Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Plant derived antimicrobial peptide Ib-AMP1 as a potential alternative drug candidate for *Staphylococcus aureus* toxins

S. Ojha, S. Deep, S. Kundu*

School of Biochemical Engineering, Indian Institute of Technology (Banaras Hindu University), Varanasi - 221005, India

Correspondence to: subirbhu@gmail.com; skundu.bce@itbhu.ac.in

Received April 6, 2016; **Accepted** May 15, 2017; **Published** July 31, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.6.11

Abstract: Due to an increase in the occurrence of multi drug resistant microorganisms a need for the development of alternative drugs comes in light. This alternative drug should be such that the microorganisms should not be able to develop resistance against them easily. Antimicrobial peptides are the most potential candidates to be developed as alternative drug. In the present study the three toxins ETA, ETB and PVL of *Staphylococcus aureus* were docked with four antimicrobial peptides, Ib-AMP1, JCPep7, Snakin2, Sesquin, derived from plants. The docking studies predict that Ib-AMP1 shows significant interactions with all these three toxins. Hence, further studies can be carried out for developing Ib-AMP1 as an alternative drug against the toxins of *Staphylococcus aureus*.

Key words: *Staphylococcus aureus* toxins; Alternate drug; Antimicrobial peptide; Docking.

Introduction

The drug resistant microorganisms have been posing a great threat to the medical world since last few decades (1). People consume the doses of antibiotics carelessly, sometimes overuse or underuse of such antibiotics gives rise to drug resistant microorganisms (2). These drug resistant microorganisms adapt several mechanisms to resist the conventional antibiotics, like causing several adaptations in their binding site to prevent the antibiotics from binding or deactivation of the antibiotics itself (3). These drug resistant microorganisms make the treatment of diseases difficult. Hence a need for development of alternative drug comes in light. A lot of work is being done on antimicrobial peptides to develop them as alternative drug (4).

Antimicrobial peptides are a part of innate immune system of all the classes of organisms such as from prokaryotes to eukaryotes. These are small length peptides (5, 6, 7). Due to several incidences of drug resistant microorganisms coming in light, the antimicrobial peptides are being developed as alternative drug. The mechanism of action of the antimicrobial peptides is by interacting with the membranes of the microorganisms. The microorganisms will have to modify a large part of their membranes to become resistant towards these antimicrobial peptides as these antimicrobial peptides are larger. This will be very energy consuming process. Hence, the microorganisms are less likely to develop resistance against antimicrobial peptides (8).

In the present study four antimicrobial peptides of plant origin and three toxins of *staphylococcus aureus* were docked. The details of antimicrobial peptides used are shown in Table 1.

Several problems in humans like scalded skin syndrome, superficial infections, endocarditis etc are caused by human pathogen *staphylococcus aureus*. The two exfoliative enzymes (ETA and ETB) are responsible for scalded skin syndrome. The ETA is heat stable and located on chromosome. The ETA consists of 242 amino acids and has molecular mass of 26,950 Da. The ETB is heat labile and located on plasmid. The ETB consists of 246 amino acids and has molecular mass of 27,274 Da (13). PVL is an exotoxin responsible for necrotizing disease. These above three toxins of staphylococcus aureus were used for the present docking studies (14).

Materials and Methods

In the present study exfoliative toxin A (ETA), exfoliative toxin B (ETB) and Panton-Valentine leukocidin (PVL), the three toxins of *Staphylococcus aureus*, were

Table 1. This table shows the source, length and sequence of the plant derived antimicrobial peptides used for the present study.

