Exploring the Stereochemistry of CXCR4-Peptide Recognition and Inhibiting HIV-1 Entry with d-Peptides Derived from Chemokines*

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Chemokine receptor CXCR4 plays an important role in the immune system and the cellular entry of human immunodeficiency virus type 1 (HIV-1). To probe the stereospecificity of the CXCR4-ligand interface, d-amino acid peptides derived from natural chemokines, viral macrophage inflammatory protein II (vMIP-II) and stromal cell-derived factor-1α (SDF-1α), were synthesized and found to compete with 125I-SDF-1 and with much higher biological stability than L-counterparts, the d-peptides showed significant activity in inhibiting replication of CXCR4-dependent HIV-1 strains. These results show the remarkable stereochemical flexibility of the CXCR4-peptide interface. Further direct binding experiments using d-peptide labeled with fluorescein (designated as FAM-DV1) demonstrated that d- and L-peptides shared similar or at least overlapping binding site(s) on the CXCR4 receptor. Structure-activity analyses of related peptide analogs of mixed chiralities or containing alanine replacements revealed specific residues at the N-terminal half of the peptides as key binding determinants. Acting as CXCR4 antagonists and with much higher biological stability than L-counterparts, the d-peptides showed significant activity in inhibiting the replication of CXCR4-dependent HIV-1 strains. These results show the remarkable stereochemical flexibility of the CXCR4-peptide interface. Further direct binding experiments using d-peptide labeled with fluorescein (designated as FAM-DV1) demonstrated that d- and L-peptides shared similar or at least overlapping binding site(s) on the CXCR4 receptor. Structure-activity analyses of related peptide analogs of mixed chiralities or containing alanine replacements revealed specific residues at the N-terminal half of the peptides as key binding determinants. 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Inhibitory activity of peptides was assessed by analytical reverse phase high performance liquid chromatography, capillary electrophoresis, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. All peptides were at least 95% pure.

To prepare FAM-DV1, a DV1 peptide labeled with fluorescein (coupled to the NH₂ group of the side chain of K-17 of DV1 sequence), the K-17 side chain was protected with (4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl and the N-terminal leucine was protected with tert-butoxycarbonyl. The (4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl group was removed at the end of the synthesis from the DV1 peptide-resin by treatment with 20% hydrazine in DMF (3 min at room temperature, three times). The resin was then washed with DMF and treated at room temperature with 5-carboxyfluorescein (2.5 eq.), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (2.5 eq.), N-hydroxybenzotriazole (HOBT) (2.5 eq.), and diisopropylethylamine (DIEA) (5 eq) in DMF (minimum to cover the resin) overnight. When necessary, the coupling was repeated to obtain a negative Kaiser test. The resin was finally washed with DMF and dichloromethane, cleaved and purified as described above.

Flow Cytometry—Following the procedure described in our recent publication (35), Sup T1 cells (2 × 10⁶) were incubated with an anti-CXCR4 monoclonal antibody (mAb) 12G5 (10 μg/ml) and various concentrations of peptides for 40 min at 4 °C, then with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Southern Biotechnology Associates, Inc., Birmingham, AL) 40 min at 4 °C. Finally, the cells were fixed in 2% paraformaldehyde in PBS and analyzed on a FACSscan flow cytometer (Coulter Electronics, Beckman Coulter, Hialeah, FL). 12G5 Competitive Binding to CXCR4—Sup-T1 cells were incubated with anti-CXCR4 mAb 12G5 (10 μg/ml) in the presence of different concentrations of the peptide at 4 °C for 40 min. The cells were washed twice with the binding buffer and stained with fluorescein isothiocyanate-labeled secondary antibody at 4 °C for another 40 min. Finally, the cells were washed two to three times and fixed with the fixing buffer. As a negative control, cells were stained only with secondary antibody. The final data points were taken as the mean fluorescence intensity of the peptide sample subtracted by that of the control. The IC₅₀ values of the peptides in competing with 12G5 binding to CXCR4 were calculated by using the software GraFit.

125I-DVF-1 Competitive Binding to CXCR4—CEM-T4 cells were harvested and washed twice with PBS. Competition binding experiments were performed using a single concentration (0.2 nM) of 125I-DVF-1 in the presence of increasing concentrations of unlabeled ligands in a final volume of 100 μl of binding buffer (50 mM HEPES, pH 7.4, 1 mM CaCl₂, 5 mM MgCl₂, 0.1% bovine serum albumin) containing increasing concentrations of test peptide. Nonspecific binding was determined by preincubation of 100 μM unlabeled DVF-1. Samples were incubated for 60 min at room temperature. The incubation was terminated by separating the cells from the binding buffer by centrifugation and washing once with 500 μl of cold binding buffer. Bound ligands were quantitated by counting γ emissions.

125I-MIP-1β Competitive Binding to CCR5—Following an experimental procedure similar to that described above, 293 cells transfected with CCR5 and 125I-MIP-1β were used to determine the specific binding activity of peptides to CCR5.

