

A Tyrosine-sulfated Peptide Based on the N Terminus of CCR5 Interacts with a CD4-enhanced Epitope of the HIV-1 gp120 Envelope Glycoprotein and Inhibits HIV-1 Entry*

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The sequential association of the human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein gp120 with CD4 and a seven-transmembrane segment coreceptor such as CCR5 or CXCR4 initiates entry of the virus into its target cell. The N terminus of CCR5, which contains several sulfated tyrosines, plays a critical role in the CD4-dependent association of gp120 with CCR5 and in viral entry. Here we demonstrate that a tyrosine-sulfated peptide based on the N terminus of CCR5, but not its unsulfated analogue, inhibits infection of macrophages and peripheral blood mononuclear cells by CCR5-dependent, but not CXCR4-dependent, HIV-1 isolates. The sulfated peptide also inhibited the association of CCR5-expressing cells with gp120-soluble CD4 complexes and, less efficiently, with MIP-1 α . Moreover, this peptide inhibited the precipitation of gp120 by 48d and 23e antibodies, which recognize CD4-inducible gp120 epitopes, but not by several other antibodies that recognize proximal epitopes. The ability of the sulfated peptide to block 48d association with gp120 was dependent in part on seven tropism-determining residues in the third variable (V3) and fourth conserved (C4) domains of gp120. These data underscore the important role of the N-terminal sulfate moieties of CCR5 in the entry of R5 HIV-1 isolates and localize a critical contact between gp120 and CCR5.

The envelope glycoprotein of the human immunodeficiency virus, type 1 (HIV-1)¹ mediates fusion of the viral and cellular membranes and entry of the virion into its target cell (1). The envelope glycoprotein is composed of two subunits, a surface

(gp120) and a transmembrane (gp41) glycoprotein that are processed from a gp160 precursor. Viral entry is initiated when gp120 binds the cellular receptor CD4 (2, 3). This association induces a conformational change in gp120 that allows it to associate with a G protein-coupled, seven-transmembrane segment coreceptor; the chemokine receptors CCR5 and CXCR4 have been identified as the major coreceptors for HIV-1 isolates (4–7). Association with the chemokine receptor is thought to promote a global rearrangement in the envelope glycoproteins that allows the interaction of the gp41 N-terminal fusion peptide with the target cell membrane, leading to the fusion of the viral and cellular membranes and entry of the viral capsid into the cell (8, 9).

HIV-1 isolates that differ in coreceptor preference are associated with different stages of infection *in vivo*. Horizontally transmitted viruses and those that predominate in natural infections, so-called R5 isolates, utilize CCR5 as a coreceptor (10). Coincident with a decline in immune function, R5X4 isolates emerge that utilize CXCR4 in addition to CCR5 as a coreceptor (10). HIV-1 isolates (X4 isolates) adapted to immortalized cell lines typically use CXCR4 as their primary or sole coreceptor (4).

Evidence in support of an *in vivo* role for CCR5 and CXCR4 in HIV-1 infection has been obtained (11–14). In addition to CCR5 and CXCR4, a number of seven-transmembrane segment receptors have been shown to support the entry of one or more HIV-1, HIV-2, or simian immunodeficiency virus isolates *in vitro* (5, 15–19). These seven-transmembrane segment receptors, many of which are also chemokine receptors, share little sequence similarity in their external domains. They do, however, have in common an N-terminal region rich in tyrosines and acidic amino acids (20). This region of CCR5 plays a central role in its ability to support entry of R5 isolates (21–24). We have shown that some or all of the tyrosines in this region are post-translationally modified by the addition of a sulfate moiety and that these sulfate moieties contribute substantially to the association of gp120-soluble CD4 (sCD4) complexes with CCR5 and to the ability of CCR5 to support HIV-1 infection (20).

Here we report that a peptide derived from the N terminus of CCR5 in which two of its four tyrosines have been modified by sulfate, but not an equivalent peptide lacking these sulfates, interacts in a specific manner with CCR5 ligands, including HIV-1 gp120. Moreover, by using gp120-directed antibodies and a chimeric gp120 glycoprotein, we have localized the site on gp120 with which the sulfated CCR5 peptide interacts.

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¹ The abbreviations used are: HIV-1, human immunodeficiency virus, type 1; R5, CCR5-utilizing; X4, CXCR4-utilizing; R5X4, utilizing both CCR5 and CXCR4; sCD4, soluble CD4; V3, third variable; C4, fourth conserved; PBMC, peripheral blood mononuclear cells; CAT, chloramphenicol acetyltransferase; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; MIP, macrophage inflammatory protein.

