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Hemolysin of *Vibrio* Species

Tamaki Mizuno, Anusuya Debnath and Shin-ichi Miyoshi

Abstract

Hemolysin is one of the major pathogenic factors among *Vibrio* species, which shows hemolytic activity against erythrocytes. It is associated with different *Vibrio* spp. that manifest either wound infection or intestinal infection as their clinical symptom. *V. vulnificus* and *V. alginolyticus* are well-known causative organisms for wound infection, whereas the gastrointestinal infection is caused by *V. cholerae*, *V. mimicus*, and *V. parahaemolyticus*. There are two major groups of hemolysins in *Vibrio* spp.: the thermostable direct hemolysin (TDH) from *V. parahaemolyticus* and the HlyA (El Tor hemolysin) from *V. cholerae*. These hemolysins have homology in certain degrees; however, the essential amino acids for the activity are variable depending on the species. This chapter summarizes the functions and features of hemolysins from *Vibrio* species, which has been reported so far.

Keywords: thermostable direct hemolysin, El Tor hemolysin, *Vibrio parahaemolyticus*, *vibrio cholerae*, *vibrio mimicus*, *vibrio vulnificus*

1. Introduction

The genus *Vibrio* is comprised of facultative, anaerobic, Gram-negative, curved-rod bacteria that are widely found in natural aquatic environments such as marine, estuarine, and freshwater [1]. More than 100 species have been currently described in this genus, and at least 12 species represented by *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* cause a variety of clinical symptoms in human (Table) [1–5]. In addition, species such as *V. metoecus* and *V. navarrensis* are among the newly isolated species from human, and it is strongly suggested that they are human pathogens of *Vibrio* spp. [6, 7]. On the other hand, the major pathogenic *Vibrio* for aquatic vertebrates or invertebrates are *V. anguillarum*, *V. harveyi*, *V. ordalii* etc., responsible for fatal hemorrhagic septicemic disease called vibriosis in marine animals [5, 8–10]. *Vibrio* spp. prefer warm water temperature (15–35°C), so they are likely to flourish more with rising environmental water temperature due to global warming and thus the probability of infections caused by them.

Human diseases caused by pathogenic *Vibrio* spp. can be divided into two major types based on symptoms: intestinal infection and non-intestinal infection [1, 3]. The intestinal infection includes gastroenteritis and cholera, whereas non-intestinal infection includes septicemia and wound infection (**Table 1**). Cholera is caused by ingestion of food and drinking water contaminated with *V. cholerae* O1/O139 that produces cholera toxin (CT) as a major virulence determinant and characterized by severe diarrhea that rapidly leads to dehydration [11, 12]. Till date it remains a major public health disease with estimated 2.9 million cases and 95,000 deaths annually worldwide [13]. There are many clinical cases of gastroenteritis by *V. parahaemolyticus* due to ingestion of raw fish and shellfish [14, 15].

Species	Diseases	Hemolysin family
V. alginolyticus	Wound infection, otitis media	TDH
V. carchariae	Wound infection	
V. cincinnatiensis	Meningitis	
V. cholerae		
O1/O139	Cholera	HlyA
non-O1/non-O139	Gastroenteritis, wound infection	HlyA, TDH
V. damsela	Wound infection	HlyA
V. fluvialis	Gastroenteritis	HlyA
V. furnissii	Gastroenteritis	
V. hollisae	Gastroenteritis, septicemia	TDH
V. metschnikovii	Cholecystitis	
V. mimicus	Gastroenteritis	HlyA, TDH
V. parahaemolyticus	Gastroenteritis, wound infection	TDH
V. vulnificus	Septicemia, wound infection	HlyA

HlyA, El Tor hemolysin group; TDH, thermostable direct hemolysin family.

Table 1.
Pathogenic Vibrio species for human and hemolysins produced by them [1, 3].

The other species such as *V. cholerae* non-O1/non-O139, *V. mimicus*, and *V. fluvialis* are known as agents of foodborne illness [3, 16–19]. On the other hand, *V. vulnificus* is the most studied among *Vibrio* spp. as a causative bacterium of wound infections, though the clinical cases by *V. damsela* and *V. alginolyticus* are also reported [3, 20–24]. *V. vulnificus* is an opportunistic pathogen and poses a threat to individuals with compromised immunity because it can also cause septicemia, which leads to high lethal rates [24–26].

