Molecular modelling and docking analysis of pleurocidin (an antimicrobial peptide) like peptides with enterotoxin H from Klebsilla pneumonia

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Abstract: Enterotoxin H is a key molecular target for replication and establishment of Klebsilla pneumonia in the host. Therefore, it is of interest to study the interaction of enterotoxin H with pleurocidin like peptides using molecular modelling (template PDB ID: 1YCE), Lig-Plot (ligand construction) and docking tools for therapeutic consideration. The hydrophobic pocket and the active site residues (Val 13, Met 16, Gly 25, Ala 25, and Ile 28) were identified using Cast P, Molegro and Sitehound tools. Docking results show that the pleurocidin like peptides interacts with the active sites of enterotoxin H with 300.96 docking score with optimal binding features.

Keywords: Enterotoxin H protein, Klebsilla pneumonia; pleurocidin, anti microbial peptide, modeling, docking

Background: Enterotoxin H is a key molecular target for replication and establishment of Klebsilla pneumonia in the host [1-3]. They are associated with endophthalmitis and urinary tract infection (UTI) [4]. A detailed understanding of the molecular structure and function of Enterotoxin H is highly relevant [5-8]. Therefore, it is of interest to study the interaction of enterotoxin H with pleurocidin like peptides using molecular modelling (template PDB ID: 1YCE), Lig-Plot (ligand construction) and docking tools for therapeutic consideration. The use of molecular docking tools such as DOCK [9-11], FlexX [12], GOLD [13], and ICM [14] in drug discovery has become routine in recent years. The search methods and score functions of various docking tools are known [15]. We describe the optimal features that support pleurocidin like peptide interaction with Enterotoxin H from Klebsilla pneumonia.

Methodology

Target peptide sequence: The pleurocidin like peptide MALDI TOF sequence from Clarias batrachus is given below is shown in Figure 1.

MKFTATFLVSLVVLMAEPGECFLGALIKG
PDB ID: 1YCE
>1YCE:A|PDBID|CHAIN|SEQUENCE
Sequence Details

Figure 1: MALDI TOF peptide sequence from Clarias batrachus

Protein template:
The 3D structure of the template membrane protein (Research Collaboration for Structural Biology (RCSB) Protein Data Bank (PDB) ID: 1YCE) is shown in Figure 2.

Figure 2: The 3D structure of a template membrane protein (PDB ID: 1YCE) generated using the modeller software.

Ligand data:
The ligand sequence data given below for enterotoxin H from K. pneumonia is downloaded from NCBI.

Enterotoxin type H [Klebsiella pneumonia subsp. rhinoscleromatis ATCC 13884]:
>gi|262043668|ref|ZP_06016777.1| enterotoxin type H [Klebsiella pneumonia subsp. rhinoscleromatis ATCC 13884]
MSGLTRKAVLLELRTCEGVTSSAEVMYSGLRSTVFFILDSSLKDNLIFRAHNETGRNKRRYFPTAEKFSGKIKPSKRESFFDS
CRRHSKYMITLRRSAR QPPKEENQ

MODELLER software:
The MODELLER software package is used for homology or comparative modelling of protein 3D structures using default parameters [8, 9].

Ligplot:
The LIGPLOT program was used for showing the 2-D representation of protein-ligand interactions in standard PDB data format.

GOLD - protein-ligand docking:
The GOLD protein ligand docking package was used for molecular docking analysis.

Table 1: Enterotoxin H active site amino acids with cluster number used in molecular docking

| Cluster Number | Amino acid Residue |
|----------------|-------------------|
| 1              | VAL 9             |
| 2              | LEU 10            |
| 3              | ALA 17            |
| 4              | ALA 26            |
| 5              | ILE 28            |
| 6              | LV 29             |

Table 2: Ligand binding site data of pleurocidin like peptide

| Cluster | Total Energy | Cluster Center Coordinates (x, y, z) | Cluster Volume |
|---------|--------------|--------------------------------------|----------------|
| 1       | -318.728     | -6.121 8.713 32.918 29              |
| 2       | -318.33      | -3.337 9.975 40.22 28              |
| 3       | -254.794     | 3.397 16.216 35.589 23             |
| 4       | -155.598     | -17.28 5.964 37.409 14             |
| 5       | -155.238     | -10.385 0.648 37.555 15             |
| 6       | -62.492      | -10.945 17.735 32.253 8             |
| 7       | -96.544      | -21.281 14.542 33.702 7             |
| 8       | -90.139      | 4.56 18.053 40.417 6               |
| 9       | -20.309      | 1.37 4.06 34.299 2               |

Table 3: Ligand transformation energy for docking of pleurocidin like peptide with enterotoxin H

| LIGPLOT | SiteHOUND | SITEHOUND |
|---------|-----------|-----------|
| 1       | 10970     | -2.94     |
| 2       | 10046     | -3.19     |
| 3       | 10022     | 0.03      |
| 4       | 9880      | -3.20     |
| 5       | 9752      | -2.82     |
| 6       | 9600      | -3.40     |
| 7       | 9752      | 3.73      |
| 8       | 9410      | 2.60      |
| 9       | 9342      | -2.92     |
| 10      | 3914      | -3.51     |
| 11      | 9224      | -3.31     |

SITEHOUND:
This tool identifies ligand binding sites by computing interactions between a chemical probe and a protein structure using PDB input data.
Model validation:
Model validation was completed using the Ram Page server and CE.

