The M-T Hook Structure Is Critical for Design of HIV-1 Fusion Inhibitors*

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Background: We recently found that N-terminal residues Met-626 and Thr-627 of HIV-1 fusion inhibitor CP621-652 adopt a unique hook-like structure, termed the M-T hook.

Results: The structure and function of the M-T hook have been characterized.

Conclusion: The M-T hook is critical for the stability and antiviral activity of HIV-1 fusion inhibitors.

Significance: Our data provide important information for designing novel HIV-1 fusion inhibitors.

CP621-652 is a potent HIV-1 fusion inhibitor peptide derived from the C-terminal heptad repeat of gp41. We recently identified that its N-terminal residues Met-626 and Thr-627 adopt a unique hook-like structure (termed M-T hook) thus stabilizing the interaction of the inhibitor with the deep pocket on the N-terminal heptad repeat. In this study, we further demonstrated that the M-T hook structure is a key determinant of CP621-652 in terms of its thermostability and anti-HIV activity. To directly define the structure and function of the M-T hook, we generated the peptide MT-C34 by incorporating Met-626 and Thr-627 into the N terminus of the C-terminal heptad repeat-derived peptide C34. The high resolution crystal structure (1.9 Å) of MT-C34 complexed by an N-terminal heptad repeat-derived peptide reveals that the M-T hook conformation is well preserved at the N-terminal extreme of the inhibitor. Strikingly, addition of two hook residues could dramatically enhance the binding affinity and thermostability of 6-helix bundle core. Compared with C34, MT-C34 exhibited significantly increased activity to inhibit HIV-1 envelope-mediated cell fusion (6.6-fold), virus entry (4.5-fold), and replication (6-fold). Mechanistically, MT-C34 had a 10.5-fold higher increase than C34 in blocking 6-helix bundle formation. We further showed that MT-C34 possessed higher potency against T20 (Enfuvirtide, Fuzeon)-resistant HIV-1 variants. Therefore, this study provides convincing data for our proposed concept that the M-T hook structure is critical for designing HIV-1 fusion inhibitors.

The envelope (Env) glycoprotein complex of human immunodeficiency virus type 1 (HIV-1), consisting of a trimer containing three gp120 surface subunits and three gp41 transmembrane subunits, is responsible for viral attachment to target cells and the subsequent fusion of viral and cellular membranes. Binding of the gp120 to the CD4 receptor and a coreceptor (CCR5 or CXCR4) triggers large conformational changes within a trimeric complex that activate the fusion machinery of gp41, which is originally sheltered by gp120 (1–4). Structurally, the gp41 can be divided into multiple functional domains as follows: a hydrophobic fusion peptide at the N terminus; an N-terminal heptad repeat (NHR); a loop with a disulfide bond at its basis; a C-terminal heptad repeat (CHR); a membrane proximal external region; a transmembrane domain (TM), and a long cytoplasmic tail (Fig. 1). Upon receptor binding, the gp41 undergoes a dramatic transition from its metastable native state into an extended pre-hairpin intermediate, in which the fusion peptide is released allowing its insertion into the targeting cell membrane. Subsequently, three cognate CHRs fold onto the trimeric coiled coil of NHR to form a stable six-helix bundle (6-HB) that bridges the viral and cellular membranes for fusion (1, 2, 4). The crystal structure of 6-HB reveals a highly conserved hydrophobic pocket in the C-terminal portion of NHR helices, which is penetrated by three hydrophobic residues (Trp-628, Trp-631, and Ile-635) from the pocket-binding domain (PBD) of the CHR helix (5–7). It is proven that the interaction between the PBD and the pocket plays an essential role in 6-HB stabilization and viral fusion, thus serving as an attractive target for development of HIV-1 entry inhibitors that block virus-cell fusion (8, 9).

T20 (Enfuvirtide, Fuzeon), derived from the wild-type CHR sequence of HIV-1 HXB2 strain, is the first and only clinically approved fusion inhibitor that is being used as a salvage therapy of HIV/AIDS patients (10–12). The mechanism of T20 inhibition involves competitive binding to the exposed NHR that pre-
vents the viral gp41 from folding into the hairpin structure, although its multifaceted modes of action have been suggested (13, 14). Unsatisfactorily, T20 requires frequent high dose administration and has a low genetic barrier for drug resistance (15–17). Indeed, the emergence and spread of T20-resistant HIV-1 variants resulted in an increased number of patients failing to respond to T20 treatment. To overcome these challenges, a number of modified second- and third-generation peptide inhibitors have been developed with improved stability and potency (18–21). Notably, these peptides were primarily derived from the gp41 CHR region, not including the PBD upstream sequence. In particular, the CHR-based peptide C34 (amino acids 628–661) has been widely used as a design template. However, we recently found that the 621QIWNNMT627 motif upstream of PBD plays important roles for the 6-HB formation and viral fusion (22). The peptides containing this motif, CP621–652 and its derivative CP32M, showed potent activity against the wild-type and T20-resistant HIV-1 strains (22–24). Very recently (25), we determined the high resolution crystal structure of CP621-652 complexed by NHR-derived peptide T21. It was found that the N-terminal 621QIWNMNT627 motif of CP621–652 is highly flexible, but the residues Met-626 and Thr-627 adopt a unique hook-like structure (termed M-T hook), which may stabilize the 6-HB conformation thus conferring the antiviral activity of the inhibitor. In this study, we focused on addressing the structure and function of the M-T hook by several approaches. The presented data demonstrate that the M-T hook is a conserved structural feature of peptide inhibitors, and it plays a critical role in binding stability and anti-HIV activity. Promisingly, the M-T hook confers the inhibitor to overcome drug resistance. Therefore, we conclude that the M-T hook structure is a critical determinant for designing HIV-1 fusion inhibitors, and its discovery provides important information for understanding the mechanism of gp41-dependent fusion and inhibition.

