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In Silico Analysis of Prospective Influence of Staphylococcus Aureus Enterotoxin a on Selected Neurotransmitters

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ABSTRACT: A principal agent involved in staphylococcal food poisoning, Staphylococcus aureus produces staphylococcal enterotoxins of which SEA are very important. Bacterial toxins can act on neuronal cells. Some of them are multifunctional and can recognize wide range of cell types along with neuronal cells. Some toxins are said to have the capacity to pass the blood brain barrier. We cannot downplay the possibility of these toxins interacting with various neurotransmitters. Along with probing the mechanism that causes diarrhea, we can also investigate if they affect various neurotransmitters alone or in tandem to cause cognitive dysfunctions in various neurodevelopment disorders like autism where gastrointestinal problems are frequent. Docking is an effective tool for identifying their potential interaction efficiency with various neurotransmitters and a study pertaining to this objective is undertaken here.

KEYWORDS: Staphylococcus aureus, SEA, Diarrhea, Somatostatin, Vasopressin, Autodock, Docking.

I. INTRODUCTION

Staphylococcal food poisoning is rated as one of the most economically significant food-borne diseases (1). A study by Holmberg & Blake et al reports that 68-88% of the victims with staphylococcal food poisoning also have associated gastroenteritis (2). Often asymptomatic (3), Staphylococcus aureus is known to cause nosocomial diarrhea and vomiting (4). An opportunistic pathogen, its colonization is thought to affect individuals who are immune compromised. There are more than 20 Staphylococcal enterotoxins, thus far, characterized of which SEA and SEB are best studied with SEA denoted as most common toxin associated with Staphylococcal food poisoning (5). Meanwhile, it is interesting to note that a case of Staphylococcus aureus enterocloitis has been reported (6). Also significant is the interaction of various enterotoxins with the enteric nervous system as these toxins can stimulate afferent neurons or induce neurotransmitter release facilitating vomiting, diarrhea and intestinal inflammation (7).

Taken into account all the above said factors, it could be right and fitting to consider the prospective influence of Staphylococcus aureus Enterotoxin A in various neurodevelopment disorders like autism where gastrointestinal problems are recurrent. This has encouraged us to craft an in silico study on the role of Staphylococcus aureus Enterotoxin A as a prospective cause of autistic enterocolitis and their indicative role in inversely affecting certain neurotransmitters which might trigger enterocolitis and in parallel some cognitive dysfunctions.

The neurotransmitter candidates selected for the study were somatostatin and vasopressin. Known as an inhibitor of growth hormone release (8), role of somatostatin in inhibiting a variety of gastrointestinal processes are also well studied (9). It also has neuronal control over many physiological functions. Recent studies have come out with strong suggestions that this neuropeptide can also control social behavior (9). Vasopressin has been identified as a key mediator of complex social behaviors, including attachment, social recognition and aggression (10). There are certain neurodevelopment disorders that are associated with social deficits. This is the rationale behind this present study where in some neurodevelopment disorders like autism, gastrointestinal problems and cognitive dysfunctions co-exist.

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This solicits attention and before designing a wet lab project to identify the significance of bacterial toxins on selected neurotransmitters, it will be suitable to corroborate it with an in silico study.

II. MATERIALS AND METHODS

Sequence retrieval
Staphylococcus aureus enterotoxin A protein sequence was retrieved from NBCI. The accession number is ABP87596.

Ligand preparation
The chemical structures of vasopressin, somatostatin in sdf format were obtained from Pubchem repository. The sdf formats were converted to pdb formats using iCon fileformat converter [11].

Protein structure prediction
The enterotoxin sequence obtained was submitted to Swissmodel workspace [12]. The 3D structure predicted was retrieved in Automated mode from Swissmodel server. The Ramachandran plot of the structure was also obtained. The modeled structure was used as the protein target for the docking studies.

III. DOCKING

Autodock 4.2 Linux version was used for docking [13]. The protein target was loaded and polar hydrogen atoms were added. Ligand structures were also loaded and the torsions were chosen. For the ligands Gasteiger charges were added and nonpolar hydrogen atoms were merged. All the rotatable bonds were set to be rotatable. Grid preparation was done and the grid box with a dimension of 70 x 65 x 65 points and 0.436 Å grid spacing were used around each binding pocket. The grid parameter file specifies an AutoGrid calculation, counting the size and position of the grid, the atom types that will be used, the coordinate file for the rigid receptor, and other parameters for calculation of the grids [14]. Rigid docking was carried out using Lamarckian Genetic Algorithm. Autogrid file and Autodock docking parameter files were generated for all the three ligands. After docking searches were finished, the best conformation was selected from the most populated cluster with the least binding energy. The interaction of docked protein-ligand complex conformations, including hydrogen bond and other interactions were analyzed.

