Specific interaction of CCR5 amino-terminal domain peptides containing sulfotyrosines with HIV-1 envelope glycoprotein gp120

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The HIV-1 envelope glycoprotein gp120 interacts consecutively with CD4 and the CCR5 coreceptor to mediate the entry of certain HIV-1 strains into target cells. Acidic residues and sulfotyrosines in the amino-terminal domain (Nt) of CCR5 are crucial for viral fusion and entry. We tested the binding of a panel of CCR5 Nt peptides to different soluble gp120/CD4 complexes and anti-CCR5 mAbs. The tyrosine residues in the peptides were sulfated, phosphorylated, or unmodified. None of the gp120/CD4 complexes associated with peptides containing unmodified or phosphorylated tyrosines. The gp120/CD4 complexes containing envelope glycoproteins from isolates that use CCR5 as a coreceptor associated with Nt peptides containing sulfotyrosines but not with peptides containing sulfotyrosines in scrambled Nt sequences. Finally, only peptides containing sulfotyrosines inhibited the entry of an R5 isolate. Our data show that proper posttranslational modification of the CCR5 Nt is required for gp120 binding and viral entry. More importantly, the Nt domain determines the specificity of the interaction between CCR5 and gp120s from isolates that use this coreceptor.

HIV-1 entry into target cells is mediated by the successive interaction of the envelope glycoprotein gp120 with CD4 and a coreceptor belonging to the seven-transmembrane G protein-coupled chemokine receptor family (1). Binding of gp120 to CD4 exposes or creates a coreceptor binding site on gp120 (2, 3). CCR5 and CXCR4 are the most physiologically relevant and widely used HIV-1 coreceptors (4). CCR5 mediates the entry of R5 isolates, and CXCR4 mediates the entry of X4 isolates. So-called R5X4 isolates are able to exploit both coreceptors (1). We and others have demonstrated that specific amino acids, including acidic residues and tyrosines located within the CCR5 amino-terminal domain (Nt, amino acids 2–31), are essential for CCR5-mediated fusion and entry of R5 and R5X4 HIV-1 strains (5–8). More recently, Farzan *et al.* demonstrated that tyrosine residues in the CCR5 Nt are sulfated (9). Tyrosine sulfation is a widespread posttranslational modification catalyzed in the Golgi apparatus by tyrosylprotein sulfotransferase (10). Tyrosine-sulfated proteins participate in protein–protein interactions that depend highly on the recognition of the sulfate groups (11).

Inhibition of cellular sulfation pathways, including tyrosine sulfation, by sodium chlorate greatly decreased soluble gp120/CD4 complex binding to $CCR5^+$ cells as well as the entry of R5 and R5X4 HIV-1 strains (ref. 9, E.G.C., unpublished data). A number of reports have implicated a role for sulfate moieties in HIV-1 entry. Several sulfated compounds, such as dextran sulfate, can inhibit HIV-1 entry by associating with CD4 or gp120 (12, 13). Sulfated proteoglycans have been shown to bind to HIV-1 gp120 at or near its third variable (V3) loop, which also determines coreceptor usage (14, 15). It is therefore conceivable that sulfotyrosines in the CCR5 Nt also interact with gp120, increasing its affinity for CCR5. The reduction in soluble gp120/CD4 binding and HIV-1 entry caused by the pretreatment of target cells with sodium chlorate, however, cannot be attributed formally to a reduction in CCR5 tyrosine sulfation (ref. 9, E.G.C., unpublished data). Chlorate anion is a sulfate mimic that inhibits proteoglycan and tyrosine sulfation (16, 17). We therefore sought to study the role and relevance of CCR5 tyrosine sulfation in HIV-1 entry by a more direct experimental approach.

The region of the CCR5 Nt spanning amino acids 2–18 contains all of the residues that were shown to be important for viral entry (5–8). We demonstrated previously that Tyr-3, -10, and -14 were required for optimal coreceptor function, whereas Tyr-15 was interchangeable with Phe-15 (6). Based on these results and the findings by Farzan *et al.* (9), this suggested that Tyr-3, -10, and -14, but not Tyr-15, might be sulfated. Alternatively, Tyr-15 sulfation could play no role in HIV-1 entry. We therefore explored the role of sulfotyrosines in positions 3, 10, and 14 by synthesizing peptides corresponding to amino acids 2–18 of the CCR5 Nt and carrying different tyrosine modifications. We first tested the ability of the Nt peptides to inhibit binding of soluble gp120/CD4 complexes and anti-CCR5 mAbs to $CCR5^+$ cells (18). The specific association of certain peptides with soluble gp120/CD4 complexes or with anti-CCR5 mAbs was confirmed further by surface plasmon resonance analysis. Inhibition of HIV-1 entry by the CCR5 Nt peptides was also tested. Our results suggest that amino acids 2–18 of the CCR5 Nt compose a gp120-binding site that determines the specificity of the interaction between CCR5 and gp120s from R5 and R5X4 isolates. Posttranslational sulfation of the tyrosine residues in the CCR5 Nt is required for gp120 binding and may modulate critically the susceptibility of target cells to HIV-1 infection *in vivo*.

