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Homology modelling of Frizzled 1 CRD and understanding the mechanism of Wnt mediated dimerization

Pankaj Sharma[1], Mahesh Kulharia[2] and Manvender Singh[3]
[1]Deptt of Biotech, N.C. College of Engg, Israna Panipat, INDIA
[2]Centre of Bioinformatics, Central Univ of Punjab, Bhatinda, INDIA
[3]Deptt of Biotech, UIET, MDU, Rohtak, INDIA

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Abstract

Frizzled is a family of G protein-coupled receptor serve as receptors in the Wnt signaling pathway. The usual length of frizzled proteins is about 600 amino acids. The N- terminus comprises of cysteine rich domain (CRD) and is extracellular. The CRD is followed by a lipopathic flexible region of 40-100 residues. The transmembrane part of frizzled protein consists of seven transmembrane α-helices that form hydrophobic domains and are considered as a typical G protein-coupled receptors (GPCRs). The Frizzled and Wnt interaction is imperative for the signal transduction involving catenin protein. To understand the Wnt and Frizzled interaction a protocol was developed for the comparative model generation. The human Frizzled receptor (Fz1) has been studied and homology based structure model is proposed. The model was exhaustively checked on various parameters and was found to be of good quality. On the surface of Cysteine Rich Domain important sites were identified that could potentially play a role in Wnt mediated dimerisation of Frizzled receptors.

Keywords: Cysteine-rich domain, β-catenin, Dishevelled, Homology modeling, PSI blast.

Introduction

Frizzled protein family belongs to the group of G protein-coupled receptor proteins. These proteins act as receptors in the Wnt signalling cascade[1]. It also regulates tissue homeostasis in many different organs in the adult and further implicated in adult tissue repair and regeneration[2,3,4]. Frizzled proteins are around 500-700 amino acids with extracellular N-terminus domain rich in cysteine residues (Cysteine Rich Domain - CRD) *)* and is extracellular. The CRD is followed by a lipopathic flexible region of 40-100 residues[5]. The transmembrane part of frizzled protein consists of seven transmembrane α-helices that form hydrophobic domains and are considered as a typical G protein-coupled receptors *(GPCRs)*[6,7].

Ligands for the extracellular CRD domain include the Wnt proteins. These are post translationally lipidated (palmitoylation) secreted proteins (length 350-400 residues) whose primary function is signal transduction[8,9]. In conjunction with the signal sequence, wnt proteins harbor a conserved archetype of 23-24 cysteine residues. These resifues serve as primary site for palmitoylation[10]. Wnt-frizzled interactions are involved in intracellular signalling. These bind to the Frizzled proteins, activating Dishevelled (DSH) proteins and finally modulating the cytoplasmic and intranuclear concentration of β-catenin[11].

When the signal is absent the degradation complex (consisting of CKIa, GSK3b, APC, Axin) hyperphosphorylates β-catenin, which is a targeted by proteosome. Wnt interaction to a Frizzled stabilizes β-catenin, which in combination with TCF/LEF activates transcription[12].

Functions: Frizzled proteins are critical for the determination of cell polarity, These proteins are also important for the development of embryo, cell division/proliferation, formation of synapses among neurons, among many other processes in developing and adult organisms. Frizzled-3 plays important role in neural crest development. Frizzled-4 mutants are responsible for familial exudative vitreoretinopathy among humans. This is a rare disease which affects the retinal cells and cause alteration in the refractivity of the clear vitreous fluid inside the eye. Wnt pathway also play important role in cancer stem cells, embryogenesis, self tissue renewal and in many developmental biological processes.

Material and Methods

Retrieved Sequence: Uniprot database sequence (accession number Q9UP38) of 647 amino acids was retrieved for Frizzled-1 protein of *Homo sapien*[13,14,15]. The N-Terminus 120 amino acids comprised the Cysteine Rich Domain (CRD). These 120 amino-acids were excised out and used as the input sequence for the model building. The sequence of the CRD is:

DGYCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQ
FYPLVKVQCSAELKFFLCSMY

APVCTVLEQALPPCRSLCERARQGCEALMNKFGFQWPD
TLKCEKFPVHGAGELCVGQN

Method for Template search for homology modelling: PSI blast was performed to produce a position-specific scoring matrix (PSSM) from a multiple sequence alignment file containing the best-scoring BLAST results to a the frizzled-1query sequence. A profile was created to identify the important locations of conserved residues in the Fz-1 sequence on the basis of scoring matrix[16,17].

