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Shiga toxin, Shiga-like toxin and Verotoxin

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Shiga toxin, Shiga-like toxin과 Verotoxin

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1. Introduction

Shiga toxin, first described in 1903 by Condradi[3], is produced by *Shigella dysenteriae, S flexneri* and *S sonnei*[30]. A closely related toxin, termed Shiga-like toxin(SLT), was described by O'Brien et al[44], who recognized that it was the same toxin as that previously described as verotoxin(VT) by Konowalchuk et al.[34] The term verotoxin was derived from its cytotoxic activity for Vero(African green monkey) cells.[33,34] High levels of SLT are produced by EHEC strains, especially *E coli* 0157 : H7 ; whereas, low levels of SLT are produced by a number of other bacteria, e.g., *Campylobacter jejuni*.[40] Since several toxins other than verotoxins are toxic for Vero cells and cytotoxicity of VT is neutralized by anti-Shiga toxin antibody, the term SLT is considered more appropriate than VT.[44]

Collectively, Shiga toxin and several antigenic types of Shiga-like toxins constitute the Shiga-like toxin "family" which includes Shiga toxin, SLT-Ⅰ SLT-Ⅱ, VT2, SLT-Ⅱ variant porcine(SLT-Ⅱvp), and SL -Ⅱ variant human(SLT- Ⅱ vh). Members of the Shiga-like toxin family have in common a basic molecular structure, single A subunit, multiple B subunits and cytotoxicity for Vero cells. The A subunit inhibits protein synthesis via its RNA N-glycosidase enzymatic activity ; whereas, the B subunit mediates receptor binding, cytotoxic specificity, and extracellular localization of the toxin[9,14,21,31,35,36,56,57] The B subunit of the Shiga toxin family has a well conserved hydrophilic sequence between amino acid residues 10 and 20, which may play a role in cytotoxic specificity.[27] All members except SLT-Ⅱvp and SLT-Ⅱvh are cytotoxic for HeLa(human cervical adenocarcinoma), and Daudi(human Burkitt lymphoma) cells, but not for Y-1(mouse adrenal) and CHO(chinese hamster ovary) cells.[8,33,34,44] Although most Vero and HeLa cells are sensitive to SLT, they sometimes become less sensitive or insensitive to SLT.[7,22]

The Shiga-like toxin family can be divided into two subfamilies, SLT-I and SLT-Ⅱ, on the basis of distinct immunological properties and nucleotide sequence homology. Members of the SLT-Ⅰ subfamily include Shiga toxin and SLT-Ⅰ, whereas, those of the SLT-Ⅱ subfamily include SLT-Ⅱ, SLT-Ⅱvp and SLT-Ⅱvh. Shiga toxin is neutralized by anti-Shiga toxin and anti-SLT-Ⅰ, and SLT-Ⅰ is neutralized by anti-SLT-I and anti-Shiga toxin, but neither are neutralized by anti-STL-Ⅱ.[58] Similarly, SLT-Ⅱ is neutralized by anti-SLT-Ⅱ and anti-SLT-Ⅱv, and SLT-Ⅱv is neutralized by anti-SLT-Ⅱv and anti-SLT-Ⅱ. Neither is neutralized by anti-Shiga toxin or anti-SLT-Ⅰ.[58] The nucleotide sequence homology between SLT-Ⅰ and Shiga toxin is nealy 100%. The only difference is at position 45 of the A subunit. The nucleotide sequence homology between SLT-Ⅱ and SLT-Ⅱv is 94% for the A subunit and 79%

for the B subunits.[59] The nucleotide sequence homology between Shiga toxin and SLT-Ⅱ is 57% for the A subunit, and 60% for the B subunit[26] ; whereas, the nucleotide sequence homology between Shiga toxin and SLT-Ⅱvp is 60% for the A subunit and 64% for the B subunit.[66] The nucleotide sequence homology between SLT-Ⅰ and SLT-Ⅱ is 57% for the A subunit and 60% for the B subunit.[26] The nucleotide sequence homology between SLT-Ⅰ and SLT-Ⅱvp is 60% for the A subunit and 64% for the B subunit.[26]

2. Shiga Toxin

Shiga toxin has been formerly designated a neurotoxin.[3] Neurologic signs are seen in mice and rabbits, but not monkeys, hamsters, rats, or guinea pigs inoculated with crude Shiga toxin preparations.[6] Shiga toxin is a chromosomally encoded, cell-associated toxin whose phenotypic expression is increased when bacteria are cultured in iron-depleted media.[43,62] Shiga toxin is relatively heat stable, with partial loss of cytotoxicity at 60℃ for 30 min and complete loss at 90℃ for 30 min.[5] Shiga toxin is neutralized by rabbit anti-SLT-Ⅰ, but not by rabbit anti-SLT-Ⅱ, rabbit anti-VT2, or rabbit anti-SLT-Ⅱvp.[38]