EXPERIMENTAL PROCEDURES

Peptides and Antibodies—The N-terminal 22 amino acid residues of CCR5 in which cysteine 20 had been altered to serine (C-peptide) and a sulfated form of this peptide (S-peptide, sulfated at tyrosines 10 and 14) were synthesized at 95% purity (American Peptide). Lyophilized peptides were dissolved in 50 mM NaCO₃/NaHCO₃ (pH 8.5) at 10 µg/µl. Anti-gp120 antibodies IgG1b12 and loop2 were kindly provided by Dr. Dennis Burton (Scripps Institute). The anti-gp120 antibody F105 was kindly provided by Dr. Marshall Posner (Dana-Farber Cancer Institute). The gp120 epitopes for the 17b, 48d, 23e, and A32 antibodies are CD4-inducible² (25, 26). 39f and loop2 are antibodies directed against the gp120 third variable (V3) loop (27), and F105 and IgG1b12 recognize the CD4-binding site of gp120 (28, 29).

Plasmids Encoding HIV-1 Envelope Glycoproteins—pSVIIIenv plasmids expressing R5 envelope glycoproteins of the ADA and YU2 HIV-1 isolates, and the X4 envelope glycoproteins of the MN and HXBc2 isolates were described previously (5, 30, 31). The chimeric envelope glycoprotein MN(ADA) was constructed by modifying a plasmid encoding the MN envelope glycoprotein, replacing the *env* sequence encoding the gp120 V3 region with that derived from the ADA gene. An additional point mutation resulting in the alteration of a fourth conserved (C4) region tropism determinant at position 440 (HXBc2 numbering) from glutamic acid (MN) to arginine (ADA) was also made. The final MN(ADA) gp120 glycoprotein differs in seven residues from the parental MN envelope glycoprotein. Both alterations were generated by polymerase chain reaction-based mutagenesis (Stratagene), and the sequence of the altered *env* gene was confirmed.

Cells—HEK293T human kidney cells and Cf2Th canine thymocytes were obtained from ATCC (ATCC CRL 11554 and CRL 1430, respectively). Cf2Th cells stably expressing CCR5 or CXCR4 have been previously described (32). Peripheral blood mononuclear cells (PBMC) were prepared by treating with phytohemagglutinin (5 µg/ml) and IL-2 (10 µg/ml) for 4 days. Macrophages were prepared by adhering PBMC to 6-well plates and removing unattached cells at 24, 48, and 72 h. Macrophages were matured for 2 weeks in conditioned media of L929 cells containing macrophage colony-stimulating factor (25 ng/ml) and granulocyte macrophage colony-stimulating factor (5 ng/ml).

HIV-1 Entry Assays—The envelope complementation assay used here has been previously described (5, 30). Briefly, HIV-1 proviral DNA lacking a functional envelope gene and encoding chloramphenicol acetyltransferase (CAT) as a reporter was cotransfected into HEK293T cells with plasmids encoding the envelope glycoproteins of ADA, 89.6, or HXBc2 HIV-1 isolates or the amphotropic murine leukemia virus or with a plasmid expressing the vesicular stomatitis virus G protein. Viruses harvested from cell supernatants corresponding to 20,000 cpm reverse transcriptase activity were incubated with 3×10^6 of the indicated target cells (PBMC or macrophages) in the presence of either 200 µM (PBMC) or 100 µM (macrophages) of the indicated peptide or peptide-free buffer. Cells were washed 6 h after incubation with virus and lysed 60 h after incubation with virus. CAT activity was measured in the target cell lysates.

Sulfated Peptide Inhibition of gp120/CD4 Binding to Cells Stably Expressing CCR5—Metabolically labeled soluble gp120 molecules were produced from HEK293T cells 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, with the indicated concentration of C- or S-peptide or buffer only in a total volume of 100 µl. Approximately 2×10^5 CCR5- or CXCR4-Cf2Th cells were pelleted and resuspended in the gp120/sCD4/peptide mix and incubated for 1 h at room temperature. Cells were washed and lysed in 200 µl of 1% *n*-dodecyl-β-D-maltoside detergent (Anatrace) with 0.1 mM phenylmethylsulfonyl fluoride and a protease inhibitor mixture (Sigma). Following centrifugation, the lysate was immunoprecipitated with 1 µl of a mixture of sera from HIV-1 infected individuals and analyzed by SDS-PAGE.

