Insilico Modeling of *Helicoverpa armegera* Aminopeptidase-N receptor and Docking Studies with *Bacillus thuringiensis* Cry1Ac Toxin

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Abstract

Bacillus thuringiensis (Bt) gene have been extensively used in the insect resistant transgenic plant development. This study is basically focused to understand the structural information of Aminopeptidase-N (APN). By considering the fact that this receptor protein participates in tight binding with Cry endo-toxins of Bt and finally results into the pore formation on insect gut membrane. This ultimately leads to insect mortality. In the present work we have selected more structurally similar TIF2 and TIF3 proteins with APN and studied their interacting motif regions with the help of sequence and structural alignments. APN transmembrane architecture was also studied and found that architecture playing a major role in the binding site availability. Docking studies helped to understand the type of bonding and interactions occurred in the process of receptor-protein binding which in turn help to design better toxins through Genetic engineering.

Key words- *Bacillus thuringiensis*, Aminopeptidage-N, Cry toxins, Transmembrane protein, Endotoxin, Tricorn Interactive Factor.

Introduction

Bacillus thuringiensis is a gram positive, aerobic spore forming bacteria which produces insecticidal crystal proteins. Transgenic insect resistant crops introduced with Cry genes in their genome lead to continuous production of crystal proteins of *"Bacillus thuringeinsis*" (Bt) (Chakrabarti *et al., 1998*; Schnepf *et al.*, 1998). These proteins are highly toxic to insect but quite safe for human beings. Generally, Bt toxins work efficiently at high lumen pH of insects. These toxins bind to a number of putative, receptor molecules and initiate pore formation on the gut epithelial membrane of insects which finally leads to their death. The receptors available on the surface of the gut epithelial and their compatibility with Bt toxin interactions plays a major role. The insect epithelial cells have two major types of receptors aminopetidase-N (APN) and cadherin (Nagamatsu *et al.,*1998; Schnepf *et al.*, 1998). One of the insect resistance development mechanisms is linked with toxin binding ability to receptors. *Plutella xylostella* and *Helicoverpa armigera* insects had already shown resistance against the Transgenic Cotton plant with Cry1Ac insert (Sayyed *et al*., 2005). The insects developed resistance against transgenic plants by using different mechanisms and one of these mechanisms is modification of receptors to avoid interactions with Bt toxins. Cry1Aa, Cry1Ac and Cry1C binding proteins are Aminopeptidase N (APN) (EC 3.4.11.2) family members (Lorence *et al.,* 1997; Luo *et al.,* 1997; Knight *et al.,* 1995; Sangadala *et al.,* 1994, Yaoi *et al.,*1997). The 120 KDa APN has a C-terminal glycosylphosphatidylinositol (GPI)

anchor (Garczynski *et al*., 1995). During the present work aminopeptidase-N is selected for detailed study investigation. In view of the above said aspects it is evident that there is a need to study *Helocoverpa armigera* APNs and CrylAc toxins to understand the structural interaction aspects, especially at the receptor active site regions. This study will help to develop new Bt toxins to prevent insect resistance development against the transgenic plants.

Material & Methods

Aminopetidase-N of *Helocoverpa armigera* (GenBank Ac No: AF521659), Tricorn Interacting Factor2 (TIF2) (GenBank Ac No: AAC98289), TIF3 (PDB ID: 1Z5H) and Cry1Ac (GenBank Ac No: AAA22331) were downloaded from NCBI databases. Multiple alignments of sequences were carried out by using ClustalW program and phylogenic tree (Phylogram) has been generated. TIF2, TIF3 structures were selected from PDB database. Protein structures were studied in detail by using SPDB viewer and Chimera visualization softwares. 3D structures of APN receptor and Cry1Ac toxin were modeled, minimized by using Biopolymer suit of Insight II (Accerlarys) software, applied CV force fields with 1 lack iterations at 25ºC. To predict the 3D homology model of APN many templates (Crystal structures of PDB IDs: 1Z5H chain B, 1GW6 chain A, 1HS6 chain A and 1H19) were used. Further the structures were validated by using Procheck program with the help of Ramachandran plot. Modeled structures were studied in detail by using SPDB viewer and UCSF Chimera visualization softwares. Active site regions of the proteins were predicted by using LigBuilder and Q-Site pocket finder programs. The APN protein models were further studied in detail for predicting the transmembrane structures by using Sosui tool and TMHMM sever to identify the external and internal exposed region of the proteins. The active site structures were aligned to understand the structural differences by using Protien-3D fit program. The shape of pockets in the 3D structure was studied further at the surface models structures by using UCSF Chimera software. APN protein was further minimized by setting the pH 9.5 and models were superimposed to study the more structural details and also RMSD was calculated. APN and Cry1Ac structures were further preceded for molecular docking studies by using GRAMM program protein-protein interactions at molecular level the binding energies were calculated by using Xscore software and also graphical interactions were viewed by using Pymol and Discovery studio.

