Structure, function, and mechanism of action for Shiga toxin

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Introduction and Background

Escherichia coli are common bacteria which can be found in all places, among humans and animals. *E. coli* can become a problem when Shiga-toxin producing *E. coli* also know as STEC, enter the body and start creating exotoxins. Pathogenic *E. coli* is known to come from bovine beef and dairy farms. There are hundreds of *E. coli* serotypes. The main being pathogenic *E. coli* is the "Big Six" and O157:H7.

Shiga toxin-producing *E. coli* (STEC) is the cause of numerous hospitalizations due to gastroenteritis and/or urethritis in the United States. STEC includes enterohemorrhagic *E. coli* (EHEC) or verotoxin-producing E. coli (VTEC). Data estimates that 265,000 STEC infections occur within the United States annually. Among those infected approximately 36% are from STEC O157. The clinical cases of *E. coli* O157:H7 infection include non-bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS). HUS is characterized by hemolytic anemia, thrombocytopenia, and renal failure. Central nervous system complications, including seizures, encephalopathy, and brain infarction also often accompany HUS. HUS is currently the most common cause of acute renal failure in children.

STEC positive can be defined as the ability to produce Shiga-toxins 1 and 2. Studies suggest that the sub-type Shiga toxin 2 (Stx2), is more commonly found, and more toxic. Both Stx1 and Stx2 share similar structures, by having A and B subunits. However, those subunits are composed differently on the two sub-types. Furthermore, there are differences in amino acid structures, protein structures, and gene sequences that influence the adhesion and virulence of the toxins.

This poster examines the relationship of the genes to the structure and function of the toxins and implications of disease.

Materials and Methods

~Collect the samples from a local bovine farm

~Enumerate total number of selected *E. coli* using diagnostic media

~Create a master plate from the MTEC plates to create a continual use for the *E. coli*

~STEC selective agar plates

~O157 selective agar plates

~Test isolates for antibiotic resistance and sensitivity

~Serotyping Prolab Kit

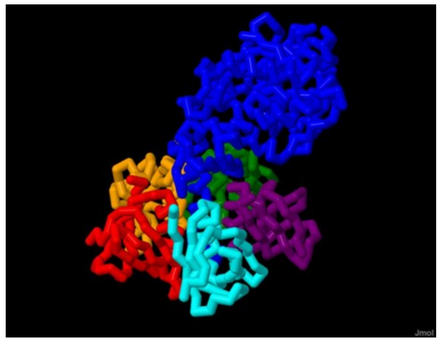

Figure 1. Jmol molecular model of gene product Stx2 gene Shiga- toxin.

Pathogenicity

STX2 is more often associated with pathogenicity. The molecular difference is found within the B-subunit. STX2B takes on multiple shapes from monomeric to pentameric as STX1B only exhibited a shape of a pentamer when undergoing mass spectroscopy. Subunit-B relates to the binding and the entry of the toxin into the target cell.
The B pentamer binds to glycolipid globotriadsylceramide (Gb3) on host cells and thereafter, subunit A is delivered into the host cell.

The A-subunit of Stx is an RNA *N*-glycosidase which cleaves a single adenine residue at position 4324 in the 28S rRNA of the 60S ribosomal subunit, preventing the ribosome from conducting protein synthesis .

Figure 3. Stx2 subunit-A, sans subunit-B
Circled is the c-terminal of Stx2, which the subunit-B surrounds and connects to.

Structure of Stx2

Shiga-toxin structure consists of two subunits, A and B.

Subunit A(Figure 1. dark blue) is the enzymatically active site.
Subunit B(Figure 2) is located at the C-terminus of subunit A and surrounds it as shown on Figure 3.

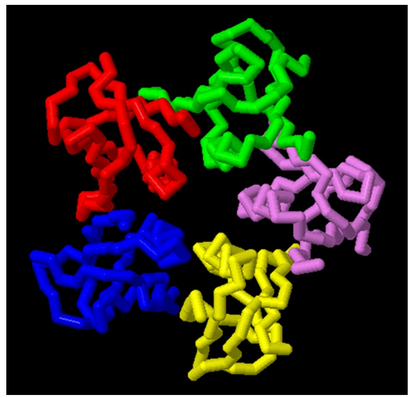

Figure 2. Subunit-B of Stx2, without subunit-A

Epidemiology

STEC is found all around the world. STEC can be exposed through farm animals, such as cattle, sheep and pigs, specifically the feces. Other ways of exposure include consumption of contaminated food or water, infected humans and sewage exposure.

The implication for epidemiology is that *E-coli* continue to be dangerous and constant. Though this is the case, prevention is still ongoing. Synsorb Pk, an orally administered, STX affinity-binding agent is given to those individuals that are infected with *E. coli* O157:H7 to prevent and lessen the symptoms of hemolytic-uremic syndrome (HUS). Other implications include using STX competitive inhibitors such as the Starfish inhibitor. Starfish functions to neutralize STX after entrance into the circulation of a person infected with enterohemorrhagic *Escherichia coli* (EHEC).

Differences between Stx1 and Stx2

There is one amino acid difference between Stx1 and Stx2 at subunit-A is a Phe30/Trp29 substitution. More differences exist at subunit-B, including Asp16/Glu15, Asn32/Ser31, Asn55/Ser54, and Ala56/Thr55, all of which are involved in hydrogen bonding interactions. Overall, there are 293 amino acid aligned on a Stx1 A-subunit molecule and 297 on an Stx2. The amino acid sequence alignment on a B-subunit displays 69 amino acids for Stx1 and 70 for Stx2.

References

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