Structurally Altered Peptides Reveal an Important Role for N-terminal Heptad Repeat Binding and Stability in the Inhibitory Action of HIV-1 Peptide DP178*

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Human immunodeficiency virus 1 gp41 folds into a six-helix bundle whereby three C-terminal heptad repeat regions pack in an anti-parallel manner against the coiled-coil formed by three N-terminal heptad repeats (NHR). Peptides that inhibit bundle formation contributed significantly to the understanding of the entry mechanism of the virus. DP178, which partially overlaps C-terminal heptad repeats, prevents bundle formation through an undefined mechanism; additionally it has been suggested to bind other ENV regions and arrest fusion in an unknown manner. We used two structurally altered DP178 peptides; in each, two sequential amino acids were substituted into their D configuration, D-SQ in the hydrophilic N-terminal region and D-LW in the hydrophobic C-terminal. Importantly, we generated an elongated NHR peptide, N54, obtaining the full N-helix docking site for DP178. Interestingly, D-LW retained wild type fusion inhibitory activity, whereas D-SQ exhibited significantly reduced activity. In correlation with the inhibitory data, CD spectroscopy and fluorescence studies revealed that all the DP178 peptides interact with N54, albeit with different stabilities of the bundles. We conclude that strong binding of DP178 N-terminal region to the endogenous NHR, without significant contribution of the C-terminal sequence of DP178 to core formation, is vital for DP178 inhibition. The finding that D-amino acid incorporation in the C terminus did not affect activity or membrane binding as revealed by surface plasmon resonance correlates with an additional membrane binding site, or membrane anchoring role, for the C terminus, which works synergistically with the N terminus to inhibit fusion.

HIV-12 utilizes a glycoprotein embedded in its outer membrane for entering its target cell. One of its subunits, gp41, enables the actual fusion action (reviewed in Refs. 1 and 2) and contains several functional regions: N-terminal fusion peptide, N-terminal heptad repeat (NHR), C-terminal heptad repeat (CHR), and the transmembrane region (Fig. 1). gp41 possesses at least three major conformational states during the fusion process: (i) a native metastable conformation in the intact virion in which gp41 is sheltered by gp120 (3, 4); (ii) the pre-hairpin conformation following receptor binding in which three NHR regions create a coiled-coil, positioning the fusion peptide close to the host membrane (5, 6); and (iii) the hairpin conformation preceding pore formation in which the six-helix bundle is formed whereby three CHR regions pack in an anti-parallel manner into hydrophobic grooves created by the central coiled-coil (7, 8). Peptides derived from the NHR and CHR regions of gp41 are known to inhibit HIV-1 fusion (4, 9). It is believed that these fusion inhibitors bind their endogenous counterparts, blocking the progression from pre-hairpin to hairpin conformation, thereby arresting the fusion process. One of these peptides, DP178 (10), was recently approved by the Food and Drug Administration and is currently part of HIV-1 treatment.

The exact extent of the six-helix bundle structure in vivo is not known; in addition, it has never been demonstrated that DP178 can actually bind the NHR region and create a six-helix bundle. Therefore, although already in clinical use, the mode of inhibition by DP178 has yet to be proven. We synthesized two structurally altered DP178 peptides and their fluorescently labeled analogs (Fig. 1). To clarify the importance of NHR binding to the inhibitory activity of DP178, we synthesized D-SQ, which includes two sequential D-amino acids in the hydrophilic N-terminal region, and to examine whether the C-terminal region of DP178 participates in binding the NHR region we synthesized D-LW, which includes two sequential D-amino acids in the hydrophobic C-terminal sequence. We reasoned that: (i) structural alterations in D-SQ will reduce its inhibitory activity by compromising its ability to bind the NHR region and create the six-helix bundle structure, and (ii) if the C-terminal part also participates in binding the NHR region then structural alterations in it will significantly reduce the inhibitory activity of the peptide as well. Importantly, we synthesized an elongated N-peptide, N54, to obtain the full docking site for DP178. We examined the inhibitory activity of the peptides by a cell-cell fusion assay and used biophysical methods to investigate their ability to bind N54 and the stabilities of these interactions. Because the C-terminal part of DP178 has been implicated in membrane binding (11), the effect of D-amino acid incorporation on the peptides binding to phospholipid membranes was also examined by using surface plasmon resonance. The results are discussed in view of the differences in the ability of DP178 N- and C-terminal regions to endure structural alterations that affect binding to the N-helix and fusion inhibition activity.