Results & Discussion

Thermoplasma acidophilum Tricorn interacting factor F2, F3 and APN of *Helocoverpa armigera* protein sequences (APN/1-1000) were multiply aligned through ClustalW1.83 and Jalveiw programs. Active site motifs were identified from the alignment by considering conserved sequence regions like 403-04, 407-17, 444-52, 517-21, 599-608, 632-38, and single amino acid regions like 596, 630 and 676 which are showing structure and sequence-based similarity for the active sites as shown in figure-1.

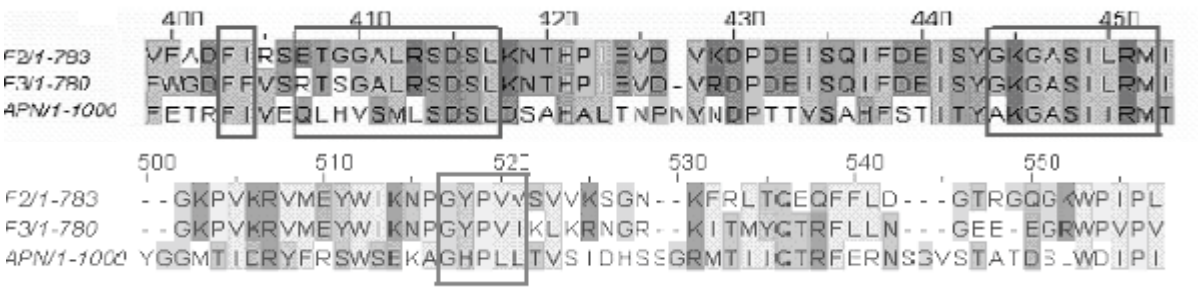


Figure 1: Multiple alignment of APN, TIF2 & TIF3 proteins shows conserved blocks.

For the above said sequences phylogenetic tree was generated by using phylogram method as shown in figure-2 and the distances showed that all the sequences are almost closely linked.

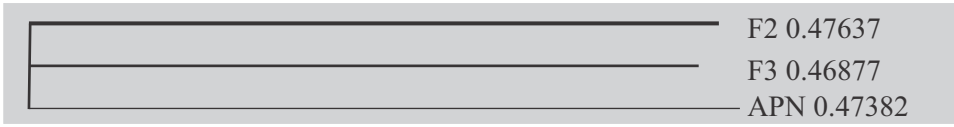


Figure 2: Phylogenetic tree showing close relationship among selected sequences.

3D models of Cry1Ac and *Heliocoverpa armigera* APN protein were generated (Pazos and Salamanca, 2008; Pardo-Lopez *et al.,* 2012). The structure of Cry1Ac was predicted using Cry1Aa (1CIY) template available in the PDB database (Berry and Crickmore, 2017). The modeled structure is shown in figure-3a. This model is evaluated by using Ramachnadran plot as shown in figure-3b. The generated model was further used to find out the interaction sites in the receptor (Dehury *et al.,* 2013).

