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Host Glycan Recognition by a Pore Forming Toxin

Samuel Bouyain[1] and Brian V. Geisbrecht[2,*]
[1]Division of Molecular Biology and Biochemistry
[2]Division of Cell Biology and Biophysics
School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO 64110, USA
*Correspondence: geisbrechtb@umkc.edu
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An exposed F-type lectin domain fused to the N-terminus of a cholesterol-dependent cytolysin scaffold allows *Streptococcus mitis* lectinolysin to cluster at fucose-rich sites on target cell membranes, thereby leading to increased pore-forming toxin activity. In this issue of *Structure*, Feil and coworkers define the structural basis for lectinolysin glycan-binding specificity.

Investigation of the manifold ways whereby pathogenic bacteria engage and disrupt host cells, tissues, and physiological systems has received considerable scientific interest throughout the last decade. Yet, one could easily argue that a number of the most intriguing developments in this area have arisen not from the identification of entirely novel classes of toxins, but through an increasingly detailed appreciation of unique twists and turns on well-established mechanistic themes. It is in this regard that the so-called cholesterol-dependent cytolysins (CDCs) have proven particularly fascinating. CDCs are a family of proteinaceous toxins that are expressed by five known genera of Gram-positive bacteria (Tweten, 2005). Whereas apparently all CDCs are secreted in a soluble, monomeric form, these toxins undergo a spontaneous self-assembly process at their target cell's surface. This assembly is not stochastic in nature, but rather consists of discrete, step-wise transitions that are predicated upon significant changes in CDC secondary, tertiary, and quaternary structures. Ultimately, this culminates in formation of a large, membrane-spanning β barrel-class pore of greater than 150 Å in diameter and with a toxin-specific stoichiometry (Tilley et al., 2005; Tweten, 2005). Precisely how these toxins contribute to establishment or propagation of infection by these bacteria remains a matter for some debate. Whereas their ability to affect potent cellular lysis seems straightforward enough, it is not clear whether host cell lysis is either beneficial or even the primary role of CDCs in promoting disease (Tweten, 2005).

Nearly 30 years of work laid the foundation for the structure/function paradigm that implied that cholesterol served as the membrane receptor for CDC toxin monomers. However, studies on *Streptococcus intermedius* intermedilysin (ILY) and *Gardnerella vaginalis* vaginolysin (VLY) demonstrated that the glycosylphosphatidylinositol-anchored protein CD59, rather than cholesterol, was responsible for initial binding of these CDCs to human cell membranes (Giddings et al., 2004). As a consequence, the general assumption that cholesterol served as a bona fide receptor for all CDCs required some careful rethinking. It is now accepted that while a large number of CDCs do utilize cholesterol as their receptor (e.g., *Clostridium perfringens* perfringolysin O), CDC's strict functional requirement for a basal percentage of membrane cholesterol more generally reflects their dependence on subtle cholesterol-dependent alterations of local membrane properties. These alterations, which are typically characteristic of lipid raft structures found in host cells, most likely promote efficient structural transitions from membrane bound monomers, to a prepore oligomer, and finally to the transmembrane pore proper (Tweten, 2005).

Considerations of the precise role of cholesterol aside, the studies on ILY and VLY raised questions as to whether these were the only CDCs that recognized CD59 as their receptor. Detailed mutational-based mapping of the ILY binding site on CD59 allowed formulation of a consensus motif that identified yet another CD59-binding bacterial protein (Wickham et al., 2011). Interestingly, this protein from *Streptococcus mitis* was originally reported as a soluble aggregation factor by virtue of its ability to alter the light scattering properties of human platelets (Ohkuni et al., 1997). Subsequent investigation of this protein demonstrated that it was instead a functional CDC (Farrand et al., 2008) and that any change in platelet behavior was most likely due to formation of large, transmembrane pores rather than aggregation as was previously believed. Curiously, Farrand et al. (2008) also observed that this *S. mitis* protein contained a lectin domain at its N-terminus; this heretofore undescribed feature in a CDC prompted them to rename this protein lectinolysin (LLY). The LLY lectin domain (denoted LLY^{lec}) is specific for difucosylated glycans found in the carbohydrate antigens Lewis b (Le^b) and Lewis y (Le^y), and its presence in LLY is not simply biological happenstance. Rather, LLY^{lec} increased the pore-forming activity of the LLY toxin by nearly an order of magnitude when compared to an LLY mutant that lacked this novel N-terminal glycan-binding domain (denoted LLY^{CDC}).

