J. of the Chosun Natural Science
Vol. 6, No. 1 (2013) pp. 16 – 20

Homology Modeling of GPR18 Receptor, an Orphan G-protein-coupled Receptor

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Abstract

G-protein-coupled receptor (GPCR) superfamily is the largest known receptor family, characterized by seven transmembrane domains and considered to be an important drug target. In this study we focused on an orphan GPCR termed as GPR18. As there is no X-ray crystal structure has been reported for this receptor, we report on a homology model of GPR18. Template structure with high homology was used for modeling and ten models were developed. A model was selected and refined by energy minimization. The selected model was further validated using various parameters. Our results could be a starting point for further structure based drug design.

Key words: Structure-Based Drug Design, Homology Modeling, GPR18, GPCR

1. Introduction

The G-protein-coupled receptor (GPCR) superfamily is the largest known receptor family, characterized by seven transmembrane domains[1,2]. GPCR's display extensive amino acid sequence similarities that allow division into several classes, each with highly characteristic conserved residues distributed throughout the molecule. GPCR's transduce a variety of extracellular signals such as photons, peptides, hormone proteins, neurotransmitters, amino acids, lipids, prostanoids, and odorants through heterotrimeric G-proteins. There are about 1000 genes encoding such receptors in the human genome, and these receptors regulate numerous physiological processes, including neuronal excitability, metabolism, reproduction, development, hormonal homeostasis, and behavior. There are more than 30% of the presently effective drugs targets are GPCR's[3].

For a considerable number of GPCR's the cognate ligand has not been identied, and these are collectively referred to as orphan GPCR's. This group of receptors with mostly unidentied function can be attractive targets for further study as GPCR's have been demonstrated to possess important functions in fundamental biological cellular processes and are successful drug targets. A series of class A orphan receptors such as GPR119, GPR55 and GPR18 have been identified and reported in the literature. In this study we focused on modeling of GPR18. GPR18 an orphan receptor was cloned and found that it was highly expressed in HUT102 cells[4].

We have already reported on many short reviews on different topics[5-9]. In this current study, GPR18 was selected for in silico studies. Till to date there is no structural report on this target. Structural information of this receptor could be useful to gain insights for further structure based drug design. The three dimensional structure of GPR18 was obtained by comparative modeling using the top template structure hit. Our results could be helpful for experimental biologists as well as for computational modelers.

2. Experimental Section

All molecular modeling calculations were performed using molecular modeling programs, Sybyl 8.1 and Modeller installed on a Linux environment.

2.1. Sequence Analysis of GPR18

The amino acid sequences of human GPR18 receptor were retrieved from the Uniprot KB/TrEMBL database. The human sequence of GPR18 receptor was fur-

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(Received : February 18, 2013, Revised : March 20, 2013, Accepted : March 25, 2013)

ther used for template identification using the basic local alignment search tool for protein (BLAST) algorithm[10,11] against the protein data bank[12]. After the search, the alignment between the template and the target sequences was performed using ClustalW 2.0[13].

2.2. Homology Modeling of GPR18

Homology modeling of GPR18 receptor was done using the Modeller9v4 program[14-16]. Ten homology models were generated with the alignment obtained using the top template structure from the PDB. Ten models were developed. Finally a model with the lower MolPdf (molecular probability density function) score and no significant main chain root mean square (RMS) deviation was selected. The selected model was further refined by simple energy minimization to remove strains and bad van der waal contacts using the energy minimization tool in Sybyl. The selected model was further validated using PROCHECK[17] and ERRAT plots[18].

