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Theoretical Modeling and Docking Studies of Silkworm Serotonin Receptor

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Abstract

The Silkworm serotonin receptor protein is an integral membrane protein which belongs to the largest superfamily of G protein-coupled receptors that communicate signals across the cell membrane through their interaction with heterotrimeric G proteins and regulate many of the physiological processes including the regulation of feeding, aggression, mood, perception, pain, anxiety etc. Despite the importance of serotonin receptor protein its structure have not yet determined experimentally due to difficulty in crystallization. The three-dimensional structure was modelled by the MODELLER program using the template rh1A which has 33% identity. The modelled structure was validated using Procheck and Ramachandran plot. It was found that the percentage of residue lying in the most favoured regions, additionally allowed regions, generously allowed regions, disallowed regions are 93.3%, 6.4%, 0% and 0.3%, respectively and it indicates reasonably good model. The overall Procheck G-factor and overall quality factor of ERRAT graph were 0.04 and 80.759, respectively. The binding site of the serotonin receptor protein was predicted using Q-Sitefinder and the interaction studies carried out with serotonin, agonist and reveals that the seven interaction residues Asp141, Val142, Gln218, Thr227, Ser230, Phe382, Leu405 of serotonin receptor protein interact with the serotonin form three hydrogen bond. This study provides a structural understanding at the atomic level of three-dimensional structure of silkworm serotonin receptor protein and their binding-sites and to elucidate of many promising active lead compounds.

Keywords: Silkworm serotonin receptor; Homology modeling; Docking studies

Introduction

Silkworm is an important domestic lepidopteran insect due to its primary role in silk production. It is also model organism for insect biochemical, genetic and genomic studies because of having strong advantages for experimental research, such as rapid development with short life cycles, ease of rearing in the laboratory, small adult size, tractability etc [1]. Silkworm products, by-products and waste products have potential medicinal value in the treatment of diabetes, bronchial asthma, primary trigeminal neuralgia, vocal nodules, facial palsy, sterility, hepatitis, acute pancreatitis, chronic nephritis, leukocytopenia, blood cholesterol and many other conditions [2]. G protein–coupled receptors (GPCRs) are largest superfamily of integral membrane proteins that communicate signals across the cell membrane through their interaction with heterotrimeric G proteins and regulate many of physiological processes of *Bombyx mori* such as neurotransmission, growth, development etc. The GPCRs of the silkworm are identified using the computational methods and they are categorized into Rhodopsin-like receptors (Class A), secretin receptors (Class B), metabotropic glutamate/pheromone receptors (Class C), fungal mating pheromone receptors (Class D), cyclic AMP receptors (Class E), and frizzled/smoothened GPCRs (Class F) [3]. GPCRs malfunctions play important role in diseases such as ulcers, allergies, migraines, anxiety, psychosis, schizophrenia, hypertension, asthma, congestive heart failure, Parkinson's, and glaucoma.

The serotonin receptor (5-hydroxytryptamine, or 5-HT) which belongs to class A (rhodopsin-like) of G protein-coupled receptors except 5-HT3 receptor is a ligand-gated ion channel. These receptor proteins are integral membrane proteins characterize of an extracellular N-terminal, an intracellular C-terminal, three intracellular loops, three extracellular loops and seven transmembrane alpha helices. It serves as neurotransmitters involved in many processes in the central nervous system, including the regulation of feeding, aggression, mood, perception, pain, anxiety etc. The mammalian families of serotonin receptors are large, and indeed it has proven to be much larger than that of any of the other GPCR-type neurotransmitter receptors, including those for dopamine, norepinephrine, glutamate, or acetylcholine.

Despite the great deal of interest, the serotonin receptor protein structure have not yet determined experimentally because these membrane-bound proteins are difficult in expression, purification and crystallization. Currently atomic-level structure has been solved only for four GPCR namely rhodopsin, the β1-adrenergic receptor, the β2-adrenergic receptor, and the A2A adenosine receptor. Consequently, there is a great need for GPCR structure predictions, for which computational methods can be much useful.

