Structure, Function and Modulation of Chemokine Receptors: Members of the G-Protein-Coupled Receptor Superfamily

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Abstract: Chemokine receptors are membrane proteins that play an important role in inflammation and the cellular entry of human immunodeficiency virus type I (HIV-1). Understanding the structure-function relationship of chemokine receptor-ligand interactions and developing novel strategies to control these interactions have important implications for therapeutic intervention of human diseases such as HIV-1 infection. This article reviews the work carried out in our laboratory in molecular modeling and site-directed mutagenesis of chemokine receptor-ligand interactions and chemical synthesis of chemokine-derived peptide agonists and antagonists. These studies demonstrate a paradigm for exploring and controlling membrane protein-protein interactions.

Keywords: Chemokines, chemokine receptors, G-protein-coupled receptor, interleukin-8 receptor, CCR5, CXCR4, SDF-1, vMIP-II, protein-protein interactions, signal transduction, drug design

INTRODUCTION

Chemokine (chemoattractant cytokine) receptors are a group of membrane proteins and belong to the superfamily of G-protein-coupled receptors (GPCRs) that possess seven transmembrane helices and transmit signals from extracellular ligands to the intracellular biological pathways *via* heterotrimeric G-proteins [1-3]. As the natural ligands of chemokine receptors, chemokines are a family of small proteins of 70-80 residues that act as chemoattractants of various types of leukocytes to sites of inflammation and to secondary lymphoid organs [4]. Based on the positions of two conserved cysteine residues in their N-termini, chemokines can be divided into four subfamilies: CC, CXC, CX3C and C [5,6]. Table 1 lists some representative chemokines and their receptors of two main subfamilies: CXC and CC. They are important for the selective activation and recruitment of a large variety of cell types in inflammation. CXC chemokines are primarily involved in the activation of neutrophils whereas CC chemokines do not affect neutrophils and generally stimulate other leukocytes such as monocytes, lymphocytes, and basophils. In addition to important roles in many physiological processes, chemokines are implicated in a wide range of human acute

Table 1 A List of Representative Chemokine Receptors and their Ligands

	Receptors	Ligands
CXC Subfamily	CXCR1 (IL-8R-α)	IL-8
	CXCR2 (IL-8R-β)	IL-8, GROα, β, γ, NAP-2, ENA78, GCP-2
	CXCR3	IP10, Mig
	CXCR4	SDF-1
CC Subfamily	CCR1	RANTES, MIP-1α, MCP-2, MCP-3
	CCR2a/b	MCP-1, MCP-2, MCP-3, MCP-4
	CCR3	Eotaxin, RANTES, MCP-3, MCP-4
	CCR4	RANTES, MIP-1α, MCP-1
	CCR5	RANTES, MIP-1α, MIP-1β

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and chronic inflammatory diseases such as acute respiratory distress syndrome, allergic asthma, psoriasis, and arthritis.

In recent years, chemokines and their receptors have also been found to be involved in the pathogenesis of human immunodeficiency virus type I (HIV-1) infection. HIV-1 enters cells through a fusion process in which the HIV-1 envelope glycoprotein gp120 binds to CD4, the main receptor for HIV-1 on the cell surface. However, it has long been known that CD4 alone is not sufficient for HIV-1 fusion and entry and that additional receptors may be needed [7,8]. In 1996 chemokine receptors CXCR4 and CCR5 were discovered to be the long-sought co-receptors for non-syncytium-inducing and syncytium-inducing HIV-1 strains, respectively [9-12]. While all HIV-1 strains appear to require either CXCR4, CCR5 or both [13,14], some strains can also use other chemokine receptors CCR3 and CCR2b as co-receptors for fusion and infection [15,16].

As shown in Fig. **1**, the viral fusion process may involve the initial binding of HIV-1 gp120 to its high-affinity receptor CD4 which results in conformational changes in gp120 and probably in CD4 [17-19] as well. The gp120-CD4 complex interacts with a chemokine coreceptor such as CXCR4 or CCR5 to form a heterotrimeric complex of gp120-CD4-coreceptor [20-22]. It has been shown that the HIV-1 envelope can bind CXCR4 independently and that this interaction is enhanced by the presence of CD4 [23]. Natural chemokines that bind CXCR4 can inhibit HIV-1 infection [24,25], probably by blocking common binding sites on CXCR4 that are required for gp120 interaction with its coreceptor and/or by inducing receptor internalization.

