Journal of Medicinal Chemistry

Letter

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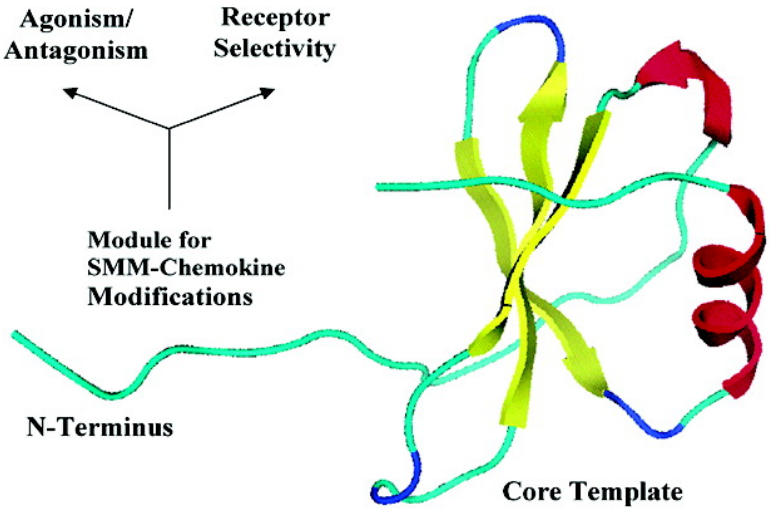
J. Med. Chem., **2005**, 48 (25), 7923-7924• DOI: 10.1021/jm050829u • Publication Date (Web): 19 November 2005



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Different Stereochemical Requirements for CXCR4 Binding and Signaling Functions As Revealed by an Anti-HIV, D-Amino Acid-Containing SMM-Chemokine Ligand

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Received August 19, 2005

Abstract: Human immunodeficiency virus type 1 (HIV-1) uses a chemokine receptor, usually CXCR4 or CCR5, for entry into the target cells. Here, we used a chemical biology approach to demonstrate that binding and signaling domains in CXCR4 are possibly distinct and separate, as the new analogue, D(1-10)-vMIP-II-(9-68)-SDF-1α (RCP222), could not activate CXCR4 despite the fact that its binding activity was comparable to that of stromal cell-derived factor (SDF)-1α, the only natural ligand of CXCR4.

Chemokine receptors CXCR4 and CCR5 are the major human immunodeficiency virus type 1 (HIV-1) coreceptors.[1−4] 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. The two main subfamilies are the CXC and CC chemokines based on the positions of two conserved cysteine residues in their amino (N)-termini.[5−8] Natural chemokines of CXCR4 or CCR5 can prevent chemokine receptor-dependent HIV-1 entry by blocking the binding of HIV-1 glycoprotein gp120 to CXCR4 or CCR5.[9,10] Alternatively, chemokines can inhibit viral entry by inducing receptor downregulation from the cell surface, thereby removing the essential coreceptors.[11,12]

Because of the importance of chemokines and their receptors in HIV-1 infection and other pathological processes, including acute respiratory distress syndrome, allergic asthma, psoriasis, arthritis, multiple sclerosis, cancer, and atherosclerosis,[5−8] we have been working toward the development of a family of unnatural chemokines, termed synthetically and modularly modified (SMM)-chemokines, or short peptides that have higher receptor binding and antiviral activities than their natural counterparts. We previously showed that D-peptides derived from the N-terminal sequence module of viral macrophage inflammatory protein (vMIP)-II display greater CXCR4 binding and antiviral activities than their parent peptides.[13]

Table 1. Amino Acid Sequences of **1** and **2** and Their Modification

Analog Designation	Modification Diagrams	Amino Acid Sequences[a]
1	1-8 / 9-68 NT | SDF-1α Core	KPVSLSYRCPCRFFESHV ARANVKHLKILNTPNCALQIV ARLKNNNRQVCIDPKLKWIQ EYLEKALNK
2	1-10 / 9-68 NT | SDF-1α Core D-vMIP-II	*LGASWHRPDK*CPCRFFESHV ARANVKHLKILNTPNCALQIV ARLKNNNRQVCIDPKLKWIQ EYLEKALNK

In this study, we applied the concept of SMM-chemokines containing D-amino acids to study the chemical basis of CXCR4-ligand interactions. Specifically, we asked the question of whether the binding and signaling functions of a chemokine ligand via its receptor are mediated by the same or distinct domains on the receptor. For this purpose, we decided to use stromal cell-derived factor (SDF)-1α (designated here as **1** (RCP211)), the only natural chemokine ligand of CXCR4,[14−16] as a model to study the chemical mechanism of chemokine ligand−receptor binding and signaling by using the above-described SMM modifications on a chemokine, particularly D-amino acid replacement. Using **1** as the target template, we replaced the N-terminal (1−8) residues of **1** with all D-forms of (1−10) sequence module of vMIP-II. As the N-termini of many chemokines, including **1**, are known to play an important role in binding and signaling via their receptors, we investigated the potential effect of the introduction of D-amino acids in the N-terminus of **1** on CXCR4 binding and signaling using this D-amino acid-containing SMM-chemokine, D(1-10)-vMIP-II-(9-68)-SDF-1α (referred to as **2** (RCP222), Table 1), as a stereochemical probe.