EXPERIMENTAL PROCEDURES

Materials—Boc and Fmoc amino acids, Boc methylbenzydrylamine resin and Fmoc Rink Amide methylbenzydrylamine resin, were purchased from Nova-Biochem AG (Laufelfinger, Switzerland). Other peptide synthesis reagents, phospholipids, cholesterol (N-[2-hydroxyethyl]

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2 The abbreviations used are: HIV, human immunodeficiency virus; NHR, N-terminal heptad repeat; CHR, C-terminal heptad repeat; PC, phosphatidylcholine; Fmoc, N-[9-fluorenylmethoxycarbonyl]; BOC, t-butoxycarbonyl; TES, 2-[(2-hydroxy-1,1-bis[hydroxyethyl]amino)ethanesulfonic acid; chol, cholesterol.
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**FIGURE 1. Top, schematic representation of HIV-1 gp120. FP, NHR, CHR, and PTM represent the fusion peptide, N-terminal heptad repeat, C-terminal heptad repeat, and pre-transmembrane domain, respectively. The sequences of the DP178 peptides are shown at the bottom. The residues substituted to their D-amino acid counterparts are in bold and underlined.**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>DP178</td>
<td>YTLHSLIEEQQEKNEGELLEDKWASLWNWF</td>
</tr>
<tr>
<td>D-LW</td>
<td>YTLHSLIEEQQEKNEGELLEDKWASLWNWF</td>
</tr>
<tr>
<td>D-SQ</td>
<td>YTLHSLIEEQQEKNEGELLEDKWASLWNWF</td>
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*piperezine-N'-[2-ethanesulfonic acid],* and *N*-octyl β-d-glucopyranoside (OG) were purchased from Sigma. All other reagents were of analytical grade. Buffers were prepared using double-glass-distilled water.

Phosphate-buffered saline was composed of NaCl (8 g/liter), KCl (0.2 g/liter), KH2PO4 (0.2 g/liter), and Na2HPO4 (1.09 g/liter) at pH 7.3. Hepes buffer was composed of 5 mM (N-[2-hydroxyethyl]piperezine-N'-[2-ethanesulfonic acid]) at pH 7.0.

*Cell Lines and Reagents—*Cell culture reagents and media were from Invitrogen. Steady-Glow Luciferase detection kit was from Promega (Madison, WI). HL2/3 cells (12) (a HeLa cell line) expressing cleaved HIV-1 molecular clone HXB2/3gpt (containing HIV-1 Gag, Env, Tat, Rev, and Nef proteins) were obtained through the AIDS Research and Reference Reagent Program from Dr. Barbara Felber and Dr. George Pavakis. The TZM-bl indicator HeLa cell line (13), which expresses high levels of CD4 and CCR5 along with endogenously expressed CXCR4, was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, National Institutes of Health from Dr. John C. Kappes, Dr. Xiaoyun Wu, and Tranzyme Inc. The cells were cultured every 3–4 days by trypsinization and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and antibiotics at 37 °C with 5% CO2 in a humidified incubator.

*Peptide Synthesis—*DP178wt, D-LW, N54, and C34 were synthesized on Rink Amide MBHA resin using the Fmoc strategy as previously described (14). They were cleaved from the resin by HF. The residues substituted to their D-amino acid counterparts are in bold and underlined.

*Fluorescent Labeling of the Peptides—*Resin-bound peptides, with their amino acid side chains fully protected, were deprotected from their N terminus protecting group and reacted with the rhodamine (emission-580 excitation-530) fluorescent probe in N,N-dimethylformamide + 2% N,N-Diisopropylethylamine solution in an overnight reaction (16). Cleavage and purification were performed according to the synthesis method.

*Preparation of Large and Small Unilamellar Vesicles—*Thin films of PC and cholesterol at a molar ratio of 9:1, respectively, were generated after dissolving the lipids in a 2:1 (v/v) mixture of CHCl3/MeOH and drying under a stream of nitrogen gas while rotating. The films were lyophilized overnight, sealed with argon gas to prevent oxidation of the lipids, and stored at −20 °C. Before the experiments, films were suspended in the appropriate buffer and vortexed for 1.5 min. The lipid suspension underwent five cycles of freezing-thawing and extrusion through polycarbonate membranes with 1- and 0.2-μm diameter pores to create large unilamellar vesicles. To prepare small unilamellar vesicles, the films were dissolved in the appropriate buffer, vortexed for 1.5 min, sonicated as previously described (17), and diluted to 0.5 mM.

