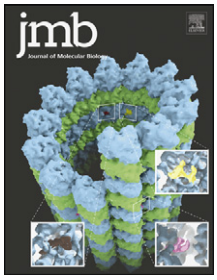

Secondary Structure Preferences of the Anthrax Toxin Protective Antigen Translocase

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Abstract

In order for many proteins to move across hydrophobic membrane bilayers, they must be unfolded and translocated by a membrane-embedded channel. These translocase channels interact with the substrate proteins they translocate via hydrophobic pore loops and cleft structures called clamps. The molecular basis for how clamps facilitate unfolding and translocation is poorly understood. Anthrax toxin is composed of three proteins, a translocase channel-forming subunit, called protective antigen (PA), and two substrate proteins, called lethal factor (LF) and edema factor. Oligomeric PA forms a large channel that contains three types of polypeptide clamp sites: an α clamp, a phenylalanine clamp, and a charge clamp. Currently, it is thought that these clamp sites operate allosterically and promote translocation via an allosteric helix compression mechanism. Here, we report on the substrate secondary structure dependence of the PA channel. Peptides derived from regions of LF with high α-helical content bound cooperatively, but those derived from β-sheet regions in LF did not, suggesting that an allosteric site preferentially recognizes α-helical structure over β-sheet structure. Peptides derived from helical sites in LF showed increasingly longer single-channel blockades as a function of peptide concentration, a result that was consistent with stronger clamping behavior and reduced backsliding. Moreover, peptides derived from helical regions of LF translocated more efficiently than peptides derived from β-sheet regions of LF. Overall, in support of the allosteric helix compression model, we find that the channel prefers α-helical sequences over β-sheet sequences.

Cells are compartmentalized by membrane bilayers. In order for proteins to circumvent these barriers, dedicated integral membrane protein transporters, or translocases, must unfold and translocate proteins across membranes. Anthrax toxin breaches the endosomal membrane bilayer by translocating large 90-kDa enzymes into the cytosol of eukaryotic host cells. The toxin [1] is composed of three individually nontoxic proteins, called protective antigen (PA), lethal factor (LF), and edema factor (EF). After PA binds to a host cell receptor, it is then cleaved by a protease into 20-kDa and 63-kDa fragments. The 63-kDa fragment self-assembles into a ring-shaped oligomer, either a heptamer [2] or an octamer [3]. The PA oligomer presents binding surfaces for three or four LF or EF to bind, depending on the oligomeric state of the PA [3–5]. The fully assembled lethal toxin (PA + LF) and edema toxin (PA + EF) complexes are then endocytosed by the host cell. Within the acidified endosome, the PA oligomer senses the low pH and converts into a membrane-inserted channel [6,7]. LF and EF are then destabilized by the low pH condition [8], and they are unfolded and translocated through the channel by means of the endosomal proton gradient [9–11]. Once in the cytosol, LF and EF catalyze reactions that disrupt normal cellular physiology. LF is a zinc metalloprotease that cleaves mitogen-activated kinase kinases [12], and EF is an adenylate cyclase that raises the cellular pool of cAMP [13].

The PA channel contains three major polypeptide clamp sites along the length of the translocation path [14,15]. These clamp sites are known to catalyze the unfolding [16] and translocation [17,18] processes.

The α clamps are deep clefts on the topmost LF/EF binding surface of the PA channel [4,19]. They form at the interfaces between PA subunits, and each α clamp can bind α-helices at about a 45° angle toward the central pore. Within the center of the PA channel is the phenylalanine clamp (φ clamp) [18]. The φ clamp is a ring of phenylalanine residues (one from each subunit) that form a 6-Å diameter opening and create a structural bottleneck inside the pore [19]. The φ clamp is absolutely required for the translocase to function [18]. Just beneath the φ clamp is a charge clamp composed of several rings of negatively charged residues [11]. The charge clamp allows the translocase to efficiently utilize the proton motive force [11]. The individual functions of the clamps are likely coordinated through a dynamic and allosteric mechanism that allows distant clamp sites to coordinate their interactions with the translocating protein [15]. Thus, it is hypothesized that through coordinated dynamics, the clamps can promote translocation while avoiding tight binding and adventitious kinetic traps [15].