We examined the effect of the D-amino acid replacement on receptor−ligand binding. We used ^{125}I-SDF-1α competition binding assays to compare the binding activity of **2** to that of its natural counterpart, **1** (Figure 1). The IC_{50} value of **2** was 2 nM, which was comparable to that of **1** (IC_{50} = 4 nM). The binding specificity of **2** for CXCR4 was also examined by testing **2** in CCR5 competition binding assays using ^{125}I-MIP-1β. No CCR5 binding of **2** was detected, as the plateau of nonspecific binding could not be reached even at 2.7 μM of **2** (data not shown).

After finding that the incorporation of D-amino acids in the N-terminus of **1** does not affect its CXCR4 binding activity, the effect of D-amino acids on CXCR4 signaling was tested using Ca^{2+} mobilization assays. In contrast to the rapid Ca^{2+} mobilization induced by **1**, **2** was not able to induce any mobilization of Ca^{2+} in Sup T1 cells expressing CXCR4 even at 1 μM, while **2** was able to reduce the signaling induced by **1** in a dose-dependent manner (Figure 2). The complete loss of signaling by **2**, in contrast with its very high receptor binding activity,

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10.1021/jm050829u CCC: $30.25
Published on Web 11/19/2005

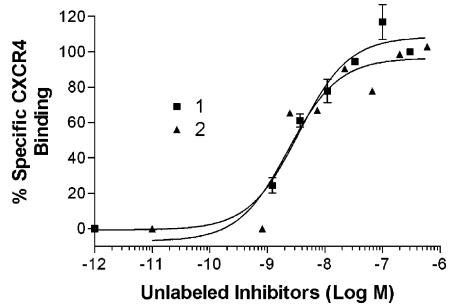

Figure 1. Binding activity of **2**. ^{125}I-SDF-1α competition binding assays were used to determine the IC_{50} value of **2**. **1** was used as a control. The binding data were analyzed using the PRISM program (GraphPad Inc., San Diego, CA). All data are shown as mean ± SD from at least three independent experiments.

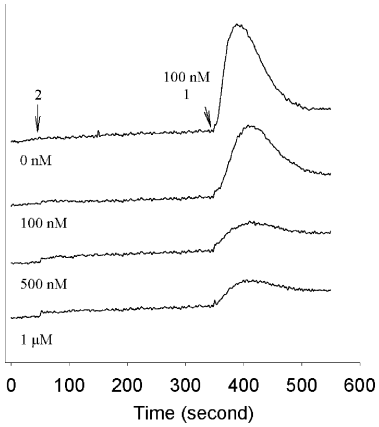

Figure 2. Signaling activity of **2**. Intracellular Ca^{2+} influx in Sup T1 cells was measured in response to **2**. For inhibition assays, Sup T1 cells were preincubated with **2** for 5 min and then stimulated with 100 nM of **1**. At least three independent experiments were performed.

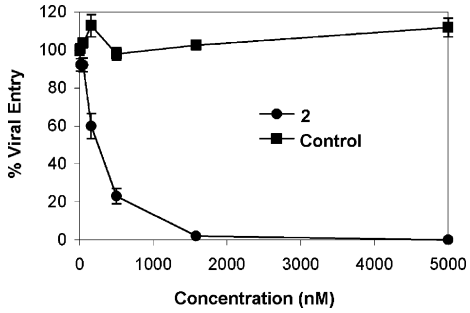

Figure 3. Antiviral activity of **2**. The antiviral activity of **2** was determined using virus infection assays. All data are shown as mean ± SD from at least three independent experiments.

clearly demonstrates that the binding and signaling functions of this chemokine involve different stereochemical requirements and plausibly distinct domains in the receptor.

