Molecular Mechanism of Cholerae Toxin *(ctx)* in Causing Diarrhea

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ABSTRACT

Vibrio cholerae is one of the pathogenic bacteria transmitted through contaminated food, especially contaminated seafood and beverages. *V. cholerae* produces *cholerae toxin (ctx)* which is encoded by the *ctx* gene located within its chromosome. This toxin has been recognized as one of the toxins responsible for cholera outbreaks. The mechanism of *ctx* gene expression is induced by environmental signals such as pH, osmolarity, temperature, bile, amino acids, and CO_2. These signals will be a positive transcriptional factor to the *ToxR* gene that regulates the biogenesis of *cholerae toxin*. After *cholerae toxin* has been successfully expressed, *V. cholerae* uses a type II secretion (T2S) pathway to deliver *cholerae* toxin to the extracellular environment. *Cholerae toxin* consists of A and B subunits. The B subunit plays a role in attaching to the receptor *Manosialosyl Ganglioside (GM_1 ganglioside)* and the A subunit plays a role in catalyzing ADP-ribosylation of G_s (stimulatory) protein and turning them into active condition. The G_s protein will convert the inactive *adenilate cyclase* (AC) into active AC. The increase of AC activity will increase the *cyclic adenosine 3′5′-monophosphate* (cAMP) concentration along the cell membrane. The cAMP then causes the active secretion of sodium (Na^+), chloride (Cl^-), potassium (K^+), bicarbonate (HCO_3^-), and water (H_2O) out of the cell into the intestinal lumen, resulting in large fluid losses and electrolyte imbalances.

Keywords: *Vibrio cholerae, cholerae toxin (ctx), ToxR* gene, type II secretion (T2S), *GM_1 ganglioside, adenilate cyclase.*

INTRODUCTION

Cholera is one of the public health problems in developing countries such as Africa, Asia and South America, although epidemiologically and bacteriologically cholera has been known since the last century (Lesmana, 2004; Ryan and Ray, 2004). The most prominent clinical features of cholera are the production of large amounts of liquid feces and the occurrence of dehydration as a result of fluid loss through feces. The incubation period of cholera can range from several hours to several days depending on the number of inoculum (Lesmana, 2004).

Vibrio cholerae can be divided into two types based on its pathogenicity, *V. cholerae* serogroup O1 / O139 and *V. cholerae* serogroup non-O1 / non-O139. Prior to 1992, only *V. cholerae* O1 was known to produce *cholerae toxin* and was the cause of endemic and epidemic outbreaks. Later, *V. cholerae* O139 is also known to produce toxins in quantities as large as serogroup O1 (Faruque, 1998, Pal, 2014). Today, *V. cholerae* serogroup O1 and O139 are considered to be pathogenic *Vibrio* groups that produce *cholerae toxin* (Dziejman et al., 2002; Ryan and Ray, 2004; Olaniran et al., 2011).

Cholerae toxin is a major toxin responsible for the occurrence of diarrhea in cholera outbreaks. This toxin is considered as a major virulence factor. Therefore, the *ctx* gene is often used to determine the pathogenicity of *V. cholerae* (Chen et al., 2004; Chomvarin et al., 2007; Huq et al., 2012). This review will discuss the molecular mechanism of *cholerae toxin* in causing diarrhea.

THE STRUCTURE OF CHOLERAE TOXIN

Vibrio cholerae produces a heat-labile enterotoxin with a molecular weight of about 84,000 Dalton comprising A and B subunits (Figure 1) (Wernick et al., 2010; Brooks et al., 2013). *Cholerae toxin* is a toxin responsible for cholera. The *cholerae toxin* belongs to pathogenic *V. cholerae* which is encoded by the *ctxA* and *ctxB* genes (Faruque, 1998; Maheshwari et al., 2011).

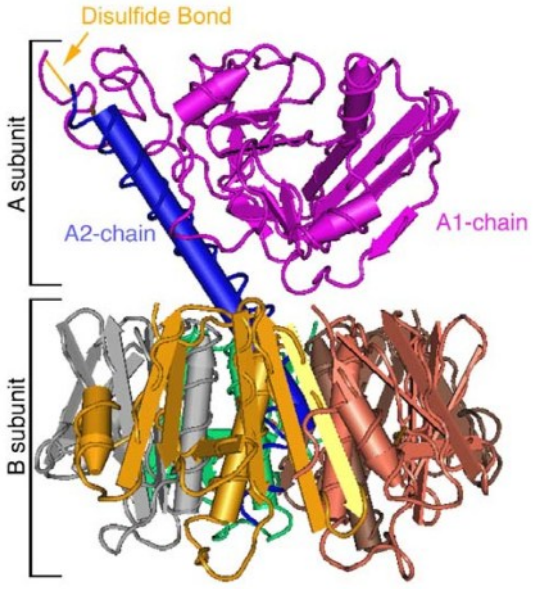

Figure 1. The three-dimensional structure of cholera toxin *(ctx)* (Wernick et al., 2010).

