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Binding modes of CCR5-targetting HIV entry inhibitors: Partial and full antagonists

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Abstract

Since the CC-chemokine receptor 5 (CCR5) was identified as a major co-receptor for human immunodeficiency virus type 1 (HIV-1) entry into a host cell, CCR5-targetting HIV entry inhibitors have been developed and some of them are currently in clinical trials. Most of these inhibitors also inhibit the physiological chemokine reaction function of CCR5, which is so far considered to be safe to patients based on the observation that individuals that naturally lack CCR5 do not show apparent health problems. Nevertheless, to minimize the toxicity and side effects, it would be ideal to preserve the chemokine receptor activity. In this work, we simulated the flexible docking of two small molecule inhibitors to CCR5 in a solvated phospholipid bilayer environment. One of the inhibitors, aplaviroc has a unique feature of preserving two of the natural chemokine ligands binding to CCR5 and subsequent activation whereas the other one, SCH-C fully blocks chemokine-CCR5 interactions. Our results revealed significantly different binding modes of these two inhibitors although both established extensive interaction networks with CCR5. Comparison of the different binding modes suggests that avoiding the deep insertion of inhibitors into the transmembrane helix bundle may be able to preserve chemokine-CCR5 interactions. These results could help design HIV co-receptor activity-specific inhibitors.

Keywords: CC-chemokine receptor 5 (CCR5); HIV entry inhibitors; Antagonists; Molecular dynamics simulation; Flexible docking

1. Introduction

Inhibitors that can prevent human immunodeficiency virus type 1 (HIV-1) from entering into host cells have emerged as a new generation of antiretroviral drugs. These HIV entry inhibitors mainly target the interactions between the viral surface glycoprotein gp120 and plasmatic membrane receptors and co-receptors of the host cell. One of such membrane co-receptors is the CC-chemokine receptor 5 (CCR5), a rhodopsin-like G-protein coupled receptor (GPCR). While CCR5 was identified as an co-receptor of HIV viral entry [1,2], it was found that individuals that naturally lack CCR5 are resistant to HIV infection and do not show apparent health problems [3,4]. This suggests that blocking the function of CCR5 or even removing CCR5 from the cell membrane by receptor internalization may provide an effective way against viral entry without producing significant health impact on patients. In fact, the first identified class of CCR5-mediated HIV entry inhibitors are the natural chemokine protein ligands of CCR5, RANTES, MIP-1α, and MIP-1β [5]. But, because protein drugs have the disadvantage of poor oral availability, the development of CCR5-targetting HIV entry inhibitors has been focused on small molecules. As a result, a considerable number of CCR5-binding small molecules have been identified to be effective for preventing viral entry and some of them have been in clinical trials [6–8]. These molecules act as dual antagonists of the chemokine receptor activity and the HIV entry co-receptor activity of CCR5. Nevertheless, the inhibition of CCR5 chemokine function is not necessary for, and does not always result in, the inhibition of the CCR5-gp120 binding because they are two independent functions of CCR5 [9]. Moreover, previous reports have shown that the viral gp120 protein and CC-chemokines bind in different regions of CCR5 [10–13]. Therefore, it should be feasible to design inhibitors that specifically disrupt CCR5-gp120 binding and viral entry but do not affect the function of CCR5 chemokine activation, namely discriminatorily against the HIV entry co-receptor activity of

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CCR5. This strategy is apparently more challenging but likely provides more clinical advantages with minimal toxicity and side effects. Encouragingly, the first few such inhibitors have been identified [14,15], which are spirodiketopiperazine derivatives with aplaviroc being the representative.

Apparently, a detailed understanding of the binding modes of the existing inhibitors would help design more potent drugs, and more important, comparison between non- or partial-antagonists and full antagonists can provide valuable insights into the structural determinants responsible for preserving the CCR5 chemokine receptor activity and thus help design more HIV co-receptor activity-specific inhibitors. Unfortunately, experimentally determined three-dimensional structure is not available for either CCR5 or CCR5-ligand complexes. Studies of the CCR5-inhibitor binding interactions have to reply on site-directed mutagenesis experiments and molecular modeling techniques. Recently, Maeda et al. [16] conducted the site-directed mutagenesis analysis of the binding of aplaviroc and two other inhibitors to CCR5 and they used the data to construct the structural models of CCR5-inhibitor complexes. In the CCR5-inhibitor complex structures constructed there, aplaviroc and the other inhibitors occupied similar binding pockets although the detailed CCR5-inhibitor interactions were different. The question about why aplaviroc is the only inhibitor able to preserve chemokine receptor activity of CCR5 while all bind to CCR5 remains open.