EXPERIMENTAL PROCEDURES

Peptide Synthesis—A panel of peptides (Fig. 1), including CHR-derived peptides CP621–652 and its mutants (Q621A, I622A, W623A, N624A, N625A, M626A, and T627A), C34, MT-C34, AA-C34, TM-C34, T20, and NHR-derived peptides N36 and N41 were synthesized by a standard solid-phase Fmoc (N-(9-fluorenyl)methoxycarbonyl) method as described previously (25). All peptides were acetylated at the N terminus and amidated at the C terminus. They were purified by reversed-phase HPLC and verified for purity and correct amino acid composition by mass spectrometry. Concentrations of the peptides were determined by UV absorbance and theoretically calculated molar extinction coefficient ε(280 nm) of 5500 M⁻¹cm⁻¹ and 1490 M⁻¹cm⁻¹ based on the number of tryptophan and tyrosine residues (all the peptides tested contain Trp and/or Tyr), respectively.

Cell-Cell Fusion Assays—To detect the inhibitory activity of CP621-652 and its mutants, cell fusion was monitored using a reporter gene assay based on activation of HIV LTR-driven luciferase cassette in TZM-bl (Target) cells by HIV-1 tat from
HL2/3 (Effector) cells (26). Briefly, the TZM-bl cells were plated in 96-well clusters (1 × 10⁴ per well) and incubated at 37 °C overnight. The target cells were cocultured with HL2/3 cells (3 × 10⁴/well) for 6 h at 37 °C in the presence or absence of a tested peptide at graded concentrations. Luciferase activity was measured using luciferase assay reagents and a Luminescence Counter (Promega, Madison, WI) according to the manufacturer’s instructions. Background luminescence in TZM-bl cells was determined without addition of HL2/3 cells. The percent inhibition of fusion by the peptides and 50% inhibitory concentration (IC₅₀) values of fusion were calculated using the GraphPad Prism software (GraphPad Software Inc., San Diego).

To compare the inhibitory activity of C34 and MT-C34, 293T effector cells seeded in 6-well plates at 4 × 10⁵ cells per well were transfected with the plasmid encoding HIV-1NL₄-3 Env. The day after transfection, the effector cells were cocultured with MT-4 cells at a ratio of 1:3 in 96-well plates in the presence or absence of tested peptides. After coculturing for an additional 48 h at 37 °C, the syncytia of each well were counted by eye. The percent inhibition and the IC₅₀ values were calculated as described above.

**HIV-1 Single-cycle Infection**—HIV-1 pseudoviruses were generated as described previously (27, 28). Briefly, 293T cells (5 × 10⁶ cells in 15 ml of growth medium in a T-75 culture flask) were cotransfected with 10 μg of an Env-expressing plasmid and 20 μg of a backbone plasmid pSG3xneo that encodes Env-defective, luciferase-expressing HIV-1 genome using Lipofectamine 2000 reagent (Invitrogen). Pseudovirus-containing culture supernatants were harvested 48 h after transfection and filtered by a 0.45-μm pore size and stored at −80 °C in 1-ml aliquots until use. The 50% tissue culture infectious dose (TCID₅₀) of a single thawed aliquot of each pseudovirus batch was determined in TZM-bl cells. The antiviral activity of CHR peptides (C34, MT-C34, AA-C34, TM-C34, T20, CP621-652, and its mutants) was determined using TZM-bl cells. Briefly, the peptides were prepared with 10 series dilutions in a 3-fold stepwise manner and mixed with 100 TCID₅₀ viruses and incubated for 1 h at room temperature. The mixture was added to TZM-bl cells (10⁴/well) and incubated at 37 °C for 48 h, and the luciferase activity was measured as described above.

**Inhibition of HIV-1NL₄-3 Replication**—The wild-type HIV-1NL₄-3 was prepared by transfection of molecular cloned pNL4-3 plasmid into 293T cells. The virus stock was harvested 48 h post-transfection and quantified for TCID₅₀. Inhibition of the peptides (MT-C34 and C34) on HIV-1NL₄-3 was performed as described for pseudovirus. In brief, 100 TCID₅₀ viruses were used to infect TZM-bl cells in the presence or absence of serially diluted peptides. Two days post-infection, the cells were harvested and lysed in reporter lysis buffer, and the luciferase activity was measured.

**Inhibition of 6-HB Formation by Peptides**—A mouse monoclonal antibody (mAb) specific for the gp41 6-HB (NC-1) was obtained from Dr. Shibo Jiang through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, National Institutes of Health. The inhibitory activity of CHR peptides on the 6-HB formation was measured by a modified ELISA-based method as described previously (22, 28). Briefly, a 96-well polystyrene plate (Costar, Corning Inc., Corning, NY) was coated with NC-1 (2 μg/ml in 0.1 M Tris, pH 8.8). A tested peptide at graded concentrations was mixed with C34-biotin (0.1 μM) and incubated with N36 (0.1 μM) at room temperature for 30 min. The mixture was then added to the NC-1-coated plate, followed by incubation at room temperature for 30 min and washing three times with a washing buffer (PBS containing 0.1% Tween 20). The streptavidin-labeled horseradish peroxidase (HRP) (Invitrogen) and the substrate 3,3,5,5-tetramethylbenzidine (Sigma) were added sequentially. Absorbance at 450 nm (A₄₅₀) was measured using an ELISA reader (Bio-Rad).

To detect the reactivity of 6-HBs with three conformation-dependent mAbs (NC-1, 2G8, 17C8) (29, 30), the isolated or mixed peptides were coated to the wells of the ELISA plate at 2 μg/ml and blocked by 3% bovine serum albumin (BSA). The anti-6-HB mAb (5 μg/ml) was added to the wells and incubated at 37 °C for 1 h. After three washes, the bound antibodies were detected by HRP-conjugated anti-mouse IgG (Sigma). The reaction was visualized by addition of 3,3,5,5-tetramethylbenzidine, and A₄₅₀ was measured.

**Circular Dichroism (CD) Spectroscopy**—CD spectroscopy was performed as described previously (22). Briefly, a CHR peptide was incubated with an equal molar concentration of NHR peptide N36 at 37 °C for 30 min. The final concentration of each peptide was 10 μM in PBS buffer, pH 7.2. The CD spectra were acquired on Jasco spectropolarimeter (model J-815) using a 1 nm bandwidth with a 1-nm step resolution from 195 to 260 nm at room temperature. The spectra were corrected by subtraction of a blank corresponding to the solvent. Data were averaged over three accumulations. The α-helical content was calculated from the CD signal by dividing the mean residue ellipticity [θ] at 222 nm by the value expected for 100% helix formation (−33,000 degrees-cm²-dmol⁻¹). The thermal denaturation experiment was performed by monitoring the change in ellipticity [θ] at 222 nm at the increasing temperature (20–98 °C) using a temperature controller. The temperature was increased at a rate of 1.2 °C per min; data were acquired at a 1-nm bandwidth at 222 nm at a frequency of 0.25 Hz. The melting curve was smoothed, and the midpoint of the thermal unfolding transition (T_m) values were taken as the maximum of the derivative d[θ]_{222}/dT. The T_m value was detected at a peptide concentration of 10 μM in PBS buffer.