Table 4: Optimized parameters for docking of pleurocidin like peptide with enterotoxin H

| Program                                         | Parameters                  |
|------------------------------------------------|-----------------------------|
| ACE Energy Term Weight (Str)                   | 1.0                         |
| COM distance Term Weight (Str)                 | 1.07                        |
| HBEnergy Term Weight (Str)                     | 1.0                         |
| Atr VdW Energy Term Weight (Str)               | 1.01                        |
| Receptor (Str)                                 | 4.01 0.02                   |
| Clustermatch (Str)                             | 0.14 0.04                   |
| Costprob Energy Term Weight (Str)              | 0.1                         |
| Desolvationparam (Str)                         | 500 0.01                    |
| dockEnergy Term Weight (Str)                   | 0.1                         |
| EnergyDockCoeff (Str)                          | 6.0                         |
| LigandName (Str)                               | Enterotoxin.pdb.ms          |
| Ligandpdb (Str)                                | Enterotoxin.Pdb             |
| LigandSeg (Str)                                | 10.0 20.0 1.5 10 10         |
| LogFile (Str)                                  | PatchDock.log               |
| LogLevel (Str)                                 | 2                           |
| MatchAlgorithm (Str)                           | 1                           |
| matchingParam (Str)                            | 1.5 1.5 0.4 0.5 0.9         |
| pLackEnergy TermWeight (Str)                   | 0.0                         |
| pUCL (Str)                                     | specific/home/cc/ux/pdpock/omegaServer/patchDock/bin/chem.lib |
| radiScalng (Str)                               | 0.8                         |
| ReceptorName (Str)                             | 0.5 0.6 0.0                 |
| receptorpdb (Str)                              | Defence.pdb.ms              |
| receptorSeg (Str)                              | 10.0 20.0 1.5 10 10         |
| repVdWEnergyTermWeight (Str)                   | 0.5                         |
| ScoreParams (Str)                              | 0.3 5.0 0.5 0.0 0.0 0.00 4 0.01 0.0 |
| scoreTormype (Str)                             | 0.3 5.0 0.5 0.0 0.0 0.0 1500 4 0.01 |

Results and Discussion:
The crystal structure of the membrane protein ATP synthase (PDB ID: 1YCE) is used as the template structure (Figure 1). The identity of the target and the template were screened to construct the model for the target pleurocidin like peptide using the protein modelling package modeller. A 40% sequence (40 %) similarity was found between target and the template. The red coloured alphabets in the alignment showed the similarity between the template and target where the conserved motif was identified (Figure 2). The solvent accessibility is one of the key factors that determines the ligand interaction and binding of the receptor - ligand complex (Figure 3). Red coloured side chains in the Figure 3 show the active solvent accessible layer.

Figure 3: Solvent accessibility and surface features of the peptide.

Figure 4: Visualization of predicted active site in enterotoxin H using Discovery Studio.

The modelled protein PL peptide was displayed in the Figure 3 which shows the superimposed secondary structure the α-helical patterns with extended sheet in the modelled structure. The exposed layer pink colour buried in the peptide chain shows the motif availed to access ligand structure (Figure 3). The catalytic active site in the ligand PLP binds the enterotoxin with the residues in the positions of Valine - 13, Methionine - 16, Glycine - 25, Alanine - 26 and Isoleucine - 28 predicted as the active residues as shown by Accelrys Discovery Studio™ (Figure 4).

This was further analyzed for the ion containing amino acid residues in the binding pocket using the tool cast-P one of online tool (Figure 5). Figure 5 indicate the aminoacid residue (binding Active site residues) for docking were four residues like Val 13, Met 16, Gly 25, Ala 25 and Ile 28. Hydrophobicity was a vital factor for structure activity relationship in binding. The Red colour buried layer in Figure 6 shows 50% hydrophobicity in the PL-peptide...
structure. The solid 3D-entity showed the highest hydrophobic interaction present in the structure, which facilitates the receptor-ligand complex of the PL-peptide and enterotoxin H complex. The RMSD (Root Mean Square Deviation) of the modelled structure is within acceptable limit.

| ID | Area | Vol |
|----|------|-----|
| 2  | 22.3 | 14.3|
| 1  | 35.1 | 18.8|

The RMSD between template and target is 2.6 Å which was below rule 5 within accepted cut-off (Figure 8). Further, the alignment of the target and template identity to validate the structure was shown in Figure 7. The chain in the template and target showed the identical motif with a perfect model. The ligplot tool was used to show the.enterotoxin H structure for the optimal preparation of the ligand enterotoxin-H. Thus, we used ligplot to show the enterotoxin H structure (Figure 9).