In this work, we combined molecular modeling and simulation techniques to study the binding of aplaviroc [14] and another inhibitor SCH-C [17] to CCR5, mainly based on the structural features of CCR5 and the inhibitors by referring to the crystal structure of the bovine rhodopsin [18]. SCH-C is an oxime-piperidine compound. Similar to aplaviroc, it binds to CCR5 with high affinities at the nanomolar level and highly effectively blocks the viral gp120-CCR5 binding with IC_{50} values of nanomolar concentrations (see Fig. 1 for the chemical structures). However, SCH-C fully blocks chemokine-CCR5 interactions whereas aplaviroc preserves the chemokine binding and signaling of CCR5 by allowing RANTES and MIP-1β binding at its anti-HIV activity-exerting concentrations. We performed flexible docking of these two inhibitors to CCR5 in a solvated phospholipid bilayer environment. The docking results reveal the different CCR5-binding modes of these two inhibitors, which are consistent with the available site-directed mutagenesis data. More importantly, comparison between the different binding models and the different properties of these two inhibitors and other inhibitors suggested the possible mechanism of preserving or blocking the physiological function of chemokine binding and activation of CCR5.

2. Methods

2.1. Model building of CCR5 with membrane and water

The modeling of CCR5 was based on a three-dimensional model built by using the threading assembly refinement method [19] and provided by Dr. Jeffrey Skolnick at Georgia Tech (http://cssb.biology.gatech.edu/skolnick/files/gpcr/gpcr.html). One of the major differences of this method from the traditional homology modeling is that it does not require a template with high sequence identity and it allows conformational changes from the template. The CCR5 model has 352 amino acid residues (SwissProt entry: P51681) and a core structure of a seven-helix bundle in the transmembrane region. There are 87.3% residues in the Ramachandran plot core region, 9.9% in the allowed region, according to the PROCHECK program [20]. The CCR5 model has a high confidence score of 2.0 according to the prediction method, indicating its high possibility of having a correct fold. The Cα atom root-mean-square deviation (RMSD) was 0.88 Å when superimposing with the bovine rhodopsin crystal structure [18] (PDB entry: 1U19 and chain A) after structure-based sequence alignment by using the Protein3Dfit program [21] (http://www.protein3dfit.uni-koeln.de/3dalign_neu/cgi-bin/3daligner.py). The hydrogen atoms were added by using the REDUCE program [22].

This initial CCR5 model was first subject to energy minimization by using the AMBER8 [23] program with the Generalized-Born implicit solvent model [24] until the energy gradient reached 10.0 kcal/mol Å. Then, to mimic the membrane environment, the energy-minimized CCR5 model was inserted into a patch of phospholipid bilayer generated by the Membrane module in the VMD1.8.5 program [25], which consisted of 112 palmitoyloleoyl-phosphatidylcholine (POPC) lipid molecules and had a dimension of ca. 75 Å × 75 Å. The POPC lipid was used because it is the main constituent of lipid bilayers. The embedded CCR5 together with the membrane was then solvated in a TIP3P water box and charge neutralized by

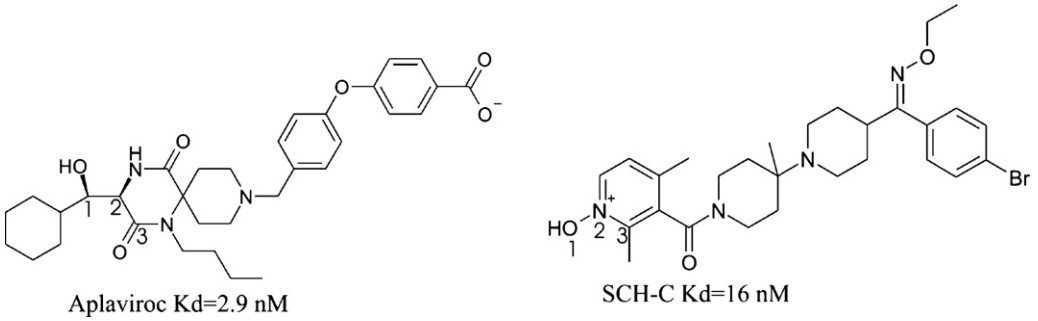

Fig. 1. Chemical structures of aplaviroc and SCH-C and their binding constants with CCR5. The labeled atoms were used for superimposition onto retinal to obtain the initial placements in CCR5.

using the tLeap module in the AMBER8 program. This added 24565 solvent water molecules and 13 chloride ions.