**Isothermal Titration Calorimetry (ITC)**—ITC assay was performed using a ITC₂₀₀₀ microcalorimeter instrument (MicroCal) as described previously (27). In brief, 1 mM N36 dissolved in double distilled H₂O was injected into the chamber containing 100 μM C34 or MT-C34. The experiments were carried out at 25 °C. The time between injections was 240 s, and the stirring speed was 500 rpm. The heats of dilution were determined in double distilled H₂O at a frequency of 0.25 Hz. The melting curve was smoothed, and the midpoint of the thermal unfolding transition (T_m) values were taken as the maximum of the derivative of the enthalpy ratio. The T_m value was detected at a peptide concentration of 10 μM in PBS buffer.

**Assembly, Crystallization, and Structure Determination**—The 6-HB containing MT-C34 and N41 was prepared by dissolving an equal amount (1:1 molar ratio) of the peptides in the denaturing buffer (100 mM NaH₂PO₄, 10 mM Tris-HCl, pH
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RESULTS

M-T Hook Structure Is a Major Determinant of CP621-652 Activity—By using the peptide CP621-652 as a model (25), we recently found that the residues Met-626 and Thr-627 adjacent to the pocket-binding sequence adopt a hook-like structure that may play critical roles for the stability and anti-HIV activity of the inhibitor. However, our studies could not rule out the possible roles of other residues within the N-terminal 621 QIWNMT627 motif. Here, we sought to determine their contributions to the peptide activity. A panel of peptides that carry single substitutions (Q621A, I622A, W623A, N624A, or N625A) was synthesized, and their antiviral activity was assessed. As shown in Table 2, all the newly designed mutations had little or marginal effects on the anti-HIV activity of CP621-652, including its inhibition on HIV-1 Env-mediated cell-cell fusion and single-cycle infection, as well as its blockage on the 6-HB formation. In sharp contrast, the peptides with M626A or T627A mutations showed dramatically decreased potency. Both M626A and T627A peptides had no inhibitory activity on the 6-HB formation at a concentration as high as 200 μM. These results demonstrated that the M-T hook residues critically determine the antiviral activity of CP621-652.

We used CD spectroscopy to examine the effect of all the mutations on the α-helicity and thermostability of the 6-HB structures. As shown in Fig. 2A, the CD spectra of an equimolar mixture of NHR-derived N36 and CP621-652 or its mutants display typical double minima at 208 and 222 nm, which indicate the formation of secondary α-helical conformation. The thermostability of each 6-HBs, defined as the midpoint of the thermal unfolding transition (Tm) values, was further measured (Fig. 2B). Compared with CP621-652 (Tm = 66.1 °C), the 6-HBs

TABLE 1
Data collection and refinement statistics

| MT-C34/N41 |            |
|------------|------------|
| Data collection |            |
| Space group | H32        |
| Cell dimensions |           |
| a, b, c | 45.26, 45.26, 209.90 Å |
| α, β, γ | 90.00, 90.00, 120.00° |
| X-ray source | RIGAKU MICROMAX-007 HF |
| Wavelength | 1.54 Å |
| Data range | 31.4 to 1.90 Å |
| Reflections unique | 6812 |
| Rfree | 0.053 (0.261) |
| R-work | 18.4 (3.2) |
| Completeness (last shell) | 98.4% (87.4%) |
| Redundancy (last shell) | 5.64 (2.52) |
| Validation |            |
| MolProbity score | 1.37, rating 98th percentile among structures of comparable resolution |
| % Favored regions and Outliers in Ramachandran plot | 100.0, 0.0, 0.0 |

α Rsyn indicates \( \sum (F_{\text{obs}}(hkl) - F_{\text{calc}}(hkl))^2 / \sum F_{\text{calc}}(hkl)^2 \) where \( F_{\text{calc}} \) is the average of symmetry-related observations of a unique reflection.

β Rwork indicates \( \sum |F_{\text{obs}}(hkl)| - |F_{\text{calc}}(hkl)| / \sum |F_{\text{obs}}(hkl)| \).

γ Rfree indicates the cross-validation \( R \) factor for 5% of reflections against which the model was not refined.

TABLE 2
Inhibitory activity of CP621-652 and its mutants on HIV-1 infection

The assays were performed in triplicate and repeated at least three times. The data are expressed as mean ± S.D.

| Peptide   | IC50 (nM) | n-Fold | IC50 (nM) | n-Fold | IC50 (μM) | n-Fold |
|-----------|-----------|--------|-----------|--------|-----------|--------|
| CP621-652 | 12.0 ± 3.6 | 1      | 6.9 ± 1.9 | 1      | 7.5 ± 2.0 | 1      |
| Q621A     | 14.3 ± 0.3 | 1.2    | 6.3 ± 0.4 | 0.9    | 4.6 ± 1.6 | 0.6    |
| I622A     | 14.3 ± 0.9 | 1.2    | 8.0 ± 0.8 | 0.9    | 4.7 ± 0.4 | 0.6    |
| W623A     | 11.1 ± 0.9 | 0.9    | 9.1 ± 0.5 | 1.3    | 6.1 ± 0.2 | 0.8    |
| N624A     | 13.4 ± 0.9 | 1.1    | 8.0 ± 0.3 | 1.2    | 3.0 ± 0.2 | 0.4    |
| N625A     | 15.9 ± 1.0 | 1.3    | 11.7 ± 0.7 | 1.7 | 11.0 ± 2.6 | 1.5    |
| M626A     | 52.0 ± 10.3 | 4.3    | 59.3 ± 1.1 | 8.6    | >200 | >26.7 |
| T627A     | 67.7 ± 8.7 | 5.6    | 163.9 ± 11.4 | 23.8 | >200 | >26.7 |

* Data were compared with the IC50 values of CP621-652.
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formed by the peptides with W623A, M626A, and T627A mutations had significantly decreased $T_m$ values (57.0, 53.1, and 54.1 °C, respectively), suggesting that these three residues are associated with the binding affinity of CP621-652 with N36. Notably, the mutations Q621A, I622A, N624A, and N625A did not significantly affect the thermostability of 6-HBs.

