Anthrax lethal toxin co-complexes are stabilized by contacts between adjacent lethal factors

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Anthrax toxin is a three-protein toxin that must first assemble before carrying out its physiological function of menacing its eukaryotic host. Much has been done, therefore, to study its assembly both in vitro and on cell surfaces. The three proteins that comprise the toxin are protective antigen (PA), lethal factor (LF), and edema factor (EF). Individually, these proteins are nontoxic, but in combination, they produce toxic complexes (Fig. 1). PA plus LF makes lethal toxin and PA plus EF makes edema toxin. To assemble, PA is first nicked by a protease to yield a 20-kD fragment and the 63-kD fragment, PA_{63} (Blaustein et al., 1989). PA_{63} fragments then self-assemble into a ring-shaped prechannel heptamer (Milne et al., 1994; Petosa et al., 1997) or octamer (Kintzer et al., 2009), which can bind to either three or four copies of LF/EF, respectively. The toxin complexes are then endocytosed into endosomes that acidify as they mature, causing the prechannel PA oligomer to convert into a membrane-inserted channel. LF and EF then unfold and translocate through this channel into the cytosol of the host cell. There in the cytosol, LF and EF carry out their physiological functions, catalyzing reactions that disrupt the cell. In this issue, Fabre et al. report a new cryo–electron microscopy (cryo-EM) structure of an anthrax lethal toxin co-complex, containing three copies of LF bound to the PA heptamer (PA_7–LF_3 complex). This new structure adds another level of complexity to our understanding of anthrax toxin assembly.

Although the current study describes a PA_7–LF complex, a previous structure of PA in complex with a fragment of LF (LF_N) revealed an octameric PA_8–LF_4 stoichiometry (Fig. 1, inset; Feld et al., 2010). Although both complexes are stable and both complexes are observed on cells (Kintzer et al., 2009), an understanding of which complex predominates during each stage of toxicity is lacking. A novel observation reported in Fabre et al. (2016) is that of interactions between adjacent LF subunits around the ring. Specifically, the C-terminal domains of one LF contact the neighboring LF on its N-terminal domain. The authors speculate that this interaction would stabilize the complex. Because the structure of the octameric complex contained the shortened LF_N (and not full-length LF), interactions between LF subunits were not observed (Feld et al., 2010). However, modeling the full-length LF into this complex (based on the new structure by Fabre et al. [2016]) reveals that LFs would similarly make contacts in the octameric complex, and in fact, these contacts would be more extensive than those observed in the heptameric complex. More specifically, the PA_7–LF_3 co-complex reveals contacts between the first and second and second and third LFs, whereas modeling predicts contacts all the way around the PA oligomer ring in the PA_8–LF_4 complex. Thus, the octamer would be expected to form a more stable co-complex.

How might the presumed stabilization of the lethal toxin co-complex be important to toxicity? Two obvious possibilities are stabilization of the prechannel oligomer and stabilization of the channel co-complex. These are not mutually exclusive, of course. It makes sense that the toxin would want to stabilize itself at the prechannel stage; such stabilization would save the assembled toxin from proteolysis and other potential insults. Studies of the toxin have shown that there exist two potential assembly pathways: one that occurs on the cell surface and another that occurs in solution. The solution assembly pathway favors the octamer over the heptamer in the sense that stability of the prechannel oligomer prevents premature channel formation in solution (Kintzer et al., 2010). Premature channel formation, which happens more readily in heptameric PA complexes with LF at neutral pH and body temperature (Kintzer et al., 2010), leads to aggregation of the membrane-spanning hydrophobic domains and thus heptamers precipitating out of solution to leave the soluble octamers behind. Furthermore, in addition to the avoidance of premature channel formation, the octameric prechannel would have additional LF contacts that could serve to stabilize the prechannel and lessen dissociation of LF from the complex.

The presumed stabilization by LF interactions might also be mechanistically important for stabiliz-

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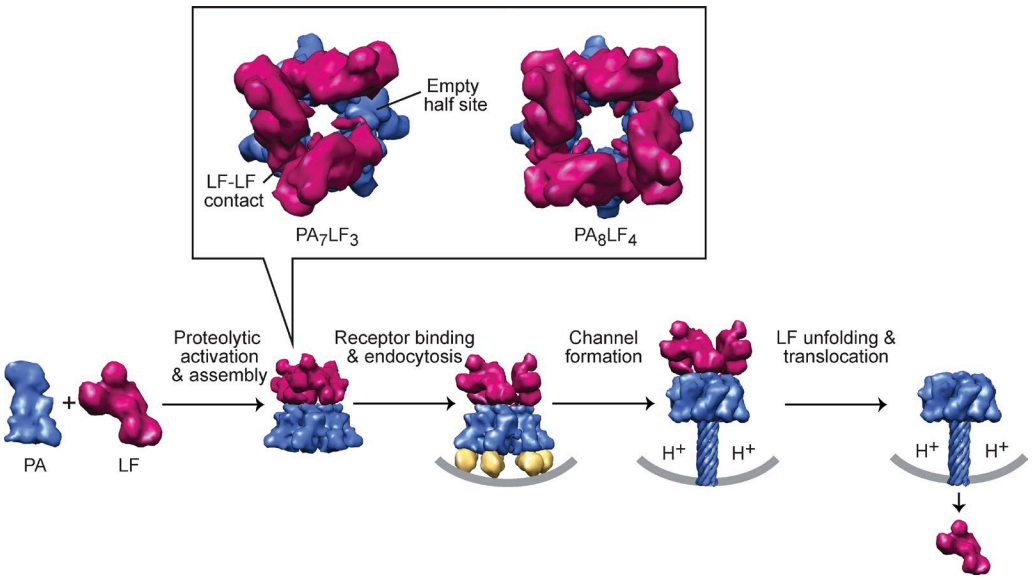