These pathogenic *Vibrio* have been reported to produce various virulence factors, including enterotoxin such as CT produced by *V. cholerae* O1/O139 [12, 27], hemolysin, and Type III secretion system (T3SS) in *V. parahaemolyticus* [28, 29] and extracellular protease in *V. vulnificus* [30]. This chapter has mainly summarized how hemolysins play an important role in the pathogenicity of Vibrio spp. based on studies till date.

2. Hemolysins

Hemolysin is a toxin that attacks membranes of mammalian erythrocyte and causes cell lysis called hemolysis. It is reported that hemolysins are produced by different species of bacteria like *Escherichia coli*, *Staphylococcus aureus*, and *Vibrio* [31–34]. In most cases, the evidence based either on in vivo experiments or clinical reports that suggests the involvement of hemolysins in the pathogenicity is reported [33, 35]. This toxin plays certainly an important role in the infection process initiated by *Vibrio* spp. Hemolysin from *Vibrio* spp. can be classified mainly into two groups, thermostable direct hemolysin (TDH) from *V. parahaemolyticus* and El Tor hemolysin (HlyA) from *V. cholerae*. Even though these toxins partially share the sequence homology, the essential amino acids for the activity, structural features, and function are different between TDH and HlyA.

2.1 Thermostable direct hemolysin (TDH), TRH, and others

2.1.1 Thermostable direct hemolysin (TDH)

V. parahaemolyticus was first isolated by Fujino et al. as a causative agent of food poisoning in Osaka, Japan [14]. The pathogenicity of *V. parahaemolyticus* is determined by multiple virulence factors including adhesins, thermostable direct hemolysin (Vp-TDH), TDH-related hemolysin (Vp-TRH), and two type III secretion systems (T3SS), T3SS1 and T3SS2 [28]. It has been reported that the clinical isolates of *V. parahaemolyticus* show β-hemolysis activity on Wagatsuma blood agar medium [36], whereas almost all non-clinical isolates are non-hemolytic. This hemolytic activity has been given a specific term known as Kanagawa phenomenon (KP), and it is due to Vp-TDH encoded by the *tdh* gene [37, 38]. Thus, Vp-TDH has been considered as an important virulence factor in gastroenteritis cases and KP reaction as a good marker for the identification of pathogenic strains. Thermostable direct hemolysin, Vp-TDH, was named so because of its characteristics. These characters include persistence of activity even after heating at 100°C for 10 minutes and the ability to act directly on erythrocytes with no enhancement in activity level even by the addition of lecithin [39]. This purified toxin has numerous biological activities such as hemolytic activity for erythrocytes of various species, cytotoxic activity for some mammalian cells, and enterotoxic activity measured by fluid accumulation (FA) in the rabbit ileal loop test [33, 40–42].

The mature form of Vp-TDH consists of 165 amino acids and is approximately of 19 kDa. It exists as a tetramer in solution, which is responsible for the membrane disruption [43, 44]. This is a pore-forming toxin, but it has no similarities with other bacterial pore formers except Vp-TDH homologs like Vp-TRH and TDH-like toxins from *V. cholerae* non-O1/non-O139, *V. mimicus,* and *V. hollisae* [45–49]. Vp-TDH forms pores of approximately 2 nm in diameter on erythrocyte membrane that results into colloidal osmotic lysis [50]; however, the exact mechanism of pore formation is not yet identified. The reactivity of Vp-TDH against erythrocytes from various animal species showed variability; for example, it causes hemolysis of erythrocytes from rat, human, rabbit, and sheep but not horse [51]. It is reported that the amino acid residues, Arg^{46}, Gly^{62}, Trp^{65}, and Gly^{90}, are critical for the hemolysis; in fact, the substitution of residue Arg^{46} by site-directed mutagenesis inhibits the formation of tetramer [33, 44, 52].

