The structure of the ligand-binding domain of neurexin 1β: regulation of LNS domain function by alternative splicing

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Introduction

Neurexins are brain-specific cell-surface proteins that are synthesized in hundreds of isoforms [1, 2, 3]. The neurexin family is thought to play a role in neuron:neuron recognition and neuron:neuron adhesion as different isoforms bind different protein ligands. The extracellular regions of neurexins contain epidermal growth factor (EGF)-like repeats and LNS domains (laminin-neurexin-sex hormone binding globulin domains). LNS domains are present in many extracellular proteins including agrin, slit, perlecan, and the G domain of laminins [3]. One species of neurexin, neurexin 1β, contains a single LNS domain in its extracellular domain and its structure is reported here.

The primary transcripts of neurexins are alternatively spliced generating different isoforms, and three out of the five known sites are found in LNS domains (sites #2, #3, and #4). As a result of the splicing event, insertions of up to 30 amino acids are incorporated in the protein. Three ligands for neurexins have been identified, all of which interact with LNS domains: α-latrotoxin (a neurotoxin), neuroligins (brain-specific cell adhesion molecules), and neurexophilins (brain-specific molecules resembling neuropeptides) [4, 5, 6]. Binding of neuroligin and α-latrotoxin has been shown to be tightly controlled by alternative splicing at site #4, which is localized in the single LNS domain common to both neurexin 1β as well as the larger multidomain α-neurexins.

Alternative splicing of LNS domains in agrin, a protein involved in neuromuscular junction formation, also regulates function [7]. Insertion of short amino acid sequences at site z in the third LNS domain of agrin by alternative splicing activates acetylcholine-receptor clustering by promoting protein complex formation [8, 9]. Splicing at site y in agrin, located in the second LNS domain, alters its glycan/proteoglycan specificity [7].

Methods and Materials

The extracellular domain of neurexin 1β without an insert at site #4 and without its signal sequence (residues 47 through 302), numbered according to Ushkaryov *et al*. [10], was overexpressed in *E. coli*, purified, and crystallized as described elsewhere [11]. Single crystals grew as very thin plates with average dimensions of 0.3 mm x 0.25 mm x 0.005 mm. The crystals have the symmetry of space group $P2_12_12$, with cell constants of a = 116.6 Å, b = 195.9 Å, and c = 103.6 Å.

The largest crystals diffract very weakly to 3.5 Å Bragg spacing when using a conventional rotating anode x-ray source. Therefore, the use of synchrotron radiation was essential for the structure determination of neurexin 1β. At the Advanced Photon Source (APS) beamline 19-ID, our crystals diffract to 2.6 Å resolution using comparatively long exposure times of close to one minute. Initial phases were obtained from a multiwavelength anomalous dispersion (MAD) experiment, also carried out at 19-ID on the seleno-methionine variant of neurexin 1β, which mass spectroscopy had confirmed to contain two selenomethionyl residues per molecule. The experimental phases yielded an uninterpretable electron density that could, however, be improved by four-fold density averaging once noncrystallographic symmetry operators were derived from eight selenium sites and four palladium sites (from a nonisomorphous derivative) See Figure 1.

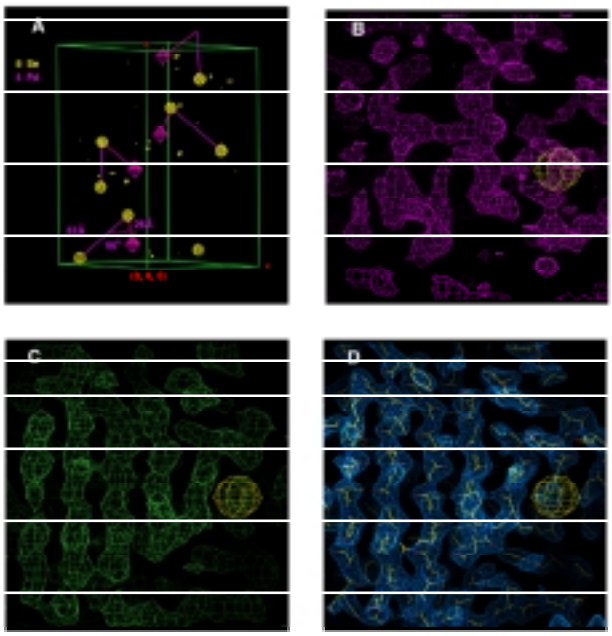

Figure 1: a) Eight selenium sites (yellow) and four palladium sites (magenta) were used in the structure determination, b) experimental electron density from the selenium phases, c) electron density after four-fold averaging, and d) electron density calculated from the final refined model.