Tyrosine-sulfated Peptides Functionally Reconstitute a CCR5 Variant Lacking a Critical Amino-terminal Region*

Michael Farzan‡, Susan Chung‡, Wenhui Li‡, Natalya Vasilieva§, Paulette L. Wright‡, Christine E. Schnitzler‡, Robb J. Marchione‡, Craig Gerard§, Norma P. Gerard§, Joseph Sodroski¶, and Hyeryun Choe§

From the §Department of Cancer Immunology and AIDS, Dana Farber Cancer Institute, Department of Pathology, Division of AIDS, Harvard Medical School, Boston, Massachusetts 02115, the ¶Perlmutter Laboratory, Children’s Hospital, Department of Medicine, Division of AIDS, Harvard Medical School, Boston, Massachusetts 02115, and the ©Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts 02115

Entry of most primary human immunodeficiency virus, type 1 (HIV-1) isolates into their target cells requires the cellular receptor CD4 and the G protein-coupled chemokine coreceptor CCR5. An acidic, tyrosine-rich, and tyrosine-sulfated domain of the CCR5 amino terminus plays a critical role in the ability of CCR5 to serve as an HIV-1 coreceptor, and tyrosine-sulfated peptides based on this region physically associate with the HIV-1 envelope glycoprotein gp120 and slow HIV-1 entry into CCR5-expressing cells. Here we show that the same tyrosine-sulfated peptides, but not their unsulfated analogs, can restore the HIV-1 coreceptor activity of a CCR5 variant lacking residues 2–17 of its amino terminus. Additionally, these sulfated peptides restored the ability of this CCR5 variant to mobilize calcium in response to the chemokines macrophage inflammatory factors 1α and 1β. These observations show that a tyrosine-sulfated region of the CCR5 amino terminus can function independently to mediate association of chemokines and the HIV-1 envelope glycoprotein with the remaining domains of CCR5.

Entry of the human immunodeficiency virus, type 1 (HIV-1)1 into its target cells requires the cell surface expression of the receptor CD4 and a G protein-coupled chemokine coreceptor such as CCR5 or CXCR4 (1–4). The entry process is initiated when the HIV-1 surface envelope glycoprotein gp120 associates with CD4 (3). This association induces in gp120 a conformational change that allows for a subsequent association with CCR5 (5, 6). Association with CCR5 is thought to promote a global rearrangement of the transmembrane component of the HIV-1 envelope glycoprotein gp41 that allows the amino-terminal fusion peptide of gp41 to interact with the target cell membrane. Subsequent conformational changes in gp41 are proposed to induce the fusion of the viral and cellular membranes and the entry of the viral capsid into the target cell (7).

HIV-1 isolates differ in their coreceptor usage, and these differences are associated with different stages of infection in vivo (8). Horizontally transmitted viruses and those that predominate in natural infection, so-called R5 isolates, utilize CCR5 as a coreceptor. Coincident with a decline of immune function, R5X4 isolates may emerge that utilize CXCR4 in addition to CCR5 as a coreceptor. HIV-1 isolates (X4 isolates) adapted to immortalized cell lines typically use CXCR4 as their primary or sole coreceptor (4). The natural chemokine ligands of CCR5: MIP-1α, MIP-1β, RANTES, block the association with CXCR4 of gp120 derived from R5 isolates and inhibit the infection of CCR5-expressing target cells by R5 isolates (9). Similarly, stromal derived factor-1, the sole identified ligand of CXCR4, inhibits HIV-1 infection of CXCR4-expressing target cells by X4 isolates (10, 11).

An in vivo role for CCR5 and CXCR4 in HIV-1 infection has been supported by studies of chemokine and chemokine receptor polymorphisms in humans (12–15). In addition to these coreceptors, a number of other G protein-coupled seven transmembrane segment receptors have been shown to support the entry of at least one HIV-1, HIV-2, or SIV (simian immunodeficiency virus) isolate in cell culture systems (1, 16–20). These additional coreceptors, most of which are chemokine receptors, share little obvious sequence similarity in their external domains. They do, however, have in common an amino-terminal region rich in tyrosines and acidic amino acids (21). This region of CCR5 plays a critical role in its ability to support the entry of R5 isolates (22). The tyrosine residues in the CCR5 amino terminus are post-translationally modified by sulfate, and these sulfate moieties contribute substantially to the ability of CCR5 to support HIV-1 entry (21). Sulfated peptides based on this tyrosine-sulfated domain of the CCR5 amino terminus, but not their unsulfated analogs, can slow HIV-1 entry and also, less efficiently, block the association of MIP-1α with CCR5 (23, 24).