S. No.	Name of antimicrobial peptide	Source of antimicrobial peptide	Length of antimicrobial peptide	Sequence of Antimicrobial peptide	Reference
1.	Ib-AMP1	Impatiens balsamina [Balsam]	20	QWGRRCCGWGPGRRYCVRWC	9
2.	JCpep7	Jatropha curcas	7	KVFLGLK	10
3.	Sesquin	Vigna unguiculata subsp. sesquipedalis [Cowpea]	10	KTCENLADTY	11
4.	Snakin-2	Solanum tuberosum [Potato]	15	SYKKIDCGGACAARC	12

Table 2. This table represents the global energy and number of hydrogen bonds formed by the docking interactions of the toxins ETA, ETB and PVL with antimicrobial peptides Ib-AMP1, JCPep7, Sesquin and Snakin-2.

S. No.	Antimicrobial peptide	Toxin	Global energy of the best docked complex (kcal/mol)	No. Of hydrogen bonds of the best docked complex
1.	Ib-AMP1	ETA	-23.16	2
		ETB	-17.18	4
		PVL	-22.15	3
2.	JCPep7	ETA	-21.22	1
		ETB	-18.28	2
		PVL	-7.80	0
3.	Sesquin	ETA	-22.58	1
		ETB	-13.88	2
		PVL	-11.04	2
4.	Snakin-2	ETA	-47.39	4
		ETB	-14.87	3
		PVL	-24.96	0

Table 3. This table shows the number of hydrogen bonds formed and the hydrogen donor and acceptor amino acid formed by the docking interactions of ETA, ETB and PVL with Ib-AMP1.

S.No.	Toxin	Antimicrobial peptide	No. Of Hydrogen bonds formed between Ib-AMP1 and toxin	H-Donor Amino acid	H-Acceptor Amino acid
1.	ETA	Ib-AMP1	2 conventional hydrogen bonds	His72	Gln1
				Gln1	Asp120
2.	ETB	Ib-AMP1	4 conventional hydrogen bonds	Arg218	Cys7
				Arg218	Cys6
				Cys6	Asn173
				Lys204	Lys9
3.	PVL	Ib-AMP1	1 salt bridge and 3 conventional hydrogen bonds	Gln1	Asp268
				Arg271	Gln1
				Lys155	Arg5
				Arg13	Gln104

docked with four antimicrobial peptides, i.e., Ib-AMP1, JCPep7, Sesquin and Snakin-2, of plant origin.

The PDB structures of ETA, ETB and PVL were downloaded from RCSB database (15,16). The PDB ID of ETA, ETB and PVL are 1DUA, 1DT2 and 1PVL respectively. The 3 dimensional structures of the antimicrobial peptides were modelled using PEPstr server (17).

The PDB structures of toxins were checked for the presence of water molecules and inhibitors. The water molecules and the inhibitors attached with the PDB structures were removed and hydrogens were added to these toxin structures. Then these structures were energy minimized and used for docking. The complete flexible docking was performed for the three toxins and the four antimicrobial peptides using FireDock server (22, 23). First the toxin and the antimicrobial peptide were docked using Patchdock server (18, 19, 20, 21). The solutions of this docking were further refined using Firedock server. Then the non bonding interactions were analyzed using Discovery Studio 4.1 Client viewer (24).

Results

All the various confirmations of all twelve sets of dockings performed were analyzed for the best docking confirmation with significant global energy in each docking. The hydrogen bonds and the global energy of the best confirmations of all the dockings were compared. The antimicrobial peptide giving the most significant result for all the toxins is predicted as the best one to be developed as drug against these three toxins of *Staphylococcus aureus*.

The dockings were performed first with Patchdock server. The toxins were given as the receptor input and the antimicrobial peptides were given as the ligand input. Clustering RMSD is kept at 4 Å for the docking. The solutions of these dockings were again given as input to the Firedock server for refinement. The Firedock server is specific for the protein-protein interactions. Further the refined results from the Firedock server were studies for the interactions. The Table 2 contains the most significant results of all the dockings performed. Then these results were compared for finding the most significant antimicrobial peptide against these toxins of *Staphylococcus aureus*.

