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In-silico Modeling of Chemokine Receptor CCR2 And CCR5 to Assist the Design of Effective and Selective Antagonists

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Abstract

Chemokine receptor antagonists have potential applications in field of drug discovery. Although the chemokine receptors are G-protein-coupled receptors, their cognate ligands are small proteins (8 to 12 kDa), and so inhibiting the ligand/receptor interaction has been challenging. The application of structure-based in-silico methods to drug discovery is still considered a major challenge, especially when the x-ray structure of the target protein is unknown. Such is the case with human CCR2 and CCR5, the most important members of the chemokine receptor family and also a potential drug target. Herein, we review the success stories of combined receptor modeling/mutagenesis approach to probe the allosteric nature of chemokine receptor binding by small molecule antagonists for CCR2 and CCR5 using Rhodopsin as template. We also urged the importance of recently available β2-andrenergic receptor as an alternate template to guide mutagenesis. The results demonstrate the usefulness and robustness of in-silico 3D models. These models could also be useful for the design of novel and potent CCR2 and CCR5 antagonists using structure based drug design.

Key words : CCR2, CCR5, Homology modeling

1. Introduction

The chemokine receptor family includes ~20 G-protein-coupled receptors that play a central role in leukocyte migration and activation[1]. Specific family members are also involved in viral entry and angiogenesis. Given this diverse range of important functions, they have been targeted as potential points of pharmaceutical intervention for blunting diseases as diverse as asthma, rheumatoid arthritis, multiple sclerosis, solid organ transplantation, atherosclerosis, cancer, and HIV infection[2]. Chemokines are relatively small proteins (8 to 12 kDa) that vary widely in sequence but exhibit similar tertiary structures. The typical chemokine structure consists of a disordered N-terminus (6 to 10 amino acids), the signature cysteine motif (C, CC, CXC, or CX3C), a loop region, a three-stranded beta-sheet, and a C-terminal alpha helix. Two disulfide bonds typically stabilize this tertiary structure[3].

Among those, CC chemokines are the major family which consists of the Monocyte Chemoattractant Protein-1 (MCP-1), the most characterized protein, also known as 'chemokine ligand CCL2'[4-6]. The CC family consists of several CC receptors (CCR 1-10); of which CCR2 is the primary receptor for MCP-1. Studies show that MCP-1 involves in the pathophysiology of the acute or chronic inflammatory conditions such as rheumatoid arthritis, atherosclerosis, asthma, obesity, and type-2 diabetes. Therefore, CCR2 receptor is an attractive target for the drug discovery[7-9]. CCR5 belongs to family of rhodopsin G-protein coupled receptor which is characterized by 7 transmembrane domains[10]. MIP-1α, MIP-1β and RANTES are natural ligands available for CCR5[11]. Homozygous and heterozygous genotype carriers with CCR5-32 base pair deletion have shown either resistance or prolonged progression of HIV-1 infection[12,13].

In recent years, few pharmaceutical companies are working on the antagonism of CCR2 s well as for CCR5 and several molecules were reported. CCX915 of ChemoCentryx, INCB3284 of Incyte and Pfizer, MK0812 of Merck, MLN1202 of Millennium Pharmaceuticals

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and MCP-1 antagonist of Telik) are under phase 1, phase 2 and preclinical trials for varied disease conditions such as arthritis, multiple sclerosis, and type-2 diabetes. First chemical inhibitor of CCR5 was developed by Takeda Chemicals industries in Japan[14,15]. Currently, there are only few chemical inhibitors of CCR5 are being investigated as anti-HIV agents in human clinical trials[16-21]. Till now only one drug was approved by FDA as CCR5 antagonists is Maraviroc (Selzentry) in August 2007, but it could associated with the increased risk of heart attack and liver damage.

As CCR2 and CCR5 is an important drug target, detailed information about these targets could be useful. There are so many studies regarding the modeling studies of CCR2 and CCR5 were reported in the literature. However, a review paper about these targets would be essential. In this review, we report the successful applications of molecular modeling studies reported on CCR2 and CCR5. We also demonstrate the use of β2-andrenergic receptor as alternate template and its advantage over the proposed rhodopsin template.