Materials and Methods

Reagents. Biotinylated CD4-IgG_2, soluble CD4 (sCD4), recombinant soluble gp120s from HIV-1_{LAI} (X4), HIV-1_{DH123} (R5X4), and HIV-$1_{JR\text{-}FL}$ (R5) isolates, gp120-ΔV3 from HIV-$1_{JR\text{-}FL}$ isolate, and anti-CCR5 mAbs PA8, PA10, PA12, and PA14 were provided by Progenics (Tarrytown, NY). Anti-CCR5 mAb 2D7 was purchased from PharMingen.

Peptides. Peptides containing different segments of the CCR5 Nt were custom synthesized by solid-phase fluorenylmethoxycarbonyl chemistry by using phospho- and sulfotyrosine precursors as building blocks where indicated (Table 1). Biotinylated versions of peptides S-10/14 and P-10/14 incorporated a carboxyl-

Abbreviations: Nt, amino-terminal domain; m.f.i., mean fluorescence intensity; RU, resonance units; r.l.u., relative light units.

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Table 1. CCR5 Nt peptides

Sequence	Label
Unmodified peptide	
D Y Q V S S P I Y D I N Y Y T S E	3/10/14
Sulfated peptides	
D **Y** Q V S S P I **Y** D I N **Y** Y T S E	S-3/10/14
D Y Q V S S P I **Y** D I N **Y** Y T S E	S-10/14
D Y Q V S S P I **Y** D I N **Y** Y T S E G A G K-biotin	bS-10/14
D Y Q V S S P I **Y** D I N Y Y T S E	S-10
D Y Q V S S P I Y D I N **Y** Y T S E	S-14
Y D I N **Y**	TS-10/14
Phosphorylated peptides	
D [Y] Q V S S P I [Y] D I N [Y] Y T S E	P-3/10/14
D Y Q V S S P I [Y] D I N [Y] Y T S E	P-10/14
D Y Q V S S P I [Y] D I N [Y] Y T S E G A G K-biotin	bP-10/14
Sulfated and scrambled peptides	
Y V S Q P D N T Y I **Y** S Y E S I D	SS-2/12
S I D I Y N P T **Y** V S N **Y** E S D Y	SS-10/14

terminal GAG spacer preceding a biotinylated lysine. After cleavage from the resin, peptides were purified by reverse-phase chromatography on C18 columns (Vydac, Hesperia, CA) and analyzed by HPLC and mass spectroscopy.

Binding of gp120 and mAbs to CCR5. A gp120/CD4 complex formed from monomeric gp120 (100 nM) and biotinylated CD4-IgG_2 (50 nM) was added to 1×10^6 L1.2-CCR5$^+$ cells in the presence of different concentrations of peptide (18). CD4-IgG_2 is tetrameric and therefore binds four molecules of gp120, which increases binding of the complex to CCR5 (19). The mean fluorescence intensity (m.f.i.) was measured by flow cytometry after addition of phycoerythrin (PE)-labeled streptavidin (Becton Dickinson). Residual gp120/CCR5 binding was calculated: (m.f.i. with peptide)/(m.f.i. without peptide) $\times$ 100%.

L1.2-CCR5$^+$ cells (1×10^6) were incubated with anti-CCR5 mAbs (50 nM) $\pm$ peptide (100 μM) (18). mAb binding was detected by using a PE-labeled goat anti-mouse antibody (Caltag, South San Francisco, CA). The m.f.i. value was measured by flow cytometry, and mAb binding was calculated as above.