The both of subunits have different functions, B subunit is responsible for binding on the *Manosialosyl Ganglioside (GM$_1$ Ganglioside)* and A subunit is an active subunit that activates *adenilate cyclase* in small intestinal epithelial cells (Maheshwari et al., 2011). The A subunit is divided into A-1 chain and A-2 chain, between A and B subunits are connected with disulfide bond (Wernick et al., 2010).

EXPRESSION AND SECRETION OF CHOLERAE TOXIN

The expression of the virulence genes is a major factor contributing to the pathogenicity of *V. cholerae*. Some of the virulence factors of *V. cholerae* include *ToxR regulator, cholerae toxin (ctxA* and *ctxB)*, *toxin-coregulated pilus subunit (TcpA), outer membrane protein U (ompU), outer membrane protein W (ompW), accessory cholerae enterotoxin (Ace), and zonular occludens toxin (Zot)* (Reidl and Klose, 2002; Waturangi et al., 2013; Ramazanzadeh et al., 2015). The expression of *V. cholerae* virulence factor is controlled by *ToxR regulatory* cascade which depends on environmental conditions (Figure 2) (Raskin *et al.,* 2004; Schild et al., 2008).

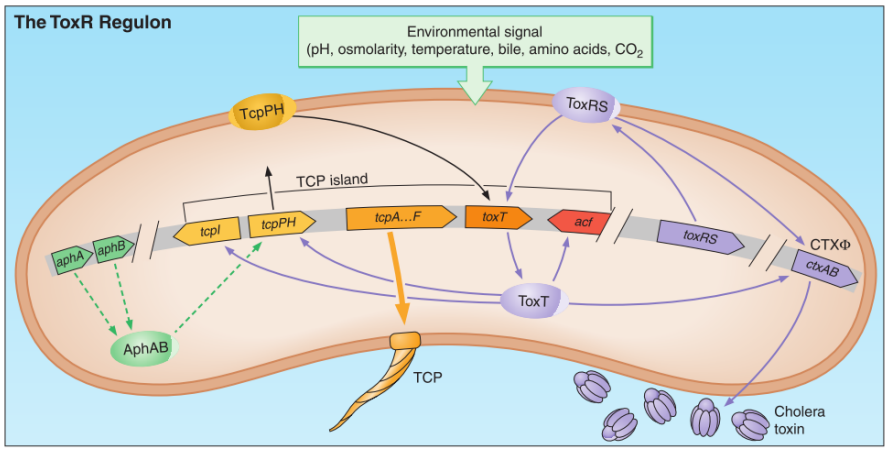

Figure 2. The mechanism of *ctx* gene activation (Raskin *et al.,* 2004).

In vivo, signals which can activate the *ToxR* gene are still unclear, but in vitro, several environmental signals such as pH, osmolarity, temperature, bile, amino acids, and CO_2 can activate the *ToxR* gene. These signals will be a positive transcriptional activator of *ToxRS* and *TcpPH*, which then activates the expression of *toxT* which is another positive transcriptional activator. *ToxT* is a protein that directly activates the biogenesis of TCP genes as well as *ctxAB* expression (Raskin et al., 2004).

Once the toxin is successfully expressed, the toxin should be secreted out to cause a disease. Most Gram-negative bacteria use the type II secretion (T2S) pathway to deliver proteins that contribute to the emergence of a disease (Korotkov et al., 2012; Green and Mecsas, 2016). The T2S system consists of two main lines: general secretion *(Sec)* and *twin arginine translocation (Tat)* pathway (Nivaskumar and Francetic, 2014; Green and Mecsas, 2016).

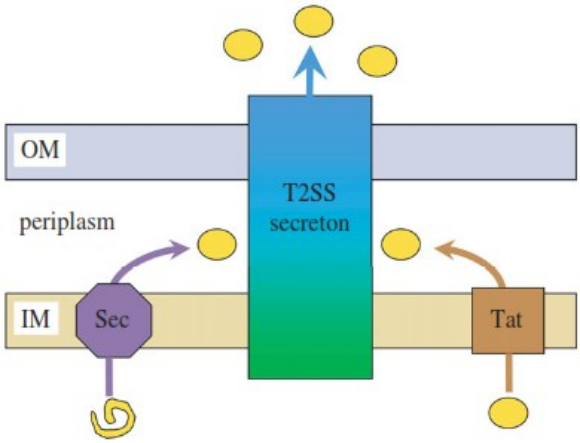

Figure 3. The secretion mechanism of the T2S system (Douzi et al., 2012).

Vibrio cholerae uses the T2S system in the *cholerae toxin* translocation process. In the transport process, *cholerae toxin* secretion uses T2S through two major steps: translocation the inner membrane through *Sec* pathway and is followed by transport of folded/oligomeric cargo protein by T2S to the extracellular environment (Douzi et al., 2012; Green and Mecsas, 2016).