Shiga toxin is cytotoxic for several culture cell lines *in vitro*, lethal for certain laboratory animals such as mice and rabbits[22] and may induce lesions in rabbit ileal loops. The toxic effects for rabbits ileal loops vary from minimal change to severe inflammation or ulceration.[1,12,16,29] Shiga toxin is cytotoxic for Vero, HeLa, KB, human liver[32,33] and Hep-2 cells[17], but not for Int 407[18], WL-38[32], CHO, L, BHK or human melanoma cells.[49]

Shiga toxin holotoxin(MW 58,000～70,000 Da ; 11) consists of an A subunit(MW 32,225 Da) and five copies of a B subunit(MW 7,700 Da ; 66). The A subunit is nicked by a protease between Ala_{235} and Ser_{254}[61], yielding two fragments, A_1(MW 27,500 Da) and A_2(MW 3,000 DA ; 50). These two fragments are linked by a disulfide bond between Cys_{243} and Cys_{261}[61]. The isoelectric point(pI) of ST holotoxin by polyacrylamide gel electrophoresis is 7.0[42], whereas, the pI for the A subunit is 11.1 and for the B subunit is 5.9.[66] The nucleotide sequence homology between Shiga toxin and SLT-Ⅰ is nearly 100%, the only difference being at position 45 of the A subunit, with threonine in Shiga toxin, versus serine in SLT-Ⅰ.[59] The nucleotide sequence homology between Shiga toxin and SLT-Ⅱ 57% for the A, and 60% for the B subunits[26], whereas, the nucleotide sequence homology between Shiga toxin and SLT-Ⅱvp is 60% for the A and 64% for the B subunits.[66]

Shiga toxin inhibits protein synthesis via serial interactions of its A and B subunits. The A subunit, especially the A_1 fragment, inhibits protein synthesis[43], while the B subunit binds to a receptor on eukaryotic(mammalian) cells.[35] The B subunit binds to a Gb_3 receptor(globotriosyl ceramide, galactose α 1-4galctose β 1-4glucose-ceramide) in eukaryotic cells[35], or both Gb_3 and β-N-acetyl-D-glucosamine in HeLa cells.[31] Following receptor binding, the intact holotoxin enters the cytoplasm by endocytosis.[43] Although the mechanism is unclear, the disulfide bond between Cys_{242} and Cys_{262} is cleaved[61] and reduced to an A_1 fragment[43], which has catalytic N-glycosidase activity.[14] The active A_1 fragment inactivates 60S ribosomal subunits[23,55] by cleaving the N-glycosidic bond of either adenosine 4324 *in vitro*[14] or adenosine 3732 *in vivo*[57] of 28S ribosomal RNA within ribosomes. Once depurinated, the 60S ribosomal subunit is inactivated and thus, no longer able to bind to elongation-factor-1-(EF-1)-dependent aminoacyl-tRNA for protein synthesis.[23,45] Eventually, Shiga toxin inhibits protein synthesis via inhibition of EF-1-dependent aminoacyl-tRNA binding to ribosomes.[14,23,45,57]

3. Shiga-like toxin-Ⅰ (SLT-I or Verotoxin 1)

SLT-Ⅰ is neutralized by rabbit anti-Shiga toxin, but not by rabbit anti-SLT-Ⅱ, rabbit anti-VT2, or rabbit anti-SLT-Ⅱvp.[38,59] SLT-Ⅰ is cytotoxic for Vero, HeLa and human umbilical cord endothelial cells[33,44,46], but not Y-1 or CHO cells.[60] SLT-Ⅰ induces fluid accumulation in ligated rabbit ileal loops, but the mechanism by which this occurs is unknown.[60] Production of SLT-Ⅰ is bacteriophage-mediated and enhanced by culture in iron-depleted media[60,65], although the latter is less essential than that required for Shiga toxin production.[65] SLT-Ⅰ is stable at 60℃ for 30 min but labile to 100℃ for 2 min.[53] Although the biological activities of SLT-Ⅰ are similar to those of SLT-Ⅱ, SLT-Ⅰ is more cytotoxic for Vero cells and less lethal for mice than SLT-Ⅱ.[60] These observations suggest that SLT-Ⅰ is a cell-associated toxin.[43]