FAM-DV1 Competitive Binding to CXCR4—Sup-T1 cells were washed twice with PBS (Ca²⁺- and Mg²⁺-free). Ligand binding experiments were performed using FAM-DV1 (200 nM) with 125I-DVF-1 (300 nM), DV1 (100 μM), or V1 (100 μM) in a final volume of 50 μl of binding buffer (50 mM HEPES, pH 7.4, 1 mM CaCl₂, 5 mM MgCl₂, 0.1% bovine serum albumin) containing 2 × 10⁵ cells. Samples were incubated for 30 min at room temperature in the dark, followed by resuspension and another 30-min incubation. The incubation was terminated by separating the cells from the binding buffer by centrifugation and washing twice with 200 μl of cold binding buffer. After the final wash, 100 μl of binding buffer was spun down for fluorescent emission analysis. Bound ligands were determined by counting fluorescent emission at 535 nm wavelength with a 1-s time delay (Wallac Victor1220). At least three independent experiments were performed. Nonspecific binding of FAM-DV1 was determined by the addition of 100 μM DV1 (500-fold in excess of 200 nM FAM-DV1 used). The fluorescence with 200 nM FAM-DV1 only was scaled to yield 100% FAM-DV1 bound. Similarly, chemical synthetic approach was used to dissect the structural and functional determinants of a number of chemokines and their binding to receptors (29). As to chemokines SDF-1α and vMIP-II, which recognize CXCR4, a large number of synthetic mutants and peptide analogs of SDF-1α were used to analyze the structure-activity relationship of different regions of SDF-1α on CXCR4 binding (30–34).

For vMIP-II, we recently found that a synthetic 21-residue peptide derived from the N terminus of vMIP-II, designated as V1, is a potent antagonist of CXCR4 and inhibits HIV-1 replication in CXCR4− /− T-cell lines (35, 36). 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. Because one important aspect of receptor-ligand interaction is the requirement of stereospecificity, we report here the synthesis and structure-function characterization of all-d-amino acid analogs of V1 peptide, designated DV1 peptides. Unexpectedly, these d-peptides display strong binding and antagonistic activity toward CXCR4, thus revealing 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 the 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 CXCR4− /− cell lines, thus demonstrating their advantage over natural l-peptides for potential clinical application.

**EXPERIMENTAL PROCEDURES**

Materials—Recombinant human chemokines SDF-1α, MIP-1β, and MIP-1β (R&D Systems, Minneapolis, MN) were lyophilized and dissolved as 1 or 2.5 μg/μl stock solutions in sterile phosphate-buffered saline (PBS) and stored at −20 °C in aliquots. The radiiodinated MIP-1β and SDF-1α were purchased from PerkinElmer Life Sciences. The specific activity of 125I-MIP-1β and 125I-SDF-1α was 2200 Ci/mmol. Cell culture media and G418 were purchased from Invitrogen. The anti-CXCR4 monoclonal antibody (mAb) was purchased from BD PharMingen (San Diego, CA). 293 cells (gift from Dr. R. Domu, University of Pennsylvania) and Sup-T1 cells (provided by the National Institutes of Health AIDS Reagent Program) were maintained in Dulbecco’s modified Eagle’s medium plus 10% fetal bovine serum. The cell lines used for the HIV-1 replication assay were kindly provided by other laboratories: MT-4 cells by Dr. N. Yamamoto (Tokyo Medical and Dental University, Tokyo, Japan), chronically HIV-1-infected H9 cells (HIV-1-H9) by the National Institutes of Health AIDS Research and Reference Reagent Program, and Sup-T1 cells by Dr. J. Hoxie (University of Pennsylvania). These cells were grown in 25 mM HEPES-buffered RPMI 1640 supplemented with 10% fetal bovine serum, 50 units/ml penicillin, 50 μg/ml streptomycin, and 0.125 μg/ml amphotericin B.

**HIV-1 was prepared from a culture supernatant of H9/H11003 cells.**

**Peptide Synthesis**—The peptides were prepared by solid phase synthesis using N-(9-fluorenylmethoxycarbonyl) (Fmoc) strategy on a 430A peptide synthesizer (Applied Biosystems, Foster City, CA) and a 9050 Pepsynthesizer Plus (Perceptive Biosystems, Cambridge, MA), as described previously (38, 39). The side chain protecting groups of N-αmoc amino acids were: Arg, 2,2,5,7,8-pentamethyl-chromen-6-sulfonyl, Asp, tert-butyl ester; Cys, triethyl; Gln, triethyl; His, triethyl; Lys, tert-butoxycarbonyl; Ser, tert-butyl ester; Tyr, tert-butyl ester; and Trp, tert-butyloxycarbonyl. In every coupling reaction step, a 4-fold excess of Fmoc amino acid, O-benzotriazol-1-yl-N,N,N′,N′-tetramethyluronium hexafluorophosphate, and 1-hydroxybenzotriazole, and 10-fold excess of diisopropylethylamine were used. The cleavage of peptides from the resin was carried out with the cleavage reagent (trifluoroacetic acid/thioanisole/ethanedithiol/trisopropylsilane, 81:5.5:5.5:2.5) for 2 h at room temperature with gentle stirring. Crude peptides were precipitated in ice-cold methanol/ethyl ether, centrifuged, and lyophilized. The crude peptides were then purified by preparative HPLC using a Dynamax 300-Å C18 column (25 cm × 21.4 mm, inner diameter) with two solvent systems of 0.1% trifluoroacetic acid/H₂O and 0.1% trifluoroacetic acid/acetonitrile. Fractions containing the appropriate peptide were pooled together and lyophilized. The purity of the final product was assessed by analytical reverse phase high performance liquid chromatography, capillary electrophoresis, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. All peptides were at least 95% pure.
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the fluorescence of each sample was adjusted to calculate the percentage of FAM-DV1 bound.