IV. RESULTS

The modeled structure of enterotoxin (Figure 1) retrieved from Swissmodel workspace had a residue range from 34 to 257 and QMEAN Z-Score: -0.975. The QMEAN4 score is a composite score consisting of a linear combination of 4 statistical potential terms (estimated model reliability between 0-1). The pseudo-energies of the contributing terms are given in Table 1 together with their Z-scores with respect to scores obtained for high-resolution experimental structures of similar size solved by X-ray crystallography:

Table 1. Scores and energies obtained for the enterotoxin model structure

Scoring function term	Raw score	Z-score
C_beta interaction energy	-105.12	-0.30
All-atom pairwise energy	-6137.83	-0.83
Solvation energy	-36.10	-1.69
Torsion angle energy	0.721	-0.97

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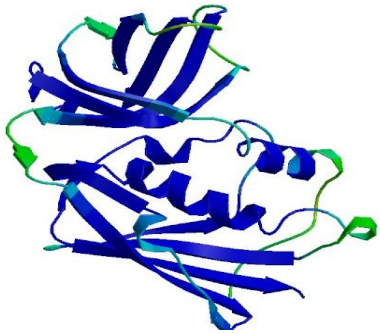

Figure 1. Enterotoxin model structure

All the docked conformations gave negative binding energy. Among the three neurotransmitters chosen for the docking study with enterotoxin structure the best i.e minimum binding energy was obtained for somatostatin. Docking results are shown in Table 2 in the increasing order of their binding energy. The aminoacids involved in the binding site of enterotoxin are Threonine 166, Serine86, Threonine83, Aspartine 84, Threonine 62, Glutamine 63, valine 219 etc. In Figure 2 and 3 the ligand is shown as ball-and-stick, surrounded by a molecular surface. The surface is colored with atomic colors in regions that contact the receptor, and gray in regions that are not in contact. Portions of the receptor that are in contact with the ligand are shown with ball-and-stick and mesh work spheres. Hydrogen bonds are shown as a string of small spheres [13]. The Binding energy was calculated based on the following formula.

Estimated Free Energy of Binding = Final Intermolecular Energy (vdW + Hbond + desolv Energy+ Electrostatic Energy) + Final Total Internal Energy + Torsional Free Energy- Unbound System's Energy where vdW is vander waals force interaction energy, Hbond is hydrogen bond energy and desolv is desolvation energy [15].

Table 2. Binding energies obtained for protein target

Sl.no.	Protein name	Ligands	Best Binding Energy (kcal/mol)	Amino acids in the binding site
1	Enterotoxin (modeled structure)	Somatostatin	-9.44	Ser86, Thr83, Asn 84, Thr 62, Glu 63
2	Enterotoxin (modeled structure)	Vasopressin	-8.26	Asp239, Ile236, Tyr220, Asn219, Val218, Ly211,

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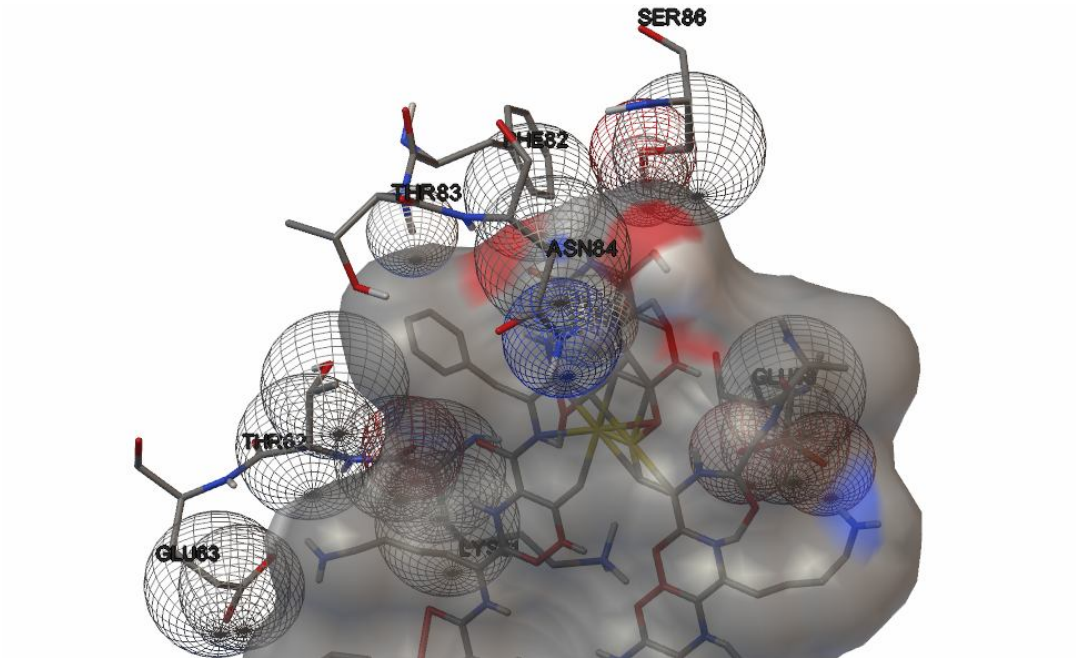