*Fusion Assay—*Fusion was monitored using a reporter gene assay based on activation of HIV long terminal repeat-driven luciferase cassette in TZM-bl (target) cells by HIV-1 tat from the HL2/3 (effector) cells. We performed fusion assay in a serum-free medium to minimize nonspecific association of peptides with serum proteins. The peptides were solubilized in Me3SO at various dilutions. The TZM-bl cells, plated in 96-well clusters (2 × 104/well) overnight at 37 °C, were placed in 50 μl of serum-free medium/well prior to fusion assays. The target cells were co-cultured with 50 μl of HL2/3 cells (2 × 104/well in serum-free medium) for 4–6 h at 37 °C in the presence or absence of various concentrations of DP178 peptides (1 μM of peptide/well). Efficiency of fusion was determined by measuring luciferase activity in the wells following the manufacturer’s instructions using the steady-glow luciferase determination kit (Promega). The light intensity was measured using a Reporter luminometer (Turner Designs, Sunnyvale, CA). Background luminescence in TZM-bl cells was determined without addition of HL2/3 cells.

*Circular Dichroism (CD) Spectroscopy—*CD measurements were performed on an Aviv 202 spectropolarimeter. The spectra were scanned using a thermostatic quartz cuvette with a path length of 1 mm. Wavelength scans were performed at 25 °C, recording time was 20 s in 1-nm steps in the range of 190–260 nm. For thermal denaturation the cores were created at 5 °C and measured in the range of 5–99 °C, 2 degrees/min rate of change, 2-min equilibration time, 2-degree steps, and 20-s recording time. Peptides and peptide mixtures (1:1 molar ratio) were scanned at a concentration of 10 μM in Hepes buffer.

*DP178-N54 Interaction, Stability, and Competition Experiments Using Fluorescently Labeled Peptides—*Interaction between the NHR region and DP178 was measured using rhodamine (Rh)-labeled peptide. A labeled DP178 peptide (Rho-DP178/Rho-D-LW/Rho-D-SQ) was dissolved and incubated, and its fluorescence was measured. Then, a 1:1 molar ratio mixture was prepared between the labeled DP178 peptide and N54 and incubated, and the fluorescence was measured. To examine six-helix bundle stability, competition experiments were performed. First, a 1:1 molar ratio mixture was prepared between the labeled DP178 peptide and N54. After incubation the peptide mixture was transferred to a 5-fold molar excess of unlabeled DP178/C34 peptide and incubated, and the fluorescence was measured. All fluorescence measurements were recorded before and after the addition of protease K (utilized in order to follow the kinetics of degradation) and followed for 10–15 min. All reactions were in Hepes buffer, and all measurements were performed at room temperature on a SLM-AMINCO Bowman series 2-luminescence spectrometer. Excitation was set at 530 nm, emission at 580 nm, band paths were 8 nm, and the voltage was 750/800 V.

*Binding Analysis by Surface Plasma Resonance Biosensor—*Biosensor experiments were carried out with a BLACore X analytical system 3000 (BLACore, Uppsala, Sweden) using L1 sensor chip (BLACore) at 25 °C. The chip contains hydrophobic aliphatic chains that include exposed polar headgroups. The applied small unilamellar vesicles interact with the polar headgroups, resulting in a bilayer lipid surface. The alterations applied to a previously described protocol (18, 19): 1) peptide solutions were 0.6–10 μM; 2) peptide solutions of 75–100 μl were injected at a 5 μl/min flow rate; 3) phosphate-buffered saline was the running and dissolving buffer. Dissociation was performed with only the running buffer, and the time corresponded to the specific peptide and its concentration. Surface plasma resonance detects changes in the refractive index of the surface layer in contact with the sensor chip.
When the peptides were measured alone, N54 exhibited a spectrum of each peptide alone (non-interacting signal) (21, 22). The two main peptides, the DP178 mixture (measured as a positive control), whereas the lowest induction signal of N54 mixture. We used as a negative control (GCN4 helical mutant with N54) that shows no difference between the experimental signal (closed squares) and the non-interacting signal (open squares). The inset represents a negative control (GCN4 helical mutant with N54) that shows no difference between the experimental signal (closed squares) and the non-interacting signal (open squares). The inset represents a negative control (GCN4 helical mutant with N54) that shows no difference between the experimental signal (closed squares) and the non-interacting signal (open squares).

