RESEARCH ARTICLE Agricultural Science Digest, Volume 44 Issue 6: 1140-1146 (December 2024)

In silico Analysis and Molecular Docking of Cry3Aa Toxin with Coleopteran Specific Midgut Receptor of ADAM10/APN Receptors

Arumugam Eniya[1], Venkatasamy Balasubramani[2,3], Marimuthu Murugan[1], Muthurajan Raveendran[2,4], Lakshmanan Pugalendhi[5], Ravikumar Caroline Nirmala[6], Rajasekaran Raghu[2], Gothandaraman Rajadurai[2]

10.18805/ag.D-5955

ABSTRACT

Background: *Bacillus thuringiensis* (*Bt*) is widely recognized as a safe and effective bioinsecticide, with Cry toxins targeting pests of various insect orders. The present study focuses on the modeling and validation of the Cry3Aa protein (deduced amino acid sequence of *cry3Aa* gene cloned from native *Bt* isolate, T121) and its interaction with midgut receptor proteins (ADAM10 and APN).

Methods: The three-dimensional structures of Cry3Aa protein and the midgut receptors ADAM10 and APN were predicted using the SWISS model server. Functional domain analysis of Cry3Aa revealed three distinct domains: N-terminal (Domain I), Central (Domain II) and C-terminal (Domain III), providing insights into their structural organization. The predicted models of Cry3Aa, ADAM10 and APN were validated using the Ramachandran plot which demonstrated structural integrity.

Result: Primary structure analysis of Cry3Aa revealed a 652 amino acid protein with a theoretical isoelectric point of 5.59, a molecular weight of 74 kDa and stable characteristics. Protein-protein docking analysis using ClusPro 2.0 showed that Cry3Aa exhibited higher level of interaction with the ADAM10 receptor than with the APN receptor. The Cry3Aa-ADAM10 docked complex demonstrated 23 hydrogen bonds, reasoning for its stability and binding affinity. These findings revealed that the Cry3Aa protein has a strong affinity against coleopteran specific midgut receptors and hence Cry3Aa has a potential to be an effective coleopteran specific insecticidal protein.

Key words: ADAM10, APN, *Bacillus thuringiensis,* Cry3Aa, Protein-protein docking.

INTRODUCTION

Bacillus thuringiensis is a Gram positive spore forming bacteria which produces parasporal crystalline inclusions such as Cry and Cyt toxins. Cry toxins are specifically toxic to insect order such as Lepidoptera, Coleoptera, Diptera and Hemiptera (De Maagd *et al*., 2001; Palma *et al*., 2014). *Bt* has been employed as a bioinsecticide for over 30 years and is considered to be safe for the environment, as being harmless to most non-target organisms, including humans and other mammals (Ibraham *et al*., 2010). Upon ingestion of Cry toxins by insects, these proteins effectively bind themselves to specific receptors in the insect midgut, resulting in paralysis of insect gut and consequently the demise of the insect (Bravo *et al*., 2007). The interaction of *Bt* toxins with the midgut of insects determines their efficacy as bioinsecticides. Various proteins have been reported to serve as receptors for the Coleopteran toxin protein family, including ADAM10 (A Disintegrin and Metalloproteinase domain-containing protein 10), Aminopeptidase N (APN), cadherin, ABC transporters and alkaline phosphatase (Velásquez *et al*., 2023).

ADAM10 and APN receptors are proteins found in the midgut of Coleopterans and identified as potential receptors for the Cry3Aa toxin. Ruiz Arroyo *et al.* (2017) substantiated the role of ADAM10 as a functional receptor for the Cry3Aa toxin in the Colorado potato beetle (*L. decemlineata*). Molecular docking analysis aims to understand the

[1]Department of Agricultural Entomology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[2]Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[3]Controller of Examinations, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[4]Directorate of Research, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[5]Department of Vegetable Science, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.
[6]Department of Plant Molecular Biology and Bioinformatics, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India.

