ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Jun; 21(6): 521~528
http://www.chinaphar.com


# Building three-dimensional structures of HIV-1 coreceptor CCR5 and its interaction with antagonist TAK779 by comparative molecular modeling[1]

HUANG Xiao-Qin, JIANG Hua-Liang[2], LUO Xiao-Min, CHEN Kai-Xian[2], JI Ru-Yun (*Shanghai Institute of Materia Medica, Shanghai 200031, China*); CAO Yang (*Chemistry Department, Suzhou University, Suzhou 215006, China*); PEI Gang (*Shanghai Institute of Cell Biology, Shanghai 200031, China*)

**KEY WORDS** TAK 779; CCR5 receptor; molecular structure; HIV-1; computer aided design

## ABSTRACT

**AIM**: To study the mechanism of interaction of CCR5 receptor with its antagonist TAK779. **METHODS**: Comparative molecular modeling has been used to develop the 3D-structural models of CCR5 receptor and its complex with TAK779. Molecular mechanics has been applied to optimize the above molecular models. Quantum mechanics has been utilized to calculate the structural information of TAK779. DOCK4.0 program is employed to dock the TAK779 molecular into the binding site of CCR5 receptor. **RESULTS**: The 3D-structural model of CCR5 receptor is constructed using the 3D-model of frog rhodopsin as a template. The binding pocket is situated in the transmembrane helices 3, 5, 6, and 7, and it is composed of conserved residues of Tyr108, Gly111, Ser114, Glu283, Gly286, and Cys290, and conservatively varied residues including Thr105, Leu107, Phe112, Gly115, Lys197, and Met287. $O_1$, $N_7$, $N_{17}$, and $O_{19}$ of TAK779 are the active center of TAK779. The pyran cycle and the aminium group of TAK779 interact with residues in the binding pocket of CCR5 receptor, the other part of TAK779 interacts with residues from the extracellular loops of CCR5. The binding energy of TAK779 with CCR5 is -51.606 kcal/mol. **CONCLUSION**: The model constructed and the interaction mode reported in the present study are useful in further understanding the molecular mechanism of receptor-virus recognition and designing new inhibitors of HIV-1 infection.

## INTRODUCTION

Recently discovered chemokine receptor CCR5 is an integral membrane protein, which promotes macrophage-tropic (M-tropic) human immunodeficiency virus type 1 (HIV-1) entry into the cell[1]. Putative molecular mechanism of this process is described as follows[2,3]: (i) The HIV-1 glycoprotein gp120 recognizes CD4 by receptor on the cell surface, and the CD4 providing a docking surface for gp120 subsequently promotes exposure of regions that can interact with the chemokine receptors; (ii) On being complexed with CD4, gp120 interacts directly with CCR5. The gp120's binding site to CCR5 is located withen the V3 loop. The interaction of gp120 with CCR5 is greatly enhanced in the presence of CD4; (iii) A conformational change occurs in the CD4-gp120-CCR5 complex leading virus glycoprotein gp41 into the cell membrane.

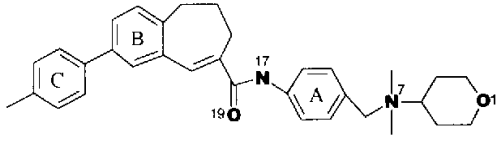
The interaction of CCR5 with the viral glycoprotein is critical for membrane fusion and virus entry. Blockade of such binding is believed to be extremely useful in the inhibition of HIV-1 infection. For this to be done, molecular mechanism accompanying the receptor-virus recognition and binding should be understood. Protein engineering approaches including modification of the functionally important residues and structural determination of gp120 complexed with CD4 receptor, have provided a framework for understanding the complex biology of HIV-1 entry into cells. The structure of a conserved HIV-1 gp120 involved in chemokine receptor CCR5 binding has also been determined recently[4]. Epitope mapping of CCR5 reveals that extracellular loop2 (EL-2) monoclone antibodies (mAbs) are more potent inhibitors of viral infection than N-terminal mAbs[5]. Thus, the

[1] Project supported by the Key Program of National Natural Science Foundation of China (Grants 29790123 and 29725203).
[2] Correspondence to Prof CHEN Kai-Xian, Prof JIANG Hua-Liang
Phn 86-21-6431-1833, ext 222. Fax 86-21-6437-0269.
E-mail kxchen@mail.shcnc.ac.cn jiang@mail.shcnc.ac.cn
Received 1999-09-29 Accepted 2000-03-15

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Jun; 21 (6)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

ability to block virus infection does not correlate with the ability to block gp120 binding. Further experiments show that a mAb named 2D7 binds to a unique antigenic determinant in the first half of EL-2 of CCR5 receptor[5]. It can be infered that the epitope recognized by 2D7 may represent a particularly attractive target for CCR5 antagonists. Thus, molecules involved in the EL-2 of CCR5 binding with the receptor may have higher efficacy in inhibiting the HIV-1 virus infection. Developing a three-dimensional (3D) model of CCR5 and delineating its interaction with antagonist TAK-779[6] may offer considerable insights into the problem of residues and structural determinants responsible for ligand-receptor binding, and therefore, the molecular modeling results may be employed in planning experiments designed to study alteration in CCR5 binding activity.

