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Molecular modeling on human CCR5 receptors and complex with CD4 antigens and HIV-1 envelope glycoprotein gp120[1]

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KEY WORDS CCR5 receptors; CD4 antigens; HIV envelope protein; HIV-1; chemokine receptors; HLA antigens; molecular models

ABSTRACT

AIM: To investigate the interaction between human CCR5 receptors (CCR5) and HIV-1 envelope glycoprotein gp120 (HIV-1 gp120) and HIV-1 receptor CD4 antigens (CD4). **METHODS**: The structurally conserved regions (SCR) of human CCR5 was built by the SYBYL/Biopolymer module using the corresponding transmembrane (TM) domain of bacteriorhodopsin (bR) as the template. The coordinates for amino-terminal residue sequence, and carboxyl-terminal residue sequence, extracellular and cytoplasmic loops were generated using LOOP SEARCH algorithm. Subsequently the structural model was merged into the complex with HIV-1 gp120 and CD4. **RESULTS**: Human CCR5 interacted with both an HIV-1 gp120 and CD4. The *N*-terminal residues (especially Met1 and Gln4) of human CCR5 contacted with CD4 residues, mainly with one span (56 – 59) of CD4 in electrostatic interaction and hydrogen-bonds. The binding sites of human CCR5 were buried in a hydrophobic center surrounded by a highly basic periphery. On the other hand, direct interatomic contacts were made between 7 CCR5 residues and 6 gp120 amino-acid residues, which included van der Waals contacts, hydrophobic interaction, and hydrogen bonds. **CONCLUSION**: The interaction model should be helpful for rational design of novel anti-HIV drugs.

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INTRODUCTION

The human immunodeficiency viruses (HIV-1 and HIV-2) and simian immunodeficiency viruses (SIV) are the etiologic agents of acquired immunodeficiency syndrome (AIDS) in their respective human and simian hosts[1]. Virus attachment also involves the interaction of the gp120 envelope glycoproteins with specific receptors the CD4 glycoprotein and members of the chemokine receptor family[2]. CCR5 and CD4 are coreceptors for immunodeficiency virus to enter into target cells. A major function of CD4 binding is to induce conformational changes in the gp120 glycoprotein that contributes to the formation and/or exposure of the binding site for the specific chemokine receptor[3]. Several primary SIV isolates no longer depend on CD4 for efficient entry, and bind to chemokine receptor without prior CD4 interaction[4]. The use of CD4 as a receptor may have evolved subsequently, allowing the high-affinity chemokine receptor-binding site of primate immunodeficiency viruses to be sequestered from host immune surveillance[1].

An X-ray crystal structure of a ternary complex that includes a gp120 core, CD4, and a neutralizing antibody (17b) has been reported. The gp120 core is composed of two domains, an inner and an outer domain, and a β sheet (the "bridging sheet"). All structural elements contribute, either directly or indirectly, to CD4 and chemokine receptor binding[5]. The 17b antibody binds to an CD4-induced (CD4i) epitope that overlaps the chemokine-receptor-binding site. Specific groups of HIV-1-neutralizing antibodies directed against the gp120 V3 loop or CD4-induced (CD4i) epitopes are able to block the binding of gp120-CD4 complexes to CCR5-expressing cells[6]. The CD4i epitopes are conserved, and discontinuous gp120 structures are better exposed after CD4 binding. And efficient CCR5

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binding is dependent on the presence of the V3 variable loop of gp120[7]. Moreover, the uncovered V3 and CD4i epitopes make up the chemokine-receptor-binding site. On the other hand, interleukin-16 shares agonistic effects on CD4- lymphocytes with anti-CD4 antibodies and HIV-1 gp120, while IL-16 and gp120 share a common receptor, CD4 glycoprotein. Unlike HIV-1 gp120, IL-16 suppresses cell proliferation without modulating CD4 from the cell surface. The activity of IL-16 is absolutely dependent upon the presence of membrane-expressed functional CD4 based on studies reported. Competition studies indicate that the IL-16 binding site on CD4 may be in close vicinity to the epitope recognized by the anti-CD4 mAb OKT4 in the V4 domain, whereas HIV-1 gp120 binds to the V1 region of CD4[8]. How about the relation between human CCR5 and IL-16?

In this report, we generated the structural model of human CCR5 using the crystal structure of bacteriorhodopsin (bR) as a template with SYBYL. Subsequent work has focused on indicating the complex model of human CCR5 with CD4 and HIV-1 gp120. It is important to reveal the action mechanism of IL-16.

