Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

CATION-PI ANALYSIS IN STAPHYLOCOCCUS AUREUS TOXINS – AN IN SILICO APPROACH

RAMADEVI MOHAN[1], RAO SETHUMADHAVAN[2] AND SUBHASHREE VENUGOPAL*

[1]Biomolecules and Genetics division, School of Bio-Sciences and Technology, VIT University, Vellore-632014, Tamilnadu, India.
[2]Bioinformatics division, School of Bio-Sciences and Technology, VIT University, Vellore-632014, Tamilnadu, India.

ABSTRACT

Structures of proteins are maintained by various non-covalent interactions, including Cation-pi interaction. In this study, we have investigated those interactions responsible for maintaining structures in toxins produced by *Staphylococcus aureus*. Toxins are potent activators of the immune system that produce a variety of diseases in humans by direct binding of major histocompatibility complex (MHC) class II molecules on Antigen presenting cells and active T cells bearing a particular T-cell receptor beta chain variable (Vβ) domain. Considering its importance in clinical aspects, we analyzed the interactions in 24 different toxins of the bacterium by *in silico* with respect to structure, stability involving secondary structures, conservation scores, solvent accessibility and stabilization centers. Out of 24 toxins investigated, we found cation-pi interactions in 22 proteins and there is a preference of Lys over Arg in cationic residues and Tyr among pi residues. The least energy value of -109.65 kcal/mol of Toxic Shock Syndrome Toxin (PDB code 2QIL) revealed its greater stability. The residues involved in forming cation-pi interactions are found buried inside the protein and highly conserved. The analysis inferred that the interaction is found common and important for maintaining the protein structure, stability to deliver the toxicity and disease mechanism in host.

KEYWORDS: Staphylococcus aureus, Toxins, Cation-pi interactions, Secondary structure, Conservation score, Stabilizing residues.

*Corresponding author

SUBHASHREE VENUGOPAL
Biomolecules and Genetics division, School of Bio-Sciences and Technology, VIT University, Vellore-632014, Tamilnadu, India

INTRODUCTION

Staphylococcus aureus is a Gram-positive spherical bacterium of approximately 1μ in diameter forming cells of grape-like clusters often found as a commensal associated with skin and mucous membranes [1]. The organism is responsible for hospital and community-acquired infections [2]. It is responsible for a large number of diseases including skin and soft tissue infections and deep seated infections such as endocarditis pneumonia and osteomyelitis by producing various virulence factors [3]. The intracellular pathogen is known to produce many extracellular toxins which contribute to the pathogenicity of the organism [4]. The toxins include exfoliative toxins, pyrogenic toxin superantigens (PTSAgs) including Staphylococcal enterotoxins (SEs) and Toxic shock syndrome toxin-1 (TSST-1), all of which produce detrimental effects on cells of the immune system [5, 6]. These toxins cause infections responsible for toxic shock syndrome, Staphylococcal scalded skin syndrome (SSSS) and food poisoning [4]. Superantigens cross-link MHC class II molecules on antigen presenting cells with T-cell receptors, leading to massive T-cell proliferation and cytokine release [7]. Enterotoxins (ET) are responsible for SSSS and bullous impetigo [8] and belong to the family of major serological types of heat-stable enterotoxins functioning both as potent gastrointestinal toxins as well as superantigens that stimulate non-specific T-cell proliferation [9]. Thus the primary function of the superantigenic toxins encoded by mobile genetic elements is to weaken the host immune system to allow the infectious pathogen to propagate and cause disease [10, 11]. Protein structures are stabilized by various non-covalent interactions such as hydrophobic, hydrogen bonds, Vander Waals and electrostatic interactions all of which are important in modern chemistry and structure stability. Cation-π interactions play an important role in biological structure and function in maintaining the stability of proteins. This type of interaction is formed between the Cation residues (Lysine (K) or Arginine (R)) and Pi residues (Phenylalanine (F) or Tyrosine (Y) or Tryptophan (W)) [12]. Many researchers had made studies on the analysis of cation-pi interactions in various groups of proteins like membrane proteins [13, 14], DNA binding proteins [15], Protein-DNA complexes [16], Sugar binding proteins [17], Antimicrobial peptides [18], therapeutic proteins [19], Laccase enzymes [20], prokaryotic and eukaryotic translation elongation factors [21] and immunoglobulin proteins [22]. Here we present the *in silico* structure, stability study on *Staphylococcus aureus* toxins which would be useful in understanding the forces responsible for maintaining the protein structure that helps the protein to exist mediating the toxin to involve in disease function by the bacterium.