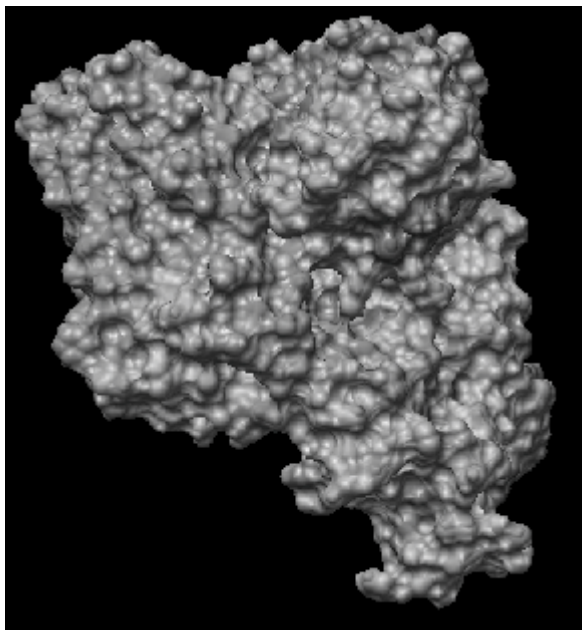


Figure 3a: Surface 3D model of Cry1Ac.

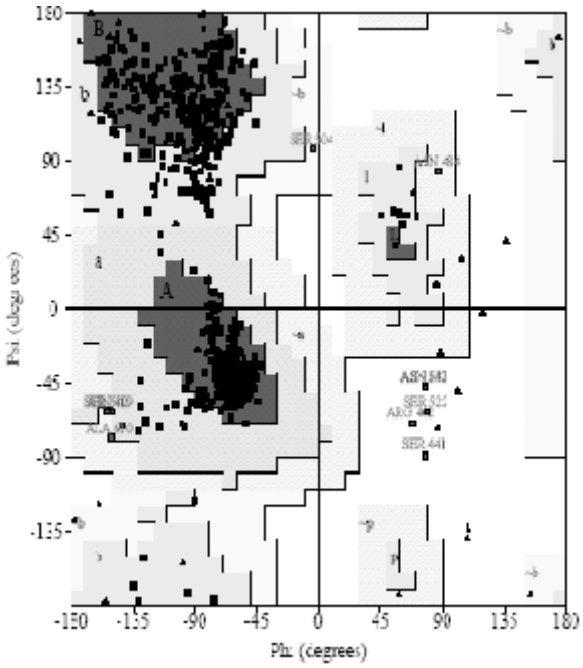


Figure 3b: Ramachandran plot for Cry1Ac.

Similarly, *Heliocoverpa armigera* APN protein was modeled by using template TIF3 whose structures are already present in the PDB. The modeled structure was validated by using Ramachandran plot as shown in figure-4 (Tajne *et al.,* 2014).

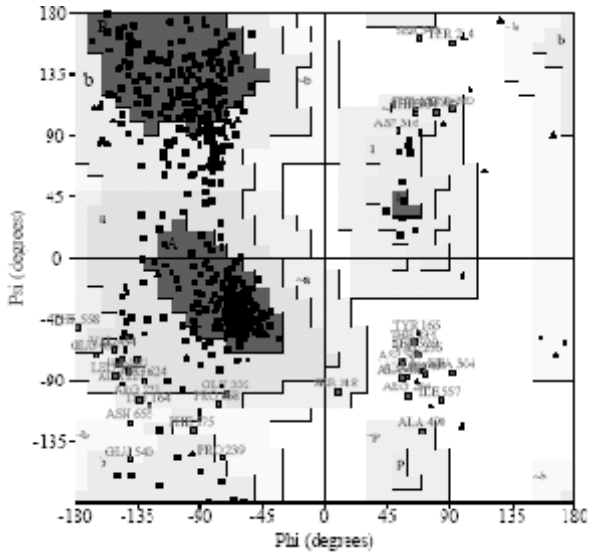


Figure 4: Ramachandran plot for modeled APN protein.

APN protein was further subjected to the LigBuilder program to find active sites. Four active site motifs and four single residue conserved active sites were traced out by LigBuilder and all the active sites together formed a cups shaped surface to bind with Cry1Ac loop regions as shown in figure-5.