2. Experimental Section

2.1. Homology Modeling of CCR2 and CCR5

The application of in-silico modeling methods to drug discovery is still considered a major challenge and proved to be useful, especially when the x-ray structure of the target protein is unknown. With the knowledge of the available rhodopsin template with low sequence identity, modeling of CCR2 and CCR5 is still possible with careful modeling analysis. Several steps needed to get a good model for further analysis. Since the template (Bovine rhodopsin) is a member of GPCR's which comprised of 7-Transmembrane domains which is similar to the topology of CCR2, modeling the transmembrane domains would be crucial. Alignment is the key step in any modeling and alignment obtained using fold recognition servers would be crucial considering the low sequence identity. The model obtained after alignment should undergo a short period of simulation to remove bad contacts and steric clashes. Several groups had performed these methodologies and developed a model for CCR2 and CCR5. The conceptual models developed by different groups were not publicly available for CCR5. However in the case of CCR2 Shi et. al. reported a modeled structure for CCR2 (PDB code: 1KP1) and it is publicly available[22]. The alignment used for the model development and the model obtained

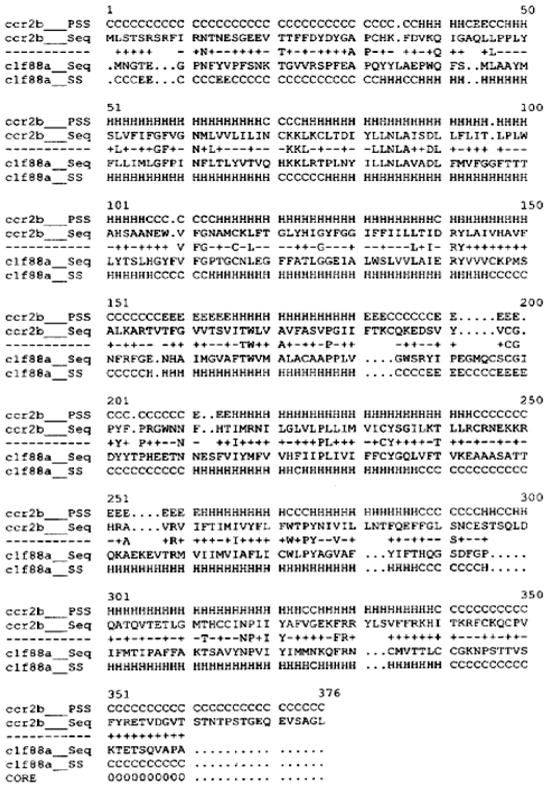

Fig. 1. Sequence alignment between the target (CCR2b) and the template (1F88) using 3D-PSSM

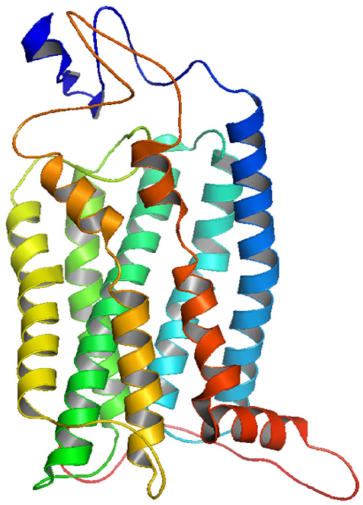

Fig. 2. The model publicly available in the protein data bank for CCR2

after MD analysis is shown in Fig. 1 and Fig. 2.

By using the rhodopsin model as template, several groups proposed combined modeling/mutagenesis studies with potent CCR2 antagonists.

2.2. Successful Application of Rhodopsin Based CCR2 Models

Different groups have published the reports of allosteric binding by CCR2 antagonists and it is summarized. Mirzadegan et al.[23] was the first to characterize that Glu291 in TM7 is an important residue in the binding site of CCR2b antagonists. They identified a family of potent CCR2 antagonists (spiropiperidines). They combined the modeling and mutational studies and found that Glu291 is a crucial residue for high affinity binding. They proposed from the model developed using Bacteriorhodopsin, the acidic residue Glu291 anchors the interaction of spiropiperidine analogs through its contact with the piperidine nitrogen.