Since CXCR4 antagonists can potentially be used as inhibitors of HIV-1 entry via CXCR4, **2** was tested for its antiviral activity using virus infection assays. The virus infection assays measure all of the early phase of infection as well as some late phase events such as transcription and tat production. Consistent with its strong binding affinity to CXCR4, **2** was effective in blocking HIV-1 entry at low concentrations (50% inhibition at 200 nM) (Figure 3). In control experiments, **2** was not able to block M-tropic HIV-1 strain that uses the other entry coreceptor, CCR5, which was consistent with its binding selectivity for CXCR4 and that its activity was not due to nonspecific cytotoxicity.

In summary, using the D-amino acid-containing SMM-chemokine analogue derived from the native SDF-1α as the chemical probe, we investigated the stereochemistry of chemokine ligand−receptor interactions and found different stereochemical requirements in binding and signaling of the ligand, as the D-amino acid replacement only affected its signaling activity but not CXCR4 binding. In addition, **2** was shown to be an effective HIV-1 inhibitor, indicating that its receptor binding, but not signaling, contributes to its anti-HIV activity. This study provides new insight into the stereochemical basis of CXCR4-ligand binding and signaling and suggests that further studies of the chemical basis of other chemokine ligand−receptor interactions may be of interest for understanding the mechanism of action of this important family of small protein ligands.

Acknowledgment. This work was supported by grants from the National Institutes of Health.

Supporting Information Available: Analytical data for peptides **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Feng, Y.; Broder, C. C.; Kennedy, P. E.; Berger, E. A. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* **1996**, *272*, 872−877.
(2) Hill, C. M.; Littman, D. R. Natural resistance to HIV? *Nature* **1996**, *382*, 668−669.
(3) Trkola, A.; Dragic, T.; Arthos, J.; Binley, J. M.; Olson, W. C.; et al. CD4-dependent, antibody-sensitive interactions between HIV-1 and its co-receptor CCR-5. *Nature* **1996**, *384*, 184−187.
(4) Wu, L.; Gerard, N. P.; Wyatt, R.; Choe, H.; Parolin, C.; et al. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. *Nature* **1996**, *6605*, 179−183.
(5) Murphy, P. M. The molecular biology of leukocyte chemoattractant receptors. *Annu. Rev. Immunol.* **1994**, *12*, 593−633.
(6) Baggiolini, M.; Dewald, B.; Moser, B. Human chemokines: an update. *Annu. Rev. Immunol.* **1997**, *15*, 675−705.
(7) Berger, E. A.; Murphy, P. M.; Farber, J. M. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Annu. Rev. Immunol.* **1999**, *17*, 657−700.
(8) Proudfoot, A. Chemokine receptors: multifaceted therapeutic targets. *Nature Rev. Immunol.* **2002**, *2*, 106−115.
(9) Bleul, C. C.; Farzan, M.; Choe, H.; Parolin, C.; Clark-Lewis, I.; et al. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* **1996**, *382*, 829−833.
(10) Oberlin, E.; Amara, A.; Bachelerie, F.; Bessia, C.; Virelizier, J. L.; et al. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature* **1996**, *382*, 833−835.
(11) Förster, R.; Kremmer, E.; Schubel, A.; Breitfeld, D.; Kleinschmidt, A. et al. Intracellular and surface expression of the HIV-1 coreceptor CXCR4/fusin on various leukocyte subsets: rapid internalization and recycling upon activation. *J. Immunol.* **1998**, *160*, 1522−1531.
(12) Zhou, N.; Luo, Z.; Luo, J.; Fan, X.; Cayabyab, M.; Hiraoka, M.; Liu, D.; Han, X.; Pesavento, J.; Dong, C. Z.; Wang, Y.; An, J.; Kaji, H.; Sodroski, J. G.; Huang, Z. Exploring the stereochemistry of CXCR4-peptide recognition and inhibiting HIV-1 entry with D-peptides derived from chemokines. *J. Biol. Chem.* **2002**, *277*, 17476−17485.
(13) Zhou, N.; Luo, Z.; Luo, J.; Fan, X.; Cayabyab, M.; et al. Exploring the stereochemistry of CXCR4-peptide recognition and inhibiting HIV-1 entry with D-peptides derived from chemokines. *J. Biol. Chem.* **2002**, *277*, 17476−17485.
(14) Nagasawa, T.; Hirota, S.; Tachibana, K.; Takakura, N.; Nishikawa, S.; et al. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature* **1996**, *382*, 635−638.
(15) Zou, Y.; Kottmann, A.; Kuroda, M.; Taniuchi, I.; Littman, D. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature* **1998**, *393*, 595−599.
(16) Tachibana, K.; Hirota, S.; Iizasa, H.; Yoshida, H.; Kawabata, K.; et al. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. *Nature* **1998**, *393*, 591−594.

JM050829U