Crystal Structure of MT-C34 Complex Reveals the M-T Hook Conformation—The above mutational analysis of the $^{621}$QIWNNMT$^{627}$ motif with CP621-652 as a template highlights the importance of the M-T hook residues for the inhibitors. To explore whether the novel structure feature of the M-T hook could be preserved in different inhibitors and whether it possesses a similar function, we generated the peptide MT-C34 by adding two hook residues to the N terminus of CHR-derived peptide C34. First, it is highly intriguing to know whether Met-626 and Thr-627 at the N terminus of PBD adopt a hook-like structure similar to the MT-hook observed in CP621-652. Briefly, Thr-627 terminates the $\alpha$-helical conformation of MT-C34 by rotating its dihedral angle $\psi$ by nearly 180°, so that the N terminus of MT-C34 turns away from the central coiled-coil trimer (Fig. 3A). The conformation of the Thr-627 is stabilized by the hydrogen bond between its side chain hydroxyl group and the backbone NH group of the downstream residue Glu-630 at $i + 3$ position (distance, 2.38 Å; angle, 167.1°). The upstream Met-626 is positioned at the top of the left side of the hydrophobic pocket on the NHR trimer, so that the hydrophobic side chain of Met-626 accommodates the hydrophobic groove between NHR and CHR helices, capping the hydrophobic pocket below. Comparing the M-T hook structures observed in CP621-652 and MT-C34, the conformation of Thr-627 and the hydrogen bonding interaction with the downstream Glu-630 are nearly identical (Fig. 4). Although residue Met-626 does not involve specific hydrogen bonding interaction, its side chain adopts a similar conformation in both peptides, suggesting that the hydrophobicity and the geometry of the methionine side chain are important for its function. Therefore, the M-T hook structure adopted by residues Met-626 and Thr-627 at the N terminus of PBD is highly preserved regardless of different peptide sequence.

M-T Hook Dramatically Stabilizes the Inhibitor-Target Interaction—We then used two biophysical approaches to characterize the function of the M-T hook in MT-C34. The CD spectroscopy was first applied to determine the $\alpha$-helicity and thermostability of the 6-HB structure formed by MT-C34 and N36 in a direct comparison with the 6-HB of C34 and N36. As shown in Fig. 5A, the CD spectra of an equimolar mixture of N36 and CP621-652 or its mutants are shown. Final concentration of each peptide in PBS is 10 μM.

The energy released by refolding of the helical NHR-CHR bundle is used to overcome the kinetic barrier and drives the gp41-dependent fusion reaction. We then applied the ITC technique to directly measure the heat released or absorbed during the interaction between the inhibitors and N36, which allows an accurate determination of binding constant ($K_i$), reaction stoichiometry ($N$), enthalpy ($\Delta H$), and entropy ($\Delta S$), thus providing a complete thermodynamic profile of the molecular interaction. As shown in Fig. 6, the formation of 6-HB between N36 and C34 or N36 and MT-C34 is a predominantly enthalpy-driven reaction, in which a large amount of heat is released. Compared with C34, the $K_i$ value of MT-C34 increased 5.4-fold (from $3.7 \times 10^5$ to $2.0 \times 10^6$ M$^{-1}$), suggesting a dramatically enhanced binding affinity. Taken together, the M-T hook structure does fortify the interaction of the inhibitors with the NHR target.
M-T Hook Structure Is Associated with the Conformation of a 6-HB Core—We were interested to know whether the M-T hook affects the conformation of a 6-HB core structure. Thus, three conformation-specific mAbs (NC-1, 2G8, and 17C8) were used to detect the 6-HBs in ELISA. As shown in Fig. 7A, these antibodies did not react with the isolated N36 or C34 but strongly reacted with their complex. In comparison, the reactivity of the N36/H18528MT-C34 complex with NC-1 obviously decreased, suggesting a changed configuration of the 6-HB that may sterically hamper the exposure of the antibody epitope. Interestingly, the conformational epitope recognized by 2G8 might be exposed more efficiently in the N36/H18528MT-C34 complex as evidenced by a significantly increased reactivity (Fig. 7B). However, the epitope for 17C8 was not affected. These data suggest that the conformation of the N36/M-T-C34 bundle may differ from that of N36/C34 bundle, implying that addition of the M-T hook residues can directly or indirectly interfere in the conformation of the 6-HB core structure.

M-T Hook Significantly Improves the Antiviral Activity of the Inhibitors—Based on the above findings, we were eager to know whether the M-T hook can enhance the antiviral activity of fusion inhibitors. We used three independent experiments to compare the anti-HIV activity of MT-C34 and C34 (Fig. 8). In the HIV-1 Env-mediated cell fusion assay, MT-C34 and C34 had their IC_{50} values at $1.6 \pm 0.6$ and $10.5 \pm 4.0$ nM, respectively, which indicates a 6.6-fold increase in potency for...
MT-C34 as compared with the parental C34. In the single-cycle entry assay, MT-C34 and C34 inhibited HIV-1NL4-3 pseudovirus with an IC_{50} of 0.6 ± 0.1 and 2.7 ± 0.1 nM, respectively, indicating a 4.5-fold increase for MT-C34. For the wild-type HIV-1NL4-3 replication, MT-C34 had an IC_{50} of 0.2 ± 0.1 nM, whereas C34 had an IC_{50} at 1.2 ± 0.3 nM, indicating a 6-fold increase for MT-C34. Therefore, we were surprised that addition of two hook residues resulted in a sharp improvement for the anti-HIV activity of inhibitor C34, which is widely used as a design template.