Enterotoxicity, which is another feature of Vp-TDH, has been evaluated by increase of FA in the rabbit ileal loop due to intestinal Cl^- secretion as a manifestation of diarrhea induced by *V. parahaemolyticus*. The Cl^- secretion from human colonic epithelial cells by Vp-TDH is caused by stimulation of Ca^{2+}-activated chloride ion channel not by pore formation on the cells [53]. Evidence suggests that Vp-TDH acts in three sequential steps: receptor binding on the epithelial cells, followed by increase in intracellular Ca^{2+} concentration due to protein kinase C activation, and finally, stimulation of Ca^{2+}-activated Cl^- channel. However, it is reported that the deletion of *tdh* only leads to partial decrease in enterotoxicity against rabbit intestinal cells, whereas cytotoxicity to Hela cells was not affected at all [54]. Moreover, a recent study provides a new evidence that Vp-TDH can also engage as an effector of T3SS and implicated to elevate FA in animal model [55]. Therefore, the reason behind pathogenicity of *V. parahaemolyticus* is perhaps not only because of Vp-TDH but also because it involves a synergistic action of multiple virulence factors including T3SS.

2.1.2 TDH-related hemolysin (Vp-TRH) and others

Vp-TRH is identified as a new hemolysin found in KP-negative strains from clinical samples, named TDH-related hemolysin (Vp-TRH) [45]. Vp-TRH protein has a conserved domain of Vp-TDH and immunologically similar to Vp-TDH. But unlike Vp-TDH, it is heat-labile and lost its activity when heated at 60°C for 10 minutes. It is reported that there are significant nucleotide differences that exist within the *trh* family of two subgroups (*trh1* and *trh2*), sharing 84% sequence identity, as opposed to the less diversity (<3.3%) of five *tdh* genes (*tdh1* to *tdh5*) [38, 56–58]. Vp-TRH also induces chloride ion secretion in human colonic epithelial cells like Vp-TDH; therefore, it is considered as one of the important virulence factors among KP-negative strains of *V. parahaemolyticus* [59].

TDH-like toxins have also been found in *V. cholerae* non-O1/non-O139, *V. mimicus*, and *V. hollisae* known as NAG-TDH, Vm-TDH, and Vh-TDH, respectively [47–49]. It is reported that all clinical isolates of *V. hollisae* possess *tdh* gene [60], whereas only some clinical strains of *V. cholerae* non-O1/non-O139 and *V. mimicus* contain *tdh* gene [46, 61]. The molecular weight of these toxins is similar to Vp-TDH and shows immunological cross-reactivity with Vp-TDH. Both NAG-TDH and Vm-TDH are stable on heating at 100°C for 10 minutes, and the hemolytic activity against erythrocytes of most animals is almost similar to Vp-TDH [47, 48]. On the other hand, Vh-TDH is a heat-labile toxin that gets inactivated by heating at 70°C for 10 minutes, unlike Vp-TDH [49]. Moreover, it is reported that *V. alginolyticus* also produce TDH-like toxin, and it shows toxicity for mouse and fish [62].

2.2 HlyA (El Tor hemolysin) and related toxins

2.2.1 HlyA of V. cholerae

V. cholerae O1/O139 is the causative agent of cholera, and its main virulence factors are cholera toxin (CT) and toxin-coregulated pilus (TcpA) [11, 12]. *V. cholerae* produces some other virulence factors such as hemolysin, hemagglutinin/protease (HA/protease), T3SS, etc., which can also serve as important elements for the pathogenesis, especially in the strains devoid of CT and TcpA [63–65]. The water-soluble cytolytic toxin produced by *V. cholerae* El Tor O1 and non-O1/non-O139 strains is known as El Tor hemolysin (HlyA)/*V. cholerae* cytolysin (VCC) [66, 67]. HlyA can facilitate lysis of erythrocytes from various animals and other mammalian cells [66, 68]. It can also exhibit potent enterotoxicity as measured by fluid accumulation in the rabbit ileal loop test. Thus, HlyA has been considered to play a crucial role in the pathogenesis of gastroenteritis caused by *V. cholerae* strains [63].