Characterizing the mechanism of biological recognition between chemokine receptors and ligands is essential for understanding the physiological or pathological processes such as HIV-1 entry that they mediate and devising novel strategies for clinical intervention. In addition, chemokine receptor-ligand complexes can serve as useful models to gain general insights into the structure-function relationship of a large number of other membrane proteins of the GPCR superfamily. In this article, we review three areas of the work carried out in our laboratory: molecular modeling of structures of chemokine receptor-ligand complexes; site-directed mutagenesis of chemokine receptors; and chemical synthesis of rationally designed receptor agonists and antagonists based on natural chemokines (Fig. **2**). These different and yet complementary approaches are combined in our research to characterize specific sites and mechanisms for the molecular interactions of chemokine receptors with their natural ligands and HIV-1 gp120. As described below, molecular modeling techniques are applied to propose plausible structural models for chemokine receptors and their complexes with ligands. Such models reveal potential sites of interaction between a receptor and a ligand. To experimentally test the putative functional role of such sites, site-directed mutagenesis of the chemokine receptor is carried out to examine the role of the predicted sites on the receptor in ligand binding and signaling. Furthermore, chemical and structure-based design approaches are applied to generate novel chemokine-derived peptides to dissect the structure-function relationship of full length chemokines and modulate interactions and functions of chemokine receptors.

MOLECULAR MODELING AND MUTATIONAL ANALYSIS OF CHEMOKINE RECEPTORS

The information about the detailed three-dimensional (3D) structure of a chemokine receptor and its complex with a ligand is critical for the understanding of the role and mechanisms of function of chemokine receptors and ligands in HIV-1 pathogenesis. However, no crystal structure is available for chemokine receptors or most of GPCRs. Since

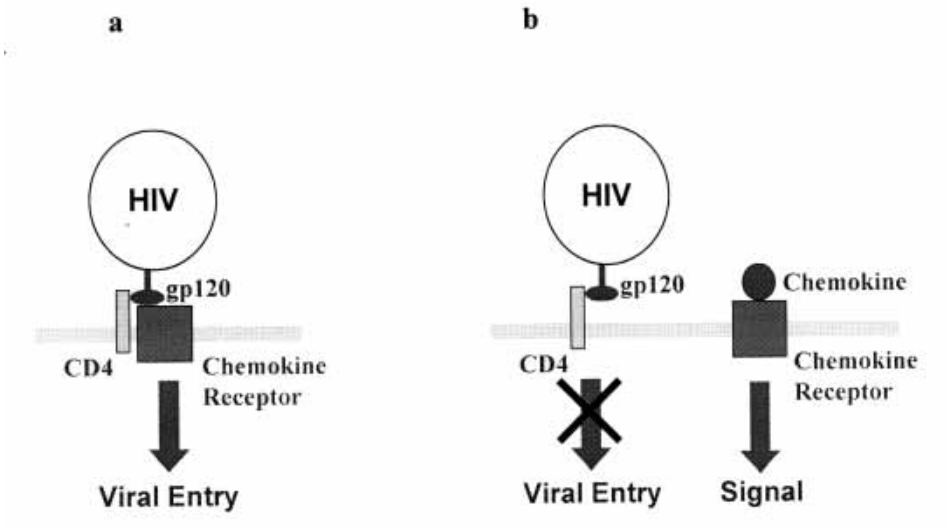

Fig. 1 (a) The interactions of HIV-1 gp120, CD4 and a chemokine coreceptor such as CXCR4 or CCR5, leading to the cellular entry of the virus. (b) The binding of a chemokine ligand (such as SDF-1α) to its receptor (such as CXCR4) blocks the association of HIV-1 gp120/CD4 with CXCR4, thus preventing the viral entry. On the other hand, the chemokine receptor-ligand interaction can activate normal signaling and biological functions (such as chemotaxis).

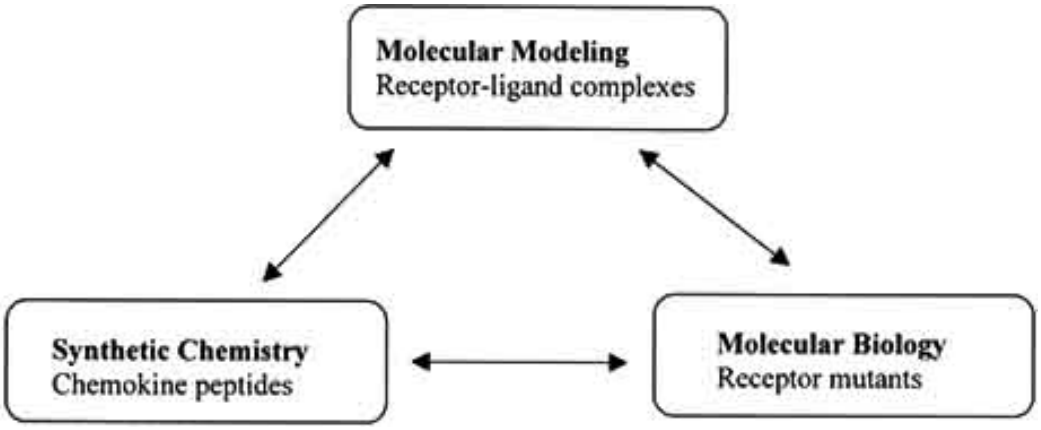

Fig. (2) An integrated approach to study chemokine receptor-ligand interactions.

the structure of the seven transmembrane helices in a similar protein, bacteriorhodopsin (bR), has been determined by electron microscopy [26], many members of the GPCR superfamily have been modeled by computational techniques based on the structure of the transmembrane segments (TMs) of bR [2,27]. We decided to extend the computational methodologies used for modeling other GPCR proteins to the family of chemokine receptors. A general approach for modeling the structure and interaction of chemokine receptors has been developed from our study of interleukin-8 receptor type β (IL-8R-β), a member of the chemokine receptor family [28].

