J Med Allied Sci 2011; 1 (1): 21-24
www.jmas.in
Print ISSN: 2231 1696 Online ISSN: 2231 170X
Journal of Medical & Allied Sciences

Original article

Homology modeling and ligand interaction of human trafficking protein particle complex subunit 3-like protein and its correlation with Alzheimer's disease

Venkata Sri Krishna K[1], Uday Kiran M[2], Siva Rama Prasad D[1], Anusha G[3], Mohana Rao V[4], Manoj G[1]

[1]Department of Biotechnology, Aurora Technological and Research Institute, Hyderabad, A.P., India.
[2]Centre for Distance Education Bioinformatics, Osmania University, Hyderabad, A.P., India.
[3]Department of Pharmacy, Sarojini Naidu Vanitha Pharmacy, Nampally, Hyderabad, A.P., India.
[4]Scientist, Monco Biotechnologies, Ameerpet, Hyderabad, A.P., India.

Article history

Received 16 January 2011
Revised 25 January 2011
Accepted 29 January 2011
Early online 30 January 2011
Print 31 January 2011

Corresponding author

Manoj G
Assistant Professor,
Department of Biotechnology,
Aurora College of Technological & Research Institute,
Parvathapur, Uppal,
Hyderabad- 39,
Andhra Pradesh, India.
Email : dhanmanu@gmail.com
Phone: +91 9966660204

Abstract

In order to understand the mechanism of molecular interactions at active sites of trafficking protein particle complex (TRAPPC) subunit 3-like protein (Accession number Q5T215) homology modeling and docking studies were taken up. We generated a three-dimensional (3D) model of target protein based on the Crystal structure of Human BET3 protein (PDB code 1SZ7) using modeller software. Under the process of homology modeling 25 models were generated, and the model having the lowest modeler objective function value was chosen for further assessment. The generated model was assessed and validated using PROCHECK software and found to be reliable.

With the generated model, we carried out a flexible docking study using the FlexX docking tool available on the Sybyl Software in order to find better antagonist site for drug binding. We carried out a flexible docking with the Palmatic acid and Donepezil as ligands; these were found to bind at LEU87, LYS84 and THR90 residues on given generated protein in our studies. We therefore concluded that the above mentioned residues were the key residue sites for ligand binding showing strong hydrogen bonding contacts.

The target protein is more prevalently seen in the amygdala region of brain which forms one of the key subunit of TRAPP complex. As per functional similarity with BET3, target protein might play key role in trafficking abnormally folded A-beta and tau proteins along endoplasmic reticulum to Golgi apparatus there by leading to formation of senile plaques in various regions of brain, whose accumulation causes Alzheimer's disease.

Key words: homology modeling, docking, modeller, FlexX, PROCHECK

© 2011 Deccan College of Medical Sciences. All rights reserved.

Trafficking protein particle complex (TRAPPC) 3-like protein is a peptide compound with 181 amino acid residues which belongs to the family of BET3. The target protein plays an important role in vesicular transport mechanism between the endoplasmic reticulum and Golgi apparatus[1,2].

The TRAPPC3 protein is expressed from BET3L gene located on Chromosome 6 at 6q22.1[3]. As per functional similarity target proteins participate in intracellular targeting; and transport of proteins in eukaryotic cells depends on a variety of proteins and protein complexes involved in the coating, budding, release, uncoating, tethering and fusion of vesicles. Vesicle budding commonly relies on formation of a protein coat around the bud and is initiated by the binding of a GTPase on the exit membrane[1,4].

The target protein is involved in coat-formation of the various proteins that are expressed in intercellular compartments, which facilitates coated proteins to interact with membrane thereby leading to polymerization and stabilization of the bud and subsequently to dissection of the vesicle from the membrane[4,5].