Corresponding Author: Venkatasamy Balasubramani, Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641 003, Tamil Nadu, India. Email: balasubramani.v@gmail.com

How to cite this article: Eniya, A., Balasubramani, V., Murugan, M., Raveendran, M., Pugalendhi, L., Caroline Nirmala, R., Raghu, R. and Rajadurai, G. (2024). *In silico* Analysis and Molecular Docking of Cry3Aa Toxin with Coleopteran Specific Midgut Receptor of ADAM10/APN Receptors. Agricultural Science Digest. 44(6): 1140-1146. doi: 10.18805/ag.D-5955.

Submitted: 30-01-2024 **Accepted:** 18-03-2024 **Online:** 03-04-2024

potential interactions between the Cry proteins and receptor proteins at the molecular level, which is crucial for elucidating the mechanism of action of the toxin and its specificity for the target insects. Homology modeling stands out as a highly dependable approach for accurately determining the three-dimensional structure of a protein, exhibiting a precision level comparable to that of a lower-resolution and experimentally determined structure. Even flawed models can prove valuable, as certain functional aspects can be anticipated based on coarse structural characteristics (Marti-Renom, 2003). The significance of studying the interaction between Cry3Aa toxin (deduced amino acid sequence of *cry3Aa* gene cloned from native *Bt* isolate, T121) and ADAM10 and APN receptors lies in the potential implications of the findings for insect pest management and the development of more targeted and effective bioinsecticides. This study focuses on the comprehensive analysis of the Cry3Aa protein, a well-known entomotoxic protein and its interactions with the receptor proteins from the Colorado potato beetle (*L. decemlineata*), specifically the ADAM10 and APN.

MATERIALS AND METHODS

Retrieval of Cry3Aa protein sequence

The Cry3Aa protein, full length amino acid sequence (Accession No. OR921179) was retrieved from National Center for Biotechnology Information (NCBI). This sequence was used as a target for modeling. The functional domains of the Cry3Aa protein were identified and analyzed using the InterPro (Paysan-Lafosse *et al*., 2023). InterPro (https://www.ebi.ac.uk/interpro/) integrates diverse protein signature databases to predict domains, homologous superfamily and functional motifs within a protein sequence.

Homology modeling of Cry3Aa protein

The receptor protein sequences pertaining to Colorado potato beetle, *Leptinotarsa decemlineata*, *viz*., APN (Accession no: ADK11709.1, consisting of 917 amino acids) and ADAM10 (Accession no: AOT22059.1, comprising 904 amino acids) were retrieved from the National Center for Biotechnology Information (NCBI). The SWISS-MODEL server (https://swissmodel.expasy.org/), a widely used tool for automated comparative protein modeling, was employed to predict the Alpha fold modeling technique for this purpose (Waterhouse *et al*., 2018). This approach based on the assumption that proteins exhibiting similar sequences also possess similar structures, allowing the construction of a model based on the known structure of a homologous protein.

Model quality assessment

The quality of Cry3Aa protein model generated via Swiss modeling server was validated using SAVESv6.0- Structure validation server (https://saves.mbi.ucla.edu/). It employs various parameters to assess the quality of the model, including ERRAT to access overall model quality, reflecting accuracy (Colovos and Yeates, 1993), PROCHECK for stereo chemical quality assessment (Laskowski *et al*., 1993) and VERIFY 3D to evaluate the compatibility of the model with its own amino acid sequences (Bowie *et al*., 1991). This validation ensures a comprehensive evaluation of the accuracy and reliability of the predicted model providing a firm basis for subsequent functional and structural analyses.

Primary structure analysis and active site prediction using CASTp

The ProtParam tool (https://web.expasy.org/protparam/) was employed to assess the primary structure of the Cry3Aa protein model, determining a range of physical and chemical parameters including Extinction Coefficient, estimated half-life, Grand average of hydropathicity (GRAVY), instability index, molecular weight and theoretical isoelectric point (pI) (Gasteiger *et al*., 2005).