The mutagenesis and the structural studies of receptor have revealed that residues in the second extracellular loop possibly concerned in ligand binding and selectivity of receptor subtype [4,5]. In previous studies, critically important extracellular loop residues involved in alpha 1-adrenergic receptor subtype-selective antagonist binding are identified [6]. An antagonist or agonist targeted at one receptor will bind to some other type of receptor and lead to unwanted side effects. Thus, three-dimensional structures are essential for the rational design of subtype-specific drugs. In rhodopsin 6th transmembrane region, phenylalanine is entirely conserved and recognized as being important for agonist activation of the receptor [7]. It has shown that the

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Rhodopsin and β2-adrenergic receptor inactive state, arginine residues of 3rd transmembrane is caged by salt bridges between Asp of 3rd transmembrane regeion and Glu at the bottom of 6th transmembrane region [8-10]. Docking studies of octopamine receptor of *Periplaneta americana* was revealed the agonist binding site of octopamine receptor and the important actively interaction residues of octopamine receptor with the agonist Octopamine [11].

In the present study, first time an effort is made to generate three-dimensional (3D) models of silkworm serotonin receptor which circumvented the absence of a crystallographic structure of silkworm serotonin receptor and docking studies with the agonist serotonin were carried out using the Autodock program. The binding site analysis and the study of these interactions will be helpful to describe its structural features, ligand binding site and to understand molecular function for the drug discovery process. In silico prediction of interactions between receptor protein and small molecules in the transmembrane ligand-binding site will be useful to decipher the function and elucidate of many promising molecule candidates for this protein.

Materials and Methods

The Silkworm, *Bombyx mori* serotonin receptor sequence with the length of 446 amino acids was collected from the SWISSPROT, a public domain protein database [12] and was retrieved in the FASTA format and utilized for further studies. The collected sequence was further characterized and the structure was determined using various computational tools. The physico-chemical properties of the serotonin receptor sequence such as theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand average hydropathy (GRAVY) were computed by the Expasy's ProtParam server [13].

Functional Characterization

The Serotonin receptor was characterized for the transmembrane regions and these regions of the sequence were predicted using the Transmembrane Hidden Markov Model (TMHMM) Program, which predicts the transmembrane regions of the sequence using of Hidden Markov Model. It can discriminate between soluble and membrane proteins with both specificity and sensitivity better than 99% [14]. The secondary structures of the protein were analyzed using the Self Optimised Prediction from Multiple Alignment (SOPMA) server which uses the nearest-neighbor approach for the prediction of secondary structure [15]. The functional domains of the serotonin receptor protein were predicted by Scanprosite program [16].

Structural Modeling and Analysis

The modelling of the three-dimensional structure of Serotonin receptor was performed using the knowledge of transmembrane regions. The templates of the Serotonin receptor was identified and selected using the criteria of higher similarity of each transmembrane region of the GPCR protein using the Basic Local Alignment Search Tool (BLAST) program [17] which was performed against Brookhaven Protein Data Bank and GPCR-SSFE database [18] to find suitable templates for homology modeling. According to the transmembrane regions of the serotonin receptor protein, the multiple templates are selected for each transmembrane region. According to the selected multiple templates of the transmembrane regions, protein were modeled with the selected templates using the MODELLER, program for comparative protein structure modelling by satisfaction of spatial restraints [19]. The constructed 3D models were energy minimized in GROMOS96 force field using steepest descent minimization algorithms [20].

The validation for structure models was performed by using SAVES server, metaserver for analyzing and validating protein structures [21], Procheck which checks the stereochemical quality of a protein structure, analysing its overall and residue-by-residue geometry [22]. Whatcheck comprises several tools for protein structure verification. This does extensive checking of many stereochemical parameters of the residues in the model [23]. Errat analyzes the statistics of non-bonded interactions between different atom types and plots the value of the error function versus position of a 9-residue sliding window, calculated by a comparison with statistics from highly refined structures and Ramachandran plot was drawn [24]. The theoretically modeled structures were validated using the Ramachandran Plot [25]. The PyMOL program was employed for interactive visualization and analysis of molecular structures [26].