The 3D structure of target protein which was generated by using BET3 protein of the same family was subjected to docking studies in order to find a potent drug which can bind to active site of target protein and play a key role in antagonism. The modeled target protein is expected to be in better spatial conformation so that any ligand can be bound at active sites through which inhibition or activation can be achieved. For carrying out further docking studies with some more ligands or drugs on modeled target protein, three important determinant residues LEU87, LYS 84 and THR90 were indentified in our study as potentially helpful. The motif region along the LEU87, LYS84 and THR90 residues resembles a pocket like structure which enables us to carry out ligand interactions with various potent drugs.

Our study assumes that by designing such a potent drug with superior binding capacity on our target protein, one can improve signal transduction pathways. Thereby altered or regulated pathways might cure different neuronal disorders[6].

Materials and methods

Sequence alignment and homology modeling

We performed sequence similarity search of target sequence using Blast tool which returned high scoring homologues sequences. Among the resultant hits, sequence with high E-value was chosen i.e., BET3 (PDB code 1sz7) to perform comparative sequence analysis and alignment with TRAPPC-3 using Clustal W tool. The program MODELLER9v1 was used for the Modeling 3D structure of target sequence. Modeller is an implementation of an automated approach to comparative modeling by satisfaction of spatial restraints[7,8]. Accordingly twenty five models were generated using the loop refinement method and the model having the lowest Modeller objective function was chosen for further validations.

Model validation

PROCHECK was used for the validation of the models generated. The main geometric parameters of the models were determined by PROCHECK[9]. Accordingly we have chosen the best model for further investigations based upon the most favorable features. In the last step of homology modeling the selected model was subjected to a series of tests for its internal consistency and reliability. Backbone conformation was evaluated by the inspection of the psi/phi Ramachandran plot obtained from PROCHECK analysis (Fig 1).

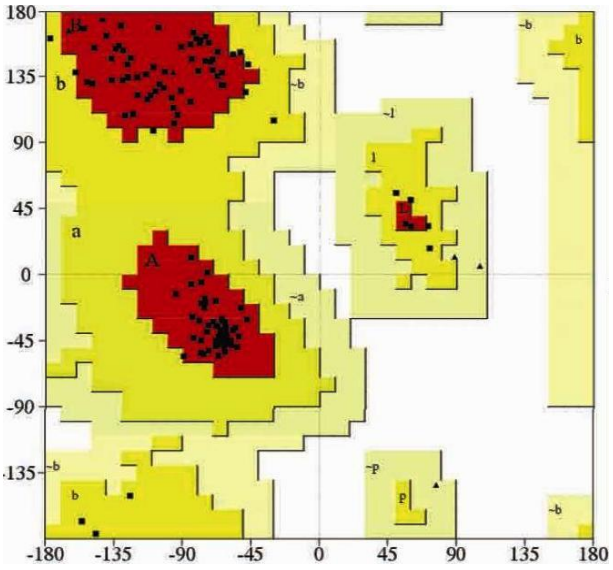

Fig 1. Ramchandran plot showing the human TRAPPC- 3 Model. The most favored regions are coloured red, additional allowed, generously allowed and disallowed regions are indicated as yellow, light yellow and white fields, respectively.

Docking

Molecular docking can fit molecules together in a favorable configuration to form a complex system. The structural information from the theoretically-modeled complex may help us to clarify the binding mechanism of ligands to the receptor. FlexX soft-

ware under Sybyl Operating System was the tool we chose to perform the automated molecular docking. All docking calculations were performed by the Lamarckian Genetic Algorithm (LGA)[10]. We ran LGA with default parameters and found insufficient sampling efficacy. Parameters of LGA were changed to obtain better result. These changes contributed higher diversity of sampled configuration of protein and allowed us to achieve sufficient sampling of the conformational space available for ligands within the binding site (Fig 2A, 2B, 3A & 3B).