Here we show that the same tyrosine-sulfated peptides, but again not their unsulfated analogs, can complement the HIV-1 coreceptor activity of a CCR5 variant lacking its amino-terminal tyrosine-sulfated domain. Tyrosine-sulfated peptides also enabled this CCR5 variant to mobilize calcium in the presence of MIP-1α and MIP-1β. We also present data demonstrating that X4 isolates are substantially less dependent on the amino terminus of CXCR4 than are R5 isolates on the analogous region of CCR5. These data underscore the functional importance of sulfate moieties in CCR5 function, highlight a distinction between CXCR4- and CCR5-mediated HIV-1 entry, and imply that a tyrosine-sulfated region of CCR5 mediates association of the natural and viral ligands of CCR5 with the remainder of the CCR5 receptor. Reconstitution of CCR5 func-
tion in this manner will be useful in further structural and functional studies of CCR5 and other G protein-coupled receptors.

EXPERIMENTAL PROCEDURES

Cells, Plasmids, and Peptides—Plasmids encoding codon-optimized CCR5 and CXCR4 in a pcDNA3.1 vector (Invitrogen) have been described previously (21). A plasmid encoding the CCR5 variant ΔNR5 was generated by deleting CCR5 codons 2–17 using the QuikChange method (Stratagene). Similarly, a plasmid encoding the CXCR4 variant ΔNX4 was generated by deleting CXCR4 codons 2–25 in the same manner. Plasmids were sequenced in their entire coding region to exclude the possibility of unintended changes.

HEK293T human kidney cells and Cf2Th canine thymocytes were obtained from the American Type Tissue Culture collection (CRL11554 and CRL1430, respectively). Cf2Th cells stably expressing CCR5 (CCR5-Cf2Th cells) or CD4 (CD4-Cf2Th cells) have been described previously (21, 25). Cf2Th cells stably expressing the CCR5 variant ΔNR5 (ΔNR5-Cf2Th cells) were made in the same way. Briefly, Cf2Th cells were transfected with a pcDNA3.1 plasmid encoding the ΔNR5 variant, selected in 1.5 mg/ml G418, and cloned by limiting dilution. Clones with high surface expression of the ΔNR5 receptors were identified by FACS analysis using the anti-CCR5 antibody 2D7 (Pharmingen).

The peptides used in these studies were synthesized and purified (>95% pure) by the American Peptide Company (Sunnyvale, CA). The amino acid sequence of the s22 peptide (MDYQQVSPIYDINYTSEPQK) is identical to the 22 amino-terminal residues of CCR5, except that cysteine 20 of CCR5 has been altered to serine; tyrosines 10 and 14 were synthesized as tyrosine sulfate, as indicated by underlining (24).

HIV-1 Entry Assays—The envelope complementation assay used here has been described previously (1, 26). Briefly, HIV-1 proviral DNA lacking a functional envelope gene and encoding chloramphenicol acetyltransferase reporter gene and pseudotyped with the envelope glycoproteins of the HIV-1 isolates YU2 (A), ADA (B), 89.6 (C), or HXBc2 (D). In parallel, aliquots of the same cells were analyzed for coreceptor surface expression by FACS using the antibodies 2D7 (CCR5 and ΔNR5, A and B) or 12G5 (CXCR4 and ΔNX4, C and D). The mean fluorescence values from FACS analysis are indicated on the horizontal axes. HIV-1 entry is shown on the vertical axes as a percentage of the chloramphenicol acetyltransferase activity observed for the highest expression level of wild-type CCR5 or CXCR4. The figure shows representative experiments of three or more experiments, depending on the isolate and receptor assayed.

