Full Length Research Paper

Homology modeling of bacterial endotoxin

Natarajan Valarmathy[2#], Nadarajan Saravanan Prabhu[1#], Kanagavel Deepankumar[1], Ravikumar Sambandam[3], Abdulrahman Alsenaidy[4] and Niraikulam Ayyadurai[1,4*]

[1]School of Biotechnology, Yeungnam University, Gyeongsan, South Korea.
[2]Department of Molecular Biology, Pusan National University, Busan, South Korea.
[3]School of Biochemical Engineering, Ulsan University, South Korea.
[4]Protein Research Chair, Department of Biochemistry, King Saud University, Riyath, Saudi Arabia.

***Bacillus thuringiensis* (or Bt) is commonly used as a biological pesticide; alternatively, the Cry toxin may be extracted and used as a pesticide. It occurs naturally in the gut of caterpillars of various types of moths and butterflies, as well as on the dark surfaces of plants. Bt crystals, sometimes referred to as insecticidal crystal proteins (ICP), are protein crystals formed during sporulation in some Bt strains. Bt produces proteins that aggregate to form a crystal. It is important to understand the mechanism of endotoxin binding or interaction with the host for the commercialization. Here, we aimed to construct computer-aided molecular model of bacterial endotoxin based comparative homology modeling, in order study the sequence in structural context and to analyze domains of the protein.**

Key words: *Bacilus thuringiensis,* biopesticide, toxin, crystal protein, entotoxin.

INTRODUCTION

Employing the biological insecticides is favorable because they destroy undesirable agricultural and human pests without introducing toxic and non biodegradable substances into the ecosystem. Among them, *Bacillus thuringiensis* (BT) is a Gram-positive, soil-dwelling bacterium, commonly used as a biological pesticide; alternatively. It produces parasporal crystal inclusions during sporulation (Hofte and Whiteley, 1989). In general, it produces two types of toxin. The main types are the Cry (crystal) toxins, and the second types are the Cyt (cytolytic) toxins which augment the Cry toxins, enhancing the effectiveness of insect control. Over 50 of the genes that encode the Cry toxins have now been sequenced and enable the toxins to be assigned to more than 15 groups on the basis of sequence similarities. The molecular mechanism of the toxin with different insect pests has been elaborately reviewed by many workers (Schnepf et al., 1998; van Frankenhuyzen, 2009).

Nowadays, the scientist delineated the toxin genes coding for different subunits of d-endotoxin to better understand the structural motifs that govern the specificity of the toxins to particular insect larvae.

The production of *B. thuringiensis* in commercial scale is economically important and in order to develop its production it is necessary to consider available human resources, technology for producing equipments and controls, adequate and cheap raw materials, adapted microorganisms and bioassay facilities (Tzeng et al., 2002; Wexler and Oppenheim, 1979). However, it is important to understand the mechanism of endotoxin binding or interaction with the host for the commercialization (Schwieters et al., 2003; 2006). Here, we aimed to construct computer-aided molecular model of Bacterial endotoxin based comparative homology modeling, in order study the sequence in structural context and to analyze domains of the protein.

METHODOLOGY

Modeller is a computer program used for comparative protein structure modeling (Marti-Renom et al., 2000). It automatically calculates a target model structure containing all non hydrogen

---

*Corresponding author. E-mail: ayyadurai@ynu.ac.kr.

# Authors contributed equally.

Table 1. Template search using BlastP database.

Structure	Identity (query coverage) (%)	Similarity (query coverage) (%)	Resolution
3EB7	49 (290/591)	66 (391/591)	2.30
1CIY	43 (258/600)	60 (258/600)	2.25
1DLC	41 (244/592)	60 (353/592)	2.50
1JI6	39 (236/602)	59 (354/602)	2.40
1W99	39 (172/570)	48 (271/570)	1.75

Sequences having above 30% identity were chosen for the multi template modeling.
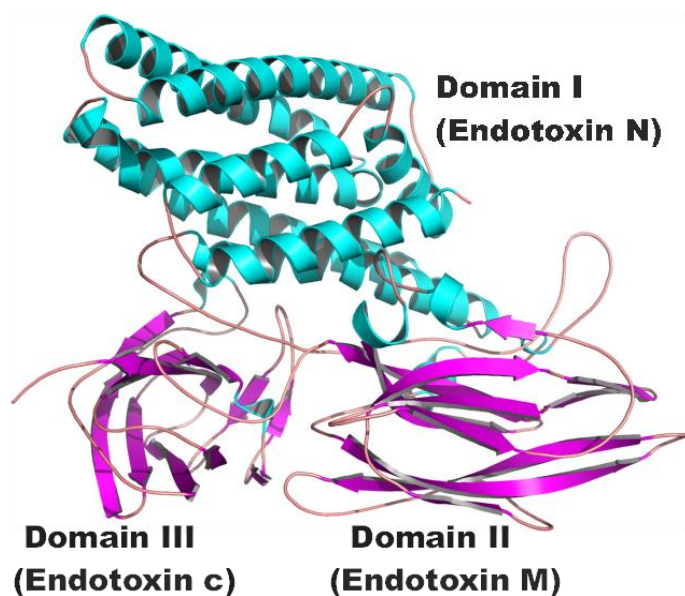