3. Results and Discussion

3.1. Sequence Analysis of GPR18

In order to identify adequate template structures for

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sp|Q14330|GPR18_HUMAN      MITLWNQDQPVPFNSSHPDEYKIAALVFYSCIFIIGLFVNITALWVFSCT 50
4EA3                       -----------------PLGLKVTIVGLYLAVCVGGLLGNCLVMYVILRH 33
                                            *   *:: : :* .: : **: *  .::*:

sp|Q14330|GPR18_HUMAN      TKKRTTVTIYMMNVALVDLIFIMTLPFRMFYYAKDEWPFGEYFCQILGAL 100
4EA3                       TKMKTATNIYIFNLALADTLVLLTLPFQGTDILLGFWPFGNALCKTVIAI 83
                           ** :*:..**::*:**.* :.::****:      . ****: :*: : *:

sp|Q14330|GPR18_HUMAN      TVFYPSIALWLLAFISADRYMAIVQPKYAKELKNTCKAVLACVGVWIMTL 150
4EA3                       DYYNMFTSTFTLTAMSVDRYVAICHP-------TSSKAQAVNVAIWALAS 126
                             :    : : *: :*.***:** :*       .:.**  . *.:* ::

sp|Q14330|GPR18_HUMAN      TTTTPLLLLYKDPDKDSTPATCLKISDIIYLKAVNVLNLTRLTFFFLIPL 200
4EA3                       VVGVPVAIMGSAQVEDEEIECLVEIPTPQDYWGP-VFAICIFLFSFIVPV 175
                           .. .*: :: .   :*.     ::*.      .  *: :  : * *::*:

sp|Q14330|GPR18_HUMAN      FIMIGCYLVIIHNLLHG---RTSKLKPKVKEKSIRIIITLLVQVLVCFMP 247
4EA3                       LVISVCYSLMIRRLRGVRLLSGSREKDRNLRRITRLVLVVVVAVFVGCWTP 225
                           :::  ** ::*:.*        *: * :  .:  *:::.::. .: *: *

sp|Q14330|GPR18_HUMAN      FHICFAFLMLGTGENSYNPWG--APTTFLMNLSTCLDVILYYIVSKQFQA 295
4EA3                       VQVFVLAQGLGVQPSSETAVAILRFCTALGYVNSCLNPILYAFLDENFKA 275
                           .:: .    **.  .* .. .   * * *  :.:**: *** ::.::*:*

sp|Q14330|GPR18_HUMAN      RVISVMLYR---NYLRSMRRKSFRSGSLRSLSNINSEML---------- 331
4EA3                       CFRLEDNWETLNDNLKVIEKADNAAQVKDALTKMRAAALDAQKATPPKLE 325
                            .     :.   : *: :.: .  :    :*::::.:  *

sp|Q14330|GPR18_HUMAN      ---------------------------------------------------
4EA3                       DKSPDSPEMKDFRHGFDILVGQIDDALKLANEGKVKEAQAAAEQLKTTRN 375

sp|Q14330|GPR18_HUMAN      ---------------------------------------------------
4EA3                       AYIQKYLPLGLKVTIVGLYLAVCVGGLLGNCLVMYVILRHTKMKTATNIY 425

sp|Q14330|GPR18_HUMAN      ---------------------------------------------------
4EA3                       IFNLALADTLVLLTLPFQGTDILLGFWPFGNALCKTVIAIDYYNMFTSTF 475

sp|Q14330|GPR18_HUMAN      ---------------------------------------------------
4EA3                       TLTAMSVDRYVAICHPIRALDVRTSSKAQAVNVAIWALASVVGVPVAIMG 525

sp|Q14330|GPR18_HUMAN      ---------------------------------------------------
4EA3                       SAEIECLVEIPTPQDYWGPVFAICIFLFSFIVPVLVISVCYSLMIRRLEK 575

sp|Q14330|GPR18_HUMAN      ---------------------------------------------------
4EA3                       DRNLRRITRLVLVVVAVFVGCWTPVQVFVLAQGLGVQPSSETAVAILRFC 625

sp|Q14330|GPR18_HUMAN      ----------------------------
4EA3                       TALGYVNSCLNPILYAFLDENFKACFRKF 654
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Fig. 1. Sequence alignment between GPR18 and A chain of the top template structure hit 4EA3.