The CASTp 3.0 server (http://sts.bioe.uic.edu/castp/index.html?4jii) was employed to analyze the pockets on protein surfaces and the internal voids within proteins. This facilitates the assessment of the accessibility of binding sites to various ligands and substrates (Tian *et al*., 2018).

Protein-protein docking of cry3Aa protein with receptor molecules

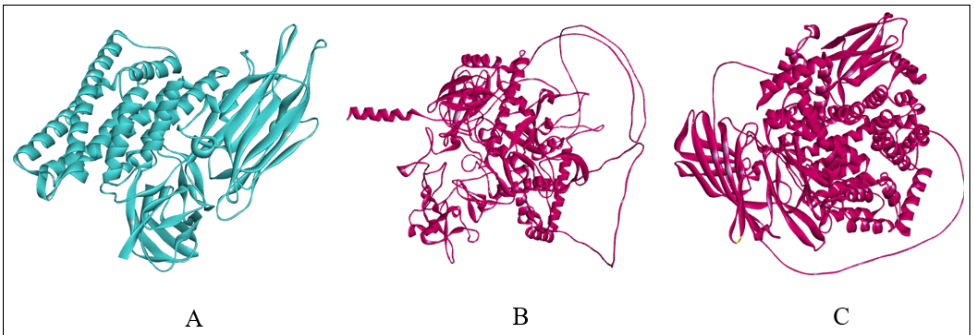
After validation of both the protein (Cry3Aa) and receptor (ADAM10, APN) model, protein- protein docking was proceeded using ClusPro 2.0 server (Kozakov *et al*., 2017). The resulting top 10 models from the ClusPro server were then retrieved in PDB format. Elucidation of the interactions between the Cry3Aa toxic protein and receptor proteins was carried out by employing Biovia Discovery Studio visualizer.

RESULTS AND DISCUSSION

Modeling and validation of Cry3Aa protein and midgut receptor protein (ADAM10 and APN)

The three dimensional structure for Cry3Aa protein and midgut receptor (ADAM10, APN) generated using the SWISS model server are given in Fig 1. The functional domain analysis was carried out using Interpro revealed three distinct domains in the Cry3Aa protein (Fig 2). The cytoplasmic domain covers amino acid residues from 1-70, N-terminal domain (Domain I) spans amino acid residues 91 to 295. The Central domain (Domain II) encompasses residues 303 to 507, while the C-terminal domain (Domain III) extends from residues 517 to 652.

The modeled Cry3Aa protein was subjected to active site prediction in CASTp server, which determined only the A chain in the protein. The Cry3Aa model quality was validated using Ramachandran plot generated with Swiss model server. Ramachandran plot revealed that 478 (92.1%) residues located in the favored region, with an additional 40 (7.7%) falling within the allowed region (Fig 3), confirming the structural integrity of model. The Ramachandran plot analysis of ADAM-10 and APN showed that 82.6% and 85.3% of its residues were in favorable regions indicating a high degree of structural stability and conformational quality (Fig 4).



(A- Cry3Aa, B- ADAM-10 (A Disintegrin and Metalloproteinase domain-containing protein 10), C- APN (Aminopeptidase- N).

Fig 1: Three dimensional protein model predicted by Swiss model server.