Theo Berkhout[24] and his group in 2003 also characterized the binding site of CCR2 antagonists from a combined receptor modeling/mutagenesis approach. The described a classical molecular modeling technique to provides the basis for the design of novel CCR2 antagonists. Initially, they developed a theoretical model of CCR2 and the docking studies was done for potent antagonists such as SB-282241, RS-504393, TAK-779, Teijin and also their own series of indole piperidine compounds. Using the model, they proposed the binding mode for each compounds and based on the results number of site directed mutagenesis studies were done. They found that Glu291, Thr292 in TM7 and Tyr120, His121 in the TM3 are crucial. They refined the theoretical model with bovine rhodopsin and the found that the ligand-docking hypotheses were in complete agreement with the results of mutational studies.

2.3. Successful Application of Rhodopsin Based CCR5 Models

As of CCR2, successful applications of modeling and mutagenesis studies were also reported for CCR5. Fano et. al.[25] in 2006 constructed a 3D model of CCR5 using Bovine rhodopsin as template and assessed through molecular dynamics. Docking simulations was done using two natural agonists and an antagonist. They found new insights into CCR5 interactions. Similarly Kondru and his group in 1998[26] developed a homology model and five CCR5 antagonists maraviroc, vicriviroc, aplaviroc, TAK-779, and TAK-220 were docked into the binding site and subsequent mutagenesis studies was also done. They found that, though the docked antagonists are structurally similar they occupy the same binding site. They also found that Glu283 is an important residue for high affinity binding except in the case of TAK-779.

Maeda et al.[27] combined the mutagenesis and modeling studies to identify the molecular interactions of CCR5 inhibitors with CCR5. They used Apalviroc, Sch-C and TAK-779 in their analyses and found that Apalviroc made extensive contacts with the extracellular loop 2 that that of Sch-C or TAK779. Moreover, a molecular modeling guided mutagenesis study of the binding pocket of CCR5 was done by Castonguay[28] and his group. 2-aryl-4-(piperidin-1-yl)butanamines and 1,3,4-trisubstituted pyrrolidines was used to develop a pharmacophore model based on the structure-activity relationships (SAR) and a human CCR5 receptor docking model using the crystal structure of rhodopsin as a template. Based on the receptor docking model, they had mapped the compounds' site of interaction with CCR5 using sitedirected mutagenesis experiments. They found that the binding site was located within a cavity near the extracellular surface formed by transmembrane helices 2, 3, 6, and 7. They also proposed some crucial residues in the binding pocket through the mutational studies.

2.4. Allosteric Binding of CCR2 and CCR5 Antagonists

A central question in the chemokine field has been whether a small molecule antagonist needs to bind orthosterically to the native ligand to be effective, or whether it can antagonize the ligand by means of allosteric binding to the transmembrane region. As summarized before, both receptor modeling and mutagenesis studies have now suggested that a variety of structurally diverse small molecule antagonists bind to specific chemokine receptors with an allosteric mechanism. When receptor mutagenesis has been used, it has suggested a small molecule ligand binding site within the transmembrane region, capped by extracellular loop 2, and frequently possessing a critical contact with a conserved glutamic acid residue located in TM7. Receptor homology modeling has extended these experimental

observations to suggest that the antagonists typically bind in an extended pocket bounded by TM2, TM3, TM5, TM6, and TM7.