The HlyA encoded by *hlyA* gene is produced in the form of 82 kDa inactive precursor, termed pre-pro-HlyA [69, 70]. This 82 kDa precursor consists of 25 amino acid long signal peptide at the N-terminal, a pro-region of 14 kDa and mature region of 65 kDa at the C-terminal. The mature form of HlyA is generated via a two-step process [71]. In the first step, the 82 kDa precursor is converted to 79 kDa pro-HlyA by cleavage of the signal peptide during its translocation through the inner membrane and then secreted extracellularly in an inactive form. In the second step, the inactive pro-HlyA is converted to active HlyA through the proteolytic removal of pro-region, usually at the bond between Ala[157] and Asn[158] (**Figure 1**; Proteolytic cleavage site). It has been found that the pro-HlyA can be activated by extracellular metalloprotease (HA/protease), a major protease of *V. cholerae* and also by other exogenous or endogenous proteases. However, the exact proteolytic cleavage site depends on the specificity of the protease, which is different compared to native processing [72]. Moreover, it is reported that pro-HlyA can
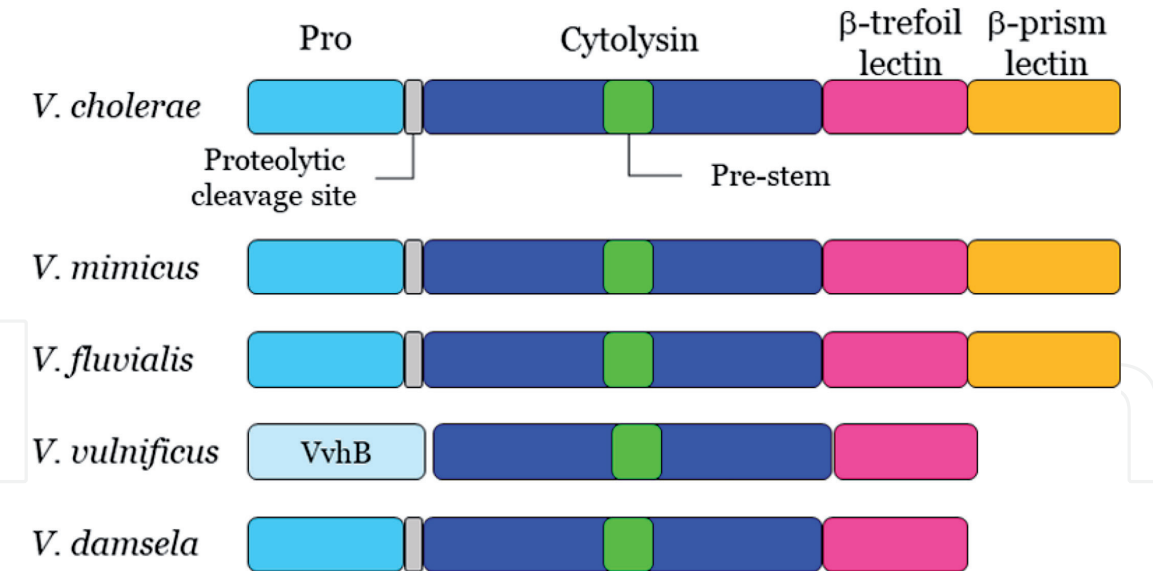
Figure 1.
Comparison between V. cholerae hemolysin (HlyA) and other Vibrio spp. hemolysins. HlyA of V. cholerae consists of pro-region (light blue), cytolysin domain (blue), β-trefoil lectin domain (pink), and β-prism lectin domain (yellow). There is a proteolytic cleavage site (gray) between pro-region and cytolysin domain for the conversion of pro-HlyA to mature HlyA. The hemolysins from V. mimicus and V. fluvialis have no significant differences in domain construction. V. vulnificus hemolysin lacks β-prism lectin domain and pro-region; instead, V. vulnificus produces VvhB that might act as chaperon-like pro-region. V. damsela produces HlyA-like hemolysin without β-prism lectin domain.

bind as a monomer to eukaryotic cell membrane, and then this bound pro-HlyA can be activated by exogenous, endogenous, extracellular, and even by cell-bound proteases [73]. It is well known that the pro-region can act as an intramolecular chaperone, an essential role of pro-region that governs the proper folding of HlyA pro-toxin [74].