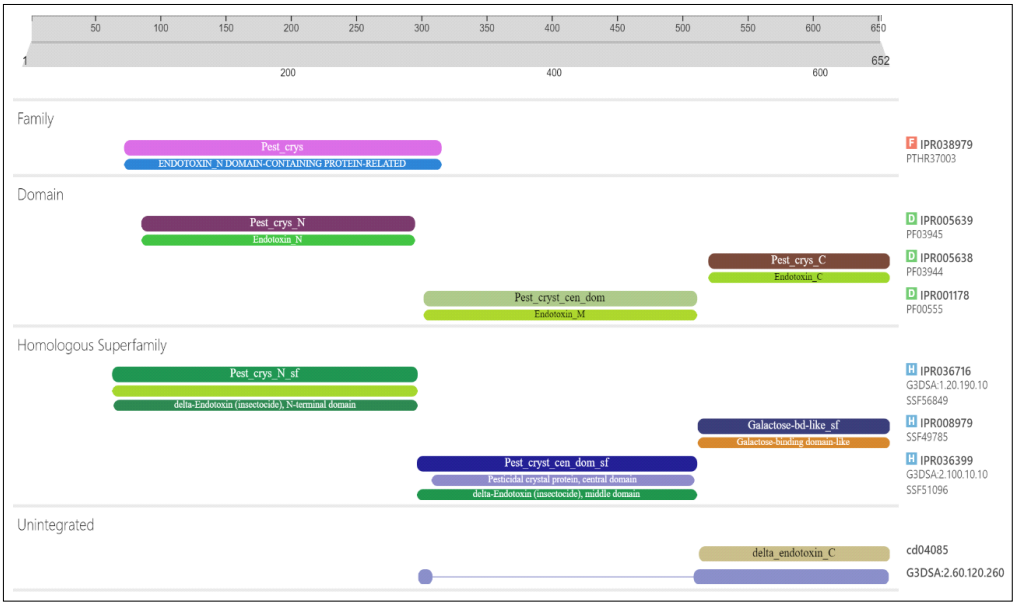


Fig 2: Functional domain analysis of Cry3Aa protein sequence through InterPro.

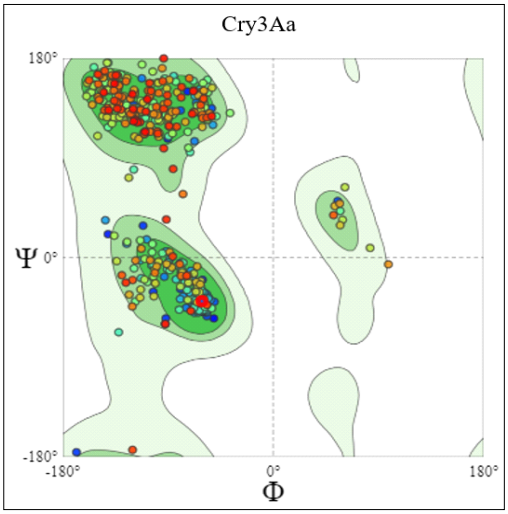


Fig 3: Ramachandran plot of Cry3Aa protein model (dihedral angle φ (phi) against ψ (psi).

Primary structure analysis of Cry3Aa protein

Analysis of primary structure of the Cry3Aa model was carried out by ProtParam. The protein model comprised of 652 amino acids with a theoretical isoelectric point (pI) of 5.59 and a molecular weight of 74 kDa. The extinction coefficient, measured to be 1.528 indicates the protein ability to absorb light at a specific wavelength, often used for protein quantification. The protein exhibited an instability index of 29.95, categorizing it as a stable. The aliphatic index, computed as 74.74 indicates the higher content of aliphatic amino acids and the GRAVY value was found to be -0.476. Furthermore, in mammalian reticulocytes, it was observed that the estimated half-life was found to be 30 h, exceeding 20 h in yeast and 10 h in *Escherichia coli*.

Protein-protein docking of Cry3Aa protein with ADAM-10 and APN receptors

The ClusPro 2.0 protein-protein docking was performed using different scoring coefficients, namely Balanced,

Electrostatic-favored, Hydrophobic-favored and VdW+Elec. The scoring coefficient used in this analysis is defined as $E = 0.40E_{rep} - 0.40E_{att} + 600E_{elec} + 1.00E_{DARS}$. This coefficient encompasses various energy components, such as repulsive energy (E_{rep}), attractive energy (E_{att}), electrostatic energy (E_{elec}) and desolvation energy (E_{DARS}). Clusters with lower energy values represent more favorable conformations of the protein-protein complexes (Table 1). The scores represent the quality of the protein-protein docking models, with lower scores indicating better conformational energies. The interactions between the Cry3Aa protein and the receptors (ADAM10 and APN) were