As shown in Fig. **3**, the structure for the transmembrane helices of a chemokine receptor is constructed based on that of bR. To further define the 3D structure of the extracellular

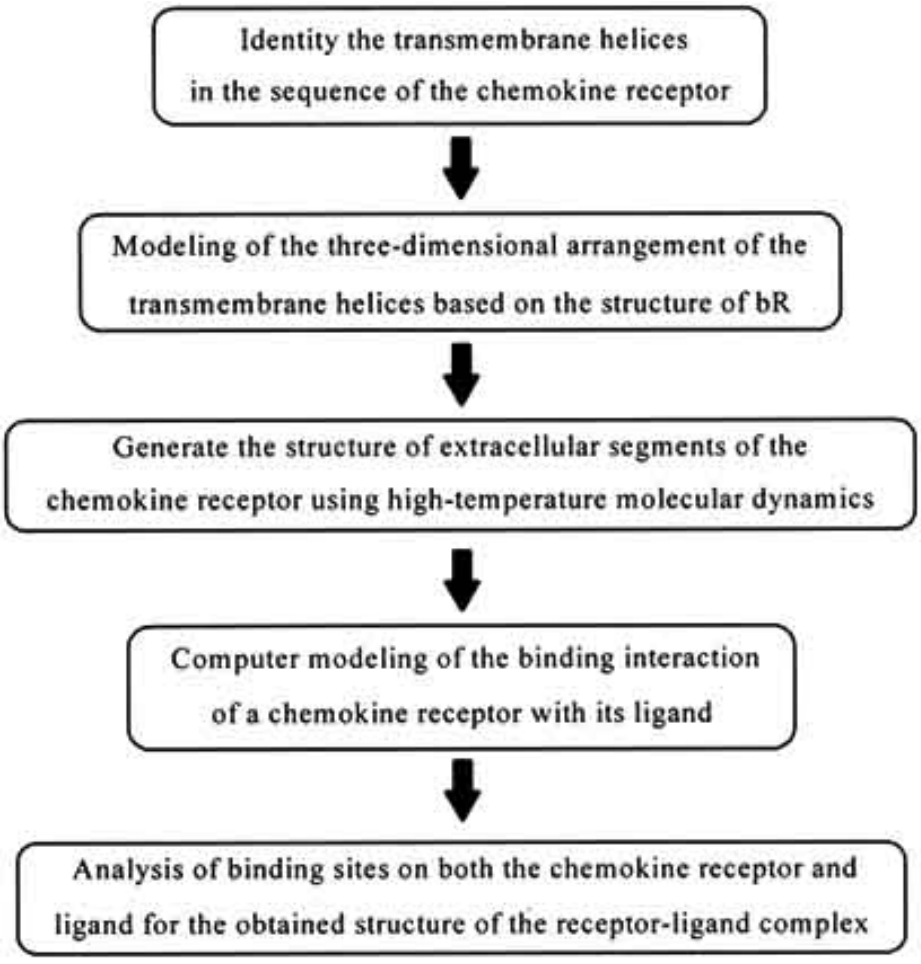

Fig. (3) The general procedure for molecular modeling of a chemokine receptor-ligand complex.

segments of the chemokine receptor and understand how they interact with a ligand, high temperature molecular dynamics simulations are performed. The 3D structure of the ligand, as determined by NMR experiment, is used as the geometric constraint in dynamics simulations. A nanosecond simulation is conducted for the receptor-ligand complex and 500 structures are extracted from the trajectory of the simulation. These structures are clustered by the similarity in their backbone structures. Plausible structural models for the receptor-ligand complex are selected based on the examination of available experimental data and binding energy of the complex. Using this approach, we proposed hypothetical structures of several chemokine receptors including interleukin-8 receptor β (IL-8R-β) or CXCR2 [28], CCR5 [29] and CXCR4 [30] and their complexes with ligands as described below.