The parameters of POPC lipid molecules were derived by using the Antechamber module in the AMBER8 program and the GAFF force field [26]. A disulfide bond was added between Cys101 and Cys178. The parameters of protein residues were assigned based on the AMBER ff03 force field [27].

2.2. Simulations of CCR5 with membrane and water to equilibrium states

We performed simulations to relax the modeled system. The simulations were carried out by using the PMEMD module in the AMBER8 program. The modeled system was first subject to a two-stage energy minimization. In the first stage of 2000 steps, CCR5 was restrained to its starting positions by a harmonic potential with a force constant of 32 kcal/(mol Å^2) while the others were unrestrained. In the second stage of 2000 steps, no restraint was applied. After energy minimization, the whole system was subjected to a gradual heating from 10 K to 300 K in 50 ps and with constant volume while CCR5 and the head groups of the lipid molecules were restrained by a force constant of 32 kcal/(mol Å^2). The system was then shifted to constant temperature of 300 K and pressure of 1.0 atm. The restraints were removed from the lipid molecules after 500 ps and from CCR5 after another 500 ps. The simulation continued for another 500 ps without any restraint. The bonds involving hydrogen atoms were constrained by using the SHAKE algorithm. The Particle Mesh Ewald (PME) method [28] was used for long-range electrostatic interactions with the default parameters. The time step was 1 fs, and the non-bonded interactions were updated every 10 time steps.

2.3. Modeling of the inhibitors

The starting structures of the inhibitors were built in the MOE software (Chemical Computing Group, Inc.) and the conformations were optimized at the HF/6-31G* level of theory by using the Gaussian03 program [29]. The carboxylate group in aplaviroc was deprotonated. The oxime oxygen atom in SCH-C was protonated, similar to salicylaldoxime that has an experimentally measured pK_a value of 9.2 [30]. The parameters of both inhibitors were derived by using the Antechamber module in the AMBER8 program and the GAFF force field [26].

2.4. Simulations of CCR5-inhibitor docking

The putative inhibitor-binding pocket of CCR5 was assumed to correspond to the retinal binding pocket in the rhodopsin crystal structure as that is the only cavity having potential to accommodate the inhibitors of the sizes of aplaviroc and SCH-C. In addition, same as in rhodopsin, this pocket is completely buried inside the transmembrane domain, enclosed by the seven transmembrane helices and the β-hairpin loop connecting helix 4 and helix 5 on the extracellular surface. We previously simulated the egress of retinal from the buried binding pocket in rhodopsin [31], but a direct simulation of ligand entry appeared to be difficult. Instead, we used modeling techniques to obtain the initial positions of the inhibitors in the putative binding pocket of CCR5. Firstly, the crystal structure of the bovine rhodopsin [18] (PDB entry: 1U19 and chain A) was superimposed onto the CCR5 structure that had been simulated and relaxed in membrane and water and then the rhodopsin protein was removed with only retinal, the Schiff base and the CE atom of Lys296 left within the CCR5 system. The retinal molecule positioned in CCR5 was then used as a starting point to place the inhibitors. Based on the assumption that the β-hydroxyl group in aplaviroc and the oxime-hydroxyl group in SCH-C should form hydrogen bonds to the only acidic residue Glu283 in the putative binding pocket, we superimposed the inhibitors onto retinal by using the three atoms labelled 1, 2 and 3 in Fig. 1, corresponding to the CE atom of Lys296, the NZ atom of the Schiff base and the C15 atom of retinal, respectively. This way of superimposition avoided interrupting the backbone structure of the protein, resulting in overlaps only with the side chains of some of the protein residues. Furthermore, the side chains of the CCR5 residues that overlapped with the inhibitors were manually adjusted, which included Tyr108, Phe109, Ser180 and Met287 for aplaviroc and Phe112, Phe113, Ile116, Trp248, Tyr251 and Met287 for SCH-C. This generated the initial orientations of the inhibitors in the putative binding pocket of CCR5 and resulted in the initial CCR5-inhibitor complex models. We then applied molecular dynamics simulations to obtain the final binding modes of CCR5 and the inhibitors. First, the whole system, including the inhibitor, the CCR5 protein, the lipid bilayer, the chloride ions and the solvent water, was energy minimized until energy gradient reached 50.0 kcal/mol Å and then was gradually heated from 0 K to 300 K in 110 ps in constant volume while the Cα atoms of the CCR5 protein were restrained by a force constant of 32 kcal/(mol Å^2). Afterwards, the system was shifted to constant pressure of 1.0 atm with constant temperature of 300 K. The restraints on the Cα atoms of the CCR5 protein were removed after 2 ns and the simulations continued for another 2 ns without any restraints. The bonds involving hydrogen atoms were constrained by using the SHAKE algorithm. The Particle Mesh Ewald (PME) method [28] was used for long-range electrostatic interactions with the default parameters. The time step was 1 fs, and the non-bonded interactions were updated every 10 time steps.

