Insilico analysis of Listeriolysin-O with its natural inhibitors

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Abstract

Present study was conducted to understand the molecular interaction of listeriolysin-O with its natural inhibitors such as Fisetin and Lutein. The crystal structure of protein listeriolysin-O was revealed and molecular docking study was conducted by AutoDock tool. The result was analysed in terms of binding energy and position of binding. Further the interacting of amino acid residues was analysed. The energy obtained from docking study of Lutein was -6.05 whereas for fisetin was -4.75. It was identified that, both inhibitors possessed the strong interaction with listeriolysin-O. Hence lutein and fisetin may represent promising therapeutic agent for treatment of Listeriosis.

Keywords: listeriosis, listeriolysin-O (LLO), fisetin, lutein, docking energy

1. Introduction

Listeriosis is an important food borne zoonotic disease of public health significance caused by pathogenic strains of Listeria spp. particularly Listeria monocytogenes [1] and Listeria ivanovii [2]. It is an infectious and fatal disease of animals, birds, fish, crustaceans and humans where septicaemia and encephalitis are predominantly observed [3, 4, 5, 6, 7]. In humans, the young ones, especially new-borns, elderly persons, pregnant women, immunocompromised and immature individuals are at higher risk of acquiring listeriosis [8, 9, 10]. In India, L. monocytogenes is one of the major etiological agents in the causation of abortions and premature births in human [11]. For diagnoses of Listeriosis, molecular techniques are extremely accurate, sensitive and specific. Now-a-days Polymerase chain reaction (PCR) is the widely used molecular tool for identification of Listeria at genomic level [12]. The most common method of treatment is prescription of antibiotics.

Listeriolysin O (LLO) is one of the most important virulence factor produced by L. monocytogenes essential for promoting the intracellular growth of bacteria. LLO encoded by cholesterol-dependent cytolysin toxin encoding gene (hlyA) present only in virulent strains of the species [13]. LLO is a (58-kD) secreted protein that can be easily detected with the use of blood agar or haemolysis assays. It contained 529 amino acids and showed strong regional homologies to Streptolysin and pneumolysin-O [14]. LLO has been proposed to be a promising target for the development of anti-listeriolis drugs. Screening of inhibitors of LLO would facilitate the process of development of anti-LLO drugs. There are many natural proteins which inhibit the virulent action of LLO. Lutein, a natural small molecule existing widely in fruits and vegetable is demonstrated to be an effective inhibitor of LLO that works by blocking its oligomerization during invasion, thus showing significant bacteriostatic effect [15]. Fisetin, another natural flavonoid, is a potent antagonist of LLO mediated haemolysis. Fisetin inhibits L.monocytogenes infections in both tissue culture and animal infection models [16]. Molecular modelling and mutational analysis revealed that, fisetin directly engages the Loop2 and Loop3 residues of L.O with its selective inhibitors.
2. Materials and Methods

2.1 Protein sequence and structure retrieval

The target protein Listeriolysin-O primary sequence was retrieved from UniProt with the accession ID as P13128. The sequence was from 39 to 526 amino acids long. Protein crystal structure was retrieved from the Protein Data Bank (PDB) with the PDB ID 4CDB. It was reported as x-ray diffraction methods and the resolution was 2.15 Å.

2.2 Ligand preparation

Various ligands suitable for this target protein (Listeriolysin-O) were searched from different literatures. The selected ligands which also take part to inhibit the natural protein were Fisetin and Lutein. These were retrieved from the PubChem database with a compound ID of 5281614 and 5281243 respectively.

2.3 Molecular Docking

To know the binding site and binding position, molecular docking was performed with the help of AutoDock tool. During protein preparation, the polar hydrogen and suitable collman charges were added to the protein 3D structure (Fig-1). The torsion angle and the torsion tree were taken for consideration during ligand preparation. Grid box was set on the whole surface of the protein in order to perform a blind docking (Figure-2).