HlyA belongs to bacterial β-barrel pore-forming toxins (β-PFTs) family that includes α-hemolysin of *Staphylococcus aureus* and aerolysin of *Aeromonas hydrophila* [75–77]. Consistent with generalized mode of action by β-PFT, the pore formation mechanism of HlyA has been proposed to follow three distinct steps (**Figure 2**); binding as a water-soluble monomer onto the target cell membrane, formation of pre-pore oligomeric intermediates by the self-assembly of toxin monomer, and finally insertion of the pore-forming stem-loop into the membrane, resulting into the formation transmembrane heptameric β-barrel pores on the cell membrane [78–80]. HlyA causes colloid osmotic lysis of mammalian cells by forming transmembrane pores on the target cell membranes [81, 82], which causes not only hemolysis but also potent cytotoxic effect such as vacuolation [83] and apoptosis [84, 85] of epithelial and immune cells.

The PFTs show affinity for a wide range of cell surface molecules such as cholesterol [86], glycosylphosphatidylinositol-anchored glycoproteins [87], and the human complement receptor CD59 [88]. In case of human erythrocyte membrane, glycophorin B has been reported to be a receptor for HlyA [89]. The hemolysis of rabbit erythrocytes by HlyA is competitively inhibited by asialofetuin and glycoproteins with multiple β_1-galactosyl residues [90]; this provides an evidence that cell surface carbohydrates are acting as functional receptors. *V. cholerae* cytolysin also shows strong preference for cholesterol- and sphingolipid-rich vesicles [91]. So, it can be said like other PFTs, HlyA also shows affinity for multiple cell surface receptor.

The mature HlyA is composed of three distinct domains: a central cytolysin domain and two lectin-like domains with β-trefoil and β-prism folds. The β-trefoil and β-prism domains exhibit structural homology to the carbohydrate-binding
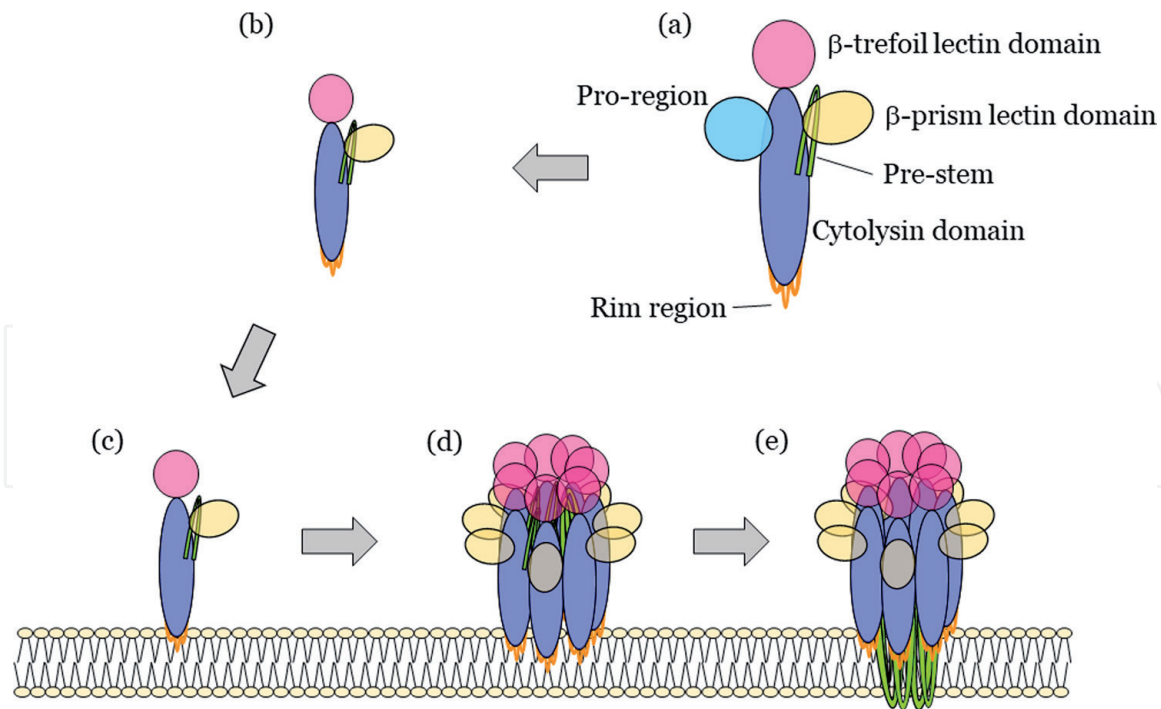
Figure 2.
Mechanism of transmembrane heptameric pore formation by HlyA. (a) Pro-HlyA structure. (b) Secreted pro-HlyA is activated through the removal of pro-region by protease. (c) HlyA monomer binds to the target membrane by using a rim region and/or β-prism lectin domain with membrane component such as cholesterol and carbohydrate receptor, respectively. (d) HlyA monomer assembles to heptameric pre-pore oligomeric intermediates. (e) The pre-stem of HlyA is inserted into the membrane, resulting into the formation of transmembrane heptameric β-barrel pores.