Based on a large amount of biophysical and other experimental data available for G-protein coupled receptors (GPCR), we have constructed the 3D-structural model of CCR5 which belongs to the superfamily of GPCRs by using the method of comparative molecular modeling. A ligand-receptor complex model relating to TAK-779 (scheme 1), which is a small molecule and is a nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity, is obtained by using DOCK4.0 program[12].




**Scheme 1. Chemical structure of TAK779.**

## COMPUTATIONAL METHODS

The modeling process was done in three stages: (1) modeling the transmembrane α-helix bundles (7TMs) of CCR5 receptor; (2) modeling the extracellular loops of CCR5 receptor; (3) docking TAK779 into constructed receptor model.

**Modeling the TM bundles of CCR5 receptors** The primary amino acid sequence of CCR5 was imported manually. According to the hydrophobicity analysis using Kyte-Doolittle scale[7], and the conservative residues in two subfamilies (protein, peptide receptors) of GPCRs and in CCR receptors, the putative transmembrane regions of CCR5 receptor corresponding to 7 TM regions of frog rhodopsin (FRh)[8] were determined. The initial coordinates of the proposed TM residues were taken from FRh's structural model. The normal dihedral angles and hydrogen bond distances of helical backbone were used as geometrical restraints in the structural modeling.

The crude structure of CCR5 was relaxed in all the sequence conserved regions (SCRs) by using the Homology module of InsightII[9]. Some structural flaws were found and corrected.

The final model was achieved with the Sybyl6.5[10]. The interactive modeling and display were performed on SGI XZ10000 workstation. The receptor model was optimized with the following parameters: a distance-dependent dielectric constant of 5.0, nonbonded cutoff 8 Å, Tripos force field with kollman-all-atom charges, conjugate gradient minimization till the energy gradient RMS < 0.05 kcal $(mol \cdot Å)^{-1}$, the whole receptor was minimized to convergence.

**Modeling the extracellular loops** The extracellular domain of CCR5 consists of three loops (EL-1, EL-2, EL-3), whose tentative structures are modeled. The N-terminus is not considered. EL-2 connects helices 4 and 5 and is attached to the end of EL-1 by a conserved disulfide bond between Cys101 and Cys178. Based on the sequence analysis and alignment of the extracellular domain of CCR5 receptor, the conformation of this longest loop was searched through a database composed of more than 700 loops and proteins from Brokenheaven Data Bank, and the InsightII package with medium to high sequence identity. An appropriate loop conformation was chosen from the top 10 candidates that had the lowest RMS values and considerable geometrical compatibility with the TM core.

Further structural modifications to the loop and side chain conformations of residues in the TM core were performed to avoid poor steric contacts and the trap of local energy minimum. The final model refining was done after all the loop sections had been assembled. The optimization was carried out with same parameters as described above.

**Ligand docking** The docking conformation of TAK779 resulted from geometrical optimization using Gaussian98 program at the level of HF/6-31G**[11], and the net atomic charge was presented in Tab 1. After the inspection of CCR5 receptor model, the possible binding pocket was revealed in a cavity formed by helices 3, 5, 6, and 7. The positively charged nitrogen core of

TAK779 was assumed to interact with the side chain of negatively charged residues situated in the inside of 7 TM bundles. In order to obtain corrective and reasonable docking results, the advanced program DOCK4.0[12] was used to tackle the interaction mode of this small molecule with CCR5 receptor. The ligand was docked into the binding site represented by spheres with anchor-first search algorithm, the ligand-receptor matching quality was scored principally based on their mutual van der Waals and electrostatic interaction. The most negative score was considered the best and written in the form of probable interactive conformation of the ligand. The model of TAK779-CCR5 receptor complex was energitically minimized after the docking was finished.

**Tab 1. The net atomic charges of TAK779 calculated by quantum mechanics method at HF/6-31G'' level (sum of Mulliken charges = 1.00000, with hydrogens summed into heavy atoms).**

