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Crystallographic analysis of extracellular fragments of mammalian plexin-A1

Tsubasa TANAKA[1], Makiko NEYAZAKI[1] and Terukazu NOGI[1,*]
[1]Graduate School of Medical Life Science, Yokohama City University,
1-7-26 Suehiro-cho, Tsurumi-ku, Yokohama, 230-0045, Japan

1 Introduction

Semaphorin has been identified as an axon guidance cues to transduce a repulsive signal via the interactions with a cell surface receptor plexin on neurons. Furthermore, it has been found that the semaphorin-plexin signaling is implicated in a wide variety of biological phenomena such as immune responses, bone homeostasis, and angiogenesis. It is also known that semaphorins are relevant to cancer and autoimmune diseases. In vertebrates, the semaphorin family contains 21 members belonging to 5 distinct classes (3 to 7) while the plexin family is constitued by nine members, which are classified into four types (A, B, C and D). All semaphorins and plexins commonly possess a sema domain, which assumes a seven-bladed β-propeller fold, in the N-terminus of the extracellular region. It is established that semaphorins and plexins bind with each other via their sema domains. Furtheremore, each semaphorin can bind to specific members of the plexin family during the signal transduction. For instance, Sema3A, which is a founding member of the semaphorin family, can signal through all of the four type-A plexins (A1, A2, A3 and A4) although neuropilin is required as a co-receptor for stable complex formation. In contrast, class 6 semaphorins bind to specific partners belonging to the type-A plexins without assistance from neuropilins. Sema6A shows higher affinities for plexin-A2 and A4 while sema6D binds more stably with plexin-A1 than other type-A plexins. In this study, we have worked on crystallographic analysis of an extracellular domain of plexin-A1 to elucidate the structural basis for the binding specificity.

2 Experiment

The extracellular region of plexin-A1 contains many post-translational modifications such as disulfide bonds and glycosylations. We therefore utilized mammalian expression system to overproduce a recombinant protein that was properly folded and glycosylated for structural analysis. Specifically, we used a mutant cell line that has deficiency in glycosylation processing pathway, termed as HEK293S GnT1(-), so as to reduce heterogeneity of N-glycosylations. On the other side, the plexin-A1 fragment was expressed as a fusion protein with a polyhistidine tag under the regulation of the CMV promoter.

To obtain a sufficient amount of protein sample for crystallization, we established a cell line that stably expresses the plexin-A1 fragment and culture it using a high-density cell culture system. As the plexin-A1 fragment was secreted from the cell, we collected culture supernatant and purified the fragment using the affinity chromatography and gel filtration. On average, several hundred micrograms of the plexin-A1 fragment were purified from one liter of culture supernatant.

The initial crystallization condition was searched using a commercial screening kit where the solutions were dispensed by a crystallization robot. Obtained crystals were soaked in cryoprotectant and then flash-frozen by liquid nitrogen. The cryoprotectant was prepared by mixing the crystallization buffer and ethylene glycol at a volume ratio of 4 : 1. X-ray diffraction experiments were performed at the beamline PF BL-17A. The diffraction data were processed using HKL2000. Structure determination was performed using the CCP4 program suite.

3 Results and Discussion

We obtained bipyramidal-shaped crystals of the plexin-A1 fragment (Fig. 1). According to the diffraction data, the crystal was estimated to belong to the tetragonal space group *I*422. We attempted to determine initial phases by molecular replacement method using the mouse plexin-A2 fragment, which was determined by our previous structural analysis. [1] Surprisingly, the results indicated that the solvent content of the crystal was extremely high and estimated over 80%. The high solvent content might be due to the presence of N-glycosylation, which should lower the chance of rigid molecular contacts.

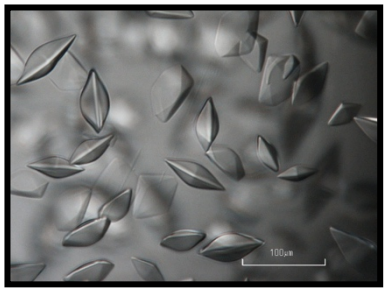

Fig. 1: Crystals of plexin-A1 fragment.

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References

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* nogi@yokohama-cu.ac.jp