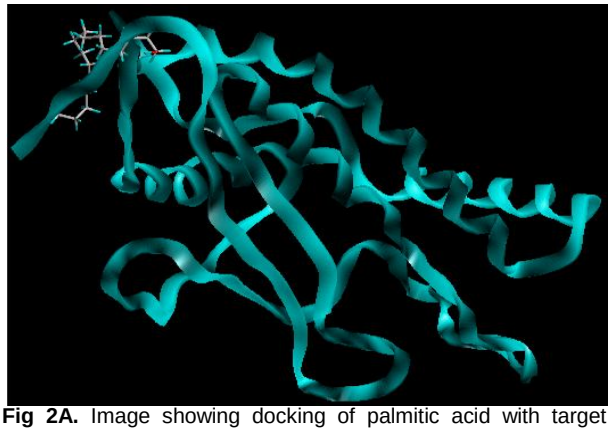

Fig 2A. Image showing docking of palmitic acid with target protein

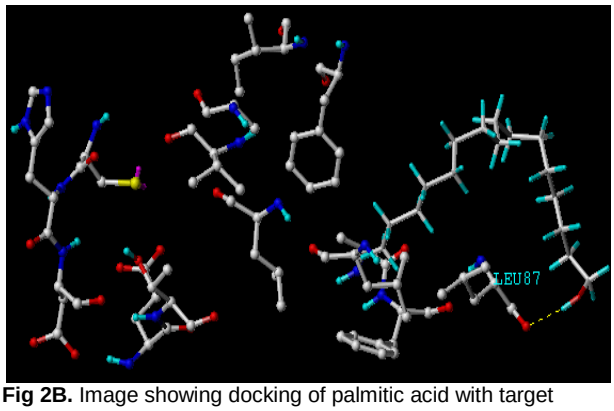

Fig 2B. Image showing docking of palmitic acid with target protein under ball and stick model

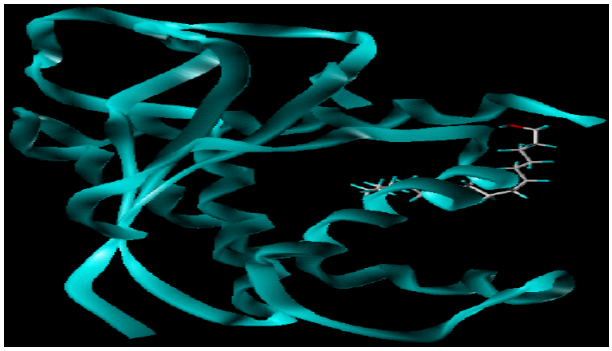

Fig 3A. Image showing docking of donopezil acid with target protein

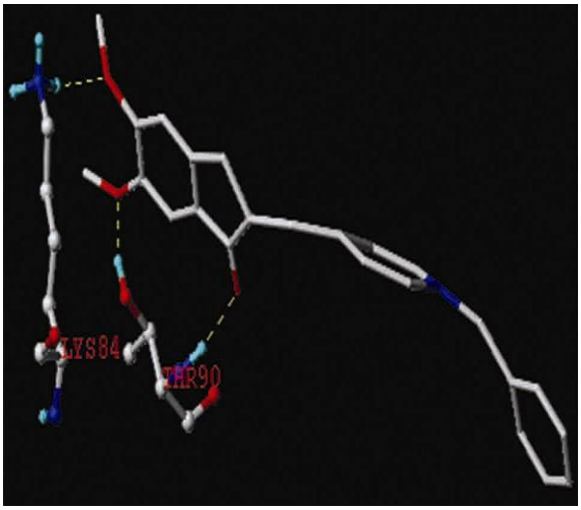

Fig 3B. Image showing docking of Donepezil with target protein under ball and stick model

Results

Homology modeling and docking

The final alignment of the TRAPPC-3 to that of BET3 was carefully checked in TM regions. We found that all the critical structural elements involved in the binding of their natural substrates were intact. Therefore, we conclude that this alignment could be used to construct reliable 3D models. Validation of the homology models involved two independent tests. The first test was to compare the residue backbone conformations in our model with the preferred values obtained from the Protein Data Bank of known structures. The results of PROCHECK analysis indicate that none of the residues were falling under disallowed ranges and the quality of Ramchandran plots is acceptable for generated model. The percentage of residues in the "core" region of modeled TRAPPC-3 was found to be 90.7. The stereo chemical quality of the models was found to be satisfactory.