Interaction between the DP178 Peptides and N54 and Its Stability Determined by CD Spectroscopy—To find out the reason for the differences in the inhibitory activities of the three C-peptides, we tested their capacity to bind the N-heptad repeat utilizing CD spectroscopy. The interaction between peptides can be assessed by comparing the spectrum of the mixed peptides (experimental signal) with the average spectrum of each peptide alone (non-interacting signal) (21, 22). The two signals are identical if the peptides do not interact with each other. When the peptides were measured alone, N54 exhibited α-helical signal whereas the C-peptides were predominantly unstructured in solution. Their "non-interacting" signals are presented in Fig. 3A by the gray curves. The actual experimental signal for each peptide mixture, recorded following the C and N peptide co-incubation, is presented in Fig. 3A by the black curves. In comparison with the non-interacting signals, the experimental signals of all the peptide mixtures demonstrated induction of α-helical signal (probably due to secondary structure induction of the C-peptides), indicating interaction between the C-peptides (C34 and the three DP178 peptides) with N54. These results correlate with the creation of six-helix bundle structures (21, 23). The highest induction of α-helical signal was observed for the C34+N54 mixture (measured as a positive control), whereas the lowest induction was observed for the d-SQ+N54 mixture. We used as a negative control the combination between N54 and an unrelated peptide, GCN4 helical mutant. The data shown in the inset of Fig. 3A demonstrate that the experimental and non-interacting signals are identical. A similar result was observed following the co-incubation of DP178wt with GCN4 helical mutant (figure not shown). To test the stabilities of the different bundles we performed thermal denaturation. The peptide mixtures were incubated, and their signal at 222 nm was monitored while the temperature was slowly raised. The melting curves for the three peptide mixtures are presented in Fig. 3B. The Tm values for the N54 mixtures with DP178wt, D-LW, and D-SQ were ~58.5, 55, and 51 °C, respectively. To conclude, all the C-peptides examined interact with N54, probably creating a six-helix bundle structure, albeit with different stabilities.

DP178 Peptides and N54 Interaction and Stability Determined by Utilizing Fluorescently Labeled Peptides—To further confirm our CD results, the different DP178 peptides were labeled with rhodamine, a fluorescent probe known to undergo self quenching. Rhodamine labeling does not interfere with the function of DP178. We measured the fluorescence, before and after the addition of proteinase K, of rhodamine-labeled C-peptides alone and of preincubated peptide mixtures between labeled C-peptides and N54. We reasoned that the C-peptides interacting with N54 peptides, creating a bundle structure, would have reduced initial fluorescence due to higher proximity of the fluorescent probes. In addition, their cleavage by proteinase K would be slower in comparison with the C-peptides alone, because the interaction with the N54 would partially protect them. As shown in Fig. 4, the signals of the C-peptides alone reveal that they are all partially aggregated in solution.
DP178wt aggregated to the highest degree; nevertheless, all the peptides were cleaved readily and almost immediately by the enzyme, as illustrated by the sudden increase of the signal following the addition of the enzyme. When each C-peptide+N54 mixture was measured, we observed an initial quenching of the signal, indicating interaction. Following enzyme addition, the D-SQ+N54 mixture exhibited the highest initial fluorescence increase and the fastest degradation kinetics, whereas the DP178wt+N54 and D-LW+N54 mixtures exhibited intermediate behavior. The C34+N54 mixture, analyzed as a control for a known stable six-helix bundle, demonstrated the smallest initial fluorescence increase and the slowest kinetic degradation. Thus, all the C-peptides interact with N54, albeit with different stabilities.