When Ib-AMP1 is docked with the three toxins ETA, ETB and PVL, the best docked complexes have -23.16 kcal/mol with 2 hydrogen bonds, -17.18 kcal/mol with 4 hydrogen bonds and -22.15 kcal/mol with 3 hydrogen bonds respectively. The best docked complexes after docking JCPep7 with ETA, ETB and PVL have -21.22 kcal/mol with 1 hydrogen bond, -18.28 kcal/mol with 2 hydrogen bonds and -7.8 kcal with no hydrogen bond respectively. ETA, ETB and PVL after docking with Sesquin gave best docked complexes as -22.58 kcal/mol with 1 hydrogen bond, -13.88 kcal/mol with 2 hydrogen bonds and -11.04 kcal/mol with 2 hydrogen bonds. The best docked complexes after dockingSnakin-2 with ETA, ETB and PVL have -47.39 kcal/mol with 4 hydrogen bonds, -14.87 kcal/mol with 3 hydrogen bonds and -24.96 kcal/mol with no hydrogen bond.

Discussion

The best docking energy of docked complexes of ETA with all the four antimicrobial peptide was with Sankin-2. The docked complex has -47.39 kcal/mol with 4 hydrogen bonds formed between ETA and Snakin-2. The best docking energy of docked complexes of ETB with all four antimicrobial peptide was with JCPep7. The docked complex has -18.28 kcal/mol with 2 hydrogen bonds formed between ETB and JCPep7. The best docking energy of docked complexes of PVL with all the four antimicrobial peptide was with Sankin-2. The docked complex has -24.96 kcal/mol but no hydrogen bond formed between PVL and Snakin-2.

The present study was aimed at predicting an antimicrobial peptide which could be developed as drug against *Staphylococcus aureus* as it would give significant docking results with all its three toxins ETA, ETB and PVL. After the analysis of all the docking results of the toxins and antimicrobial peptides, it was predicted that Ib-AMP1 gives most significant docking results with all the three toxins ETA, ETB and PVL. The interactions are shown in Table: 3. The docked complex of Ib-AMP1 and ETA has -23.16 kcal/mol and two hydrogen bonds are formed between ETA and Ib-AMP1. The docked complex is shown in Figure: 1. The docked complexes of ETB and PVL with Ib-AMP1 have -17.18 kcal/mol with 4 hydrogen bonds between ETB and Ib-AMP1 and -22.15 kcal/mol with 3 hydrogen bonds and one salt bridge between PVL and Ib-AMP1 respectively. The docked complexes of ETB and Ib-AMP1 and PVL and Ib-AMP1 are shown in Figure: 2 and Figure: 3 respectively. Thus, Ib-AMP1 gives significant docking energies and also significant intermolecular interactions between toxins and antimicrobial peptides. Further work can be conducted to develop Ib-AMP1 as an alternative drug against the toxins of *Staphylococcus aureus*.

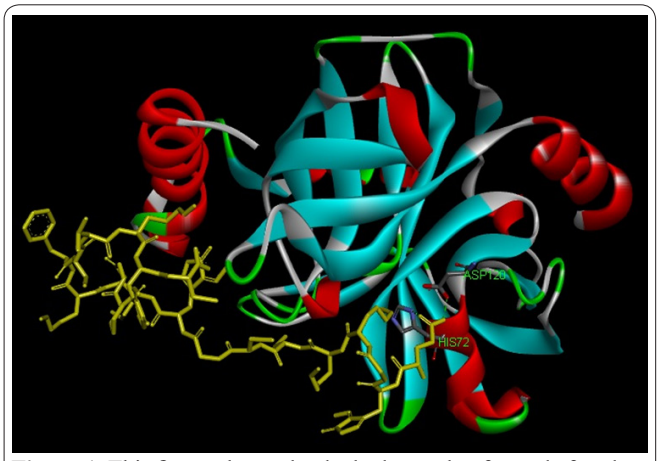

Figure 1. This figure shows the docked complex formed after docking ETA with Ib-AMP1 and the interacting residues of ETA.

