SorLA Vps10p domain directly recognizes Amyloid-β peptide

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1 Introduction

SorLA is a single-pass transmembrane protein containing a large (~2,000 a.a) multi-domain extracellular region. It is abundantly expressed in neurons while its physiological function is still incompletely understood. SorLA has been known to be genetically linked with Alzheimer's disease (AD) and its protein level is reduced in the brain of some AD patients [1]. It was reported that sorLA directly interacts with amyloid precursor protein (APP), a precursor of the pathogenic amyloid-β (Aβ) peptide, through its LA domain [2], which led to a hypothesis that sorLA would re-route APP away from the amyloidogenic pathway. Previously, we reported that sorLA directly binds to Aβ peptide via the Vps10p domain and plays an important role in the catabolism Aβ clearance in living cells [3]. We also determined the structure of Vps10p domain of sorLA, both as ligand free form and as a complex with its own propeptide. Our structural and functional study strongly suggest that sorLA Vps10p domain may play a central role in its Aβ-lowering function in neurons. In the current study, we obtained the structure of human sorLA Vps10p domain in complex with Aβ peptide fragment, and identified the Aβ peptide binding site at the inner tunnel of the β-propeller fold.

2 Experiment

Recombinant human sorLA Vps10p domain (753 a.a) with C-terminal His-tag was produced using CHO lec 3.2.8.1 cell [4]. The secreted protein was purified from the culture supernatants using Ni-NTA resin. C-terminal His-tag was removed by TEV protease cleavage, and N-linked glycans were trimmed by Endoglycosidase H (New England Biolab). Further purification was performed by cation exchange chromatography under acidic condition, and purified protein sample was concentrated to approximately 10.0 mg/mL. The co-crystal with Aβ fragment was obtained under the condition of 0.1 M Tris buffer pH 7.8, 1.6 M sodium acetate with 1 mM synthesized 10-mer peptide fragment.

X-ray diffraction data sets were collected at the beam line BL-17A of Photon Factory. The data were processed using *HKL2000* program package [5], and initial phase was accomplished by molecular replacement method using *PAHSER* [6]. Ten-bladed β-propeller portion of sorLA Vps10p domain co-crystallized with its own propeptide was used as search model. The structure models were built using *COOT* [7] with model refinement cycle with *phenix.refine* [6]. The structure models were validated using the program *MOLPROBITY* [8].

3 Results and Discussion

Previously, we determined the structure of human sorLA Vps10p domain, which consists of a ten-bladed β-propeller domain (86-622) followed by two small Cys-rich domains designated as 10CC-a (623-675) and 10CC-b (676-751). Also, we successfully determined the structure of propeptide complex, where the peptide bound at the inner wall of the central tunnel by extending the "blade 1" β-sheet. From these results, we speculate that sorLA is able to capture Aβ peptide (which is known to possess β-sheet forming propensity). We also identified several Aβ fragments that exhibited sub-micromolar affinity toward sorLA Vps10p domain through a biochemical binding assay. One of them, $A\beta_{6-15}$ (10-mer), was used for co-crystallization, and diffraction quality crystals were obtained. We determined the structure at 3.2Å resolution by molecular replacement. As for the sorLA structure, the β-propeller domain and 10CC-a domain are essentially identical to that of propeptide complex, while 10CC-b domain is disordered in the $A\beta_{6-15}$ complex. Importantly, the ligand $A\beta_{6-15}$ bound inside the β-propeller tunnel, at the exact same position to the propeptide binding site, using the same "strand extension" mode (figure). Because the electron density was visible only for a part (~7 residues) of the 10-mer peptide and its quality was relatively poor compared to the rest of the molecule, we could not confidently build structural model of the bound peptide in the density. This poor electron density suggests a partial occupancy and/or a mobility of the Aβ peptide, and indicate the transient nature of the interaction.

Our current results provide strong evidence for the direct interaction between human sorLA Vps10p domain and the Aβ peptide, as well as its "strand-extension" binding mode. The structural detail of the Aβ-binding activity of sorLA, when combined with the fact that it functions as a sorting receptor in cells [9], helps to understand the sorLA's protective effect against AD by decreasing the cellular level of toxic Aβ peptide.

stick model : propeptide

mesh : Fo-Fc map (2.5σ)
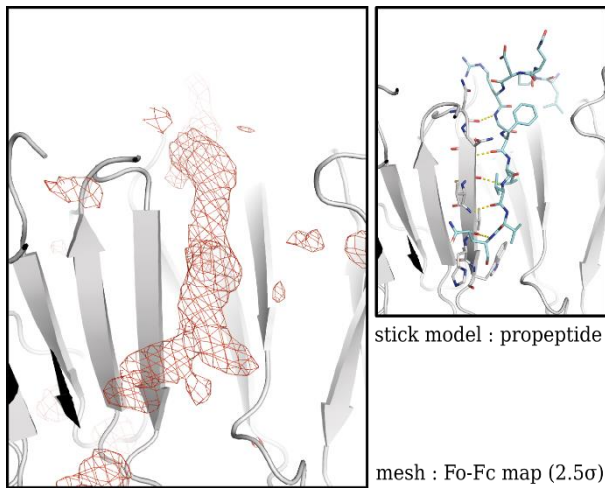
Left figure shows the structure of sorLA Vps10p domain in the Aβ6-15 complex, overlaid with the *Fo-Fc* omit map contoured at 2.5 σ (orange wireframe). Right figure shows the corresponding region of the structure of Vps10p domain in complex with the propeptide.

References

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