MATERIALS AND METHODS

(i) Toxins Dataset

The study included 24 different types of toxins of *Staphylococcus aureus* bacterium. We used the toxins for the analysis whose protein structures were resolved in more than 2Aº. The Cartesian-coordinate files of those proteins were downloaded from Protein Data Bank (PDB) database (http://www.rcsb.org/). The details of the toxins are tabulated in Table 1. The heteroatoms and the residues involved in forming complexes were removed from the proteins and their structures were refined by $KoBa^{MIN}$ server (http://csb.stanford.edu/kobamin/). The server uses energy minimization using knowledge-based potential of mean force and stereochemistry correction for the proteins by bringing them closer to the native-like conformation. It uses CαRMSD, GDT-HA and GDT-TS calculations for refinement and Biopython to check structural integrity [23].

(ii) Delineation of cation-pi interactions

The cation-pi interactions in each protein are analyzed by the CAPTURE program (http://capture.caltech.edu/) developed by Gallivan and Dougherty [24] in which the interactions are based on two geometric approach (i) cation-pi pairs (K or R to F, Y or W residues) within 10A˚ of each other and not

having a gap to insert a water molecules and (ii) the electrostatic energy is less than -2 kcal/mol or both electrostatic energy and Vander Waals energies are less than -1 kcal/mol. The percentage composition of a specific amino acid residue contributing to cation-pi interactions is obtained by the equation $Comp_{cat-\pi}(i) = n_{cat-\pi}(i) \times [100/n(i)]$, where 'i' denotes the five residues (K, R, F, Y, W), $n_{cat-\pi}$ is the number of residues involved in forming cation-pi interactions and n(i) stands for the number of residues of type 'i' in the protein.

(iii)Energetic contribution due to Cation-Pi interactions

The energetic contribution of cation-pi interactions was calculated for each toxin and for all possible pairs of positively charged-aromatic aminoacids. The total cation-pi interaction energy ($E_{cat-\pi}$) is the sum of electrostatic (E_{es}) and Vander Waals energy (E_{vw}) which were obtained by the program CAPTURE that has implemented a subset of OPLS force field [25] which is known as Optimized Potentials for Liquid Simulations. The force field is used to describe the interaction between two atoms and it has both i) United Atom (OPLS-UA) - with only polar hydrogens and ii) (OPLS-AA) - with all atoms including non-polar hydrogens to calculate the energies. The electrostatic energy (E_{es}) is calculated using the equation

$E_{el}=\sum q_i q_j e^2/r_{ij}$

where q_i and q_j are the charges for the atoms i and j, respectively and r_{ij} is the distance between them. The Van der Waals energy is given by

$E_{vw} = 4\varepsilon_{ij}[(\sigma_{ij}^{12}/r_{ij}^{12})-(\sigma_{ij}^{6}/r_{ij}^{6})]$

where $\sigma_{ij} = (\sigma_{ii}\sigma_{ij})^{1/2}$ and $\varepsilon_{ij} = (\varepsilon_{ii}e_{jj})^{1/2}$; σ and ε are the Van der Waals radius and well depth, respectively.

