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Crystal structure of full-length ApoER2 ectodomain in complex with its ligand reelin

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1 Introduction

Reelin is a large extracellular protein that plays a critical role in brain development. Reelin binds to apolipoprotein E receptor 2 (ApoER2) and very low-density lipoprotein receptor (VLDLR) on the surface of neurons to induce tyrosine phosphorylation of Dab1 associated with the cytoplasmic domain of these receptors, resulting in the proper migration of the neurons during the brain development [1, 2].

ApoER2 and VLDLR are members of the low-density lipoprotein receptor (LDLR) family. Their extracellular regions consist of seven or eight LDLR class A (LA) modules, three epidermal growth factor (EGF) modules, and a YWTD β-propeller domain.

We have previously reported the crystal structure of the biologically active reelin fragment (R5-6, 725 residues) in complex with the minimum ligand-binding fragment of ApoER2 (LA1, 44 residues), which clarified the binding mechanism involving the Ca^{2+}-coordinated acidic residues in the receptor LA1 module to capture a single Lys residue of reelin (K2467) [3]. While LA1 is sufficient to mediate stable binding of reelin to the receptor, we find that the R5-6 exhibits much higher affinity toward the full-length ApoER2 ectodomain (ApoER2ec, 571 residues) than the LA1 fragment, suggesting the presence of additional binding interface(s).

In the present study, we have solved the crystal structure of the complex formed between R5-6 and ApoER2ec at 3.2Å resolution.

2 Experiment

Recombinant ApoER2ec was expressed as a soluble secreted protein fused with human growth hormone (hGH) at its N-terminal, using CHO-Lec3.2.8.1 cells [4, 5]. The culture supernatants containing secreted hGH-ApoER2ec was concentrated using Aquacide II and purified using Ni-NTA agarose. The N-terminal hGH-tag was removed by TEV protease cleavage and further purification was performed by Mono-Q anion exchange column chromatography.

Selenomethionine (SeMet) labeled R5-6 fragment was produced by CHO-Lec3.2.8.1 stable cell line as described [6].

Purified ApoER2ec and R5-6 were concentrated by ultrafiltration, respectively, and then mixed at a molar ratio of 1: 1.25 for complex formation and subjected to crystallization trials.

Crystals with bi-pyramid shape were formed under the condition of 20 mM Hepes-NaOH pH7.5, 200-250 mM sodium thiocyanate, and 11-12.5%(w/v) PEG 3350. Crystals were cryoprotected by transferring into the crystallization solution supplemented with 20%(v/v) ethylene glycol. The X-ray diffraction data sets were collected at the beam line BL-5A and BL-17A of Photon Factory. The data were processed using *HKL2000* program package [7], and initial phases were determined by SeMet single-wavelength anomalous diffraction using *SHLEX* [8] and *SHARP* [9]. The structural models were built using *COOT* [10] with refinement cycle with *Refmac5* and *phenix.refine* [11,12]. Quality of the models was validated using the program *MOLPROBITY* [13].

3 Results and Discussion

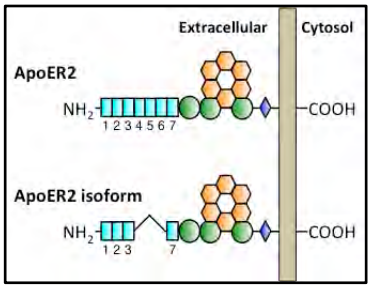
We have previously clarified the basic recognition mechanism of reelin by ApoER2, by solving the structure of complex between R5-6 and the minimum binding unit within the receptor (LA1) [3]. However, the structure did not give insights about how the regions outside this “core” binding interface may contribute to the recognition specificity/affinity. Therefore, we aimed at obtaining structural information of the complex between R5-6 and the multi-domain, full-length receptor ectodomain fragment. To facilitate crystallization, we used the shortest and thus supposedly less flexible isoform of ApoER2 comprising four LA modules (LA1, 2, 3, and 7), three EGF modules (EGF-A~C), and a YWTD β-propeller domain. This isoform is in fact expressed most abundantly in brain [14].

Based on the experimental electron density map, we were able to build a structural model of the complex between ApoER extracellular domain and R5-6. Due to the lack of clear electron density, we could not model the structure for LA3 and LA7. However, the positions for the ApoER2 LA1 domain and the R5-6 ligand were essentially unchanged from the structure of the “minimum” complex determined previously.

Furthermore, we identified two additional interaction sites that have not been appreciated before.

The first new site (called site 2) corresponds to an interaction between LA2 and Reelin repeat 5. A Lysine residue from repeat 5 (Lys-2194) is recognized by both acidic and aromatic residues of LA2 in a canonical fashion. The second new site (called site 3) is composed of a polymorphic Reelin loop in repeat 5 that extends into the inter-domain cleft between EGF-A and B in ApoER2 to make direct contact.

Our result not only provides more detailed view as to the molecular mechanism of reelin recognition by the neuronal receptor, but also gives a first glimpse of multipoint ligand recognition by the LDLR family member receptors.



Domain architectures of ApoER2
ApoER2 consists of LA modules (cyan rectangles), EGF modules (green ovals) and a YWTD beta-propeller domain (orange hexagons). The long and short isoforms are shown at top and bottom, respectively.

Crystal structure of ApoER2ec–R5-6 complex
R5-6 displayed as a ribbon model (gray). While, ApoER2ec displayed as a cartoon model. LA1-2 are colored in cyan, a cluster of EGF-motif are colored in light green, and a YWTD beta-propeller domain is colored in orange.

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