Identification and Characterization of the Plasticity-Relevant Fucose-α(1-2)Galactose Glycoproteome from Mouse Brain

Thesis by

Heather Elizabeth Murrey

In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

2009

(Defended 4 August 2008)

…for my family and friends who have helped me over the years…

Acknowledgments

Without the help and support of many people, both scientific and personal, the work in this thesis would not have been possible. I would first and foremost like to thank my advisor, Professor Linda Hsieh-Wilson, for her advice and guidance throughout my years here at Caltech. She has helped me develop as a scientist in all aspects of research, from experimental design to written and oral communication skills.

I would also like to thank the members of my committee, Professor Dennis Dougherty, Professsor David Chan, and Professor Paul Patterson. During my candidacy and yearly meetings, I have received excellent feed-back that has furthered my research. I'd also like to thank the rest of the Dougherty lab, especially Erik Rodriguez for help with scientific discussions and experimental troubleshooting. Erik has also been one of my closest friends outside of work and we've shared many memorable (and crazy!) moments together.

I would not be in the position I am in today without the support of my undergraduate advisor, Professor Irwin Levitan, who gave me an opportunity to start working in the lab as a freshman that knew nothing about scientific research. Yi Zhou was a post-doc in the lab who took me under his wing and taught me almost everything I know about molecular biology. We worked together for three and half years, during which time I learned many experimental techniques that I have carried through my graduate career.

I am also indebted to Professor's Peter S. Kim and Harvey F. Lodish at MIT, where I worked for two years after finishing my undergraduate research. I learned almost everything I know about protein biochemistry from my work in the Kim lab. I'd also like

to give a special thanks to Dr. Tsu-Shuen Tsao from the Lodish lab. He was the first person to let me take what I had learned over the years, and apply it to a new area of research. His belief in me and my ideas, built my confidence as a scientist, and our work together led to the publication of multiple papers. In addition to his help in my development as a scientist, he is also a great friend outside of the lab and is still someone I look to for advice.

I'd like to thank all members of the Hsieh-Wilson lab, both past and present, especially Cristal Gama. Cristal has been one of my best friends, and has helped me in the lab as well as outside. We have shared many wonderful as well as difficult times together, and without her help and the help of her family, I would not be where I am today. I'll always remember making tameles (did I spell it right??), eating posole (I'm sure I didn't spell that right…) and spending holidays with her and her family, they always made me feel at home and I really appreciate everything they have done for me over the years. I'd also like to thank past lab members Sarah Tully, Callie Bryan, Katie Saliba, and Tammy Campbell, all of whom I spent many a night with outside of Caltech. I'll miss pumpkin carving at the Bryan household! That was always a lot of fun. I'd like to thank newer lab members Young-In Oh, Arif Wibowo, and especially Chithra Krishnamurthy. They have become my good friends outside of the lab, and Chithra has been a wonderful partner to work with on the fucose project.

I am also indepted to the help from the animal facility here at Caltech, especially Jennifer Alex, Ana Colon, Gwen Williams, Karen Chase, and Janet Baer, all of whom have helped me maintain and care for the animals, as well as learn new experimental

techniques. I also need to thank my collaborators at GNF, Dr. Eric C. Peters and Dr. Scott Ficarro for all the hard work they put in on my experiments.

There are also many people I would like to thank outside of Caltech, including my family. My mother, sisters Kelly and Colleen, as well as my brother-in-law Matt and nephew Brody have always given me their support and have helped my tremendously over the years. In addition, my friend Cynthia Capdeville has been a great friend since high school, and continues to support me throughout my scientific endeavors. Lastly, I'd like to thank Christian St. James, for being there for me during one of the most difficult times in my life, and giving me the love and support I need to be where I am today. Thanks everyone!