3. Results and discussion

3.1. Putative inhibitor-binding pocket of CCR5

The putative inhibitor-binding pocket of CCR5 was assumed to correspond to the retinal-binding pocket in the rhodopsin crystal structure [18] as that is the only cavity having potential to accommodate the inhibitors of the sizes of aplaviroc and SCH-C. This pocket is also in the region where amino acid substitutions showed significant effects on inhibitor binding and viral entry [16,32–34]. In addition, same as in rhodopsin, this pocket is completely buried inside the transmembrane domain, enclosed by the seven transmembrane helices and the

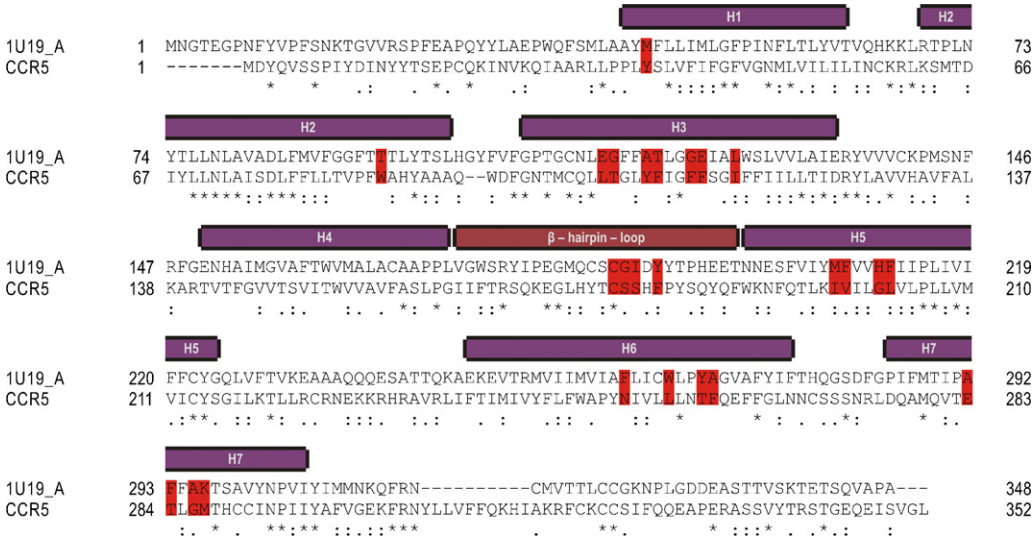

Fig. 2. Sequence alignment of CCR5 with bovine rhodopsin (PDB entry: 1U19 and chain A). The residues in 3 Å distance to retinal in rhodopsin and the corresponding residues in CCR5 are colored red.

β-hairpin loop connecting helix 4 and helix 5 on the extracellular surface. The sequence alignment of CCR5 with bovine rhodopsin is shown in Fig. 2 and the residues in 3Å distance to retinal in rhodopsin and the corresponding residues in CCR5 are colored red. We can see that several residues corresponding to those lining the binding pocket of retinal in rhodopsin are large aromatic residues in CCR5, including Tyr37 in helix 1, Trp86 in helix 2, Tyr108, Phe109, Phe112 and Phe113 in helix 3, Phe260 in helix 6. Moreover, Tyr108, Phe109, Phe112, Phe113 protruded into the pocket and formed tight aromatic packing together with Tyr251, Trp248 during the equilibrium simulation of CCR5 in the solvated phospholipid bilayer. As a result, the pocket was too small to accommodate the inhibitors (Fig. 3) but could be enlarged by rotating some of the side chains. To accommodate aplaviroc and SCH-C, considering the specific shapes of these two ligands, the following residues were adjusted, Tyr108, Phe109, Ser180 and Met287 for aplaviroc and Phe112, Phe113, Ile116, Trp248, Tyr251 and Met287 for SCH-C, respectively. The enlarged pocket (Fig. 3) avoided the overlap between the inhibitors and the backbone of the protein, providing the starting points for docking simulations allowing flexibility of both inhibitor and protein.