Surface Plasmon Resonance Measurements (Biacore). Streptavidin-coated sensor chips (Biacore) were divided into four consecutive surfaces, each with a separate flow chamber. The flow chambers could be independent or connected. The entire sensor chip surface was conditioned with five injections of regeneration solution (1 M NaCl, 50 mM NaOH) and equilibrated with 10 mM Hepes/150 mM NaCl/3 mM EDTA/0.005% polysorbate 20 (HBS-EP) as recommended by the manufacturer. Four hundred resonance units (RU) of biotinylated peptide was bound per sensor chip surface by injection of peptide (100 nM) in HBS-EP buffer, followed by an injection of regeneration solution and equilibration with HBS-EP buffer. Phosphorylated and sulfated peptides were immobilized on the second and third sensor chip surfaces, respectively. Control surfaces were treated with the HBS-EP buffer only, followed by an injection of regeneration solution and equilibration with HBS-EP buffer. The first and fourth sensor chip surfaces were always used as controls. Proteins (100 nM) in HBS-EP were flowed consecutively over the four sensor chip surfaces and washed out by HBS-EP buffer. Surface plasmon resonance was monitored and displayed in arbitrary RU as a function of time. Similar RU values measured for the two control surfaces confirmed that the protein solutions were not significantly depleted while flowing over the peptide-containing chip surfaces. After each set of measurements, the chip was regenerated and equilibrated as described above. The following proteins were tested for binding to the peptides: gp120, sCD4, gp120/sCD4, PA8, PA10, and 2D7.

Single-Cycle HIV-1 Entry Assay. NLluc$^+$env$^-$ particles pseudotyped with envelope glycoproteins from MuLV, HTLV-1, and HIV-1 strains JR-FL, HxB_2, DH123, Gun-1 were made as described (5). Target cells (HeLa-CD4$^+$CCR5$^+$ or U87-CD4$^+$CCR5$^+$) were incubated with virus-containing supernatant fractions (100 ng/ml p24) $\pm$ peptide (100 μM) for 4 h. After 48 h, the cells were lysed, and luciferase activity (relative light units, r.l.u.) was measured by using a standard kit (Promega) as described (5). Viral entry was calculated: (r.l.u. with peptide)/(r.l.u. without peptide) $\times$ 100%.

Results

Inhibition of gp120/CD4 Binding to CCR5 by Nt Peptides. Table 1 describes the different peptides that were used in this study. We first tested whether tyrosine-sulfated peptides spanning amino acids 2–18 of the CCR5 Nt could inhibit binding of the $gp120_{JR\text{-}FL}$/CD4-IgG_2 complex to CCR5$^+$ cells. The HIV-$1_{JR\text{-}FL}$ isolate uses CCR5 exclusively as a coreceptor (20). Only peptides S-3/10/14 and S-10/14 inhibited complex binding to the cells in a dose-dependent manner (Fig. 1*a*). Peptides S-10 and S-14 had no inhibitory activity, even at the highest concentrations (Fig. 1*a*). Peptide TS-10/14, spanning amino acids 10–14, did not inhibit $gp120_{JR\text{-}FL}$/CD4-IgG_2 binding to CCR5$^+$ cells, despite the presence of two sulfotyrosine residues (Fig. 1*b*). The unmodified peptide 3/10/14 increased binding of the complex to the CCR5$^+$ cells by about 5-fold at the highest concentration (data not shown). This increase in binding was not specific, because it also occurred when cells were incubated with gp120 from X4 HIV-1 isolates or CD4-IgG_2 alone (data not shown).

Tyrosine-phosphorylated peptides P-10/14 and P-3/10/14 did not inhibit $gp120_{JR\text{-}FL}$/CD4-IgG_2 binding to CCR5$^+$ cells (Fig. 1*b*). As further specificity controls, we synthesized peptides containing the first 17 residues of the CCR5 Nt in random order with sulfotyrosines in positions 10 and 14 (SS-10/14) or in positions 2 and 12 (SS-2/12). Neither one of these peptides reduced $gp120_{JR\text{-}FL}$/CD4-IgG_2 binding to CCR5$^+$ cells, even at the highest concentrations (Fig. 1*b*). SS-10/14 increased binding of the complex to the cells, just like the wild-type peptide containing unmodified tyrosines (data not shown).

Association of the $gp120_{JR\text{-}FL}$/CD4 Complex with CCR5 Nt Peptides. Biotinylated peptides were attached to the streptavidin-coated gold surface of a sensor chip and solutions containing no protein, sCD4, gp120, or gp120/sCD4 complexes were flowed over the immobilized peptides. Adsorption of the proteins to the sensor chip due to protein/peptide binding was detected by an increase in surface plasmon resonance signal (RU), which reports changes in the effective refractive index very near the gold surface of the sensor chip (21). All buffer and temperature conditions being equal, for proteins of similar size such as the different gp120/sCD4 complexes, RU plateau values are directly proportional to the amount of protein bound to the peptide.