Sulfated Peptide Inhibition of Chemokine Binding to CCR5-expressing Cells—An assay to measure binding of chemokines to Cf2Th-CCR5 cells has been previously described (21, 22). Briefly, 2×10^5 Cf2Th cells stably expressing CCR5 or CXCR4 were incubated for 30 min at 37 °C with 0.2 nM [¹²⁵I]-MIP-1α and 200 µM S-peptide, C-peptide, or buffer only or 300 nM unlabeled MIP-1α in a volume of 100 µl. Cells were washed and bound [¹²⁵I] was counted in a gamma counter.

Sulfated Peptide Inhibition of gp120/sCD4 Binding to Anti-gp120 Antibodies—Labeled cell supernatant, prepared as described above and containing approximately 30 ng of gp120, was incubated with 300 ng of sCD4 in a total volume of 50 µl in the presence of C-peptide, S-peptide, or buffer only at 37 °C for 30 min. Then 30 ng of the indicated antibodies in 10 µl were added to this preincubated mix together with 10 µl of 10% protein A-Sepharose beads (Amersham Pharmacia Biotech). After incubation for an additional hour at room temperature, the precipitates were analyzed by SDS-PAGE. The gp120 bands were quantified by phosphorimaging (Bio-Rad).

RESULTS

Our previous work demonstrated that the N terminus of CCR5 contains 2–4 sulfate moieties that modify tyrosine 3 and 1–3 tyrosines at residues 10, 14, and 15 (20). The study of CCR5 variants in which all but one tyrosine were altered to aspartic acid revealed that constructs containing tyrosine 3, 10, or 14 were most efficiently sulfated. In contrast, when the same constructs were assayed for their coreceptor activity, constructs containing tyrosines at positions 10, 14, or 15 were shown to be most efficient at supporting HIV-1 entry. Thus, because pure preparations of sulfated peptides could be obtained best with peptides containing two or fewer non-adjacent sulfates (data not shown), we assayed a peptide that included the first 22 residues of the N terminus of CCR5, in which tyrosines 10 and 14 were sulfated and cysteine 20 had been altered to a serine. We refer hereafter to this peptide as the S-peptide, indicating that it is tyrosine sulfated. We also synthesized an unsulfated analogue of this peptide, which we refer to as the C-peptide, for the fact that it is a CCR5-derived control for the S-peptide.

We first assayed the ability of these peptides to inhibit the entry of recombinant HIV-1 viruses pseudotyped with the envelope glycoproteins of the R5 isolate ADA, the dual tropic (R5X4) isolate 89.6, and the X4 isolate HXBc2. As controls, we used recombinant HIV-1 viruses pseudotyped with the envelope glycoproteins of either the amphotropic murine leukemia virus or the vesicular stomatitis virus G protein. As shown in Fig. 1A, entry of the various pseudotyped viruses into human PBMC was not significantly altered by incubation with the C-peptide as compared with peptide-free buffer alone. However, the S-peptide substantially inhibited the entry of viruses pseudotyped with the ADA envelope glycoproteins. No consistent effect of the S-peptide was observed for viruses pseudotyped with the HXBc2 envelope glycoproteins or with the amphotropic murine leukemia virus envelope glycoproteins. The inability of the S-peptide to inhibit entry of viruses pseudotyped with the envelope glycoproteins of the HXBc2 isolate is consistent with the inability of this isolate to utilize CCR5 as a coreceptor.

The infection of primary human macrophages by viruses pseudotyped with the R5 ADA or the R5X4 89.6 envelope glycoproteins was inhibited by the S-peptide but unaffected by the C-peptide (Fig. 1B). The control virus pseudotyped with the vesicular stomatitis virus G protein was not affected by either peptide. We conclude that the entry of R5 HIV-1 isolates could be inhibited by the S-peptide but not the C-peptide; thus, the sulfate moieties on the S-peptide are critical for the ability of this peptide to block CCR5-dependent HIV-1 entry.

Complexes of sCD4 and the gp120 molecules of R5 or R5X4 isolates specifically bind CCR5-expressing cells (6, 7, 33). We incubated metabolically labeled gp120 from the R5 HIV-1 isolates ADA and YU2 with sCD4 in the presence of either the S-peptide or the C-peptide (Fig. 2, A and B). The S-peptide inhibited the ability of ADA gp120/sCD4 complexes to associate with CCR5-expressing Cf2Th cells, whereas no significant inhibition was observed with the C-peptide (Fig. 2A). Approximately 50% inhibition was observed by phosphorimaging at an

² S. Xiang, unpublished data.

