Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Structural and Biochemical Characterisation of Utrophin and Dystrophin Spectrin Repeat Domains

by

Muralidharan Muthu

Submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Biochemistry

Massey University,

Palmerston North, NZ

2012

Abstract

Duchenne and Becker muscular dystrophies are muscle-wasting disorders caused by mutations in the X-linked dystrophin gene. Dystrophin is a large cytoskeletal protein belonging to the spectrin superfamily, that links intracellular F-actin to the extracellular matrix *via* a membrane-associated glyco-protein complex thus maintaining structural rigidity and flexibility. Utrophin is a widely expressed protein that has been shown to functionally compensate for dystrophin in cultured muscle cells as well as in the muscular dystrophy mice model. Both utrophin and dystrophin share a similar domain architecture with N-terminal actin-binding domains and C-terminal variable domains separated by 22 or 24 spectrin-like repeats respectively. Therapeutic strategies to replace individuals having defective dystrophin with utrophin require full characterisation of these proteins.

In this thesis, high-resolution structures of the N-terminal first spectrin repeat domains from utrophin and dystrophin have been determined by x-ray crystallography to 1.95 and 2.3 Å. Despite multiple structures of spectrin repeats in the Protein Data Bank from α-actinin, spectrin and plectin these are the first structures determined for any of the spectrin repeats from utrophin and dystrophin. These structures are similar to one another and display a three-helix bundle spectrin repeat fold. The repeat domain structure reveals the relationship between the canonical spectrin repeat domain sequence motif and the structural domain for utrophin and dystrophin, showing the N-terminal first spectrin repeat to be extended at the C-terminal end. Earlier biochemical studies revealed that the extension at the C-terminus was required for the protein's stability. Studies have also shown that spectrin repeats of utrophin are required for a higher affinity interaction of the actin-binding domain with F-actin. However, it was unclear whether the N-terminal repeat domain has an intrinsic affinity for F-actin. In the present study the actin-binding properties of these spectrin repeats are elucidated.

Previous experiments using molecular dynamic simulations and atomic force microscopy of tandem spectrin repeat domains from erythroid spectrin and α-actinin suggested that flexibility of multiple repeats depends upon the linker region. However

under higher extension, the triple helical domain further undergoes unfolding and refolding and thus functions as an elastic element within the cell. Studies using steered molecular dynamics suggested that the force required for unfolding the N-terminal first spectrin repeat domain from utrophin is higher in comparison to that of the N-terminal first spectrin repeat from dystrophin.

Keywords

Dystrophin, Utrophin, F-actin, Spectrin repeats.

Acknowledgements

First and foremost, I would like to thank my supervisor, Dr. Andrew J Sutherland-Smith for his support and patience during my PhD. I feel incredibly lucky and honoured to have been able to work for him. I have learned so much while working in his laboratory. He gave me the opportunity to do amazing research and also supported me for various conferences throughout my PhD and for that, I am truly thankful.

I want to thank Prof Geoff B. Jameson, who taught me an "Advanced Chemistry" course and the course during the NZ Synchrotron workshop, these courses provided me with a much broader understanding of x-ray crystallography and synchrotron radiation. I would like to thank Dr Steven Pascal and his group for their help to understand concepts of NMR spectroscopy and the incredible things that one can do with it. Thanks to Dr Jen Hsin at Stanford University for conducting simulation experiments and scientific advice on molecular dynamics.

I am truly thankful to Prof. Barry Scott and Assoc. Prof. Kathryn Stowell, for their support and advice during my PhD and providing a pleasant research environment in IMBS. I would like to thank Dr Gill Norris and Dr Mark Patchett for letting me use some of their chemicals and instruments when required. I would also like to thank Dr Greg Sawyer for his assistance with the cloning, protein purification and assays. I would like to thank Simon Oakley and Bryan Anderson for their help to understand crystallography software and visualisation tools. I would like to thank past and present members in structural biology for their support and providing me with a great atmosphere especially to Matt, Trevor, Alice, Judith, Jana, Jan and Komala. I would also like to express my gratitude to Massey University for a Doctoral Scholarship. I would like to thank my friends Srinu, Keerti, Sridhar, Pradeep and Harinath for their incredible support for many years.

Lastly but not least, I would like to thank my parents Mr. Muthu and Mrs. Bhuvana, grandparents Mr. Bangaru and Mrs. Kasthuri, and my wife Kavitha for their love and support throughout my life.

Contents

List of Figures

List of Tables

List of Abbreviations

ABA	actin binding assay
ABD	actin binding domain
ABS	actin binding sequence
AFM	atomic force microscopy
Amp	ampicillin
APS	ammonium persulphate
ATP	adenosine triphosphate
AWCGS	Allan Wilson Centre Genome Service
BMD	Becker muscular dystrophy
BSA	bovine serum albumin
CBB	Coomassie brilliant blue
CCP4	Collaborative Computational Project No. 4
CCP4MG	Collaborative Computational Project No. 4 Molecular Graphics
CD	circular dichroism
CH	calponin homology
COOT	Crystallographic Object-Oriented Toolkit
CV-SMD	constant velocity-steered molecular dynamics
DAP	dystrophin-associated protein
DAPC	dystrophin-associated protein complex
DIW	deionised water
dko	double knockout
DMD	Duchenne muscular dystrophy
DSR1	dystrophin N-terminal first spectrin repeat (residues 338 to 456)
DSR12	dystrophin N-terminal first two spectrin repeat (residues 338 to 567)
DTT	dithiothreitol
Dys	dystrophin
EM	electron microscopy
EDTA	ethylenediaminetetaacetic acid
F-actin	filamentous actin

HCl	hydrochloric acid
IDT	Integrated DNA Technologies
IPTG	isopropyl-β-D-thiogalactopyranoside
MCS	multiple cloning site
MD	molecular dynamics
MPD	2-methyl-2, 4-pentanediol
MR	molecular replacement
MTJ	myotendinous junction
NaCl	sodium chloride
NAMD	Not (just) Another Molecular Dynamics program
Ni-NTA	nickel nitrotriacetic acid
NMJ	neuromuscular junction
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PDB	protein data bank
PEG	polyethylene glycol
Pfam	database of Protein families
PMSF	phenylmethanesulfonyl fluoride
pN	pico Newton
rTEV	tobacco etch virus, recombinant
s/n	supernatant
SMART	Simple Modular Architecture Research Tool
SR	spectrin repeat
TCEP	tris [2–carboxyethyl] phosphine
TEMED	N,N,N',N'-tetramethylethylenediamine
Tris	tris(hydroxymethyl)aminomethane
USR1	utrophin N-terminal first spectrin repeat (residues 308 to 425)
USR12	utrophin N-terminal first two spectrin repeats (residues 308 to 537)
Utr	utrophin
Ve	elution volume
Vo	void volume
VMD	visual molecular display

Xp21	short arm(p) of the X-chromosome at position 21
Å	Ångström or Angstrom
λ	wavelength
θ	angle of reflection
a, b, c	axial lengths of a unit cell along x, y and z coordinates
α, β, γ	interaxial angles between b & c, c & a, and a & b respectively

Related Publications

Part of the work presented in this thesis is in the following publication. I am very grateful to all co-authors of these papers.

Muthu M, Richardson KA, Sutherland-Smith AJ, (2012). The crystal structures of dystrophin and utrophin spectrin repeats: Implications for domain boundaries. *PLoS ONE 7(7): e40066. Doi:10.1371/journal.pone.0040066.*

PDB Coordinates: 3UUL, 3UUM, 3UUN