Active Site Analysis and Docking

By using the modelled structures of the GPCR proteins, the active binding site of these proteins were identified using the Q-Sitefinder Program, which can identify pockets on protein surface to predict ligand-binding sites [27]. Serotonin receptors expressed throughout the brain and they are responsible for cognition and basic brain functions. This serotonin receptor was docked with the agonist serotonin using the Autodock program [28]. The docking energy and KI was obtained for the docked molecules. The interaction studies were carried out between the agonist and the receptor protein. In this analysis, the interacted residues and conformation of the transmembrane regions of the molecules were predicted.

Results and Discussion

The Serotonin receptor protein was retrived from Swissprot and the physico-chemical properties of the protein was computed by Expasy Protparam tool is presented in Table 1. The PI of the protein was 8.95 and it reveals that the protein is basic in nature. The instability index, which provides an estimate of the stability of protein in a test tube of protein was 54, that was more than 40, so it indicates the membrane protein was unstable protein. The Grand average of hydropathicity (GRAVY) value of protein (0.306), it indicates the protein has less interaction with water. The aliphatic index was 103.45, which indicates the protein may be stable for a wide range of temperature.

The serotonin receptor protein was characterized of seven transmembrane helices and these regions are identified using the program THMMM is shown in Table 2. The seven transmembrane regions of the serotonin protein are 65-87, 99-121, 136-157, 177-199, 224-246, 368-390 respectively were predicted using the THMMM program. Disulphide bridges between Cys134 and Cys214 was predicted which is used to determine the thermostability of protein and the fucntional domains of these protein, G_Protein_ReCep_F1 was identified using Scanprosite. The secondary structure of the protein are determined using the SOPMA Program were represented in the Figure 1. The result shows the Alpha helices are dominated among other secondary elements, followed by random coil, extended strand and beta turn respectively.

The three-dimensional structure of this protein was not available due to the difficulty in crystallization, and so the homology modelling was carried out using the templates, identified by BLAST program.

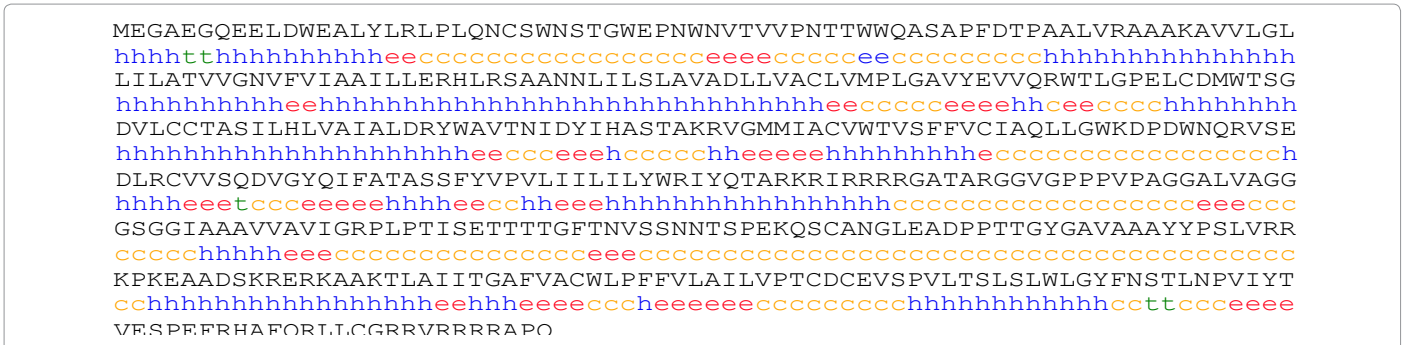
Accession No.	Length	M.wt	pI	-R	+R	EC	II	AI	GRAVY
Q17239	446	48599.4	8.95	31	41	102620 101870	54.00 unstable.	103.45	0.306

Table 1: Physico-chemical properties of Serotonin Receptor protein.

