doi:10.1016/j.jmb.2008.04.046 *J. Mol. Biol.* (2008) **379**, 981–990

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Solution Structure of the Inner DysF Domain of Myoferlin and Implications for Limb Girdle Muscular Dystrophy Type 2B

Pryank Patel[1], Richard Harris[2], Stella M. Geddes[1], Eugen-Matthias Strehle[1,3], James D. Watson[4], Rumaisa Bashir[5], Katharine Bushby[3], Paul C. Driscoll[2] and Nicholas H. Keep[1]*

[1]*Institute of Structural and Molecular Biology and School of Crystallography, Birkbeck University of London, London WC1E 7HX, UK*

[2]*Institute of Structural and Molecular Biology and Department of Biochemistry and Molecular Biology, University College London, London WC1E 6BT, UK*

[3]*Institute of Human Genetics, University of Newcastle, Newcastle-upon-Tyne NE1 3BZ, UK*

[4]*European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK*

[5]*School of Biological and Biomedical Sciences, South Road, Durham DH1 3LE, UK*

Received 15 February 2008; received in revised form 11 April 2008; accepted 21 April 2008
Available online 26 April 2008

Edited by M. F. Summers

Mutations in the protein dysferlin, a member of the ferlin family, lead to limb girdle muscular dystrophy type 2B and Myoshi myopathy. The ferlins are large proteins characterised by multiple C2 domains and a single C-terminal membrane-spanning helix. However, there is sequence conservation in some of the ferlin family in regions outside the C2 domains. In one annotation of the domain structure of these proteins, an unusual internal duplication event has been noted where a putative domain is inserted in between the N- and C-terminal parts of a homologous domain. This domain is known as the DysF domain. Here, we present the solution structure of the inner DysF domain of the dysferlin paralogue myoferlin, which has a unique fold held together by stacking of arginine and tryptophans, mutations that lead to clinical disease in dysferlin.

Keywords: muscular dystrophy; dysferlin; myoferlin; Fer domain; NMR

Introduction

Limb girdle muscular dystrophy (LGMD) describes a range of progressive muscle-wasting diseases, which begin with the arms and pelvic girdle and are subclassified based on the underlying genetic defect. Miyoshi myopathy[1] is an autosomal recessive muscle-wasting disease that begins in the distal, posterior

**Corresponding author.* E-mail address: n.keep@mail.cryst.bbk.ac.uk.

Abbreviations used: LGMD, limb girdle muscular dystrophy; PDB, Protein Data Bank; HSQC, heteronuclear single quantum coherence; NOE, nuclear Overhauser enhancement; TSR, thrombospondin repeat; NOESY, nuclear Overhauser enhancement spectroscopy.

leg muscles. The dysferlin gene was identified by reverse genetics as the locus of mutations that lead to both autosomal recessive LGMD type 2B[2] and Myoshi myopathy.[3] Dysferlin was so named as it showed homology to the *Caenorhabditis elegans* gene *fer1*. Mutations in fer1 cause defects in spermatozoa motility due to a failure of the immature spermatid to fuse with membranous organelles.[4]

The ferlin family of proteins is characterised by a number of C2 domains, normally labelled A, B, and so forth, starting from the N-terminus, and a C-terminal membrane-spanning helix. C2 domains were originally characterised as the second homologous region amongst isoforms of Ca^{2+}-dependent protein kinase C and were subsequently identified in a wide range of proteins (for a review, see Ref. 5). C2 domains consist of around 130 residues, which form two 4-stranded antiparallel β-sheets, with two different topologies known.[6,7] C2 domains classically bind phospholipids in a Ca^{2+}-dependent manner, although some domains have been reported to bind to phospholipids independent of calcium ions and others have not had any binding properties demonstrated for them.[8] C2 domains also form protein–protein interactions and have been shown to have more than one interaction interface.[9]