**Intracellular Calcium Measurement**—Sup T1 cells and CCR5 transfected 293 cells were used to measure the intracellular calcium influx. [Ca\(^{2+}\)]\(_c\) was measured using excitation at 340 and 380 nm on a fluorescence spectrometer (PerkinElmer LS50). Calibration was performed using 0.033% Triton X-100 for total fluorophore release and 1.66 mM EGTA to chelate free Ca\(^{2+}\). Intracellular Ca\(^{2+}\) concentrations were calculated using the fluorescence spectrometer measurement program.

**Stability of Peptides in Human Serum**—Pure human serum (kindly provided by Robert Kornfeld, Thomas Jefferson University, Philadelphia, PA) was diluted with PBS to 80% serum solution. DV1 and V1 peptides were dissolved in the serum solution with a concentration of 10 mM. Samples were collected at different time points of incubation at room temperature and subjected to HPLC analysis with the injection of 10-μl peptide samples (Microsorb-MV C18 5-μm, 25 cm × 4.6 mm, 80% CH\(_3\)CN with 0.1% trifluoroacetic acid, UV 220 nm, 1 ml/min). The stability of the peptides was calculated based on the changes in the intensity of UV absorbance of the peptides.

**Circular Dichroism (CD) Spectrum**—CD spectra of DV1 and V1 peptides were measured using a JASCO J-810 spectropolarimeter. The CD spectra of peptide solutions were recorded between 190 and 260 nm with a scanning speed of 100 nm/min, a bandwidth of 1.0 nm, and an integration time of 0.5 s. The CD spectra were corrected for protein concentration.

**Results**

**D-Peptides Derived from vMIP-II N Terminus Bind CXCR4**—In this study, we used synthetic all-D-amino acid peptides to explore the stereosepecificity of the molecular recognition between the CXCR4 receptor and its ligands. Based on our previous finding that V1 peptide derived from the N-terminus (residues 1–21) of viral chemokine vMIP-II antagonizes CXCR4 function and blocks HIV-1 entry via CXCR4 (35, 36), we synthesized DV1, an all-D-enantiomer of V1 peptide that has the identical amino acid sequence as V1 but the chirality of all residues changed to d-configuration (Table 1). DV1 showed a circular dichroism (CD) spectrum that is the mirror image of V1 peptide (Fig. 1a). In addition, the spectra displayed negative intensity bands near 220 nm for V1 and DV1, suggesting that both peptides adopted a random conformation in aqueous solution. If DV1 and V1 are mirror images of each other as suggested by CD, there is significant conformational difference between them, as highlighted by distinct amino acid side chain orientations in their structural models (Fig. 2).

**DV1 peptide was tested for CXCR4 binding affinity in a competitive binding assay using anti-CXCR4 mAb 12G5 following the procedure described previously (35). Surprisingly, despite the changes in chirality of all amino acids and thus extensive conformational difference from parent V1 peptide as described above, DV1 peptide showed strong binding affinity for CXCR4 (Fig. 3a). Furthermore, the CXCR4 binding affinity of DV1 peptide was much higher than that of V1 peptide, as shown by an IC\(_{50}\) of 32 nM for DV1 as compared with that of 456 nM for V1 in competing with the specific CXCR4 binding by mAb 12G5 (Table I). Using another assay method, DV1 was shown to compete with 125I-SDF-1\(_{a}\) in CXCR4 binding with an IC\(_{50}\) of 13 nM as compared with that of 218 nM V1 peptide (Fig. 3b). Because vMIP-II binds other receptors such as CCR5, we tested the activity of DV1 peptide in CCR5 binding using the 125I-MIP-1\(_{B}\) competitive binding method. In contrast to its strong CXCR4 interaction, DV1 peptide did not show any CCR5 binding even at a much higher concentration of 100 μM (Fig. 3c).

**Table I**

| Peptides | Sequence\(^a\) | IC\(_{50}\) as characterized by 12G5 Competitive binding (nM) |
|----------|----------------|---------------------------------------------------------|
| DV1      | L.GASWHPDKCCGLYKRPPL | 32 |
| V1 (all l-amino acids) | L.GASWHPDKCCGLYKRPPL | 456 |
| V1-DCl\(^b\) | L.GASWHPDKCGLYKRPPL | 36 |
| V1-1CDP | L.GASWHPDKCCGLYKRPPL | 279 |
| DV3 | L.GASWHPDK | 439 |
| DV1-L1A\(^c\) | A.GASWHPDKCCGLYKRPPL | 23,700 |
| DV1-W5A | L.GASWHPDKCCGLYKRPPL | 1,076 |
| DV1-R7A | L.GASWHPDKCCGLYKRPPL | 2,455 |
| DV1-K10A | L.GASWHPDKCCGLYKRPPL | 288 |
| DV1-C11A | L.GASWHPDKCCGLYKRPPL | 21 |
| DV1-Q16A | L.GASWHPDKCCGLYKRPPL | 64 |
| DV1-R18A | L.GASWHPDKCCGLYKRPPL | 202 |
| DV1-C11A12A | L.GASWHPDKCCGLYKRPPL | 106 |
| DS1 | L.SYRCPRFF | 4,579 |
| SI (all l-amino acids) | L.SYRCPRFF | 3,470 |

\(^a\) Unless specified otherwise (for V1 and S1), all residues are of D-configuration.