	TM1	TM2	TM3	TM4	TM5	TM6	TM7
Region	65-87	99-121	136-157	177-199	224-246	368-390	400-422
Template	2VT44 (62.1)	2RH1 (62.5)	2VT4 (74.3)	2Z73 (44)	1U19 (44.4)	2VT4-2RH1 (63.6)	3EML (54.2)

Note: TM1-7 refers transmembrane regions of this protein. Figures in parenthesis denotes % of similarity with the template.

Table 2: Templates for the transmembrane regions.

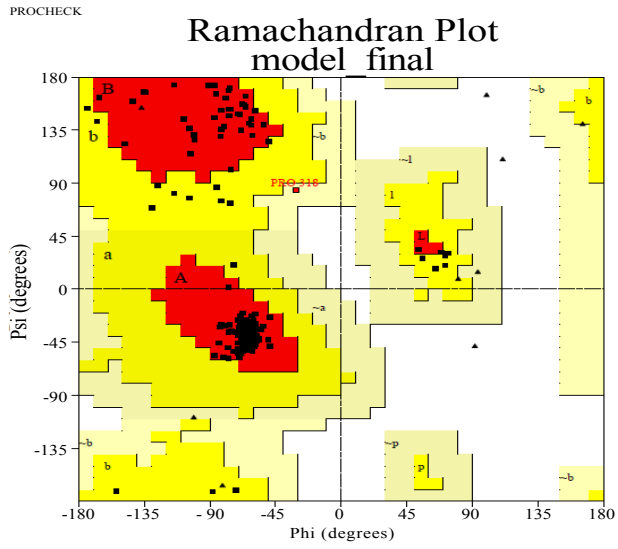


The Percentage of Alpha helix (Hh), Random coil (Cc), Extended strand (Ee), Beta turn (Tt) is 41.93, 40.58, 15.25, 2.24 respectively. 310helix (Gg), Pi helix (Ii), Beta bridge (Bb), Bend region (Ss) is 0.00%

Figure 1: Secondary Structure prediction.

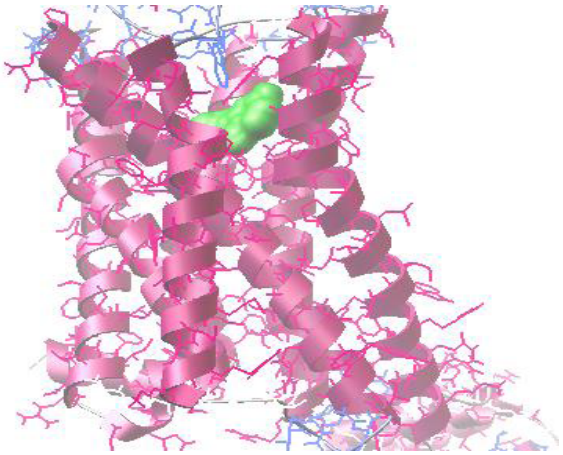
This protein was dominated by transmembrane regions; the template which has more homology with the each transmembrane regions of the protein was identified, presented in the (Table 2) and with the template rh1A which has 33% identity with the serotonin receptor protein. The three-dimensional structure of this protein was modelled using these templates by the MODELLER Program which was shown in Figure 2. The modelled structure was superimposed on the template structure and the Root Mean Square Deviation RMSD between the modeled structure and the template is 0.78 Å. The modeled structure was validated using the saves server, which has Procheck, Whatcheck, Errat and Ramachandran Plots. By using Procheck, the Ramachandran Plot was mapped, depicted in Figure 3. In the Ramachandran plot analysis, it was found that the percentage of residue lying in the most favoured regions, additionally allowed regions, generously allowed regions and disallowed regions are 93.9%, 6.1%, 0% and 0% respectively. The procheck G-factor for dihedrals and overall PG-factor was 0.17 and 0.04, respectively. In the ERRAT graphs, the overall quality factor was 81.572 and this modeled structure indicates reasonably good model. Further the refined model was validated by the WHATCHECK program in which the z-scores of bond lengths, bond angles, omega angle restraints, side chain planarity, improper dihedral distribution, inside/outside distribution are 0.963, 1.205, 0.670, 0.214, 0.904, and 1.159, respectively. The refined model structure was acceptable because all the scores are positive; positive is better than average in these program.

