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Review

Diverse binding modes, same goal: The receptor recognition mechanism of botulinum neurotoxin

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ABSTRACT

Botulinum neurotoxins (BoNTs) are among the most deadly toxins known. They act rapidly in a highly specific manner to block neurotransmitter release by cleaving the soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) complex at neuromuscular junctions. The extreme toxicity of BoNTs relies predominantly on their neurotropism that is accomplished by recognition of two host receptors, a polysialo-ganglioside and in the majority of cases a synaptic vesicle protein, through their receptor-binding domains. Two proteins, synaptotagmin and synaptic vesicle glycoprotein 2, have been identified as the receptors for various serotypes of BoNTs. Here, we review recent breakthroughs in the structural studies of BoNT–protein receptor recognitions that highlight a range of diverse mechanisms by which BoNTs manipulate host neuronal proteins for highly specific uptake at neuromuscular junctions.

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1. Introduction

Botulinum neurotoxins (BoNTs) are designated as Tier 1 select agents by the Centers for Disease Control and Prevention (CDC). With an estimated lethal dose for human at ~1 ng per kg of body weight (Gill, 1982), BoNTs are among the most life threatening natural substances that raise serious concern of a possible bio-warfare use (Arnon et al., 2001; Bigalke and Rummel, 2005; Binz et al., 1990). There are seven major serotypically distinct BoNTs, termed BoNT/A to BoNT/G, which comprise at least 40 different subtypes. They are also structurally and functionally related to tetanus toxin (for a recent review see (Rossetto et al., 2014)). The eighth serotype, BoNT/H, has recently been proposed but remains to be verified experimentally (Barash and Arnon, 2014; Dover et al., 2014). Interestingly, the double-faced BoNTs, especially BoNT/A, are among the top-selling drugs as prescription medicines in clinic and facial rejuvenation agents in cosmetic industries (Bigalke, 2013).

Abbreviations: botulinum neurotoxin, BoNT; light chain, LC; heavy chain, HC; N-terminal domain of HC, H_N; C-terminal domain of HC, H_C; N-terminal subdomain of H_C, H_{CN}; C-terminal subdomain of H_C, H_{CC}; synaptotagmin, Syt; synaptic vesicle glycoprotein 2, SV2.

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BoNT is synthesized as a ~150 kDa protein and then proteolytically cleaved into two chains, an N-terminal ~50 kDa light chain (LC) and a C-terminal ~100 kDa heavy chain (HC), which are linked by an essential disulfide bridge. Crystal structures of BoNT/A1, BoNT/B1, and BoNT/E1 all exhibit a similar tri-modular architecture (Kumaran et al., 2009; Lacy et al., 1998; Swaminathan and Eswaramoorthy, 2000). LC is a Zn^{2+}-metalloprotease, whereas HC comprises an N-terminal ~50 kDa translocation domain (H_N) and a C-terminal ~50 kDa receptor-binding domain (H_C). H_C is further divided into an N-terminal (H_{CN}) and a C-terminal (H_{CC}) subdomains (Fig. 1A). Other BoNT serotypes are expected to adopt a similar architecture.

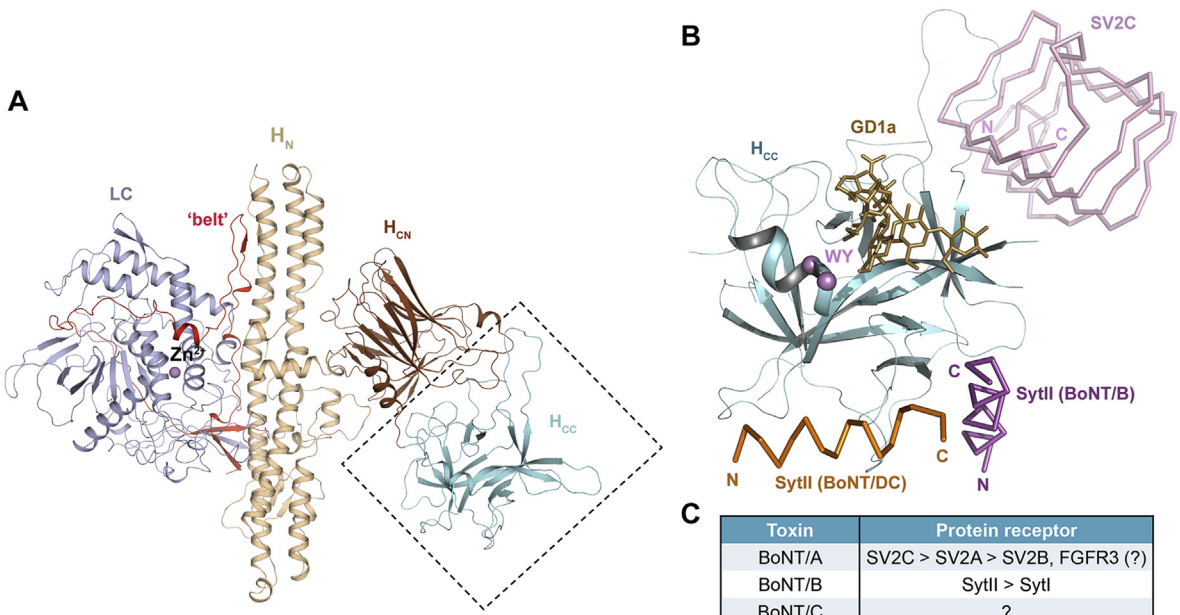
The modular structures of BoNTs are highly adapted for their potent neuron-specific toxicity. In foodborne or intestinal botulism, BoNTs are secreted in the form of large progenitor toxin complexes composed of non-toxic non-hemagglutinin protein and other auxiliary proteins, which are essential for the absorption of BoNTs in the intestine to enter the general circulation (Fujinaga et al., 2013; Gu and Jin, 2013; Gu et al., 2012; Lee et al., 2013, 2014). BoNTs then travel to the neuromuscular junction, where the H_C domain specifically targets presynaptic motoneurons and toxins are endocytosed through synaptic vesicle recycling. Upon vesicular acidification, the H_N domain undergoes a conformational change to form a protein channel that allows translocation of LC to the cytoplasm. LC specifically cleaves the soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) complex that forms a crucial vesicle fusion machinery. The cleavage terminates neurotransmitter release and paralyzes the affected muscle (Blasi et al., 1993; Schiavo et al., 1992, 2000).