GPR18, Blast search was done. The search using Blast algorithm against the PDB revealed many potential templates for modeling. The Uniprot sequence of GPR18 (Q14330) was retrieved from Uniprot database. Among the template hits, the top template structure was selected. The top template structure used for modeling was N/OFQ Opioid Receptor (PDB code: 4EA3)[19]. The sequence identity between GPR18 and N/OFQ Opioid Receptor was found to be 25% with query coverage of 82%. The sequence conservation and the alignment were obtained using ClustalW and are shown in Fig. 1.

3.2. Homology Modeling of GPR18

The coordinates of the crystal structure of respective template hit (PDB code: 4EA3) was used to build the homology model of GPR18. The three dimensional homology models of GPR118 was generated using Modeller9v4 program. Ten models were developed. In these models, the seven trans-membrane (TM) helices have been properly transformed according to that of the template structure. The unique feature of chemokines is the presence of disulphide bonds and in the case of GPR18 the disulphide bond between 94 and 172 is crucial. So, we investigated that whether the models retained the crucial disulphide bond and it was observed to be there in all the models. Finally the model with the lowest MolPdf as well as with the lowest RMSD was selected. The selected model was further subjected to energy minimization to remove strains and the energy minimized model is shown in Fig. 2. The selected models were further validated stereochemically using additional parameters such as the PROCHECK and ERRAT plots. We have observed that 89.8% of the residues are in favored regions, 9.8% of residues are in additionally allowed regions and 0.3% of residues are in generously allowed regions. The overall quality factor observed by ERRART is 80.317. The results indicate that the selected model is statistically valid. The PROCHECK and ERRAT plots for GPR18 is shown in Fig. 3 and Fig. 4.

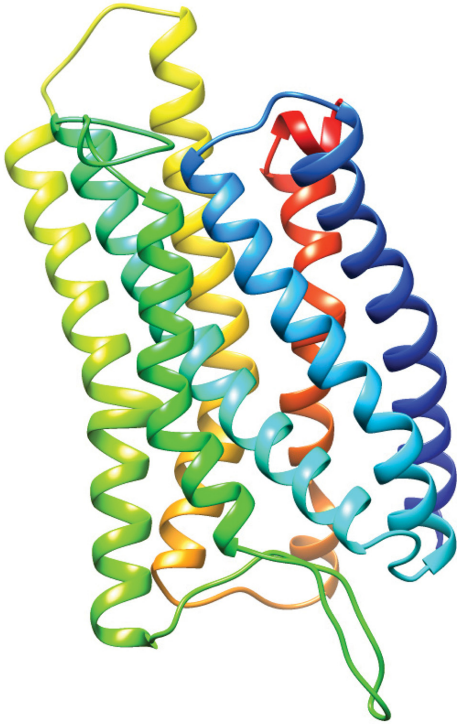

Fig. 2. Homology model of GPR18 receptor structure. Seven transmembrane helices are colored in different color coding. N-Terminal and C-Terminal loop regions are deleted for clarity

