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Chih-chin Huang
National Institutes of Health

Son N. Lam
National Institute of Diabetes and Digestive and Kidney Diseases

Priyamvada Acharya
National Institutes of Health

Min Tang
National Institutes of Health

Shi-Hua Xiang
Harvard Medical School

See next page for additional authors

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Structures of the CCR5 N Terminus and of a Tyrosine-Sulfated Antibody with HIV-1 gp120 and CD4

Chih-chin Huang,1,* Son N. Lam,2* Priyamvada Acharya,1 Min Tang,1 Shi-Hua Xiang,3 Syed Shahzad-ul Hassan,2 Robyn L. Stanfield,4 James Robinson,5 Joseph Sodroski,1 Ian A. Wilson,4 Richard Wyatt,† Carole A. Bewley,1† Peter D. Kwong1†

The CCR5 co-receptor binds to the HIV-1 gp120 envelope glycoprotein and facilitates HIV-1 entry into cells. Its N terminus is tyrosine-sulfated, as are many antibodies that react with the co-receptor binding site on gp120. We applied nuclear magnetic resonance and crystallographic techniques to analyze the structure of the CCR5 N terminus and that of the tyrosine-sulfated antibody 412d in complex with gp120 and CD4. The conformations of tyrosine-sulfated regions of CCR5 (α-helix) and 412d (extended-loop) are surprisingly different. Nonetheless, a critical sulfotyrosine on CCR5 and on 412d induces similar structural rearrangements in gp120. These results now provide a framework for understanding HIV-1 interactions with the CCR5 N terminus during viral entry and define a conserved site on gp120, whose recognition of sulfotyrosine engenders posttranslational mimicry by the immune system.

E ntry of human immunodeficiency virus type 1 (HIV-1) into host cells requires its gp120 envelope glycoprotein to bind to two cell-surface receptors, CD4 and a co-receptor, either CCR5 or CXCR4 [reviewed in (1, 2)]. CCR5 and CXCR4 are members of a family of chemokine receptors that are G protein-coupled receptors (3) characterized by seven transmembrane helices, an extracellular N terminus, which is variable in length, and three extracellular loops (ECLs) (Fig. 1A). The structure of the co-receptor has not been determined, but some insight has come from the crystal structures of other family members (4).

Elements critical to interactions with HIV-1 are located in the co-receptor N terminus and around its second extracellular loop (ECL2) (5–8). The co-receptor N terminus interacts with a highly conserved 4-stranded bridging sheet in gp120, which assembles upon CD4 binding, whereas the ECL2 region of the co-receptor interacts with the tip of the immunodominant V3 loop in gp120. Considerable distance separates these two interactive regions, which suggests that they are independent (9–12).

The N-terminal interaction of co-receptor with HIV-1 requires an unusual posttranslational modification, O-sulfation of tyrosine (13). On CCR5, tyrosines at residues 3, 10, 14, and 15 may be O-sulfated, but sulfations at residues 10 and 14 are sufficient to facilitate interaction with HIV-1 (14). Interestingly, many CD4-induced antibodies that react with the bridging sheet region are also modified by O-sulfation (15). To define structurally the interaction of HIV-1 with the N terminus of CCR5 and to understand the molecular details of the mimicry of this interaction by CD4-induced antibodies, we used a combination of nuclear magnetic resonance (NMR) and x-ray crystallography to determine the structures of the N terminus of CCR5 and of a functionally sulfated antibody, 412d, in complex with HIV-1 gp120. Analysis of these structures, combined with molecular docking and saturation transfer difference NMR, identified a conserved site on gp120, which recognizes sulfotyrosine with high selectivity.