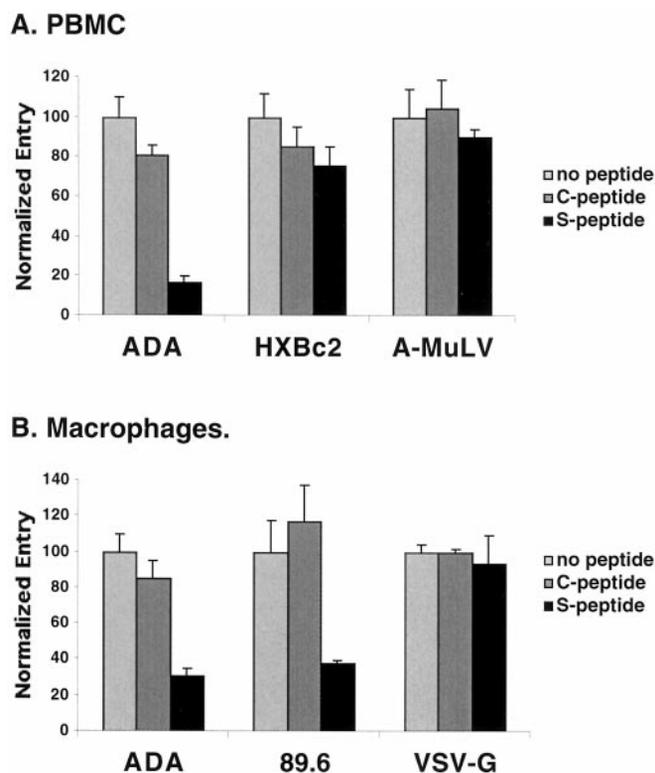


FIG. 1. The sulfated N-terminal CCR5 peptide inhibits HIV-1 infection of primary cells. Approximately 3×10^6 phytohemagglutinin-stimulated primary human PBMC (A) or macrophages (B) were incubated with peptide-free buffer (no peptide), 200 μM C-peptide or S-peptide (for PBMC), or 100 μM C-peptide or S-peptide (for macrophages) together with 20,000 *RT* units of a recombinant CAT-expressing HIV-1 pseudotyped with the indicated envelope glycoproteins. The CAT activity in the target cells 3 days after infection is shown, expressed as a percentage of CAT activity observed for cells incubated in peptide-free buffer.

S-peptide concentration of 50 μM , suggesting that the affinity of this peptide for gp120/sCD4 complexes is in this range. Similar data was obtained using gp120 molecules of the R5 HIV-1 isolate YU2 (Fig. 2B). No binding of the YU2 gp120 molecule to Cf2Th-CXCR4 cells, which do not express CCR5, was observed, even in the presence of sCD4. We conclude that the S-peptide inhibits the association of gp120/sCD4 complexes with CCR5.

We also assessed the ability of the S-peptide to block the association of MIP-1 α , a natural chemokine ligand of CCR5, with CCR5-expressing cells (Fig. 2C). Radiolabeled MIP-1 α (0.2 nM) was incubated with Cf2Th-CCR5 cells in the presence of peptide-free buffer, 200 μM C- or S-peptide, or 200 nM unlabeled MIP-1 α . Cf2Th-CXCR4 cells were also included as a control and bound minimal amounts of radiolabeled MIP-1 α . The C-peptide had no effect on the ability of radiolabeled MIP-1 α to bind CCR5-expressing cells, whereas the S-peptide partially inhibited this association. Inhibition of MIP-1 α binding to Cf2Th cells by the S-peptide was much less efficient than that by unlabeled MIP-1 α .