THE ACTION OF CHOLERAE TOXIN

A person who has normal stomach acid should swallow 10^8-10^{10} organisms in the water to get infected and become ill, because the bacteria are very sensitive to the acidic environment. If the mediator is food, as many as 10^2-10^4 bacteria are needed because of the sufficient buffer capacity of the food. Some medications and conditions that may lower the acid levels in the stomach make a person more sensitive to *V. cholerae* infection (Brooks et al., 2013).

Vibrio cholerae colonizes in the intestinal epithelium but is not invasive or causes structural changes of the epithelium (Lesmana, 2004). The main effect of *V. cholerae*

infection is the actively increasing secretion of chloride, sodium, potassium, bicarbonate and water. This event occurs through the activity of *cholerae toxin* (Ryan and Ray, 2004).

There are very important *V. cholerae* surface proteins related to the life cycle and pathogenesis of cholera, namely *N-acetyl-D-glucosamine binding protein (GbpA)* and *hemagglutinin / protease (HapA)*. *GbpA* is associated with the ability of *V. cholerae* to attach to the chitin surface and also to the mucin lining the intestinal epithelial cells (Kirn et al., 2005). Based on molecular analysis, *GbpA* has 4 domains that generally relate to the ability to attach to the chito-oligosaccharides. Additional function of domain 1 is related to attachment to mucin while domain 2, 3 along with domain 1 helps bacteria to colonize the rat baby's small intestine (Wong et al., 2012). *Hemagglutinin / protease (HapA)* acts as a proteolytic enzyme that can lyse existing substrates in the intestinal environment such as ovomucin, fibronectin, and lactoferrin. *HapA* helps *V. cholerae* to penetrate more deeply and degrade the mucus layer of the intestine. Therefore, *cholerae toxin* can bind with *GM_1 ganglioside* receptors as well as for detachment processes (Sikora, 2013).

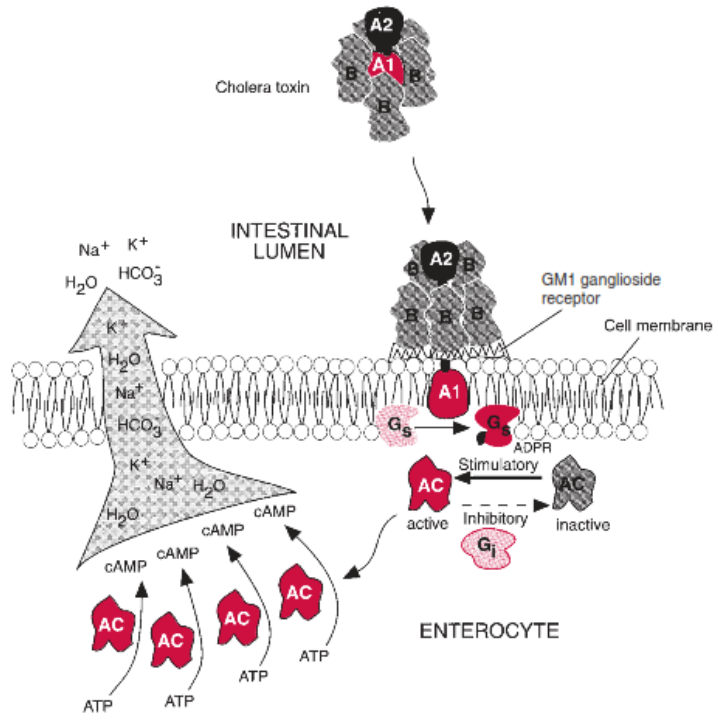

Figure 4. The action of cholera toxin (Ryan and Ray, 2004).

The complete *cholerae toxin* consisting of A and B subunits is released by *V. cholerae* and then B subunit binds to *GM_1 ganglioside* receptors on the intestinal epithelial mucosal

surface and A subunit which is an active part of the toxin catalyzes ADP-ribosylation of G (stimulatory) protein and converts it become active. The G_s protein plays a role in converting the inactive adenilate cyclase (AC) into active AC then the increase of AC activity will increase the cyclic adenosine 3'5'-monophosphate (cAMP) concentration along the cell membrane. Furthermore, cAMP causes active secretion of sodium (Na^+), chloride (Cl^-), potassium (K^+), bicarbonate (HCO_3^-), and water (H_2O) exit from cells to the intestinal lumen resulting in large fluid losses and electrolyte imbalances (Ryan and Ray, 2004; Thiagarajah and Verkman, 2005; Lima and Fonteles, 2015). The clinical features of cholera are diarrhea and the occurrence of dehydration as a result of fluid loss through feces. Diarrhea is often followed by vomiting, especially early in the disease and if left untreated can cause death (Lesmana, 2004).

CONCLUSION

Vibrio cholerae O1 and O139 are pathogenic serogroups known to produce *cholerae toxin* which is a major toxin in causing diarrhea in humans. The mechanism in causing diarrhea caused by *cholera toxin* is through increased adenilate cyclase. Since *cholerae toxin* is an important virulence factor, therefore the *ctx* gene is a target gene that is often used to determine the pathogenicity of *V. cholerae.*

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