(iv) Conservation of amino acid residues

Consurf server which is available at http://consurf.tau.ac.il/ was used for identifying the conservation score of cation-Pi interacting amino acid residues for each toxin in the dataset. The server compares the sequence of a PDB chain with the proteins deposited in Swiss-Prot and calculates the homologues for the PDB sequence. The number of PSIBLAST iterations and the cutoff e-value used were 1 and 0.001 respectively. The resulting sequences were used for multiple alignments. Using the alignments, the residues are classified into nine categories from highly variable to highly conserved based on these protein sequence alignments. Residues with a score of 1 are considered highly variable and residues with a score of 9 are considered highly conserved [26].

(v) Location of cation-pi residues based on secondary structure and solvent accessibility

Investigating the structural significance of secondary structure is important for understanding the structure and functions of proteins. So we analyzed each of the cation-pi residues by locating their presence in different secondary structures and solvent accessibility for toxins of the *S. aureus*. Solvent accessibility is the ratio between the solvent accessible surface area of a residue in three-dimensional structure of a protein and in an extended tripeptide conformation. We calculated the secondary structure of the residues involved in cation–π pairs from the information available in the Protein Data Bank [27] and solvent accessibility of different cation–π residues by program GETAREA available at http://curie.utmb.edu/getarea.html. The GETAREA program is based on an analytical calculation of the accessible surface areas and their gradients with respect to atomic coordinates provided in a PDB file of the protein. The program finds solvent exposed vertices of intersecting atoms, and thereby avoids calculating buried vertices which are not needed to determine the accessible surface area by the Gauss–Bonnet theorem and hence is more accurate in finding the solvent accessibility [28].

(vi) Prediction of stabilization centers

Stabilization centers (SCs) are important for protein stability involved in contacts composed of certain clusters of residues. They are expected to stabilize protein structures by preventing decay of folded structure [29]. Two

residues are considered to be Stabilization centers, if atleast one atom-atom distance between the two residues show shorter than the sum of Van der Waals radii plus 1 A°, or if they are separated by atleast 10 residues in the structure, or if it is possible to select one-one residues from both flanking tetrapeptides of the two residues making atleast seven contacts between these two triplets [30]. SCide program which is available at http://www.enzim.hu/scide was used to find the stabilization centers in the proteins maintaining the stability in structure.

RESULTS

(i) Preference of cationic and aromatic residues involved in cation-pi interaction in S.aureus toxins

The preference of aminoacid residues that are formed in cation-pi interactions in the toxins used in the study is analyzed and the results are given in the Table 2. We found that in these toxins, Tyr has the highest occurrence among the aromatic pi residues involved in cation-pi interactions. Moreover, less than 50% of Phe and Trp residues are involved in these cation-pi interactions as compared to Tyr. Lysine is higher than Arginine amongst the cationic residues in the set of toxins studied. This trend is similar to those seen in transmembrane proteins [13], DNA [15] and RNA-binding proteins [31]. The number of cation-pi interaction in *S. aureus* toxins in the studied data ranges from 1 to 15 except two proteins namely 1XXG and 1QTF in which there are no cation-pi interactions. The number of cation-pi interactions found in each of the toxin is shown in Table 2. There are six cation-π interacting pairs seen normally in cation-pi interactions, in which 2 of the cationic residues (Lys and Arg) form pairs with pi residues (Phe, Tyr, Trp). They are Lys-Phe, Lys-Tyr, Lys-Trp, Arg-Phe, Arg-Tyr and Arg-Trp. The Pymol view of Arg-Tyr and Lys-Phe interacting pairs for the protein 1D5X_C is shown in the Fig. 1. The Arg-Phe interacting pair in 1EXF toxin visualized in Pymol is shown in the Fig. 2. In our investigation, among the cation-pi interactions involving Arg residues, the number of Arg-Tyr interacting pairs of aminoacids were found to be greater with maximum number of 44 pairs and the least was Arg-Trp with 1 interacting pair. Thus, Arg-Tyr interacting pairs may be quite important for maintaining the stability in these toxins. Among Lys residues involved in cation-pi interactions, Lys-Trp residue was greater than the Lys-Phe and Lys-Tyr interacting pairs. Overall, there are about 143 cation-π interacting pairs present in 24 proteins.