Exploring DP178 Peptides and N54 Interaction and Stability by Performing Competition Experiments with Fluorescently Labeled Peptides—To further clarify the differences in the stabilities of the different peptide bundles created by the different DP178 peptides, a competition strategy was employed. First, we incubated a labeled DP178 peptide with N54, and then a five molar excess of unlabeled DP178wt was added. Following incubation, the fluorescence before and after the addition of proteinase K was measured. We reasoned that the level of labeled DP178 peptides displaced would depend on the stability of the original bundle created. The results are presented in Fig. 5A. The labeled DP178wt+N54 mixture gives the same fluorescence signal with or without excess unlabeled DP178, indicating that DP178wt interacts strongly with N54, hardly allowing its replacement. In contrast, the initial fluorescence of the labeled D-SQ+N54 with excess unlabeled DP178wt mixture is significantly higher than without the peptide, demonstrating displacement of the labeled D-SQ peptide by unlabeled DP178wt peptide. Moreover, there was an initial high increase of the signal, as well as faster kinetics of degradation following the addition of the enzyme. Taken together, these observations support the conclusion that the D-SQ+N54 interaction is less stable than DP178wt+N54. The D-LW+N54 mixture demonstrated an intermediate state. The initial signal had an intermediate intensity; nevertheless, its degradation kinetics more resembled the pattern displayed by DP178wt+N54. To verify our results, we performed competition experiments again, with an excess of unlabeled C34 peptide (which overlaps the N-terminal sequence of DP178), and the same pattern was revealed (Fig. 5B). Thus, we conclude that the interaction between N54 and the DP178 peptides differs in its stability, specified from the most stable to the least: DP178wt, D-LW, and D-SQ.

Binding of the Peptides to PC/Chol Membranes Determined by Surface Plasmon Resonance—The C-terminal region of DP178 is vital for its activity (21, 24), and it presumably involves membrane binding (11). To examine whether the structural alterations in this region influenced the ability of the peptides to bind membranes, we performed a binding assay using a BIAcore biosensor. The DP178 peptides at varying concentrations of 0.6 to 10 μM were allowed to interact with PC/Chol bilayers on the surface of an L1 chip. The profiles of the different peptide sensorgrams were very similar, indicating similar binding capabilities (Fig. 6). For quantitative results we analyzed each sensogram alone by curve fitting using numerical integration analysis (20). We attempted analysis with two different reaction models; the "1:1 reaction model asserts only one step, which involves binding of the peptide to the lipids, whereas the "two-state reaction" model asserts an additional step involving either aggregation or partial insertion of the peptide into the lipid bilayer (19). The model that exhibited the best fit and the highest consistency was the 1:1 binding reaction model; based on it, binding constants were...
DISCUSSION

The discovery of fusion inhibitors led to the current accepted fusion model, demonstrating the utility of fusion inhibitors as tools for deciphering HIV-1 fusion mechanism. In this study, by utilizing DP178 diastereomeric peptides and DP178 full docking site, we were able to demonstrate the importance of the interaction with the NHR region, and the stability of the bundle formed, to the inhibitory activity of DP178.

It has been implicated that DP178 has an additional mode of action involving its C terminus, as will be elaborated below, and that this region has a helical structure (25). The purpose of the study was to distinguish between the contributions of the different regions to the inhibitory activity of DP178. Therefore, we chose residues in the C- and N-terminals for substitution to their D-amino acid counterparts. In the D-SQ mutant the specific location was chosen because the serine residue is known to disrupt the stability of the N36+C34 six-helix bundle (26). The D-amino acids in the D-LW mutant replaced known hydrophobic residues that are critical for its inhibitory activity (21). Because it has been demonstrated previously that insertion of D-amino acids into peptides disrupts their helical structure (27), we can assume that the DP178 mutants are structurally altered.

Interestingly, when the activity of the DP178 peptides was analyzed by a cell-cell fusion assay, we found that D-LW exhibited similar inhibitory activity to DP178wt despite the presence of two D-amino acids, whereas the activity of D-SQ was significantly reduced. By using CD spectroscopy and by utilizing fluorescently labeled C-peptides, we show that all the DP178 peptides interact with an elongated N-peptide, N54, and form bundles. However, the stabilities of the various bundles (also observed by CD spectroscopy (Fig. 3) and by fluorescently labeled C-peptides (Figs. 4, 5)), demonstrated a gradient in which DP178wt forms the most stable complex, followed by D-LW, and D-SQ. The considerable reduction in the inhibitory activity of D-SQ, combined with its reduced bundle stability, demonstrates the importance of the interaction and its stability between the N-terminal region of DP178 and the NHR to DP178wt inhibitory activity. In contrast, interfering with the structure of the C-terminal region of DP178 in D-LW results in a slight reduction in inhibitory activity combined with intermediate bundle stability. Therefore, we suggest that this region enlarges the interacting interface without participating in the six-helix bundle complex. However, the fluorescence studies showed a difference between the stabilities of DP178wt and D-LW bundles that should probably affect the inhibitory activity more than 2-fold. This might suggest that in the presence of cells there is an additional target for the C-terminal part of DP178 as elaborated below.