Molecular Modeling of Interleukin-8 Receptor β

Interleukin 8 (IL-8), one of the CXC chemokines, is a potent neutrophil chemoattractant which is expressed by many cell types in response to inflammatory stimuli [31]. Two human IL-8 receptors were cloned and characterized, designated as type α (IL-8R-α) and type β (IL-8R−β) receptors, respectively [32,33]. To simulate the interaction of IL-8 with its receptor IL-8R−β, the crystal structure of IL-8 [34] was placed on top of the extracellular domains of IL-8R−β. This initial structure of the complex was extensively energy-minimized and then subjected to molecular dynamics simulation. A high temperature (900 K) and a long simulation time (1 ns) were used. Also, during the dynamics simulation, the transmembrane core helices were fixed, and the conformation of IL-8 was constrained in order to maintain its X-ray crystal structure. These procedures were used in order to search more effectively the conformations of the extracellular loops of IL-8R-β which are important for ligand binding.

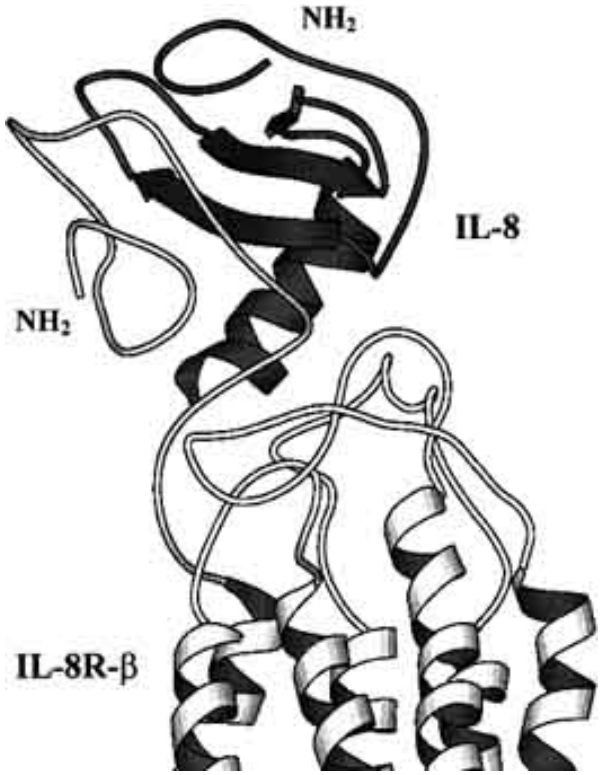

Fig. (4) A plausible structural model of IL-8R−β in complex with IL-8. The figure was generated by using the MOLSCRIPT program [81].

500 structures of the IL-8 and IL-8R−β complex were extracted from the molecular dynamics trajectory at a time interval of every 2 ps. All structures were energy-minimized, and the interaction energy (binding energy) between IL-8 and IL-8R−β was calculated for each energy-minimized structure. These structures were clustered into about 100 conformational families based on their backbone structural similarities. A representative structure for each family was analyzed according to the known mutation data and binding energy. Five structures were finally selected as possible models for the complex, including the structure with the most favorable binding energy (Fig. **4**). In all five models, the IL-8 molecule covers its receptor like a lip, and only the residues involved in hydrogen bond formation are different. Hydrogen bond formation between IL-8 and IL-8R−β was observed in all of these models. The modeling results are in agreement with available experimental data [35-37].

Molecular Modeling and Site-directed Mutagenesis of CCR5

The entry of HIV-1 into the target cell is mediated by CD4 as the primary receptor [38,39], as well as chemokine receptors such as CCR5, CXCR4, CCR3 and CCR2b, as necessary coreceptors [9-12]. Macrophage-tropic (M-tropic) strains of HIV-1 use CCR5, whereas T-cell-tropic (T-tropic) strains use CXCR4. Dual-tropic strains are those HIV-1 isolates that are capable of using both CCR5 and CXCR4. M-tropic viruses that use CCR5 are involved in sexual transmission and are the predominant virus type during asymptotic stages of the disease [40]. The crucial importance of CCR5 in HIV-1 transmission and feasibility of CCR5-specific agents to inhibit viral infection were further demonstrated by the observation that individuals with CCR5 mutations appear to be both healthy and highly resistant to HIV infection [41]. The CC chemokine ligands of CCR5 include macrophage inflammatory protein 1 β (MIP-1β), macrophage inflammatory protein 1 α (MIP-1α), and regulated on activation normal T cell expressed and secreted (RANTES). Among these CCR5 ligands, RANTES and MIP-1α can bind to other CC chemokine receptors while MIP-1β is known to be most specific for CCR5 [5].

The characterization of structural and functional determinants of CCR5 for its ligand binding activity and HIV-1 coreceptor function is essential for understanding mechanisms of HIV-1 viral entry and developing novel therapeutic agents. To this end, many studies have recently been carried out by a number of laboratories using chimera receptors [42-48] and site-directed mutagenesis [49-51]. Despite such a wealth of information from genetic and molecular biological experiments, the detailed structure-function relationships of CCR5 are yet to be defined in the absence of the 3D structure.