In addition, similar to rhodopsin, there are very few charged residues in the transmembrane region. Glu283 in helix 7 is the only acidic residue in the transmembrane region close to the extracellular side and it lacks a counterion in its vicinity. Glu283 tended to point towards the extracellular side and in a 6 Å distance to Arg274 during the equilibrium simulation. It could be a reasonable assumption that Glu283 may form hydrogen bonds to inhibitors.

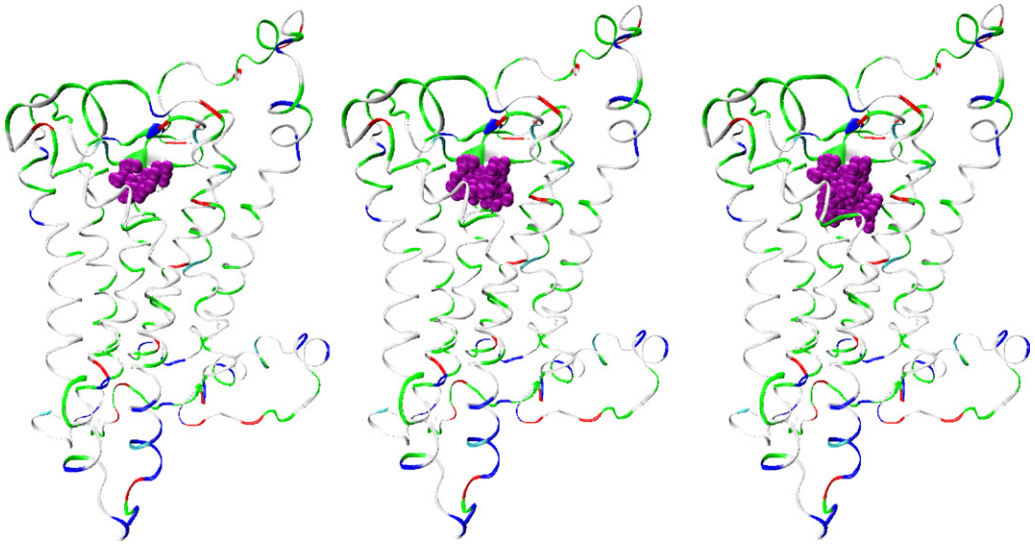

Fig. 3. The purple balls show the putative inhibitor-binding pockets of CCR5, detected by the PASS [44] program, which is buried in the interface region between the transmembrane domain and the extracellular β-hairpin loop. (Left) The last snapshot structure of CCR5 in the equilibrium simulation. (Middle) Side chains of Tyr108, Phe109, Ser180 and Met287 were adjusted for docking aplaviroc. (Right) Side chains of Phe112, Phe113, Ile116, Trp248, Tyr251 and Met287 were adjusted for docking SCH-C.

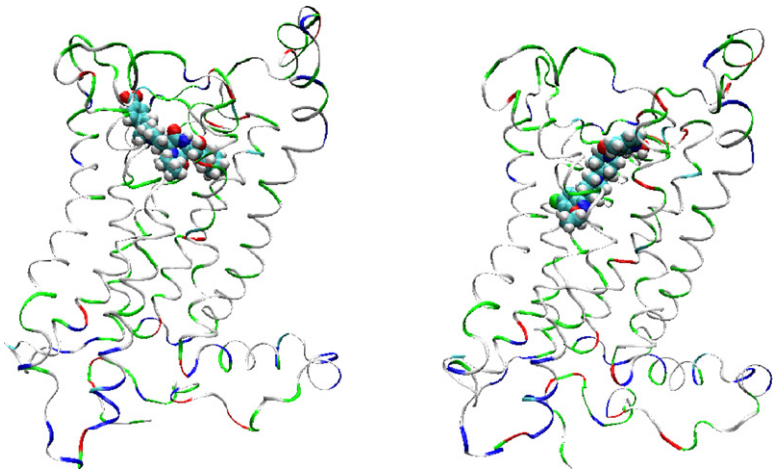

Fig. 4. Structures of the CCR5-inhibitor complexes after the 4.11 ns molecular dynamics simulations. The CCR5 proteins are shown in ribbon and the inhibitors are highlighted by the Van der Waals sphere representations. (Left) Aplaviroc; (Right) SCH-C. Aplaviroc bound in a shallow pocket underneath the extracellular β-hairpin loop while SCH-C bound in a pocket spanning into the middle of the transmembrane bundle.

3.2. Binding modes of aplaviroc and SCH-C with CCR5

During the 4.11 ns molecular dynamics simulations, the relative orientations of the inhibitors in CCR5 were explored and optimized, accompanied by conformational changes in the inhibitors and CCR5, mainly the protein side chains in the proximity. This established extensive, however, significantly different CCR5-inhibitor interaction networks for aplaviroc and SCH-C. Fig. 4 illustrates the docked inhibitor positions and Fig. 5 shows the structures of the binding pockets.