FIG. 1. Sequences of receptor amino termini and peptides used. A, sequences of the amino-terminal domains of CCR5, the CCR5 variant ΔNR5, CXCR4, and the CXCR4 variant ΔNX4. Tyrosine residues known to be sulfated (21, 45) are indicated (SO3−). B, sequences of synthetic peptides used in this study. The sulfated tyrosines are indicated (SO3−).

FIG. 2. HIV-1 entry into cells expressing CCR5, but not CXCR4, is strongly dependent on the coreceptor amino terminus. Cf2Th-CD4 cells transiently transfected with varying amounts of plasmid encoding CCR5, ΔNR5, CXCR4, or ΔNX4 receptors were incubated with HIV-1 virus containing a chloramphenicol acetyltransferase reporter gene and pseudotyped with the envelope glycoproteins of the HIV-1 isolates YU2 (A), ADA (B), 89.6 (C), or HXBc2 (D). In parallel, aliquots of the same cells were analyzed for coreceptor surface expression by FACS using the antibodies 2D7 (CCR5 and ΔNR5, A and B) or 12G5 (CXCR4 and ΔNX4, C and D). The mean fluorescence values from FACS analysis are indicated on the horizontal axes. HIV-1 entry is shown on the vertical axes as a percentage of the chloramphenicol acetyltransferase activity observed for the highest expression level of wild-type CCR5 or CXCR4. The figure shows representative experiments of three or more experiments, depending on the isolate and receptor assayed.
the CCR5 variant ΔNR5, or the CXCR4 variant ΔNX4. Experiments that included the s22, s13, and c22 peptides were performed by incubating target cells with a mixture of viruses and peptide at the indicated concentrations in a total volume of 150 µl in 48-well plates. The cells were washed following overnight incubation and lysed 60 h later. Chloramphenicol acetyltransferase activity was then measured in cell lysates.

Calcium Mobilization Assays—CF2Th-ΔNR5 or CF2Th-CCR5 cells were plated onto a 384-well plate at 12,000 cells/well. After 24 h, the cells were loaded with the no-wash dye in loading buffer (Molecular Devices) for 1 h at 37 °C in a total volume of 40 µl, as recommended by the manufacturer. The measurements of calcium mobilization in response to MIP-1α or MIP-1β, in the presence or absence of the indicated peptides, were performed by injecting 10 µl of the chemokine/peptide mixture onto the loaded cells, using FLIPR (Molecular Devices).

Binding of HIV-1 Envelope Glycoproteins to CCR5 or ΔNR5-expressing Cells—Metabolically labeled soluble gp120 molecules derived from the YU2 and ADA HIV-1 isolates were produced from HEK293T cells that were transfected with gp120 expressor plasmids and labeled with [35S]cysteine and [35S]methionine. Radiolabeled cell supernatants were centrifuged to remove debris, and gp120 content was quantified by Western blotting with sera from HIV-1-infected individuals using purified gp120 as a standard. Supernatant containing 50 ng of labeled gp120 was incubated for 30 min at 37 °C with 500 ng of soluble CD4 and the indicated concentrations of s22, s13, or c22 peptide or buffer only, as indicated. Transient mobilization of calcium was measured using FLIPR (Molecular Devices). B, experiment similar to that in A except that CF2Th-ΔNR5 cells were pulsed with 500 nM MIP-1α and varying concentrations of the s22 peptide as indicated. Calcium measurements for A and B were performed simultaneously but separated for clarity. The experiment is representative of two such experiments.

FIG. 3. Sulfated peptides reconstitute the ability of ΔNR5 to support HIV-1 entry. CF2Th-CD4 cells transiently transfected with a plasmid encoding ΔNR5 were incubated with HIV-1 pseudotyped with the envelope glycoproteins of the YU2 (A) or ADA (B) isolates and the indicated concentrations of the s22, s13, and c22 peptides. The entry values represent percentages of entry observed with cells expressing wild-type CCR5 and were derived from a plot of entry versus expression using wild-type CCR5, as shown in Fig. 2. C, CF2Th-CD4 cells transiently transfected with plasmids encoding either ΔNR5 or ΔNX4, as indicated, where incubated with HIV-1 pseudotyped with the envelope glycoproteins of ADA, 89.6, and HXBc2 in the presence of 137 µM c22 (open symbols) or s22 (filled symbols). Entry is again reported as a percentage of that observed for cells expressing wild-type CCR5 (ΔNR5) or CXCR4 (ΔNX4). The error bars in these figures indicate the range observed for two independent infections of ΔNR5- or ΔNX4-expressing cells.