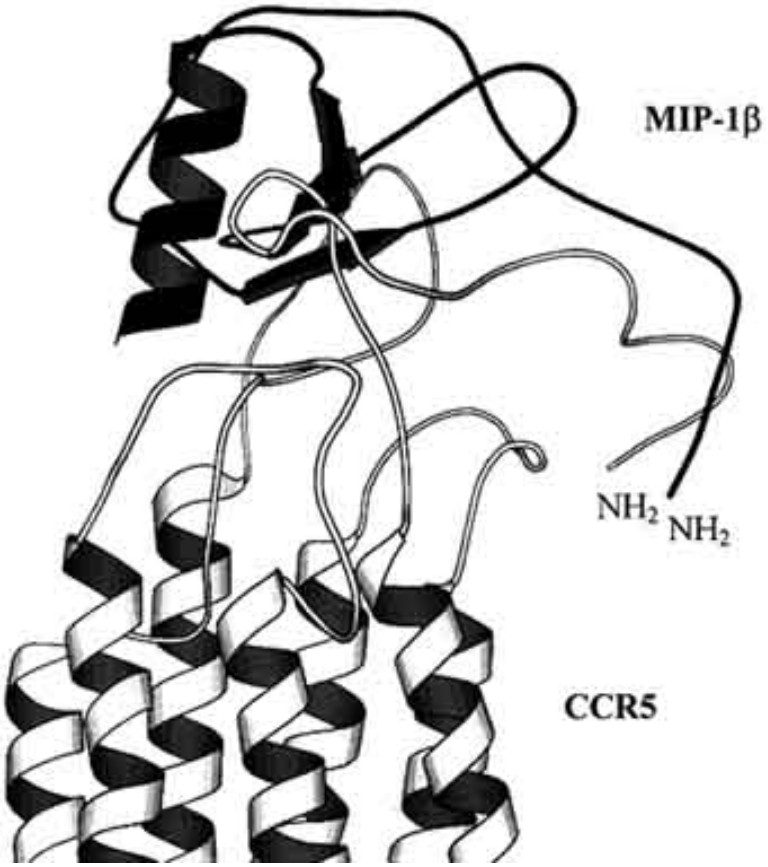

Fig. (5) A plausible structural model of CCR5 in complex with MIP-1β.

In view of this, we used an approach combining molecular modeling and experimental validation, similar to that developed in the study of IL-8R-β [28], to propose plausible 3D structural models of CCR5 and its complex with MIP-1β (Fig. **5**). These models rationalize the available data from chimeric and mutational experiments and provide a possible framework to understand the structural basis of CCR5 interactions with natural chemokine ligands and HIV-1 envelope glycoprotein gp120. Furthermore, based on the molecular modeling results, we carried out site-directed mutagenesis studies of the amino (N)-terminus and the second extracellular loop of CCR5 to test specific structure-function hypotheses of our models. This lead to the novel observation that certain residues, such as Tyr10 and Lys26, in the N-terminus of CCR5 play a critical structural role for ligand binding and signaling [29]. Single glycine substitution of these residues significantly decreases chemokine binding and signal transduction. These results provide new insight into the structural basis for CCR5 receptor-ligand interaction and may guide the design of the novel inhibitors.

Molecular Modeling and Site-directed Mutagenesis of CXCR4

The stromal cell-derived factor-1 (SDF-1α) is one of the CXC chemokines, which plays critical roles in the migration, proliferation, and differentiation of leukocytes. SDF-1α is the only known natural ligand of CXCR4 receptor [24,25]. CXCR4 can also be recognized by an antagonistic ligand, the viral macrophage inflammatory protein-II (vMIP-II) encoded by the Kaposi's sarcoma-associated herpes virus [52]. vMIP-II displays a broader spectrum of receptor activities than any mammalian chemokine as it binds with high affinity to a number of both CC and CXC chemokine receptors including CXCR4 and CCR5 and inhibits cell entry of HIV-1 mediated by these receptors [53,54]. Studies with knockout mice of CXCR4 have demonstrated that this molecule plays an important role in immunomodulation, organogenesis, hematopoiesis, and derailed cerebellar neuron migration [55-57]. CXCR4 has also been identified as one of the co-receptors for HIV-1 [9]. CXCR4 mediates infection of T-cell-line tropic HIV-1 strains and has also been found to be used by human immunodeficiency virus type II (HIV-2) strains adapted to replication in CD4-negative cell-lines [58].