Specific association of the $gp120_{JR\text{-}FL}$/sCD4 complex with the sulfotyrosine-containing peptide bS-10/14 was accompanied by a significant increase in RU (Fig. 2*a*). The signal plateau but not the shape of the sensorgrams varied with $gp120_{JR\text{-}FL}$/sCD4 concentration, indicating that the peptide/complex interaction was dose dependent (Fig. 2*a*). The sensorgram obtained with bP-10/14 is similar to the one obtained in the absence of peptide, indicating a complete lack of association of the phosphorylated peptide with the protein complex (Fig. 2*a*). Neither $gp120_{JR\text{-}FL}$ nor sCD4 alone produced a significant increase in RU, indicating that they did not associate with the immobilized peptides. (Fig.

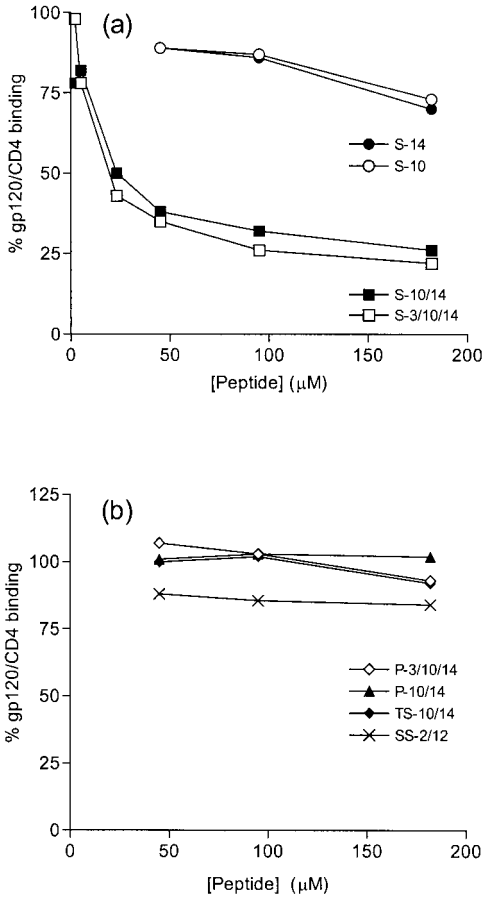

Fig. 1. Effect of peptides on $gp120_{JR\text{-}FL}$ binding to CCR5. L1.2-$CCR5^+$ cells were incubated with the biotinylated $gp120_{JR\text{-}FL}$/CD4-IgG_2 complex in the presence of different concentrations of peptides (*a*) S-3/10/14, S-10/14, S-10, S-14, or (*b*) P-3/10/14, P-10/14, SS-2/12, SS-10/14, TS-10/14. The extent of complex binding in the absence of peptide was defined as 100% (m.f.i. ≈40 ± 5). Binding in the presence of peptide is expressed as a percentage of control. When CCR5-negative cells were used, binding of the $gp120_{JR\text{-}FL}$/CD4-IgG2 complex was negligible (≈10%, m.f.i. ≈2 ± 1). The values shown are from a sample experiment.

2 *b* and *c*). The gp120-$\Delta V3_{JR\text{-}FL}$/sCD4 complex was also unable to associate with the peptides (Fig. 2*d*).

To ascertain further the specificity of the peptide/complex association, we performed Biacore analyses by using envelope glycoproteins from HIV-1_{DH123}, an R5X4 isolate, and HIV-1_{LAI}, an X4 isolate (5). $gp120_{DH123}$/sCD4 associated specifically with the sulfated peptide, although the plateau RU values were lower than those observed with $gp120_{JR\text{-}FL}$/sCD4 (Fig. 2*e*). We detected no binding of $gp120_{DH123}$/sCD4 to the phosphorylated peptide (Fig.2*e*), nor did $gp120_{DH123}$ alone associate with the peptides (Fig. 2*f*). Finally, $gp120_{LAI}$ with or without sCD4 was not able to associate with either one of the peptides (Fig. 2 *g* and *h*).