(ii) Energetic contribution of cation-π interacting pairs

The cation-pi interaction energy differs significantly in the studied toxins which ranged from -8 to -110 kcal/mol and the results are given in Table 3. The results revealed that among the 24 toxins studied, the protein TSST (PDB code: 2QIL) possessed the highest stability which had an energy value of -109.65 kcal/mol. The next stable toxin was 1TS2 (a mutant of the wild TSST toxin mutated at T128A) which possessed energy value of -101.23 kcal/mol. Of the 22 proteins possessing cation-pi interactions analyzed, 50% of the proteins showed caton-pi energy value less than -20 kcal/mol and the other 50% of the proteins showed energy value greater than -20 kcal/mol.

(iii) Secondary structure of cation-pi interaction forming residues

We have computed the preference of cation-pi interaction forming residues in different secondary structures and the results are shown in Table 4. It was found that among cationic residues, Arg found to be in helix regions, whereas Lys residues preferred to be in β-strands. In the pi residues, Phe preferred to be in strands and Tyr and Trp preferred to be in helix.

(iv) Solvent accessibility of the cation-pi interacting residues in therapeutic proteins

We used GETAREA program to estimate the solvent accessibility of the residues involved in cation-pi interactions. The results are shown in the Table 4 which revealed that 37.76% and 70.62% of cation and pi forming residues involved in cation-pi interactions were found to

be buried inside the protein and 25.17% and 10.48% of cation and pi residues exposed to the surface.

(v) Conservation score analysis

We used Consurf server to compute the conservation score of aminoacid residues involved in cation-pi interactions in toxins and the results are shown in Table 4. The analysis revealed that 31.46 % and 45.45% of aminoacid residues are ≥5 score and 69.23% and 55.24% aminoacids of cation and pi forming residues are ≤5 which meant they are highly conserved.

(vi) Stabilization centers analysis

We computed the stabilization centers for the residues involved in cation-pi interactions in *S. aureus* toxins using SCide program and the results are shown in Table 5. Atleast one stabilization center was found in the toxins involving cation-pi interactions except 1F77. The percentage occurrence of stabilization center residues involved in the cation-pi forming residues was 30.76% and 27.97% for cation and pi residues respectively.

Table 1
Toxins involved in the study

PDB	Toxin	Resolution (A°)
1SXT	Staphylococcal enterotoxin A	2.7
1D5X	Staphylococcal enterotoxin B	2.45
1I4X	Staphylococcal enterotoxin C2	2.4
1JWM	Staphylococcal enterotoxin C3	2.7
1XXG	Enterotoxin G	2.2
1F77	Enterotoxin H	2.4
1EXF	Exfoliative toxin A	2.1
1QTF	Exfoliative toxin B	2.4
2QIL	TSST-1	2.07
2QK7	γ-hemolysin	2.4
2LKF	Leukocidin F	2.5
1I4H	Staphylococcal enterotoxin A	2.9
1L05	Enterotoxin A D227A mutant	3.2
1JWS	Enterotoxin C3 variant 3B1	2.6
1JWU	Enterotoxin C3 variant 3B2	2.3
1KLG	Enterotoxin C3 variant 3B2	2.4
2IPK	Enterotoxin C3 variant 3B2	2.3
1PYW	Enterotoxin C3 variant 3B2	2.1
1DUE	Exfoliative toxin A S195A mutant	2
1QIL	TSST-1 mutant	2.5
1TS2	TSST-1 mutant T128A	2.3
1TS3	TSST-1 mutant H135A	2
1TS4	TSST-1 mutant Q139K	3.4
1TS5	TSST-1 mutant I140T	3.1