\(^b\) Those l-amino acids residues in the sequence are indicated by underlines, whereas the rest are D-amino acids.

\(^c\) The position for alanine replacement is indicated by bold and underlined type.
CXCR4-transfected 293 cells was inhibited by the addition of SDF-1α (300 nM), DV1 (100 μM), or V1 (100 μM) (data not shown).

*N-terminal Half of β-Peptides Is the Major Determinant of CXCR4 Binding*—As described above, the chirality change in all amino acid residues of V1 peptide resulted in DV1 peptide with significantly increased CXCR4 affinity. To further explore the effect of chirality on receptor binding, peptides of hybrid chiralities were synthesized. By using Cys-11 as the central point of V1 peptide, we changed the chirality of residues before (residues 1–10) and after Cys-11 (residues 12–21) to D-configuration, respectively (Table I). Two peptides synthesized accordingly were therefore designated as V1-DCL and V1-LCD that were composed of mixed D- and L-amino acids. V1-DCL peptide has D-configuration for the N-terminal half and L-configuration for the C-terminal half, whereas V1-LCD peptide has the opposite. Both peptides were subjected to anti-CXCR4 mAb 12G5 competitive binding assay. V1-DCL displayed CXCR4 binding activity comparable with DV1, and a similar observation was made for V1-LCD and V1 (Table I and Fig. 5a). These results further confirmed the ability of CXCR4 in recognizing peptide ligands of opposite or mixed chiralities. In addition, they suggested that CXCR4 binding affinity of these peptides was mainly determined by the chirality of their N-terminal half with D-residues at this part interacting with CXCR4 receptor better than L-residues. Apparently, the first 10 residues of DV1 or V1 are the major determinant of activity. In contrast, chirality change in the C-terminal half had much less effect on CXCR4 binding activity.

To further address the role of the C-terminal half, we synthesized a truncated DV1 analog, designated as DV3, in which C-terminal residues (residues 11–21) of DV1 were removed. Although retaining CXCR4 binding, DV3 was less potent than DV1 (Table I and Fig. 5a). This is consistent with previous study of V3 peptide, a truncated V1 analog that also contains only first 10 residues and shows much decreased CXCR4 binding as compared with V1 (35). These results indicated that, although the N-terminal half (residues 1–10) is most important, the C-terminal half (residues 11–21) also plays a role in receptor binding.

### Specific Residues in β-Peptides Important for CXCR4 Recognition

To further characterize the structure-activity relationship of DV1 peptide, eight mutant peptides containing alanine replacement at various positions of DV1 were synthesized (Table I). Consistent with the important role of the N-terminal half of DV1 peptide as discussed above, mutations in this part had more significant effect on receptor binding than mutations in the other part. Mutant peptides DV1-L1A, DV1-W5A, and 17479

![Fig. 1](http://www.jbc.org/)

*a*, CD spectra of DV1 and V1 peptides. *b*, CD spectra of DV1 and mutant DV1-L1A peptides.
chosen as representatives and their opposite chiralities, only the side chains of Leu-1 and Trp-5 that chains in these two peptides display different orientations because of DV1 and V1 peptides. With the same main chain direction but different side chain orientations.

Because both DV1 and V1 peptides recognize CXCR4 despite their very different side chain orientations caused by changes in chirality, this raised the question of whether these peptides may bind the receptor through nonspecific interactions. A diverse group of peptides and organic molecules with a high positive charge (+8 or +9) are known to bind CXCR4, presumably through electrostatic interactions with the negatively charged surface (−9) of CXCR4 (45–48). It can be imagined that these interactions are likely less sensitive to changes in the stereochemistry of ligands so long as the overall positive charge is maintained. However, this should not be the case for DV1 peptide because it has an overall charge of only +3.5, much lower than other highly positively charged CXCR4 ligands described above. In addition, mutant peptides DV1-K10A and DV1-R18A, in which positively charged side chains of Lys-10 and Arg-18 were removed, respectively, have an even lower charge of +2.5, yet retain significant CXCR4 binding activity. Most importantly, as discussed above for mutants DV1-L1A and DV1-W5A, uncharged hydrophobic side chains (most notably the side chain of Leu-1) of DV1 peptide play an essential role in receptor recognition and are responsible for different receptor affinities of up to 741-fold. A similar conclusion was drawn in previous analysis of V1 and mutant peptides (36). Taken together, these data clearly demonstrated that, despite the seemingly contradictory notion of the nonspecificity of CXCR4 toward the stereochemistry of ligands, CXCR4 interacts with both DV1 and V1 peptides with a high degree of sensitivity for specific side chain groups in these peptides.

