

**Stony Brook University
The Graduate School**

Doctoral Defense Announcement

Abstract

Membrane Topology of the Amphiphilic and Semi-Hydrophobic Helices in the T Domain of
Diphtheria Toxin

By

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Diphtheria toxin (DT), a protein synthesized by *Corynebacterium diphtheriae*, is composed of a catalytic domain (A chain), a membrane-inserting domain (T domain) and a receptor-binding domain (R domain). The T domain is thought to be primarily responsible for insertion and translocation of the catalytic domain. Exposure to the low pH in the lumen of endosomes aids translocation by triggering a conformational change that exposes hydrophobic sequences in the T domain, thus inducing membrane insertion. Previously, the membrane-inserted T domain has been shown to exist in shallowly (P state) and deeply (TM state) inserted conformations. Further defining the structure of the membrane-inserted T domain is likely to provide insights into the mechanism of A chain translocation.

The first study focused on the hydrophilic N-terminal helices of the T domain (TH1-TH3). Both the changes in conformation triggered by exposure to low pH and topography upon membrane insertion were studied. Using fluorescence techniques, we found that upon low pH treatment, TH 1-3 insert into the bilayer, but shallowly, which is in contrast to the deep insertion of T domains hydrophobic helices TH 5-9. Further, unlike what is observed with TH 5-9, there was no significant change in TH1-TH3 insertion depth when the T domain switched between P- and TM- state. The behavior of TH 1-3 raises the possibility that they do not interact with TH 5-9. Instead, TH1-3 aid in translocation by acting as an A chain-attached flexible tether. In a second study the topology of A-T protein (toxin A chain connected to T domain) was compared to the previously characterized topology of the isolated A chain and isolated T-domain. Fluorescence studies showed that several parts of the T domain insert more deeply into the membrane in the A-T protein than they do in isolated T domain. TH5-7 appears to form a transient transmembrane structure in the A-T protein, in contrast to the non-transmembrane state observed in the isolated T domain. This suggests that TH5-7 have an important role in translocation, and suggest a new model for the translocation mechanism.

Date: May 1st, 2007

Time: 3:45pm

Place: Life Science Building, Rm038

Program: Chemistry

Dissertation Advisor: Dr. Erwin London