To study regions on the HIV-1 gp120 glycoprotein that interact with the CCR5 N terminus, we measured the ability of the S- and C-peptides to block the precipitation of gp120 by anti-gp120 antibodies. It has been shown that antibodies such as 17b and 48d bind to discontinuous, CD4-induced gp120 epitopes near the CCR5-binding domain of gp120 (6, 25, 33). As shown in Fig. 3, A–C, the S-peptide, but not the C-peptide, interfered with the ability of the 48d antibody to precipitate the gp120 envelope glycoproteins of the R5 HIV-1 isolates ADA and YU2, but not those of the X4 isolate HXBc2. This inhibition was

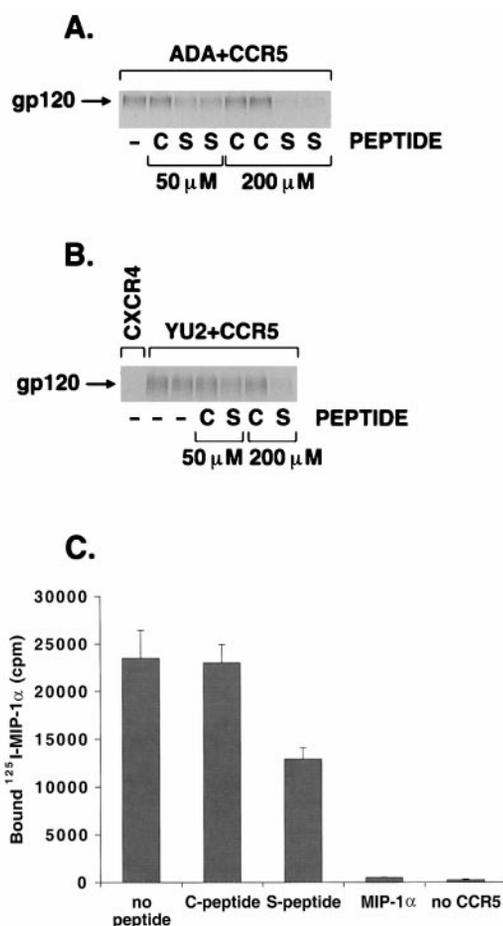


FIG. 2. The sulfated N-terminal CCR5 peptide blocks association of the gp120 glycoprotein of R5 HIV-1 isolates with CCR5. A and B, 50 ng of radiolabeled gp120 from the R5 isolates ADA (A) or YU2 (B) and 500 ng of sCD4 were incubated with the indicated concentrations of the S-peptide (S) or the C-peptide (C) or with peptide-free buffer (–). The peptide/gp120/sCD4 mixtures were then incubated with Cf2Th-CCR5 or Cf2Th-CXCR4 cells (indicated by +CCR5 or CXCR4, respectively). Cells were washed and lysed, and radiolabeled gp120 was precipitated with a mixture of sera from HIV-1 infected individuals and analyzed by SDS-PAGE. C, Cf2Th-CCR5 cells were incubated with 0.2 nM ^{125}I -MIP-1 α and peptide buffer alone (no peptide), 200 μM C-peptide, 200 μM S-peptide, or 200 nM unlabeled MIP-1 α . As a control, 0.2 nM ^{125}I -MIP-1 α was also incubated with Cf2Th-CXCR4 cells lacking CCR5 (indicated as no CCR5). Cells were washed, and bound ^{125}I was quantified by scintillation counting.

observed in the presence and, to a lesser degree, in the absence of sCD4 (Fig. 3, A and B).

A panel of antibodies was similarly examined for inhibition by the S-peptide, using the gp120 envelope glycoprotein of the ADA isolate. As shown in Fig. 4A, neither the S-peptide nor the C-peptide interfered with the ability of the IgG1b12 and F105 antibodies, which are directed against the CD4-binding site (28, 29), to precipitate gp120. Similarly, neither peptide interfered with the precipitation of gp120 by the loop2 and 39f antibodies directed against the tip of the gp120 V3 loop (27). Among the set of antibodies (17b, A32, 48d, 23e) whose epitope is enhanced by sCD4 association with gp120, only precipitation of gp120 by the 48d and 23e antibodies could be inhibited by the S-peptide. In both cases the association of gp120 with sCD4 enhanced this inhibition. Other CD4-induced antibodies, including A32 and, surprisingly, 17b, precipitated gp120 efficiently in the presence of the S- and C-peptides. Because all of the monoclonal antibodies tested exhibit similar affinities for soluble gp120 monomers (26), we conclude that the binding site of the S-peptide overlaps the 48d and 23e epitopes, but not

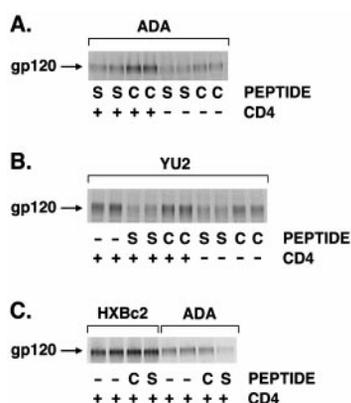


FIG. 3. Binding of the CD4-induced antibody 48d is blocked by the tyrosine-sulfated peptide. Radiolabeled gp120 from the R5 isolates ADA and YU2 and the X4 isolate HXBc2 was incubated with or without soluble CD4 as indicated and 200 μ M S-peptide (S) or C-peptide (C) or with peptide-free buffer (-). Mixtures were then precipitated with the 48d antibody, and precipitates were analyzed by SDS-PAGE. Separate experiments are shown in A, B, and C.

those of the other antibodies tested.

