The functional studies of amyloid fibrils and their toxicity

By

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A thesis submitted for the Degree of

Doctor of Philosophy

in the

School of Chemistry and Physics

The University of Adelaide

January 2015

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ABSTRACT

Amyloid fibrils are a form of highly ordered, β-sheet protein structure found in many sites in the body. Fibril formation occurs when intermediates along the protein-folding pathway irreversibly enter the off-folding pathway to form highly ordered amyloid fibrils. Amyloid fibril formation is of considerable research interest because of its intimate association with a wide range of debilitating diseases, including Alzheimer's, Huntington's and Parkinson's diseases and type II diabetes. Recently, it has been found that amyloid fibrils enhance human immunodeficiency virus (HIV-1) infection. Semen contains a fibril forming component that significantly increases the ability of HIV-1 to infect cells. This component is associated with peptide fragments of prostatic acid phosphatase and has been termed semen-derived enhancer of viral infection (SEVI). SEVI acts at the virus entry stage and only boots infectivity when the peptide has folded into highly structured arrays of amyloid fibrils. The work presented in this thesis describes the broader roles of SEVI in HIV-1 infection including its toxicity to neuronal and epithelial cells as well as the toxicity of α_{s2}-casein (milk fibril forming protein).

Firstly, the amyloidogenic regions of SEVI are identified by the use of computer algorithms. Accordingly, these regions were synthesised to examine their individual fibril-forming propensity. Fragments from the central regions formed fibrils of similar morphology to SEVI at physiological pH and temperature. Fibril formation was assessed via thioflavin T assay, circular dichroism spectrometry and transmission electron microscopy (TEM). In this study the toxicity of SEVI and its amyloidogenic fragments to neuronal and epithelial cells was investigated. SEVI and its fragments were toxic to neuronal cells but not to confluent epithelial cells.

Secondly, the coreceptors used by SEVI and its amyloidogenic fragments are identified. HIV enters the cell by the interaction of glycoprotein (gp) 120 envelope with the cellular

differentiation (CD) 4 protein and secondary coreceptors. Affinofile assays showed that SEVI and its fragments use CCR5 and CXCR4 secondary coreceptors to enhance HIV-1 entry to the host cells. Additionally the ability of clusterin to inhibit fibril formation by SEVI was investigated. Clusterin inhibited fibril formation by SEVI in a concentration dependent manner thereby inhibiting the cytotoxicity associated with the fibrils.

Lastly, work detailing the toxicity of fibrils formed by milk protein α_{s2}-casein is presented. α_{s2}-Casein forms fibrils spontaneously under physiological conditions. These fibrils have been found in *corpora amylacea*, an amyloid condition that infrequently develops within the mammary tissue of cows. The use of cell toxicity assays show that fibrils formed by α_{s2}-casein were toxic to pheochromocytoma (PC) 12 cells. Furthermore, the use of Thioflavin T assay and TEM showed that a polyphenol epigallocatechin-3-gallate (EGCG), a component found in green tea extracts, inhibits fibril formation by α_{s2}-casein. Previous research has found that, EGCG can reduce fibril formation and cellular toxicity of various fibril-forming proteins. EGCG has also been shown to inhibit SEVI enhancement of HIV infection in a manner dependent on the ability of EGCG to disrupt SEVI fibril formation, providing proof of principle for the potential of anti-fibril agents as inhibitors of HIV infection.

DECLARATION

I certify that this work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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31st January 2015

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor Professor John Carver, you have been amazing. I will forever be grateful for your dedication, support and supervision over the past four years. Being in the Carver laboratory was a blessing because of you. You have a good heart and pray that God blesses everything you do.

I would also like to thank my co supervisor, Ian Musgrave, a man who is always happy, thank you for your help. It has been a great pleasure and honour working under your direction and supervision over the past four years.

Special thanks to Drs Yanqin Liu, Daniella Williams and Antonio Calabrese and the rest of Carver Laboratory team, Katy, Nick and Ranzhao for your technical assistance and the positive influence. Your support and friendship has been invaluable.

To my family, my man and kids, thank you for your support, believe and allowing me the opportunity to fulfil my dream. You have been a source of strength and I will forever love you. I look forward to spending more quality time with you.

To my late Papa and Mama, I am proud and forever thankful for what you have done for me. Thank you for your sacrifices so that I can have better education. I believe I have made you proud. I will always love you and may souls rest in peace. Thanks also to by two sisters Mosarwana and Kerapetse and my brother Outlwile for unconditional love and encouragement.

Finally, thanks be to God who gives victory through our Lord Jesus Christ. This work has been possible because of your grace, mercies and blessings.

Abbreviations

α_{s2}-CN	alpha$_{S2}$-casein
κ-CN	kappa-casein
AIDS	acquired immunodeficiency syndrome
CACO-2	epithelial colon carcinoma cells
CA	*corpora amylacea*
CA	caspid
CCR5	C chemokine receptor 5 (R5)
CXCR4	CXC chemokine receptor 4 (X4)
CD4	cluster of differentiation
CD	circular dichroism
CLI	complement-lysis inhibitor
CMA	chaperone-mediated autophagy
CMV	cytomegalovirus
CSF	cerebrospinal fluid
DNA	deoxy ribonucleic acid
DMEM	Dulbecco's modified eagle medium
DMSO	dimethyl sulfoxide

EGCG	(-)-epigallocatechin-3-gallate
Env	envelope
ER	endoplasmic reticulum
ERAD	endoplasmic reticulum-associated protein degradation
FPLC	fast protein liquid chromatography
gp	glycoprotein
HIV	human immune virus
HPLC	high performance liquid chromatography
HSPs	heat-shock proteins
LAMP2A	lysosome-associated membrane protein type-2A
MA	matrix
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NC	nucleocaspid
PAP	prostatic acid phosphatase
PC12	pheochromocytoma-12
PCD	protein conformational disorders
PCP	*Pneumocystis carinii pneumonia*
PR	protease
QTOF2	quadruple time of flight

RCM_K-CN reduced and carboxymethylated kappa-casein

RNA ribonucleic acid

RT reverse transcriptase

SAP serum amyloid P component

SD standard deviation

SEVI semen-derived enhancer of viral infection

TEM transmission electron microscopy

ThT thioflavin T

UV ultraviolet