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Structural study on the F-spondin reeler domain revealed a deformable disulfide-bonded loop in the β-sandwich structure

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Introduction

F-spondin is a secreted and extracellular matrix-attached protein implicated in axonal pathfinding during neural development as well as vascular remodeling in adult tissues. F-spondin has 807 amino acids divided into eight domains: N-terminal reeler domain, a spondin domain, and six C-terminal thrombospondin type-1 repeat domains. The N-terminal reeler domain of F-spondin shares amino acid sequence similarity with reelin, which is a large secreted glycoprotein that guides migrating neurons during cortical development.

F-spondin is processed to two fragments in vivo. Recent analyses have shown that N-terminal fragment binds to the central domain of amyloid-β precursor protein (APP) in a calcium dependent manner and prevent the initial β-secretase cleavage of APP. The C-terminal fragment is further processed to produce shorter fragments with opposing biological functions (i.e., repulsive and adhesive properties toward neurons), of which spatial presentation are controlled by cell surface receptors that belong to lipoprotein receptor family.

In this study, we have determined the three-dimensional structure of the N-terminal reeler domain by x-ray crystallography. Our results have revealed that the reeler domain adopts a nine-stranded β-sandwich fold similar to immunoglobulin domains, but it also shows unique structural features including an N-terminal disulfide-bonded loop with unusual structural plasticity.

Experimental procedure and Results

Structure determination of FSP198, the longer fragment of the F-spondin reeler domain

To obtain the structural data on the F-spondin reeler domain, we first designed a construct including N-terminal 198 residues of this domain (FSP198). The FSP198 fragment was recombinantly produced in mammalian expression system and subjected to crystallization. Diffraction quality crystals were obtained at 293K using 0.2 M Bis-tris (pH 5.5), 0.4 M ammonium acetate, 25% polyethylene glycol (PEG) 3350 as precipitant. The native data set was collected at the beamline PF-AR NW-12A. The crystal diffracted x-ray up to 2.7 Å resolution, and it belongs to the tetragonal space group $P4_3$ with unit cell dimensions of $a = b = 57.6$ Å and $c = 190.9$ Å. Initial phases were determined with the single wavelength anomalous scattering method by using the Pt-derivative crystal. The derivative data were collected at the beamline BL-17A. After iterative model building and refinement, the crystallographic R-factor and free R-factor were reduced to 24.7 % and 31.4 %, respectively. FSP198 adopts an immunoglobulin-like nine-stranded β-sandwich fold, and four molecules are present in the asymmetric unit. Interestingly, two different conformations, designated as A and B, were observed in the N-terminal region. The N-terminal region forms a long flexible loop, which is anchored to the main body of the β-sandwich with a disulfide bond. In molecule A, the loop makes long excursion and overpasses the ridge of the front sheet. In contrast, the same loop in molecule B shifts more than 10 Å and makes a short parallel β-sheet with a part of the front sheet.

Structure determination of FSP145, the shorter fragment

We next designed a new construct including the core 145 residues (FSP145) to improve the accuracy of the structure and to identify the actual native structure of the reeler domain. High quality crystals were obtained at 293 K using 0.1M Tris-HCl (pH 8.0), 0.2M trimethylamine N-oxide and 15% PEG 3350. X-ray diffraction experiment was performed at the beamline PF-AR NW-12A, and the native data set was collected up to 1.45 Å resolution. The crystal belongs to the monoclinic space group $C2$ with unit cell dimensions of $a = 93.3$ Å, $b = 50.5$ Å, $c = 63.2$ Å, and $\beta = 100.4°$. Two FSP145 molecules are present in the asymmetric unit. Their overall structures are similar to that of the molecule A of FSP198, but the disulfide-bonded loops assume different conformations. The loop wraps around the front face of the β-sheet like molecule A, but with a different path. The loop does not pack against the hydrophobic ridge of the β-sheet but instead makes salt bridge. Our results have shown that the N-terminal disulfide bonded loop has unusual structural plasticity, suggesting a specialized function of this region in mediating protein-protein interactions.

References

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