METHODS

Molecular modeling of the three-dimensional structure of human CCR5 was performed on a Silicon Graphics Iris Indigo (SGI Inc, Silicon CA, USA) workstation using the BIOPOLYMER module of the commercial software packages Sybyl 6.3 (Tripos Inc, St Louis MO, USA).

Molecular modeling of human CCR5 One high-resolution X-ray crystal structure of bR (PDB file-code 2BRD) was used as the template structure to create human CCR5 model. According to the 7 TM domains of human CCR5 from amino acids sequence database (Swiss-Port card: O14699), the sequences of the SCR of human CCR5 were aligned with bR (Tab 1). The structure of the SCR of bR was extracted and used as the template for positioning of the α-helices main axes using the Biopolymer module within SYBYL software. Automatic adjustments were made to remove the unfavorable steric interactions. The geometry of the protein was optimized for 200 steps with the steepest descent minimization and subsequently for 2000 steps with the conjugate gradient minimization, using the Amber force field with Kollmann all-atom charges. A cutoff of 0.8 NM was used, while dielectric constant was set 5.0 and dependent on the distance.

We generated the *N*-terminal, *C*-terminal, and extracellular and cytoplasmic loop segments using LOOP SEARCH algorithm in Biopolymer module, and formed two disulfide bridges exist at extracellular domain (Cys20 – Cys269 and Cys101 – Cys178 of human CCR5, Fig 1).

Tab 1. Alignment of the helical sequences between human CCR5 and bacteriorhodopsin.

Helical name	Name	SP	Residue sequences	TP
Helix-1	bR	8	EWIWALGTALMGLGTLYFLVKG	29
	hCCR5	31	RLLPPLYSLVFIFGFVGNMLVI	52
Helix-2	bR	36	DAKKFYAITTLVPAIAFTMYLSMLL	60
	hCCR5	67	IYLLNLAISDLFFLLTVPFWAHYAA	91
Helix-3	bR	75	PIYWARYADWLFTTPLLLLDLALL	98
	hCCR5	102	QLLTGLYFIGFFSGIFFIILLTID	125
Helix-4	bR	103	QGTILALVGADGIMIGTGLVGAL	125
	hCCR5	143	TFGVVTSVITWVVAVFASLPGII	165
Helix-5	bR	132	RFVWWAISTAAMLYILYVLFFGFT	155
	hCCR5	192	NFQTLKIVILGLVLPLLVMVICYS	215
Helix-6	bR	164	EVASTFKVLRNVTVVLWSAYPVVWL	188
	hCCR5	232	RAVRLIFTIMIVYFLFWAPYNIVLL	256
Helix-7	bR	201	IETLLFMVLDVSAKVGFGLILLRS	224
	hCCR5	280	VTETLGMTHCCINPIIYAFVGEKF	303

Note: bR means bacteriorhodopsin and hCCR5 means human CCR5. SP and TP respectively express the starting points and the terminal points of seven α-helical fragments.

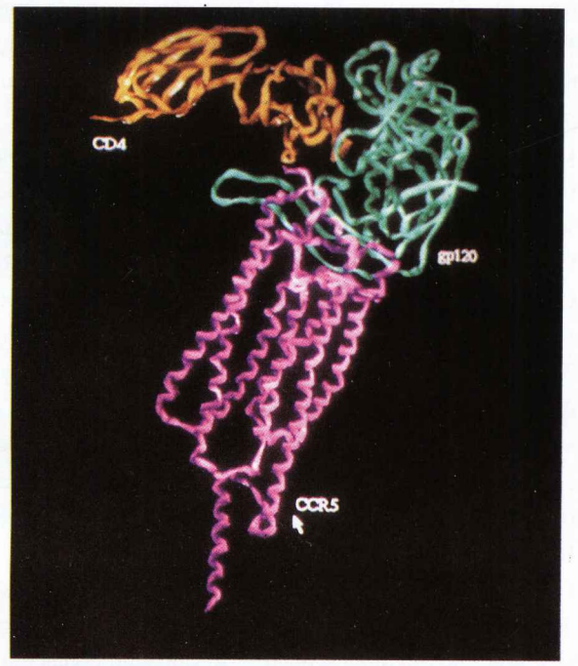

Fig 1. The complex model of human CCR5 with HIV-1 gp120 and CD4 glycoprotein. These proteins are displayed by colored shaded ribbons. Human CCR5, gp120, and CD4 are colored by purple, green, and orange, respectively.