Given that the orientations of side chains in DV1 and V1 peptides are not the same because of their different d- and L-configurations (Fig. 2), one should expect that corresponding residues in DV1 and V1 peptides have distinct interactions with CXCR4 and thus contribute differently to receptor binding. To verify this, we compared the changes in receptor binding affinity caused by mutations in DV1 and V1 peptides (Table II). The largest difference in residue contribution to receptor binding between DV1 and V1 peptides was found for those residues at the N-terminal half, e.g. Leu-1 was the most critical binding determinant and Lys-10 the least important one for DV1, whereas the corresponding Lys-10 was most critical for V1. By contrast, the difference in residue contribution to CXCR4 affinity between these two peptides was much smaller for the C-terminal half, which was consistent with the less important role of the C-terminal half in receptor recognition.

d-Peptides Inhibit SDF-1α Signaling through CXCR4—DV1 peptide was tested for activity in inducing signal via CXCR4 as measured by intracellular calcium influx in Sup T1 cells expressing the receptor. DV1 did not show any activity in triggering CXCR4 signal transduction but blocked SDF-1α signaling via CXCR4, thus indicating that it is an antagonist of CXCR4 function (Fig. 6). This is consistent with a previous observation that V1 peptide is also a CXCR4 antagonist (35). For those DV1 mutant peptides that had some binding activity for CXCR4 (Table I), their ability to induce signal or interfere with SDF-1α signaling via CXCR4 was also verified by a different binding method using 125I-SDF-1α (data not shown). These results further supported the notion that CXCR4 receptor can accommodate the changes in amino acid chirality of different peptide ligands.

d-Peptides Are Highly Stable in Human Serum—The incorporation of d-amino acids into a peptide sequence should enhance its resistance to proteolytic degradation because unnatural residues are less likely to be recognized by natural enzymes. To test this, the biological stability of DV1 and V1 peptides was studied in human serum. The peptides were mixed with human serum and degraded products of peptides monitored by HPLC for a period of time. V1 peptide was rapidly degraded, and by 24 h the peptide peak on HPLC disappeared completely (data not shown). In contrast, DV1 peptide did not...
show any degradation during the same period and even after 72 h of prolonged incubation with human serum. These results were consistent with the notion that DV1 peptide has much higher biological stability than V1 peptide, which should be of advantage for any potential clinical application.

**D-Peptides Inhibit HIV-1 Replication in CXCR4 Cell Lines**—Having shown that DV1 peptide is a potent CXCR4 antagonist and highly stable in biological conditions, we reasoned that it could inhibit the replication of CXCR4-dependent HIV-1 strains by blocking the entry of viruses via CXCR4 co-receptor. By measuring CAT activity in activated human PBMC infected with a recombinant HIV-1 containing the HXBc2 envelope glycoproteins, we found that DV1 peptide was a potent inhibitor of infection. By contrast, a control mutant peptide DV1-L1A, which contains a single alanine substitution at Leu-1 but substantially loses CXCR4 binding, did not show any activity (Fig. 7a). The DV1 peptide was completely inactive in control experiments measuring CAT activity in CCR5+CD4+ C2Th cells infected with the dual tropic 89.6 HIV-1 strain (Fig. 7b). These data are in agreement with other results described

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

**Fig. 3.** a and b, CXCR4 binding of peptides DV1 (□) and V1 (○) as well as controls vMIP-II (●) and SDF-1α (▲) as characterized by anti-CXCR4 mAb 12G5 competitive binding assay (a) and by 125I-SDF-1α competitive binding assay (b). c, CCR5 binding of peptides DV1 (□) and V1 (○) as well as control vMIP-II (●) as characterized by 125I-MIP-1β competitive binding assay.

**Fig. 4.** CXCR4 binding of FAM-DV1 (200 nM) competed by SDF-1α (300 nM), DV1 (100 μM), and V1 (100 μM) on Sup T1 cells.
above and demonstrate that DV1 peptide is a selective inhibitor of HIV-1 co-receptor function of CXCR4 but not CCR5. Note that two different cells (activated PBMC and CCR5/H11001 CD4/Cf2Th cells) were used to test the inhibition by the peptide of HIV-1 entry via CXCR4 and CCR5, respectively. Activated PBMC were used because they are the most relevant target cells for HIV-1 infection in vivo. Because activated PBMC express both CXCR4 and CCR5, we used an X4 virus, which utilizes only CXCR4 as a coreceptor. To demonstrate that entry of the virus is not inhibited by the peptide when CCR5 is used as a coreceptor, one needs to switch both target cells and virus. For this reason, we used Cf2Th cells that express CD4 and CCR5 and can be utilized by an R5 X4 virus that uses both CCR5 and CXCR4.