Aplaviroc bound in a shallow pocket underneath the extracellular β-hairpin loop. The protein residues that are in 3 Å distance to the inhibitor are Tyr37 in helix 1, Pro84 in helix 2, Thr105, Tyr108, Phe109 and Phe112 in helix 3, Pro162 and Gly163 in helix 4, Phe166, Arg168, Thr177, Ser180 and Phe182 in the extracellular β-hairpin loop, Lys191 in helix 5, Pro250, Tyr251, Val254, Leu255, and Asn258 in helix 6, Arg274 in the extracellular loop between helix 6 and helix 7, Met279, Glu283 and Met287 in helix 7. Glu283 and Arg274 formed hydrogen bonds to the β-hydroxyl group of the inhibitor at one end and Lys191 formed a hydrogen bond to the carboxyl group of the inhibitor at the other end. The alanine mutation of Glu283 and Lys191 have been shown to lead to the drastic loss of CCR5-aplaviroc binding [16], which could be due to the loss of the hydrogen bonds. No site-directed mutagenesis data are yet available for Arg274. Glu283 and Arg274 also formed an intramolecular hydrogen bond between each other, further stabilizing the intermolecular hydrogen bonding network. The mutation of Gly163 to arginine was observed to diminish inhibitor binding [16], which could be explained by the largely reduced size of the binding pocket in the mutant. The only residue which alanine scanning resulted a drastic loss of binding but not on the above 3 Å-distance list was Cys178 in the extracellular β-hairpin loop. Cys178 was presumed to form disulfide bond to Cys101 in helix 3 and its importance for inhibitor binding may arise from its crucial role on the conformation of CCR5 [35]. In addition to the hydrogen bonding interactions, aplaviroc has substantial hydrophobic contacts with CCR5, including the face-to-face π-stacking between the two phenyl rings of the inhibitor and Phe166 and Phe182 as well as the face-to-face hydrophobic packing between the cyclohexyl group of the inhibitor and Tyr108. In addition, four solvent water molecules entered the binding pocket and in 3 Å distance to aplaviroc, which established a hydrogen bonding network among the inhibitor, Ser180 and the water molecules. The aromatic cluster of Phe109, Phe112, Phe113 and Tyr251 was beneath the inhibitor. The overall orientation of aplaviroc is similar to the model constructed by Maeda et al. [16] but with more extensive CCR5-inhibitor interactions.

Different from aplaviroc, SCH-C bound in a pocket spanning into the transmembrane bundle up to the middle of helix 3. The protein residues that are in 3 Å distance to the inhibitor are Tyr108, Phe109, Phe112, Phe113, Ser114, Gly115, Ile116 and Phe117 in helix 3, Glu172, Gly173, Thr177, Cys178 and Ser179 in the extracellular β-hairpin loop, Ile198, Gly202, and Leu203 in helix 5, Trp248, Tyr251, Asn252, Val254 and Leu255 in helix 6, Ala278, Met279, Thr282 and Glu283 in helix 7. The hydrophobic end of the inhibitor containing the bromobenzene group and the ethoxyl group deeply inserted into the transmembrane bundle, surrounded by Phe109, Phe112, Phe113, Ile116, Ile198, Trp248, Tyr251 and Leu255. The observed greater effects of mutations of Phe112, Phe113, Ile198 on the binding of SCH-C than aplaviroc [16] could be due to the tighter contacts between SCH-C and these residues. Similar to aplaviroc, SCH-C formed a hydrogen bond with Glu283 through its oxime-hydroxyl group, which could explain the dramatic loss of binding affinity when Glu283 was mutated to alanine [16]. The other terminal oxygen atom of Glu283 formed two intramolecular hydrogen bonds to the hydroxyl groups of Thr177 and Tyr108, respectively. The oxime-piperidine ring of the inhibitor fitted into a hydrophobic pocket created by the side chains of Glu172 and Arg274 that formed a salt link between each other. In addition, two solvent water molecules were in the 3 Å distance to the inhibitor. The binding mode of CCR5-SCH-C obtained

