In silico modelling and docking studies of natural flavonoid derivatives as tetanus inhibitors

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ABSTRACT

The availability of experimental (X-ray, NMR) structures is very less. To overcome this problem the structures have been determined theoretically, especially those determined by homology modeling techniques. Protein-Ligand docking is increasingly used in Drug Discovery. The present study explains computational methods to design tetanus toxin light chain [synthetic construct], using the crystal structure available from Protein Data Bank (PDB ID: 1ZB7). Model was generated by using MODELLER9.16. After designing the model, functional effect was confirmed in terms of protein ligand binding by molecular docking using Autodock4.2. The docking investigation of modelled AAC37720 protein with natural flavonoid derivatives using Autodock4.2 software was performed. Out of 15 compounds Glycyrrhizastragaloside compound shows highest binding energy of -9.87 kCal/mol. Two compounds shows interactions with four amino acids, seven compounds are involved in interacting with three amino acids with good binding energy. Rest of the compounds shows two interactions with good binding energy. More importantly, homology modelling provides awareness into understanding and appropriately interpreting the data produced by these methods.

Keywords: Homology modelling; Natural flavonoid derivatives; Modeller 9.16; Autodock4.2; tetanus toxin.

INTRODUCTION

Tetanus is an acute disease exhibited by motor system and autonomic nervous system instability (Owusu-Darko S 2012). Tetanus toxin is produced by Clostridium tetani, an anaerobic bacillus. Tetanus toxin is responsible for the development of tetanus. Tetanus toxin is a zinc-dependent metalloproteinase that targets vesicle associated membrane protein, is responsible for neurotransmitter from nerve endings (Bjornar Hassel 2013). Tetanus can affect anyone, infants and children. Approximately the molecular weight of the tetanus toxin is 1,50,000 for single polypeptide (Halpern JL 1990). Computer aided approach is a novel platform to screen drug targets and design potential inhibitors. Intracellular toxin of tetanus may be obtained by disturbing the fermentation prior to lysis (Helting TB 1979).

In order to understand the possible binding modes of natural flavonoid derivatives at the tetanus toxin light chain [synthetic construct] receptor (Eisel U 1993) we have conducted a docking study. Since the crystal structure of the AAC37720 receptor has not been solved. Hence, our approach necessitated the use of a homology modeling paradigm. We decided to build a homology model using in silico tools in order to determine which model was best in line with our in vitro data. A model was built based on Crystal Structure of Botulinum Neurotoxin Type G Light Chain (PDB code 1ZB7) as template using molecular modeling – MODELLER9.16 program. We then performed docking/scoring experiments, using three docking programs: Autodock4.2.

In the present study, we determined that the homology model built by using MODELLER9.16, based on Crystal Structure of Botulinum Neurotoxin Type G Light Chain as a template. Together with an Autodock4.2 is confirmed the best agreement with our in silico results. These modelling and docking studies have provided useful insights into the possible binding modes of camptothecin derivatives at the AAC37720 receptor.

METHODOLOGY

Homology modelling

The amino acid sequence of tetanus toxin light chain [synthetic construct] was retrieved from NCBI. A sequence similarity search was performed by using Protein BLAST (Altschul SF 1990) tool for identifying templates for homology model building. The sequence was searched for their structural similarity with the query mutant protein by running NCBI protein BLAST against Protein Data Bank (PDB). The template was identified...
<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Binding energy</th>
<th>$K_i$</th>
<th>Interactions</th>
</tr>
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<td>Meciadonol</td>
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<td>57.78nM</td>
<td>ASP226, ARG372(II), LYS370</td>
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<tr>
<td>Hypolatein-8-glucoside</td>
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</table>
on the basis of maximum score, smaller the e-value, >30% identity. 1287 protein was selected as a template for modelled protein with 49% identity. A relative sequence alignment was accomplished with the template structures using ClustalX and ClustalW tools (Larkin MA 2007).

MODELLER 9.16 was used to develop a model. It is an automated approach to comparative modeling by gratification of spatial restraints to develop perfect models.
Manually modifying the input alignment file in MODELLER 9.16 (Webb B 2014) to match the template sequence and query sequence and generated 20 models. By using MODELLER9.16 auto model class, calculated three dimensional models of the target automatically. The Lowest Objective Function is used to select the best model by the smallest value of normalized Discrete Optimized Molecule Energy (DOPE) score. These generated models were then cross checked in detail for protein structure stereochemistry including Ramachandran plot and Psi/Phi angles using PROCHECK (Las-kowski R.A 1993).