The characterization of structural and functional determinants of CXCR4 for its ligand binding activity and HIV-1 coreceptor function is essential for understanding mechanisms of HIV-1 viral entry and developing novel therapeutic agents for HIV-1 infection. To this end, several studies have recently been carried out by a number of laboratories using chimeric chemokine receptors and mutants to demonstrate that multiple domains of CXCR4 are

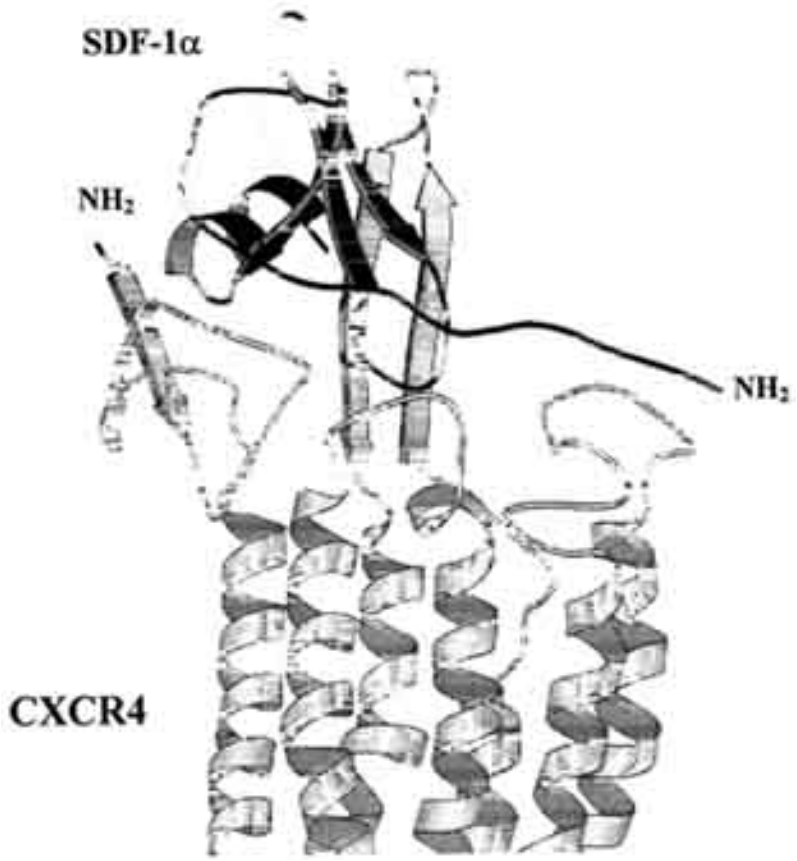

Fig. (6) A plausible structural model of CXCR4 in complex with SDF-1α.

required for HIV-1 co-receptor activity [59-67]. Furthermore it has been demonstrated that the N-terminal domain of CXCR4 was a determinant in SDF-1α binding and that the second extracellular loop of CXCR4 was involved in receptor signaling [61,64,66].

To better understand CXCR4 receptor and coreceptor functions, we used an approach combining molecular modeling and experimental validation, similar to that developed in our earlier studies of IL-8R-β [28] and CCR5 [29], to propose plausible three-dimensional structural models of CXCR4 and its complex with SDF-1α or vMIP-II (Fig. **6**) [30]. These models rationalize the available data from chimeric and mutational experiments and provide a possible framework for understanding the structural basis of CXCR4 interactions with chemokine ligands and the HIV-1 envelope glycoprotein gp120. Furthermore, based on the molecular modeling results, we carried out site-directed mutagenesis studies on the N-terminus, the second extracellular loop, and the third extracellular loop of CXCR4 to test specific structure-function hypotheses from our structural models. These structural and mutational studies provide valuable information regarding the structural basis for CXCR4 activity in chemokine binding and HIV-1 viral entry, and could guide the design of novel targeted inhibitors.

STUDIES OF SYNTHETIC PEPTIDES RELATED TO CHEMOKINE LIGANDS

Synthetic Peptides as Mimics of SDF-1α

Complementary to the above described molecular modeling and mutational studies of chemokine receptors, we used synthetic peptides mimicking specific regions of a chemokine ligand as templates to study the structure-function relationship of the native molecule and develop novel agonists or antagonists of chemokine receptors. For example, SDF-1 is the ligand for CXCR4 [68,69]. The N-terminus of SDF-1 has been shown to be essential for CXCR4 recognition and signal transduction by studies of synthetic peptides and SDF-1 mutants [70,71]. In contrast, the role of other domains of SDF-1, such as a central core region of three antiparallel β-strands following the SDF-1 N-terminus, are less clear. Since this central core region contains a number of positively charged residues, it has been suggested that these residues may interact with the negatively charged residues in the extracellular domains of CXCR4 [72]. In addition, peptides and organic compounds of high positive charges such as ALX40-4C [73], T22 [74], and AMD3100 [75] are found to have high affinity for CXCR4. Taken together, these observations tend to indicate that the electrostatic interaction may play a role in CXCR4 recognition.

