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Building-block architecture of botulinum toxin complex: Conformational changes provide insights into the hemagglutination ability of the complex

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ABSTRACT

Clostridium botulinum produces the botulinum neurotoxin (BoNT). Previously, we provided evidence for the "building-block" model of botulinum toxin complex (TC). In this model, a single BoNT is associated with a single nontoxic nonhemagglutinin (NTNHA), yielding M-TC; three HA-70 molecules are attached and form M-TC/HA-70, and one to three "arms" of the HA-33/HA-17 trimer (two HA-33 and one HA-17) further bind to M-TC/HA-70 via HA-17 and HA-70 binding, yielding one-, two-, and three-arm L-TC. Of all TCs, only the three-arm L-TC caused hemagglutination. In this study, we determined the solution structures for the botulinum TCs using small-angle X-ray scattering (SAXS). The mature three-arm L-TC exhibited the shape of a "bird spreading its wings", in contrast to the model having three "arms", as revealed by transmission electron microscopy. SAXS images indicated that one of the three arms of the HA-33/HA-17 trimer bound to both HA-70 and BoNT. Taken together, these findings regarding the conformational changes in the building-block architecture of TC may explain why only three-arm L-TC exhibited hemagglutination.

1. Introduction

Clostridium botulinum produces botulinum neurotoxin (BoNT), the most potent toxin in nature. This neurotoxin is a causative agent of human and animal botulism disease and can be classified into seven serotypes (A–G) [1]. Serotypes A, B, E, and F are associated with human botulism, whereas serotypes C and D predominantly cause animal and avian disease. BoNT is a metalloendopeptidase having a zinc ion in its molecule [2]. BoNT targets nerve endings at the neuromuscular junction and enters into nerve cells via endocytosis, after which BoNT cleaves the specific site in soluble NSF attachment protein receptors (SNAREs) associated with neurotransmitter release [3–5]. This process results in muscle paralysis and may cause death in both humans and animals.

In culture fluids and contaminated foods, BoNT is present as a part of a toxin complex (TC) through its associations with auxiliary nontoxic proteins, i.e., nontoxic nonhemagglutinin (NTNHA) and three types of hemagglutinin (HA; HA-70, HA-33, and HA-17). BoNT is susceptible to digestion in the gastrointestinal tract of humans and animals; therefore, its toxicity is decreased when it is exposed to digestive juices. However, when part of the TC, BoNT is stable, even in the presence of digestive juices [6]. Thus, nontoxic proteins play a role in protection of BoNT against digestive conditions. Furthermore, the HA protein may facilitate transepithelial transport at the intestinal wall [7]. Recent studies have shown that the serotype A TC disrupts the E-cadherin-mediated intercellular barrier of the intestinal epithelia and facilitates the paracellular absorption of the TC [8].

In the TCs produced by serotype C and D strains, there are two types of TCs: hemagglutination-negative and hemagglutination-positive TCs [9,10]. Hemagglutination-negative TCs are referred to as M-TCs and include BoNT and NTNHA, whereas hemagglutination-positive TCs are referred to as L-TCs and include M-TC and HA proteins. Therefore, hemagglutination is thought to be caused by HA proteins [9]. However, we have previously identified an intermediate TC species [11]. M-TC is composed of a single molecule of BoNT and a single molecule of NTNHA. The HA-70 trimer is attached to the M-TC molecule via an interaction between NTNHA and HA-70, yielding the M-TC/HA-70 complex. Additionally, three "arms" of the HA-33/HA-17

Abbreviations: BoNT, botulinum neurotoxin; TC, toxin complex; NTNHA, nontoxic nonhemagglutinin; HA, hemagglutinin; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SAXS, small-angle X-ray scattering; QCM, quartz crystal microbalance; DAM, dummy atom model; TEM, transmission electron microscopy