Anti-CCR5 mAb Interactions with the Nt Peptides. We determined whether the CCR5 Nt peptides could inhibit binding of a panel of anti-CCR5 mAbs to $CCR5^+$ cells. Only PA8 binding was reduced significantly by all wild-type peptides containing amino acids 2–18, regardless of tyrosine modification (Fig. 3). Biacore analysis confirmed that PA8 similarly and specifically associated with both sulfated and phosphorylated peptides (Fig. 4). Mutational analysis predicted that the PA8 epitope lies exclusively in the Nt of CCR5 (18), and the present data confirmed that conclusion. The PA12 epitope was also predicted to be exclusively within the Nt (18), but binding of PA12 to CCR5 was not inhibited by any of the peptides (Fig. 3). PA10 binding to CCR5 was inhibited only by S-3/10/14 (Fig. 3), yet Biacore analysis showed that PA10 also associated with bS-10/14 and to a lesser extent with bP-10/14 (Fig. 4). Biacore analysis is probably more sensitive than the gp120/CCR5-binding assay, which would account for this discrepancy. Our previous work predicted that the PA10 epitope spans residues in the Nt and ECL2 (18). In light of the ability of PA10 to interact with the sulfated peptides, we now propose that ECL2 residues only weakly influence PA10 interactions with CCR5. Binding of 2D7 to CCR5 was not inhibited by any of the peptides (Fig. 3). Biacore analysis showed no association of 2D7 with the sulfated and phosphorylated peptides (Fig. 4). The 2D7 epitope lies exclusively in ECL2 (18).

Inhibition of HIV-1 Entry by CCR5 Nt Peptides. The ability of different CCR5 Nt peptides to inhibit HIV-1 entry into $CD4^+CCR5^+CXCR4^+$ cells was tested by using a luciferase-based single round of entry assay (5). Only peptides S-10/14 and S-3/10/14 inhibited the entry of the R5 isolate HIV-$1_{JR\text{-}FL}$ by approximately 50% in HeLa-$CD4^+CCR5^+$ and U87MG-$CD4^+CCR5^+$ (Fig. 5 and data not shown). We were unable to inhibit the entry of the R5X4 isolates HIV-1_{DH123} and HIV-$1_{Gun\text{-}1}$, or of the X4 isolate HIV-1_{HxB2}. Entry of R5X4 isolates was also unaffected in cell lines that express CD4 and CCR5 but not CXCR4 (data not shown). The entry of MuLV and HTLV pseudotypes was also unaffected by the peptides (Fig. 5).

Discussion

We synthesized tyrosine-modified peptides spanning the region of the CCR5 Nt that contains all of the residues that are important for viral entry (5–8). Interactions between the Nt peptides and gp120/CD4 complexes were characterized. A minimum of two sulfotyrosines in positions 10 and 14 were required for efficient inhibition of soluble $gp120_{JR\text{-}FL}$/CD4 complex binding to CCR5. Substitution of the sulfate groups for phosphates, which are also negatively charged at physiological pH, rendered the Nt peptides inactive. Inhibition of gp120/CCR5 binding depended, therefore, on the presence of sulfate moieties and was not simply caused by nonspecific electrostatic interactions between the peptide and the gp120/CD4 complex or the peptide and the cell surface. Inhibition of gp120/CCR5 binding also depended on the primary structure surrounding the sulfotyrosines, because peptides with random sequences of CCR5 amino acids 2–18 had no inhibitory activity. The entire Nt sequence spanning amino acids 2–18 was also required because a shortened peptide containing amino acids 10–14 was unable to inhibit gp120/CD4 binding, despite the presence of two sulfotyrosines. Most polysulfated compounds that inhibit HIV-1 entry exert their effect by binding to gp120 or CD4 through nonspecific electrostatic interactions (12, 13, 22). Some, however, may mimic the structure of the CCR5 Nt and thus inhibit gp120/coreceptor binding.

Qualitative Biacore analyses allowed us to demonstrate a highly specific CD4-dependent interaction between a tyrosine-sulfated Nt peptide and $gp120_{JR\text{-}FL}$. No binding of the protein complex to a tyrosine-phosphorylated peptide was observed. Only gp120s derived from isolates that use CCR5 as a coreceptor associated with the sulfated peptide. $gp120_{DH123}$/CD4 binding was weaker than $gp120_{JR\text{-}FL}$/CD4 binding, suggesting that envelope glycoproteins from R5X4 isolates have a lower apparent affinity for CCR5 than envelope glycoproteins from R5 isolates. $gp120_{LAI}$, derived from an isolate that uses only CXCR4, did not bind to the sulfated peptide. The shape of the sensorgram obtained for the binding of the $gp120_{JR\text{-}FL}$/sCD4 complex to bS-10/14 is indicative of pseudo-first-order binding kinetics. The apparent on and off rates of gp120/CD4 binding to the sulfated