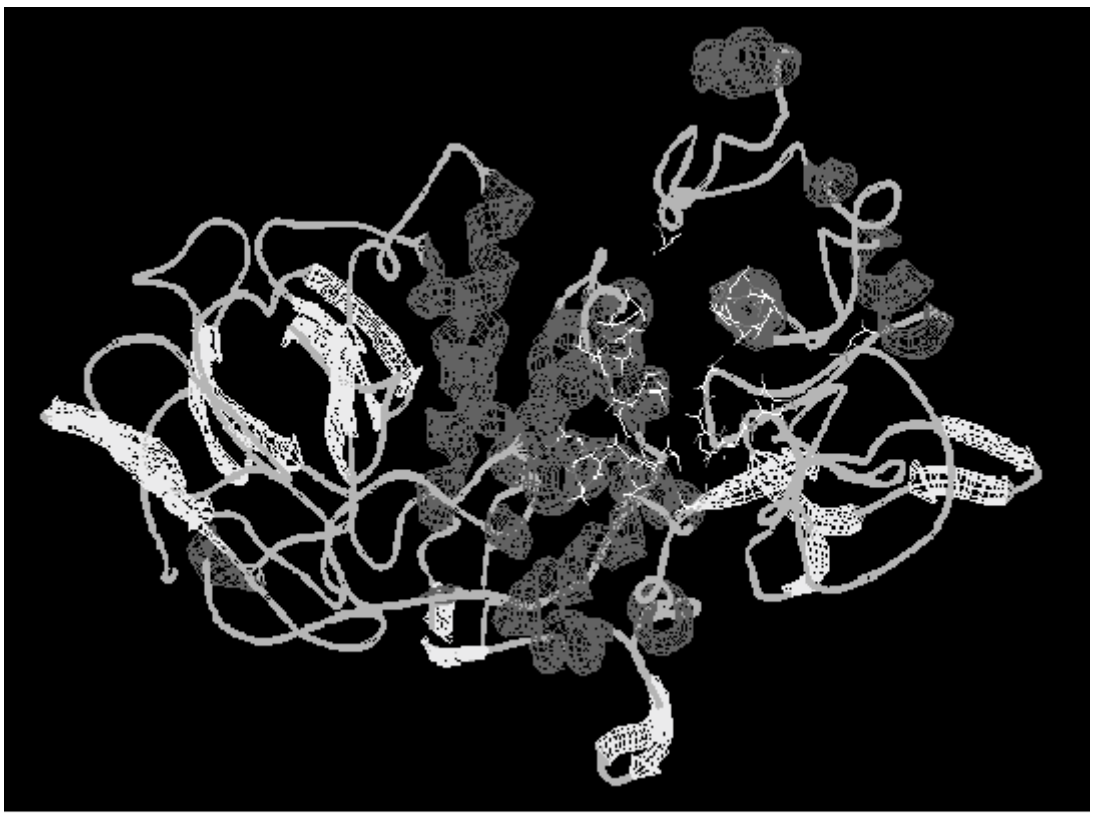


Figure 5: Main active sites predicted by LigBuilder highlighted (arrows pointed towards active sites).

These motifs and residues were highlighted based on the ribbon structure of the APN, however the main active sites were found to be surrounded by a cup shaped receptor structure containing with many active sites. This provides complementary binding space to the Cry 1Ac.

The structures were modeled both at neural pH 7.0 and basic pH 9.5 simultaneously. The X-ray crystallographic structures of Cry1Aa, TIF1 and TIF2 & TIF3, available in PDB were determined at pH 7.0, but the insect gut has pH range of 7.5-10.5 whereas mid gut region has pH of 9.5 or more than that. Bt protoxins interacts with proteases and converts into the toxins (Van Rie *et al.,* 1990). At simulated high pH environment APN protein displayed different conformation and was compared with the structure available at natural pH. The structural changes are clearly visible by looking at the ribbon structures pair-wise alignment of the APN at pH 7.0 & 9.5 as shown in figure-6. The structures are approximately close and properly aligned. It suggests that Bt toxins 3D model predictions and interactions (Docking) must be carried out at a specific pH. Similar studies were carried out in Cockroach pheromone binding proteins and found structural changes occur at different pH (Lartigue *et al.,* 2003).