Accumulating evidence suggests that DP178 has an additional inhibitory mode of action involving its C-terminal region and membrane binding (11, 23, 28, 29). The membrane binding can refer to specific binding interactions or simply anchoring to the membrane. This alternative mode of action might involve interfering with aggregation of gp41 trimers (by binding the pre-transmembrane region region), thus preventing pore expansion and arresting the fusion process. Membrane binding experiments were performed to examine whether the insertion of D-amino acids in the C-terminal region of DP178 resulted in reduced membrane binding ability. The DP178 peptides exhibited very similar affinities to PC:Chol membranes as measured by surface plasmon resonance. Taking into account previous reports demonstrating that incorporation of D-amino acids within membrane-bound peptides do not significantly affect their structure in the membrane or their ability to recognize their counterpart (30–32), we attribute the inhibitory activity contribution of the C-terminal region to structural tolerance of the C terminus of DP178 when bound to the cell membrane.

Interestingly, differences in the aggregation states of the isolated DP178 peptides in solution were also detected. The diastereomeric peptides demonstrated reduced aggregation in comparison to DP178wt.
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(Fig. 4). This observation might have important therapeutic implications because one of the major problems involving treatment with DP178 is its fast aggregation complicating its administration and efficacy.

Stability of the six-helix bundle has traditionally been assessed using N36+C34 combinations, specifically with respect to binding of C34 to the pocket in the NHR trimer. However, the N36+C34 do not provide complete answers to the issue of six-helix bundle stability because other regions may also be involved. For instance, N51+C43 six-helix bundle has a much higher melting temperature than the N36+C34 counterpart (33). We found in this study that stable bundles could be formed between N54 and DP178, although DP178 does not include the residues that bind the pocket. Because we were interested in the effects of DP178 mutations close to the membrane proximal region, we used the N54+DP178 complexes as our base line.

Creation of a six-helix bundle structure between DP178 and N-peptides has not been shown directly to date. In 1996, Lawless et al. (21) demonstrated, by CD, interaction of DP178 with another N-peptide, DP107 (illustrated in Fig. 7), but their CD results included reduction of the experimental signal in comparison to the non-interactive signal. Another study (23) examined six-helix bundle creation between DP178 and four different N-peptides (illustrated in Fig. 7). N36-F10+DP178 exhibited a small induction of the experimental signal, whereas DP107+DP178 (similarly to Lawless et al., Ref. 21) and N46+DP178 exhibited reduction of the experimental signal. Liu et al. also verified their CD results by fluorescent native gels and concluded that DP178 binds the N-terminal side of the NHR region, but only to a weak extent, and that the interaction with the N-peptides mainly creates insoluble complexes. Our data emphasize the importance of obtaining the full overlap between the N- and the DP178 peptides to obtain a soluble, stable bundle.

When DP178wt (Fig. 5) or C34 (data not shown) interacts with N54, the C-peptide is hardly displaced by exogenous addition of C-peptides. In contrast, when the initial interaction is created with one of the two diastereomers, it can be displaced, and the extent of displacement is dependent on the position of the d-amino acids. This phenomenon can explain the time window of DP178 activity; it can inhibit HIV-1 fusion after exposure of the prehairpin conformation, but before creation of the hairpin conformation, because it cannot displace the endogenous CHR once the six-helix bundle complex has been formed (22).

Taken together, we conclude that strong binding of DP178 N-terminal to the endogenous NHR region, without significant contribution of the C-terminal sequence of DP178 in core formation, is vital for DP178 inhibition. In addition, the finding that d-amino acid incorporation in the C terminus did not significantly affect the activity correlates with an additional membrane binding site, or membrane anchoring role, for the C terminus of DP178 that works synergistically with the N terminus to inhibit fusion.

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