The modelled proteins were evaluated using threading to validate the constructed model PL-peptide. So the constructed structure was analyzed by the Ram page server and Swiss pdb viewer [16-18]. The Ram page server validated the structure with allowed number of aminoacid in the favoured region (above 94%). The PL peptide in Figure 7 shows 96% allowed region in the model. This indicates that the constructed PL-peptide model was well constructed and perfectly assigned in the structural and geometric entity.

The structure of target was developed using the template and docked using the gold docking software. The docking of the PL-peptide and enterotoxin H was well docked (Figure 10). The ligand enterotoxin H was bonded in the active site. The amino acid residues involved in the docking of the ligand enterotoxin H was analyzed using the cluster of site Hound web server were (VAL9, LEU10, VAL13, VAL14, ALA17, ALA26, ILE28, LYS29 and LEU 27) as shown in Table 1. The cluster coordinates and the total energy for docking in coordinates were obtained (-500) which shows good receipting energy level - 318.728, -318.330, -254.794, -155.508, 155.238, -82.492, -66.544, -59.139, -20.309 (Table 2). After the docking the best ligand transformation energy was found as -7.50834 65.33267 20.29962, and the docking score was noted as -300.96 (Table 3). It is known that values below 1 are considered as good docking score in the Gaussian docking rules as shown in Table 4.
The antimicrobial peptide was targeted to produce a peptide therapeutic. Therefore, it is of interest to understand the PL-peptide structure and the receptor activity with the pathogenic toxin from *Klebsiella pneumoniae*. The PL-peptide was modelled and it was optimized for the docking process followed by virtual screening as described elsewhere [19, 20]. It was found that glycosyl amines are suitable drugs to halt the growth of *M. tuberculosis* [21]. The modelling of PL-peptide by the modeller packages shows the three possible entities with values 157.9, 121.8 and 138.3. Data with the lowest value of 121.8 for conformation is used further as described by Kuntz *et al.* [9]. Alignment was performed for conserved motif to assess similarity between the target and the template. A 40% identity was found between template and target. Thus, the model was constructed using the template membrane protein with PDB ID: 1YCE.

The constructed model was evaluated by the ram page server and the combinatorial extension. The Ram page server validated the structure and reports the number of aminoacid in the favoured region (above 94%). Similarly, the number of allowed regions and the outlier region was expected as less than 3% and more than 1% the PL-peptide showed with an allowed region (2%) and outlier region (3.1%). This indicated that the constructed PL-peptide model was well modelled and perfectly assigned for structural and geometric analysis. The RMSD between template & target is 2.6 Å which was below allowed cut off limit. Then the modelled PL-peptide was docked with the ligand enterotoxin H receptor. The enterotoxin H receptor was protein toxin secreted by the *Klebsiella pneumoniae*. Hence, it is of interest to study the interaction between a peptide and the enterotoxin-H using docking tools. The PL-peptide is a peptide antibiotic against *Klebsiella pneumoniae* known by *in vitro* and *in vivo* studies in the mice. There is a strong evidence for receipting activity of PL-peptide with enterotoxin H.

The important docking parameters such as solvent accessibility, binding site prediction, hydrophobicity were analyzed in the receptor, the ligand and optimized for the docking as described elsewhere [22-28]. Active binding sites were predicted using the Cast P tool for studying the receptor protein ligand interactions [29-31]. Similarly, the active site binding sites of PL-peptide were predicted using the AccelrySTM and Cast P web tools. The amino acid residue such as Val 13, Met 16, Gly 25, Ala 25 and Ile 28 were predicted in the pocket of binding site. In the PL-peptide the Ligand enterotoxin H were optimized to find the active site of ligand enterotoxin H. The site hound web tool was used for predicting active sites in the ligand (VAL9, LEU10, VAL13, VAL14, ALA17, ALA26, ILE28, LYS 29 and LEU 27). The cluster coordinates and the total energy for docking were calculated using the gold package as -500 which shows good receipting energy level such as 318.728, -318.330 , -254.794 , -155.508 , -155.238 , -82.492 , -66.544 , -59.139 , 20.309. The best ligand transformation energy was noted as -7.50834 65.33267 20.29962, and the docking score was -300.96. Thus,
we report data to support the optimal binding of PL-peptide with the enterotoxin H from K. pneumonia for further consideration.

**Conclusion:**
We report the molecular modelling and docking analysis data (300.96 docking score and -7.50834 ligand transformation energy) of pleurocidin like peptide (an antimicrobial peptide) with enterotoxin H from Klebsilla pneumonia for further consideration as a therapeutic agent.

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