We used NMR techniques that exploit the transfer of information from bound to ligand-free states (16, 17) to analyze the interactions of a 14-residue peptide (CCR52–15), which consisted of residues 2 to 15 of CCR5 with sulfotyrosine (Tys) at positions 10 and 14 (Fig. 1) (18). We collected two-dimensional (2D) nuclear Overhauser enhancement spectroscopy (NOESY) spectra of solutions containing CCR52–15 either free or in the presence of gp120, CD4, or a gp120-CD4 complex (peptide:protein ratio of 40:1). Whereas spectra containing free CCR52–15 or CCR52–15 with either gp120 or CD4 contained few cross peaks, CCR52–15 in the presence of the gp120-CD4 complex gave rise to high-quality spectra containing numerous NOEs (Fig. 1B and fig. S1). Complete 1H, 13C, and 15N assignments of CCR52–15 (table S1) were made on the basis of standard 2D homonuclear and heteronuclear NMR experiments that measure scalar and dipolar couplings.

The NOE data of CCR52–15 in the presence of gp120-CD4 (Fig. 1B) were sufficient for calculating a high quality ensemble of NMR structures (Fig. 1C). Structure calculations were carried out on the ordered region comprising residues 7 to 15. A total of 70 distance restraints (corresponding to 35 intraresidue and 35 interresidue NOEs), and 56 dihedral angle restraints were included in the final round of structure calculations, which gave rise to an ensemble of 40 structures with a backbone root-mean-square deviation (rmsd) of 0.46 Å and an rmsd of 1.39 Å for all atoms in the ordered region (residues 9 to 14) (table S2). Superpositions of the final ensemble defined a helical conformation for residues 9 to 15, which deviated from the ideal by a backbone rmsd of only 0.26 Å (Fig. 1D). Sulfotyrosines 10 and 14 extended from the same face of the helix, with sulfate moieties separated by ~10 Å and an ~90° rotation around the helix axis.

We were unable to obtain crystals of CCR52–15 in complex with HIV-1 gp120-CD4, and the size and glycosylation of the ternary complex hindered direct determination by NMR. We were, however, able to obtain ~3.5 Å diffraction from crystals of the antigen-binding fragment (Fab) of the 412d antibody, in complex with gp120 (core with V3, CCR5-dependent isolate YU2) and CD4. The 412d antibody is functionally tyrosine-sulfated, binds to a CD4-induced epitope that overlaps the site of co-receptor binding on HIV-1 gp120, and recognizes preferentially CCR5-dependent strains of HIV-1 gp120 (15). Moreover, the tyrosine-sulfated region of 412d can be sub-
We solved the 412d-gp120-CD4 structure by molecular replacement. Despite less than optimal resolution and completeness, initial unbiased maps showed clear definition of important antibody features (fig. S2). Structure refinement resulted in an R_{cryst} of 20% (R_{free} 27%) (Fig. 2, table S3, and fig. S3). The overall mode of binding of 412d resembles that of 17b, which shares a heavy chain of similar genomic origin (fig. S4) (20). A hydrophobic interaction pins the second complementarity-determining region of the heavy chain (CDR H2) to a conserved hydrophobic surface on the bridging sheet of gp120, whereas the acidic CDR H3 binds a basic gp120 surface. Antibody 412d, however, interacts with a much larger overall surface area than either 17b or X5 (fig. S4). The increased 412d interaction surface is due primarily to an increase in buried surface associated with its CDR H3. Comparison of free (20) and bound structures of 412d shows that extensive ordering occurs in CDR H3 when bound to gp120 (fig. S5).

The two sulfotyrosines in the CDR H3 region of 412d bind to gp120 in quite different ways (fig. 2). The sulfotyrosine at residue 100 of 412d (Tys 100c^{412d} [Kabat numbering (21)]) is mostly exposed, with its aromatic ring making π-cation interactions with the guanidinium of Arg 327^{gp120} and its sulfate group making only peripheral electrostatic interactions. By contrast, the side-chain of Tys 100c^{412d} is mostly buried, with Ile 322, and Ile 326 embracing one face of the tyrosine ring, while the aliphatic base of Arg 440^{gp120} supports the other. Together, the two sulfotyrosines account for about 20% of the total buried surface on 412d, with almost 100 Å² derived from Tys 100c^{412d}.

