Mass spectrometry analysis of non-protein amino acid misincorporation and proteomic changes in neurotoxicity-related cellular pathways

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Certificate of Original Authorship

I, Joel Ricky Steele declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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Abstract

Neurodegenerative diseases cause significant morbidity and mortality globally, with the prevalence continuing to rise due to prolonged life expectancy. Many neurodegenerative disorders share a common pathology that involves protein misfolding, aggregation and deposition in the brain. Dietary intake of non-protein amino acids has previously been linked to such proteinopathies, with indirect evidence indicating potential misincorporation of non-protein amino acids into growing protein chains. Phenotypic and proteomic investigations could provide more direct evidence of misincorporation and further elucidate the role that non-protein amino acids may play in neurodegenerative disease. The aim of this work was to determine if non-protein amino acids incorporate into the human proteome at a level detectable by mass spectrometry, with a focus on the amino acids L-DOPA, BMAA, and azetidine 2-carboxylic acid. An enzymatic method for the conversion of tyrosine residues to L-DOPA was successfully developed, providing a basis for studying the incorporation of L-DOPA into proteins. L-DOPA incorporation into proteins was also detected following treatment of human neuronal cells *in vitro*, with quantitative proteomics revealing activation of the unfolded protein response, evidence of oxidative stress, and changes in pathways involved in neurodegenerative diseases. Meta-analysis of proteomics datasets revealed a significant effect of sample preparation on the oxidation of samples, which could potentially mask true *in vivo* oxidation. Labelling techniques and mass spectrometer resolution were also found to be important for the identification of unique peptides and modifications, including misincorporated amino acids. The treatment of human neuronal cells with BMAA *in vitro* induced proteomic changes indicating a profile of toxicity like that previously reported for glutamate-mediated excitotoxicity, but the incorporation of BMAA into proteins was not detected. Conversely, the incorporation of azetidine 2-carboxylic acid into proteins was readily detectable following *in vitro* treatment of cells, importantly in proteins involved in cell proteostasis. Azetidine 2-carboxylic acid also resulted in quantitative proteomic changes, including an increased abundance of protein folding machinery and a decreased abundance of translational machinery. The significant proteomic changes in neuronal cells following exposure to all three non-protein amino acids investigated indicated changes in pathways potentially related to neurodegeneration and neurotoxicity, indicating a potential role in such pathologies that should be further explored. This thesis also provided direct evidence that certain non-protein amino acids can be incorporated into human proteins at a level detectable by mass spectrometry, paving the way for future studies to further investigate the role of such amino acids in human disease.

Contents

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Publications associated with this thesis

Steele, J.R., Italiano, C.J., Phillips, C.R., Violi, J.P., Pu, L., Rodgers, K.J. & Padula, M.P. (2021), 'Misincorporation proteomics technologies: a review', *Proteomes*, vol. 9, no. 1, p. 2.

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Quinn, A.W., Phillips, C.R., Violi, J.P., **Steele, J.R.**, Johnson, M.S., Westerhausen, M.T. & Rodgers, K.J. (2021), 'β-Methylamino-L-alanine-induced protein aggregation in vitro and protection by L-serine', *Amino Acids*, vol. 53, no. 9, pp. 1351-9.

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Rodgers, K., Chan, S. & **Steele, J.** (2017), 'Administration of L-tyrosine with levodopa could be neuroprotective in Parkinson's disease', Journal of Neurochemistry, vol. 142, Wiley 111 River St, Hoboken 07030-5774, NJ USA, pp. 245. **(Conference article)**

Rodgers, K., **Steele, J.** & Padula, M. (2017), 'A novel approach to detect the presence of levodopa (lDOPA) in the polypeptide chains of proteins', Journal of Neurochemistry, vol. 142, Wiley 111 River St, Hoboken 07030-5774, NJ USA, pp. 164. **(Conference article)**

Chen, H., **Steele, J.**, Li, G., Chan, Y., Oliver, B., Saad, S. & Machaalani, R. (2019), 'E-vapour inhalation–How does it affect memory?', IBRO Reports, vol. 6, pp. S208-S9. **(Conference article)**

Poster presentations

2017

22nd Annual Lorne Proteomics Symposium (Lorne, Australia)

Title: Using proteomic analysis to uncover the mechanisms of non-protein amino acids attributed to neurological diseases (#37). Authors: **Joel Steele**, Matt Padula, Kenneth Rodgers. Presented a lightning talk for this abstract, the poster also won an award.

2018

23rd Annual Lorne Proteomics Symposium (Lorne, Australia)

Title: Non-protein amino acids and neurological disease: their detection in human proteins and effects. Authors: **Joel Steele**, Matt Padula, Kenneth Rodgers.