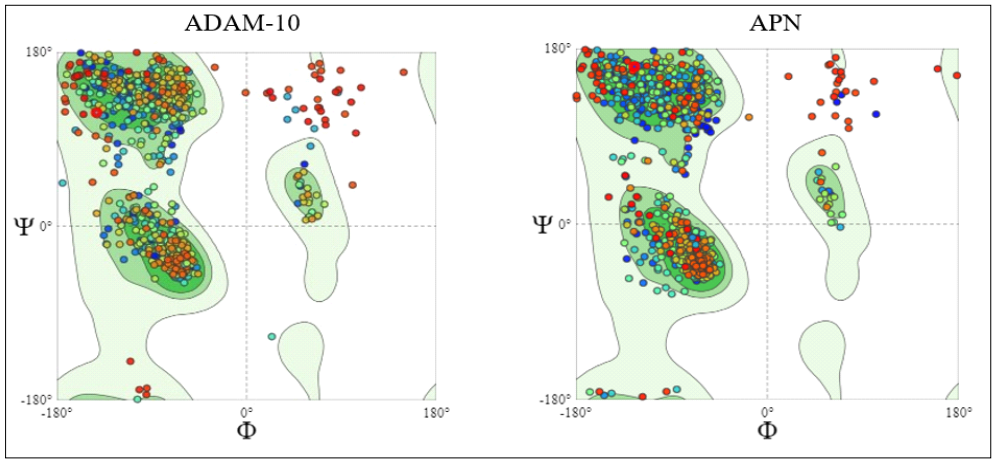


Fig 4: Ramachandran plot of ADAM-10 and APN protein models [dihedral angle φ (phi) against ψ (psi)].



Fig 5: Interaction of Cry3Aa protein with ADAM10 receptor visualized through Biovia Discovery studio, where yellow and green represent the pocket atoms of Cry3Aa and ADAM10 respectively.

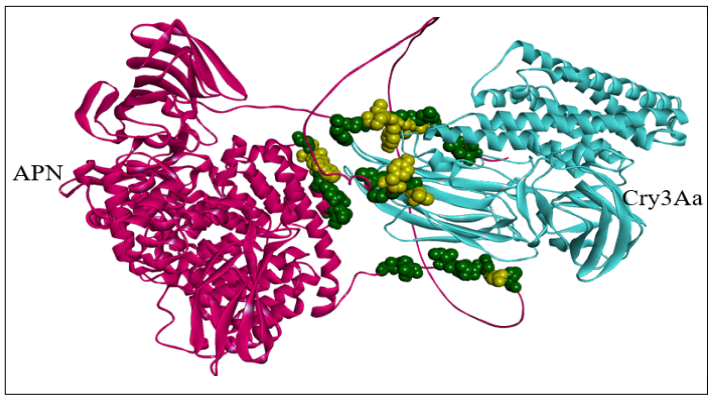


Fig 6: Interaction of Cry3Aa protein with APN receptor visualized through Biovia Discovery studio, where yellow and green represent the pocket atoms of Cry3Aa and APN respectively.

identified using Biovia discovery studio visualizer (Fig 5 and 6). The results of the docking studies were illustrated in Table 2 and Table 3 revealing the interaction between the receptor (ADAM10 and APN) and Cry3Aa protein.