The anti-HIV mechanism of HIV-1 fusion inhibitors is through binding to the exposed NHR or CHR of gp41 during its pre-hairpin conformation thus blocking the formation of 6-HB core in a dominant-negative manner. We previously established an ELISA-based method to determine whether a peptide or small molecule-based fusion inhibitor can physically block the 6-HB formation, in which the 6-HB-specific mAb NC-1 was coated to the plate as a capture and the biotinylated-C34 was used for signal detection (22, 28). By applying this approach, we showed that MT-C34 could efficiently block the 6-HB formation with an IC_{50} of 0.2 ± 0.0 μM, whereas C34 had an IC_{50} value at 2.1 ± 0.2 μM (Fig. 8D). Therefore, the MT hook structure may confer the capacity of the peptide inhibitor to compete off the viral CHR thereby improving its anti-HIV activity.

To verify the specificity of the M-T hook conferring the stability and antiviral function, we synthesized two control peptides, AA-C34 and TM-C34 (Fig. 1). In comparison, addition of double alanines or the M-T hook residues in a reverse order to the N terminus of C34 had no obvious roles in stabilizing the 6-HB and enhancing the anti-HIV activity (Table 3). Therefore, the peptide size and/or nonspecific interaction cannot explain the antiviral function of the M-T hook structure.

**Potent Activity of MT-C34 against T20- and C34-resistant HIV-1 Variants**—Does the M-T hook-modified peptide MT-C34 possess an improved activity against T20-resistant HIV-1 strains? We therefore constructed a panel of HIV-1 pseudoviruses that carry single or double T20-resistant mutations. As shown in Table 4, these HIV-1 variants also conferred high cross-resistance to C34. Excitingly, MT-C34 had dramatically enhanced potency against these resistant viruses. For example, although C34 inhibited the V38A variant with an IC_{50} of 26.5 nM, MT-C34 had an IC_{50} at 1.2 nM, indicating a 22-fold increased potency for MT-C34. Particularly, although C34 showed dramatically reduced activity to inhibit HIV-1 strains with double mutations (I37T/N43K or V38A/N42T), MT-C34 remained highly active. These results strongly suggested that the M-T hook can significantly improve the inhibitors to overcome the problem of drug resistance.

**DISCUSSION**

In our previous study (25), we found the M-T hook structure adopted by two N-terminal residues (Met-626 and Thr-627) of the fusion inhibitor CP621-652 in the 6-HB core. Here, we have finely characterized its structure and function by several approaches. Based on the CP621-652 as a template, our mutational analysis demonstrated that the M-T hook is a key determinant for the anti-HIV activity of CP621-652. The crystal structure of MT-C34-based 6-HB reveals that the conformation of the M-T hook is well preserved at the N terminus of the inhibitor C34. Based on MT-C34 as a model, we convincingly showed that the simple incorporation of the M-T hook to the inhibitor can dramatically increase the binding affinity and the antiviral potency and contribute to overcome the drug resistance.

In the last 2 decades, the fusion protein gp41 of HIV-1 has been extensively explored. However, the structure for an intact gp41 molecule or its full-length ectodomain is still lacking (5–7, 31). Alternatively, three pioneering crystal structures of gp41
were determined by using synthesized or biosynthetic peptide fragments limited to the putative NHR and CHR regions, whereas the unstructured loop region and the hydrophobic fusion peptide were eliminated (5–7). Recently, the most complete structure of HIV-1 gp41 was reported with the sequences extended to the fusion peptide proximal region and the membrane proximal external region but lacking the loop region and the fusion peptide (31). However, our recent studies demonstrated that the upstream sequence of PBD in the CHR region of gp41, which is located closely to the loop region, plays critical roles for gp41-dependent fusion and inhibition (22). In particular, the peptide CP621-652 containing the 621QIWNNMT627 motif showed significantly increased thermostability in the presence of the NHR-derived peptide T21. The crystal structure of CP621-652 complexed by T21 surprisingly identified that the N-terminal residues Met-626 and Thr-627 of the inhibitor adopt a hook-like conformation, in which Thr-627 redirects the peptide chain to position Met-626 above the left side of hydrophobic pocket on the NHR trimer, and the side chain of Met-626 caps the hydrophobic pocket thereby stabilizing the interaction between the pocket and the PBD. Given that these two residues are connected to the first pocket-inserting residue Trp-628 and both are highly conserved among all HIV-1 strains, it is conceivable that they play important roles for the function of gp41. Indeed, our mutagenesis studies verified their essential roles for HIV-1 Env-mediated cell fusion and virus entry (25). Prominently, single mutations of the M-T hook residues could completely disrupt the infectivity of virus. However, our previous studies could not fully rule out the possibility that the M-T hook structure and its interaction with the NHR pocket could be the result of a thermodynamic stabilization due to the crystal formation. The flexibility of the upstream 621QIWNN627 sequence could indeed favor such a stabilizing interaction, especially in the context of peptides. In this study, our mutational analysis provided insight that the M-T hook is a major determinant of CP621-652 in terms of its thermostability and anti-HIV activity.

**FIGURE 6. Biophysical characterization of MT-C34 and C34 by ITC assay.** 1 mM N36 dissolved in double distilled H2O was injected into the chamber containing 100 μM C34 (A) or MT-C34 (B). The experiments were carried out at 25 °C. Data acquisition and analysis were performed using MicroCal Origin software (version 7.0).

**FIGURE 7. Reactivity of the 6-HBs with conformation-dependent mAbs.** The reactivity of anti-HB mAbs NC-1 (A) and 2G8 and 17C8 (B) with the isolated or mixed peptides was tested by ELISA. The final concentration of a tested mAb was 5 μg/ml.