The ability of DV1 peptide to block HIV-1 replication was also confirmed in another assay measuring the production of viral p24 antigen in MT-4 cells after being infected with HIV-1 isolated from the supernatant of H9/IIIb cells. DV1 strongly

![Graph of CXCR4 binding](image)

**TABLE II**

| Decrease in CXCR4 binding (x-fold) |
|------------------------------------|
| L1A      | W5A   | R7A   | K10A  | C11A  | C11A, C12A | Q16A   | R18A  |
| DV1      | 741   | 34    | 77    | 9     | 0.8 (incr)<sup>a</sup> | 3      | 2     | 6     |
| V1(28)   | 29    | 4     | 65    | >156  | 8 (incr)<sup>a</sup>   | 3      | 0.8 (incr)<sup>a</sup> | 1.5   |

<sup>a</sup> incr, the value shown is the increase in CXCR4 binding.

![Graph of CXCR4 binding](image)
inhibited p24 production as compared with control mutant DV1-L1A (Fig. 7c). Consistent with this observation, the cell viability assay showed that DV1 protected MT-4 cells from the cytopathic effects of HIV-1 infection (data not shown). It was noted that, when V1 peptide was used for comparison, it was active during the initial period and gradually lost effect over time, whereas DV1 peptide maintained its activity throughout the course of experiments. This is consistent with the higher biological stability of DV1 peptide as described above and supports the notion that peptides of d-amino acids have advantages for therapeutic application.

DISCUSSION

CXCR4 plays an essential role in many physiological functions as the receptor for chemokines such as SDF-1α. In addition, CXCR4 is involved in the pathogenesis of HIV-1 infection by serving as the co-receptor for the cellular entry of T and dual tropic virus strains. CXCR4 belongs to the GPCR superfamily, which contains a large number of membrane proteins that are important therapeutic targets but whose structures and interactions with their ligands need to be further elucidated. In this study, we explored the mechanism of CXCR4-ligand recognition with synthetic chemokine-derived peptides containing d-amino acids in an attempt to gain insights into the structure and function of CXCR4 and design novel inhibitors of HIV-1 entry. Through structure and function analyses of a series of d-peptides, d- and l-hybrid peptides, and mutant analogs derived from vMIP-II and SDF-1α, we found that the CXCR4 binding surface is tolerant of changes in peptide stereochemistry. These peptides containing some or all d-amino acids display significantly different topologies on side chains from l-counterparts and yet retain or even enhance their highly specific interactions with the receptor. This seems to be contrary to the notion that, in general, receptor-ligand interface is highly sensitive to changes in stereo configurations of ligands.

ALX40–4C, a peptide of nine d-Arg residues, is known to bind CXCR4 (45). However, this peptide, like other highly positively charged molecules such as T-22 (46) and AMD3100 (47), most likely acts through overall charge-charge electrostatic interactions (48). This is different from the d- and l-peptides reported here for which specific interactions of uncharged hydrophobic side chains of the peptides with the

FIG. 6. Intracellular calcium influx in Sup T1 cells. DV1 peptide with the indicated concentrations and SDF-1α (100 nM) were sequentially used to treat Sup T1 cells.

FIG. 7. Inhibition of HIV-1 replication by DV1 peptide (○) and a negative control mutant DV1-L1A (●). a, inhibition by peptides of CAT activity in target PBMC incubated with recombinant HIV-1 expressing CAT and containing the X4 HXB2 envelope glycoproteins. b, inhibition of CAT activity by peptides at 20 μM in CCR5+ CD4+ Cf2Th cells infected with recombinant virus containing the envelope glycoproteins of the dual tropic 89.6 HIV-1 strain. c, inhibition of p24 production by peptides in MT-4 cells infected with HIV-1 isolated from the supernatant of H9/IIIB cells.
Inhibition of HIV-1 Entry with d-Peptides from Chemokines

The understanding of the mechanism and structural basis for the remarkable property of the CXCR4 binding surface described here will have important implications for the design of novel ligands. Receptors and proteins mediating the HIV-1 entry event have become important targets for drug discovery, as agents blocking the virus from entering cells will serve as a new type of therapeutic that augments the arsenal of current drugs targeting the replication of the virus inside the cell. Small molecule inhibitors of HIV-1 entry via two principal co-receptors CXCR4 and CCR5 have been reported (32, 45–47, 55). In addition, HIV-1 entry inhibitors have also been developed to target the HIV-1 envelope glycoprotein gp41 (56). In this study, by taking advantage of the stability of unnatural amino acids, DV1 peptide shows significant anti-HIV-1 activity in biological conditions and thus may be a desirable lead for potential therapeutic development. As shown by this d-peptide, the apparent lack of stereospecificity of the CXCR4 binding surface as observed here might be exploited for the design of other highly stable and potent ligand molecules with novel topological features.