domain of the plant lectin ricin and jacalin, respectively [78]. In fact, the 15 kDa β-prism lectin domain has carbohydrate-binding activity [92], and the deletion of 15 kDa β-prism lectin domain generates a 50 kDa variant (HlyA50) with no effect on the global conformation of the monomer, but the hemolytic activity reduced by approximately 1000-fold [93, 94]. The β-prism domain has been shown to promote self-assembly of the toxin monomer in carbohydrate-independent manner, suggesting the hemolytic activity of HlyA50 is compromised due to reduction in pre-pore oligomeric intermediates [95]. Another study proposed the role of β-trefoil domain and showed that it is critical for the folding of cytolysin domain to its active conformation [96]. Recently, it is reported that the three loop sequences located in the bottom tip of the cytolysin domain play a critical role in the initial interaction with membrane lipid bilayer. This study showed that the replacement of the amino acid residues in the three loop sequences designated as "rim region" compromises the specific interaction of HlyA monomer with membrane lipid bilayer and blocks the pore formation process. Thus, it leads to repression in the lysis of human erythrocytes and reduced cytotoxic activity for HT-29 human colorectal adenocarcinoma cells [97]. In the next step that is pre-pore oligomerization, it has been shown that alteration of key amino acids affects not only the formation of oligomeric intermediates but also the subsequent formation of functional transmembrane pore [98]. Finally, pre-pore oligomeric intermediates lead to the formation of transmembrane β-barrel pore. Paul et al. confirmed that the transmembrane stem region plays a significant role in the functional pore formation. However, the deletion of "pre-stem" loop of cytolysin domain does not affect the membrane binding and pre-pore heptamer formation [99]. Therefore, it is considered that each step of HlyA pore formation mechanism plays an indispensable role in the generation of functional transmembrane pore in the target cell and thus enhances the virulence of *V. cholerae*.

2.2.2 Other related El Tor hemolysin of Vibrio species

Several studies have reported that other *Vibrio* species such as *V. mimicus*, *V. vulnificus*, and *V. fluvialis* also produce hemolysin that shares some common structural features with HlyA [100–102].

V. mimicus, a species closely related to *V. cholerae*, is a causative agent of human gastroenteritis [103]. Pathogenic strains of *V. mimicus* exhibit various clinical symptoms from watery to dysentery-like diarrhea [104]. This pathogen produces many kinds of virulence factors such as CT-like enterotoxin and heat-stable enterotoxin [105–108], with Vm-TDH as a causative factor in some clinical strains. However, most clinical strains lack the ability to produce any of these toxins. The heat-labile hemolysin/cytolysin (*V. mimicus* hemolysin; VMH) is thought to be the most common virulent enteropathogenic factor [109, 110]. In fact, VMH induces FA in a ligated rabbit ileal loop in dose-dependent manner, and the antibody against VMH apparently reduces enterotoxicity by *V. mimicus* in the living cells [100, 111]. These findings indicate that VMH is potently related to pathogenesis of this pathogen. The enterotoxic activity of VMH might be due to intestinal Cl^- secretion caused by the activation of both Ca^{2+}-dependent and cyclic AMP-dependent Cl^- secretion systems [111, 112]. Similar to HlyA, it has been indicated that VMH is also a pore-forming toxin. This toxin can disrupt various mammalian erythrocytes including bovine, rabbit, sheep, human, and mouse in colloid osmotic manner, and it shows the highest sensitivity for the horse erythrocytes [100].