Figure 6: Superimposed structures of *Helocoverpa armigera* APN at different pH.

The Transmembrane nature of the receptor proteins was studied in order to understand the exact location of the active sites and their availability to the toxins binding on the receptor. The receptor proteins were submitted to the TMHMM and Sosui transmembrane protein prediction programs. Results are quite interesting that the N terminal lies outside in extra cellular region and some region behind N-terminal is buried inside transmembrane while APN C' terminal region is buried and anchored to the membrane and major portion of the protein chain is exposed and present in the outer region as shown in figure-7.

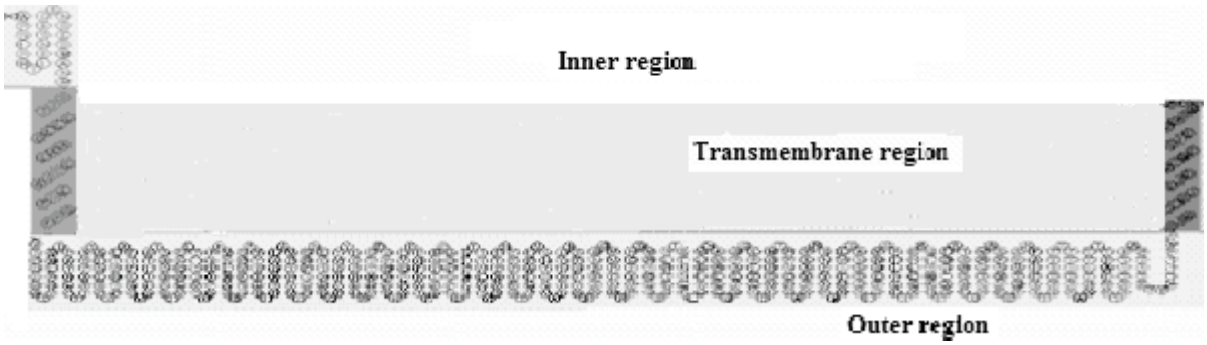


Figure 7: Transmembrane structure of (N-terminal falling in transmembrane and the C-terminal lies in the inner site).

APN and Cry1Ac structures were further preceded for molecular docking studies by using GRAMM v1.03 software to study the protein-protein interaction at molecular level (Ahmad *et al.,* 2015). The docked structure is shown in figure-8.

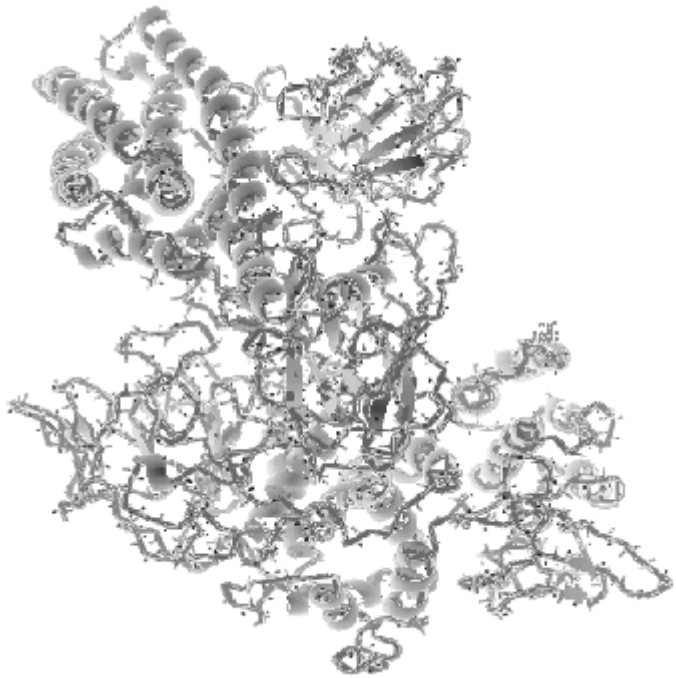
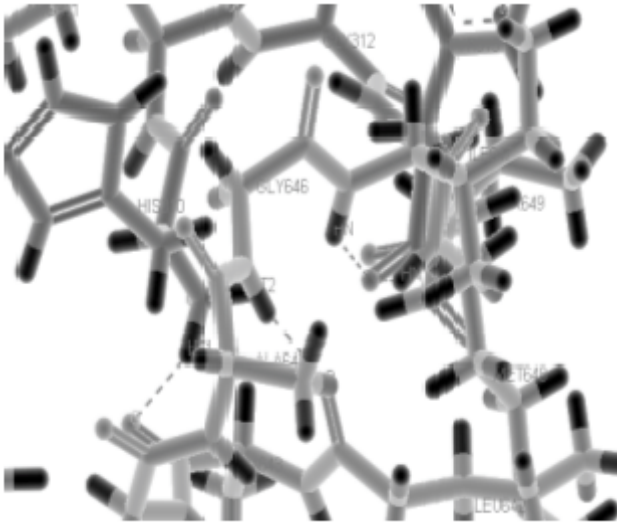


