyn attachment to human lens proteins.

In Chapter 3, α -crystallin was also incubated with 3OHKyn under low oxygen tension, and the findings from this study showed that 3OHKyn modified the Cys residue in α A-crystallin. In addition, oxidation of methionine and tryptophan was observed. Age-related nuclear cataract is associated with colouration, insolubilisation and extensive oxidation of Cys and methionine residues. It appears that 3OHKyn in the lens may promote the oxidation and modifications of proteins, and may contribute to oxidative stress in the human lens.

In Chapter 4, the aim was to examine if 3OHKyn could act as a crosslinker of cataract lens proteins. 3OHKyn is known to readily oxidise and yield highly reactive species. It was therefore proposed that 3OHKyn bound to lens proteins could promote crosslinking, insolubilisation and colouration of lens proteins following formation of oxidised species. 3OHKyn amino acid adducts were incubated with excess amino acids, and the resulting products examined. These compounds may be analogous to those that would form in a cataract lens. In addition, 3OHKyn-modified protein was incubated and the products were examined by SDS-PAGE, fluorescence spectroscopy and mass spectrometry. Results showed that 3OHKyn, under the conditions used, does not crosslink lens protein. Proof of the hypothesis that 3OHKyn crosslinks proteins in the lens requires the isolation of characteristic chemical markers from cataract lens proteins that contain the modified 3OHKyn molecules.

In Chapter 5, the aim was to isolate novel compounds from the hydrolysates of human cataract lens proteins and to determine their chemical properties.

Overall this thesis provides evidence that 3OHKyn plays a role in the post-translational modification of normal human lens proteins, and it also provides preliminary data on the role of 3OHKyn in human cataract.