2.5. Use of β2-Adrenergic Receptor Structure as an Alternative Template

As we mentioned earlier, several groups demonstrate the use of the rhodopsin receptor as a template for chemokine receptor modeling with manipulation. An alternate template recently became available with the release of the β2-adrenergic/carazolol crystal structure by Cherezov et al. in 2007[29] warranting a comparison of models based on this new template to the existing rhodopsin models to assess its relevance in chemokine modeling. In contrast to the rhodopsin crystal structure, the β2-adrenergic ligand-binding site is larger and more open because of differences in helical and extracellular loop 2 placement. Because of this, the initial chemokine receptor models constructed from the β2-adrenergic template showed binding sites within the transmembrane region of a size sufficient to dock ligands without manipulation. However modeling the extracellular loop 2 would be crucial to avoid steric clashes in the binding site. The alignment between the target sequences (CCR2 and CCR5) and the template (PDB code: 2RH1) is shown in Fig. 3.

```
           1                                                   50
2rh1-align ..........  ..........  ..........  .......DEV WVVGMGIVMS
CCR2       MLSTSRSRFI RNTNESGEEV TTFFDYDYGA PCHKFDVKQI GAQLLPPLYS
CCR5       ..........  ..MDYQVSSP IYDINYYTSE PCQKINVKQI AARLLPPLYS

           51                                                  100
2rh1-align LIVLAIVFGN VLVITAIAKF ERLQTVTNYF ITSLACADLV MGLAVVPFGA
CCR2       LVFIFGFVGN MLVVLILINC KKLKCLTDIY LLNLAISDLL F.LITLPLWA
CCR5       LVFIFGFVGN MLVILILINC KRLKSMTDIY LLNLAISDLF F.LLTVPFWA

           101                                                 150
2rh1-align AHILMKMWTF GNFWCEFWTS IDVLCVTASI ETLCVIAVDR YFAITSPFKY
CCR2       HSAANE.WVF GNAMCKLFTG LYHIGYFGGI FFIILLTIDR YLAIVHAVFA
CCR5       HYAAAQ.WDF GNTMCQLLTG LYFIGFFSGI FFIILLTIDR YLAVVHAVFA

           151                                                 200
2rh1-align QSLLTKNKAR VIILMVWIVS GLTSFLPIQM HWYRATHQEA INCYAEETCC
CCR2       LKARTVTFGV VTSVITWLVA VFASVPGIIF TKCQKE.... ....DSVYVC
CCR5       LKARTVTFGV VTSVITWVVA VFASLPGIIF TRSQKE.... ....GLHYTC

           201                                                 250
2rh1-align DFFT...... ..NQAYAIAS SIVSFYVPLV IMVFVYSRVF QEAKRQLKF.
CCR2       GPYFP....R GWNNFHTIMR NILGLVLPLL IMVICYSGIL KTLLRCRNE.
CCR5       SSHFPYSQYQ FWKNFQTLKI VILGLVLPLL VMVICYSGIL KTLLRCRNE.

           251                                                 300
2rh1-align .CLKEHKALK TLGIIMGTFT LCWLPFFIVN IVHVIQD... ......NLIR
CCR2       ..KKRHRAVR VIFTIMIVYF LFWTPYNIVI LLNTFQEFFG LSNCESTSQL
CCR5       ..KKRHRAVR LIFTIMIVYF LFWAPYNIVL LLNTFQEFFG LNNCSSSNRL

           301                                                 350
2rh1-align KEVYILLNWI GYVNSGFNPL IYCR.SPDFR IAFQELLCL. ..........
CCR2       DQATQVTETL GMTHCCINPI IYAFVGEKFR RYLSVFFRKH ITKRFCKQCP
CCR5       DQAMQVTETL GMTHCCINPI IYAFVGEKFR NYLLVFFQKH IAKRFCKCCS

           351                                                 400
2rh1-align ..........  ..........  .......
CCR2       VFYRETVDGV TSTNTPSTGE QEVSAGL
CCR5       IFQQEAPERA SSVYTRSTGE QEISVGL
```

Fig. 3. The alignment between the target sequences (CCR2 and CCR5) and the template (PDB code: 2RH1)

3. Conclusion

As discussed previously, numerous contributions have been made that have used site-directed mutagenesis and receptor homology modeling as a template for rationalization of the binding of known ligands. With the knowledge of mutagenesis studies it is possible to provide the binding of ligands that are consistent with experimental results. By performing the modeling studies using the available templates is it good enough' to allow for SAR-guided docking in the absence of other experimental evidence. However, no articles have described the use of homology models to drive progress in a program in the absence of mutagenesis data. Further studies focusing on these issues would be encouraging. Moreover combination of insilico methodologies (QSAR, Comparative modeling and Docking) with the experimentalist could be really successful and provide a starting point for the synthesis of more potent and selective antagonists.

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