Figure 8: Docked structure of APN receptor and Cry1Ac toxin

The binding energy was calculated using Xscore software which was found to be -6.34 and deviation –log [kd] is 2.37. The graphical molecular interactions between APN active sites and Cry1Ac loop-I, II and III were also generated as shown in figure-9a, 9b and 9c respectively.

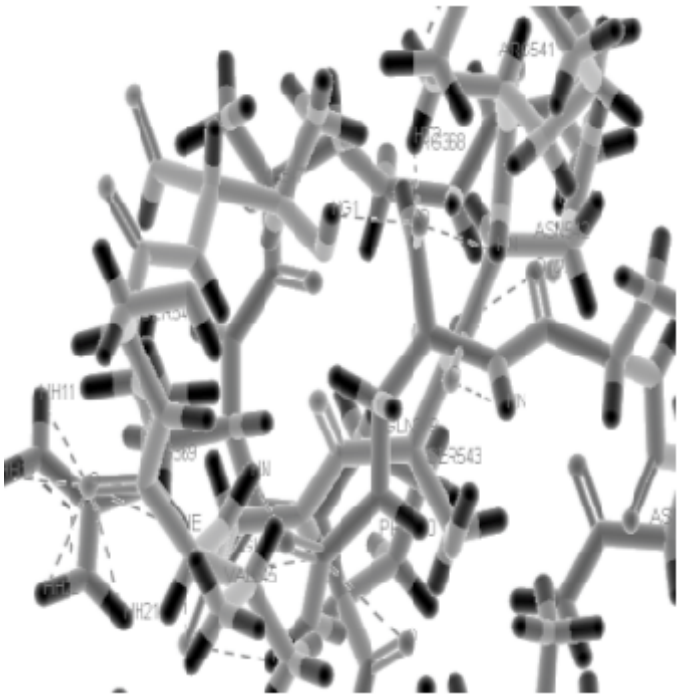
Interaction between APN and Bt Cry1Ac loop- 1 region



Cry Ac (toxin)	APN (Receptor)
310 HIS-HT2	O-LEU 642
310HIS-HT1	O-ALA 643
TYR313-O	HN-MET 648
TVR313-O	HN-LEU 647

Figure 9a: Interaction between APN active site-I and Bt Cry1Ac loop-region

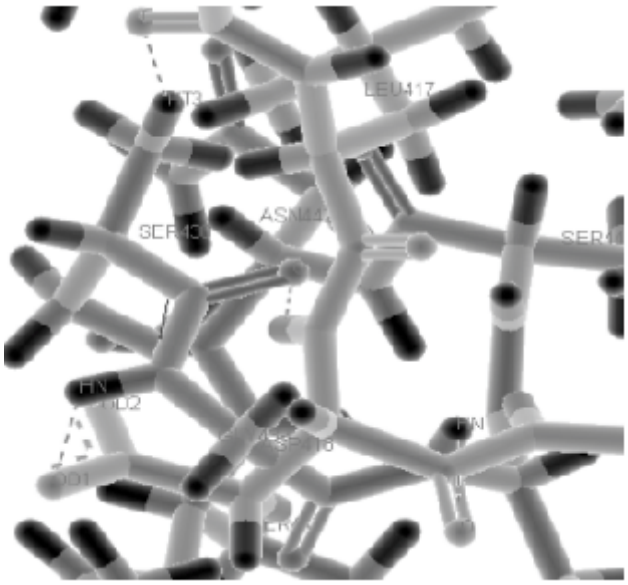
Interaction between APN and Bt Cry1Ac loop- II region



