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The structural basis for the 2D assembly of a β-neurexin 1/neuroligin 1 complex

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Introduction

Neurexins (Nrxs) and Neuroligins (NLs) are synaptic cell adhesion proteins that are thought to be involved in presynaptic and postsynaptic differentiation. Ectodomains of presynaptic Nrxs and postsynaptic NLs interact and assemble with each other across the synaptic cleft. This triggers the further assembly and rearrangement of several synapse-associated molecules through protein-protein interactions in the cytoplasmic region, resulting in the functional specialization of asymmetrical synapse [1]. The molecular mechanism of cell-cell adhesion mediated by Nrxs and NLs has been revealed through the X-ray structure, while it remains unclear if and how the trans-cellular complex can promote recruitment of cytoplasmic binding partners.

Here we report the crystal structure of Nrx1β and NL1 [2]. This structure exhibits the unique structural feature; the complexes assemble laterally into a "layer", that the spatial distribution is compatible with the trans-synaptic complex on adhered neurons.

Experimental Procedure

Recombinant NL1 ectodomain was produced as a fusion protein of the C-terminal segment of human growth hormone (hGH) in mammalian cell line CHOlec3.2.8.1 [3]. Nrx1β was overexpressed in E.coli as a fusion protein of glutathione S-transferase (GST). NL1 and Nrx1β were purified using affinity chromatography with Ni-NTA and immobilized glutathione respectively. Both proteins were treated with TEV protease to cleave fusion proteins and forther purified by ion-exchange chromatography. Equal molar mixture of both purified proteins was concentrated to approximately 10mg/ml for crystallization.

Unexpectedly, we observed the formation of crystalline precipitant immediately after addition of 2mM Ca^{2+} into the mixture. Finally, we obtained the crystal for X-ray diffraction analysis by hanging drop vapor diffusion. The crystal was grown under condition of 5 mM $CaCl_2$ in 10 mM Tris-Cl (pH 8.0) and 0.1 M NaCl at 293K, followed by the treatment of 10 mM Tris-Cl (pH 8.0), 0.1 M NaCl, and 20 % PEG400 for dehydration and cryoprotection.

Diffraction data were collected using synchrotron radiation beamline BL-17A of Photon Factory. The data were processed using the *HKL*-2000 program package [4]. Phase determination was accomplished by molecular replacement method using MOLREP [5]. The structure model was built using COOT [6] with cycles of model refinement with REFMAC5 [7]. This model structure was validated using the program MOLPROBITY [8].

Results and Discussion

The Nrx1β/NL1 complex comprises a heterotetramer in which two Nrx1β protomers bind to distal ends of NL1 homodimer. Additionally, the heterotetrameric complexes are regularly assembled into a plane in the crystal, forming a highly unique sheet-like structure (Figure 1). In this sheet, heterotetramers oriented in the same direction are aligned along the crystallographic *a*- and *c*-axis, and the most C-terminal tails of Nrx1β and NL1 are all pointing toward opposite membrane. This sheet mimics the situation that Nrx1β encounters and assembles with NL1 at the synaptic cleft. In fact, electron microscopic analysis revealed the 2D sheet-like structure at the site of cell-cell contact [2]. These findings indicate a mechanism in which Nrx1β and NL1 spontaneously assemble into the 2D sheet under physiological condition in the intact cell.

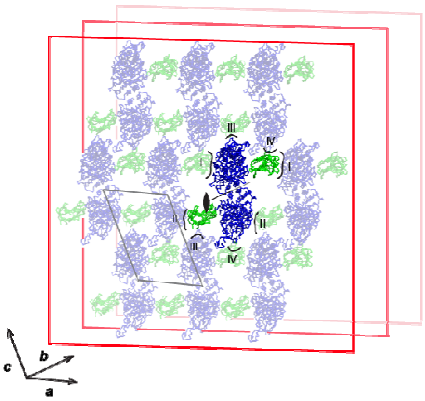

Figure 1 The 2D-assembled structure of Nrx1β/NL1 complex.

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