Homology Building: Yasara structure which features a complete homology modeling module that has automated protocol to obtain a refined high-resolution model using a CASP validated and approved protocol [18]. The Q9UP38 was reformated in fasta format (using inhouse perl scripts). The structure of CRD of FZ1 protein was modelled using CRD of FZ8 as template (PDB ID = 1ijx) with yasara structure software package.

Results and Discussion

Secondary Structure Prediction: The prediction of secondary structure was carried out using a program called "Define Secondary Structure of Proteins" (DSSP) as depicted below (strand (S), helix (H), turn (T), overwound or 3/10-helix (3), and coil are shown in the figure 1.

Swapping of NH1 and NH2: The arginine residues at 2, 84, 89, 91, 136, 218, 223, 225 had their different N-H- and N-H swapped.

Validation of structural model of Frizzled 1: The overall deviation in bond lengths (less than 0.667) were calculated and were found to be less than the generally accepted values as compared to the mean accepted values for standard bonds. Bond angle validation was also carried out using what check program and only three bond angles listed in the table-1 below were found to deviate significantly from standard bond angles. This is due to the effect of thermal fluctuations set during molecular dynamics simulations and mimics real structure of a protein.

Backbone conformation Score validation: The Ramachandran plot closely adhered to the permissive values as seen in figure 2. The average score of all residues corresponded to the allowed areas in the Ramachandran plot.

Secondary structure alignment

```
                  10          20          30          40          50          60
                   |           |           |           |           |           |
  1 -  60 ERGISVPDHGYCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSAE
( 105)-( 164)      TT   SS   333TTTT  SS TT TT   THHHHHHHH333HHHHHHTT TT
                  70          80          90          100         110         120
                   |           |           |           |           |           |
  61 -  120 LKFFLCSMYAPVCTVLEQALPPCRSLCERARQGCEALMNKFGFQWPDTLKCEKFPVHGAG
( 165)-( 224)HHHHHHHHHHT    TTT     HHHHHHHHHHHHHHHHHHTT    333 333T  TTTT
                         130
                          |
  121 -  134 ELCVGQNTSDKGTP
( 225)-( 238)T        TT

                  140         150         160         170         180         190
                   |           |           |           |           |           |
  135 -  194 ERGISVPDHGYCQPISIPLCTDIAYNQTIMPNLLGHTNQEDAGLEVHQFYPLVKVQCSAE
( 105)-( 164)      TT   SS   333TTTT  SS TT TT   THHHHHHHH333HHHHHHTT TT

                  200         210         220         230                 240     250
                   |           |           |           |                   |       |
  195 -  254 LKFFLCSMYAPVCTVLEQALPPCRSLCERARQGCEALMNKFGFQWPDTLKCEKFPVHGAG
 ( 165)-( 224)HHHHHHHHHHT    TTT    HHHHHHHHHHHHHHHHHHTT    333 TTTT  TTTT
                         260
                          |
  255 -  268 ELCVGQNTSDKGTP
( 225)-( 238)T    T    TT
```

Figure-1
Depiction of secondary structure of protein of Frizzled 1

Histidine Residue Protonation: Histidine protonation state determination is a very difficult and cumbersome task. Histidine residues can be protonated on ND1 (HIS-D) or protonated on NE2 (HIS-E), or even protonated on both ND1 and NE2 (HIS-H) and lastly positively charged). The determination of exact state of protonation was made on the basis of hydrogen bond network. An alternative assignment was made on the basis of best fit to the structure. In the table 2 below all normal histidine residues are listed. The assignment based on the geometry of the residue is listed first, together with the RMS Z-score for the fit to the Engh and Huber parameters. For all residues where the H-bond assignment is different, the assignment is listed in the last columns, together with its RMS Z-score parameters.

