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Research Paper

Tyrosine-sulfated V2 peptides inhibit HIV-1 infection via coreceptor mimicry

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ABSTRACT

Tyrosine sulfation is a post-translational modification that facilitates protein-protein interaction. Two sulfated tyrosines (Tys173 and Tys177) were recently identified within the second variable (V2) loop of the major HIV-1 envelope glycoprotein, gp120, and shown to contribute to stabilizing the intramolecular interaction between V2 and the third variable (V3) loop. Here, we report that tyrosine-sulfated peptides derived from V2 act as structural and functional mimics of the CCR5 N-terminus and potently block HIV-1 infection. Nuclear magnetic and surface plasmon resonance analyses indicate that a tyrosine-sulfated V2 peptide (pV2α-Tys) adopts a CCR5-like helical conformation and directly interacts with gp120 in a CD4-dependent fashion, competing with a CCR5 N-terminal peptide. Sulfated V2 mimics, but not their non-sulfated counterparts, inhibit HIV-1 entry and fusion by preventing coreceptor utilization, with the highly conserved C-terminal sulfotyrosine, Tys177, playing a dominant role. Unlike CCR5 N-terminal peptides, V2 mimics inhibit a broad range of HIV-1 strains irrespective of their coreceptor tropism, highlighting the overall structural conservation of the coreceptor-binding site in gp120. These results document the use of receptor mimicry by a retrovirus to occlude a key neutralization target site and provide leads for the design of therapeutic strategies against HIV-1.

1. Introduction

Progressive refinement of the molecular anatomy of the native HIV-1 envelope spike, which represents the sole target of neutralizing antibodies, is accruing critical information for the rational design of effective inhibitors and vaccine immunogens (Burton et al., 2012; Mascola and Haynes, 2013; Wyatt and Sodroski, 1998). The envelope spike displayed on the surface of infectious virions is a homotrimer of gp120:gp41 heterodimers (Wyatt and Sodroski, 1998). Due to inherent difficulties in crystallizing the native envelope trimer, most high-resolution structures were initially obtained with monomeric gp120 stabilized in complex with soluble CD4 (sCD4) and/or specific monoclonal antibodies (mAb) (Chen et al., 2009; Huang et al., 2005, 2007; Kwon et al., 2012; Kwong et al., 1998, 2000; Pancera et al., 2010). Recently, important progress has been made with the design of soluble, cleaved, disulfide bridge-stabilized gp140 trimers (SOSIP.664), which display several features of the pre-fusion envelope spike (Sanders et al., 2013), although the stabilizing mutations were shown to abrogate fusion competence and to induce some alterations of the antigenic profile (Alsahafi et al., 2015). The structure of several SOSIP.664 trimers has been resolved by crystallization or high-resolution cryogenic electron microscopy (cryoEM), providing valuable information on the pre-fusion conformation of the envelope trimer (Bartesaghi et al., 2013; Julien et al., 2013; Kwon et al., 2015; Garces et al., 2015; Lyumkis et al., 2013; Pancera et al., 2014; Stewart-Jones et al., 2016). Additional insights have come with the progressive increase in cryoEM imaging resolution of *bona fide* native trimers expressed on virion or cellular surface membranes (Hu et al., 2011; Lee et al., 2016; Liu et al., 2008; White et al., 2010; Wu et al., 2010). Despite these remarkable advancements, however, several aspects of the structure-function relationships in the HIV-1 envelope spike remain to be defined, which may be critical for the design of effective inhibitors targeting functional elements of the HIV-1 envelope spike.

