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Antibody-driven crystallization of sorLA Vps10p domain

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1. Introduction

We have recently solved the crystal structure of sorLA Vps10p domain in its ligand-free form and in two liganded forms with different peptides. SorLA is a huge single-pass transmembrane protein containing a multi-domain extracelluar region. It is expressed in neurons, and has been known to be genetically linked with Alzheimer's disease (AD) [1, 2, 3]. Although its physiological function has not been entirely clear, we discovered that sorLA directly binds amyloid-β peptide through its N-terminal Vps10p domain. Starting from this discovery, we conducted a series of investigations using mouse models and cultured cell system in collaboration with Dr. Thomas E. Willnow's group (Max Delbrück Center for Molecular Medicine, Berlin), resulting in a proposal that sorLA functions as Aβ clearance receptor [4].

Following this study, we have tried to unravel the molecular mechanism of Aβ recognition by sorLA Vps10p domain. As a result of biochemical assays and crystal structure determinations, we concluded that sorLA Vps10p domain recognizes each ligand peptide specifically, whereas the amino acid sequences of ligand peptides do not contribute much to the binding specificity. The cyrstal structure revealed that sorLA Vps10p accomplishes this moderate binding specificity by utilizing β-extention mechanism. Finally, we suggested that sorLA Vps10p may recognize amyloidogenic peptides while they are monomeric inside the cells, and prevent them from becoming neurotoxic by forming insoluble oligomers [5].

As a next stage of sorLA Vps10p study, we have tried to crystallize several variants of sorLA Vps10p domains such as mutants and complexes with compounds. SorLA Vps10p domain exhibits difficult crystallization property; it requires very long crystallization period (several months) with rather low reproducibility. To improve this poor crystallization character, we decided to utilize a structure-dependent antibody as crystallization chaperon.

2. Experiment

Recombinant human sorLA Vps10p domain lacking propeptide portion with C-terminal His-tag was produced using CHO lec 3.2.8.1 [6]. The secreted protein in the culture medium was purified using Ni-NTA resin. C-terminal His-tag was cleaved off by TEV protease, and N-linked glycans were trimmed by Endoglycosidase H (New England Biolab). After changing the pH value of solution from 8.0 to 4.5, protein was purified by cation exchange chromatography under acidic condition. Finally, the pH value of solution was reverted to 8.0, and the sample solution was concentrated by ultrafilteration.

Anti-sorLA Vps10p antibody (Mouse $IgG_{1,\ \kappa}$) was purified from the hybridoma culture supernatants and the puriried IgG was digested with papain-agarose (Thermo scientific) to produce Fab fragment. The F_c fragment was removed from the digestion product though the protein A-Sepharose resin (GE healthcare). The purified F_{ab} fragment was mixed with sorLA Vps10p protein, and further purified by size exclusion chromatography. The complete shift in the elution position indicated the stable interaction between sorLA Vps10p and F_{ab} fragment, and the stoicheometric complex was isolated. The complex was concentrated with the ultrafilteration devise to approximately 10.0 mg/mL.

The purified sorLA Vps10p:F_{ab} complex was screened for its crystallization condition in the presence of 15-residue propeptide and its Se-Met derivative (denoted as LPP15 and LPP15Se). The initial condition including 0.1 M cacodylate buffer pH 6.5, 0.2 M $(CH_3COO)_2Ca$, 20 % PEG 8,000 was optimized and finally we could obtain diffraction quality crystals with LPP15Se under the condition of 0.1 M MES buffer pH 6.5, 0.2 M $(CH_3COO)_2Ca$, 12 % PEG 8,000 for several days. Collected images were processed by *HKL2000*[7] at the resolution of 3.2 Å, and the initial phases were calculated by molecular replacement with *PHSER*[8] using our sorLA Vps10p domain structure complexed with LPP15 (PDB ID: 3WSY) and anti-progesterone antibody F_{ab} fragment (Mouse $IgG_{1,\ \kappa}$ PDBID: 1DBA) as search models. Initial rigid-body refinement and subsequent refinment procedure by *phenix.refine*[8] resulted in the R_{work}/R_{free} factor of 0.208 / 0.260 for two pairs of sorLA Vps10p complexed with F_{ab} fragment in the asymmetric unit.

3. Result and Discussion

By utilizing specific antibody against sorLA Vps10p domain, we successfully obtain the crystal of the Fab complex which was of appropriate diffraction quality within several days, in a highly reproducible manner. This F_{ab} fragment did not interfere with the ligand binding of sorLA Vps10p, and strongly drove the crystallization. Using this F_{ab} fragment, it will be possible to crystallize many kinds of sorLA Vps10p variants, thereby facilitating our further understanding of the molecular mechanism behind its biological function.

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