| Atom | Charge | Atom | Charge | Atom | Charge |
|---|---|---|---|---|---|
| $O^{1}$ | −0.607933 | $C^{13}$ | −0.143061 | $C^{25}$ | −0.043017 |
| $C^{2}$ | 0.102489 | $C^{14}$ | 0.339291 | $C^{26}$ | −0.127349 |
| $C^{3}$ | −0.276276 | $C^{15}$ | −0.185182 | $C^{27}$ | −0.160201 |
| $C^{4}$ | 0.022395 | $C^{16}$ | −0.131389 | $C^{28}$ | −0.132875 |
| $C^{5}$ | −0.280220 | $N^{17}$ | −0.826261 | $C^{29}$ | 0.029396 |
| $C^{6}$ | 0.102876 | $C^{18}$ | 0.790330 | $C^{30}$ | −0.138200 |
| $N^{7}$ | −0.621230 | $O^{19}$ | −0.597127 | $C^{31}$ | −0.035559 |
| $C^{8}$ | −0.172172 | $C^{20}$ | −0.098189 | $C^{32}$ | −0.137357 |
| $C^{9}$ | −0.173674 | $C^{21}$ | −0.250319 | $C^{33}$ | −0.156680 |
| $C^{10}$ | −0.065817 | $C^{22}$ | −0.196468 | $C^{34}$ | −0.005886 |
| $C^{11}$ | −0.092377 | $C^{23}$ | −0.243938 | $C^{35}$ | −0.160155 |
| $C^{12}$ | −0.151834 | $C^{24}$ | −0.006617 | $C^{36}$ | −0.140563 |

**Binding energy calculation** The binding energy ($E_{binding}$) of TAK779 with CCR5 receptor was calculated as equation 1:

$$E_{binding} = E_{complex} - (E_{ligand} + E_{receptor}) \quad (1)$$

where $E_{ligand}$ was the energy of the lowest-energy conformation of ligand, $E_{receptor}$ was the energy of optimized receptor. The calculations were performed with a distance dependent dielectric constant of 5r. The calculation method and parameters used were set up as described above.

## RESULTS AND DISCUSSION

**3D-model of CCR5 receptor** Fig 1 represents the results of sequence alignment between CCR5 receptor and the FRh template[8]. This alignment is chosen not only according to the alignment score but also on the basis of the conserved residues in two subfamilies (protein, peptide receptors) of GPCR and CCR receptors. The 3D-model of CCR5 receptor is constructed and shown in Fig 2. The model is primarily based on the TM helical bundles of FRh template which was built by Herzyk and Hubbard in 1998[8]. By combining biophysical and biochemical information with the electron density projection map of FRh at 6 Å solution, this template is more reasonable than the bovine rhodopsin model, so it can be used as a template to model the structure of CCR5 receptor. The atomic RMS difference over 190 $C^{\alpha}$ atoms comprising the TMs of CCR5 is 1.8 Å compared with the starting template. The TMs arrangement is in accordance with the helical conformation of GPCRs. The EL-1 loop arches between helices 2 and 3 with Cys101 of the conserved disulfide bridge located at conjunction with helix 3. Asp95, which is a potential binding site for an antibody (eg mAb45523) seems to be outside the orientation and is highly accessible, and in close positional proximity with Lys171 and Glu172. The longest EL-2 erects around the outside edge of the binding pocket, and it is able to control the entrance of small ligands and the binding of endogenous peptides like RANTES, MIP-1β. Residues Tyr184 to Phe189, which are oriented outside in our model, could form an accessible face of CCR5 for EL-2 mAbs. This is in consistance with the experimental results which state that these residues contribute to the recognization of epitopes by EL-2 mAbs. Furthermore, as shown in Fig 2, residues Lys171 and Glu172 are also accessible to all multidomain (MD) mAbs. This point has been testified by the epitope map of CCR5 receptor where Lys171 and Glu172 are presumably required to be in contact with all MD mAbs[5]. The EL-3 forms a flat span between helices 6 and 7, and Cys269 probably forms disulfide bond with Cys20 at the N-terminus.

The geometrical distances between $C^{\alpha}$ atoms of the most conserved residues are listed in Tab 2 and labeled in black in Fig 1. These data are in accordance with the orientation constraints of the template. As these conserved residues play a very important role in the formation of 7 TMs and in the determination of the relative position of these α-helix bundles, it can be said that this data not only offers validation of our constructed model but also provides a valuable check on the relative positions of the helices and the integrity of the receptor bundle.

One of the important characteristics of CCR5 receptor model is that there are many hydrogen bonds formed by residues in the TM helices and extracellular domains.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Jun; 21 (6)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

These hydrogen bonds are summarized in Tab 3.