VMH encoded by *vmhA* gene is predicted to be of 83 kDa with 82% similarity with *V. cholerae* HlyA. VMH is also secreted as 80 kDa precursor known as pro-VMH [113], which is then converted to 66 kDa mature toxin through the removal of N-terminal propeptide by trypsin-like protease of *V. mimicus* between the amino acid residues Arg^{151} and Ser^{152} [114, 115]. It has been assumed that VMH might be processed in a two-step reaction just like HlyA and pro-toxin can be activated by various proteases such as trypsin, chymotrypsin, and metalloprotease [115, 116]. Similar to 50 kDa variant of HlyA, mature VMH can be converted to 51 kDa of VMH (designated VMH51) through the removal of 15 kDa from C-terminal end by metalloprotease of *V. mimicus*. VMH51 almost showed no lytic activity toward horse erythrocytes because it lost the binding affinity toward erythrocyte membrane [116]. However, the VMH51 can associate with sheep erythrocyte membranes though the affinity is reduced as compared with intact VMH, suggesting that the truncated toxin interacts with other components in sheep erythrocyte membrane. It might be concluded that the 15 kDa C-terminal domain of VMH is functionally similar to β-prism lectin domain of HlyA.

V. fluvialis is one of the foodborne pathogens which can cause clinical symptoms similar to *V. cholerae* [117–119]. *V. fluvialis* secrets El Tor-like hemolysin, designed as *V. fluvialis* hemolysin (VFH), which can elicit lysis of erythrocytes from various animal. In addition to hemolytic activity, VFH can also trigger cytotoxicity toward Chinese hamster ovary (CHO) cells and induction of fluid accumulation in suckling mouse [102]. The purified VFH has molecular weight of 63 kDa, whose N-terminal amino acid sequence shares homology to HlyA from *V. cholerae* and VMH from *V. mimicus* [102]. It is suspected that VFH might play an important role in *V. fluvialis* pathogenicity.

V. vulnificus was first isolated from a leg ulcer, and it was wrongly reported as *V. parahaemolyticus* [120]. Later, it was found that some characters were different from *V. parahaemolyticus* such as positive lactose fermentation, so subsequently it was termed as *V. vulnificus* [20]. *V. vulnificus* can cause two types of illness, the primary septicemia and the wound infection [24]. The former is remarkable for its high fatality rate (over 50%). The primary septicemia is caused by the consumption

of raw seafood, especially shellfish such as oyster contaminated by *V. vulnificus*, and it is reported that 95% of all seafood-related deaths are caused by *V. vulnificus* in the United States [121, 122]. Because most septicemia patients have an underlying disease such as hepatic cirrhosis, hepatitis, or diabetes, the septicemia by *V. vulnificus* is considered as an opportunistic infection [24]. Wound infections characterized clinical symptoms are edema, erythema, or necrosis and occurred after exposure to contaminated seawater or marine products. However, gastrointestinal symptom like diarrhea is very rare due to *V. vulnificus* infection [25, 26]. *V. vulnificus* produces various extracellular virulence factors such as hemolysin or protease [123, 124]. Hemolysin secreted by *V. vulnificus* called as *V. vulnificus* hemolysin (VVH) is also a toxin that can form pore on the target membranes of various mammalian cells. Purified VVH exhibits lytic activity against erythrocytes of various mammals and cultured cells such as CHO, mast, and pulmonary endothelial cells [101, 125–127]. In addition, it is reported that the sublytic doses of hemolysin can trigger apoptotic signaling pathway in human vascular endothelial cell line, ECV304 cells [128], and oligomerization of VVH is essential for the apoptotic activity in CHO cells [129].