FIG. 4. Sulfated peptides reconstitute the ability of ΔNR5 to signal in response to MIP-1α. A, CF2Th-ΔNR5 or CF2Th-CCR5 cells expressing comparable levels of receptor were loaded with no-wash dye (Molecular Devices) and pulsed with 500 nM (CF2Th-ΔNR5 cells) or 50 nM (CF2Th-CCR5 cells) MIP-1α premixed with 200 µM s22, s13, or c22 peptide or buffer only, as indicated. Transient mobilization of calcium was measured using FLIPR (Molecular Devices). B, experiment similar to that in A except that CF2Th-ΔNR5 cells were pulsed with 500 nM MIP-1α and varying concentrations of the s22 peptide as indicated.

Binding of Chemokine to CF2Th-CCR5 Cells—Approximately 2 × 10⁵ CF2Th-CCR5 cells were incubated for 30 min at 37 °C with 0.2 µM...
The Amino Terminus of CCR5, but Not That of CXCR4, Is Critical for HIV-1 Infection—We have previously demonstrated that specific tyrosines and acidic residues in the amino terminus of CCR5 are critical to the ability of the R5 isolates ADA and YU2 and the R5X4 isolate 89.6 to infect CCR5-expressing target cells (22). We have also shown that sulfate moieties at tyrosines 10, 14, and 15 of CCR5 contribute to the efficiency of infection by the same isolates (21). To further assess the role of the CCR5 amino-terminal region that includes these tyrosines, a construct, denoted ΔNR5 (Fig. 1A), was generated that lacks residues 2–17 but retains the first cysteine of the CCR5, which is important for efficient receptor expression (28).

Fig. 2 demonstrates that HIV-1 viruses pseudotyped with the envelope glycoproteins of the R5 isolates YU2 and ADA could efficiently enter Cf2Th-CD4 cells transfected with varying amounts of plasmid encoding wild-type CCR5 (Fig. 2, A and B, circles). However, these same viruses were markedly less efficient in infecting Cf2Th-CD4 cells transfected with a plasmid encoding ΔNR5 (Fig. 2, A and B, squares). Both R5 viruses infected ΔNR5-expressing Cf2Th-CD4 cells at less than 2% the efficiency of cells expressing the equivalent levels of wild-type CCR5. Moreover, viruses pseudotyped with the envelope glycoproteins of the R5X4 isolate 89.6 did not detectably infect Cf2Th-CD4 cells expressing ΔNR5, although they efficiently infected Cf2Th cells expressing wild-type CCR5 (data not shown). In contrast, as shown in Fig. 2 (C and D), Cf2Th-CD4 cells transfected with plasmids encoding ΔNX4, a CXCR4 variant analogous to ΔNR5 (Fig. 1A), could be efficiently infected by viruses pseudotyped with the envelope glycoproteins of the R5X4 isolate 89.6 or the X4 isolate HXBc2 (19 and 44%, respectively, of infection observed for cells expressing equivalent levels of wild-type CXCR4). These data underscore the major contribution of the CCR5 amino terminus to the ability of R5 and R5X4 isolates to enter target cells and highlight the less significant contribution of the amino terminus of CXCR4 to infection by X4 and R5X4 isolates.

Tyrosine-sulfated Peptides Enhance Entry of HIV-1 Isolates into Cells Expressing the CCR5 Variant ΔNR5—Tyrosine-sulfated peptides based on the CCR5 amino terminus specifically block HIV-1 infection of CCR5-expressing target cells at micromolar concentrations and inhibit the association of CCR5 with gp120/sCD4 complexes and with the chemokine MIP-1α (23, 24, 29). In particular, a peptide (denoted here as s22; Fig. 1B) corresponding to the first 22 residues of the CCR5 amino terminus, with sulfated moieties present at tyrosines 10 and 14, blocked the entry into peripheral blood mononuclear cells and macrophages of viruses pseudotyped with the ADA, YU2, and 89.6 envelope glycoproteins (24). A peptide (denoted as c22; Fig. 1B) with the identical 22 residues but lacking the two sulfate moieties had no effect on HIV-1 entry or CCR5 association with gp120 or MIP-1α. These same peptides, as well as a smaller peptide (denoted here as s13; Fig. 1B) corresponding to residues in the immediate vicinity of tyrosines 10 and 14, were assayed for their effect on the ability of HIV-1 to enter cells expressing ΔNR5.