To characterize the binding site of the S-peptide further, we assayed its ability to block precipitation of the gp120 glycoprotein from an X4 isolate, MN, and from an MN variant, MN(ADA), by the 48d antibody. MN(ADA) is a variant of MN that had been converted to an R5 phenotype by the substitution of seven gp120 residues in the V3 and C4 regions with those of the ADA isolate³ (Fig. 4C). The 48d antibody efficiently precipitated the gp120/sCD4 complexes derived from the ADA and MN isolates and the MN(ADA) chimera (Fig. 4B). The S-peptide did not interfere with the precipitation of the MN envelope glycoprotein complexed with sCD4. By contrast, the S-peptide inhibited the precipitation by the 48d antibody of the MN(ADA) gp120/sCD4 complexes, although less efficiently than it interfered with precipitation of ADA gp120/sCD4 complexes. These data indicate that the binding of the S-peptide to gp120/sCD4 complexes depends on a small number of gp120 residues in the N- and C-terminal bases of the third variable loop and/or a single residue in the C4 region. Notably, of these changes, the only alteration to a positive charge, which could complement the net four negative charges on the S-peptide, occurs in the C4 residue (glutamic acid 440 to arginine) (Fig. 4C).

DISCUSSION

The N terminus of CCR5, in particular a tyrosine-rich region in this N terminus, plays a critical role in the ability of HIV-1 to enter CCR5-expressing cells (20–24, 34). Some or all of the tyrosines in this region are modified by the addition of a sulfate group, and we have previously demonstrated that the presence of these sulfate moieties substantially contributes to the ability of CCR5 to support viral entry (20).

The ability of a sulfated peptide derived from the N terminus of CCR5, but not its unsulfated analogue, to inhibit the association of HIV-1 gp120 with CCR5 and to block HIV-1 entry supports the assertion that sulfated tyrosines and the sulfate moieties themselves contribute to a specific interaction of the CCR5 N terminus with gp120. Similar conclusions were reached by Cormier *et al.* (36), who reported that CCR5-derived peptides containing at least two sulfated tyrosines could inhibit the entry of an R5 HIV-1 isolate. Of interest, a CCR5 peptide in which a single tyrosine was modified by sulfate either at position 10 or 14 did not inhibit the association of gp120/CD4 complexes with CCR5 (36), indicating that both sulfate moi-

eties of the S-peptide contribute to the inhibitory activities described here.

The relatively high concentrations necessary for observable inhibition of gp120-CCR5 interactions suggest a low affinity ($\sim 50 \mu$ M) of the peptide for gp120/sCD4 complexes. This affinity is approximately 10^3 - 10^4 -fold lower than that measured for the gp120-sCD4 interaction with native CCR5 on the cell surface (6, 7). This affinity difference is consistent with additional roles for other CCR5 domains in the interaction with gp120 (24) and with the high entropic barrier that a relatively disordered peptide must overcome in binding gp120. Compared with inhibition of gp120-CCR5 binding, even higher concentrations of S-peptide were required to inhibit the entry of R5 viruses. This probably reflects the fact that virus entry, unlike the gp120-CCR5 binding assay, involves oligomeric gp120 contacts with multiple CCR5 proteins and is assisted by virus association with the cell surface via CD4 and possibly other adhesion molecules.

Similar concentrations of the S-peptide less efficiently inhibited the association of the chemokine MIP-1 α with CCR5, as compared with gp120-CCR5 inhibition. This may be a consequence of the lower intrinsic affinity (apparently $>200 \mu$ M) of the S-peptide for MIP-1 α or may reflect the possibility that S-peptide/MIP-1 α complexes retain the ability to bind CCR5. The observed inhibition is consistent with a role for sulfated tyrosines in the MIP-1 α /CCR5 association (20). The less effective inhibition is consistent with a greater dependence of MIP-1 α binding, relative to that of gp120, on the second extracellular loop of CCR5 (35).