| | | | | |
|---|---|---|---|---|
| TM1 | FRh | 38 | SMLAAYMFLLIMLGFPINFLVLVVTV | 63 |
| | CCR5 | 31 | *RLLPPLYSLVFIFGFVGNMLVILILI* | 56 |
| TM2 | FRh | 70 | TPLNYILLNLAVADLFMVFGGFTTTL | 95 |
| | CCR5 | 63 | *SMTDIYLLNLAISDLFFLLTVPFWAH* | 88 |
| TM3 | FRh | 111 | NLEGFFATLGGEIALWSLVVLAIERYVVVC | 140 |
| | CCR5 | 102 | *QLLTGLYFIGFFSGIFFIILLTIDRYLAVV* | 131 |
| TM4 | FRh | 151 | NHAIMGVAFTWVMALACAAPPLVGWS | 176 |
| | CCR5 | 143 | *TFGVVTSVITWVVAVFASLPGIIFTR* | 168 |
| TM5 | FRh | 202 | SPVIYMFVVHFIIPLIVIFFCYGQLVFTVK | 231 |
| | CCR5 | 193 | *FQTLKTVILGLVLPLLVMVICYSGILKTLL* | 222 |
| TM6 | FRh | 250 | VTRMVIIMVIAFLICWLPYAGVAFIY | 275 |
| | CCR5 | 233 | *AVRLIETIMIVYFLFWAPYNIVLLLN* | 258 |
| TM7 | FRh | 286 | IFMTIPAFFAKTSAVYNPVIYIMMNK | 311 |
| | CCR5 | 277 | *QAMQVTETLGMTHCCINPIIYAFVGE* | 302 |
| EL-1 | CCR5 | 89 | YAAAQWDFGNTMC | 101 |
| EL-2 | CCR5 | 169 | SQKEGLHYTCSSHFPYSQYQFWKN | 192 |
| EL-3 | CCR5 | 259 | TFQEFFGLNNCSSSNRLD | 276 |

**Fig 1. Alignment of CCR5 receptor with Frog Rhdopsin (FRh) structural model template[13] in the transmembrane helices (TM1-TM7) and determination of the EL-loops (EL1-EL3). Residues, which are all not the same in the family of CCR coreceptors, are shown in the italic style. Residues which are in arial black are the most conserved residues that are from two subfamilies (protein, peptide receptors) of rhodopsin-like GPCRs[12]. Cys101 and Cys178 which form the disulfide bond between the end of EL-1 loop, and EL-2, Cys269 which probably forms a disulfide bond with Cys20 at the N-termini are also labeled in black.**

Tab 3 shows that the hydrogen bond cluster in the EL-2 makes it more stable in structure and it can act potently as a filter for peptidal ligands to control their entrance into the binding pocket situated in the 7 TM bundles. The key role of this loop has been proved by a lot of structure-activity studies relating to the binding of endogenous peptidal ligands with CCR5 receptor[13,14]. The hydrogen bonds network (Tab 3) formed between three extracellular loops and the extracellular end of helix bundles indicates that these loops may take a well-packed conformational state. In other words, the interaction of N-terminus with HIV-1 glycoprotein gp120[4,15] must be strong enough to induce a conformational change in the extracellular loops for the resulting HIV-1 virus fusion into the cell. The hydrogen bonds formed near the intracellular end would be one of the reasons that the helical end towards the cytoplasmic is packed more tightly than the other end. These hydrogen bonds exist mainly among the residues of Asn48, Asp125, Arg126, Gly145, Ser149, and some other residues from the intracellular loops.

In spite of some degree of approximations made in the molecular modeling of CCR5 receptor, there are at least two evidences in support of our model. First of all, the 3D-structural model of CCR5 is in agreement with certain general principles of protein structure, it provides satisfactory distance constraints between residues of different helices. The hydrogen bonds listed in Tab 3 ellucidate that spatially close side chains are necessary for maintaining the close packing within the transmembrane domain and extracellular loops. The second proof for the validation of our structural model stems from the excellent agreement of FRh model with a sample of structural and experimental data available for this prototypical GPCR[8].

**Interaction mechanism of TAK779 with CCR5 receptor** The constructed CCR5 receptor has a deep binding cavity which is situated in the extracellular side of the transmembrane domain between helices 3, 5, 6, and 7. This cavity is partly covered by the extracellular loops, particularly the EL-2. The binding pocket is composed of conserved residues of Tyr108, Gly111, Ser114, Glu283, Gly286, and Cys290, and conservatively varied residues including Thr105, Leu107, Phe112, Gly115, Lys197, and Met287. Most of the residues in the binding pocket have fixed side chain orientations. The 3D model of ligand-receptor complex is shown in Fig 3, and Fig 4 represents the interaction mode of TAK779 with CCR5 receptor. The energy of TAK779-CCR5 receptor complex is $-336.985$ kcal/mol, CCR5 $-317.839$ kcal/mol, and TAK779 in the lowest-energy conformation 32.46 kcal/mol, so the binding energy of TAK779 with CCR5 receptor is $-51.606$ kcal/mol calculated according to equation 1. This result demonstrates that TAK779 strongly and perfectly interacts with CCR5 receptor.