SLT-Ⅰ holotoxin has a MW of approximately 70,000 Da, and consists of an A subunit(MW 32,211 Da) and five copies of a B subunit(MW 7,690 Da ; 26, 54). The isoelectric point(pI) of SLT-Ⅰ holotoxin is 7.0 by polyacrylamide gel electrophoresis.[48] The pI for the A subunit is 11.1 whereas, the pI for the B subunit is 5.9.[26]

Similar to Shiga toxin, SLT-Ⅰ inhibits protein synthesis via the serial interactions of its A and B subunits. The B subunit binds to a Gb_3 receptor, and mediates uptake of the A subunit into the cytoplams.[36] In the cytoplasm, the A subunit is nicked by a protease between Ala_{253} and Ser_{254}, yielding A_1 and A_2 fragments, and reduced to an active A_1 fragment.[61] The amino acid sequence 202 through 231 of the A_1 fragment seems to play a central role in cytotoxicity, as mutation in this region abolishes cytotoxicity.[25] The enzymatically active A_1 Gragment inactivates the 60S ribosomal subunit[23] via depurination of either adenosine 4324 *in vitro*[21] or adenosine 3732 *in vivo*[57] in 28S rRNA of ribosomes. Glutamic acid 167 in the A subunit of SLT-I is an active site residue for depurination of adenosine 4324 in 28S rRNA of ribosomes *in vitro*[11] The inactivated 60S ribosomal subunit is no longer able to bind to EF-1-dependent aminoacyl-tRNA for protein synthesis.[23] Eventually, SLT-I inhibits protein synthesis via blockage of EF-1-dependent aminoacyl-tRNA binding to ribosomes.[21,23,57]

4. Shiga-like toxin-Ⅱ(SLT-Ⅱ)

SLT-Ⅱ is cytotoxic for Vero and HeLa cells[33,44], but not Y-1 or CHO cells.[60] SLT-Ⅱ is also enterotoxigenic for ligated rabbit ileal loops.[60] The biological activities of SLT-Ⅱ are neutralized by rabbit anit-SLT-Ⅱ, anti-SLT-Ⅱ vp, and partially by anti-VT2, but not by anti-Shiga toxin or anti-SLT-Ⅰ.[13,38,58] Although production of SLT-Ⅱ, like SLT-Ⅰ, is bacteriophage-mediated, unlike SLT-Ⅰ, the level of production is not increased by culture in iron-depleted media.[66] The cytotoxicity of SLT-Ⅱ is stable at 60℃ for 10 min, but labile at 80℃ for 10 min.[68] SLT-Ⅱ is more lethal for mice, but less cytotoxic for Vero cells than SLT-Ⅰ.[43,60]

SLT-Ⅱ holotoxin is approximately 60 kDa(*E coli* 0157 : H7 strain 3657 by gel filtration ; 10) to 64 kDa(*E coli* 0157 : H7 strain 932 by SDS-PAGE ; 51), and consists of an A subunit and three copies of a B subunit. The A subunit ranges from 32 kDa[*E coli* strain K-12(pEBI) ; 47] to 35 kDa(*E coli* 0157 : H7 strain J-2 ; 67, 68), whereas, the molecular weight of the B subunit, as determined by SDS-PAGE, ranges from 10.2kDa [strain K-12(pEBI) ; 47] to 10.7 kDa(strain J-2 ; 67, 68). The pI of SLT-Ⅱ holotoxin and its subunits varies. The pI for holotoxin was 5.0 for strain 3657[10], 5.2 for strains K-12(pEBI) and 932[13,51] and 4.1 for strain J-2.[67,68] The pI for the A and B subunits of various strains has ranged from 8.1 to 9.8 for the A subunit, and 4.1 to 5.8 for the B subunit.[24,26]

There are at least three regions in the A and B subunits of SLT-Ⅱ which mediate cytotoxic activity. Mutation causing loss of four amino acids at the carboxyl terminus of the 70-amino acid mature B subunit resulted in loss of cytotoxic activity.[52] Deletion of the region coding for amino acids 3 through 18 of the 296-amino acid mature A subunit resulted in complete loss of cytotoxicity.[52] Substitution of aspartic acid for glutamic acid 166 decreases the capacity of the SLT-Ⅱ to inhibit protein synthesis at least 100-fold.[25]

