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Permanent Inhibition of Viral Entry by Covalent Entrapment of HIV gp41 on the Virus Surface

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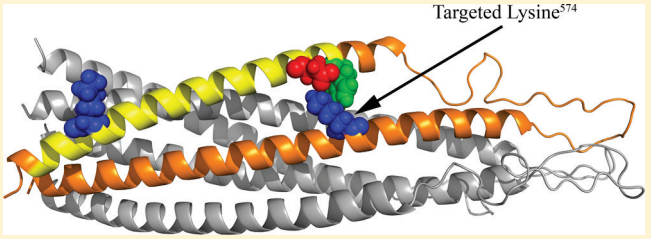
ABSTRACT: HIV entry occurs by concerted conformational changes in the envelope protein complex on the surface of the virus. This complex is made up of a trimer of heterodimers of two subunits: surface subunit, gp120, and transmembrane subunit, gp41. Conformational changes in the envelope complex allow gp41 to mediate membrane fusion leading to exposure of two gp41 regions: N-heptad repeat (NHR) and C-heptad repeat (CHR). Peptides from the NHR or the CHR have been found to inhibit HIV entry. Herein we show that we can covalently inhibit HIV viral entry by permanently trapping the gp41 intermediate on the virus surface using a covalently reactive group on inhibitory peptides. This is evidence showing that vulnerable conformational intermediates exist transiently during HIV viral entry, and the details presented herein will facilitate development of envelope as a target for therapeutics and potential chemopreventive agents that could disable the virus before contact with the host cell.



According to the most recent data from UNAIDS, ~33.3 million people are living with HIV and in 2009 there were 1.8 million AIDS-related deaths. Because of the global significance of HIV-1 infection, there have been strong efforts to develop treatments for HIV/AIDS and to develop chemopreventive agents to prevent HIV-1 infection. As one of the efforts, several peptide entry inhibitors have been under development, and one of them is in use in the clinic (T20, brand name Fuzeon, generic name enfuvirtide). HIV peptide entry inhibitors are synthetic peptides ~30 amino acids in length which mimic sequences of the transmembrane subunit, gp41, of the HIV-1 envelope protein complex (for review see ref 1).

The envelope spike on the surface of the virus is made of two protein subunits, expressed as a precursor protein, gp160. gp41 is the transmembrane subunit which is noncovalently associated with the surface subunit, gp120. gp41 and gp120 form a trimer of heterodimers on the virus or cell surface. gp41 can be divided into functional domains including the fusion peptide, necessary for membrane fusion, at the N-terminus, two helical heptad repeat (HR) regions which are designated N-terminal heptad repeat (NHR) and C-terminal heptad repeat (CHR), a loop region, a membrane proximal region (MPER), and a transmembrane domain (TM) followed by a C-terminal cytoplasmic region. Entry of HIV-1 begins with binding of gp120 to the CD4 receptor on the target cell membrane followed by conformational changes in gp120 which allow binding to the HIV coreceptor, either CCR5 or CXCR4. This leads to further conformational changes that facilitate gp41-mediated membrane fusion.[2] Entry inhibitor studies have been very important in helping to shed light on how gp41 mediates membrane fusion.[3−12] It is known from these studies that receptor binding leads to exposure of specific regions of gp41 and insertion of the fusion peptide into the host cell membrane followed by formation of a hairpin-like six-helix bundle (6HB) conformation of gp41 at which point the two membranes fuse as the virus core enters into the host cell. The regions of gp41 that are exposed in the intermediate conformation are very important targets for inhibition of viral entry and cell−cell fusion.

Several structural studies of what is presumed to be the post-fusion structure of the 6HB due to extreme stability (T_m > 100 °C) have shown that three antiparallel domains of NHR aggregate to form a core 3-helical bundle and three CHR regions wrap around the NHR core forming the 6HB.[13−20] Thus, it is proposed that peptide inhibitors made up of sequences of the CHR or NHR bind to form heterogeneous 6HB molecules, preventing collapse of gp41 to the stable 6HB conformation and thereby inhibiting membrane fusion and viral entry or cell−cell fusion. Various fusion inhibitors have been under development.[21−25] The advantages of fusion inhibitors are that (1) inhibitors can potentially disable native virus before initial infection occurs and (2) the targeted regions are vital to viral function and are highly conserved.