Table 2
Cation-pi interaction analysis in toxins of Staphylococcus aureus

PDB	K-F	K-Y	K-W	R-F	R-Y	R-W
1SXT_A	K41-F57	K37-Y88	K103-W63		R160-Y30, R161-Y31, R181-Y231	
1SXT_ B		K35-Y88			R160-Y30, R181-Y231, R211-Y108	
1D5X_C	K221-F196				R165-Y28	
1I4X	K170-F166, K221-F196	K56-Y32, K65-Y94, K108-Y94			R164-Y28	
1JWM_D		K57-Y32, K162-Y85			R164-Y28	
1XXG						
1F77_A					R143-Y21	
1F77_B					R143-Y21	
1EXF				R67-F34		
1QTF						
2QIL_A	K70-F83	K178-Y51	K67-W12, K171-W12		R134-Y13	
2QIL_ B	K70-F83	K178-Y51	K67-W12, K171-W12		R134-Y13	
2QIL_ C	K70-F83	K178-Y51	K67-W12, K171-W12		R134-Y13	
2QK7_A		K70-Y190			R71-Y237, R188-Y60	
2QK7_B	K270-F269			R272-F30		
2LKF				R272-F30	R197-Y188	R197-W176
1I4H_A					R160-Y30, R161-Y31, R181-Y231	
1I4H_ B			K103-W63		R214-Y32, R160-Y30, R161-Y31	

1L05_D		K166-Y167			R160-Y30, R161-Y31, R181-Y231
1JWS_D	K108-F95	K57-Y32, K162-Y85			R164-Y28
1JWU_D		K57-Y32, K162-Y85			R164-Y28
1KLG_D	K108-F95	K57-Y32, K162-Y85			R164-Y28
2IPK_D	K108-F95	K76-Y77, K162-Y85			R164-Y28
1PYW_D	K65-F44, K170-F166	K57-Y32			R164-Y28
1DUE		K13-Y187		R87-F50	
1QIL_A	K70-F83	K178-Y51	K67-W12, K171-W12		R134-Y13
1QIL_B	K70-F83	K178-Y51	K67-W12, K171-W12		R134-Y13
1QIL_C	K70-F83	K178-Y51	K67-W12, K171-W12		R134-Y13
1TS2_A	K70-F83	K178-Y51	K67-W12, K171-W12		R134-Y13
1TS2_B	K270-F283	K378-Y251	K267-W212, K371-W212		R334-Y213
1TS2_C	K470-F483	K578-Y451	K467-W412, K571-W412		R534-Y413
1TS3_A	K70-F83		K67-W12, K171-W12		R134-Y13
1TS3_B	K270-F283	K378-Y251	K267-W212, K371-W212		R334-Y213
1TS3_C	K470-F483		K467-W412, K571-W412		R534-Y413
1TS4_A	K70-F83	K139-Y115	K67-W12, K171-W12		R134-Y13
1TS4_B	K270-F283	K378-Y251	K267-W212, K371-W212		R334-Y213
1TS5_A	K70-F83	K178-Y51	K67-W12, K171-W12		R134-Y13
1TS5_B	K267-F331, K270-F283	K378-Y251	K267-W212, K371-W212		R334-Y213

Table 3
Energetic contribution of cation-pi interactions in toxins of Staphylococcus aureus

PDB	E (cat-π) kcal/mol
1SXT_A	-45.96
1D5X_C	-10.12
1I4X	-22.61
1JWM	-15.84
1XXG	-
1F77_A	-13.28
1EXF	-16.72
1QTF	-
2QIL	-109.65
2QK7	-20.5
2LKF	-29.13
1I4H_A	-40.89
1L05_D	-10.43
1JWS_D	-18.96
1JWU_D	-16.23
1KLG_D	-19.19
2IPK	-17.35
1PYW_D	-12.46
1DUE	-8.36
1QIL	-90.76
1TS2	-101.23
1TS3	-83.3
1TS4	-42.53
1TS5	-69.77

Table 4
Cation-π residue location, secondary structure, conservation score and solvent accessibility in S. aureus toxins