The molecular sites of activity of SLT-Ⅱ are both similar and different from other members of the Shiga-like toxin family. Similar to Shiga toxin, SLT-Ⅰ, and VT2, SLT-Ⅱ binds to a Gb_3 receptor in eukaryotic cells[1,56] ; but, unlike Shiga toxin and SLT-Ⅰ, SLT-Ⅱ is not nicked, and therefore, not reduced to activated fragments.[24] SLT-Ⅱ inactivates 60S ribosomal subunits via depurination of adenosine 4324 in 28S rRNA[14,17], which prevents the 60S subunits from binding to EF-1-dependent aminoacyl-tRNA for protein synthesis.[14] Eventually, SLT-Ⅱ inhibits polypeptide chain elongation by blocking EF-1 dependent aminoacyl-tRNA binding to ribosomes.[47]

5. Verotoxin 2(VT2)

Most authors consider the terms, SLT-Ⅱ and VT2 synonymous[60] ; however, there are antigenic and biological differences. VT2, which is produced by *E coli* 0157 : H⁻ strain E32511, is antigenically distinct from SLT-Ⅱ.[19] *E coli* 0157 : H⁻ strain E32511 is the only isolate known to produce VT2. VT2 is partially neutralized by rabbit anti-SLT-Ⅱ, whereas SLT-Ⅱ is completely neutralized by rabbit anti-VT2.[19] VT2 is more labile to heat than SLT-1(VT1). Approximately 50% of the cytotoxic activity of VT2 is lost at 60℃ for 30 min.[20] The molecu-

lar weight of the A subunit of VT2, on the basis of SDS-PAGE, is 35 kDa.[41] Although the molecular weight of the A subunit is nearly identical between VT2 and SLT-Ⅱ, the isoelectric point for VT2 holotoxin(pI 6.5) is much higher than for SLT-Ⅱ(pI 4.1～5.2 ; 20). Like Shiga toxin and SLT-Ⅰ, binds to a Gb_3 receptor.[64]

6. Shiga-like toxin-Ⅱ variant(SLT-Ⅱv)

Two types of LST-Ⅱv, -Ⅱvp and -Ⅱvh, have been reported. SLT-Ⅱvp is produced by certain serogroups of *E coli*, typically 0138, 0139, and 0141, which cause edema disease in pigs.[37,39] SLT-Ⅱvh is produced by *E coli* 091 : H21 strain B2F1 isolated from some patients with HUS.[28] Production of SLT-Ⅱvp is not increased by culture in iron-depleted media.[66] SLT-Ⅱvp is cytotoxic for Vero[39], Y-1[2], PK 15, Madin-Darby bovine, and canine kidney[38] cells, but not for HeLa[2,39] cells. Like SLT-Ⅰ and -Ⅱ, SLT-Ⅱvp is lethal for mice and enterotoxigenic for ligated rabbit ileal loops.[38] The cytotoxic effects of SLT-Ⅱvp are neutralized by rabbit anti-SLT-Ⅱvp and rabbit anti-SLT-Ⅱ, but not by rabbit anti-Shiga toxin or anti-SLT-Ⅰ.[28,38,39] SLT-Ⅱvp is more heat labile than SLT-Ⅰ and SLT-Ⅱ, as it is completely inactivated at 75℃ for 30 min.[15] The molecular weight of SLT-Ⅱvp by DNA sequence analysis is 33,050 Da for the A and 7,565 Da for the B subunits[66] ; by SDS-PAGE it is 33,000 Da and 7,500 Da for the A and B subunits, respectively.[38] The pI for the A subunit is 8.7 and for B subunit is 10.2.[66] Either Gb_4 (globotetraosyl ceramide, GalNAc β 1-3Gal α 1-4Gal β 1-4Gal β 1-1Cer)or Gb_5(galactosylglobotetraosyl ceramide, Gal β 1-3GalNAc β 1-3Gal α 1-4Gal β 1-4Glc β 1-1Cer ; 9, 56)can serve as the B subunit receptor for SLT-Ⅱvp. SLT-Ⅱvp inhibits protein synthesis via cleavage of the N-glycosidic bond of adenosine 3732(depurination of adenosine3732) in 28S rRNA of ribosomes.[57] Although SLT-Ⅱvp is antigenically related to SLT-Ⅱ and its molecular mode of action similar, its receptor(Gb_4 and Gb_5) and cytotoxic activities differ(i.e., cytotoxic for Y-1 cells, but non-cytotoxic for HeLa cells ; 2, 9, 39, 56, 57).

SLT-Ⅱvh differs from SLT-Ⅱ, SLT-Ⅱvp, Shiga toxin and SLT-Ⅰ by antigenic, molecular and biologic characteristics. SLT-Ⅱvh is cytotoxic for Vero cells, the PI of its holotoxic is 6.1[28] and its B subunit receptor for SLT-Ⅱvh is Gb_3(galabiosyl ceramide, Gal α 1-4Gal β 1-1Cer) and Gb_3[56]

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