The whole protein structural model was optimized by molecular dynamics and molecular mechanics. Following each dynamics run, the total energy was minimized via molecular mechanics using a steepest descent algorithm and a subsequent conjugate gradient method.

Molecular modeling of human CCR5 protein in complex with the CD4 receptor and an HIV gp120 envelope glycoprotein One high resolution X-ray crystal structure of an HIV gp120 envelope glycoprotein was in complex with the CD4 receptor and a neutralizing human antibody (PDB filecode 1GC1) was used as a template structure to create the complex model with human CCR5 and gp120 CD4. The models were constructed using the following methodology. Firstly, the binding site of human CCR5 interacting with an HIV gp120 core envelope glycoprotein was compared with that of a neutralizing human antibody (17b) which binds to a CD4-induced site on gp120 (CD4i)[15]. Secondly, the 17b antibody was replaced by human CCR5. Thirdly, crystallographic coordinates of the V3 variable loop of the HIV gp120 surface glycoprotein and the CD4i epitopes were extracted and docked onto the corresponding human CCR5 regions using the Biopolymer module within SYBYL software. Finally, molecular dynamics and molecular mechanics were used to optimize the model. Following each dynamics run, the total energy was minimized via molecular mechanics using a steepest descent algorithm and a subsequent conjugate gradient method.

RESULTS AND DISCUSSION

Knowledge, both from the three-dimensional (3D) structures of homologous proteins and from the general analysis of protein structure, is of value in modeling a protein of known sequence but unknown structure. Many models are constructed by homologous modeling on graphics devices, but automated procedures have come into greater use[9]. Tang Yun *et al* ever used the crystal structure of bR as a template to build 3D structures of μ opioid receptor[10]. Here we built molecular model of human CCR5 with the same method, knowledge-based protein homologous modeling.

Molecular modeling of human CCR5 Our studies show the 3D structure of human CCR5 including helical motifs and loop segments. The fifteen helical portions exist at TM domain, intracellular domain, and extracellular domain. As the G protein-coupled receptor (GPCR) family, a seven-helix hydrophobic sequence is located at the TM domain, but some of the hydrophobic helical fragments cross membrane into intracellular domain or extracellular domain, such as helix-4, helix-5, and helix-7. Additionally, three helixes, helix-A (Ser7-Tyr10), helix-B (Ile12-Tyr15), and helix-C (Lys26-Ala30), are situated at the *N*-terminal loop and helix-H (Pro332-Ser349) at the *C*-terminal loop; two helixes, helix-D (Leu257-Glu262) and helix-E (Phe264-Asn268), lie to the third extracellular loop; and yet helix-F (Lys59-Lys62) is located at the first intracellular loop and helix-G (Lys228-Arg230) at the third intracellular loop. There are twelve cysteine residues in human CCR5, three cysteine residues in the fifth and seventh helix TM (Cys213, Cys290, and Cys291 in human CCR5). Particularly, there are two highly conserved cysteine residues in the second and third extracellular loops of human CCR5 (Cys101 and Cys178) as β-adrenergic receptor, two cysteine residues of that in the first and fourth extracellular loops (Cys20

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and Cys269) are very important. Similar to β-adrenergic receptor, two disulfide bridges exist at extracellular domain (Cys20-Cys269 and Cys101-Cys178 of human CCR5). The other cysteine residues are located at the intracellular domain, which may not be considered temporarily due to the active sites of human CCR5 location at the extracellular domain (Tyr14, Gln93, and Pro183 of human CCR5).

Unlike other GPCR, the active site of CCR5 is situated at the extracellular regions, including *N*-terminal residues and the intervening first extracellular loop and the second extracellular loop. By using chimera constructions and site directed mutagenesis, Kuhmann *et al* found and proposed that these key amino acids (Y14, Q93, and P183) on the extracellular surface of CCR5 were nevertheless critical for infections by independent isolates of macrophage-tropic HIV-1[11]. In our results, the tyrosine-rich motif Tyr10-Asp-Ile-Asn-Tyr-Tyr15 has been showed to be essential for coreceptor function, especially replacement of the 14th tyrosine residue by asparagine residue caused some change in steric and electric character. This brings a serious problem whether the substitution of hydrophobic residue tyrosine with hydrophilic residue asparagine would influence the interaction between human CCR5 and HIV-1 gp120 envelope glycoprotein, which was supported by Kuhmann *et al*[11]. Since CD4 and other chemokine receptors (CCR5 and CXCR4) are needed to help HIV-1 infection, HIV-1 can not enter into target cells when gp120 can not contact with CCR5. This may be the reason that people surrounded by highly dangerous patients do not infect AIDS, probably these people have a natural CCR5 deficiency disease. Obviously, the active sites of human CCR5 are very important in inhibiting infections by independent isolates of M-tropic HIV-1. However, HIV-1 entry into target cells needs CD4 participation, and the apparent affinity of the interaction between human CCR5 and HIV-1 gp120 is dramatically lower in the absence of soluble CD4 because the CCR5-binding site on HIV-1 gp120 is fully formed or exposed only after CD4 binding[3].