To test this hypothesis, two studies were carried out using synthetic peptides [76]. In the first study, peptide analogs possessing amino acid sequences from both the N-terminus and the β-sheet region of SDF-1 were used as models to study the functional role of the β-sheet region of SDF-1 (Table 2). The attachment of positively charged residues to the N-terminal peptide sequence of SDF-1 (as in SD-2 and SD-3 analogs) was found to enhance the ability of the peptides in CXCR4 binding and inhibiting CXCR4-mediated T-tropic HIV-1 entry. In the second study, two peptides containing nine arginines and the N-terminal signal sequence of SDF-1 were used as models to study the receptor binding mechanism of CXCR4 antagonists of high positive charges such as ALX40-4C (Table 2). One peptide (SD-5) did not show signaling activity as indicated by the lack of calcium influx while another peptide (SD-4) induced unusual calcium influx distinct from that induced by the SDF-1 N-terminal peptide. In addition, the signal induced by the SDF-1 N-terminal peptide was inhibited by ALX40-4C. Therefore, the first study provides experimental support for the role of the highly positive β-sheet region of SDF-1 in CXCR4 binding. The second study suggests that the binding site of ALX40-4C in CXCR4 may partially overlap with that of the SDF-1 N-terminal peptide. Both findings should be valuable for the design of SDF-1 agonists and antagonists.

Table 2. The sequence of the Designed Peptides

	Sequence
N-term	KPVSLSYRCPCRFF
SD-2	KPVSLSYRCPCRFF-AAAA-RARLKAKHLK
SD-3	KPVSLSYRCPCRFF-RRRRRR
SD-4	KPVSLSYRCPCRFF-GGGG-RRRRRRRRR
SD-5	KPVSLSYR-GGGG-RRRRRRRRR
ALX40-4C	CH_3CO-$(dR)_9$-$CONH_2$

In addition to the central β-sheet region, the functional role of the C-terminal helix of SDF-1 was studied by using synthetic peptide models containing both N- and C-terminal regions of SDF-1 [77] (Fig. **7**). The attachment of the C-terminus of SDF-1, which by itself had no activity in receptor binding and signaling, dramatically increased the effect of the N-terminal fragment in inducing chemotaxis and intracellular calcium influx in sup T1 cells as compared with the peptide containing only the N-terminal sequence. The enhancement in activity was not due to the increase in receptor affinity as the N, C-terminal peptide did not show higher CXCR4 binding than the N-terminal peptide. On the other hand, the intracellular calcium influx activated by the N, C-terminal peptide, but not the N-terminal peptide, was completely abolished by the addition of heparin, suggesting that the C-terminal fragment of the peptide binds glycosaminoglycans (GAGs) and exerts an effect to modulate biological activity. These data raise the possibility that the C-terminus in native SDF-1 is one of interaction sites with GAGs and may be associated with biological function of SDF-1. Furthermore, this study demonstrates an approach for the design of novel agonists or antagonists of other chemokine receptors that possess enhanced biological activity.

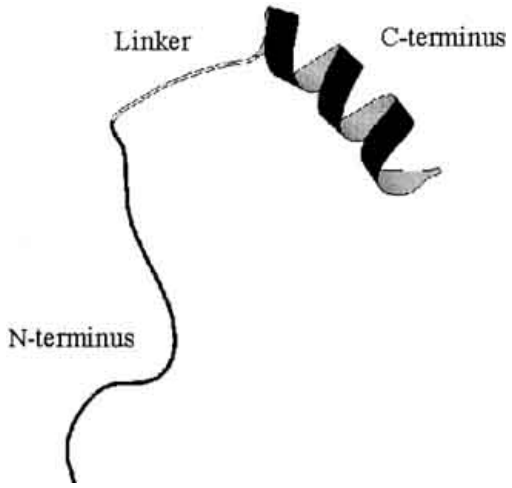

Fig. (7). The schematic structure of NCT-tide. NCT-tide consists of N- (residues 5-14) and C-termini (residues 55-67) of SDF-1, which is linked by four glycines. The amino acid sequence of the peptide is as follow: LSYRCPCRFF-GGGG-LKWIQEYLEKALN.Twocontrol peptides NT-tide and CT-tide were synthesized containing the N- (LSYRCPCRFF) and C-termini (LKWIQEYLEKALN) of SDF-1α, respectively.

Peptides and Peptidomimetics Derived from vMIP-II as Antagonists of CXCR4

The viral macrophage inflammatory protein-II (vMIP-II) encoded by Kaposi's sarcoma-associated herpesvirus is unique among all known chemokines in that vMIP-II shows a broad-spectrum interaction with both CC and CXC chemokine receptors, particularly CCR5 and CXCR4 [53,54]. To elucidate the mechanism of the promiscuous receptor interaction of vMIP-II, we studied synthetic peptides derived from the N-terminus of vMIP-II (Table 3) [78]. In contrast to the full-length protein that recognizes both CXCR4 and CCR5, a peptide designated as V1 corresponding to residues 1-21 of vMIP-II was shown to strongly bind CXCR4, but not CCR5. This peptide selectively prevented CXCR4 signal transduction and coreceptor function in mediating the entry of T- and dual-

Table 3. Sequences of vMIP-II and N-terminal Peptide of vMIP-II

vMIP-II	LGASWHRPDKCCLGYQKRPLPQVLLSSWYPTSQLCS KPGVIFLTKRGRQVCADKSKDWVKKLMQQLPVTAR
V1 (residues 1-21 of vMIP-II)	LGASWHRPDKCCLGYQKRPLP

tropic HIV-1 isolates, but not those of CCR5. Further analysis of truncated peptide analogs revealed the importance of first several residues for the activity with CXCR4. These results suggest that the N-terminus of vMIP-II is essential for its function *via* CXCR4. In addition, they reveal a possible mechanism for the distinctive interactions of vMIP-II with different chemokine receptors, a notion that may be further exploited to dissect the structural basis of its promiscuous biological function.