PDB	Cation				π			
	Residue	Str	C	ASA	Residue	Str	C	ASA
1SXT	K41	E	0	I	F57	S	6	I
	K37	E	1	Nil	Y88	E	4	I
	K103	H	5	O	W63	S	1	O
	R160	H	9	I	Y30	H	8	I
	R161	H	1	Nil	Y31	T	1	Nil
	R181	E	8	I	Y231	E	0	Nil
	K35	E	3	Nil	Y88	E	4	I
	R160	H	9	I	Y30	H	8	I
	R181	E	8	Nil	Y231	E	1	Nil
	R211	G	5	Nil	Y108	E	9	I
1D5X	K221	NIL	7	Nil	F196	E	7	I
	R165	H	9	I	Y28	H	9	I
1I4X	K170	H	1	O	F166	H	1	Nil
	K56	Nil	1	Nil	Y32	NIL	1	Nil
	K65	Nil	8	I	Y94	NIL	6	I
	K108	E	5	Nil	Y94	NIL	6	I
	R164	E	9	I	Y28	H	9	I
	K221	Nil	7	Nil	F196	E	8	I
1JWM	K57	T	6	Nil	Y32	NIL	1	Nil
	K162	H	7	Nil	Y85	E	6	I

	R164	H	9	Nil	Y28	T	8	I
1F77	R143	H	9	I	Y21	H	8	Nil
	R143	H	9	I	Y21	H	8	Nil
1EXF	R67	E	8	O	F34	E	1	Nil
2QIL	K70	E	4	O	F83	E	4	I
	K178	NIL	8	O	Y51	S	2	O
	K67	E	6	I	W12	H	8	I
	K171	NIL	6	Nil	W12	H	8	I
	R134	H	9	I	Y13	H	9	I
	K70	E	4	O	F83	E	4	I
	K178	NIL	8	Nil	Y51	T	2	O
	K67	E	6	I	W12	H	8	I
	K171	NIL	6	Nil	W12	H	8	I
	R134	H	9	I	Y13	H	9	I
	K70	E	4	O	F83	E	4	I
	K178	NIL	8	Nil	Y51	T	2	O
	K67	E	6	I	W12	H	8	I
	K171	NIL	6	O	W12	H	8	I
	R134	H	9	I	Y13	H	9	I
2QK7	K70	E	3	I	Y190	G	7	I
	R71	E	1	Nil	Y237	E	2	Nil
	R188	G	1	Nil	Y60	E	5	I
	K270	E	1	Nil	F269	E	2	Nil
	R272	E	4	I	F30	T	3	I
2LKF	R272	E	1	I	F30	T	1	I
	R197	S	9	O	Y188	T	1	I
	R197	S	9	O	W176	S	3	O
1I4H	R160	H	9	I	Y30	H	8	I
	R161	H	2	Nil	Y31	T	1	Nil
	R181	E	7	I	Y231	E	3	Nil
	K103	NIL	4	O	W63	S	1	Nil
	R214	G	3	Nil	Y32	T	1	I
	R160	H	9	I	Y30	H	8	I
	R161	H	2	Nil	Y31	H	1	I
1L05	K166	H	1	O	Y167	H	6	I
	R160	H	9	I	Y30	H	8	I
	R161	H	1	O	Y31	T	1	Nil
	R181	E	8	I	Y231	E	4	I
1JWS	K108	E	5	Nil	F95	NIL	4	I
	K57	T	6	Nil	Y32	NIL	1	Nil
	K162	H	7	Nil	Y85	E	6	I
	R164	H	9	I	Y28	T	9	I
1JWU	K57	T	6	Nil	Y32	NIL	1	Nil
	K162	H	7	Nil	Y85	E	6	I
	R164	H	9	Nil	Y28	H	8	I
1KLG	K108	E	5	Nil	F95	NIL	4	I
	K57	T	6	Nil	Y32	Nil	1	Nil
	K162	H	7	Nil	Y85	E	6	I
	R164	H	9	I	Y28	H	8	I
2IPK	K108	E	5	Nil	F95	NIL	4	I
	K76	H	1	O	Y77	H	1	I
	K162	H	7	Nil	Y85	E	6	I
	R164	H	9	I	Y28	S	8	I
1PYW	K65	E	8	I	F44	S	5	Nil
	K170	H	9	O	F166	H	6	Nil
	K57	T	6	Nil	Y32	NIL	1	I
	R164	H	9	I	Y28	H	9	I
1DUE	K13	H	1	Nil	Y187	E	9	I
	R87	E	2	I	F50	E	1	Nil
1QIL	K70	E	4	O	F83	E	4	O
	K178	NIL	8	Nil	Y51	S	1	O
	K67	E	6	I	W12	H	7	I
	K171	NIL	6	Nil	W12	H	7	I
	R134	H	9	I	Y13	H	9	I
	K70	E	4	O	F83	E	4	O
	K178	NIL	8	Nil	Y51	S	1	O
	K67	E	6	I	W12	H	7	I
	K171	NIL	6	Nil	W12	H	7	I
	R134	H	9	I	Y13	H	9	I
	K70	E	4	O	F83	E	4	O
	K178	NIL	8	Nil	Y51	S	1	O
	K67	E	6	I	W12	H	7	I
	K171	NIL	6	Nil	W12	H	7	I
	R134	H	9	I	Y13	H	9	I
1TS2	K70	E	3	O	F83	E	4	I