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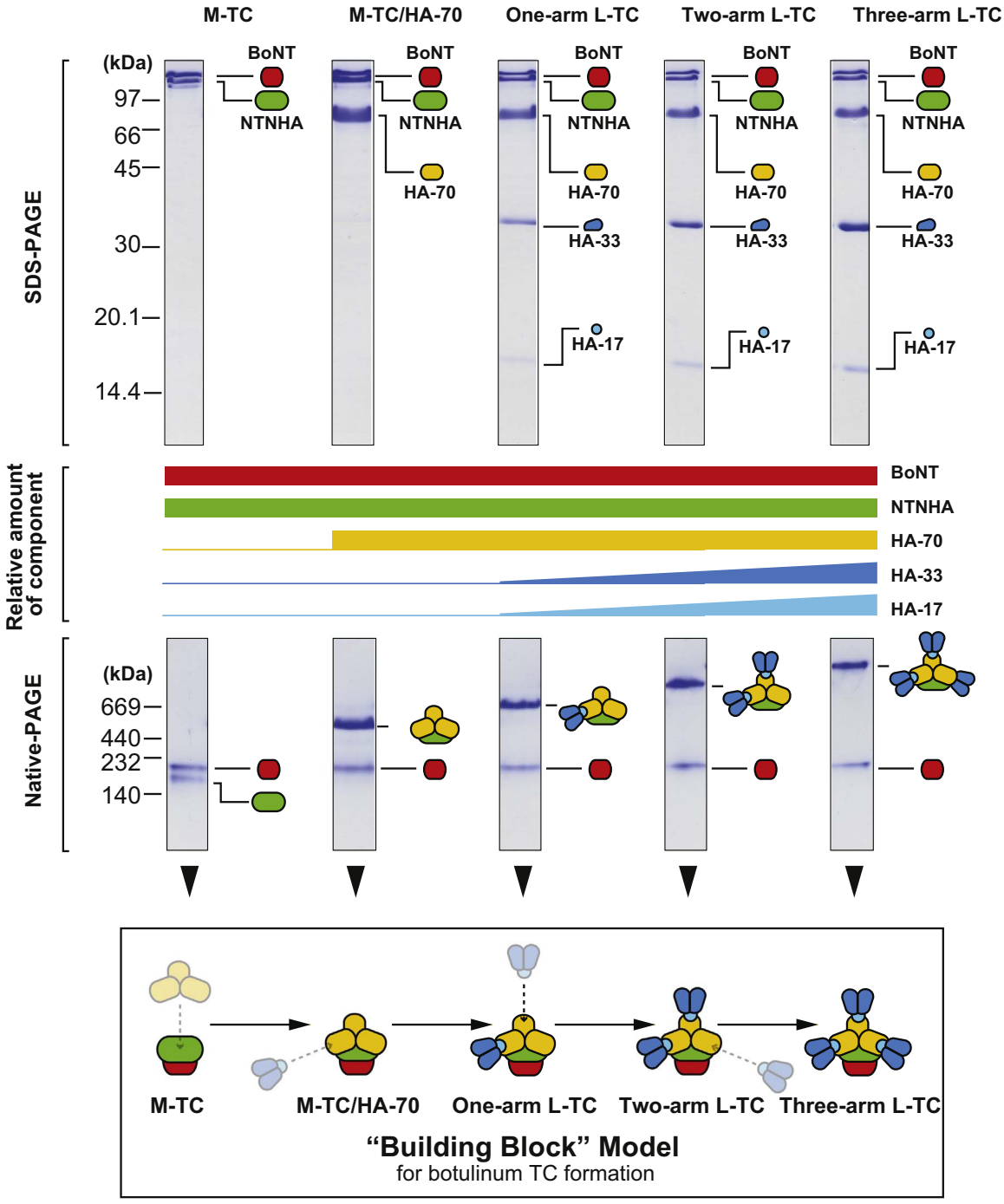

Fig. 1. Building-block architecture model for the botulinum toxin complex (TC). All TC species were developed on the SDS-PAGE and native PAGE as shown in supplementary fig. 1. Relative amounts of each component in the TC species are represented by the width of the ribbon. As shown in the "building-block" model in the bottom of the figure, the M-TC matured into the three-arm L-TC via intermediate TC species, including M-TC/HA-70, one-arm L-TC, and two-arm L-TC.

trimer (a complex of two HA-33 proteins and one HA-17 protein) are bound to the M-TC/HA-70, forming mature three-arm L-TC [12]. As intermediates between the M-TC/HA-70 to three-arm L-TC, one- and two-arm L-TCs, which have only one or two "arms" of the HA-33/HA-17 trimer, respectively (see Fig. 1), have also been observed. The botulinum TC is not formed by random association of the component proteins, but is constructed through a specific mechanism; thus the construction of botulinum TC is a sort of "building-block architecture". Interestingly, hemagglutination occurs only by the three-arm L-TC, but not by intermediate TC species, although these species also contain HA proteins [11].

In this study, we aimed to determine the solution structure of the "building-block" TC species, including the M-TC, M-TC/HA-70, one-arm L-TC, two-arm L-TC, and three-arm L-TC. Furthermore, we discussed the relationship between the molecular conformation of the TC species and hemagglutination.

2. Materials and methods

2.1. Production and purification of TC species

C. botulinum serotype D strain 4947 was cultured anaerobically for 5 days using dialysis [13]. Crude TC was precipitated with 60% (w/v) saturated ammonium sulfate, dialyzed against 50 mM acetate buffer

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