The possible binding sites of serotonin receptor protein were searched using Q-Sitefinder program. It was found that the residues such as Trp137, Thr138, Asp141, Val142, Cys145, Thr146, Gln195, Lys200, Val216, Gln218, Tyr222, Gln223, Ile224, Ala226, Thr227, Ser230, Phe231, Trp379, Phe382, Phe383, Ala386, Leu405, Tyr409 in site 1, and this region was considered as most favorable binding site of protein for docking. The efforts were made to obtain the entire orientation of the ligand inside the binding pocket. Docking of serotonin, agonist of serotonin receptor protein was performed using the Autodock program and docked with the docking energy and KI were -4.83 and 288.7uM shown in Figure 4. Disulphide bridge was formed between the Cys134 of TM3 region and highly conserved residue Cys214 of 2nd Extracellular region (EL2) and it also observed in the structures of rhodopsin and the β2-adrenergic receptor [29]. Here



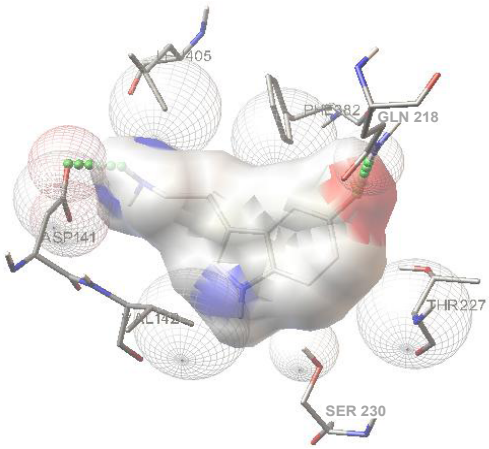
The Percentage of Favoured regions, Additional allowed regions are 93.9, 6.1, respectively.

Figure 2: Ramachandran Plot.



The three-dimensional modelled structure bound with the serotonin, agonist. The upper portion extracellular region and the lower portion intracellular region. The TM 3, TM 5, TM6, TM7, EL2 regions has interaction with the agonist.

Figure 3: The modelled Structure of Serotonin receptor.



Asp141 and Gln218 residues has form hydrogen bonds with the agonist with the distance of 2, 2.3, 3.2 respectively.

Figure 4: Interaction of Serotonin with the receptor.

the interaction include the 3, 5, 6, 7 transmembrane (TM) region and the EL2 region of the protein with the agonist. It clearly shows that the accessibility of the ligand to the ligand binding site is mainly enabled by EL2 region [29].

Serotonin receptor has highly conserved three residues aspartic acid, arginine, tyrosine (DRY) located at the boundary between TM3 and intracellular loop 2 and the previous studies shown that ERY sequence in rhodopsin referred to as an arginine cage [30]. Docking studies implies that the seven residues of the serotonin receptor protein such as Asp141, Val142, Gln218, Thr227, Ser230, Phe382, Leu405 interact with the serotonin in which Gln218 was EL2 region and Phe382, Leu405 residues are TM6 and TM7 regions. Here Asp141, Val142 and Thr227, Ser230 belongs to the TM3 and TM5 region respectively. Upon binding the agonist serotonin can potentially form the hydrogen bonds with the receptor at the three positions of residues Asp141 and Gln218. This is the first report on structural analysis and docking studies of silkworm serotonin receptor. These interaction studies provides the guidance for ensuring the experimental studies and provides an improved structural understanding at the atomic level of three-dimensional structure of silkworm serotonin receptor protein and the binding-sites for agonist, serotonin. It might also be useful to design and develop novel agonist that would bind to the serotonin receptor protein for better productivity.

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