BL-17A/2011G027

Structural basis for peptide ligand recognition by SorLA Vps10p domain

Yu Kitago[1], Zenzaburo Nakata[1], Masamichi Nagae[1], Terukazu Nogi[2], and Junichi Takagi[1*]
[1]Osaka University, Suita, Osaka 565-0871, Japan
[2]Yokohama City University, Yokohama, Kanagawa 230-0045, Japan

1 Introduction

SorLA is a single-pass transmembrane protein containing a large (~2,000 a.a) multi-domain extracellular region and is expressed abundantly in neurons. It belongs to both LDL receptor family and Vps10p family based on its domain architecture, and its physiological function is incompletely understood. SorLA has been known for its strong genetic link with Alzheimer's disease (AD) because of the reduced level in the brain of AD patient [1]. Furthermore, it was reported that SorLA directly interacts with amyloid precursor protein (APP), a precursor for the pathogenic amyloid-β (Aβ) peptide [2]. However, it is not clear if the APP binding ability of SorLA can be solely accounted for its protective effect against AD. To shed lights into the direct role of SorLA in AD pathogenesis, we conducted a structural study of Vps10p domain located at the N-terminal of SorLA predicted to possess peptide binding ability.

2 Experiment

Human SorLA Vps10p domain (753 a.a) with C-terminal His-tag was produced recombinantly using CHO lec 3.2.8.1 cell [3]. The secreted protein was purified from the cultured medium supernatant using Ni-NTA resin. C-terminal His-tag was removed by TEV protease cleavage, and N-linked glycans were trimmed by Endoglycosidase H. Further purification was performed by cation exchange chromatography under acidic condition, and purified protein sample was concentrated to approximately 10.0 mg/mL. The ligand free protein crystal was grown under the condition of 0.1 M sodium acetate buffer pH 4.5 and 1.2 M sodium dihydrogen phosphate that gave 2.35 Å resolution diffraction using cryoprotectant solution including 20 % (v/v) ethylene glycol. The co-crystal with its own propeptide fragment was obtained under the condition of 0.1 M 2-morpholineethanesulfonic acid monohydrate (MES) buffer pH 6.5, 1.6 M sodium acetate with 1 mM 15-mer propeptide fragment giving 3.10 Å resolution diffraction.

X-ray diffraction data sets were collected at the beam line BL-17A of Photon Factory. The data were processed using *HKL2000* program package [4], and initial phase was accomplished by molecular replacement method using *MOLREP* [5]. Human sortilin Vps10p domain structure (3F6K) was used as search model for the ligand free SorLA structure. The resultant SorLA Vps10p structure was then used for the propeptide complex as a search model. The structure models were built using *COOT* [6] with model refinement cycle with *REFMAC5* [7]. The structure models were validated using the program *MOLPROBITY* [8].

3 Results and Discussion

SorLA Vps10p domain consists of ten-bladed β-propeller (86-622) having a large tunnel at the center, followed by two small Cys-rich domains designated as 10CC-a (623-675) and 10CC-b (676-751). A 15-mer propeptide fragment (LPP15) that was shown to exhibit sub-micromolar affinity by biochemical binding assay was used for co-crystallization. In the complex structure, LPP15 was bound at the inner surface of the β-propeller central tunnel, such that it formed additional strand of a propeller β-sheet.

The crystal structures suggest that SorLA Vps10p domain may generally recognize peptide ligands in a "strand-extension" mode. In separate biochemical experiment, we found that SorLA Vps10p ligand binding ability was pH-dependent, where Vps10p domain bound ligands at neutral condition and released them at acidic condition. This explains why the ligand free form crystallized only at acidic condition and propeptide complex crystallized at neutral condition. From these results, we speculate that SorLA is able to capture Aβ peptide (which is known to possess β-sheet forming propensity) and dump it in the lysosome where the internal environment is acidic. This hypothesis is consistent with the proposed function of SorLA as a sorting receptor in cells [9], and can explain the SorLA's protective effect against AD by decreasing the cellular level of toxic Aβ peptide.

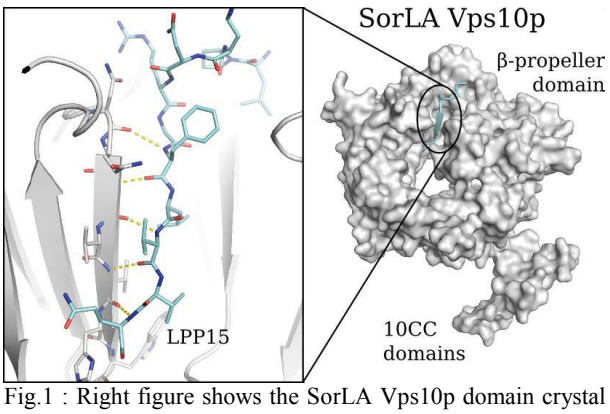

Fig.1 : Right figure shows the SorLA Vps10p domain crystal structure represented as gray surface with its propeptide fragment (LPP15) as cyan cartoon. The left close up view

shows that LPP15 binds to Vps10p domain by the parallel β-strand addition mode.

Acknowledgement

We would like to thank Drs L. M. G. Chavas, Y. Yamada, N. Matsugaki and N. Igarashi of the Photon Factory for providing data-collection facilities and for support

References

[1] Scherzer, C. R. *et al.* (2004). *Archives of Neurology* **61** : 1200-1205.

[2] Andersen, O. M. *et al.* (2006). *Biochemistry* **45** : 2618-2628.

[3] Stanley, P.(1989). *Mol Cell Biol* **9** : 377-383.

[4] Otwinowski, Z. and Minor, W.(1997). *Methods in enzymology* **276** : 307- 326.

[5] Vagin, A. and Teplyakov, A.(2010). *Acta Crystallogr D Biol Crystallogr* **66** : 22-25.

[6] Emsley, P. and Cowtan, K.(2004). *Acta Crystallogr D Biol Crystallogr* **60** : 2126-32.

[7] Murshudov, G. N.; Vagin, A. A. and Dodson, E. J.(1997). *Acta Crystallogr D Biol Crystallogr* **53** : 240-55.

[8] Chen, V. B. *et al.* (2010). *Acta Crystallogr D Biol Crystallogr* **66** : 12-21.

[9] Andersen, O. M. *et al.* (2005). *Proc Natl Acad Sci U S A* **102** : 13461-13466.

* takagi@protein.osaka-u.ac.jp