were determined by using synthesized or biosynthetic peptide fragments limited to the putative NHR and CHR regions, whereas the unstructured loop region and the hydrophobic fusion peptide were eliminated (5–7). Recently, the most complete structure of HIV-1 gp41 was reported with the sequences extended to the fusion peptide proximal region and the membrane proximal external region but lacking the loop region and the fusion peptide (31). However, our recent studies demonstrated that the upstream sequence of PBD in the CHR region of gp41, which is located closely to the loop region, plays critical roles for gp41-dependent fusion and inhibition (22). In particular, the peptide CP621-652 containing the 621QIWNNMT627 motif showed significantly increased thermostability in the presence of the NHR-derived peptide T21. The crystal structure of CP621-652 complexed by T21 surprisingly identified that the N-terminal residues Met-626 and Thr-627 of the inhibitor adopt a hook-like conformation, in which Thr-627 redirects the peptide chain to position Met-626 above the left side of hydrophobic pocket on the NHR trimer, and the side chain of Met-626 caps the hydrophobic pocket thereby stabilizing the interaction between the pocket and the PBD. Given that these two residues are connected to the first pocket-inserting residue Trp-628 and both are highly conserved among all HIV-1 strains, it is conceivable that they play important roles for the function of gp41. Indeed, our mutagenesis studies verified their essential roles for HIV-1 Env-mediated cell fusion and virus entry (25). Prominently, single mutations of the M-T hook residues could completely disrupt the infectivity of virus. However, our previous studies could not fully rule out the possibility that the M-T hook structure and its interaction with the NHR pocket could be the result of a thermodynamic stabilization due to the crystal formation. The flexibility of the upstream 621QIWNN627 sequence could indeed favor such a stabilizing interaction, especially in the context of peptides. In this study, our mutational analysis provided insight that the M-T hook is a major determinant of CP621-652 in terms of its thermostability and anti-HIV activity. In other words, the upstream
621QIWNN625 residues have little or a minor effect on the peptide except for Trp-623, which can affect the thermostability of 6-HB core as measured by CD spectroscopy. Nonetheless, the alanine substitution of Trp-623 does not affect the antiviral activity of inhibitor. Given that the conformation of Trp-623 could not be defined due to its flexibility in the CP621-652-based 6-HB (25), it is hard to explain the discordance. But one can speculate that the Trp-623-mediated nonspecific hydrophobic interaction may cause this phenomenon.

To delineate the structure-activity relationship of the MT-hook residues, we used the CHR-derived peptide C34 as a model. This peptide was applied to solve the first structure of gp41 thus representing the core CHR sequence (5). More importantly, it is widely used as a template for inhibitor design (18, 19, 21, 32–34). Our crystal structure of MT-C34 in the 6-HB reveals that the incorporated methionine and threonine at the N terminus of the peptide do present as a hook-like conformation like that found in CP621-652. The significance of this observation is multifaceted as follows: 1) it has ruled out the possible role of other residues in the 621QIWNNMT627 motif for the formation of the M-T hook structure; 2) it has swept out our concern over the difficulty of two residues to adopt the hook structure while positioning at the N-terminal extreme of the highly helical peptide C34; and 3) importantly, the M-T hook may be a favored structural feature adopted by the residues Met-626 and Thr-627 of CHR-based peptides thus serving as a universal strategy for designing HIV-1 fusion inhibitors.

We recently discovered that the residues Met-626 and Thr-627 of HIV-1 fusion inhibitor CP32M do not adopt the M-T hook structure as its parental peptide CP621-652 or MT-C34, instead, the “VEWNEMT” motif folds into the helical conformation (24). However, when the helical conformation of the VEWNEMT motif is disrupted by crystal packing interactions, Met-626 and Thr-627 of CP32M can also form an M-T hook conformation. Therefore, this region is highly mobile and could assume the M-T hook formation if the preferred /H9251 -helical conformation is disrupted.

During the gp41-mediated fusion process, the helical interaction between the NHR and CHR is essential for HIV-1-mediated cell fusion and infection (35–38). Importantly, each of the grooves on the surface of the trimeric NHR coiled coils has a particularly deep cavity that accommodates three hydrophobic residues from each CHR helix (Ile-635, Trp-631, and Trp-628), which exerts a pivotal role for the stability of the 6-HB core. However, it is astonishing that the high affinity interaction of NHR-CHR peptides can be further dramatically fortified by the simple addition of two hook residues. The CD spectroscopy data indicate that the M-T hook can increase the melting temperature (Tm) of 6-HB by 10 °C, while the ITC data indicate a greater than 5-fold enhancement in binding affinity, which

\[ \text{TABLE 3} \]

| Peptide | Tm°C | IC50 HIV-1 entry | IC50 6-HB formation |
|---------|------|----------------|-------------------|
| C34     | 65.2 | 1.5 ± 0.1 M    | 2.3 ± 0.1 μM      |
| MT-C34  | 75   | 0.4 ± 0.0 M    | 0.4 ± 0.0 M       |
| AA-C34  | 65.3 | 1.3 ± 0.1 M    | 3.4 ± 0.3 M       |
| TM-C34  | 65.2 | 1.4 ± 0.2 M    | 3.8 ± 0.2 M       |
M-T Hook for HIV-1 Fusion Inhibitors

TABLE 4
Inhibitory activity of MT-C34 against T20- and C34-resistant HIV-1 variants

| HIV-1NL4–3 | IC50 (nM) | n-Fold | WT | 2.2 ± 0.1 | 1 | 0.5 ± 0.1 | 1 |
|------------|-----------|--------|----|------------|---|-----------|---|
| I37T       | 51.1 ± 12.4 | 10.6 | I37T | 30.8 ± 4.8 | 14 | 2.7 ± 0.5 | 4.5 |
| V38A       | 17.46 ± 80.4 | 34.2 | V38A | 26.5 ± 7.7 | 12.1 | 1.2 ± 0.1 | 2 |
| V38M       | 37.02 ± 61.2 | 7.2 | V38M | 19.3 ± 3.2 | 9.1 | 1.9 ± 0.3 | 3.2 |
| Q40H       | 124.0 ± 120.4 | 24.3 | Q40H | 68.0 ± 7.2 | 30.9 | 3.4 ± 0.5 | 5.7 |
| N43K       | 316.9 ± 51.3 | 6.2 | N43K | 28.8 ± 6.7 | 13.1 | 2.0 ± 0.5 | 3.3 |
| I37T/N43K  | >2250 | >44 | I37T/N43K | 1087.0 ± 491.0 | 494.1 | 12.7 ± 0.8 | 21.2 |
| V38A/N42T  | >2250 | >44 | V38A/N42T | 339.7 ± 15.4 | 154.4 | 13.8 ± 0.9 | 23 |

* Data were compared with the IC50 values of WT HIV-1NL4–3.

could translate into an extremely stable helical interaction. Therefore, we speculate that the M-T hook can tightly "hook" the hydrophobic NHR pocket thus critically conferring the binding force of the inhibitor to the target. The addition of the M-T hook to C34 might also cause a conformational change of the 6-HB as detected by three conformation-dependent mAbs (NC-1, 2G8, and 17C8). All three mAbs were originally generated by the 6-HB containing N36/34 as immunogens, showing the reactivity with the N36-C34 complex but not the isolated peptides (29, 30). Interestingly, the significantly reduced reactivity of NC-1 with the N36-MT-C34 complex implies a possible role of the M-T hook during the formation of the gp41 pre-hairpin intermediate, as the recent studies indicate that the target motif of NC-1 must be the outer surface-exposed region of the coiled-coil N-helix trimer (39, 40). Therefore, the structure and function relationship of the M-T hook in the context of the pre-hairpin and 6-HB core conformations during the gp41-mediated fusion definitely need further characterizations.