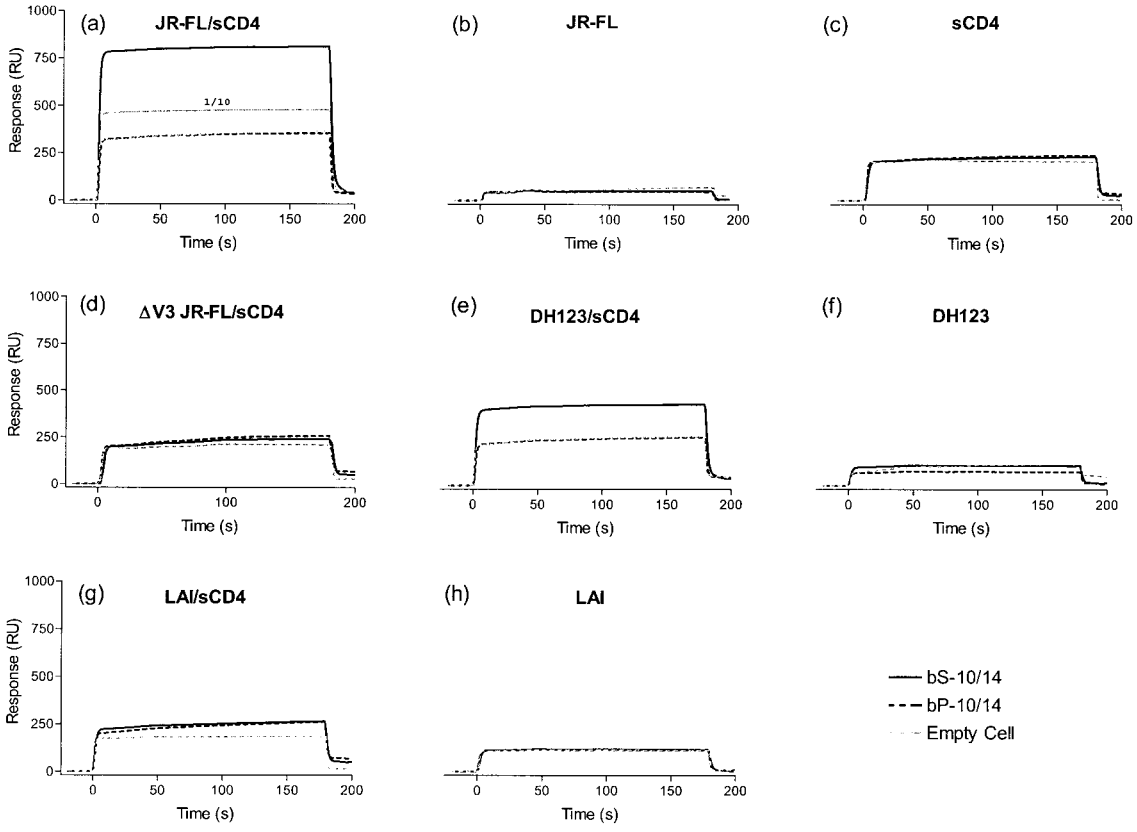

Fig. 2. Binding of the gp120/sCD4 complex to sulfated and phosphorylated peptides. The association of different gp120/sCD4 complexes with immobilized biotinylated peptide bP-10/14 (dotted black lines) or bS-10/14 (solid black lines) was analyzed by Biacore. RU values as a function of time were also measured in the absence of peptide (dotted gray lines). Binding studies were performed with the following proteins (100 nM): (*a*) $gp120_{JR\text{-}FL}$/sCD4, (*b*) $gp120_{JR\text{-}FL}$, (*c*) sCD4, (*d*) gp120-$\Delta V3_{JR\text{-}FL}$/sCD4, (*e*) $gp120_{DH123}$/sCD4, (*f*) $gp120_{DH123}$, (*g*) $gp120_{LAI}$/sCD4 and (*h*) $gp120_{LAI}$. A 10-fold dilution (10 nM) of $gp120_{JR\text{-}FL}$/sCD4 was also tested (solid gray line in *a*). Representative examples of RU measurements are shown.

peptide are unusually rapid and outside the range that the Biacore can measure accurately (23). To measure precisely the kinetic parameters of the complex/peptide interaction, additional analyses that rely on stopped flow methodology will be required.