As shown in Fig 4, the pyran cycle of TAK779 binds in a conserved region, which is the inner part of binding pocket. The oxygen atom in the pyran cycle forms a hydrogen bond with the side chain of residues Ser114, we believe that this hydrogen bond is an imputant factor which influences the whole orientation of TAK779 in the binding pocket. The whole pyran cycle is surrounded by residues of Gly111, Ser114, and Gly115 from TM3, and residues of Gly286, Met287, and Cys290 of TM7. The interaction style between the pyran cycle and the surrounding residues is mainly hydrophobic. The aminium nitrogen may interact with the

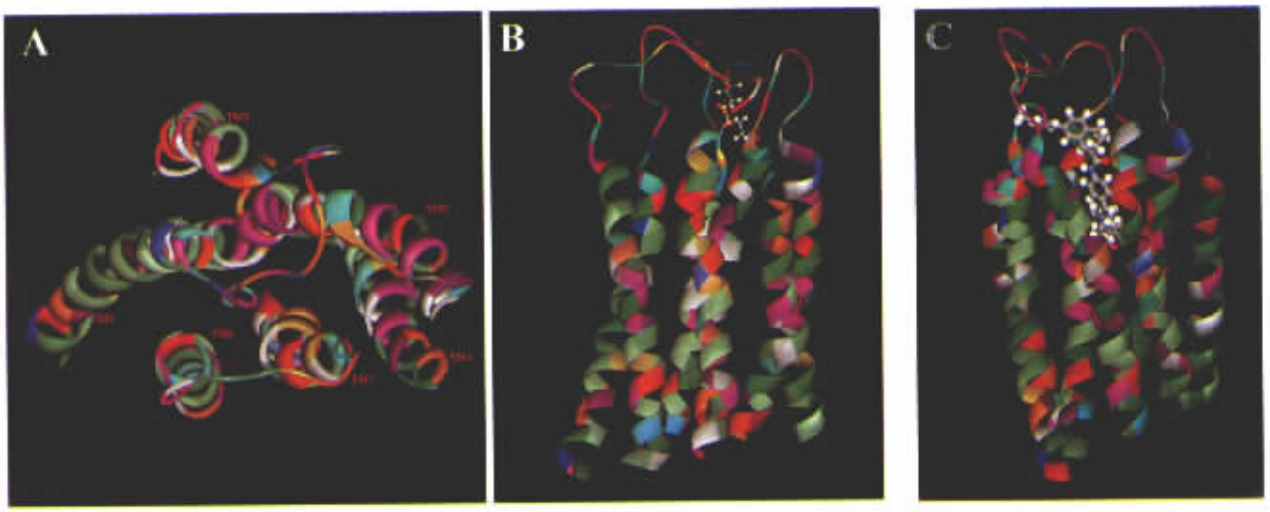



**Fig 2.** Cartoon representation of transmembrane helices and extracellular loops of CCR5 receptor model. Top view (A) and side view (B) from the extracellular surface, the disulfide bond formed by side chains of Cys101 and Cys178 is shown in ball-and-stick style.

**Fig 3.** Molecular model of TAK775-CCR5 receptor complex. TAK775 is shown in ball-and-stick style, the CCR5 receptor is represented as a flat ribbon.