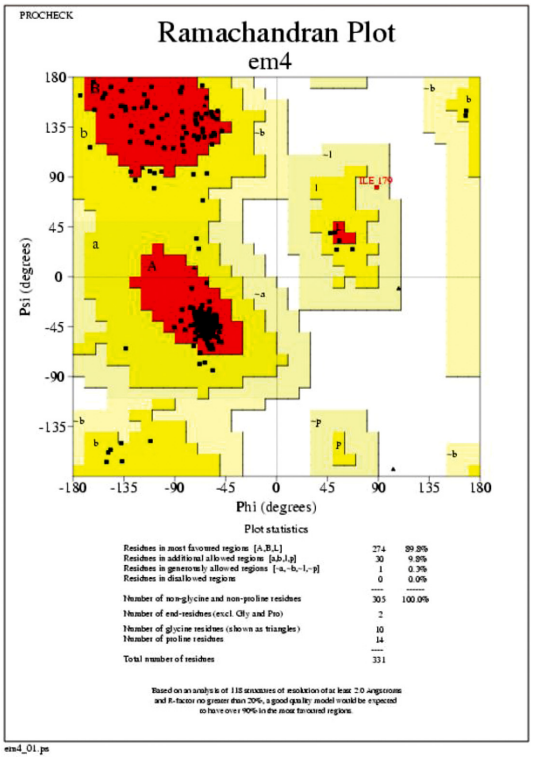

Fig. 3. Ramachandran plot of energy minimized structure of GPR18 receptor.

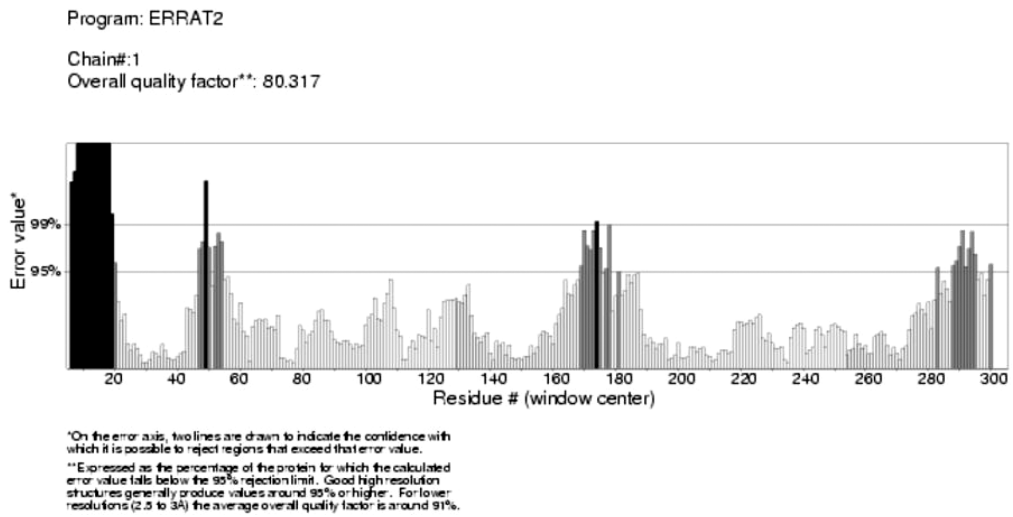

Fig. 4. ERRAT plot of energy minimized structure of GPR18 receptor.

3.3. Discussions

GPR18 is reported to be a class A orphan receptor. The highly expressed orphan GPCR, has intrinsic constitutive activity and could play a role in inhibition of apoptosis in melanoma metastases may also be relevant for other malignancies[20]. As the function of this GPCR is largely unknown, structural information of GPR18 could be helpful and this intended us to initiate this study. Domains that are conserved in the proteins of the same family will have more similar functions. Thus, the model was built with more confidence by selecting the template (GPCR) which will be more reliable, not only structurally but also functionally. It is possible to use multiple templates for modeling. However to retain the crucial disulphide bond between 94 and 172 of GPR18, single template structure was used. We also tried with multiple template structures for modeling. As we couldn't able to get the disulphide bond we stick with the top template for modeling. Ten homology models were developed. Finally, a model with low MolPdf and lower rmsd values was selected. The model was further subjected to energy minimization to remove strains and bad van der waal contacts. The energy minimized model was further validated using PROCHECK and ERRAT plots and the selected model was found to be satisfactory. Our results could be useful for further structure based drug design targeting GPR18.

4. Conclusion

In this short communication, homology modeling of GPR18 was done. Homology model of GPR18 was done using human nociceptin/orphanin receptor as template. Models were developed and one with lower RMSD and MolPdf values was selected and refined. The selected model was further validated using PROCHECK and ERRAT plots. We think our results could be useful.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012001465).

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