2. Dual receptor model

The molecular mechanisms by which BoNTs specifically target motoneurons have attracted great attention in recent decades. It is believed that most BoNTs possess two independent binding regions in H_{CC} for polysialo-gangliosides and neuronal protein receptors to achieve high binding affinity and specificity (Montecucco, 1986). H_{CC} adopts a β-trefoil fold containing 12 core β-strands. Structure-based sequence alignment of H_{CC} from different BoNT serotypes displays a low sequence conservation of ~31% on average. The conserved residues are mainly in the core of H_{CC} that are important for maintaining the protein fold, while the surface loops connecting the β-strands are highly variable (Ginalski et al., 2000).

Polysialo-gangliosides, such as GD1a and GT1b, are a large family of glycosphingolipids that are present abundantly on the outer leaflet of the presynaptic membrane and are organized in microdomains together with some glycoproteins. BoNT/A, /B, /E, /F, and /G have a conserved ganglioside-binding site in H_{CC} composed of a "E(Q) … H(K) … SXWY … G" motif (Fig. 1B), whereas BoNT/C, /D, and /DC display two independent ganglioside-binding sites (for a more detailed review of the BoNT–ganglioside interaction, see (Rummel, 2013)). BoNT–ganglioside interaction ensures an effective initial capture and enrichment of the scarcely distributed BoNTs to the presynaptic nerve terminus, preceding the engagement of the protein receptor.

In contrast to the conserved ganglioside-binding mode, BoNTs recognize their protein receptors in a serotype-specific manner, even though only two protein receptors, synaptotagmin (Syt) and synaptic vesicle glycoprotein 2 (SV2), have been identified (Fig. 1C).



C

Toxin	Protein receptor
BoNT/A	SV2C > SV2A > SV2B, FGFR3 (?)
BoNT/B	SytII > SytI
BoNT/C	?
BoNT/D	SV2B > SV2C > SV2A
BoNT/DC	Syt
BoNT/E	SV2A > SV2B
BoNT/F	SV2
BoNT/G	SytI ~ SytII
TeNT	Nidogen, SV2

Fig. 1. The conserved structure of BoNTs and their diverse receptor-binding modes. (**A**) The conserved architecture of BoNTs: LC (light blue), H_N (wheat), the belt region connecting LC and H_N (red), H_{CN} (brown), and H_{CC} (cyan) (BoNT/B, PDB ID: 2NP0). (**B**) The H_{CC} is a versatile receptor-binding domain. The model is built based on superposition of the structures of H_CB–SytII (PDB ID: 4KBB), H_CDC–SytII (PDB ID: 4ISR), and H_CA–SV2C (PDB ID: 4JRA). $H_{CC}B$ is represented as cartoon (cyan) and the view direction is similar to that shown in panel (A). The BoNT/B-bound SytII (magenta), BoNT/DC-bound SytII (orange), and BoNT/A-bound SV2C (pink) are drawn in ribbon. GD1a, representing the polysialo-ganglioside receptor, is represented as sticks (gold). The highly conserved ganglioside-binding residues (WY) are highlighted in purple. (**C**) A summary of the protein receptors of various BoNT serotypes and tetanus toxin (TeNT). The preferred receptors of BoNT/A, /B, /D, and /E are listed in the order of descending affinities. SytI and SytII show similar binding affinity towards BoNT/G.

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