Complex model of human CCR5 with an HIV-1 gp120 and CD4 These studies result a conclusion that human CCR5 may interact with both an HIV gp120 envelope glycoprotein and CD4 receptor (Fig 1). It is different from the complex structure of gp120/CD4/17b where there are no direct CD4-17b contacts and none of the gp120 residues contacts both 17b and CD4[3]. As a coreceptor of gp120, human CCR5 also contacts with the other receptor, CD4 glycoprotein, which was confirmed by Wu L *et al*[12]. Here the *N*-terminal residues, especially Met1 and Gln4, of human CCR5 involve the sites that contact with CD4 residues, mainly with one span (56 – 69) of CD4. Met1 of human CCR5 contacts with Arg56 of CD4 with electrostatic interaction, while Gln4 interacts with the critical CD4 residues Arg58 and Arg59 (Fig 2). The caroxylate group of Gln4 makes double hydrogen bonds with the guanidinium *N* atoms of Arg58 and Arg59. The binding sites of CCR5 were buried in a hydrophobic center surrounded by a highly basic periphery (3 Arg, and no Asp or Glu). On the other hand, direct interatomic contacts are made between 7 CCR5 residues and 6 gp120 amino-acid residues. These include van der Waals contacts, hydrophobic interaction, and hydrogen bond. Residues in contact are concentrated in three dispersed regions of human CCR5 (Fig 3): the tyrosine-rich motif, the span from 22 to 29 and Phe96 residue; and they are distributed over three segments of gp120, one residue from the V3 loop, strand β20 and β21. Nearly 70 % of the gp120 residues that make contacts with human CCR5 do so only through main-chain atoms of gp120, and 40 % of human CCR5 contacts are made by gp120 main-chain atoms (Fig 3).

The positive amine group of Lys22 of human CCR5 makes a direct hydrogen bond with the carbonyl group of Ala299 in the V3 variable loop of gp120. Similarly, the carboxylate group of Asp11 makes a salt bridge with the amine group of Lys421. Tyr15 and Thr16 make hydrogen bonds with Gln422 and Arg419 at strand β20 of gp120, respectively. The amide groups of these residues (Phe96, Ile28, and Ala29) of CCR5 make three hydrogen bonds with the corresponding residues (Tyr435 and Pro438) at strand β21 of gp120.

The recent research results show that the structural domains of an extended active site which are responsible for molecular recognition of large lipid substrates, such as the β-chemokines MIP-1α, MIP-1β, and RANTES, are different from the hydrophobic domains in our human CCR5 model binding the different portions of gp120, while can be settled by site-directed mutagenesis and structure-activity experiments[13]. The site of interaction between the chemokine receptor CCR5 and

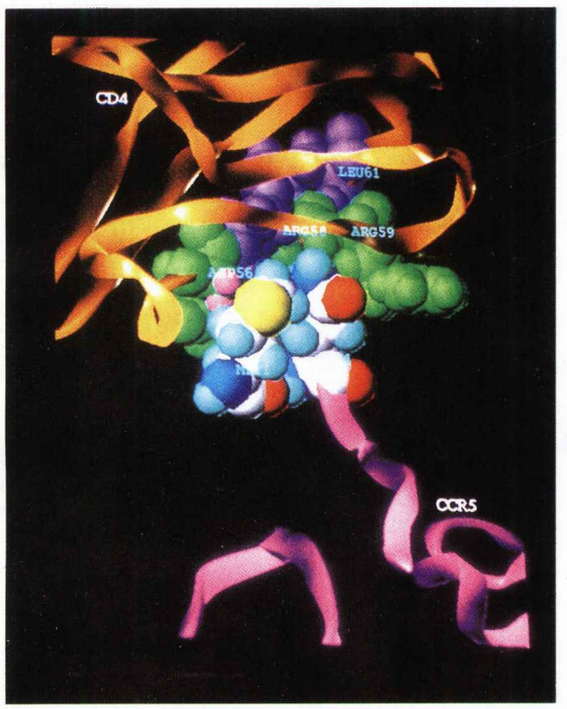

Fig 2. The interaction of human CCR5 and CD4 glycoprotein. The binding sites are displayed by space fill balls and colored by atoms. The human CCR5 appears as purple shaded ribbon, and the CD4 glycoprotein appears as orange shaded ribbon.