To facilitate interactions with the sulfotyrosines in 412d, the V3 stem is rearranged. The conserved Arg 298^{ gp120} and Pro 299^{ gp120} at the base of the V3 loop are mostly unchanged, but the subsequent Asn residues at 301^{ gp120} and 302^{ gp120} shift ~7 Å to form one wall of the Tys 100c^{412d} sulfate-binding pocket. Residue 301^{ gp120} is N-glycosylated, but the glycan faces solvent, and its presence should have little impact on the ability of the binding pocket to form. Meanwhile, in the returning strand (22), Ile

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**Fig. 1.** Structure of the tyrosine-sulfated N terminus of CCR5 in the gp120-bound conformation. (A) CCR5 sequence and schematic of its insertion in the cell membrane. Sequence letters in purple correspond to residues in CCR5^{2-15}, with sulfotyrosines (Tys) critical for interaction with HIV-1 highlighted in black. ECLs are labeled, and disulfide bridges (-SS-) depicted. (B) 2D NOEY spectra for CCR5^{2-15} free in solution (left) and in the presence of gp120-CD4 (right). NMR samples (20 mM phosphate, 50 mM NaCl, pH 6.85) contained 800 μM CCR5^{2-15} in the presence of 20 μM gp120-CD4 and were recorded at 500 MHz, 300 K, mixing time = 150 msec. Sequential NH(0)-COH(1) NOEs were observed between every residue, thereby confirming sequential assignments, and predicted intraresidue NOEs were observed for all residues. No correlations beyond sequential NOEs were observed between residues 2 and 7, indicating that this region of CCR5 was extended or disordered. In contrast, NOEs from COH(0) to NH(1) + 1,2,3 and from NH(0) to NH(1) + 1,2,3 were observed for residues 9 to 15 (fig. S1), indicating an ordered α-helical structure (33). (C) Structure of the ordered region of gp120-bound CCR5^{2-15}. Stereoview (left) of 25 lowest energy-simulated annealing structures superimposed by fitting to the backbone of residues 9 to 15. Structural statistics are provided in table S2. Backbone appears in blue, amide hydrogens (9 to 15) in blue, side chains (11 to 13) in green, and Tys 10 and Tys 14 in red. Ribbon diagram (right) of restrained minimized mean structure with side chains in stick representations.
**Fig. 2.** Structure of the tyrosine-sulfated antibody 412d in complex with HIV-1 gp120 and CD4. (A) Ribbon representation. CD4 is yellow, the heavy chain of Fab 412d is dark blue, the light chain is cyan, and gp120 is gray, except for the V3 loop, which is orange. The CDR H3 loop of 412d is red, with sulfotyrosines depicted in stick representation. (B) Close-up, with molecular surface of gp120 in gray and sulfotyrosines of 412d (red labels) and select residues of gp120 (black labels) in stick representation. Dotted lines represent coordinating hydrogen bonds between gp120 and the sulfate group of Tys100C412d. The sulfate of Tys100C412d makes a full complement of ionic interactions: a salt bridge to Arg298gp120 and hydrogen bonds to the side-chain nitrogen of Asn302gp120, the side-chain hydroxyl of Thr303gp120, and the main-chain amides of 302gp120, 303gp120, and 441gp120 (34).

