Structural and functional analysis of two small leucine-rich repeat proteoglycans, fibromodulin and chondroadherin

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Abstract

The small leucine-rich proteoglycans (SLRPs) are important regulators of extracellular matrix assembly and cell signalling. We have determined crystal structures at ~2.2 Å resolution of human fibromodulin and chondroadherin, two collagen-binding SLRPs. Their overall fold is similar to that of the prototypical SLRP, decorin, but unlike decorin neither fibromodulin nor chondroadherin forms a stable dimer. A previously identified binding site for integrin α2β1 maps to an α-helix in the C-terminal cap region of chondroadherin. Interrogation of the Collagen Toolkits revealed a unique binding site for chondroadherin in collagen II, and no binding to collagen III. A triple-helical peptide containing the sequence GAOGPSGFQGLOGPOGPO (O is hydroxyproline) forms a stable complex with chondroadherin in solution. In fibrillar collagen I and II, this sequence is aligned with the collagen cross-linking site KGHR, suggesting a role for chondroadherin in cross-linking.

Introduction

The small leucine-rich proteoglycans (SLRPs) constitute a family of secreted proteins with important roles in extracellular matrix assembly [1,2] and cell signalling [3]. SLRPs are divided into five classes based on sequence homology. Despite their name, not all SLRPs are modified with glycosaminoglycan chains, and several of them are additionally modified by tyrosine sulphation [1,2]. The biological functions of SLRPs have been studied in knockout mice and the findings so far suggest tissue-specific functions of each SLRP, conveyed through defined temporospatial expression patterns and interactions with collagen. The data also point to differential roles of SLRPs in organising collagens into tissue-specific supramolecular structures [1,2].

Fibromodulin, a class II SLRP, is associated with dense collagen matrix in tendons and ligaments, as well as in fibrotic tissues, tumors and atherosclerotic plaques [4–6]. In fibromodulin-deficient mouse tendons, collagen fibrillogenesis is dysregulated: collagen fibrils are misassembled [7], collagen α chains are aberrantly cross-linked, and collagen C-telopeptides are excessively oxidised by the collagen cross-linking enzyme, lysyl oxidase [8]. A possible mechanism for fibromodulin's role in collagen fibrillogenesis is its recruitment to collagen cross-linking sites and also its interaction with, and apparent effect on, lysyl oxidase [9].

One of the more distant homologues of fibromodulin is chondroadherin, a class IV SLRP found in cartilage and bone [10]. Chondroadherin-deficient mice have thinner cortical bones and longer growth plate proliferation zones [11], as well as mechanically softer knee surface cartilage [12]. Chondroadherin interacts with collagen II [13], and mediates cell-matrix interactions through binding to integrin α2β1 [14] and heparan sulphate [15].

The SLRPs belong to the large superfamily of leucine-rich repeat (LRR) proteins [16,17], which

Table 1. Crystallographic statistics.

	Fibromodulin	Chondroadherin
Data collection		
Beamline	I04-1	I24
Wavelength (Å)	0.9282	1.739
Resolution range (Å)	65.9–2.21 (2.27–2.21)	58.4–2.17 (2.23–2.17)
Space group	*C*2	*C*2
Unit cell dimensions		
a, *b*, *c* (Å)	108.11, 98.93, 111.34	215.45, 60.70, 57.66
α, β, γ (°)	90, 107.39, 90	90, 100.60, 90
Unique reflections	55,434	37,325
Multiplicity	6.8 (6.9)	5.8 (3.9)
Completeness (%)	99.0 (99.1)	95.8 (75.3)
Mean I/σ(I)	9.5 (1.1)	9.3 (1.6)
$CC_{1/2}$	0.998 (0.523)	0.987 (0.506)
R_{merge}	0.111 (1.81)	0.148 (0.840)
Refinement		
Protein atoms	5268	5210
Solvent atoms	566 H_2O, 2 Ni^{2+}, 2 Cl^-, 1 SO_4^{2-}	412 H_2O, 2 Ni^{2+}, 1 Cl^-, 3 PO_4^{3-}
R_{work}	0.179	0.188
R_{free}	0.216	0.240
R.m.s.d. bonds (Å)	0.003	0.003
R.m.s.d. angles (°)	0.76	0.69
Ramachandran plot		
Favoured (%)	95.1	92.8
Outliers (%)	0	0

includes not only secreted proteins but also a large number of cell surface receptors, such as the Toll-like receptors involved in innate immunity [18], the platelet von Willebrand factor receptor, glycoprotein Ib [19,20], and proteins involved in neural development [21,22]. The defining feature of these proteins is the presence of multiple repeats of 20–30 amino acids in length and starting with the consensus sequence LxxLxLxxNxL (L can be substituted by I, V or other hydrophobic residues). The folding principle of LRR proteins was revealed by the crystal structure of ribonuclease inhibitor [23]: the conserved leucine residues form the hydrophobic core of a curved solenoid structure that is characterised by an inner concave face composed of parallel β-strands and an outer convex face composed of variable structure. In proteins with long LRRs, such as ribonuclease inhibitor, the outer face consists of α-helices and the solenoid is highly curved; in proteins with short LRRs, the helices are replaced by loops and turns, which reduces the solenoid's curvature [16].

Interaction partners of LRR proteins frequently bind to the concave face [16]. Indeed, several modelling and mutational studies have implicated the concave face of SLRPs in collagen binding [4,24–29]. The crystal structure of the prototypical class I SLRP, decorin, revealed a tight dimer in which most of the concave face is occluded [30]. This finding led to a controversy about the physiological state of decorin and other SLRPs [31–33], which was only recently resolved by the demonstration that decorin

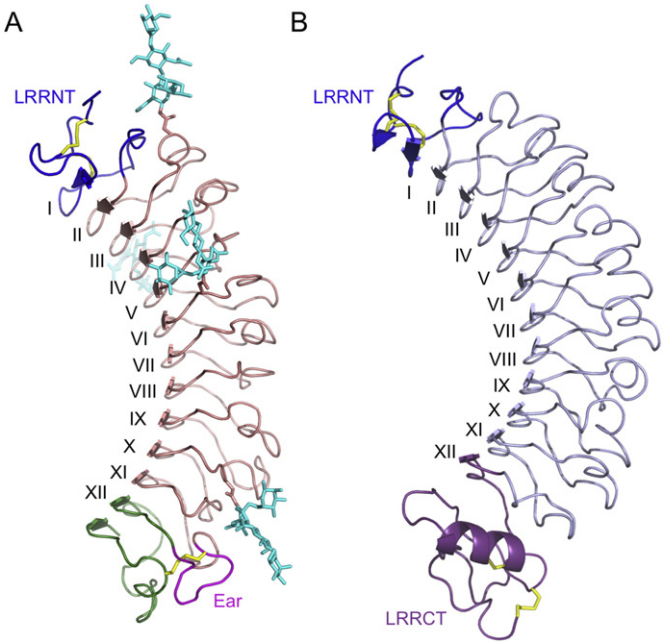

Fig. 1. Crystal structures of (A) fibromodulin and (B) chondroadherin. The LRRNTs are coloured blue in both proteins and disulphide bonds are shown as yellow sticks. The C-terminal cap of fibromodulin is in green, with the ear loop highlighted in magenta. The four *N*-linked glycans of fibromodulin are shown as cyan sticks. The LRRCT of chondroadherin is in purple. The LRRs are labelled with roman numerals (see Fig. S1 for sequences).