The findings of present study indicated that the strong interactions of GLU441 of the Cry3Aa protein with LYS790 residues of the *L. decemlineata* ADAM10 receptor with a distance of 1.4+Å, emphasizing the strength of the interaction essential for the stability and functionality. These results align with previous studies, with ADAM10 as a functional receptor and Cry3Aa as toxin in *L. decemlineata* (Ochoa-Campuzano *et al*., in 2007, Ruiz-Arroyo *et al*., in 2017). The results provided relevant information about the functional significance of the Cry3Aa-ADAM10 interaction. APN receptors have been identified as functional receptors for Cry3Aa toxins in coleopteran insects like *Rhynchophorus ferrugineus* (Wang *et al*., 2023). But the specific increase in aminopeptidase activity in the resistance strain of *L. decemlineata* suggests that aminopeptidase-N may play a role in the adaptive mechanisms that confer resistance to *Bt* toxins, particularly Cry3Aa toxin. This finding highlights the potential significance of aminopeptidase-N in the context of insect resistance to *Bt* toxins and the survival of *L. decemlineata* on *Bt*-potato plants (Loseva *et al*., 2002). Guo *et al.* (2020) identified a 107 kDa aminopeptidase N (APN) as a binding protein for Cry3Aa toxin in the brush border membrane vesicles (BBMVs) of *Monochamus alternatus* larvae. Ahmad *et al*., (2015) reported that Vip3Aa- Cry1Ac fusion protein has a strong affinity against lepidopteran pests. Ser290, Ser293, Leu337, Thr340 and Arg437 residues of fusion protein are involved in the interaction with insect receptors.

In the current study, the Cluster score was found to be lowest in the Cry3Aa_ADAM10 (-1302.0) when compared with the Cry3Aa_APN (-1113.3), indicating that the Cry3Aa protein had better interaction of Cry3Aa with the ADAM10 compared to the APN receptor. The docked complex of Cry3Aa_ADAM exhibits a total of 23 hydrogen bonds, highlighting the significant molecular interactions contributing to the stability and binding affinity of this

Table 1: Cluster scores of ADAM10 and APN.

Receptors	Representative	Weighted score
Cry3Aa_ADAM10	Center	-1027.8
	Lowest energy	-1302.0
Cry3Aa_APN	Center	-750.0
	Lowest energy	-1113.3

Table 2: ADAM10 receptor interactions with Cry3Aa protein.

Interactions		Distance
Position of amino acids and hydrogen bonds in ADAM 10	Position of amino acids and hydrogen bonds in Cry3Aa	(Å)
LYS 790 (HZ1)	GLU 441 (OE2)	1.72
LYS 790 (HZ3)	GLU 441 (OE1)	1.72
ARG 821 (HH22)	ASN 626 (OD1)	1.79
TYR 825 (HH)	SER 566 (OG)	1.81
PRO 816 (O)	ARG 375 (HH11)	1.84
ARG 824 (HH21)	GLU 622 (OE1)	1.89
LEU 813 (O)	ASN 403 (HD21)	1.90
LEU 820 (O)	TYR 558 (HH)	1.94
ARG 821 (HE)	ASN 626 (OD1)	2.02
ARG 824 (HE)	VAL 563 (O)	2.02
TYR 825 (HN)	SER 564 (O)	2.06
GLU 184 (OE1)	TYR 359 (HH)	2.09
PRO 816 (O)	ARG 375 (HH21)	2.13
ARG 824 (HH21)	GLN 567 (O)	2.35
GLY 403 (HN)	TYR 358 (OH)	2.40
MET 777 (SD)	LYS 71 (HZ1)	2.45
ARG 824 (HH22)	TYR 565 (O)	2.66
ARG 824 (HE)	TYR 565 (O)	3.08
ARG 821 (HH11)	ASP 588 (OD1)	3.09
CYS 791 (SG)	ALA 442 (O)	3.24
GLU 173 (OE2)	SER 421 (CB)	3.29
THR 402 (CB)	TYR 358 (OH)	3.36
ARG 824 (CA)	SER 564 (O)	3.42

Table 3: APN receptor interactions with Cry3Aa protein.

Interactions		Distance
Position of amino acids and hydrogen bonds in APN	Position of amino acids and hydrogen bonds in Cry3Aa	(Å)
TYR 14 (HH)	SER 425 (OG)	1.88
GLN 857 (HE21)	TYR 358 (O)	1.94
TYR 994 (OH)	ASN 331 (HD22)	1.94
THR 15 (O)	ARG 323 (HH22)	1.95
THR 15 (O)	ARG 323 (HE)	1.96
ILE 10 (O)	ASN 320 (HD21)	2.01
SER 861 (O)	TYR 358 (HH)	2.21
TYR 14(HN)	ASN 321 (OD1)	2.43
ASP 914 (OD1)	ARG 375 (HH22)	2.46
ILE 998 (O)	GLY 390 (HN)	2.46
PHE 12 (O)	ASN 321 (HN)	2.51
ASP 914 (OD1)	ARG 375 (HH21)	2.55
TYR 14 (O)	ARG 323 (CA)	3.79