**Fig. 3.** Interaction of the N terminus of CCR5 with HIV-1 gp120-CD4. (A) Molecular docking. The 20 lowest energy structures (black) from 200 docking runs of CCR52-15 are shown in stick representation. Despite initial random orientations, all favorable docking solutions had Tys14 binding at the bridging sheet-V3 interface; none had Tys10 at this cleft. Ribbon representations illustrate CD4 in yellow, gp120 in gray (with V3 in orange), and the lowest energy structure of CCR52-15 in purple. (B) Close-up, with molecular surface of gp120 in gray and select residues of gp120 (black labels) and CCR5 (purple labels) in stick representation. (C) Saturation transfer difference NMR spectrum of CCR52-15 in the presence of gp120–CD4 (red) overlaid on a control 1H spectrum (black). Experimental conditions were identical to those used for NOE experiments, except that the carrier was set at −1 and 50 parts per million for on- and off-resonance saturation, respectively. The intensities of the most strongly enhanced peaks (Tys14 and Tyr15) have been normalized to the corresponding signals in the control spectrum. Peak assignments made by 2D NMR (table S1) appear above their corresponding doublet signals. Tys14 and Tyr15 show strong saturation transfer difference effects, whereas Tys10 shows a medium effect and Tyr3 a very weak effect. These effects correlate directly with the buried surface area of each tyrosine ring in the docked structure. See fig. S9 for overlaid spectra employing 1 to 7 s saturation. (D) Effect of CCR52-15 on the proteolytic sensitivity of the V3. Electrophoresis on an 8 to 25% gradient SDS polyacrylamide gel shows the results of thrombin digestion on gp120 (core with V3; YU2 RS strain of HIV-1) alone, or in the presence of sCD4 or sCD4 and CCR52-15 (35). (E) Structural intermediates of HIV-1 entry. At far left, a single monomer of unliganded gp120 (gray) is shown with separated β-hairpins. The threefold axis, from which gp41 interacts in the functional oligomer, is labeled with the number 3. In the CD4-bound state, the bridging sheet assembles, and the V3 (orange) is exposed and flexible. The next state involves either (upper pathway) the interaction of the CCRS-ECL2 region with the V3 tip or (lower pathway) the interaction of the CCRS N terminus, which induces rigidification of the V3 stem. Engagement of CCR5 at both N terminus and ECL2 region triggers additional conformational changes leading to HIV-1 entry.
The V3 stem of gp120 is highly flexible and can adopt various conformations. We used saturation transfer difference NMR to study the conformational changes in the V3 loop upon binding of CCR5. The results showed that the V3 loop is highly conformationally flexible, with a conserved sulfotyrosine binding pocket that is formed upon binding to CCR5.

The V3 loop was modeled using molecular dynamics simulations, and the results showed that the V3 loop is flexible and can adopt multiple conformations. The binding of CCR5 to the V3 loop was modeled using computational methods, and the results showed that the CCR5 N terminus forms a rigid β-hairpin upon binding to the gp120 V3 loop.

The CCR5 β-hairpin provides an explanation for the observed lack of cross-reactivity between 412d and CCR5, as the sulfotyrosine binding pocket of 412d is not present in CCR5.

Overall, the results of this study provide a deeper understanding of the conformational changes in the V3 loop of gp120 upon binding to CCR5, and highlight the importance of the sulfotyrosine binding pocket in the recognition process.
Several peptides were tested in initial NMR titration experiments, wherein line broadening of peptide signals was monitored upon addition of CD4. Although a peptide corresponding to CCR5 residues 2 to 18 (25) showed tighter binding than 2 to 15, the shorter peptide exhibited superior quality NOESY spectra. See also (22).

18. Stem movements are somewhat imprecise because the returning V3 stem exhibits considerable disorder before interaction with the N terminus of CCR5 (21).

22. The nascent β-hairpin in the V3 stem extends ~15 Å further extension is interrupted by a lattice contact, which occurs with the outgoing portion of the V3 stem. In the absence of this lattice contact, the stem β-hairpin may extend farther, perhaps joining with the V3 tip to zip up much of the V3 loop.

24. Data incompleteness and resolution (~3.5 Å) made delineation of hydrogen bonds problematic. The current designation is consistent with the substitutional mutagenesis experiments (fig. S10); alternatively, discrimination between sulfoxotyrosine and phosphotyrosine suggests complete sulfate coordination by hydrogen bond acceptors (U2), with the side-chain nitrogen of Asn 302 donating a hydrogen bond instead of the hydroxyl of Thr 303.

35. The 24-hour time point that is shown clearly depicts the protective effect of CCR5Δ32 with sCD4. CCR5Δ32 without sCD4 does not show this effect, and shorter incubations show that sCD4 enhances V3 cleavage.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/317/5846/1930/DC1

Materials and Methods

Figs. S1 to S10

Tables S1 to S3

References

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