REFERENCES

1. Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, S., Kitamura, Y., Yoshida, N., Kikutani, H., and Kishimoto, T. (1996) Nature 382, 635–638
2. Zou, Y., Kottmann, A., Kuroda, M., Tanisch, I., and Littman, D. (1998) Nature 393, 595–599
3. Ma, Q., Jones, D., Borghesani, P. R., Segal, R. A., Nagasawa, T., Kishimoto, T., Bronson, R. T., and Springer, T. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9448–9453
4. Feng, Y., Broder, C. C., Kennedy, P. E., and Berger, E. A. (1998) Science 272, 752–757
5. Dragic, T., Litwin, V., Allaway, G. P., Martin, S. R., Huang, Y., Nagashima, K. A., Cavanah, C., Maitland, P. J., Kup, C. A., and Paxton, W. A. (1998) Nature 391, 667–673
6. Deng, H., Liu, R. K., Ekins, P., Vine, J., Unutmaz, D., Burkhart, M., Marzio, P. D., Marmon, S., Sutton, R. H., Hill, C. M., Davis, C. B., Peiper, S. C., Schall, T. J., Littman, D. R., and Lanier, L. L. (1996) Nature 378, 611–616
7. Alving, G., Comodiare, C. B., Fent, Y., Kennedy, P. E., and Murphy, P. M. (1996) Science 272, 1955–1958
8. BLEU, C. C., Farzan, M., Cho, H., Parolin, C., Clark-Lewis, I., and Sodroski, J. (1996) Nature 382, 829–833
9. Oberlin, E., Amara, A., Bacherer, F., Bessia, C., Virelizier, J. L., Arzana-Seisdedos, F., Schwartz, O., Heald, J. M., Clark-Lewis, I., Legler, D. F., Loetscher, M., Baggiolini, M., and Moser, B. (1996) Nature 382, 833–835
10. Moore, P. S., Boshoff, C., Weiss, R. A., and Chang, Y. (1996) Science 274, 1739–1744
11. Boshoff, C., Endo, Y., Collins, P. D., Takeuchi, Y., Reeves, J. D., Schweickart, V. I., Stuits, M. A., Sasak, T., Williams, T. J., Gray, P. W., Moore, P. S., Chang, Y., and Weiss, R. A. (1997) Science 278, 290–294
12. Kledal, N. T., Rosenkilde, M. M., Coulin, F., Simmons, G., Johnsen, A. H., Alouani, S., Power, C. A., Lattichia, H. R., Gerstof, J., Clapham, P. R., Clark-Lewis, I., Wells, T. N. C., and Schwartz, T. W. (1997) Science 277, 1656–1659
13. Murphy, P. M. (1994) Annu. Rev. Immunol. 12, 593–633
14. Strader, C. D., Fong, T. M., Tota, M. R., and Underwood, D. (1994) Annu. Rev. Biochem. 63, 101–132
15. Picard, L., Wilkinson, D. A., Pirot, J., Peiper, S. C., and Moser, B. (1996) Virology 231, 105–111
16. Breart, A., Heveker, N., Pleinko, O., Seli, N., and Alizon, M. (1997) J. Virol. 71, 4744–4751
17. Liu, Z., Berson, J. F., Chen, Y., Turner, D. J., Zhang, T., Sharron, M., Jenks, M. L., Wang, Z., Kim, J., Bucker, J., Hoe, M., Peiper, S. C., and Doms, R. W. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6426–6431
18. Wang, Z. X., Berson, J. F., Zhang, T. Y., Chen, Y. H., Sun, Y., Sharron, M., Lu, Z. H., and Peiper, S. C. (1997) Blood 89, 2378–2384
19. Chabot, D. J., Zhang, F. P., Quinnan, G. V., and Broder, C. C. (1999) J. Virol. 73, 6598–6609
20. Doranz, B. R., White, W. J., Hammond, B., Berson, J., Peiper, S. C., Peiper, S. C., and Moser, B. (1999) J. Virol. 73, 2755–2761
21. Chabot, D. J., and Broder, C. C. (2000) J. Biol. Chem. 275, 23774–23782
22. Breart, A., Heveker, N., Montes, M., and Alizon, M. (2000) J. Biol. Chem. 275, 23786–23794
23. Kajumo, F., Thompson, D. A., Guo, Y., and Dragic, T. (1996) Virology 211, 249–257
24. Luo, Z., Butcher, D., and Huang, Z. (1997) Protein Eng. 10, 1029–1045
25. Zhou, N., Luo, Z., Hall, J. W., Lu, J., and Wang, H., and Zou, Y. (2000) Eur. J. Immunol. 30, 164–173
26. Lee, B., Sharron, M., Blainpain, C., Doranz, B. J., Vakili, J., Seth, P., Berg, E., Liu, G., Guy, H. R., Durell, S. E., Parmentier, M., Chang, C. N., Price, K., Tsang, M., and Doms, R. W. (1999) J. Biol. Chem. 274, 9617–9626
27. Chabot, D. J., Chen, H., Dimitrov, D. S., and Broder, C. C. (2000) J. Virol. 74, 4404–4413
28. Zhou, N., Luo, Z., Liu, J., Liu, D., Hall, J. W., Pomerantz, R. J., and Huang, Z. (2001) J. Biol. Chem. 276, 42826–42833
29. Clark-Lewis, I., Kim, K., Rajarathnam, K., Gong, J. H., Dewald, B., Moser, B., Baggiolini, M., and Sykes, B. D. (1995) Leukocyte Biol. 57, 703–714
30. Crump, M. P., Gong, J. H., Loetscher, P., Rajarathnam, K., Amara, A., Arzana-Seisdedos, F., Virelizier, J. L., Baggiolini, M., Sykes, B. D., and Clark-Lewis, I. (1997) EMBO J. 16, 6996–7007
31. Loetscher, M., Gong, J. H., Baggiolini, M., and Clark-Lewis, I. (1998) J. Biol. Chem. 273, 22279–22283
32. Heveker, N., Montes, M., Germeroth, L., Amara, A., Trautmann, A., Alizon, M., and Schneider-Mergener, J. (1998) Curr. Biol. 8, 369–376
33. Luo, Z., Zhou, N., Liu, J., Hall, J. W., and Huang, Z. (1999) Biochem. Biophys. Res. Commun. 263, 689–695
34. Luo, Z., Liu, J., Zhou, N., Hall, J. W., and Huang, Z. (1999) Biochem. Biophys. Res. Commun. 264, 42–47
35. Zhou, N., Luo, Z., Luo, J., Hall, J. W., and Huang, Z. (2000) Biochemistry 39, 3782–3787
36. Luo, Z., Fan, X., Zhou, N., Hiraoka, M., Luo, J., Kaji, H., and Huang, Z. (2000) Biochemistry 39, 13545–13550
37. Endres, M. F., Clapham, P. R., Marsh, M., Aboja, M., Turner, J. D., McKnight, A., Thomas, J. F., Stoebenhaus-Haggary, B., Cho, S., Vanes, P., Wells, T. N., Power, C. A., Sutterwala, S. S., Doms, R. W., Landau, N. R., and Hoxie, J. A. (1996) Cell 87, 745–756
38. Sato, T., Arumini, J. M., Li, S., Friedman, T. M., Gao, J., Edling, A., and