	K178	NIL	8	O	Y51	T	2	O
	K67	E	6	I	W12	H	7	I
	K171	NIL	6	Nil	W12	H	7	I
	R134	H	9	I	Y13	H	9	I
	K270	E	3	O	F283	E	4	I
	K378	NIL	8	O	Y251	S	2	O
	K267	E	6	I	W212	H	7	I
	K371	NIL	6	Nil	W212	H	7	I
	R334	H	9	I	Y213	H	9	I
	K470	E	3	O	F483	E	4	I
	K578	NIL	8	O	Y451	T	2	O
	K467	E	6	I	W412	H	7	I
	K571	NIL	6	Nil	W412	H	7	I
	R534	H	9	I	Y413	H	9	I
1TS3	K70	E	4	O	F83	E	4	I
	K67	NIL	6	I	W12	H	7	I
	K171	NIL	6	Nil	W12	H	7	I
	R134	H	9	I	Y13	H	9	I
	K270	S	4	O	F283	E	4	I
	K378	NIL	8	Nil	Y251	S	1	O
	K267	E	6	I	W212	H	7	I
	K371	NIL	6	Nil	W212	H	7	I
	R334	H	9	I	Y213	H	9	I
	K470	E	4	O	F483	E	4	I
	K467	E	6	I	W412	H	7	I
	K571	NIL	6	Nil	W412	H	7	I
	R534	H	9	I	Y413	H	9	I
1TS4	K70	E	3	O	F83	E	4	Nil
	K139	T	3	O	Y115	S	2	Nil
	K67	E	6	I	W12	H	7	I
	K171	NIL	6	O	W12	H	7	I
	R134	H	9	O	Y13	H	9	I
	K270	S	3	O	F283	E	4	Nil
	K378	NIL	8	Nil	Y251	S	1	I
	K267	E	6	I	W212	H	7	Nil
	K371	NIL	6	Nil	W212	H	7	Nil
	R334	H	9	I	Y213	H	9	I
1TS5	K70	E	3	O	F83	E	4	I
	K178	NIL	8	Nil	Y51	S	2	I
	K67	E	6	I	W12	H	7	I
	K171	NIL	6	O	W12	H	7	I
	R134	H	9	I	Y13	H	9	I
	K267	E	6	Nil	F331	H	6	Nil
	K270	E	3	O	F283	E	4	I
	K378	NIL	8	Nil	Y251	S	2	I
	K267	E	6	I	W212	H	7	I
	K371	NIL	6	O	W212	H	7	I
	R334	H	9	I	Y213	H	9	I

Note: Str Secondary structure, E β-strand, T Turn, Nil Not assigned, G 3/10 helix, S Bend, H α-helix; ASA accessible surface area or solvent accessibility, I buried (<20), M (20-50), E exposed (>50); Cons conservation score (1=highly variable, 9= highly conserved).