2019

18th Human Proteome Organization World Congress – HUPO (Adelaide, Australia)

Three abstracts were awarded a poster presentation:

- Proteomic mapping of chemical warfare agent exposed plasma abs# 856.
- Mapping hydroxylated tyrosine in the human brain proteome: The formation and incorporation of L-DOPA abs# 857
- The neurotoxin β-Methylamino-L-alanine and its incorporation into proteins abs# 858

Abbreviations

α-2M	Alpha-2-macroglobulin
aaRS	Aminoacyl tRNA synthetase
AD	Alzheimer's disease
AEG	N-(2-aminoethyl) glycine
ALS	Amyotrophic lateral sclerosis
ALS-PDC	Amyotrophic lateral sclerosis-Parkinson's Dementia complex
AMBIC	Ammonium bi-carbonate
AQS	6-aminoquinolyl-N-hydroxysuccnimidyl-carbate
AZE	Azetidine-2-carboxylic acid
BCA	Bicinchonic acid
BMAA	β-methylamino-L-alanine
BOAA	β-N-oxalyl-α,β-L-diaminopropionic acid
BSA	Bovine serum albumin
CDC42	Cell division control protein 42 homolog
CHOP	CCAT-enhancer-binding protein homologous protein
CID	Collisional induced dissociation
CNS	Central nervous system
CSF	Cerebral spinal fluid
Da	Dalton
DAB	L-2,4-diaminobutyric acid
DDA	Data dependent analysis
D-DOPA	D-3,4-dihydroxyphenylalanine
DENR	Density-regulated protein
DIA	Data independent analysis
DMEM	Dulbecco's Modified Eagles' Medium
DTT	Dithiothreitol
EBT	1,1'-ethylidene-bis[L-tryptophan]
EDTA	Ethylenediaminetetraacetic acid
EMS	Eosinophilia Myalga Syndrome
ER	Endoplasmic reticulum
FDR	False discovery rate
FLD	Fluorescence detector
FL-HPLC	high-pressure liquid chromatography-fluorescence platform

GAPDH	glycerol-3-phosphate dehydrogenase
GC	Gas chromatography
GNB2	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2
HBB	Haemoglobin
HEPES	(2-hydroxyethyl)-1-piperazine ethanesulfonic acid
HNRNPD	Heterogeneous nuclear ribonucleoprotein D0
IAA	iodoacetamide
IDA	Intelligent data Acquisition
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography tandem mass spectrometry
L-DOPA	L-3,4-dihydroxyphenylalanine
LFQ	Label free quantification
LOPIT	Localisation of organelle proteins by isotope tagging
m/z	Mass-to-charge ratio
MBP	Myelin basic protein
MEM	Minimum Essential Medium
MiP	Misincorporation proteomics
MND	Motor neuron disease
MRM	Multiple reaction monitoring
mRNA	Messenger RNA
MS	Multiple sclerosis
MS/MS	Tandem mass spectrometry
NBT	Nitroblue tetrazolium
NPAA	Non-protein amino acid
OST	Oligosaccharyl transferase complex
Ox-Met	Oxidised methionine
Ox-Phe	Oxidised phenylalanine
PB-DOPA	Protein bound L-DOPA
PBS	Phosphate-buffered saline
PD	Parkinson's disease
PMI	Post mortem interval
PPIA	Peptidyl-prolyl cis-trans isomerase A
PRM	Parallel reaction monitoring
PRMT1	Protein arginine N-methyltransferase

PSM	Peptide spectral match
PTM	Post translational modification
PVDF	Polyvinylidene fluoride
ROS	Reactive oxygen species
RTS-SPS-MS3	Real time search enabled SPS-MS3
SART1	U4/U6.U5 tri-snRNP-associated protein 1
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	standard error of the mean
SILAC	Stable isotope labelling by amino acids in cell culture
SLE	Systemic lupus erythematosus
SNRPG	Small nuclear ribonucleoprotein G
SNRPGP15	Putative small nuclear ribonucleoprotein G-like protein 15
SPRM1	Serine/arginine repetitive matrix protein 1
SPS-MS3	synchronous precursor selection based MS3
SRM	Single reaction monitoring
SRPR	Signal recognition particle receptor subunit alpha
TAILS	N-terminal isotopic labelling of substrates
TCA	Trichloroacetic acid
TCEP	tris(2-carboxyethyl)phosphine
TDP-43	TAR DNA-binding protein 43
TMT	Tandem Mass Tags
TOF	Time of flight
tRNA	Transfer RNA
UPR	Unfolded protein response
UTC-7	7M urea, 2M thiourea, 0.1% C7BzO

Thesis organisation

The organisation of this thesis is outlined below:

- Chapter One: This introduction frames the research questions for this thesis.
- Chapter Two: Published critical review of the literature concerned with NPAAs, the methods used to study their role and effect on an organism's proteome, and establishment of the formal pursuit of proteomic incorporation of NPAAs, with technologies and considerations outlined to advance the field of NPAA study.
- Chapter Three: A method for the enzymatic conversion of proteomes to contain L-DOPA to create reference mass spectra for analysis of samples that potentially contain proteoforms with L-DOPA incorporated, as well as providing evidence for L-DOPAs *in vitro* toxicity, and the parallels of the toxicity to a state of neurodegeneration highlighted.
- Chapter Four: Meta-analysis of publicly available data for the presence of proteoforms/peptidoform incorporated L-DOPA to establish a baseline of L-DOPA presence in the human proteome. The draft map of the human proteome (brain subset) was analysed as a baseline for control. A Parkinson's disease TMT labelling experiment on the substantia nigra was also analysed and finally a label free LC-MS/MS dataset of the proteome of the olfactory lobes of Parkinson's sufferers.
- Chapter Five: The effect of BMAA and Azetidine 2-carboxylic acid on the neuronal proteome of SH-SY5Y cells and their incorporation.
- Chapter Six: General discussion, future directions and concluding remarks.
- Appendices
- References