Figure 1. **Lethal toxin assembly and translocation mechanism.** PA (blue) and LF (magenta) coassemble after PA is proteolytically nicked. The assembled lethal toxin complexes bind to a cell surface receptor (gold) and are endocytosed. The endosome acidifies, transforming the prechannel PA into a membrane-inserted channel. LF unfolds and translocates through the PA channel. (inset) Two possible oligomeric stoichiometries of the lethal toxin co-complex: PA_7–LF_3 and PA_8–LF_4. Because two PA subunits create a binding site for each LF, then the PA heptamer contains only three LFs, and the octamer contains four LFs. As a result, for the heptamer, there is an empty half-site, where the LF–LF contacts are interrupted. The octamer, in contrast, forms LF–LF contacts completely around the ring.

ing the channel complex. This stabilization would maintain the integrity of the channel complex with LF as it traffics through the endosomal compartment and minimize the effects of proteolysis within the endosome/lysosome. Fabre et al. (2016) speculate further that channel state stabilization, created by contacts between neighboring LFs, might dictate the mechanism by which LF is translocated through the channel. Specifically, they propose a mechanism that would affect the order in which the LF domains translocate through the PA channel (Fig. 2). In their model, the LF with the least number of stabilizing contacts with neighboring LFs would translocate first, followed by the LF relieved of its contacts with the now translocated LF. Although this model is feasible, an argument can also be made that LFs translocate randomly (Fig. 2). All LFs have identical N-terminal leader sequences and therefore have identical probabilities of reaching the central pore and being translocated first. Certainly, if the more stabilized LF were to translocate first, then it would translocate slower than the less-well-stabilized LF. But this slower rate is not insurmountable, as demonstrated by experiments on the PA_8–LF_4 complex (Kintzer et al., 2009). Here, only stabilized LFs would be available for the first translocation event because, unlike the heptamer, LFs would make head to tail contacts all the way around the ring of the octamer (Fig. 1, inset). We know from experiments that LFs in the octamer translocate efficiently (Kintzer et al., 2009). Therefore, in support of the "random" translocation mechanism, the most stabilized LF is able to translocate first in the octameric lethal toxin complexes.

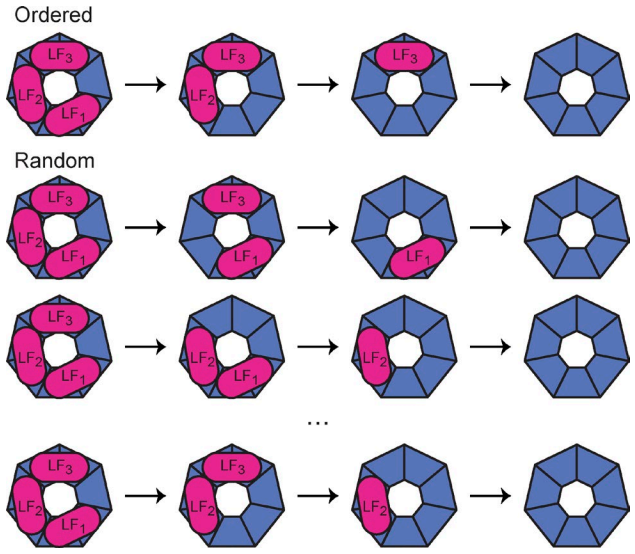

Figure 2. **Ordered and random lethal toxin translocation mechanisms.** The presumed stabilizing contacts between adjacent LFs may dictate order that the individual LFs translocate. Shown are possible translocation pathways, where the PA heptamer is colored blue and LF is colored magenta. The ordered mechanism translocates the least encumbered LF first (LF_1). This translocation is followed by LF_2 and then LF_3 in an ordered pathway. The random mechanism translocates the three LFs in any order. Shown are three of the six possible random pathways.

Another context in which to consider the stability of anthrax toxin complexes is the bloodstream. Recent work has shown that toxin complexes can assemble in the blood (Ezzell and Abshire, 1992; Ezzell et al., 2009; Kintzer et al., 2010), but this assembly pathway is distinct from cell surface assembly. Presumably, neutralizing antibodies to the toxin would need to target these assembled complexes, but to do so they would need to be designed to target accessible epitopes. Depending on the type of lethal toxin complex, different epitopes would present themselves in this context. Because one of the major approved treatments of anthrax toxemia is neutralizing antibodies, this highlights one of the important potential applications of the cryo-EM structure of anthrax lethal toxin of Fabre et al. (2016).

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