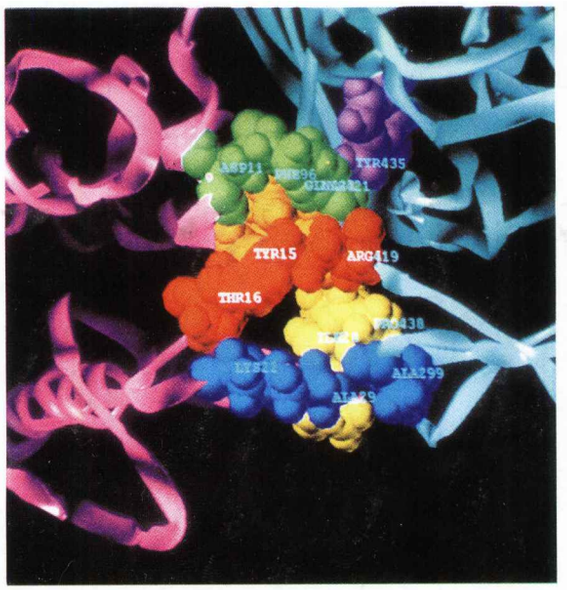

Fig 3. The interaction of human CCR5 and HIV gp120. The human CCR5 appears as purple shaded ribbon. HIV-1 gp120 glycoprotein appears as bluegreen shaded ribbon. The binding sites were displayed by space fill balls and colored by residue type.

glycoprotein gp120 overlaps with the 17b epitope, which lies across the base of the four-stranded bridging sheet. Mutational analysis showed that the basic and polar gp120 residues (Lys121, Arg419, Lys421, Gln422) were important for CCR5 interaction. The chemokine-receptor-binding site overlapped with the CD4-induced gp120 epitope and also contained V3 loop of gp120[1]. Moreover, the CCR5-binding site on HIV-1 gp120 is fully formed or exposed only after CD4 binding, and efficient CCR5 binding is dependent on the presence of the V3 variable loop of gp120. The research results showed that a complex of gp120 and soluble CD4 interacted with CCR5 and inhibited the binding of the natural CCR5 ligands. The apparent affinity of the interaction between gp120 and CCR5 was dramatically lower in the absence of soluble CD4. Additionally, in the absence of gp120, an interaction between a two-domain CD4 fragment and CCR5 was observed. A gp120 fragment retaining the CD4-binding site and overlapping epitopes was able to interact with CCR5 only if the V3 loop, which can specify HIV-1 tropism and chemokine receptor choice, was also present on the molecule. Neutralizing antibodies directly against either CD4-induced or V3 epitopes on gp120 blocked the interaction of gp120-CD4 complexes with CCR5. These results suggest that HIV-1 attachment to CD4 create a high-affinity binding site for CCR5, leading to membrane fusion and virus entry[3].

This work above showed that it would be a key cycle in inhibiting HIV infection and that the interaction of CCR5 with gp120 was blocked. Moreover, our studies supported the viewpoint above. And it has a source of inspiration for new drug development and research.

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人 CCR5 受体与 CD4 抗原和 HIV-1 包膜糖蛋白 gp120 复合物的分子模型[1]

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关键词 CCR5 受体; CD4 抗原; HIV 包膜蛋白质; HIV-1; 趋化因子受体; HLA 抗原; 分子模型

目的: 研究人 CCR5 与 CD4 抗原和 HIV-1 包膜糖蛋白 gp120 的相互作用. **方法**: 人 CCR5 受体的结构保守区由 SYBYL 软件中的 Biopolymer 模块建立, 非保守区由 LOOP SEARCH 方法建立. 将得到的结构模型与 CD4 抗原和 gp120 结合形成复合物. **结果**: 人 CCR5 受体既可以与 CD4 抗原相互作用, 也可以和 gp120 相互作用, 其 *N*-末端残基通过静电和氢键方式与 CD4 相互作用, 并深埋在一个疏水中心里, 被碱性基团包围. 而且有 7 个氨基酸残基通过范德华作用、疏水性作用和氢键与 6 个 gp120 残基相互作用. **结论**: 该模型将有助于设计作用更强的抗艾滋病药物.

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