We recently reported the identification of two sulfated tyrosines (Tys173 and Tys177) within the second variable (V2) domain of HIV-

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1 gp120, showing that tyrosine sulfation modulates HIV-1 neutralization sensitivity and, thereby, may facilitate immune evasion (Cimbro et al., 2014), an art mastered by HIV-1 to limit the immune system ability to recognize conserved neutralization epitopes (Chen et al., 2009; Kwong et al., 2002; Liu et al., 2011; Pancera et al., 2010; Pinter et al., 2004). Tyrosine sulfation was documented in gp120 from multiple HIV-1 strains grown in primary human $CD4^+$ T cells, including primary isolates minimally passaged *ex vivo*, thus corroborating its physiological relevance. Remarkably, the V2 sulfotyrosines are identically spaced as two critical sulfated tyrosines within the N-terminal domain of the CCR5 coreceptor, which were shown to bolster the interaction of CCR5 with the base of the gp120 third variable (V3) domain, facilitating HIV-1 entry (Farzan et al., 1999; Huang et al., 2007). In line with these observations, tyrosine-sulfated peptides derived from the CCR5 N-terminus were shown to specifically inhibit infection by CCR5-tropic HIV-1 isolates (Cormier et al., 2000; Cormier et al., 2001; Farzan et al., 2000). Likewise, a chimeric molecule encompassing a tyrosine-sulfated peptide inserted at the C-terminus of CD4-Ig was recently reported to possess potent antiviral activity against a broad range of HIV-1 isolates (Gardner et al., 2015).

Based on these observations, we hypothesized that the sulfated region of the V2 loop could functionally mimic the sulfated N-terminus of CCR5 and establish an intramolecular interaction with the coreceptor-binding site at the base of the V3 loop. In the unliganded envelope, this interaction could serve as a structural constraint to stabilize the closed pre-fusion conformation of the trimer. Consistent with this hypothesis, recent crystallographic structures obtained with soluble SOSIP trimers show the V2 tyrosines positioned in close proximity to the CCR5-binding site (Julien et al., 2013; Kwon et al., 2015; Pancera et al., 2014; Garces et al., 2015; Stewart-Jones et al., 2016). In the present study, we utilize sulfated V2 mimetic peptides to provide a structural and functional characterization of the sulfotyrosine-mediated intramolecular interaction between V2 and V3. Moreover, we show that tyrosine-sulfated V2 mimetic peptides display potent and broad-spectrum anti-HIV-1 activity, opening new perspectives for the design of effective HIV-1 entry inhibitors.

2. Materials and methods

2.1. Peptides and proteins

Tyrosine-sulfated and control, non-sulfated mimetic peptides were produced using solid-phase synthesis technology by American Peptide Company. Each peptide was purified to >95% and extensively quality controlled by HPLC and Mass Spectrometry Electro-Spray. The peptide aa. sequences were the following: Lys-Val-Gln-Lys-Glu-Tyr(SO_3H)-Ala-Leu-Phe-Tyr(SO_3H)-Glu-Leu-Asp-Ile-Val-Pro-Ile-Asp (pV2α-Tys); Lys-Lys-Gln-Lys-Val-His-Ala-Leu-Phe-Tyr(SO_3H)-Lys-Leu-Asp-Ile-Val-Pro-Ile-Lys (pV2αE-Tys); Met-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr(SO_3H)-Asp-Ile-Asn-Tyr(SO_3H)-Tyr-Thr-Ser-Glu-Pro-Ser-Gln-Lys (pCCR5-Tys); Asp-Pro-Val-Leu-Glu-Phe-Tyr(SO_3H)-Lys-Ile-Asp-Tyr(SO_3H)-Ile-Glu-Leu-Ala-Val-Gln-Lys (pSCR-Tys). The peptides were dissolved in 10 nM NH_4OH at 10 mg/mL and stored frozen until use. Recombinant monomeric gp120 (strain BaL) was obtained from the NIH AIDS Reagent Program; the recombinant SOSIP trimer (strain BG505) was produced in 293FS cells and purified by lectin-affinity and size-exclusion chromatography as described (Julien et al., 2013); 2-domain sCD4 was obtained from the NIH AIDS Reagent Program; the CD4-M48U mini-protein (Martin et al., 2003) was a gift of Loic Martin.