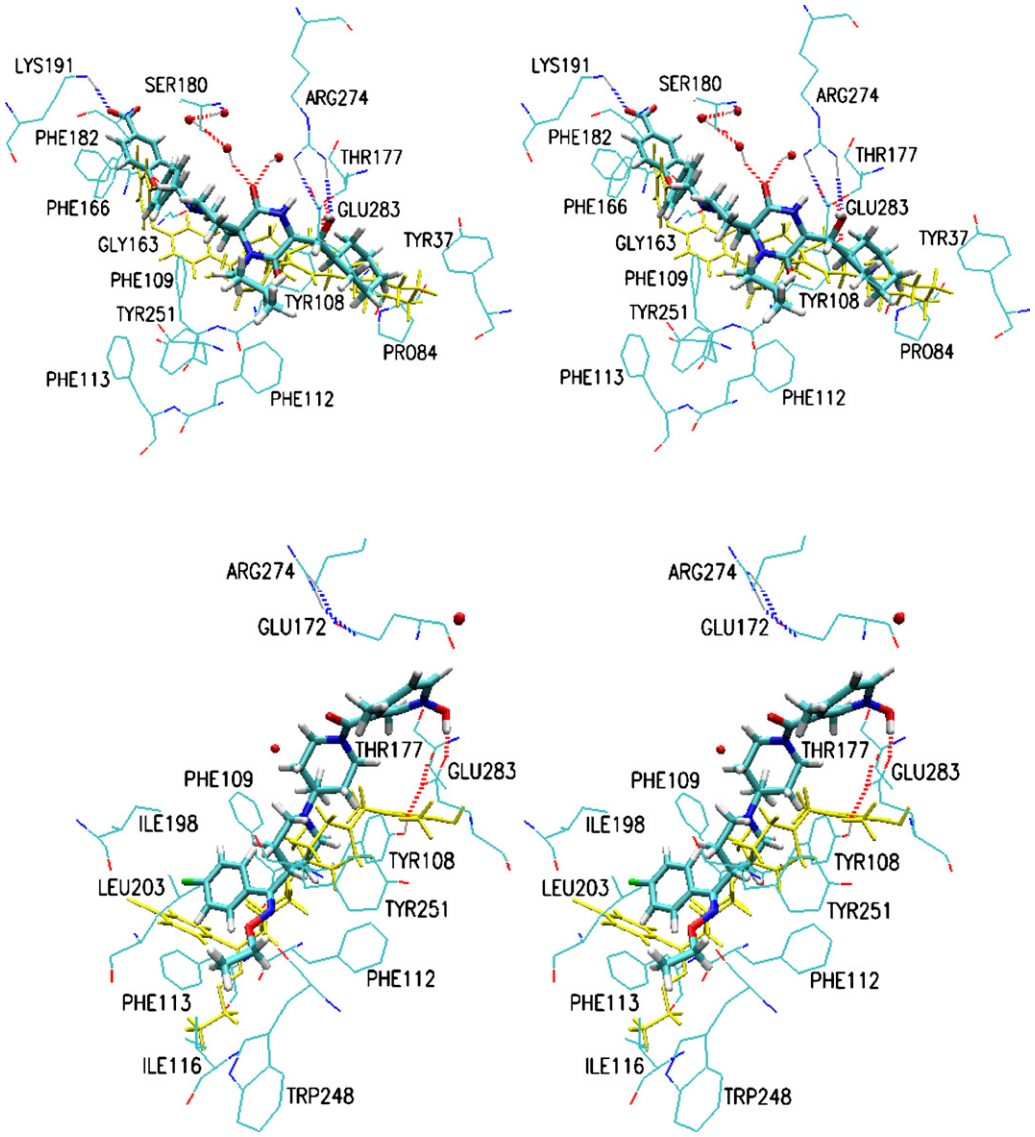

Fig. 5. Stereoviews of the interaction networks between CCR5 and the inhibitors, established during the flexible docking simulations. The inhibitors aplaviroc and SCH-C are shown in sticks and their starting conformations were colored yellow. The protein residues showing important interactions with the inhibitors are shown by lines. For clarity, some residues in 3 Å distance to the inhibitors are not shown, see text for the complete lists. The water molecules in 3 Å distance to the inhibitors are shown in red balls. The hydrogen bonds are shown in dashed lines. The pictures were made from the same view after superimposing the protein onto the starting conformations. (upper): aplaviroc; (lower): SCH-C.

here is different from the model constructed by Maeda et al. [16], where SCH-C was located almost horizontally beneath the extracellular β-hairpin loop. Moreover, because the oxime oxygen atom was treated deprotonated in that model, it formed a hydrogen bond to Tyr37 instead of Glu283, which was used to explain the dramatic loss of binding affinity when Tyr37 was mutated to alanine. However, in a recent report by Seibert et al. [34], the mutations of Tyr37 to phenylalanine or tryptophan resulted in little or no impact on inhibition of HIV-1 entry. This indicated that Tyr37 contributed to the inhibitor binding most likely by its aromatic ring rather than forming a hydrogen bond to the inhibitor. In our model, Tyr37 and nearby residues consisted of a hydrophobic cluster and its hydroxyl group formed a hydrogen bond to the backbone oxygen atom of Glu283, which may play a critical role in stabilizing the CCR5 conformation required for SCH-C binding.