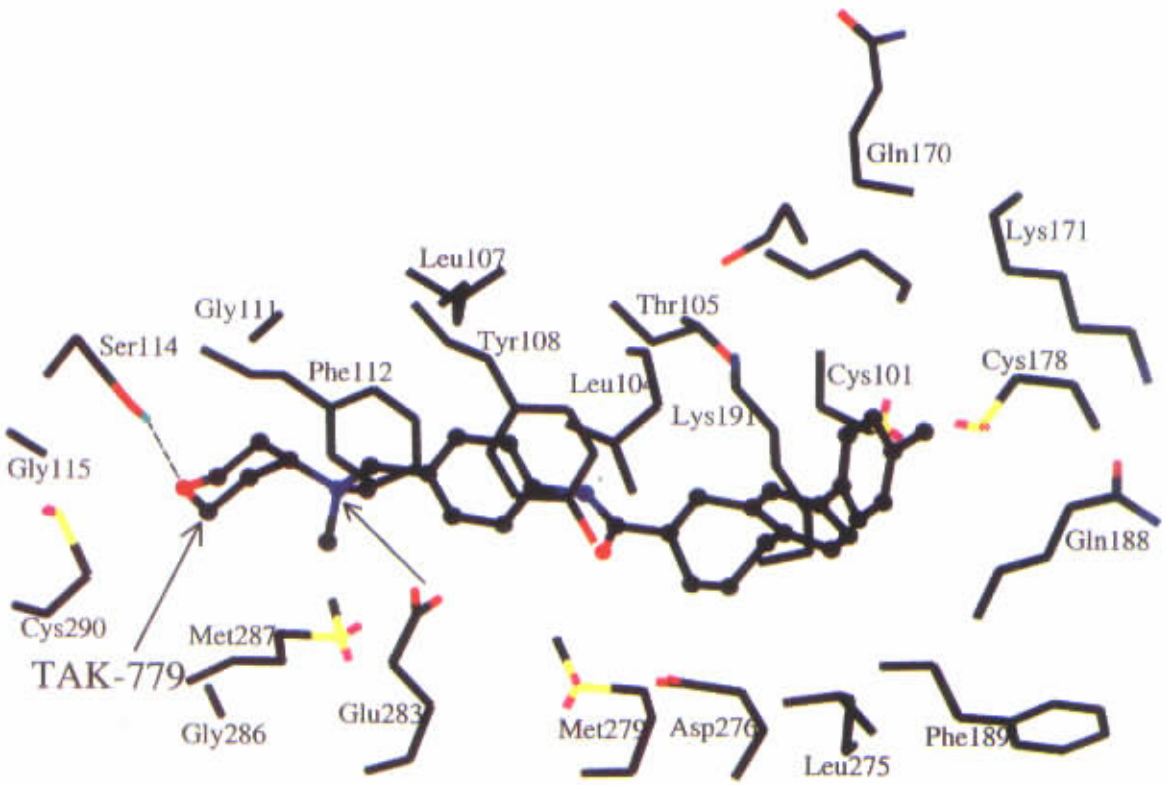



**Fig 4.** Binding mode of TAK775 with CCR5 receptor, the intermolecular hydrogen bond is represented by the dashed line. Only the side chains of residues of CCR5 receptor (within 5 Å around the ligand) interacting with TAK775 are shown by atom type. Electrostatic interaction may exist between the positively charged nitrogen of TAK775 and negatively charged side chain of Glu283.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Jun; 21 (6)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

**Tab 2. The geometrical distances between $C^{\alpha}$s of conserved residues in the transmembrane helix bundles of CCR5 receptor.**

| $C_1^{\alpha}$ | $C_2^{\alpha}$ | Distance (Å) | Location | $C_1^{\alpha}$ | $C_2^{\alpha}$ | Distance (Å) | Location |
|---|---|---|---|---|---|---|---|
| Tyr37 | Leu285 | 10.875 | TMs1 – 7 | Phe247 | Cys291 | 10.794 | TMs6 – 7 |
| Tyr37 | Asp76 | 11.848 | TMs1 – 2 | Tyr244 | Asn293 | 5.674 | TMs6 – 7 |
| Gly44 | Asp76 | 6.436 | TMs1 – 2 | Tyr244 | Pro294 | 10.591 | TMs6 – 7 |
| Gly47 | Ala73 | 8.329 | TMs1 – 2 | Tyr297 | Tyr244 | 9.325 | TMs7 – 6 |
| Asn48 | Leu72 | 5.910 | TMs1 – 2 | Leu122 | Tyr214 | 12.512 | TMs3 – 5 |
| Val51 | Leu69 | 7.054 | TMs1 – 2 | Asp125 | Tyr214 | 12.566 | TMs3 – 5 |
| Asn48 | Asp76 | 7.651 | TMs1 – 2 | Arg126 | Ile217 | 9.180 | TMs3 – 5 |
| Tyr37 | Gly286 | 11.626 | TMs1 – 7 | Tyr127 | Ile217 | 8.082 | TMs3 – 5 |
| Gly44 | Cys291 | 6.113 | TMs1 – 7 | Leu122 | Trp153 | 12.883 | TMs3 – 4 |
| Asn48 | Asn293 | 11.650 | TMs1 – 7 | Leu122 | Asn71 | 10.659 | TMs3 – 2 |
| Tyr297 | Val51 | 13.295 | TMs7 – 1 | Asp125 | Leu69 | 11.894 | TMs3 – 2 |
| Asn71 | Pro294 | 11.067 | TMs2 – 7 | Arg126 | Leu72 | 16.340 | TMs3 – 2 |
| Leu69 | Tyr297 | 10.330 | TMs2 – 7 | Pro206 | Aala156 | 13.121 | TMs5 – 4 |
| Pro250 | Leu285 | 7.343 | TMs6 – 7 | Pro206 | Pro162 | 17.298 | TMs5 – 4 |
| Gly286 | Trp248 | 7.798 | TMs4 – 6 | Asn71 | Trp153 | 10.570 | TMs2 – 4 |
| Leu69 | Trp153 | 14.576 | TMs2 – 4 | Ala73 | Pro294 | 10.711 | TMs2 – 7 |
| Leu122 | Tyr244 | 10.749 | TMs3 – 6 | Asp125 | Tyr297 | 12.851 | TMs3 – 7 |

**Tab 3. Some hydrogen bonds (besides the backbone hydrogen bonds) formed by residues from TM helices and extracellular loops in CCR5 receptor.**