Fig. 3 demonstrates that both s22 and s13 substantially enhanced the ability of the ADA and YU2 viruses to infect Cf2Th-CD4 cells expressing ΔNR5. Entry of the YU2 virus was nearly 30% of that observed for Cf2Th-CD4 cells expressing equivalent levels of wild-type CCR5 at the highest concentration of s22 used (137 μM). Entry of the ADA virus was similarly enhanced by s22, to about 25% of entry observed for wild-type CCR5-expressing cells (Fig. 3). The s13 peptide was less efficient than the s22 peptide at enhancing the entry of the YU2 virus but enhanced the entry of the ADA virus only slightly less efficiently than the s22 peptide. The c22 peptide had no effect on the ability of either the ADA or YU2 viruses to infect the same cells. None of the peptides were capable of enhancing the entry of HIV-1 pseudotyped with the envelope glycoprotein of the R5X4 89.6 isolate (Fig. 3C), a result consistent with the greater sensitivity of this isolate to amino-terminal perturbations of CCR5 (21, 22, 30). These peptides also had no effect on infection by viruses pseudotyped with the envelope glycoproteins of 89.6 or with the X4 isolate HXBc2 of Cf2Th-CD4 cells expressing either wild-type CXCR4 or ΔNX4, a CXCR4 variant lacking residues 2–25 (Fig. 3C). None of the peptides enhanced entry of the ADA, YU2, 89.6, or HXBc2 viruses into Cf2Th-CD4 cells lacking coreceptor molecules (data not shown). These data show that the amino terminus of CCR5 provided in trans can complement the inability of cells expressing ΔNR5 to support efficient HIV-1 infection by R5 isolates.

Tyrosine-sulfated Peptides Reconstitute the Ability of ΔNR5 to Mobilize Calcium in Response to Chemokine—Cf2Th cells expressing CCR5 mobilize calcium in response to its natural ligands MIP-1α, MIP-1β, and RANTES (31, 32). We assayed the ability of these cells stably transfected with plasmids encoding ΔNR5 or CCR5 (Cf2Th-ΔNR5 or Cf2Th-CCR5 cells, respectively) to mobilize calcium in response to MIP-1α and MIP-1β in the

![Fig. 5. The peptides s22 and s13 block gp120/CD4 and MIP-1α association with CCR5. A, approximately 50 ng of metabolically radiolaabeled gp120 of the HIV-1 isolates ADA or YU2 premixed with 500 ng of soluble CD4 was incubated with Cf2Th-CCR5 or Cf2Th cells in the presence of 100 μM of the indicated peptide or with buffer only (no). The cells were washed and lysed, and the lysates were immunoprecipitated with sera from HIV-1-infected individuals. The immunoprecipitates were analyzed by SDS-PAGE. B, Cf2Th-CCR5 or Cf2Th cells were incubated with 0.1 nM [3H]MIP-1α and 200 μM of the indicated peptide or with buffer only. The cells were washed, and bound [3H]MIP-1α was quantified by γ counting.](image-url)
presence and absence of the s22, s13, and c22 peptides.

Fig. 4A demonstrates that C2fTh-ΔNR5 cells did not detectably mobilize calcium in the presence of high concentrations (500 nM) of the chemokine MIP-1α. However, when 200 μM of the s22 peptide was introduced with the MIP-1α to C2fTh-ΔNR5 cells, an efficient calcium flux could be detected (Fig. 4A). This response was comparable with or higher than the response of C2fTh-CCR5 cells when pulsed with 50 nM of MIP-1α. The s13 peptide allowed only a modest mobilization of calcium in C2fTh-ΔNR5 cells pulsed with 500 nM MIP-1α. No detectable response was observed with 500 nM MIP-1α and 200 μM of the unsulfated c22 peptide (Fig. 4A) or in the absence of chemokine (not shown). Qualitatively identical results were obtained using the chemokine MIP-1β in the same experiments (data not shown). Fig. 4B demonstrates that the ability of the s22 peptide to enable MIP-1α to mobilize calcium in C2fTh-ΔNR5 cells is dependent on the concentration of s22 used, with 50 μM of s22 enabling a substantially lower signal than with higher concentrations of s22. These results demonstrate that, at the concentrations indicated, sulfated CCR5-derived peptides can reconstitute the ability of ΔNR5 to respond to natural ligands of CCR5.