The sulfated CCR5 peptide also interfered with the ability of the antibody 48d to precipitate gp120. Notably, this competition was observed, albeit to a lesser degree, in the absence of soluble CD4, suggesting that the N terminus of CCR5 can interact to some extent with gp120 in the absence of CD4. Using qualitative BIAcore analysis, Cormier *et al.* (36) did not detect the binding of sulfated CCR5 N-terminal peptides to R5 gp120 glycoproteins in the absence of sCD4. This discrepancy probably reflects differences in the sensitivity of the two assays for detection of peptide binding.

Our results provide clues to the location of the S-peptide-binding site on the HIV-1 gp120 glycoprotein and, by inference, to the CCR5-interactive region on the viral envelope glycoproteins. The S-peptide competed specifically for the binding of the 48d and the 23e antibodies to R5 gp120 glycoproteins. Interestingly, the sulfated peptide did not compete with 17b, an antibody that recognizes a gp120 epitope closely related to the 48d epitope. Binding of the 17b antibody, like that of the 48d antibody, is enhanced by the association of gp120 with CD4, and these antibodies compete efficiently with one another and with surface-expressed CCR5 for gp120 association (6, 7, 26). However, the 48d and 17b antibodies are distinguished by several properties. First, 48d, but not 17b, competes efficiently with antibodies to the V3 loop of gp120 (26). Second, 48d, but not 17b, requires the presence of the base of the V3 loop to associate with gp120 in the absence of CD4 (37). Third, 48d, but not 17b, is sensitive to gp120 residue changes at positions 256(Ser \rightarrow Tyr), 262(Asn \rightarrow Thr), 384(Tyr \rightarrow Glu), and 421(Lys \rightarrow Leu) (25). The latter alteration also ablates the ability of 48d, but not 17b, to neutralize HIV-1 infection (25). Combined with the x-ray structure of gp120 complexed with sCD4 and the 17b antibody (38), these data locate the 48d epitope to a region between the 17b epitope and the V3 loop, partially overlapping both. Another antibody that recognizes a CD4-induced gp120 epitope, 23e,⁴ was also partially inhibited by the S-peptide. Although the

³ H. Choe, unpublished data.

⁴ S. Xiang, J. Robinson, and J. Sodroski, manuscript in preparation.

