THE BOVINE SPLICEOSOMAL U1 SMALL NUCLEAR RIBONUCLEOPROTEIN PARTICLE: A STUDY OF ITS AUTOANTIGENICITY AND BIOCHEMICAL PROPERTIES

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry at Massey University, Palmerston North, New Zealand.

Andrew James Robertson
2006

Abstract

Despite individual autoimmune diseases being relatively rare, collectively these diseases afflict 8 % of the population according to the American Autoimmune Related Diseases Association. With over 75 % of those affected being women, autoimmune disease has been recognised, by the World Health Organisation and the US National Institutes of Health, as a major global women's health issue. One third of autoimmune sufferers have a rheumatological disorder, which commonly affect the joints, muscle, skin, salivary glands and kidneys. Antibodies against nuclear antigens are a serological hallmark of these diseases. Detection of these antibodies is used in the diagnosis and prognosis of the disease. The sensitivity and specificity of the test, of which the antigen is a key component, is pivotal to correct disease diagnosis and management.

The relationship between circulating autoantibodies and the target antigen is complex. Improving the effectiveness of a test to assist in diagnosis and prognosis comes from characterisation and understanding these complex relationships.

This thesis compares bovine spliceosomal U1 small nuclear ribonucleoprotein particle (U1 snRNP) complex with its human equivalent, and examines the validity of using this bovine derived autoantigen in the diagnosis of the human autoimmune diseases, systemic lupus erythematosus and mixed connective tissue disease.

Differences between bovine and human U1 snRNP composition were characterised using a combination of electrophoretic, immunoassay and mass spectrometry techniques. Although the U1C protein could not be identified in bovine U1 snRNP, all other specificities were present. U1A remained intact, whilst the U1 snRNP specific 68K protein was dephosphorylated and a large C-terminal domain was removed, such that 68K migrated as a 30-36 kDa cluster on SDS-PAGE. Bovine SmD proteins, present in U1 and non-U1 snRNPs,

were unaffected, whereas, SmB′/B was truncated to a 12 kDa peptide, which interestingly, was no longer reactive with anti-RNP sera in western blot.

The recognition of human SmB′/B protein by anti-RNP sera in western blot was further examined. A technique was developed to immunoaffinity purify tryptic digests of SmB′/B which could then be analysed by mass spectrometry. Interestingly, the human replication element protein (HREP) was tentatively identified, rather than SmB′/B as expected. It may be possible, therefore, that anti-RNP sera may be reacting with a protein other than SmB′/B.

To examine the contribution of the individual U1 snRNP proteins to anti-RNP and anti-Sm sera reactivities, a method was developed to dissociate bovine U1 snRNP and to purify the individual component antigens. It was demonstrated both empirically and through anecdotal feedback from a commercial diagnostic kit producer that patient sera respond better to purified Sm-free 68K than the recombinant 68K antigen.

The effect of commercial processing of bovine thymus, the source for U1 snRNP antigen, was determined. In this study, variables that may be controlled during processing, such as temperature, protease activity and pH, were investigated. Hydrolysis of the intact human 68K protein with the necrotic protease, cathepsin L, produced 38 and 25 kDa fragments, whereas exposure to ambient temperature and low pH produced 32 kDa peptide fragments similar to those observed in purified bovine 68K. It was therefore proposed that 68K protein may undergo autocatalytic hydrolysis during necrotic cell death.

Thorough characterisation of the bovine spliceosomal U1 snRNP proteins has not only validated their use as diagnostic reagents in autoimmune disease but also provided some insight into the inactivation of U1 snRNP function during early cell death.

Acknowledgements

I would like to thank the many people who have encouraged and assisted me during this study.

Special thanks are extended to my supervisors, Dr. Gillian Norris of the Institute of Molecular BioSciences, Massey University and Dr. Neil Cook, AroTec Diagnostics Limited, for their patient guidance and support.

I must also thank some people who have worked with me during the time spent on this thesis: Tim Catchpole, Rebecca Wymer, Erin Boshier and Amelia Knight at AroTec Diagnostics for keeping me sane. A particular mention should go to Greta Moraes of Industrial Research Limited for her empathy and encouragement. Thanks also to those in the Norris Lab at Massey University, particularly Dr. Santanu Deb Choudhury for his assistance with the operation of the MALDI mass spectrometer and Trevor Loo for his general helpfulness.