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Townsend, R., Germann, M. W., Korngold, R., and Huang, Z. (1997) J. Biol. Chem. 272, 12175–12180

39. Li, S., Choksi, S., Shan, S., Gao, J., Korngold, R., and Huang, Z. (1998) J. Biol. Chem. 273, 16442–16445

40. Butcher, D. J., Kowalska, M. A., Li, S., Luo, Z., Shan, S., Lu, Z., Niewiarowski, S., and Huang, Z. (1997) FEBS Lett. 408, 183–187

41. Thali, M., Furman, C., Ho, D. D., Robinson, J., Tilley, S., Pinter, A., and Sodroski, J. (1991) J. Virol. 66, 183–187

42. Choe, H., Farzan, M., Konkel, M., Sun, Y., Mareon, L., Cayabyab, M., Berman, M., Dorf, M. E., Gerard, N., Gerard, C., and Sodroski, J. (1998) J. Virol. 72, 6113–6118

43. Helseth, E., Kowalski, M., Gabuzda, D., Olshovsky, U., Haseltine, W., and Sodroski, J. (1990) J. Virol. 66, 2416–2420

44. Fujihashi, T., Sakata, T., Kaji, A., and Kaji, H. (1995) AIDS Res. Hum. Retroviruses 11, 461–471

45. Doranz, B. J., Gevirtz-Perbas, K., Sharren, M. P., Mao, S. H., Goetz, M. B., Daar, E. S., Doms, R. W., and O’Brien, W. A. (1997) J. Exp. Med. 186, 1395–1400

46. Murakami, T., Nakajima, T., Koyanagi, Y., Tachibana, K., Fuji, N., Tamamura, H., Yoshida, N., Waki, M., Matsumoto, A., Yoshie, O., Kishimoto, T., Yamamoto, N., and Naganawa, T. (1997) J. Exp. Med. 186, 1389–1393

47. Schols, D., Struyf, S., van Damme, J., Este, J. A., Henson, G., and De Clercq, E. (1997) J. Exp. Med. 186, 1383–1388

48. Dealwis, C., Fernandez, E. J., Thompson, D. A., Simon, R. J., Siani, M. A., and Lolis, E. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6941–6946

49. Chorev, M., and Goodman, M. (1993) Acc. Chem. Res. 26, 266–273

50. Fisher, P. J., Prendergast, F. G., Ehrhardt, M. E., Urbauer, J. L., Wand, A. J., Sedarounis, S. S., McCormick, D. J., and Buckley, P. J. (1994) Nature 368, 651–653

51. Blondelle, S. E., Crooks, E., Aligue, R., Agell, N., Bachs, O., Esteve, V., Tejero, R., Celda, B., Pastor, M. T., and Perez-Paya, E. (2000) J. Pept. Res. 53, 148–162

52. Miles, A. J., Skubitz, A. P., Furcht, L. T., and Fields, G. B. (1994) J. Biol. Chem. 269, 30939–30945

53. Li, C., McCarthy, J. B., Furcht, L. T., and Fields, G. B. (1997) Biochemistry 36, 15404–15410

54. Feifel, B., Schönfeld, H.-J., and Christen, P. (1998) J. Biol. Chem. 273, 11999–12002

55. Baba, M., Nishimura, O., Kanzaki, N., Okamoto, M., Sawada, H., Iizawa, Y., Shirataki, M., Aramaki, Y., Okonogi, K., Ogawa, Y., Meguro, K., and Fujino, M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 5698–5703

56. Sodroski, J. G. (1999) Cell 99, 243–246
Exploring the Stereochemistry of CXCR4-Peptide Recognition and Inhibiting HIV-1 Entry with d-Peptides Derived from Chemokines

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