| Location | Donor | | Acceptor | | Location | Donor | | Acceptor | |
|---|---|---|---|---|---|---|---|---|---|
| | Residue | Group | Group | Residue | | Residue | Group | Group | Residue |
| Helix1 – 2 | Asn48 | $-N^{\delta 2}H$ | $^{(-)}O^{\delta 2}-$ | Asp76 | EL2 | Ser169 | -NH | O = C < | Phe166 |
| Helix3 – 3 | Arg126 | $-^{(+)}N^{\eta 1}H$ | $^{(-)}O^{\delta 1}-$ | Asp125 | EL2 | Gln186 | $-N^{\varepsilon}H$ | OH | Thr187 |
| Helix4 – 4 | Ser149 | -OH | O = C < | Gly145 | EL2 | His181 | = NH | O = C < | Ser179 |
| Helix3 – 2 | Asp125 | -NH | -OH | Tyr68 | EL3 | Phe264 | -NH | O = C < | Glu262 |
| Helix3 – 3 | Arg126 | $-^{(+)}N^{\eta 2}H$ | $^{(-)}O^{\delta 1}-$ | Asp125 | EL3 | Asn267 | $-N^{\delta}H$ | O = C < | Gln261 |
| EL1 | Asn98 | -NH | O = C < | Phe96 | EL3 | Asn273 | $-N^{\delta}H$ | -OH | Ser272 |
| EL1 | Ala91 | -NH | $O^{\varepsilon}$ = C < | Gln93 | EL3 | Asn268 | $-N^{\delta}H$ | $O^{\varepsilon}-$ | Asn268 |
| EL1 | Gln93 | $-N^{\varepsilon}H$ | O = C < | Tyr89 | EL3 | Arg274 | -NH | O = C < | Ser272 |
| EL1 | Thr99 | -NH | O = C < | Gly97 | EL3 | Gln280 | -NH | O = C < | Asp276 |
| EL2 | Lys171 | $-^{(+)}N^{\zeta}H$ | $O^{\varepsilon}$ = C < | Gln188 | EL3 | Gln280 | $-N^{\delta}H$ | $^{(-)}O^{\delta 1}-$ | Asp276 |
| EL2 | Ser180 | -NH | $O^{\varepsilon}$ = C < | Gln188 | EL3 | Asn267 | -NH | $O^{\varepsilon}$ = C < | Asn268 |
| EL2 | His181 | $-N^{\varepsilon}H$ | O = C < | Ser180 | EL3 – 2 | Asn268 | $-N^{\delta}H$ | -OH | Ser185 |
| EL2 | Trp190 | -NH | O = C < | Gln188 | EL3 – 1 | Asn273 | $-N^{\delta}H$ | $O^{\varepsilon}$ = C < | Asn98 |
| EL2 | Thr177 | -NH | O = C < | Glu172 | EL2 – 3 | Gln186 | $-N^{\varepsilon}H$ | O = C < | Gly265 |
| EL2 | Thr177 | -NH | O = C < | Leu174 | EL2 – 3 | Trp190 | $-N^{\varepsilon}H$ | $O^{\varepsilon}$ = C < | Asn267 |
| EL2 | Ser179 | -NH | -OH | Thr177 | EL1-Helix2 | Gln93 | $-N^{\varepsilon}H$ | O = C < | His88 |
| EL2 | Lys191 | -NH | O = C < | Thr187 | Helix5-EL2 | Phe193 | -NH | O = C < | Trp190 |
| EL2 | Gln188 | -NH | O = C < | Gln186 | Helix3-EL2 | Gln102 | $N^{\varepsilon}H$ | O = C < | Lys171 |
| EL2 | Gln188 | -NH | $O^{\varepsilon}$ = C < | Gln188 | Helix4-EL2 | Arg168 | $-N^{\eta}H$ | $O^{\delta}$ = C < | Gln170 |
| EL2 | Lys191 | $-^{(+)}N^{\zeta}H$ | -OH | Ser169 | Helix7-EL3 | Ala278 | -NH | O = C < | Arg274 |

negatively charged side chains of residue Glu283 at TM7 through electrostatic interaction, although the distance between them is longer than the normal length of a hydrogen bond, which results from the steric hindrance of twomethyl groups connecting the aminium nitrogen of TAK779. The phenyl ring A of TAK779 interacts with the side chains of residues Leu104, Leu107, Tyr108, Phe112, and Met279 through hydrophobic interaction, especially the phenyl rings at the side chains of Phe108 and Phe112 run parallel with the phenyl ring A of TAK779. This indicates that there is a *kind of* $\pi$-$\pi$ stacking interaction between them. The other part of

TAK779 is situated in the extracellular domain of CCR5 receptor, and interacts with the residues mainly from extracellular loops, especially from the EL-2. This part of TAK779 including two other phenyl rings (B and C) connecting the amido group interacts with the side chains of many residues through hydrophobic interactions. These interacting extracellular residues are Cys101, Ser169, Gln170, Lys171, Cys178, Gln188, Phe189, Leu275, and Asp276. Gln102 and Thr105 also take part in the interaction with TAK779, particularly the side chain of Thr105 can form a hydrogen bond with the amido group of TAK779. Residues Cys101 and Cys178, which form the conserved disulfide bond of CCR5, also play an important role in the ligand-receptor interaction. Residue Lys171, which has been proved as one of the binding sites for all multidomain mAbs, is also within the 5 Å environment around TAK779. This demonstrates that part of the EL-2 loop is indeed involved in the interaction with ligand, especially the endogenous peptides like RANTES. Based on above docking results and discussion, it can be concluded that the EL-2 together with EL-3 control the conformational change of CCR5 before and after binding with the ligands.