My gratitude also to family and friends, particularly my partner Liz Doolin without whose love, devotion and sacrifice I could not have completed this thesis. Also our gorgeous children, Gemma, Campbell and Matthew, whose cuddles, giggles and smiles make everything worth while.

Finally, many thanks to Technology New Zealand and Arotec Diagnostics Limited for their financial support.

.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

2A2MP	2-amino-2-methylpropanol
2D	two dimensional
aDMA	asymmetrical dimethylarginine
ANA	anti-nuclear antibody
BCIP	5-bromo 4-chloro 3-indolylphosphate
BCR	B-cell receptor
BSA	bovine serum albumin
CD	cluster designation
CHAPS	3-(3-cholamidopropyldimethyl ammonio) propanesulfonic acid
CHT	ceramic hydroxyapatite
CIE	counterimmunoelectrophoresis
Da	dalton
DID	double immunodiffusion
DIG	digoxigenin
DNA	deoxyribonucleic acid
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
EIA	enzyme-linked immunoassay
ELISA	enzyme linked immunosorbent assay
ENA	extractable nuclear antigen
FN	false-negative
FP	false-positive
HeLa	cervical carcinoma cell line (Helen Lane)
HEPES	N-(2-hydroxyethyl) piperazine N′(2-ethanesulfonic acid)
HPLC	high performance liquid chromatography
HREP	human replication element protein
HRP	horse radish peroxidase
HT	hydroxyapatite
IEF	isoelectric focussing
Ig	immunoglobulin

IIF	indirect immunofluorescence
IPG	immobilised pH gradient
kDa	kilodalton
m^7G	monomethylguanosine
MALDI-TOF	matrix assisted laser desorption ionisation time of flight
MCTD	mixed connective tissue disease
MFG-E8	milk fat globule epidermal growth factor–8
MHC	major histocompatibility complex
mRNA	messenger ribonucleic acid
NBT	nitrobenzamidine triazine
NMWCO	nominal molecular weight cut off
nt	nucleotides
O-GalNAc	O-linked β-N-acetylgalactosamine
O-GlcNAc	O-linked β-N-acetylglucosamine
PBS	phosphate buffered saline
PBST	phosphate buffered saline containing 0.02 % v/v Tween 20
pI	isoelectric point
PIE	polyadenylation inhibitory element
PMSF	phenylmethylsulfonyl fluoride
PP	protein phosphatase
r68K	recombinant 68K protein
RA	rheumatoid arthritis
RNA	ribonucleic acid
RNAse	RNA nuclease
RNP	ribonucleoprotein particle
RP-HPLC	reverse phase HPLC
RRM	RNA recognition motif
sDMA	symmetrical dimethylarginine
SDS	sodium dodecyl sulfate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SF-A	U1 snRNP-free U1A
SLE	systemic lupus erythematosus
SmB^{trunc}	truncated SmB protein

SMN	survival of motor neuron complex
snRNA	small nuclear ribonucleic acid
snRNP	small nuclear ribonucleoprotein particle
SPE	SP-Sepharose column elution
TBE	tris borate EDTA
TCR	T-cell receptor
TEMED	N,N,N′,N′-tetramethylethylenediamine
TLR	toll-like receptor
TMG	trimethylguanosine
Topo I	DNA topoisomerase I
Triton x100	4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol
Tween-20	polyethylene glycol sorbitan monolaurate
UV	ultra violet
v/v	volume/volume
w/v	weight/volume

Abbreviations for amino acids

Amino acid	Three-letter abbreviation	One-letter symbol
alanine	ala	A
arginine	arg	R
asparagine	asn	N
aspartic acid	asp	D
cysteine	cys	C
glutamine	gln	Q
glutamic acid	glu	E
glycine	gly	G
histidine	his	H
isoleucine	iso	I
leucine	leu	L
lysine	lys	K
methionine	met	M
phenylalanine	phe	F
proline	pro	P
serine	ser	S
threonine	thr	T
tryptophan	trp	W
tyrosine	tyr	Y
valine	val	V