VVH (VvhA) precursor has molecular weight of 51 kDa encoded by the structure gene *vvhA*, which constitutes an operon with *vvhB* gene. The *vvhB* gene is present upstream of *vvhA* and encodes 18 kDa protein VvhB. The VvhA precursor is composed of a signal peptide (20 amino acid residues) and cytolysin domain (Gln^1 to Arg^{318}) including a putative pre-stem and β-trefoil lectin-like domain (His^{319} to Leu^{451}) (**Figure 1**); the pro-region and β-prism lectin domain are absent as compared with HlyA precursor [78, 130]. Although the function of VvhB is unknown, it might act as a chaperon in the absence of the pro-region like HlyA. This speculation is supported by the fact that even though VvhA is expressed in the absence of *vvhB* in vitro, the hemolytic activity cannot be detected [131]. Although VVH lacks β-prism lectin domain, the β-trefoil lectin domain has displayed binding capability for glycerol, N-acetyl-D-galactosamine, and N-acetyl-D-lactosamine unlike HlyA [92, 130]. In fact, VVH exhibits decreased ability to bind CHO cells when preincubated with methyl-beta-cyclodextrin, an oligosaccharide, and, thus, inhibition of its cytotoxic effect [132]. Similar to HlyA, it is believed that the VVH monomer binds to the cell membrane and forms oligomers [101, 133, 134] and the crystal structure of β-trefoil lectin domain of VVH reveals a heptameric ring arrangement [130]. It is strongly suggested that cholesterol is the receptor for VVH and facilitates conversion of monomer to oligomer [133, 135]. In addition, it is reported that Thr^{438} in the β-trefoil lectin domain is responsible for binding to cholesterol [131]. On the other hand, Phe^{334} in cytolysin domain that is located near the joint of two domains is essential for oligomerization of toxin monomer [136]. Moreover, it is shown that the mutation of Leu^{451} causes inhibition of hemolytic activity without reducing the membrane binding ability; this suggests that the Leu^{451} is essential for the oligomer formation [137]. Recently, a study showed that properties such as polarity and indole ring of amino acid Trp^{246} are essential for the binding of toxin to the target membrane [138]. It is assumed that hemolytic process of VVH is almost similar to HlyA though there are some differences in the function and structure of VVH.

It has been reported that a heat-labile hemolysin purified from *V. tubiashii*, a pathogen of juvenile bivalve, is similar to VVH [139]. Like VVH, this toxin has showed competitive inhibition by cholesterol and can lyse erythrocytes. In addition, the toxin exhibits cytotoxicity to CHO, Caco-2, and Atlantic menhaden liver cells in tissue culture.

V. damsela has been reported to cause wound infection by handling of fish, exposure to seawater and marine animals, and ingestion of raw seafood [21, 140–143]. It has been considered that there is not any other hemolysin in this bacterium, except of a hemolysin with phospholipase D activity known as damselysin [144].

Recently, it is reported that this bacterium possesses HlyA-like hemolysin encoded within a new virulence plasmid pPHDD1. The characteristics of this new HlyA-like hemolysin from *V. damsela* are not yet identified, but the predicted amino acid sequences show 69% similarity with HlyA of *V. cholerae*, missing the β-prism lectin-like domain (**Figure 1**) [145].

3. Conclusion

This chapter is focused on the hemolysins produced by *Vibrio* species, especially the human pathogens. Hemolysins are classified into two groups, namely, thermo-stable direct hemolysin (TDH) and El Tor hemolysin (HlyA). This chapter pays attention to Vp-TDH (*V. parahaemolyticus*), HlyA (*V. cholerae*), VMH (*V. mimicus*), and VVH (*V. vulnificus*) because these are well studied in terms of the toxin structure and their relation with the pathogenesis. The mechanism of action by HlyA and the essential amino acid residues have been clarified through the crystal structure of HlyA pro-toxin and the transmembrane heptameric oligomer over the past decade. Although the crystal structure has revealed the structural information about Vp-TDH and VVH, the exact mechanism of pore formation in the target membrane is yet to be studied. Several studies have indicated the involvement of novel virulence factors in pathogenesis like T3SS, but still Vp-TDH and Vp-TRH are considered to be the major virulence factors of *V. parahaemolyticus*, one of the important food poisoning bacteria in Japan and other eastern and Southeast Asian countries. HlyA is thought to be a major factor in CT-negative strains (e.g., *V. cholerae* non-O1/non-O139) that can cause diarrhea because it can induce enterotoxicity as well as apoptosis. *V. mimicus* hemolysin, VMH, is just one of the many enterotoxic factors. Even though there is detailed information about structural composition and mode of action of some of the hemolysin such as Vp-TDH and HlyA, still there is a lack of information about other hemolysins. Therefore, it is necessary to further enhance our knowledge regarding these toxins in order to thoroughly understand the mechanism of pathogenesis for the prevention of endemic infectious diseases associated with these pathogens.

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