T-20 is a peptide sequence from the CHR/MPER region of gp41, which was approved by the FDA as an injectable HIV-1 fusion inhibitor in 2003. Many other peptides have shown great promise and are in continuing development (for recent review,

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see ref 1). Even though there are NHR-based peptides such as N36, N42, and N36F10, investigators have placed greater focus on the development of CHR-based peptides.[26−28] The primary reason is that C-peptide inhibitors are monomeric in solution and show a higher potency, whereas N-peptides tend to aggregate in solution and exhibit lower potency.

We had previously shown in cell−cell fusion studies[12] that C34-based peptides with a maleimide moiety will bind covalently to the fusogenic subunit, gp41, of the HIV-1 envelope complex on the cell surface, permanently inhibiting cell−cell fusion. The goal was to show that peptides with a specific gp41 sequence, HXB2 C34, from the CHR region, could be designed with a reactive moiety strategically placed in order to optimize covalent entrapment of the vulnerable HIV gp41 fusion intermediate. Placement of the reactive group was based upon the atomic level structures of the HIV gp41 ectodomain which are considered to be the postfusion structure based on stability[18−20] (Figure 1). Being able to trap the fusogenic intermediate of gp41 will advance mechanistic studies of viral entry and a trapped gp41 intermediate also has the potential to elicit novel immune responses, studies of which are ongoing in our laboratories. Herein, we report the first evidence that covalent peptide inhibitors are able to trap the fusogenic intermediate of HIV-1 gp41 on the virus surface and inhibit viral entry in a permanent manner complementing our previous findings in cell−cell fusion.