Table-1
Bold angles deviating from the usual

Amino acid Number	Amino acid name	Chain ID	Connecting atoms 1	Connecting atoms 2	Atom Type	Bond angle
36	HIS (140-)	A	NE2	CD2	CG	110.84
146	CYS (116-)	B	CA	CB	SG	103.65
215	PRO (185-)	B	CD	N	CA	105.96 -

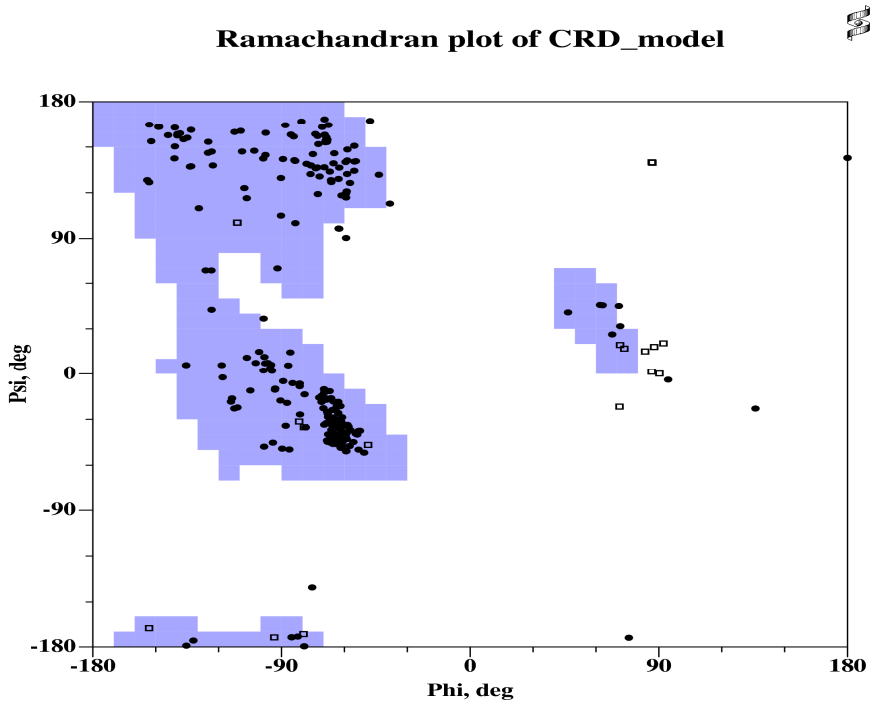

Figure-2
Ramachandran plot of CRD

Table-2
Deviation of Bond angles

Amino acid Number	Histidine assignment	Chain ID	Histidine assignment	Zscore	Histidine assignment	Zscore
9	HIS (113-)	A	HIS-D	0.40	HIS-E	0.83
36	HIS (140-)	A	HIS-D	1.28	HIS-E	1.71
47	HIS (151-)	A	HIS-H	0.46	HIS-D	0.66
117	HIS (221-)	A	HIS-D	0.42		
143	HIS (113-)	B	HIS-D	0.43	HIS-E	0.85
170	HIS (140-)	B	HIS-D	0.40	HIS-E	0.75
181	HIS (151-)	B	HIS-D	0.52		
251	HIS (221-)	B	HIS-D	0.43	HIS-E	0.82

Overall summary of the results: The overall quality of the structure vis-a-vis the available good structures is good as shown in table-1 and table-2. This useful as the structure can serve a starting point for further investigations that is for modelling calculations. The summary of the data given in table-3 indicates that the structure of the model of Frizzled-1 is reasonable.

Table-3
Z score values of various parameters

Parameter	Zscore
RMS Z-score for bond lengths	0.400
RMS-deviation in bond distances	0.010
RMS Z-score for bond angles	0.737
RMS-deviation in bond angles	1.632
Ramachandran Z-score	-0.240
1st generation packing quality	-3.697
2nd generation packing quality	-2.610
Ramachandran plot appearance	-0.240
chi-1/chi-2 rotamer normality	3.719
Backbone conformation	-1.801

Conclusion

G-protein coupled receptors (GPCRs) are an important therapeutic target group. The members of GPCR gene superfamily are integral trans-membrane proteins that are classified in three separate families viz Rhodopsin like family; secretin like family and metabotropic glutamate receptor. Frizzled (Fz) receptors also constitute a separate group in the GPCR superfamily even though the interaction of Fz with G-protein is still not directly confirmed. Targeting Fz-wnt interaction is very important to modulate the signaling process involved in cell-fate assignation and cytoskeletal organization. Here the techniques of comparative modeling was used to obtain a model of CRD of FZ1. The high resolution structure of extracellular "cysteine rich domain" (CRD) of Fz-8 was used to obtained the models of corresponding domain of Fz-1. These models were rigorously examined for structural errors and the models were validated.

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