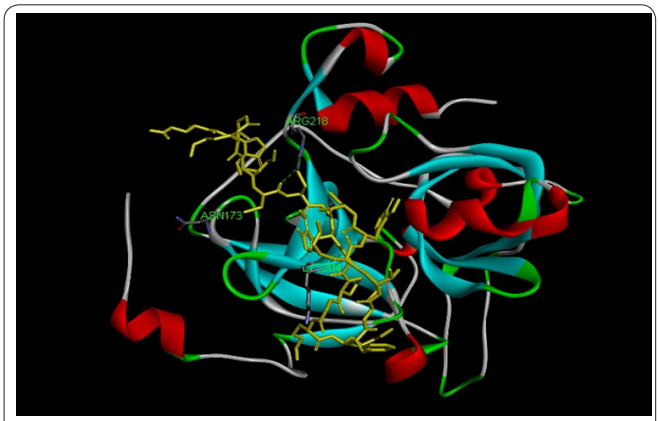

Figure2. This figure shows the docked complex formed after docking ETB with Ib-AMP1 and the interacting residues of ETB.

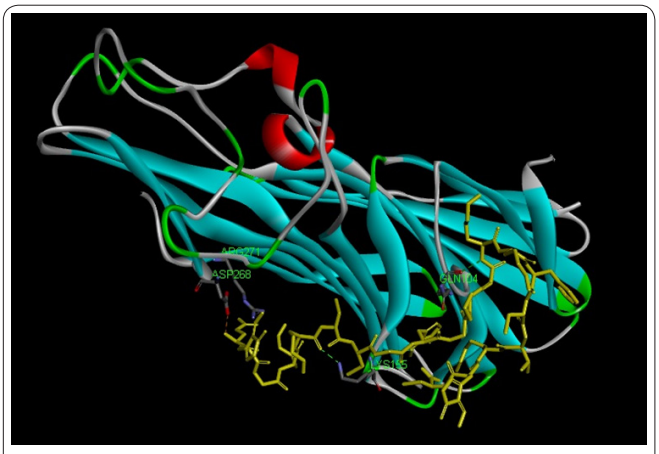

Figure 3. This figure shows the docked complex formed after docking PVL with Ib-AMP1 and the interacting residues of PVL.

References

1. Hancock REW and Patrzykat A. Clinical Development of Cationic Antimicrobial Peptides: From Natural to Novel Antibiotics. Current Drug Targets - Infectious Disorders 2002; 2: 79-83.
2. Davies J and Davies D. Origins and Evolution of Antibiotic Resistance. Microbiol. and Molecular Biol. Reviews 2010; 74 (3): 417-433.
3. Giedraitienė A, Vitkauskienė A, Naginienė R, Pavilonis A. Antibiotic Resistance Mechanisms of Clinically Important Bacteria. Medicina 2011; 47(3): 137-146.
4. Guilhelmelli F, Vilela N, Albuquerque P, Derengowski LS, Silva-Pereira1 I, Kyaw CM. Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance. Frontiers in Microbiology | Antimicrobials, Resistance and Chemotherapy 2013; 4:353: 1-12.
5. Sang Y and Blecha F. Antimicrobial peptides and bacteriocins: alternatives to traditional antibiotics. Animal Health Research Reviews 2008; 9(2): 227–235.
6. Butu M and Butu A. Antimicrobial peptides – natural antibiotics. Romanian Biotechnological Letters 2011; 16(3): 6135-6145.
7. Giuliani A, Pirri G, Nicoletto SF. Antimicrobial peptides: an overview of a promising class of therapeutics. Central European Journal of Biology 2007; 2(1): 1–33.
8. Baltzer SA and Brown MH. Antimicrobial Peptides – Promising Alternatives to Conventional Antibiotics. Journal of Molecular Microbiology and Biotechnology 2011; 20: 228–235.
9. Tailor RH, Acland DP, Attenborough S, Cammue BPA, Evans IJ, Osborn RW, Ray JA, Rees SB, Broekaert WF. A Novel Family of Small Cysteine-rich Antimicrobial Peptides from Seed of Impatiens balsamina Is Derived from a Single Pre-cursor Protein. The Journal of Biological Chemistry 1997; 272(39): 24480–24487.
10. Xiao J, Zhang H, Niu L, Wang X. Efficient Screening of a Novel Antimicrobial Peptide from Jatropha curcas by Cell Mem-brane Affinity Chromatography. Journal of Agricultural and Food Chemistry 2011; 59: 1145–1151.
11. Wong JH, and Ng TB. Sesquin, a potent defensin-like antimicrobial peptide from ground beans with inhibitory activities toward tumor cells and HIV-1 reverse transcriptase. Peptides 2005; 26:1120–1126.