Figure 1. Ribbon representation of the Cry delta endotoxin of *B. thuringenesis*. Structures were highlighted based on the secondary structure. (Helices-Cyan, Beta sheet-magenta and coil-light pink color). Model Structure stored in Protein model database (PMDB id-PM0078047).

atoms using the target-template sequence alignment and template atomic coordinate as inputs. The input is sequence alignment file of target and template atomic coordinates of the template and a simple script file. This software automatically calculates atomic coordinate of all non-hydrogen atoms of the protein sequence.

Template search and template validation

The potentially related sequences for our query sequence were identified by sequence alignment against PDB database using NCBI-BlastP program. Out the 11 templates Chain A of BT-Insecticidal toxin (1CIY) is the best aligned protein sequence shows 73% identity with a expected value of 0.0 chosen as template for modeling studies (Table 1).

Molecular modelling

Based on the sequence alignment and the 3D structure of template, models were generated for the query sequence (Chung and

Subbiah, 1996; Venclovas and Margelevicius, 2005). The N-terminal and C-terminal overhangs were removed during the modeling step. The constructed model structure was energy minimzed at steepest descent minimization around 200 steps and evaluated for the structure quality using SAVES server. The root mean square deviation (RMSD) values were calculated between the modeled structures and its corresponding template structures to check the accomplishment of the modeling process. The quality of molecular models of toxin was determined by examining the distribution of amino acid residues in the Ramachandran plot.

RESULTS AND DISCUSSION

Based on the sequence analysis with Pfam database using CDART represents that the protein belongs to Delta endotoxin family of *Bacillus thuringensis* (Bravo, 1997). This family of protein are said to be insecticidal toxins produced by Bacillus species of bacteria. During spore formation, the bacteria produce crystals of this protein. When an insect ingests these proteins, they are activated by proteolytic cleavage. The N terminus is cleaved in all of the proteins and C-terminal extension is cleaved in some members. Once it is activated, the endotoxin binds to the gut epithelium and causes cell lysis leading to death. This activated region of the delta endotoxin is composed of three structural domains. The N-terminal helical domain is involved in membrane insertion and pore formation. The second and third domains are involved in receptor binding (Figure 1).

On the basis of quality of sequences, we chose the first sequences as template for the comparative modeling purpose. Our protein blast results shows that out of the 14 Blast hits the best kit was observed with the 1CIY because it shows 74% identity with the target sequence with E-value 0.0 covering 442 amino acids of the query (N-terminal and C-terminal overhangs removed). The template contains forty two amino acids of protein sequence. Further the sequence was pair wisely aligned with our target sequence to generate model structures.