Abstract

Fucα(1-2)Gal carbohydrates have been implicated in cognitive processes such as learning and memory. However, a molecular level understanding of their functions has been lacking. This thesis describes multiple chemical and biological approaches that we have undertaken to elucidate the molecular mechanisms by which fucosyl sugars mediate neuronal communication. We demonstrate that Fucα(1-2)Gal carbohydrates play an important role in the regulation of synaptic proteins and neuronal morphology. We identify synapsins Ia and Ib as prominent Fucα(1-2)Gal glycoproteins in rat hippocampus, and fucosylation protects synapsin I from proteolytic degradation by the calcium-activated protease calpain. Synapsin fucosylation has important consequences on neuronal growth and morphology, with defucosylation leading to stunted neurites and delayed synapse formation. In addition, we identify the Fucα(1-2)Gal proteome from mouse olfactory bulb using lectin affinity chromatography. We discover four major classes of Fucα(1-2)Gal glycoproteins, including the immunoglobulin superfamily of cell adhesion molecules, ion channels and solute carriers/transporters, ATP-binding proteins, and synaptic vesicle-associated proteins. Protein fucosylation is regulated by FUT1 in mouse olfactory bulb, and olfactory bulb development is impaired in FUT1-deficient mice. In particular, FUT1 KO animals exhibit defects in the olfactory nerve and glomerular layers of olfactory sensory neurons expressing the fucosylated cell adhesion molecules NCAM and OCAM. Lastly, we explore the molecular mechanisms of protein fucosylation by metabolic labeling with alkynyl- and azido-fucose derivatives. We demonstrate that fucosylated glycoconjugates are present along both axons and dendrites

of developing neuronal cultures, as well as in the Golgi body. We identify the fucosylated proteome from cultured cortical neurons, and demonstrate that proteins such as NCAM, the MARCKS family of proteins, and the inositol 1,4,5 triphosphate receptor are fucosylated. In addition, we can label fucosylated glycans *in vivo*, which will have important consequences for studies on the dynamics of protein fucosylation in living animals. Cumulatively, our studies suggest important functional roles for fucosyl-carbohydrates in the nervous system, and implicate an extended role for fucose in the molecular mechanisms that may underlie synaptic plasticity and neuronal development.

Table of Contents

List of Figures

Chapter 3

Chapter 4

Chapter 5

Appendix 1

Appendix 2

Appendix 3

List of Tables

List of Abbreviations

1D	one-dimensional
2D	two-dimensional
2-dGal	2-deoxy-D-galactose
2-fucosyllactose	L-fucoseα(1-2)galactoseβ(1-4)glucose
3-dGal	3-deoxy-D-galactose
4-dGal	4-deoxy-D-galactose
6-dGal	6-deoxy-D-galactose
2-dGlc	2-deoxy-D-glucose
AAA	*Anguilla anguilla* agglutinin
Ab	antibody
Ac	acetyl, acetate
$AgNO_3$	silver nitrate
AOB	accessory olfactory bulb
aq	aqeous
ATP	adenosine triphosphate
Baf. A1	bafilomycin A1
BCA	bicinchoninic acid
β-Gal	β-galactosidase

BSA	bovine serum albumin
°C	degree Celsius
$CaCl_2$	calcium chloride
Cacna2d1	alpha 2/delta subunit of the dihyrdopyridine-sensitive channel
cAMP	cyclic adenosine monophosphate
CAMs	cell adhesion molecules
CDG	congenital disorder of glycosylation
CH_3CN	acetonitrile
$CHCl_3$	chloroform
CHO	Chinese hamster ovary
CMF-HBSS	Calcium and Magnesium Free Hank's Balanced Salt Solution
CNS	central nervous system
CO_2	carbon dioxide
CRMP-2	collapsin response mediator protein
CV	column volume
ddH_2O	double distilled water
D-Gal	D-galactose
DIV	days in vitro
DMEM	Dulbecco's Minimal Eagle's medium
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
E18	embryonic day 18
EBI-IPI	European Bioinformatics Institute-International Protein Index
ECL	enhance chemiluminescence
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol tetraacetic acid
Endo H	endoglycosidase H
ER	endoplasmic reticulum
FCS	fetal calf serum
Fuc	L-fucose
Fucα(1-2)Gal	fucose α(1-2) galactose
Fucα(1-3)Gal	fucose α(1-2) galactose
Fucα(1-4)Gal	fucose α(1-4) galactose
Fucα(1-6)GlcNAc	fucose α(1-6) *N*-acetylglucosamine
FUT1	α(1-2) fucosyltransferase 1
FUT2	α(1-2) fucosyltransferase 2
FUT3	α(1-3,4) fucosyltransferase 3
FUT4	α(1-3,4) fucosyltransferase 4
FUT5	α(1-3) fucosyltransferase 5
FUT6	α(1-3) fucosyltransferase 6
FUT7	α(1-3) fucosyltransferase 7
FUT8	α(1-6) fucosyltransferase 8
FUT9	α(1-6) fucosyltransferase 9
FUT10	putative α(1-3) fucosyltransferase 10