2.2. NMR spectroscopy

Peptide pV2α-Tys labeled with ^{15}N was synthesized by American Peptide Company. All NMR samples were prepared by dissolving the tyrosine-sulfated pV2α-Tys in aqueous buffer containing 20 mM sodium phosphate pH 6.8, 50 mM sodium chloride, 0.02% sodium azide and 10% deuterium oxide. Binary complexes of either 2-domain sCD4 or recombinant gp120 (BaL) with pV2α-Tys and the ternary sCD4:gp120:pV2α-Tys peptide complex were prepared by combining all components and incubating on ice for 1 h. The complexes were then exchanged into NMR buffer and concentrated to a final volume of 200 μL. The final concentrations of sCD4, gp120 and pV2α-Tys were 20 μM, 20 μM and 800 μM, respectively. All NMR spectra were collected at 30 °C on a Bruker DRX 600 MHz NMR spectrometer equipped with a ^{1}H/^{15}N/^{13}C cryoprobe.

Peptide assignments were completed using 2D TOCSY and NOESY experiments. NOESY spectra were acquired with τ_{mix} of 100, 250 and 400 milliseconds (ms). A τ_{mix} of 250 ms was found to give optimal NOE cross peak intensities. To assist in assignment process, we chemically synthesized pV2α-Tys with ^{15}N–labeled Ala, Ile, Leu, Phe, and Val at all 8 occurrences of those amino acids. The ^{15}N–labeled pV2α-Tys peptide, at a concentration of 0.8 mM, was used to collect 2D ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC), 3D ^{15}N–edited TOCSY-HSQC and 3D ^{15}N–edited NOESY-HSQC spectra. trNOESY spectra of pV2α-Tys in the presence of 20 μM sCD4–gp120 were acquired using the same NOESY pulse scheme and parameters.

NMR structures were computed using the torsion angle dynamics program Cyana 3.0. NOE assignments were obtained using an iterative combination of the NOEASSIGN automated peak assignment feature of Cyana and manual editing. A total of 272 unambiguous, non-redundant distance constraints were employed in calculation of the final ensemble of 20 structures, which had an average target function of 0.85 ± 0.11 and average RMSD to the mean of 0.41 ± 0.17 Å (backbone) and 1.24 ± 0.21 Å (heavy atoms).

Competition between pCCR5-Tys and pV2α-Tys for binding to the sCD4:gp120 complex was assessed using 2D NOESY, 2D TOCSY, 2D HSQC and 3D ^{15}N-edited NOESY-HSQC spectra using ^{15}N–labeled V2 peptide. The sample conditions were the same as described above except that the V2 peptide concentration was 0.4 mM. Experimental data sets were collected with 0, 0.8 mM and 1.6 mM unlabeled pCCR5-Tys.

2.3. Circular dichroism spectroscopy

Far-UV CD spectra were measured and recorded on a Jasco-810 Spectropolarimeter (Jasco Inc., Easton, MD) equipped with a temperature controller. Spectra were recorded using a 0.1 cm quartz cuvette, at 0.3 mg/mL (137 μM), with a scan speed of 50 nm/min, response time of 1 s, band width of 4 nm and averaging of 5 scans. Peptide concentrations were determined spectrophotometrically in 30% acetonitrile at 280 nm using a molar extinction coefficient of 2980 M^{-1} cm^{-1}. The raw CD data were analyzed by Dichroweb and using the Contin-LL algorithm (http://dichroweb.cryst.bbk.ac.uk/html/home.shtml), and reference set 3 for secondary structure prediction.

2.4. Surface plasmon resonance

Peptide pV2α-Tys was immobilized on a Biacore CM5 sensor surface. Recombinant gp120 from HIV-1 BaL (at 25-400 nM) or SOSIP trimer (at 12.5–200 nM) were pre-incubated with or without a 2-fold molar excess of CD4-M48U miniprotein (at 200 nM) for 2 min at 25 μL/min in HBS supplemented with 3 mM EDTA and 0.005% Tween-20, followed by a 2 min dissociation phase on a Biacore 3000 instrument (GE Healthcare). Surfaces were regenerated after each cycle by a brief injection of 4.5 M $MgCl_2$.

2.5. Clustal omega alignment

The alignment of V2 domain from 6 different HIV-1 subtypes (A to F) was performed using all the sequences deposited in the Los Alamos HIV Sequence Database and selecting one sequence for each patient isolate. A total of 191 sequences from subtype A, 3306 from subtype B, 2160