With the protein held static throughout the simulation, the antagonist molecule was manipulated to fit inside the cavity, largely by applying a random displacement to each degree of freedom, i.e., translation of its centre of gravity, orientation and rotation around each of its flexible internal dihedral angles. The docked complexes of receptor-ligand were selected according to the criteria of interacting energy combined with geometrical matching quality. Accordingly the binding confirmations were evaluated and ranked. The most probable residues involved in antagonist binding were determined using the evaluations of interaction energies. The Table I explains the docking scores of ligand interaction with target protein.

Table I. Docking scores of ligand interaction with target protein

Compound	Interacting amino acids	Docking score	Distances
Donepezil	LYS 84, THR 90	-7.038	2.130Ang, 2.096Ang
Palmatic acid	LEU 87	-6.764	2.079Ang

Discussion

Homology modeling was done to construct 3D model of target protein towards ligand interaction. The models generated were assessed by PROCHECK. The stable structure was further used to perform the docking to identify the role of several amino acids in agonist or antagonist binding. Selective and potent antagonists and natural ligands were used for docking studies. We found that several potential aromatic compounds interact with the lipophilic side of the antagonist binding with hydrophobic residues.

A binding interaction between a small molecule ligand and a enzyme or protein may result in activation or inhibition of the enzyme. If the protein is a receptor, ligand binding may result in agonism or antagonism. Docking is most commonly used in the field of drug design - most drugs are small organic molecules and docking may be applied to: Hit identification. Docking combined with a scoring function can be used to quickly screen large databases of potential drugs *in silico* in order to identify molecules that are likely to bind target protein of interest.

The absence or malfunction of one or other subunit complexes in TRAPP could lead to pumping misfolded protein such as tau, A-Beta protein, prions to various brain regions leading to syndromes like trisomy 21, Creutzfeldt–Jakob or malfunctioning of some of vital neuronal activities[4-6].

Target protein expressed in the central nervous system helps in vesicular coat formation of various proteins thereby we infer it may have a significant role in trafficking misfolded protein to various brain regions thereby leading to formation of senile plaques[8], which hinder memory-processing functions.

We conclude that as target protein presence is seen in amygdala region, its malfunctioning there, could lead to anterograde and retrograde conditions resulting in dementia, as in Alzheimer's disease.

Conflict of interest: None

Acknowledgments: None

References

1. Kaiser C, Novick SF. Transport from the endoplasmic reticulum to the Golgi. Current Opinion in Cell Biology, 1998; 10 (4):477-482.
2. Ostermanna J, Stauberb T, Nilsson T. 16-Vesicular Transport. Protein Targeting, Transport, and Translocation 2002:377-401.
3. http://www.uniprot.org/docs/humchr06
4. Sztula E, Lupashinb V. Role of vesicle tethering factors in the ER–Golgi membrane traffic. FEBS letters 2009; 23(3):3770-3783.
5. Turnbull AP, Kümmel D, Prinz B, Holz BC, Schultchen J, Lang C, et al. Structure of palmitoylated BET3: insights into TRAPP complex assembly and membrane localization. EMBO J 2005; 24(5):875–884.
6. Shorta B, Haasa A, Barr FA. Golgins and GTPases, giving identity and structure to the Golgi apparatus. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 2005; 1744 3(10):383-395.
7. Sali A and Blundell TL. Comparative protein modeling by satisfaction of spatial restraints. J. Mol. Biol. 1993; 234:779-815.
8. Fiser, Do RK, Sali A. Modeling of loops in protein structures, Protein Science 2000; 9:1753-1773.
9. Luthy R, Bowie JU, Eisenberg D. Assessment of Protein Models with Three-Dimensional Profiles. Nature 1992; 356:83-85.
10. Thomsen R. Flexible ligand docking using evolutionary algorithms: investigating the effects of variation operators and local search hybrids. Bio Systems 2003; 72:57–7