Finally, we generated the model which contains 17 helices and 28 beta strands. The N-terminal helical domain represented in red involves in membrane insertion and pore formation which contain11 helices and 2 beta sheets other two domain represented in yellow color involves in receptor binding (that is) they bind to the Gut epithelial receptor of insect after activation by the gut
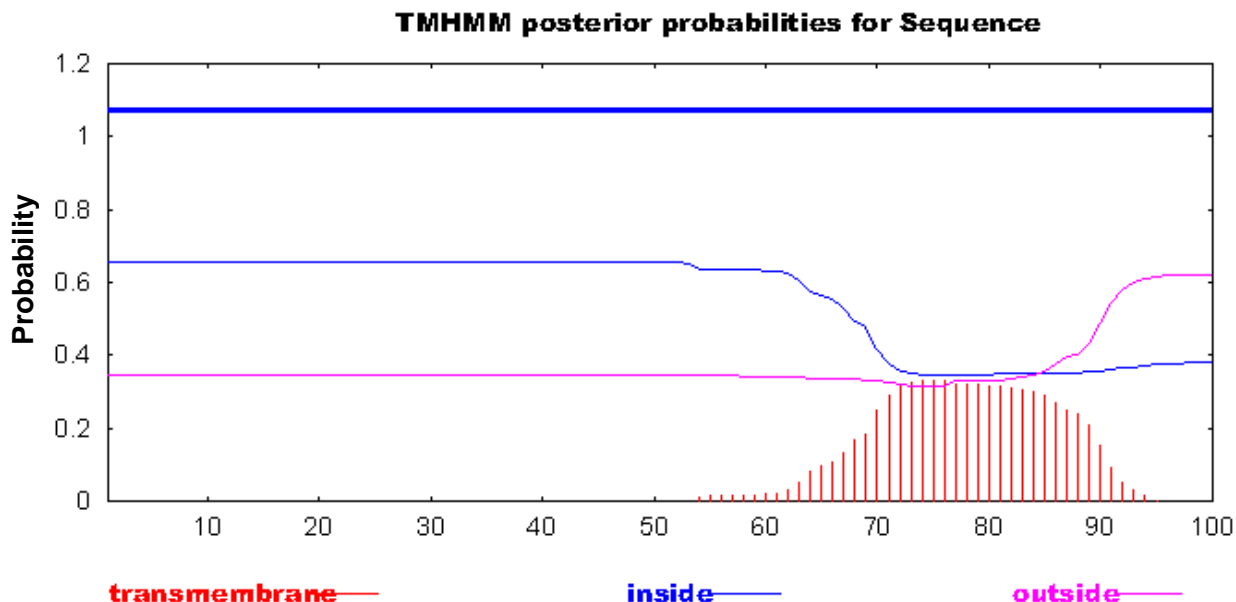
Figure 2. Transmembrane prediction of the bacterial endotoxin. The first 100 amino acid of Protein shows a transmembrane depicting that the domain I is transmembrane domain and other two domain lie outside.

proteases. Nevertheless, the residues located in the Cry1 AC protein fall in the disallowed region of the Ramachandran plot. Ramachandran plot statistics given by Procheck show clearly that 89.1% of the residues are in allowed region and 0.2% of residues in disallowed region.

Remarkably, it has been reported that the cry 1AC protein after digested by insect gut protease became activated and inserted into the plasma membrane and form pores that lead to cell death through osmotic lysis. In this study, we predicted the transmembrane of the protein using TMHMM. Transmembrane prediction for the whole sequence shows no Transmembrane helix in the protein but predicted results for every 100 amino acids shows that expected number of amino acids in the transmembrane helix are from 46 to 68. These residues found in Domain I of cry toxin Protein. Based on this predicted result, we come to a conclusion that the Domain I (46 to 68 amino acids) of Cry 1Ac involve in insertion into the gut epithelial plasma membrane of insect and leads to the pore formation (Figure 2).

In conclusion, we predicted the structure of protein and the domain that are involve in the membrane pore formation predicting the protease.

ACKNOWLEDGMENT

REFERENCES

Hofte H, Whiteley HR (1989) Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiol. Rev., 53(2): 242-255.

Van Frankenhuyzen K (2009). Insecticidal activity of *Bacillus thuringiensis* crystal proteins. J. Invertebr. Pathol., 101 (1) :1-16.

Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR, Dean DH (1998). *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev., 62 (3): 775-806.

Marti-Renom MA, Stuart AC, Fiser A, Sanchez R, Melo F, Sali A (2000). Comparative protein structure modeling of genes and genomes. Annu. Rev. Biophys. Biomol. Struct., 29 (3): 291–325.

Chung SY, Subbiah S (1996). A structural explanation for the twilight zone of protein sequence homology. Structure, 4 (1): 1123–1127.

Venclovas C, Margelevicius M (2005) Comparative modeling in CASP6 using consensus approach to template selection, sequence-structure alignment, and structure assessment. Proteins, 61(S7): 99–105.

Bravo (1997). Phylogenetic relationships of *Bacillus thuringiensis* delta-endotoxin family proteins and their functional domains. J. Bacteriol., 179: 2793–2801.

Tzeng YL, Datta A, Kolli VK, Carlson RW, Stephens DS (2002) Endotoxin of *Neisseria meningitidis* composed only of intact lipid A: inactivation of the meningococcal 3-deoxy-D-manno-octulosonic acid transferase. J. Bacteriol., 184 (9): 2379–2388.

Wexler H, Oppenheim JD (1979). Isolation, characterization, and biological properties of an endotoxin-like material from the Gram-positive organism *Listeria monocytogenes*. Infect. Immun., 23 (3): 845–857.

Schwieters CD, Kuszewski JJ, Tjandra N, Clore GM (2003) The Xplor-NIH NMR Molecular Structure Determination Package. J. Magn. Res., 160: 66-74.

Schwieters CD, Kuszewski JJ, Tjandra N, Clore GM (2006) Using Xplor-NIH for NMR molecular structure determination. Progr. NMR Spectroscopy, 48: 47-62.