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Ph-Dependent Conformational Changes and Insertion of Diphtheria Toxin Adsorbed to Lipid Membranes by Neutron and X-Ray Reflection. M.S. Kent, H. Yim, S. Satija, I. Kuzmenko, Sandia National Laboratories, Albuquerque, NM.

Several important bacterial toxins, such as diphtheria, tetanus, and botulinum, invade cells through a process of high affinity binding, internalization via endosome formation, and subsequent membrane penetration of the catalytic domain activated by a pH drop in the endosome. These toxins are composed of three domains: a binding domain, a translocation domain, and an enzyme. In each case, the translocation process is not well understood with regard to the detailed conformational changes that occur at each step or how the relatively large catalytic domains can pass through the membrane. Several groups have recently advanced the hypothesis that the catalytic domains unfold and the translocation domains chaperone their refolding into translocation competent conformations. To begin to address this, we performed neutron reflectivity measurements for diphtheria toxin bound to lipid membranes as a function of pH. Work to date has focused on the early stages. In particular, we show that no adsorption occurs at pH = 7.6, but strong adsorption occurs over at a pH range from 6.5 to 6.0. Upon binding at pH 6.5, the toxin can be modeled as a single layer. However, upon further acidification at least two domains can be resolved and these undergo distinctly different conformational changes with pH. At least two stages of conformational change occur, as the thickness of the outer layer increases from pH 6.3 to 5.3 and then decreases from pH 5.3 to 4.5. In addition, the extent of segment insertion into the membrane was determined at various pH values by X-ray reflection and grazing incidence X-ray diffraction.