Cry 1Ac (toxin)	APN (Receptor)
368 ARG-HH11	OD1-ASN542
369 AGR-HE11	O-VAL545
369 AGR-HE12	O-VAL545
369 ARG-HE22	O-VAL545
369 ARC-HE	O-VAL545
369 ARC-HE	O-LN544
369ARG -O	HN-GLN544
370PRO-O	HN-GLN544
GLN 378-O	NH-543
GLN379-O	NH-ARG541
GLN379-O	NH-ARG541
GLN379-O	HGI-RG541
GLN379-NH	0-ASN542
GLN379-HE22	O-GLN544
OGLN379-OE1	NH-VAL545

Figure 9b: Interaction between APN active site-II and Bt Cry1Ac loop-II region.

Interaction between APN and Bt Cry1Ac loop- III region



Cry 1Ac (toxin)	APN (Receptor)
STR438-HT3	O-SETR416
SER438-O	HN-ASP418
GLY439-NH	OD1-SP410
GLY439-NH	OD2-ASP418

Figure 9c: Interaction between APN active site-III and Bt Cry1Ac loop-III region.

The interaction between APN active site-I and Bt Cry1Ac Loop-I forms four hydrogen bonds, APN active site-II and Bt Cry1Ac Loop-II forms fifteen hydrogen bonds and APN active site-III and Bt Cry1Ac Loop-III forms four hydrogen bonds. The hydrogen bonds decide the strength of the binding between two proteins which lead to the toxic activity enhancement. These interaction studies help to understand the toxicity improvement of the Bt toxins which in turn help to develop new mutations of amino acids at gene level. These genes can be used further in the transgenic plant development.

Conclusions

Though APN, TIF2, TIF3 proteins have similar motifs, sequence similarity, however it is also observed that they do have structural similarity. The active sites falling within the cup shaped binding region of APN facilitate tight binding to the Cry1Ac toxins and leads to the pore formation on the cell membrane. This study clearly indicates that pH plays a major role in the structural conformational changes and the active site architecture of APN. Transmembrane architecture also clearly predicts that the active sites regions are easily available for the toxin binding. These results are substantiated with appropriate docking studies which will open doors to the experimental biologist to explore more about receptor-toxin interactions.

References

Ahmad, A., Javed, M.R., Rao, A., Khan, M.A., Ahad, A., Shahid, A.A., Husnain, T. 2015. In-silico determination of insecticidal potential of Vip3Aa-Cry1Ac fusion protein against Lepidopteran targets using molecular docking. Front. *Plant Sci.*, 6, 1081.

Berry, C., Crickmore, N. 2017. Structural classification of insecticidal proteins–towards an in-silico characterization of novel toxins. *J. Invert. Pathol.*, 142, 16–22.

Chakrabarti, S.K., Mandaokar, A.D., Kumar, A.P., Sharma, R.P. 1998. Synergistic effect of Cry1Ac and Cry1F delta-endotoxons of Bacillus thuringiensis on cotton bollworm, *Helicoverpa armigera*. *Curr. Sci.*, 75, 663–664.

Dehury, B., Sahu, M., Sahu, J., Sarma, K., Sen, P., Modi, M.K., Choudhury, M.D. 2013. Structural analysis and molecular dynamics simulations of novel endotoxin Cry1Id from *Bacillus thuringiensis* to pave the way for development of novel fusion proteins against insect pests of crops. *J. Mol. Model.*, 19, 5301–5316.