The s13 Peptide Is Less Efficient than the s22 Peptide in Blocking the Association of gp120 and MIP-1α with CCR5—The s13 peptide was consistently less efficient than the s22 peptide in the above assays, although it encompasses the region shown to be most critical to the coreceptor function of CCR5. To determine the relative ability of the s13 peptide to associate with gp120/CD4 complexes, this peptide was assayed for its ability to block the association of CCR5 with gp120/CD4. Fig. 5A shows that the s13 was ~10–20% less efficient than the s22 peptide in blocking the association of complexes of CD4 and the ADA or YU2 gp120 glycoproteins with C2fTh-CCR5 cells.

We previously demonstrated that the s22 at high concentrations can specifically interfere with the association of MIP-1α and CCR5 (24). We assayed the relative ability of s22 and s13 to block the ability of MIP-1α to bind C2fTh-CCR5 cells. As shown in Fig. 5B, the s22 was more efficient than the s13 peptide in blocking the association of MIP-1α with CCR5. The relative difference between the two sulfated peptides appeared to be more pronounced in the blocking the association of CCR5 to MIP-1α than to gp120/CD4 complexes. It is also worth noting that no binding of gp120/CD4 complexes or MIP-1α to C2fTh-ΔNR5 cells could be detected in the presence or absence of any of the peptides assayed here (data not shown). We conclude that the s22 peptide is slightly more efficient than the s13 peptide in interfering with the ligation of gp120/CD4 complexes and MIP-1α to CCR5.

**DISCUSSION**

We have shown here that deletion of the critical tyrosine-sulfated region of CCR5 dramatically interferes with HIV-1 entry but that an analogous deletion of CXCR4 has a much less pronounced effect. These data are consistent with other observations suggesting a major role for the amino terminus of CCR5, but not that of CXCR4, in HIV-1 coreceptor function (21, 22, 27, 33–37). We have also shown that two tyrosine-sulfated peptides (s22 and s13) based on the amino terminus of CCR5 can complement the inability of a CCR5 variant lacking residues 2–17 (ΔNR5) to support efficient HIV-1 infection or to mobilize calcium in response to chemokine. These data are the first that demonstrate the complementation of a defective G protein-coupled receptor variant with a soluble fragment of that receptor.

We and others have previously demonstrated a physical association between the HIV-1 envelope glycoprotein gp120/CD4 complexes and s22 and similar peptides. For example, immuno precipitation of gp120/CD4 complexes by the CD4 induced anti-gp120 antibodies 48d and 23e was blocked by s22 (24), and multimeric gp120/CD4 complexes could bind sulfated CCR5-derived peptide in an enzyme-linked immunosorbent assay-based assay (29). Fig. 6 shows schematically a model of reconstitution that best reflects these observations and those in this study. In the presence of CD4 and a fully functional CCR5 molecule, the s22 peptide binds a portion of the CCR5-binding domain of HIV-1 gp120, sterically impeding association of gp120 with CCR5 (Fig. 6, top panels). The ΔNR5 molecule, lacking its critical amino terminus, cannot associate with the CD4-bound HIV-1 envelope glycoprotein and cannot mediate entry (Fig. 6, bottom left panel). However, the s22 peptide bound to the HIV-1 envelope glycoprotein can transiently associate with ΔNR5 receptor to mediate entry (Fig. 6, bottom...
right panel). s22 cannot enhance the entry observed for CXCR4 and ΔNX4 because the envelope glycoproteins of X4 isolates cannot bind s22 (23, 24) and presumably because it cannot mediate association of these gp120 molecules with these CXCR4-based receptors.