In this issue of *Structure*, Feil et al., (2012) report the crystal structures of LLY^{lec} from *S. mitis* in its apo form, bound to fucose and to the difucosylated tetrasaccharides Le^b and Le^y in an effort to explain how the presence of the lectin domain in LLY promotes its recognition of target cell membranes. This domain belongs to the F-type family of lectins and is most similar to the fucose-specific agglutinin from *Anguilla anguilla* (Bianchet et al., 2002) and the carbohydrate-binding module SpX-1 found in the virulence factor *sp*GH98 from *Streptococcus pneumoniae* (Boraston et al., 2006). These lectin domains superimpose with Rms deviations of 0.6–1.5 Å and adopt a jellyroll β-barrel fold. The glycan-binding site consists of five loops connecting the

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strands at one extremity of the barrel in an arrangement reminiscent of the one found in complementarity-determining regions of variable immunoglobulin domains. This satisfying structural analogy was first elaborated for F-type lectin domains by Bianchet and coworkers nearly 10 years ago (Bianchet et al., 2002).

Binding of LLY to the glycans Le^b and Le^y is primarily mediated by a terminal α1,2-fucose residue, which anchors the tetrasaccharide to the protein. The remaining sugar moieties form a network of water-mediated hydrogen bonds with the lectin to mediate specific binding to Le^b and Le^y. The terminal fucose makes exquisitely complementary interactions with the LLY lectin domain as hydrophobic residues surround its aliphatic portion, while residues His85, Arg112, and Arg120 form hydrogen bonds with the hydroxyl groups. The position of the anchoring fucose moiety is only compatible with a terminal α1,2 linkage and accounts for the selectivity of LLY toward Le^b and Le^y along with the water-mediated hydrogen bonds formed between the protein and the remaining galactose and fucose moieties. This sugar-binding mode is conserved in the SpX-1/Le^y complex structure, in which the anchoring α1,2-fucose is found in the same pocket as in LLY, while the remaining hexose moieties form a network of water-mediated hydrogen bonds with SpX-1 residues. Not surprisingly, SpX-1 conserves the His/Arg/Arg triad that locks fucose into the binding pocket (Boraston et al., 2006). Given such similar binding modes, does LLY discriminate between Le^b and Le^y glycans? Feil et al., (2012) argue that Le^b is expressed much more widely than Le^y, the expression of which is restricted to tissues that *S. mitis* invades. This suggests, at least at first glance, that Le^y is the preferred LLY ligand within the host.

Previous studies suggested that LLY^{lec} might remain masked within the context of a soluble monomer, yet became exposed following LLY binding to target cell surface since its presence enhanced LLY activity relative to LLY^{cdc} (Farrand et al., 2008). In light of more recent data, however, this model is somewhat perplexing since LLY^{cdc} is now known to contain a functional membrane-targeting CD59-binding motif (Wickham et al., 2011). To address this paradox from a structural perspective, the authors carried out a small angle X-ray scattering (SAXS) study on full-length LLY to determine the location of LLY^{lec} relative to the other four domains found within its CDC core. Their analysis of the molecular envelope reconstructed from the SAXS data suggests that all five domains of LLY lie largely in the same plane, with only a slight kink between the N-terminal LLY^{lec} region and the remainder of the toxin. This sort of conformation appears to accommodate the rather small, twelve-residue peptide sequence that links these two functionally isolable regions quite well. Not only that, when viewed in light of previous electron microscopy data, it also strongly suggests that the LLY^{lec} glycan binding domain is exposed on the outside of the fully formed LLY pore (Tilley et al., 2005). Given these results, it now seems that the function of LLY^{lec} may lie in the earliest membrane targeting events, rather than after the initial interaction has occurred.

What remains to be fully understood is the nature of the fucosylated ligands recognized by LLY under physiological situations. Are there simply fucose-rich regions in the vicinity of the cholesterol-rich lipid rafts necessary for pore formation by LLY? Or could the purpose of the LLY^{lec} domain be to promote binding to a specific membrane bound protein? Feil et al., (2012) point out that CD59 presents difucosylated ligands via its N-linked glycans, implying that a cooperative interaction between these two different CD59 binding modes is theoretically possible. These considerations aside, the authors also raise another intriguing possibility regarding glycan binding by LLY. Since Le^y is expressed by cells found in only a limited number of tissue types but is highly expressed in certain types of cancers (Yuriev et al., 2005), could the combined pore-forming and Le^y binding properties of the toxin be harnessed therapeutically? The fact that LLY was originally isolated from the serum of patients infected by *S. mitis* (Ohkuni et al., 1997) suggests that LLY is at least somewhat stable in human circulation. While such a far-reaching application clearly remains a long way off, the work presented herein provides important foundational knowledge for moving forward.

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