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Elucidating the structural features of the signaling molecule reelin by x-ray crystallography

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Introduction

Reelin, a gigantic extracellular glycoprotein, plays a central role in cortical layer formation during mammalian brain development. Reelin was originally identified as a gene product absent in reeler mice exhibiting malformations of the cerebral cortex. Reelin acts on migrating neuronal precursors, and regulates correct cell positioning in the cortex and other brain structures. It is now accepted that reelin binds to the LDLR family receptors such as ApoER2 or VLDLR on neurons and initiates a signaling cascade involving phosphorylation of the adaptor molecule Dab-1.

Reelin is a modular protein and has a characteristic repeat structure termed as the reelin repeat. Reelin contains eight repeats in total, and each repeat comprises a central epidermal growth factor (EGF) module flanked by two homologous subrepeats of 150-190 amino acids. The EGF-like module is ubiquitous among extracellular proteins, but the two subrepeats are unique to reelin and fail to show any sequence similarities to other protein families. Thus, the structural data were not at all available for the reelin repeat as well as the full-length reelin molecule. In this study, we tried to elucidate the structural features of the reelin molecule by x-ray crystallography.

Experimental procedure and Results

Structure determination of one-repeat fragment, R3

To obtain the structural data on the reelin repeat, we first tried to crystallize the third reelin repeat, which is designated as R3. The R3 fragment was recombinantly produced in mammalian expression systems and subjected to crystallization. Diffraction quality crystals were obtained at 293K using 12-20 %(w/v) PEG3350, 200-400 mM $MgCl_2$, 100 mM Tris-Cl pH 8.5 as precipitant. Preliminary crystallographic data were collected at the beamlines BL-6A and PF-AR NW-12A. The crystal belongs to the rhombohedral space group $R32$ with unit cell dimensions of $a = b = 129.93$ Å and $c = 122.49$ Å in the hexagonal setting. Since attempts to produce heavy atom derivatives have failed, initial phases were determined with the single wavelength anomalous scattering method by using the Se-Met mutant. The resulting structure showed a compact, horseshoe-like arrangement of the three modules; subrepeat A, EGF-like module and subrepeat B. The two subrepeats showed structural similarities to carbohydrate-binding proteins, which had not been anticipated from primary sequence analysis.

Structure determination of two-repeat fragment, R5-6

Our biochemical analysis has indicated that the two-repeat fragment comprising the fifth and sixth repeats, R5-6, is a minimum signaling competent unit. Hence, we next worked on the structure determination of R5-6. The R5-6 fragment subjected to crystallization was also produced in mammalian cells. Optimized crystallization conditions are as follows; 4-7% PEG3350, 75-140 mM ammonium acetate, 100 mM HEPES-Na pH 7.0. The crystal belongs to the monoclinic space group $P2_1$ with unit cell dimensions of $a = 61.01$ Å, $b = 70.95$ Å, $c = 94.77$ Å and $\beta = 93.6$ °. Phases were determined with the molecular replacement method by using the R3 structure above as a search model. The most striking structural feature of the R5-6 fragment is the unusual spatial arrangement of R5 and R6. These two repeat units are arranged side-by-side and related by almost perfect translation.

Identification of zinc ions in R5-6 structure

In the course of model building, we found two metal ions on the molecular surface of R5-6. These two ions were presumed to be Zn^{2+} ions based on the geometry of the metal and the ligands. The ions are coordinated with His and Glu side chains and the coordination numbers are 4 and 5, which is consistent with the typical coordination geometry of Zn^{2+}. To confirm the binding of Zn^{2+}, we collected diffraction data at two different wavelengths; 1.27911 Å (near the zinc absorption edge) and 1.29000 Å (as the lower energy remote), and calculated anomalous difference Fourier maps. Data collections were performed at the beamline BL-5A. As expected, strong peaks from zinc anomalous dispersion appeared at the two metal-binding sites in the electron density map at 1.27911 Å and they diminished at 1.29000 Å. Taken together, we have concluded that the observed two metal ions are both Zn^{2+}.

References

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