Several models have been proposed for how proteins translocate through the PA channel. One model is a proton-driven, extended-chain [20] Brownian ratchet [9,21–23]. In this model, the translocating chain contains acidic residues along its length. These acidic residues are protonated, neutralizing the negative charge in the chain. Brownian motion allows the extended chain to diffuse past an anionic-repulsion (or cation-selectivity) charge-clamp site in the channel. Then, the chain deprotonates down the proton gradient, allowing the chain to become negatively charged. The deprotonated chain is less likely to retrotranslocate back into channel because the charge-clamp site in the channel repels anionic charge. Also, the proton concentration is lower on the high pH side of the membrane, limiting the reprotonation of the chain that has translocated. The cycle can then repeat on the next section of translocating chain. Key features of the mechanism include the fact that the translocating polypeptide is in the extended chain conformation. Also, the various peptide clamp sites in the channel are in fixed, static conformations during translocation.

Another model, the allosteric helix compression model, retains the basic core of the Brownian ratchet but considers that the channel is not a static structure [15]. In the allosteric helix compression mechanism, the translocating chain compresses from the extended state to a helical one upon protonation. To accommodate the helix, the φ clamp may convert to a wider diameter opening. The compression from extended chain to helix generates a power stroke because the end-to-end distance contracts about 2 Å per residue. As helix forms and populates within the α clamp, it allosterically triggers the φ-clamp site to contract to the narrower diameter state, which only accommodates the extended-chain state of the translocating chain. This contraction of the φ clamp causes the translocating chain to expand past the charge clamp. The translocating chain then deprotonates down the gradient and is then trapped electrostatically by the charge clamp. The deprotonation causes the φ clamp to reset back to its lower affinity helix accepting state, allowing the cycle to repeat on the next section of the translocating chain.

There are many unanswered questions concerning anthrax toxin translocation. How does the translocase perform on peptide sequences with high β-sheet content? Does the channel use the Brownian ratchet mechanism over the helix compression mechanism when helix formation is too energetically costly? Or does the channel use the helix compression mechanism even when the translocating chain has high β-sheet content?

Results

Secondary structure preferences of the cooperative binding activity

We first asked whether there was a secondary structure preference for the allosteric sites in the PA channel. Previously, we characterized a 50-residue peptide from LF, residues 1–50, called αL(1–50), which was derived from a region of LF that contains an α-helix [24]. To test for the secondary structure dependence of binding and translocation, five additional ~50-residue peptides were selected from LF that had α-helical or β-sheet secondary structure (Fig. 1a and b; each peptide is named by the range of residue numbers that it spans). In total, there are now three peptides derived from α-helical regions of LF and three peptides derived from β-sheet regions of LF (Supplementary Table S1).

As a previous study revealed that αL(1–50) bound to PA with high cooperativity [24], the other α-helical and β-sheet peptides were tested in a similar binding assay to assess their binding cooperativity. An ensemble of PA channels was formed in symmetric pH 5.6 buffer, and then, each peptide was titrated across a range of concentrations that would block the conductance through the channel (Supplementary Fig. S1A and B). The fraction of blocked PA channels as a function of peptide concentration was then fit by the cooperative Hill model and the Monod–Wyman–Changeux (MWC) allostery model. A significant cooperativity was determined when the Hill coefficient, n, was greater than unity and when there was a large ratio of the MWC binding dissociation constants for the taut (K_T) and relaxed (K_R) states of the system. For the α peptides, αL(1–50), αL(139–190), and αL(370–419), their K_T/K_R ratios were 330 (±40), 290 (±60), and 300 (±30) fold, respectively, while their n values were 3.1 (±0.3), 2.3 (±0.3), and 2.2 (±0.2), respectively (Fig. 1c and Supplementary Table S2). For the β peptides,