Discovery of T20 did open a bright avenue not only for exploring the mechanism of virus-cell fusion but also for developing the antiviral drugs. Based on the structural information of gp41, a number of strategies have been applied to design novel HIV-1 fusion inhibitors with improved anti-HIV activity and pharmaceutical profiles (18–21), in which the peptide C34 was largely used as a design template. The rationale is that C34 can interact with the NHR pocket through its N-terminal PBD thus having high binding stability and anti-HIV potency. Typically, the peptides are engineered to stabilize the helical conformation by introducing the salt bridges or alanine residues, which are usually mapped to the 36–45-amino acid region of the peptide-binding site in the NHR domain of the viral gp41, with the 36GIV38 motif being a hot spot for resistance (14–17). During this study, it was another surprise to find that addition of two hook residues can significantly improve the peptide to overcome the drug resistance. In most of the cases, MT-C34 was able to efficiently inhibit T20-resistant HIV-1 variants that are frequently emerged in T20-treated patients (Table 4). More promisingly, MT-C34 had potent activity against HIV-1 strains having cross-resistance to C34. Therefore, the M-T hook structure should be considered in the future design of HIV-1 fusion inhibitors that may help to overcome the problem of drug resistance.

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REFERENCES
1. Eckert, D. M., and Kim, P. S. (2001) Mechanisms of viral membrane fusion and its inhibition. Annu. Rev. Biochem. 70, 777–810
2. Colman, P. M., and Lawrence, M. C. (2003) The structural biology of type 1 viral membrane fusion. Nat. Rev. Mol. Cell Biol. 4, 309–319
3. Zhu, P., Liu, J., Bess, J., Jr., Chertova, E., Lifson, J. D., Grisé, H., Ofek, G. A., Taylor, K. A., and Roux, K. H. (2006) Distribution and three-dimensional structure of AIDS virus envelope spikes. Nature 441, 847–852
4. Harrison, S. C. (2008) Viral membrane fusion. Nat. Struct. Mol. Biol. 15, 690–698
5. Chan, D. C., Fass, D., Berger, J. M., and Kim, P. S. (1997) Core structure of gp41 from the HIV envelope glycoprotein. Cell 89, 263–273
6. Tan, K., Liu, J., Wang, J., Shen, S., and Lu, M. (1997) Atomic structure of a thermostable subdomain of HIV-1 gp41. Proc. Natl. Acad. Sci. U.S.A. 94, 12303–12308
7. Weissenhorn, W., Dessen, A., Harrison, S. C., Skehel, J. J., and Wiley, D. C. (1997) Atomic structure of the ectodomain from HIV-1 gp41. Nature 387, 426–430
8. Chan, D. C., Chutkowski, C. T., and Kim, P. S. (1998) Evidence that a prominent cavity in the coiled coil of HIV type 1 gp41 is an attractive drug target. Proc. Natl. Acad. Sci. U.S.A. 95, 15613–15617
9. Chan, D. C., and Kim, P. S. (1998) HIV entry and its inhibition. Cell 93, 681–684
10. Kilby, J. M., Hopkins, S., Venetta, T. M., DiMassimo, B., Cloud, G. A., Lee, J. Y., Aldredge, L., Hunter, E., Lambert, D., Bolognesi, D., Matthews, T., Johnson, M. R., Nowak, M. A., Shaw, G. M., and Saag, M. S. (1998) Potential...
suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. *Nat. Med.* 4, 1302–1307
11. Lalezari, J. P., Henry, K., O’Hearn, M., Montaner, J. S., Piliero, P. J., Trottier, B., Walmsley, S., Cohen, C., Kuritzkes, D. R., Eron, J. J., Jr, Chung, J., DeMasi, R., Donatucci, L., Drobnes, C., Delestanty, J., and Salgo, M. (2003) Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. *N. Engl. J. Med.* 348, 2175–2185
12. Lazzarin, A., Clotet, B., Cooper, D., Reynolds, J., Arasteh, K., Nelson, M., Katlama, C., Stellbrink, H. J., Delfraissy, J. F., Lange, J., Huson, L., DeMasi, R., Wat, C., Delestanty, J., Drobnes, C., Salgo, M., and TORO 2 Study Group (2003) Efficacy of enfuvirtide in patients infected with drug-resistant HIV-1 in Europe and Australia. *N. Engl. J. Med.* 348, 2186–2195
13. Wild, C. T., Shugars, D. C., Greenwell, T. K., McDanal, C. B., and Matthews, T. J. (1999) Peptides corresponding to a predictive α-helical domain of human immunodeficiency virus type 1 gp41 are potent inhibitors of virus infection. *Proc. Natl. Acad. Sci. U.S.A.* 96, 9770–9774
14. Ashkenazi, A., Wexler-Cohen, Y., and Shai, Y. (2011) Multifaceted action of Fuzon as virus-cell membrane fusion inhibitor. *Biochim. Biophys. Acta* 1808, 2352–2358
15. Rimsky, L. T., Shugars, D. C., and Matthews, T. J. (1998) Determinants of virus infection. *Antimicrob. Agents Chemother.* 42, 1896–1905
16. Greenberg, M. L., and Cammac, N. (2004) Resistance to enfuvirtide, the first HIV fusion inhibitor. *J. Antimicrob. Chemother.* 53, 333–340
17. He, Y. (2012) Broad antiviral activity and crystal structure of HIV-1 fusion inhibitor. *J. Biol. Chem.* 287, 11126–11134
18. Weng, Y., and Weiss, C. D. (1998) Mutational analysis of residues in the coiled-coil domain of human immunodeficiency virus type 1 transmembrane protein gp41. *J. Virol.* 72, 9676–9682