3. Results and Discussion

The crystal structure of protein listeriolysin-O was reported (Fig-1) as x-ray diffraction method and the resolution were 2.15 Å and 0.246 as R-free value [15]. Analysis of crystal structure reveals that the structure consists of polypeptide chain A having sequence length 488 residues [17].

In order to find out the molecular mechanism of protein listeriolysin-O with the selected two ligands, molecular docking study was conducted by AutoDock tool. The result was analysed in terms of binding energy/docked energy and position of binding. Best binding position of Listeriolysin-O with Lutein (Fig-3) and Listeriolysin-O with fisetin (Fig-5) was revealed. The energy was obtained from docking study of Lutein was -6.05. It also indicates the best rank within the total surface of target protein for the ligand binding. Similarly, for fisetin, the best binding pose was generated. The docking energy between target protein listeriolysin-O and fisetin was evaluated as -4.75. As the Autodock tool indicate more effective binding as more negative score, hence it was identified that, both inhibitors were possessing the strong interaction with listeriolysin. Further the interacting amino acid residues were analysed as they play significant role in binding mechanism. These amino acid residues between listeriolysin-O and Lutein within 4Å were GLU139, SER135, HIS79, TRY78, VAL77, VAL75, ALA286, TYR70AND SAP69(Fig-4).Similarly for fisetin PRO502, LEU503, ARG475, TYR444, ASN473, VAL438, TYR98 and TYR440 (Fig-6) residues are playing important role in binding of protein with in its suitable best pose on the surface of listeriolysin-O. The results obtained in accordance with the finding of Wang et al., 2015 [16].

Antibiotics are used rampantly in most of the bacterial infection. More than 200 antibiotics have been utilised for treatment of both human and animals. Now-a-days, the negative side of this rampant uses of antibiotics draws attention of researcher. The gram positive bacteria Listeria monocytogenes has not been escaped from this global problem of antibiotic resistance. According to recent reports, Drug resistance Listeria monocytogenes have been identified in many times [18, 19, 20]. Therefore it is the essential to identify new method of treatment of bacterial infection without inducing selective pressure unlike antibiotics. In this regard, Lutein and fisetin were identified as potent inhibitor of listeriolysin-O [15, 16]. Hence the study described about the molecular mechanism of target protein Listeriolysin-O with its two selective inhibitors which may lead a step towards the clear understanding of their interactions in a more deeply manner. The interactions of inhibitor with listeriolysin-O were supported by the finding of Loose et al., 2015; Wang et al 2015; Liu et al., 2017 [15, 16, 21]. According to study fisetin protects against L. monocytogenes infection by reducing the production of listeriolysin-O also it reduces L. monocytogenes-mediated cell death but not toxinmediate cell death. Which indicate significant decreases in the haemolytic activity without hampering the growth of bacteria [21]. Whereas Lutein inhibits intracellular growth of L. monocytogenes but also block L. monocytogenes escape from phagocytic vacuole which inhibits cell to cell migration [15]. This present study explained the effectiveness of natural compound in inhibiting the activity of LLO which create a new way for future use of fisetin and lutein as highly potent LLO-specific therapeutic drugs.
These compounds have shown antilisterial effects by inhibiting the target protein LLO. These compounds have shown specific binding position to listeriolysin-O. Further their binding energy with listeriolysin was scored negatively. Lutein has more negative binding energy as compare to fisetin, which indicates lutein bind more strongly to listeriolysin than fisetin. Hence lutein and fisetin may represent and novel therapeutic agent for treatment of Listeriosis. In this context, consumption of selected fruits and vegetables that contain large quantity of lutein and fisetin or adding those supplements may directly play a role in preventing listeria infection in susceptible animal and human population. However, extensive molecular studies necessary before administrating these compounds in clinical setting.

5. Conflict of Interest
There is no conflict of interest for this Article.

6. References