Table 5
Prediction of stabilization centers in cation-pi forming residues

PDB	Stabilization centers
1SXT	K41, F57, K35, R181, Y231, R181, Y231
1D5X	Y28
1I4X	K65, Y94, K108, Y94, Y32
1JWM	Y32, Y85
1EXF	R67
2QIL	K178, Y51, K67, K178, Y51, K67, K178, Y51, K67
2QK7	F269
2LKF	R197
1I4H	R181, Y231, K103
1L05	R181, Y231

1JWS	Y32
1JWU	Y32, Y85
1KLG	K108, F95, Y32, Y85
2IPK	K108, F95
1PYW	Y32
1DUE	Y187, F50
1QIL	K178, K67, Y51, K178, K67, Y51, K178, K67, Y51
1TS2	K178, K67, Y51, K378, K267, Y251, K578, K467, Y451
1TS3	K67, K267, Y251, K467
1TS4	K67, F83, K378, K267, F283, Y251
1TS5	K178, K67, Y51, Y251, K267, K378, K267

Fig. 1 Pymol view of cation-π pair of Arg-Tyr and Lys-Phe in 1D5X_C
Fig. 2 Pymol view of cation-π pair of Arg-Phe in 1EXF

DISCUSSION

The interactions formed between cations and aromatics rings are referred to as cation-pi interactions [32, 33] which is a new kind of binding force as being compared with the classical interactions such as hydrogen bonding, hydrophobic effects and ion-pairing. Cation-pi interactions play central roles in molecular recognition, stabilization of protein structures and nucleic acid structures and their biological functions [34]. Researches reveal that Trp is the most likely aromatic residue involved in cation-pi interaction [32] but our results showed that Tyr to be the most likely residue which meant that the Tyr could also be found more prevalent in energetically significant cation-pi interactions. It is well-known that cation-pi interactions play an important contribution to protein stability as revealed by the experimental studies of Prajapati et al. [35]. The virulence of *S. aureus* is multifactorial, meaning the combined action of various virulence factors but the infections and diseases due to its toxins are specific in which exfoliative toxins, TSST and enterotoxins causes Staphylococcal Skin Syndrome (SSSS), Toxic Shock Syndrome and food poisoning respectively [36]. Thus the stability imparted by cation-pi interactions in the toxins is related to virulence in host due to its presence and well-functioning of the proteins in the host.

CONCLUSION

We have analyzed the role of Cation-pi interaction in the protein structure stability and specificity of toxins of *Staphylococcus aureus* bacterium. In the dataset of 24 proteins, except two proteins (1XXG and 1QTF), we found 143 cation-pi interacting pairs which is an appreciable number of cation-π interactions found in these proteins. We found that the Lysine is preferred over Arginine in cation residues and among pi residues, Tyrosine is common. The toxin TSST with PDB ID: 2QIL was found to be the most stable protein among the studied toxins which was revealed with its low negative energy value of -109.65kcal/mol. Further, we made an attempt to evaluate the characteristic features of aminoacids involved in cation-pi interactions such as secondary structure, solvent accessibility, conservation score and stabilization centers. Secondary structure preference of the cation-pi interaction residues revealed that among cations Arg was in helix and Lys in β-strands whereas among pi residues Phe in β-strands and Tyr and Trp in helix regions. The solvent accessibility prediction revealed that most of the cation-pi interactions forming residues are found buried inside the protein and are highly conserved. The presence of atleast one stabilization center in all toxins except 1F77 further indicates the stability

in structures. The frequent number of cation-pi interactions in the studied toxins confirmed that they are found more prevalent and involve in protein stability, which plays an important role in the functioning of the toxins by its presence in the host cell system.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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