*(HE: Hydrogen atom attached to the first atom (specific amino acid residue), HE21- Hydrogen atom attached to the second atom, HN- Hydrogen atom attached to the nitrogen atom of an amino group, HH: Hydrogen atom attached to another hydrogen atom, HH11- Hydrogen atom attached to another hydrogen atom in position 11, HH21- Hydrogen atom attached to another hydrogen atom in position 21, HH22- Hydrogen atom attached to another hydrogen atom in position 22, HZ1- Hydrogen atom attached to the first atom, HZ3- Hydrogen atom attached to the third atom, O: Oxygen atom, OE1- Oxygen atom within the carboxylate group of a glutamic acid (glutamate) residue, OE2- Oxygen atom within the carboxylate group of an acidic amino acid residue, OD1- Oxygen atom within the carboxylate group of an acidic amino acid residue, OG: Oxygen atom attached to a hydroxyl group, OH- Hydroxyl group, HD22- Hydrogen atom attached to a heavy atom in position 22, HD21- Hydrogen atom attached to a heavy atom in position 21, CB- Beta-carbon atom in an amino acid residue, CA- Alpha-carbon atom in an amino acid residue).

complex. It plays a vital role in the effectiveness of Cry3Aa toxin. This finding expands the understanding of the molecular basis of toxin-receptor interactions and highlights the importance of specific protein-protein interactions in the context of insecticidal pore-forming toxins and their receptors.

CONCLUSION

In this study, three-dimensional structure of the Cry3Aa protein and its putative receptors- ADAM10 and APN was successfully modelled employing the SWISS-MODEL server. The structural integrity of the Cry3Aa model was validated through Ramachandran plot analysis. Primary structure analysis revealed key parameters, such as molecular weight, isoelectric point and estimated half-life. Protein-protein docking experiments using ClusPro 2.0 provided insights into the interactions between Cry3Aa and the receptors, emphasizing good interaction with ADAM10 over APN. The detailed analysis of hydrogen bond interactions highlighted the significance of specific residues in stabilizing the Cry3Aa-ADAM10 complex. These findings contribute to the understanding of the molecular basis of toxin-receptor interactions, a valuable insights necessary for the development of targeted and effective bio insecticides.

Conflict of interest

All authors declare that they have no conflicts of interest.

REFERENCES

Ahmad, A., Javed, M.R., Rao, A.Q., Khan, M.A., Ahad, A., Din, S.U., Shahid, A.A. and Husnain, T. (2015). *In silico* determination of insecticidal potential of Vip3Aa-Cry1Ac fusion protein against Lepidopteran targets using molecular docking. Frontiers in Plant Science. 6: 1081. https://doi.org/10.3389/fpls.2015.01081.

Bowie, J.U., Lüthy, R. and Eisenberg, D. (1991). A method to identify protein sequences that fold into a known three-dimensional structure. Science. 253(5016): 164-170.

Bravo, A., Gill, S.S. and Soberón, M. (2007). Mode of action of *Bacillus thuringiensis* cry and cyt toxins and their potential for insect control. Toxicon. 49(4): 423-435.

Colovos, C. and Yeates, T.O. (1993). Verification of protein structures: Patterns of nonbonded atomic interactions. Protein Science. 2(9): 1511-1519.

De Maagd, R.A., Bravo, A. and Crickmore, N. (2001). How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. Trends in Genetics. 17(4): 193-199.

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S. E., Wilkins, M.R., Appel, R.D. and Bairoch, A. (2005). Protein Identification and Analysis Tools on the ExPASy Server. Humana Press. pp 571-607.