Molecular docking studies

The structures of natural flavonoid derivatives shown in table 1, and was retrieved from NCBI. All the molecules were docked against Tetanus toxin light chain [synthetic construct]. Later all the 15 inhibitors were sketched in sybyl6.7 software (Gunda SK 2015) and was energetically minimized by adding Gasteiger-Huckel charges. The molecule was then saved in .mol2 format for molecular docking purpose.

The modelled protein structure was imported to Auto-dock 4.2, a protein-ligand docking tool (Gunda SK 2016) and structurally optimized by adding polar hydrogens. The model was saved in PDBQT format. Later all the ligands were docking individually. After loading the molecule ligands were prepared by optimizing the torsion angles and saving them in PDBQT format. Potential binding site for the model was identified using 3Dligand site (Wass MN 2010). A grid was generated around to identify xyz coordinates (X=-26.606, Y=59.648 and Z=8.668) around binding site of modelled Tetanus toxin light chain [synthetic construct]. Lamarckian genetic algorithm (LGA) (Morris GM 1998) was selected for freezing, docking and default parameters used in autodock4.2.
RESULTS AND DISCUSSION

Tetanus toxin light chain [synthetic construct] (AAC37720) contains sequence length of 457 aa was modelled by taking the template protein of Crystal Structure of Botulinum Neurotoxin Type G Light Chain (PDB entry: 1ZB7). The most homologous template for building a homology model for Tetanus toxin light chain [synthetic construct] was identified through protein blast. Based upon the homology search, the template 1ZB7 was selected on the basis of E-value, % identity etc., initial alignment was performed by using clustalX. Twenty models were generated using Modeller 9.16 program. The alignment file was checked manually to fit both the sequences. The modeller generated 20 models for all the primary sequences. All the models were checked with the least object function and was selected for the generation of Ramachandran plot by using procheck software. Procheck estimates protein stereochemistry evaluation (phi and psi angles).

The PROCHECK software generates Ramachandran plot and other files which list complete residue by residue data. The Ramachandran plot of the template 1ZB7 shows 320 amino acid residues (88.9%) in most favorable regions with 39 amino acid residues (10.8%) falling into additionally allowed regions and there is only one amino acid residue falling into the generously allowed (0.3%), and there is no amino acid residue fall in disallowed region. Whereas for the modelled protein shows, 362 amino acid residues (88.9%) in the most favorable region, 42 amino acid residues (10.3%) in the additionally allowed region, three amino acids in generously allowed region (0.6%), whereas there is no amino acid residue present in disallowed region. These results clearly indicate that the generated model is more conformationally superior to the template structure. Ramachandran plot of modelled protein was shown in figure 1.

Docking results

Molecular docking is the most widely used method for the calculation of protein–ligand interactions. Molecular docking studies was carried out thirty camptothecin derivatives against modelled AAC37720 protein. The binding energy, inhibition constant, hydrogen bond forming residues and interacting residues of all the compounds are shown in Table 1. The binding energy for all the molecules range from -6.98 to -9.87 KCal/mol. Compound Glycyrrhizoflavone shows three interactions with Asp226, Arg372, Lys370. Out of fifteen natural flavonoid compounds 2 compounds shows four interactions and 7 compounds show three interactions, 3 compounds show two interactions and rest of the compounds shows only one interaction. Glycyrrhizoflavone exhibit highest binding energy of -9.87 KCal/mol. The results of all the interacting amino acid residues with flavonoid derivatives are shown in table 1. All the 15 molecules interactions were shown in figure 2.

CONCLUSION

Structure-based drug design techniques were implemented in the past by the lack of a crystal structure for the target protein. Homology modeling is a powerful tool to suggest modeling of ligand-receptor interactions. The modelling studies are also useful for mutagenesis experiments, lead optimization etc., lack of crystal structure of tetanus toxin light chain [synthetic construct] motivated us to apply in silico techniques to initiate the drug discovery process for this protein. To understand the characteristics, structural features of AAC37720 and to execute the structure based drug discovery strategy we developed a model by using modeller. Several amino acid residues are involved in direct interactions with flavonoid derivatives. These studies should help to improve our knowledge of understanding the role of homology modeling and docking studies in drug discovery process.

REFERENCES