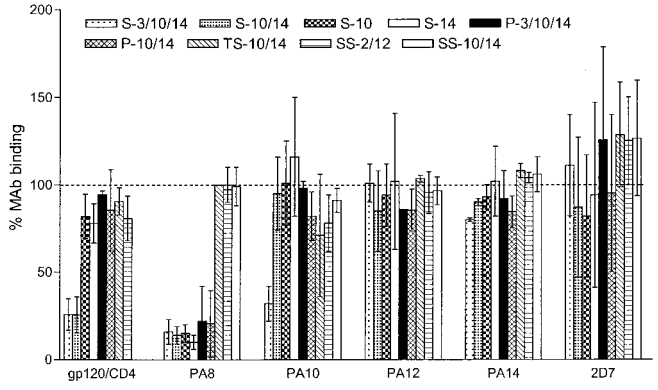

Fig. 3. Effect of peptides on mAb binding to CCR5. L1.2-$CCR5^+$ cells were incubated with the anti-CCR5 mAbs in the presence of peptides. The extent of mAb binding in the absence of peptide was defined as 100% (m.f.i. ≈50–400, depending on the mAb) and is represented by a horizontal dotted line. Binding in the presence of peptide is expressed as a percentage of control. When CCR5-negative cells were used, binding of mAbs was negligible (m.f.i. ≈2 ± 1). Each data point represents the mean ± SD of three replicates.

We also studied binding of the Nt peptides to several anti-CCR5 mAbs, all of which recognize conformational epitopes in CCR5 and inhibit gp120/CCR5 binding (18). PA12 and 2D7 did not bind to any of the peptides. Binding of PA8 to the peptides was independent of tyrosine modification, whereas PA10 associated more with the sulfotyrosine-containing peptide than with the phosphotyrosine-containing peptide. It seems, therefore, that sulfotyrosines and phosphotyrosines are relatively interchangeable for the purpose of mAb binding, whereas gp120/CD4 binding has an absolute requirement for sulfotyrosines. Relatively subtle differences in size and geometry of sulfate and phosphate groups might be relevant for binding of the CCR5 Nt to gp120, which must not only accept the negative charge but also coordinate, probably by hydrogen bonds, the tyrosine sulfate oxygens. The kinetics of mAb binding to the CCR5 Nt peptides exhibited large apparent on rates and slow apparent off rates, which also differed from our observations of gp120/CD4-binding kinetics.

None of the Nt peptides inhibited MuLV, HTLV, and HIV-1_{HxB2} envelope-mediated viral entry. Peptides S-10/14 and S-3/10/14 similarly inhibited the entry of the HIV-$1_{JR\text{-}FL}$ R5 strain in two different cell lines and did not inhibit the entry of HIV-1_{DH123} and HIV-$1_{Gun\text{-}1}$ R5X4 strains. The inhibition of HIV-1 entry by tyrosine-sulfated peptides was partial (≈50%) but nonetheless remarkable given the difficulty of blocking multimeric protein–protein interactions with short linear peptides (24–29). The inaccessibility of CCR5-binding sites on virion-associated gp120 may explain the partial inhibition of viral

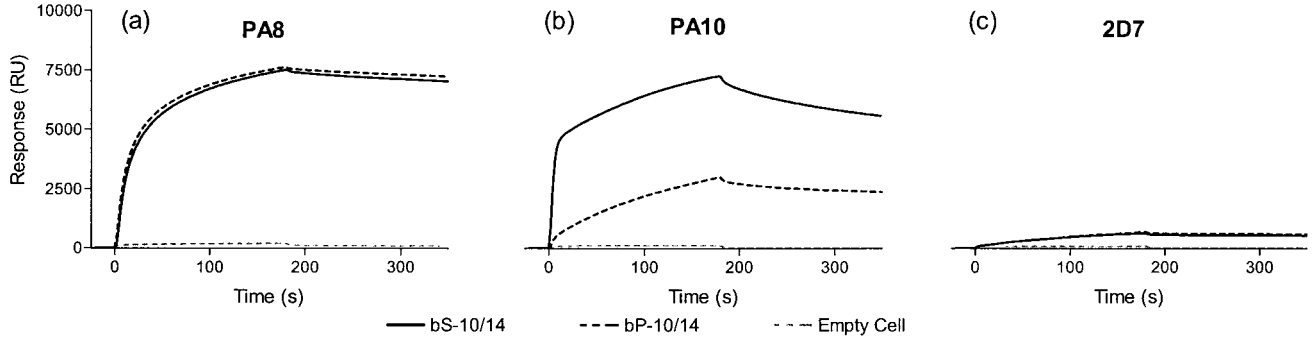

Fig. 4. Binding of mAbs to sulfated and phosphorylated peptides. The association of anti-CCR5 mAbs with immobilized biotinylated peptide bP-10/14 (dotted black lines) or bS-10/14 (solid black lines) was analyzed by Biacore. RU values as a function of time were also measured in the absence of peptide (dotted gray lines). Binding studies were performed with (*a*) PA8, (*b*) PA10, and (*c*) 2D7. Representative examples of RU measurements are shown.

entry by Nt peptides. Indeed, once gp120 interacts with membrane-bound CD4 and the CCR5-binding site is exposed or created, gp120 might interact preferentially with the membrane-bound coreceptor rather than with soluble CCR5 Nt peptides. The complete insensitivity of R5X4 entry to the peptides is probably because of the lower affinity of R5X4 gp120 for CCR5 and CCR5-derived peptides.