Garczynski, S.F., Adang, M.J., 1995. *Bacillus thuringiensis* CryIA(c) δ-endotoxin binding aminopeptidase in the *Manduca sexta* midgut has a glycosyl-phosphatidylinositol anchor. *Insect Biochem. Mol. Biol.*, 25, 409–415.

Knight, P.J., Crickmore, N., Ellar, D.J. 1994. The receptor for *Bacillus thuringiensis* CrylA(c) delta-endotoxin in the brush border membrane of the lepidopteran *Manduca sexta* is aminopeptidase. *N. Mol. Microbiol.,* 11, 429–436.

Lartigue, A., Gruez, A., Spinelli, S., Rivière, S., Brossut, R., Tegoni, M., Cambillau, C. 2003. The crystal structure of a cockroach pheromone-binding protein suggests a new ligand binding and release mechanism. J Biol Chem., 278(32), 30213-30220.

Lorence, A., Darszon, A., Bravo, A. 1997. Aminopeptidase dependent pore formation of *Bacillus thuringiensis* Cry1Ac toxin on *Trichoplusia ni* membranes. *FEBS lett.*, 414, 303–307.

Luo, K., Tabashnik, B.E., Adang, M.J. 1997. Binding of *Bacillus thuringiensis* Cry1Ac Toxin to Aminopeptidase in Susceptible and Resistant Diamondback Moths (Plutella xylostella). *Applied and Environmental Microbiology*, 63(3), 1024-1027.

Nagamatsu, Y., Toda, S., Koike, T., Miyoshi, Y., Shigematsu, S., Kogure, M. 1998. Cloning, Sequencing, and Expression of the *Bombyx mori* Receptor for *Bacillus thuringiensis* Insecticidal CryIA(a) Toxin. *Bioscience, Biotechnology, and Biochemistry*, 62(4), 727-734,

Pardo-Lopez, L., Soberon, M., Bravo, A. 2012. *Bacillus thuringiensis* insecticidal three-domain Cry toxins: Mode of action, insect resistance and consequences for crop protection. *FEMS Microbio. Rev.*, 37, 3–22.

Pazos, S.A., Salamanca, J.A. 2008. Minireview and hypothesis: Homology modeling of Spodoptera litura (*Lepidoptera: Noctuidae*) Aminopeptidase N receptor. *Rev. Acad. Colomb. Cien,*, 32, 139–144.

Sangadala, S., Walters, F.S., English, L.H., Adang, M.J. 1994. A mixture of *Manduca sexta* aminopeptidase and phosphatase enhances *Bacillus thuringiensis* insecticidal CryIA(c) toxin binding and $^{86}Rb^{+}$–K^{+} efflux *in vitro*. *J Biol Chem,* 269, 10088–10092.

Sayyed, A.H., Gatsi, R., Ibiza-Palacios, M.S., Escriche, B., Wright, D.J., Crickmore, N 2005. Common, but complex, mode of resistance of *Plutella xylostella* to *Bacillus thuringiensis* toxins Cry1Ab and Cry1Ac. *Appl Environ* Microbiol, 71(11), 6863–6869.

Schnepf, E., Crickmore, N., Van Rie J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R., Dean, D.H. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* 62, 775–806.

Tajne, S., Boddupally, D., Sadumpati, V., Vudem, D.R., Khareedu, V.R. 2014. Synthetic fusion-protein containing domains of Bt Cry1Ac and *Allium sativumlectin* (ASAL) conferred enhanced insecticidal activity against major *lepidopteran* pests. *J. Biotech.*, 171, 71–75.

Van Rie, J., Jansen, S., Höfte, H., Degheeled, D., Van Mellaert, H. 1990. Receptors on the brush border membrane of the insect midgut as determinants of the specificity of *Bacillus thuringiensis* δ-endotoxins. *Appl. Environ. Microb.*, 56, 1378–1385.

Yaoi, K., Kadotani, T., Kuwana, H., Shinkawa, A., Takahash, T., Iwahana, H., Sato, R. 1997. Aminopeptidase N from *Bombyx mori* as a candidate for the receptor of *Bacillus thuringiensis* CrylAa toxin. *Eur. J. Biochem*. 246, 652-657