3.3. Relating inhibitor-CCR5 binding modes to chemokine antagonistic activity

The precise way by which chemokines interact with and activate CCR5 is unknown. What we know from the available site-mutagenesis data on chemokine-CCR5 binding and activation, is that the core domains of chemokines bind to the extracellular domain of CCR5 while the N-terminal of chemokines interact with the transmembrane helix bundle of CCR5 [13,36–38], particularly the aromatic cluster in the middle of helix 3. This means that chemokine-CCR5 interactions may be interrupted by changes in either the extracellular domain or the transmembrane helix bundle in an inhibitor-bound CCR5. In the cases of aplaviroc and SCH-C, the binding mode of aplaviroc is characterized by an almost horizontal orientation underneath the extracellular β-hairpin

loop whereas SCH has much fewer interactions with the extracellular domain but deeply inserts into the transmembrane domain. Given the facts that aplaviroc and RANTES bind simultaneously to CCR5 [14] whereas SCH-C fully block chemokine binding, it is reasonable to infer that the deep insertion of SCH-C into the transmembrane helix bundle may be responsible for the blockage of chemokine-CCR5 interactions. This inference can be further supported by the similar binding modes of other inhibitors that also act as full antagonists of chemokine-CCR5 interactions, including the first small molecule inhibitor TAK-779 [39] and a series of 1-amino-2-pheny-4-(piperidin-1-yl)-butane analogues developed by Merck Research Laboratories. For TAK-77, site-directed mutagenesis experiments conducted by Dragic et al. [32] suggested that the long methylphenylbenzocycloheptenyl group inserted into the transmembrane hydrophobic pocket and a computational docking study by Fano et al. [40] provided an detailed structural picture of such a binding mode where Phe109, Phe112, Phe113, Ile198 and Trp248 consisted of the hydrophobic pocket. The same hydrophobic pocket accommodated the *N*-methyl-*N*-phenylsulfonylamino moiety of the Merck inhibitors in the docking models developed by Xu et al. [41]. A possible explanation could be that the occupancy of this transmembrane hydrophobic pocket by inhibitors may prevent the interactions between the N-terminal of chemokines and CCR5 and thus lead to loss of binding. Another explanation could be that the deep binding of antagonists to CCR5 induced undesirable conformational changes against chemokine binding. This is, however, not obvious in our docking simulations as no significant conformational changes were observed in CCR5. The backbone root-mean-square deviations (RMSD) of CCR5 from the inhibitor-free conformation were 2.66 Å with aplaviroc and 2.64 Å with SCH-C, respectively. In addition, the deep insertion of inhibitor into the transmembrane region may also have impacts on CCR5 activation as it can hinder the relative movement of helix 3 and helix 6, which is generally believed to be crucial for the activation of CCR5 [42,43].

4. Conclusion

By using molecular dynamics docking simulations, we obtained hypothetical models of the complex structures of CCR5 binding to two HIV entry inhibitors, aplaviroc and SCH-C in a solvated phospholipid bilayer environment, starting from the initial placements based on the structural features of both the inhibitors and CCR5. Apart from their high activities of inhibiting the viral entry co-receptor activity of CCR5, aplaviroc has a unique feature of preserving two of the natural chemokine ligands binding to CCR5 and subsequent activation whereas SCH-C fully blocks chemokine-CCR5 interactions. The obtained complex structures exhibited significantly different binding modes of the two inhibitors while both inhibitors showed extensive interactions with CCR5. Aplaviroc bound in a shallow pocket underneath the extracellular β-hairpin loop whereas SCH-C deeply inserted into the transmembrane helix bundle and its hydrophobic end was located in the inter-helical cleft between helix 3 and helix 6. We proposed that the different chemokine antagonistic activities of these two inhibitors can be related to their different binding modes. To preserve the physiological chemokine activation function of CCR5, inhibitors should bind in a way avoiding deep insertion into the transmembrane helix bundle of CCR5. This suggestion can be supported by the similar binding modes of other inhibitors that also act as full antagonists of chemokine-CCR5 interactions. Insights from this work could help design HIV co-receptor activity-specific inhibitors.

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