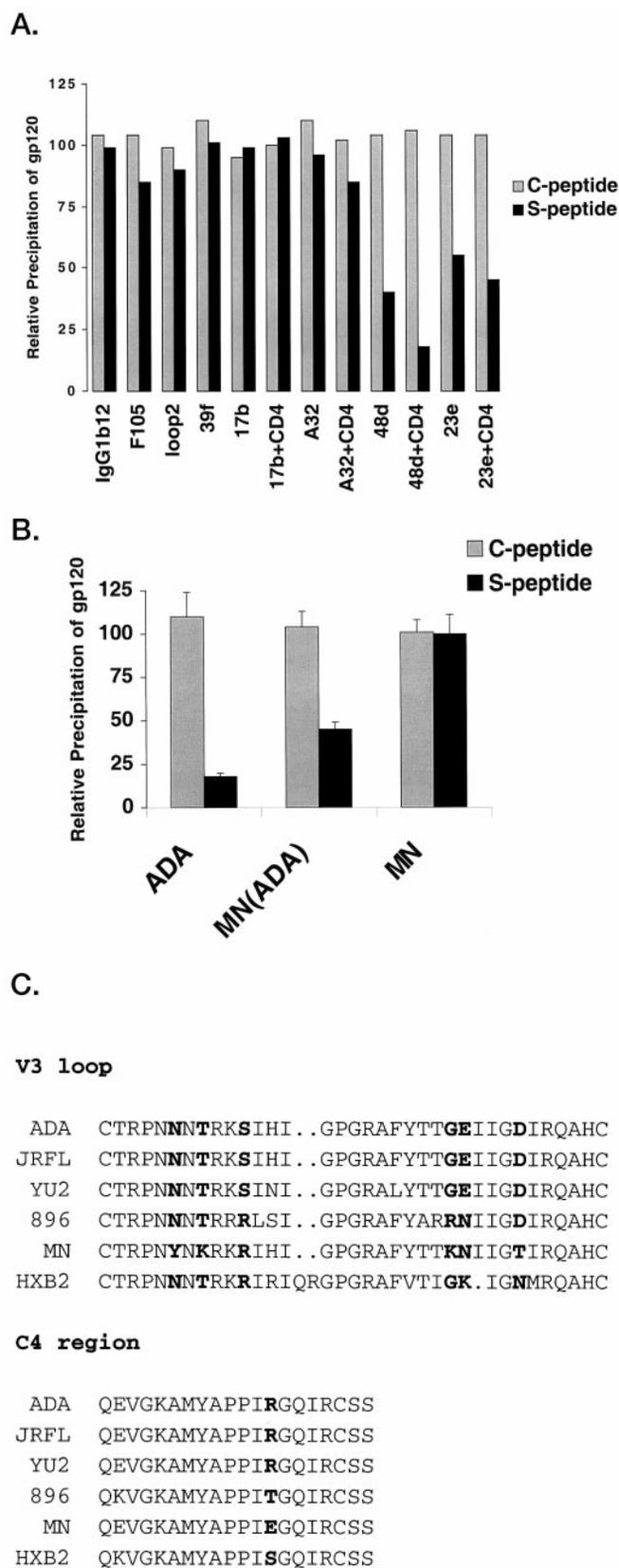


FIG. 4. The sulfated CCR5 peptide interacts with a CD4-induced epitope of gp120 that includes tropism determinants in the V3 and C4 regions of gp120. *A*, the gp120 glycoprotein of the R5 isolate ADA was incubated with the indicated antibodies in the presence of peptide-free buffer, the C-peptide (gray bars), or the S-peptide (black bars). The mixtures were then precipitated with the indicated antibodies, and precipitates were analyzed by SDS-PAGE. Precipitation by the 17b, A32, 48d, and 23e antibodies, which recognize CD4-induced gp120 epitopes, was performed in the presence or absence of excess

epitope for this antibody has not yet been mapped, such information could further localize the gp120-binding site for the CCR5 N terminus. The S-peptide did not block the binding of the A32 antibody, which recognizes a CD4-induced epitope that is adjacent to, but does not overlap, the 17b and 48d epitopes (26).

The sulfated peptide inhibited the association of the 48d antibody with gp120 of the R5 isolate ADA but not of the X4 isolates HXBc2 or MN. Alteration of six residues in the V3 loop and one in the C4 domain of MN to those found in the R5 isolate ADA switched the tropism to an R5 phenotype.⁵ Coinciding with this change in tropism, the sulfated CCR5 peptide can partially block the association of the 48d antibody with this MN gp120 variant. These data support the localization of the binding site of the CCR5 peptide is modulated by changes in the base of the third variable loop and in a single gp120 residue in the C4 region. This residue (position 440 in the prototypic HXBc2 sequence) is somewhat variable and has been shown to interact functionally with the V3 loop to modulate HIV-1 replication and tropism (39). Further characterization of this region and its interaction with the N terminus of CCR5 may lead to the development of additional sulfated compounds that inhibit the association of gp120 with CCR5.

The ability of sulfated peptides and small molecules to block ligand association with chemokine receptors is not likely to be limited to CCR5. We have previously noted that many seven-transmembrane segment receptors, including most known chemokine receptors, have N-terminal tyrosines that are likely to be sulfated (20). Investigation of the properties of tyrosine-sulfated peptides and peptidomimetics based on the N termini of these receptors will probably contribute to a better structural understanding of the specificity of these receptors as well.

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⁵ H. Choe, unpublished data.

soluble CD4 as indicated. Antibodies IgG1b12 and F105 recognize the CD4-binding site of gp120. Antibodies loop2 and 39f recognize the tip of the V3 loop of gp120. The values shown represent the percentage of gp120 precipitated relative to that observed in the presence of peptide-free buffer. *B*, the gp120 glycoproteins of the R5 isolate ADA, the X4 isolate MN, and the R5 MN(ADA) chimera were incubated with soluble CD4 in the presence of buffer alone, the C-peptide (gray bars), or the S-peptide (black bars). The mixtures were then precipitated with the anti-gp120 antibody 48d, and the precipitates were analyzed by SDS-PAGE. The values shown represent the percentage of gp120 precipitated relative to that observed in the presence of peptide-free buffer, as quantified by phosphorimaging. *C*, amino acid sequences of the V3 loop and a portion of the C4 region of the gp120 molecules of selected HIV-1 isolates. Residues that vary between the ADA and MN isolates are shown in bold.

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