19. Shu, W., Liu, J., Ji, H., Radigen, L., Jiang, S., and Lu, M. (2000) Helical interactions in the HIV-1 gp41 core reveal structural basis for the inhibitory activity of gp41 peptides. *Biochemistry* 39, 1634–1642
20. Weng, Y., Wang, Z., and Weiss, C. D. (2000) Structure-function studies of the self-assembly domain of the human immunodeficiency virus type 1 transmembrane protein gp41. *J. Virol.* 74, 5368–5372
21. Lu, M., Stoller, M. O., Wang, S., Liu, J., Fagan, M. B., and Nunn, J. H. (2001) Structural and functional analysis of interhelical interactions in the human immunodeficiency virus type 1 gp41 envelope glycoprotein by alamine-scanning mutagenesis. *J. Virol.* 75, 11146–11156
22. Sackett, K., Wexler-Cohen, Y., and Shai, Y. (2006) Characterization of the HIV N-terminal fusion peptide-containing region in context of key gp41 fusion conformations. *J. Biol. Chem.* 281, 21755–21762
23. Dimitrov, A. S., Louis, J. M., Bewley, C. A., Clore, G. M., and Blumenthal, R. (2005) Conformational changes in HIV-1 gp41 in the course of HIV-1 infection. *Proc. Natl. Acad. Sci. U.S.A.* 102, 14271–14274
24. Naito, T., Izumi, K., Kodama, E., Sakagami, Y., Kajiwara, K., Nishikawa, H., Naito, T., Izumi, K., Kodama, E., Sakagami, Y., Kajiwara, K., Nishikawa, H., and Wat, C. (2007) Structural basis of potent and broad HIV-1 fusion inhibitor CP32M. *J. Biol. Chem.* 282, 26618–26629
25. Chong, H., Yao, X., Qiu, Z., Qiu, B., Han, R., Waltersperger, S., Wang, M., He, Y., and Cui, S. (2007) Discovery of critical residues for viral entry and inhibition through structural insight of HIV-1 fusion inhibitor CP621-652. *J. Biol. Chem.* 282, 20281–20289
26. Wexler-Cohen, Y., Johnson, B. T., Puri, A., Blumenthal, R., and Shai, Y. (2006) Structurally altered peptides reveal an important role for N-terminal helical repeat binding and stability in the inhibitory action of HIV-1 fusion inhibitor DP178. *J. Biol. Chem.* 281, 9005–9010
27. He, Y., Liu, S., Jing, W., Li, J., Tian, D., Dehnath, A. K., Kirchhoff, F., and Jiang, S. (2007) Conserved residue Lys-574 in the cavity of HIV-1 Gp41 coiled-coil domain is critical for six-helix bundle stability and virus entry. *J. Biol. Chem.* 282, 25631–25639
28. He, Y., Liu, S., Li, J., Lu, H., Qi, Z., Liu, Z., Dehnath, A. K., and Jiang, S. (2008) Conserved salt bridge between the N- and C-terminal heptad repeat regions of the human immunodeficiency virus type 1 gp41 core structure is critical for viral entry and inhibition. *J. Biol. Chem.* 82, 11129–11139
29. Li, J., Chen, X., Huang, J., Jiang, S., and Chen, Y. H. (2009) Identification of critical antibody-binding sites in the HIV-1 gp41 six-helix bundle core as potential targets for HIV-1 fusion inhibitors. *Immunobiology* 214, 51–60
30. Jiang, S., Lin, K., and Lu, M. (1998) A conformation-specific monoclonal antibody reactive with fusion-active gp41 from the human immunodeficiency virus type 1 envelope glycoprotein. *J. Virol.* 72, 10213–10217
31. Buzon, V., Natajran, G., Schibili, D., Campello, F., Kozlov, M. M., and Weissenhorn, W. (2010) Crystal structure of HIV-1 gp41 including both fusion peptide and membrane proximal external regions. *PLoS Pathog.* 6, e1000880
32. Ingallinella, P., Bianchi, E., Ladwa, N. A., Wang, Y. J., Hrin, R., Veneziano, M., Bonelli, F., Ketas, T. J., Moore, J. P., Miller, M. D., and Peski, A. (2009) Addition of a cholesterol group to an HIV-1 peptide fusion inhibitor dramatically increases its antiviral potency. *Proc. Natl. Acad. Sci. U.S.A.* 106, 5801–5806
33. Stoddart, C. A., Nault, G., Galkina, S. A., Thibaudeau, B., Bakis, P., Bousquet-Gagnon, N., Robitaille, M., Bellomo, M., Paradis, V., Liscourt, P., Lobach, A., Rivard, M. E., Pak, R. G., Mankowski, M. K., Bridon, D., and Quraishi, O. (2008) Albumin-conjugated C34 peptide HIV-1 fusion inhibitor. Equipping to C34 and T-20 in vitro with sustained activity in SCID-hu Thy/Liv mice. *J. Biol. Chem.* 283, 34045–34052
34. Li, J., Chen, X., Huang, J., Jiang, S., and Zhang, L. (2008) Design and evaluation of sifuvirtide, a novel HIV-1 fusion inhibitor. *J. Biol. Chem.* 283, 11126–11134
35. He, Y., Xiao, Y., Song, H., Liang, Q., Lu, D., Chen, X., Lu, H., Jing, W., Jiang, S., and Zhang, L. (2008) Structural basis of enfuvirtide, the first HIV fusion inhibitor. *J. Biol. Chem.* 283, 10543–10550
36. Mozetič, J., Buzon, V., Natajran, G., Schibili, D., Campello, F., Kozlov, M. M., and Weissenhorn, W. (2010) Crystal structure of HIV-1 gp41 including both fusion peptide and membrane proximal external regions. *PLoS Pathog.* 6, e1000880
37. Eggink, D., Bontjer, I., Langedijk, J. P., Berkhout, B., and Sanders, R. W. (2011) Resistance of human immunodeficiency virus type 1 to a third-generation fusion inhibitor requires multiple mutations in gp41 and is accompanied by a dramatic loss of gp41 function. *J. Virol.* 85, 10785–10797
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