The purification and proteomic analysis of α-synuclein-containing inclusions in neurodegenerative disorders

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Summary

Neurodegenerative disorders affect a large proportion of the elderly population. A group of disorders, known as the α-synucleinopathies, are characterised by the presence of α-synuclein-containing protein inclusions, such as Lewy Bodies (LBs) found in neurons from Parkinson's Disease (PD) and Dementia with Lewy Bodies (DLB), and Glial Cytoplasmic Inclusions (GCIs) found in oligodendrocytes from Multiple System Atrophy (MSA). Since the discovery of LBs 100 years ago, some major inclusion proteins such as α-synuclein have been identified, but a detailed understanding of their protein composition has yet to be achieved. It is still not known how or why inclusions form and what role they play in the disease process. One hypothesis is that damaged proteins, unable to be degraded by the cell due to underlying proteasomal system or autophagic defects, are selectively targeted to inclusions via vesicle-mediated transport as a protective mechanism.

The analysis of the protein composition of inclusions has been hindered by ineffective methods for isolating the inclusions from the surrounding tissue. This was addressed in this study by optimising a published method for GCI purification by Gai *et al.* (1999) that utilises Percoll gradient density centrifugation combined with magnetic immunocapture. The optimised method gave a 28-fold increase in yield compared to the published method and a 2D-DIGE comparison showed a 3.8-fold increase in α-synuclein enrichment (the major protein in GCIs) and a corresponding 5.2-fold reduction in tubulin contamination. The optimised GCI purification method was then successfully adapted to the purification of LBs from DLB tissue.

A 2D-DIGE comparison of purified GCIs (n=6) and LBs (n=2) revealed that GCIs consist of 11.9% α-synuclein, 2.8% α-β-crystallin and 1.7% 14-3-3 proteins compared to 8.5%, 2.0% and 1.5% in LBs, respectively. A positive linear relationship was found between the relative quantities of α-synuclein and α-β-crystallin in inclusions from each case. The remaining 83-88% of inclusion proteins consists of more than 150 proteins possessing a diverse range of biological functions, including vesicle trafficking, cytoskeletal structure, protein degradation, chaperones, mitochondrial proteins and endoplasmic reticulum proteins.

The GCI protein identifications were performed by sequencing peptides using nanospray Orbitrap mass spectrometry. Peptides were obtained from complex mixtures of trypsin-digested GCI extracts purified from five MSA cases and from trypsin-digested 1-DE gel slices and 2-DE gel spots of GCI proteins. 160 proteins were identified in at least 4 out of the 5 MSA cases analysed. 21% of these 160 proteins were synaptic vesicle-related. The identification of LB proteins was performed by mass spectrometry of complex mixtures of trypsin-digested LB extracts from two DLB cases. Of the 112 proteins identified in both DLB cases and a minimum of 4 out of 5 MSA cases, 25% were synaptic vesicle-related, including synaptosomal-associated protein 25 and V-type proton ATPases.

This study has generated an optimised method for the purification of inclusions to a purity not achieved previously with a yield sufficient for multiple forms of analysis. A comprehensive characterisation of the protein composition of both GCIs and LBs has been performed, including the relative quantification of the major inclusion proteins α-synuclein and α-β-crystallin. The identification of a large set of vesicle-trafficking proteins suggests that α-synuclein may be targeted to LBs and GCIs via a common vesicle trafficking mechanism.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Amellia McCormack

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Abbreviations

%	percentage
x g	x gravity
°C	degrees Celcius
µg	microgram
µL	microlitre
µM	micromolar
1°	primary
1-DE	one-dimensional electrophoresis
2°	secondary
2-DE	two-dimensional electrophoresis
2D	two-dimensional
aa	amino acids
ACN	acetonitrile
BSA	bovine serum albumin
CHAPS	3-[3-cholamidopropyl)dimethylammonio]-1-propanesulphonate
CID	collision-induced dissociation
cM	centimeter
Da	Dalton
DAB	3,3'-Diaminobenzidine
DIGE	Difference Gel Electrophoresis
DNA	deoxyribonucleic acid
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
EtOH	ethanol
FA	formic acid
GE	General Electric
H_2O	water
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
IEF	isoelectric focusing
IPG	immobilised pH gradient

kDa	kilodalton
LB	Lewy body
M	molar
mA	milliampere
mL	milliliter
mm	millimetre
mM	millimolar
mRNA	messenger RNA
MS	mass spectrometry
MS/MS	tandem mass spectrometry
m/Z	mass-to-charge ratio
NaCl	sodium chloride
ng	nanogram
nL	nanolitre
PAGE	polyacrylamide gel electrophoresis
pH	hydrogen ion concentration
pI	isoelectric point
PMSF	phenylmethanesulfonyl fluoride
ppm	parts per million
RNA	ribonucleic acid
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
TBS	tris buffered saline
TBST	tris buffered saline Tween -20
Tris	Tris (hydroxymethyl) aminomethane
V	volts
v/v	volume per volume
W	watts
w/v	weight per volume