12. Berrocal-Lobo M, Segura A, Moreno M, Lo´ pez G, Garcı´a-Olmedo F, Molina A. Snakin-2, an Antimicrobial Peptide from Potato Whose Gene Is Locally Induced by Wounding and Responds to Pathogen Infection. Plant Physiol. 2002; 128: 951-961.
13. Ladhani S. Understanding the mechanism of action of the exfoliative toxins of Staphylococcus aureus. FEMS Immunol. and Medical Microbiol. 2003; 39: 181-189.
14. Bien J, Sokolova O, Bozko P. Characterization of Virulence Factors of Staphylococcus aureus: Novel Function of Known Virulence Factors That Are Implicated in Activation of Airway Epithelial Proinflammatory Response. Journal of Pathogens 2011;2011: Article ID 601905: 1- 13.
15. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. Nucleic Acids Research 2000; 28(1): 235- 242.
16. Rose PW, Bi C, Bluhm WF, Christie CH, Dimitropoulos D, Dutta S, Green RK, Goodsell DS, Prlic A, Quesada M, Quinn GB, Ramos AB, Westbrook JD, Young J, Zardecki C, Berman HM, Bourne PE. The RCSB Protein Dta Bank: new resources for research and education. Nucleic Acids Research, 2013; 41: Database issue: D475-D482.
17. Kaur H, Garg A, Raghava GPS. PEPstr: A de novo method for tertiary structure prediction of small bioactive peptides. Protein Pept Lett. 2007; 14: 626-630.
18. Duhovny D, Nussinov R, Wolfson HJ. Efficient Unbound Docking of Rigid Molecules. In Gusfield et al., Ed. Proceedings of the 2'nd Workshop on Algorithms in Bioinformatics(WABI) Rome, Italy, Lecture Notes in Computer Science. Springer Verlag, 2002; 2452: 185- 200.
19. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. PatchDock and SymmDock: servers for rigid and symmetric doc-king. Nucl. Acids. Res. 2005; 33: W363- 367.
20. Schneidman-Duhovny D, Inbar Y, Polak V, Shatsky M, Halperin I, Benyamini H, Barzilai A, Dror O, Haspel N, Nussinov R, Wolfson HJ. Taking geometry to its edge: fast unbound rigid (and hinge-bent) docking. Proteins
2003; 52(1): 107-112.
21. Mashiach E, Schneidman-Duhovny D, Peri A, Shavit Y, Nussinov R, Wolfson HJ, An integrated suite of fast docking algorithms. Proteins 2010; 78(15):3197-3204.
22. Andrusier N, Nussinov R, Wolfson HJ. FireDock: Fast Interaction Refinement in Molecular Docking. Proteins 2007; 69(1):139-159.
23. Mashiach E, Schneidman-Duhovny D, Andrusier N, Nussi-nov R, Wolfson HJ. FireDock: a web server for fast interaction refinement in molecular docking.Nucleic Acids Res. 2008; 36(Web Server issue):W229-232.
24. Accelrys Software Inc., Discovery Studio Modeling Environment, Release x.x , San Diego: Accelrys Software Inc. 2007.