Guo, Y., Carballar-Lejarazú, R., Sheng, L., Fang, Y., Wang, S., Liang, G., Hu, X., Wang, R., Zhang, F. and Wu, S. (2020). Identification and characterization of aminopeptidase-N as a binding protein for Cry3Aa in the midgut of *Monochamus alternatus* (Coleoptera: Cerambycidae). Journal of Economic Entomology. 113(5): 2259-2268.

Ibrahim, M. A., Griko, N., Junker, M. and Bulla, L.A. (2010). *Bacillus thuringiensis*: A genomics and proteomics perspective. Bioengineered Bugs. 1(1): 31-50.

Kozakov, D., Hall, D.R., Xia, B., Porter, K.A., Padhorny, D., Yueh, C., Beglov, D., Vajda, S. (2017). The ClusPro web server for protein-protein docking. Nature Protocols. 12(2): 255-278. Pdf.

Laskowski, R.A., MacArthur, M.W., Moss, D.S., Thornton, J.M. (1993). PROCHECK-a program to check the stereochemical quality of protein structures. Journal of Applied Crystallography. 26: 283-291.

Loseva, O., Ibrahim, M., Candas, M., Koller, C.N., Bauer, L.S. and Bulla, J. L.A. (2002). Changes in protease activity and Cry3Aa toxin binding in the Colorado potato beetle: Implications for insect resistance to *Bacillus thuringiensis* toxins. Insect Biochemistry and Molecular Biology. 32(5): 567-577.

Marti-Renom, M.A. (2003). Protein structure modelling for structural genomics. Business briefing. Future Drug Discovery. 59-63.

Ochoa-Campuzano, C., Real, M.D., Martínez-Ramírez, A.C., Bravo, A. and Rausell, C. (2007). An ADAM metalloprotease is a Cry3Aa *Bacillus thuringiensis* toxin receptor. Biochemical and Biophysical Research Communications. 362(2): 437-442.

Palma, L., Muñoz, D., Berry, C., Murillo, J. and Caballero, P. (2014). *Bacillus thuringiensis* toxins: An overview of their biocidal activity. Toxins. 6(12): 3296-3325.

Paysan-Lafosse, T., Blum, M., Chuguransky, S., Grego, T., Pinto, B.L., Salazar, G.A., Bileschi, M.L., Bork, P., Bridge, A., Colwell, L. and Gough, J. (2023). InterPro in 2022. Nucleic Acids Research. 51(D1): D418-D427.

Ruiz Arroyo, V.M., García Robles, I., Ochoa Campuzano, C., Goig, G.A., Zaitseva, E., Baaken, G., Martínez Ramírez, A.C., Rausell, C. and Real, M.D. (2017). Validation of ADAM10 metalloprotease as a *Bacillus thuringiensis* Cry3Aa toxin functional receptor in colorado potato beetle (*Leptinotarsa decemlineata*). Insect Molecular Biology. 26(2): 204-214.

Tian, W., Chen, C., Lei, X., Zhao, J. and Liang, J. (2018). CASTp 3.0: Computed atlas of surface topography of proteins. Nucleic Acids Research. 46(W1): W363-W367.

Velásquez, C.L.F., Cantón, P.E., Sanchez-Flores, A., Soberón, M., Bravo, A. and Cerón, S.J.A. (2023). Identification of cry toxin receptor genes homologs in a de novo transcriptome of *Premnotrypes vorax* (Coleoptera: Curculionidae). Plos one. 18(9): e0291546. doi: 10.1371/journal.pone.0291546.

Wang, S., Guo, Y., Sun, Y., Weng, M., Liao, Q., Qiu, R., Zou, S. and Wu, S. (2023). Identification of two *Bacillus thuringiensis* Cry3Aa toxin-binding aminopeptidase N from *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). Bulletin of Entomological Research. 113(5): 615-625.

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F.T., de Beer, T.A.P., Rempfer, C., Bordoli, L. and Lepore, R. (2018). Swiss-model: Homology modelling of protein structures and complexes. Nucleic Acids Research. 46(W1): W296-W303.