In humans, the ferlin family consists of three characterised proteins: dysferlin, myoferlin[10] and otoferlin.[11] Myoferlin has 56% sequence identity to dysferlin and is highly expressed in early differentiating myoblasts, in contrast to dysferlin, which is most highly expressed in mature myotubes.[12,13] Dysferlin knockout mice show defects in Ca^{2+}-dependent sarcolemmal membrane repair and accumulate vesicles at the sarcolemma.[14] Microarray analysis of dysferlin-deficient mice identified annexins as potential interaction partners, and the interaction was confirmed experimentally.[15] Myoferlin knockout mice show reduced muscle size and smaller myofibres, although there is some increase in dysferlin to compensate.[13] Conversely, myoferlin is upregulated in dystrophic and regenerating muscle and myoferlin and dysferlin can partially compensate for each other. Otoferlin is mutated in a non-syndromic form of deafness.[11] Otoferlin interacts with SNAP25 and SNARE proteins and probably acts as the calcium sensor that triggers membrane fusion at auditory cell synapses.[16]

Domain annotation of the various ferlin family members varies significantly between the two main sequence-based annotation databases Pfam[17] and SMART,[18] although they do cross-refer to domains annotated by the other (Fig. 1). The databases do not agree on the number of C2 domains. There are probably seven C2 domains in both myoferlin and dysferlin, but both the fifth and seventh domains are only weakly predicted by both databases with *E* values >0.001. In contrast, the C2A domains of both dysferlin and myoferlin are classical C2 domains. The C2A domain of dysferlin has been shown experimentally to be a Ca^{2+}-dependent phospholipid binding domain,[12] and the structure of the C2A domain of myoferlin [Protein Data Bank (PDB) accession code 2dmh, unpublished data] has regular type II topology (similar to phospholipase C^{δ}).

Outside the C2 domains, Pfam predicts, for both myoferlin and dysferlin, a FerI domain, which lies between the C2B and C2C domains, and FerA and FerB, which lie in the long stretch between C2C and

Table 1. Refinement statistics

	Ensemble		Lowest energy
RMSD to restraints			
Distances (Å)			
Unambiguous			
Intra (606)	0.016	±0.002	0.015
Sequential ($\|i-j\|=1$) (762)	0.025	±0.002	0.025
Short ($1<\|i-j\|<5$) (316)	0.030	±0.003	0.033
Long ($\|i-j\|>5$) (921)	0.026	±0.002	0.025
Ambiguous (205) (Å)	0.023	±0.006	0.018
Hydrogen bond (48) (Å)	0.024	±0.001	0.024
Dihedral (109) (°)	0.510	±0.111	0.540
RDC (87) (Hz)	0.216	±0.012	0.223
Number of residual restraint violations			
NOE violations >0.5 Å	0		0
Angle violations >5°	0		0
Deviations from idealised covalent geometry			
Bonds (Å)	0.008	±0.001	0.008
Angles (°)	1.202	±0.027	1.215
Impropers (°)	2.053	±0.084	2.022
PROCHECK parameters			
Most favoured (%)	75.990	2.921	77.100
Additionally allowed (%)	20.520	3.044	20.000
Generously allowed (%)	2.405	1.137	1.900
Disallowed (%)	1.110	0.996	1.000
Number of bad contacts	0.650	0.587	0.000
CHARMM Lennard–Jones energy			
E-LJ (kcal mol^{-1})	−1000	25	−1002
R-factor for RDC (%)	0.831	0.048	0.859
RMSD from average structure			
Backbone (Å)	0.744	0.106	0.746
Heavy atoms (Å)	1.140	0.147	1.054

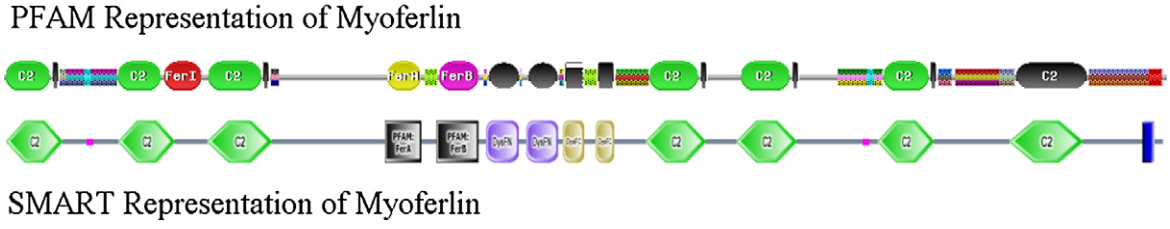

Fig. 1. Domain structure and sequence of the ferlins. Pfam and SMART domain representations of human myoferlin.