Although these peptides could complement the functional activities of ΔNR5, they could not complement the inability of ΔNR5 to detectably bind gp120/CD4 complexes or chemokine ligands. This apparent discrepancy can be readily resolved by proposing that the sulfated peptides have a rapid off-rate (24). This off-rate would prevent detection of a physical association between ΔNR5 and the ligands of CCR5 in binding assays that require bound complexes to persist through one or more wash steps. In contrast, both HIV-1 infection and chemokine signaling require only a transient association among ΔNR5, sulfated peptides, and gp120 or chemokine, respectively, and these processes, once underway, are irreversible. In the entry assays described, the HIV-1 virion is held in position on the cell surface by CD4. A relatively short-lived association of the HIV-1 envelope with peptide appears to be sufficient to mediate association with ΔNR5 and induce fusion of the virion with the target cell. Similarly, chemokine-mediated signaling is also an event that requires only a short-lived association of chemokine with its receptor, including, as these data imply, the receptor amino terminus.

This model contrasts with previous suggestions that the amino terminus of chemokine receptors is a relatively disordered domain that functions primarily to position chemokine proximal to the relatively more important extracellular loops of the molecule (38–41). Our data suggest that the CCR5 amino terminus has important contacts with the remainder of the receptor and may constitute a large portion of the ligand-accessible surface of the receptor.

Functional reconstitution of G protein-coupled receptors may be useful in further studies of these receptors and their ligands. Peptide variants of the ones used here can be used to distinguish between amino-terminal residues that interact with ligand and those that interact with the receptor body. Sulfated peptides also may be useful in the bulk synchronization of the HIV-1 fusion process, allowing the coordinated exposure of functional intermediate forms of the HIV-1 envelope glycoprotein (7). Studying this intermediate form could cast light on the mechanism and kinetics of HIV-1 fusion.

Finally, these data provide the most compelling evidence to date of the functional involvement of the sulfated moieties of the CCR5 amino terminus in both chemokine association and HIV-1 entry. Given the functional roles of amino-terminal tryptophan sulfates in CCR5, CCR2b, CXCR4, C3XCR1, and the C5a receptor (21, 30, 42–44), these observations are likely to be applicable to many chemoattractant G protein-coupled receptors.

REFERENCES

1. Cho, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P. P., Wu, L., Mackay, C. R., LaRosa, G., Newman, W., Gerard, N., and Sodroski, J. (1996) Cell 85, 1135–1148
2. Dalglish, A. G., Beverley, P. C., Clapham, P. R., Crawford, D. H., Greaves, M. F., and Weiss, R. A. (1984) Nature 312, 763–767
3. Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gueulec, J., Charra, J., and Springer, M. S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5859–5863
4. Feng, Y., Broder, C. C., Kennedy, P. E., and Berger, E. A. (1996) Science 271, 6854–6857
5. Blangy, C., Lee, B., Vakili, J., Delabays, P. A., Goyaerts, C., Mignotte, L., Sharron, M., Dupriez, Y., Vassart, G., and Peiper, S. C., Parmentier, M. (1996) J. Biol. Chem. 271, 17161–17166
6. Bieniasz, P. D., Alston, I. M., Sallusto, F., Giguere, V., and Springer, T. A. (1996) Nature 382, 335–3312
7. Korc, M., Bartsch, T., Hahnenberger, D., Goertler, J., and Springer, T. A. (1996) Nature 382, 335–3312
8. Broder, C. C., Kennedy, P. E., and Berger, E. A. (1996) Science 271, 6854–6857
9. Blangy, C., Lee, B., Vakili, J., Delabays, P. A., Goyaerts, C., Mignotte, L., Sharron, M., Dupriez, Y., Vassart, G., and Peiper, S. C., Parmentier, M. (1996) J. Biol. Chem. 271, 17161–17166
10. Bieniasz, P. D., Alston, I. M., Sallusto, F., Giguere, V., and Springer, T. A. (1996) Nature 382, 335–3312
11. Korc, M., Bartsch, T., Hahnenberger, D., Goertler, J., and Springer, T. A. (1996) Nature 382, 335–3312
12. Broder, C. C., Kennedy, P. E., and Berger, E. A. (1996) Science 271, 6854–6857