One of the results from quantum mechanics of TAK779 is the net atomic charge which is listed in Tab 1. Atoms of $O_1$, $N_7$, $N_{17}$, and $O_{19}$ have very high values of charge, which tells us that the groups containing these atoms are the active center of TAK779. There is a coherence between the results from quantum mechanics (QM) and the structural model built by molecular mechanics (MM). Thus our interaction mode of TAK779 with CCR5 receptor has testified the correctness of the QM results, which serve as a further validation of our molecular modeling results.

To conclude, we have constructed the 3D-structural model of HIV-1 coreceptor CCR5 and its complex with antagonist TAK779. The interaction mechanism of the ligand-receptor has been reasonably interpreted. These results are helpful for further work on CD4-gp120-CCR5 complex and have some significance in designing of new HIV-1 inhibitors.

## REFERENCES

1 Raport CJ, Gosling J, Schweickart VL, Gray PW, Charo IF. Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1beta, and MIP-1alpha. J Biol Chem 1996; 271: 17161 – 6.
2 Wu L, Gerard NP, Wyatt R, Choe H, Parolin C, Ruffing N, *et al*. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. Nature 1996; 384: 179 – 83.
3 Rizzuto CD, Wyatt R, Hernandez-Ramos N, Kwong PD, Hendrickson WA, Sodroski J, *et al*. A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. Science 1998; 280: 1949 – 53.
4 Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 1998; 393: 648 – 59.
5 Lee B, Sharron M, Blanpain C, Doranz BJ, Tsang M, Doms RW, *et al*. Epitope mapping of CCR5 reveals multiple conformational states and distinct but overlapping structures involved in chemokine and coreceptor function. J Biol Chem 1999; 274: 9617 – 26.
6 Baba M, Nishimura O, Kanzaki N, Okamoto M, Sawada H, Iizawa Y, *et al*. A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. Proc Natl Acad Sci USA 1999; 96: 5698 – 703.
7 Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. J Mol Biol 1982; 157: 105 – 32.
8 Herzyk P, Hubbard RE. Combined biophysical and biochemical information confirms arrangement of transmembrane helices visible from the three-dimensional map of frog rhodopsin. J Mol Biol 1998; 281: 741 – 54.
9 InsightII [computer program]. Version 97.5. California: Molecular Simulation Inc; 1997.
10 Sybyl[computer program]. Version 6.5. St Louis (MO): Tripos Associates Inc; 1998.
11 Gaussian[computer program]. Version 98. Pittsburgh (PA): Gussian Inc; 1998.
12 Ewing TJA, Kuntz ID. DOCK[computer program]. Version 4.0. California: University of California in San Diego (UCSD); 1998.
13 Samson M, LaRosa G, Libert F, Paindavoine P, Detheux M, Vassart G. The second extracellular loop of CCR5 is the major determinant of ligand specificity. J Biol Chem 1997; 272: 24934 – 41.
14 Wyatt R, Sodroski J. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science 1998; 280: 1884 – 8.
15 Farzan M, Choe H, Vaca L, Martin K, Sun Y, Desjardins E, *et al*. A tyrosine-rich region in the N terminus of CCR5 is important for human immunodeficiency virus type 1 entry and mediates an association between gp120 and CCR5. J Virol 1998; 72: 1160 – 4.

## HIV-共受体 CCR5 的三维结构及与其拮抗剂 TAK 779 相互作用的比较分子模拟[1]

黄小琴，蒋华良[2]，罗小民，陈凯先[2]，嵇汝运

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Jun; 21 (6)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

(中国科学院上海药物研究所, 上海 200031, 中国); 曹 阳 (苏州大学化学系, 苏州 215006, 中国); 裴 钢 (中国科学院上海细胞生物学研究所, 上海 200032, 中国)

**关键词** TAK 779; CCR5 受体; 分子结构; HIV-1; 计算机辅助设计

**目的**: 研究 HIV-1 的共受体 CCR5 与其拮抗剂 TAK779 的相互作用机制. **方法**: 用比较分子模拟方法建立 CCR5 受体的三维结构模型; 通过量子化学计算得到 TAK779 分子的结构参数和最优几何构型; 用 DOCK4.0 程序将 TAK779 分子对接到 CCR5 受体的结合位点上. **结果**: 通过分子力学优化得到了 CCR5 受体的三维结构模型, 配体的结合口袋位于第三、五、六、七跨膜区, 组成结合口袋的氨基酸残基主要为 Thr105、Leu107、Tyr108、Gly111、Phe112、Ser114、Gly115、Lys197、Glu283、Gly286、Met287、Cys290; TAK779 与 CCR5 受体的相互作用方式为氢键、静电和疏水作用; 配体与受体的结合能为 −51.606 kcal/mol. **结论**: 上述模型有助于进一步理解膜受体识别 HIV-1 病毒的分子机制并设计新的 HIV-1 抑制剂.

(责任编辑 朱倩蓉)