To understand the structure-function relationship of V1 peptide, its solution conformation was studied using circular dichroism spectroscopy which showed a random conformation similar to that of the corresponding N-terminus in native vMIP-II [79]. In addition, we synthesized a series of mutant analogs of V1 containing alanine, glycine or phenylalanine substitution at various positions. Residues Leu-1, Arg-7 and Lys-10 of V1 peptide were found to be critical for receptor interaction, because single alanine replacement at these positions dramatically decreased peptide binding to CXCR4. In contrast, alanine or phenylalanine substitution at Cys-11 led to significant enhancement in peptide affinity for CXCR4. Finally, we showed that V1 peptide inhibits HIV-1 replication in $CXCR4^+$ T cell lines. These studies provide new insights into the structure-function relationship of V1 peptide and demonstrate that this peptide may be a lead for the development of therapeutic agents.

Being highly amenable to chemical synthesis and modification, this V1 peptide prompted us to use chemically modified analogs of V1 as probes to study the molecular recognition of CXCR4-ligand complex. Since one important aspect of receptor-ligand interaction is the requirement of stereospecificity, we synthesized an all-D-amino acid analog of V1 peptide, designated as DV1 peptide [80]. Unexpectedly, DV1 displays high affinity and antagonistic activity toward CXCR4, despite the dramatic different conformations between DV1 and V1 as the opposite mirror images. This reveals that the peptide binding site on CXCR4 is tolerant of changes in chirality of ligands. Similar observations are also made for other D-peptides derived from the N-terminus of SDF-1α. These findings have important implications for understanding mechanism of CXCR4-ligand interaction and designing novel inhibitory molecules. Furthermore, DV1 peptides are highly resistant to proteolytic degradation and show significant activity in blocking HIV-1 replication in $CXCR_4^+$ cell lines, thus demonstrating their advantage over natural L-peptides for potential clinical application. Taken together, the various L- and D-peptide antagonists of CXCR4 described above could serve as leads for the development of new therapeutic agents for HIV-1 infection and other immune system diseases.

CONCLUSION

Chemokine receptors and their ligands play an essential role in inflammation and HIV-1 entry. Chemokine receptors are members of the GPCR superfamily that includes a large number of membrane proteins that have diverse biological functions and have been a major class of therapeutic targets. As such, understanding the structure-function relationship of chemokine receptor-ligand interactions and developing novel strategies to control these interactions have important implications for therapeutic intervention of human diseases such as HIV-1 infection and the study of protein-protein interactions involving other GPCRs. To this end, we have used an approach combining molecular modeling, chemical synthesis and molecular and cellular biology to characterize the functional determinants of the chemokine receptor-ligand interface and design chemokine receptor agonists and antagonists.

Using molecular modeling techniques, we have predicted plausible models for structures of several chemokine receptors, IL-8R-β, CCR5 and CXCR4 and their complexes with ligands. These models provide a starting point to rationalize available biological data about the receptors and understand the structural basis of their functions. Furthermore, they reveal potentially new details of receptor-ligand interactions. Based on such information, we have carried out site-directed mutagenesis of CCR5 and CCR4 to test the functional role of specific residues on the receptor as predicted by molecular modeling studies. This has led to the identification of key amino acid residues of CCR5 or CXCR4 essential for chemokine binding, interaction with HIV-1 envelope glycoprotein gp120, or signal transduction.

Finally, we have used a chemical synthetic approach to dissect the structure-activity relationship of chemokines in conjunction with structural and functional characterization of their receptors by molecular modeling and site-directed mutagenesis techniques. Synthetic peptides derived from or mimicking putative functional domains of natural chemokines, SDF-1α and vMIP-II have been studied to assess the role of these domains in receptor binding and signaling. Peptides derived from the N-terminus of vMIP-II have been shown to be potent antagonists of CXCR4 and inhibitors of HIV-1 entry *via* CXCR4. Chemical modifications of these peptides such as the incorporation of unnatural D-amino acids have resulted in analogs with significantly enhanced receptor affinity and biological stability which are advantageous for further therapeutic development. Therefore, the computational, chemical and biological studies of protein-protein interactions involving chemokine receptors and ligands demonstrate a paradigm for exploring and controlling molecular recognition of membrane proteins.

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