FUT11	putative α(1-3) fucosyltransferase 11
g	gram, gravitational force
Gal	galactose
GalNAc	*N*-acetylgalactosamine
GDP-fucose	guanosine diphosphatyl-fucose
Glc	glucose
GlcA	D-glucuronic acid
GlcN	D-glucosamine
GlcNAc	*N*-acetylglucosamine
GluR1	glutamate receptor 1
GTP	guanosine triphosphate
h	hour
H	homogenate
HIO_4	periodate
hnRNP	heterogeneous ribonucleoprotein
H_2O	water
HOAc	acetic acid
HRP	horse-radish peroxidase
Hsc/Hsp 70	heat shock chaperonin/heat shock protein 70
IACUC	institute of animal care and use committee
IgSF	immunoglobulin superfamily
IgG	immunoglobulin
IP	immunoprecipitated
K^+	potassium ion
K_{assoc}	association constant
KCl	potassium chloride
kDa	kilodalton
$K_3Fe(CN)_6$	potassium ferricyanide
KO	knockout
L	liter
LAC	lectin affinity chromatography
LAD II	leukocyte adhesion deficiency type II
LC/MS^n	liquid-chromatography mass spectrometry
LTL	*Lotus tetragonolobus* lectin
LTP	long-term potentiation
LS1	soluble fraction of synaptosome lysis
LS2	crude synaptosol
LP1	insoluble fraction of synaptosome lysis
LP2	crude synaptic vesicle preparation
M	molar
MALDI-TOF	matrix-assisted laser desorption/ionization time-of-flight
Man	mannose
MAP2	microtubule associated protein 2
MEM	Minimal Eagle's Medium
MeOH	methanol
μg	microgram

MG132	proteosome inhibitor
$MgCl_2$	magnesium chloride
min	minutes
m	milli or meter
μ	micro
μCi	micro-Curie
MOB	main olfactory bulb
mol	mole
MS	mass spectrometry
Munc18	syntaxin-binding protein
MWCO	molecular weight cut-off
n	nano
N	normal
Na^+	sodium ion
NaCl	sodium chloride
NaOH	sodium hydroxide
Na_2CO_3	sodium bicarbonate
NaN_3	sodium azide
$Na_2S_2O_3$	sodium thiosulfate
NCAM	neural cell adhesion molecule
NCBI	national center for biotechnology information
NETFD	SDS-neutralization lysis buffer
Neu5Ac	sialic acid
NH_4HCO_3	ammonium bicarbonate
NIH	national institute of health
NP-40	nonidet P-40 detergent
NPI	neuronal pentraxin I
NSF	*N*-ethylmaleimide sensitive factor
NTCB	2-nitro-5-thiocyanobenzoic acid
OCAM	olfactory cell adhesion molecule
OEt	*O*-ethyl
ONL	olfactory nerve layer
OSN	olfactory sensory neuron
P0	post-natal day 0 mouse or rat pup
P1	insoluble fraction 1
P2	insoluble fraction 2
P2’	insoluble fraction 2’; crude syanptosomes
P3	post-natal day 3 mouse or rat pup
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PEPCase	phosphoenolpyruvate carboxylase
PNGase F	*N*-glycosidase F
POFUT1	*O*-fucosylatransferase 1
POFUT2	*O*-fucosylatransferase 2
PSA	polysialic acid
PSD-95	post synaptic density protein 95

PTM	post-translation modification
PVDF	polyvinylidene difluoride
Rab-GDI	Rab guanine-dissociation inhibitor
RNA	ribonucleic acid
rpm	revolutions per minute
rt	room temperature
S1	soluble fraction 1
S2	soluble fraction 2
S2’	soluble fraction 2’
SDS	sodium dodecyl sulfate
Slc12a2	solute carrier family member 2
Sec1	noncatalytic α(1-2) fucosyltransferase
SEM	standard error of the mean
SG	sucrose-gradient purified synaptic vesicles
SNAP-25	synaptosomal-associated protein of 25 kDa
SynI	synapsin I
Syn KO	synapsin knockout
TBST	tris buffered saline with Tween-20
TCEP	tris(2-carboxyethyl)phosphine
TEAA	triethylammonium acetate
Tris-Cl	tris chloride
TRPV5	transient receptor potential cation channel
UEAI	*Ulex europeaus* agglutinin I
UDP	uridyl-diphosphate
UV	ultraviolet
VDAC1	voltage-dependent anion channel 1
vol	volume
w/v	weight per volume
WGA	wheat germ agglutinin
WT	wild type
Xyl	xylose