Structural studies of CCR5 have relied mostly on the functional characterization of point mutants and coreceptor chimeras. We and others have shown that residues in the CCR5 Nt, including Asp-2 and -11, Glu-18, Ser-17, Cys-20, and Tyr-3, -10, -14, and -15, were important for CCR5-mediated viral fusion and entry (5–8). No alanine substitution of any other single extracellular residue significantly reduced CCR5 coreceptor function. Several groups showed, however, that substitution of more than one residue in the extracellular loops of CCR5 could lead to a decrease in coreceptor function (8, 30, 31). Furthermore, chimeras formed between CCR5 and other chemokine receptors variably mediated HIV-1 entry (8, 30–36). The consensus has been, therefore, that the Nt is an important determinant of CCR5 coreceptor function, which is also modulated by other domains of the coreceptor.

This study demonstrated that the CCR5 Nt determines the specificity of the interaction between CCR5 and gp120s from isolates that use this coreceptor. The first 17 residues of the CCR5 Nt form an independent protein domain that contains a binding site for gp120. Posttranslational sulfation of the tyrosine residues in the CCR5 Nt is required for gp120 binding and may modulate critically the susceptibility of target cells to HIV-1 infection *in vivo*. The remaining residues of CCR5 might have no function other than to tether the Nt close to the plasma membrane. Alternatively, binding of gp120 to the CCR5 Nt could be followed by interactions with other domains of CCR5. If the off rate of gp120 association to membrane-bound CCR5 is also high, then such contacts may be necessary to stabilize the gp120/CCR5 complex in order for the next phase of membrane fusion to occur. We recently completed a study that supports this model. TAK-779, a small molecule inhibitor of CCR5-mediated HIV-1 entry, interacts exclusively with residues in helices 1, 2, 3, and 7 of CCR5 yet is able to inhibit gp120/CCR5 binding (37).

Crystallographic data and mutational analyses indicate that the coreceptor-binding site on gp120 has a conserved and a variable component (38, 39). The conserved component is a basic region that faces away from the virus, toward the target cell membrane (40, 41). The electrostatic potential of this region is influenced strongly by the overall charge, but not the precise structure, of the V3 loop (40, 41). A V3 loop-deleted $gp120_{JR\text{-}FL}$ did not associate with the sulfated peptide, just as this protein was unable to bind to full-length CCR5 on the cell surface (2). Because the Nt peptide clearly determines the specificity of the gp120/CCR5 interaction, and the V3 loop has been shown to determine coreceptor usage, it is likely that the V3 loop interacts directly with the CCR5 Nt. Conserved regions of gp120 that participate in coreceptor binding (38) would then interact with other domains of CCR5 to provide additional binding energy.

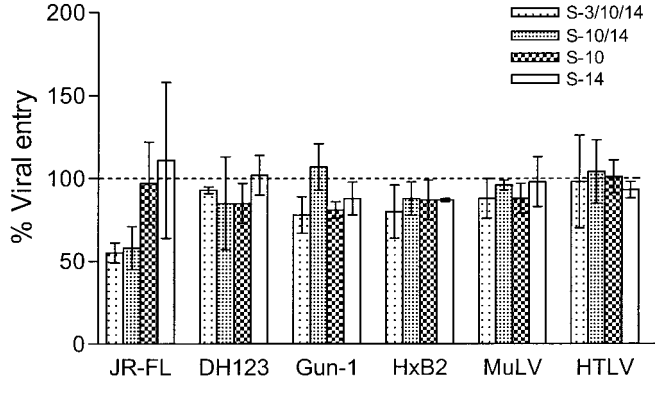

Fig. 5. Effect of peptides on viral entry. HeLa-$CD4^+CCR5^+$ cells were infected with $NLluc^+env^-$ particles pseudotyped with different viral envelopes in the presence of peptides. Luciferase activity (r.l.u.) was measured 48 h after infection. The extent of entry in the absence of peptide was defined as 100% (r.l.u. ≈25,000 ± 9,000) and is represented by a horizontal dotted line. Background r.l.u. values were ≈7 ± 2. Each data point represents the mean ± SD of three replicates.

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