Figure 2. Docking interaction of enterotoxin with somatostatin

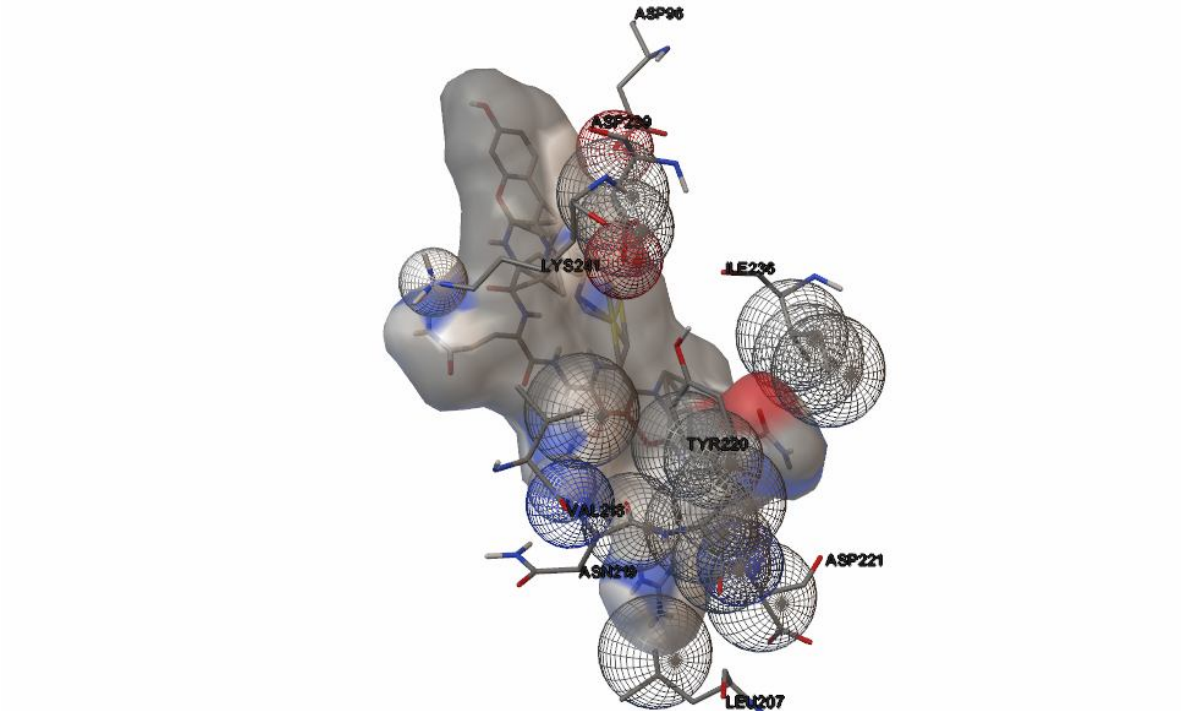

Figure 3. Docking interaction of enterotoxin with vasopressin

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AutoDock gives tools for clustering the results either at the last part of each docking or by combining together multiple docking results and re-clustering them. The single best score resulting from each cluster is shown in the output file [13]. Table 3 shows the histogram of cluster analysis of docked conformations of enterotoxin with somatostatin and Table 4 shows the root mean square deviation (RMSD) values in each cluster of docking.

Table 3. Cluster analysis of docked conformations of somatostatin

Cluster Rank	Lowest BE	Run	Mean BE	Num in clusters
1	-9.44	10	-9.44	1
2	-8.48	6	-8.48	1
3	-8.26	9	-7.55	2
4	-7.84	3	-7.84	1
5	-7.27	5	-7.27	1

BE-Binding Energy

Table 4. RMSD values in each cluster of enterotoxin with somatostatin

Run	BE	Cluster RMSD	Reference RMSD
10	-9.44	0.00	60.11
6	-8.48	0.00	59.66
9	-8.26	0.00	59.74
1	-6.84	0.58	59.66
3	-7.84	0.00	58.66

BE-Binding Energy

V. DISCUSSION

Out of the two neurotransmitters selected for the in silico study somatostatin is the most affected by the interaction with enterotoxin followed by vasopressin. Analysing the binding site aminoacid interactions of enterotoxin, serine and threonine are polar amino acids generally seen in the core, aspargine and glutamine are polar aminoacids with affinity to form hydrogen bonds. It is noteworthy to mention that serine and threonine are major proteinogenic amino acids. They are found on the surface as well as buried within proteins. Arginine is positively charged amino acid well intended to bind the phosphate anion, and is frequently found in the active centers of proteins that bind phosphorylated substrates [16]. The in silico study could analyze the binding efficacy of enterotoxin on the neurotransmitters which could also have an effect on the cognitive functions.

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