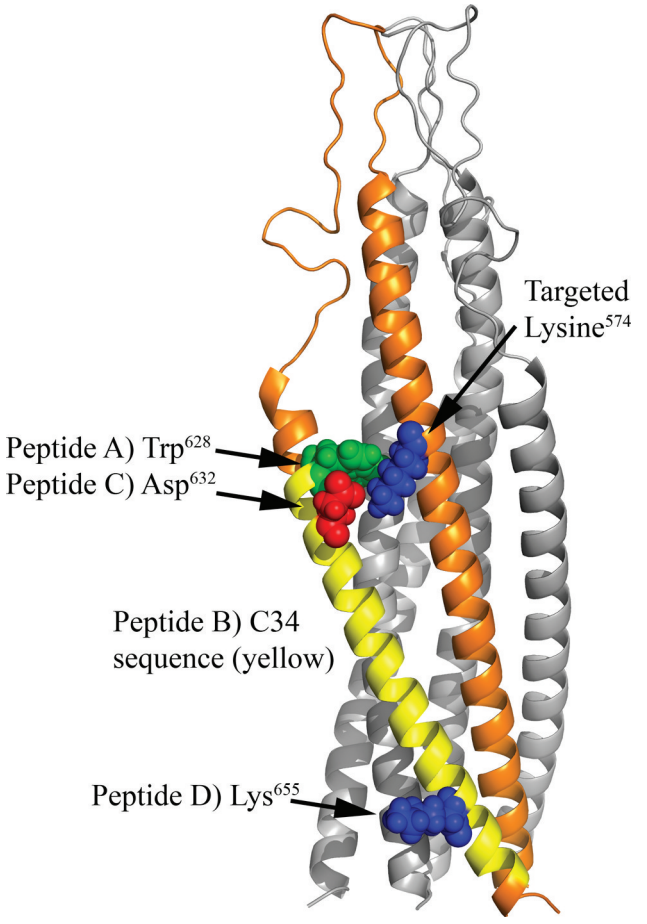

Figure 1. Three-dimensional model showing the placement of the reactive moieties on the C34 peptide sequence of HIV gp41 within the context of the six-helix bundle (6HB) structure. The amino acid Lys^{574}, which was targeted for attack by maleimide, is highlighted on the NHR. The C34 sequence on the CHR is shown in yellow. Details are highlighted on only one of the monomers for simplicity. Placement positions of the maleimide moiety on the C34 peptide are highlighted as follows: A, chemical linkage of maleimide onto the α-NH of Trp^{628}; C, insertion of a lysine in the position of Asp^{632} and chemical linkage of maleimide to its ε-NH; D, chemical linkage of maleimide onto the ε-NH of Lys^{655}. The structure was rendered with PyMOL[46] based upon the coordinates from theoretical model 1IF3.[19]

EXPERIMENTAL PROCEDURES

Covalent Peptides. Covalent and noncovalent peptides were synthesized as previously reported.[12] Briefly, the sequence of C34 peptide inhibitor was used as the peptide backbone (Figure 2). Covalent peptide A has a maleimide reactive group

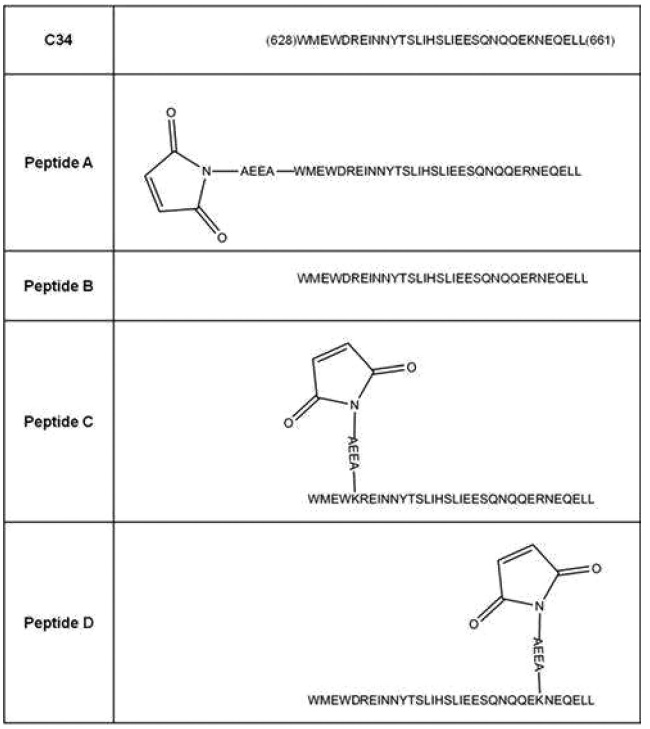

Figure 2. Diagrams of covalent inhibitors. The sequence of C34 peptide inhibitor was used as the peptide backbone. The numbers are based on the HXB2 strain. Peptide A has a maleimide reactive group at the N-terminus of the peptide (W^{628}) using the linker amino-ethoxyethoxyacetic acid (AEEA), and Lys^{655} was replaced with an arginine residue to prevent self-reactivity. Peptide B has the same sequence as native HXB2 C34 peptide, except for replacement of Lys^{655} with arginine. Peptide C has Asp^{632} substituted with a lysine in order to attach the chemical spacer and the reactive group at the Asp^{632} site. Peptide C also has an arginine substitution at Lys^{655} to avoid self-reactivity. Peptide D has the linker, AEEA, and the maleimide group attached to the native Lys^{655}.

at the N-terminus of the peptide using the linker amino-ethoxyethoxyacetic acid (AEEA, Figure 2). Lys^{655} was replaced with an arginine residue to avoid self-reactivity. For covalent peptide C, Asp^{632} was substituted with a lysine in order to attach the chemical spacer and the reactive group at the Asp^{632} site. Peptide C also has an arginine substitution at Lys^{655}. Peptide D has the linker and maleimide moiety placed at Lys^{655}, near the C-terminus of the peptide. Noncovalent peptide B was designed to mimic C34, but with the Arg^{655} substitution that was necessary for peptides A and C in order to

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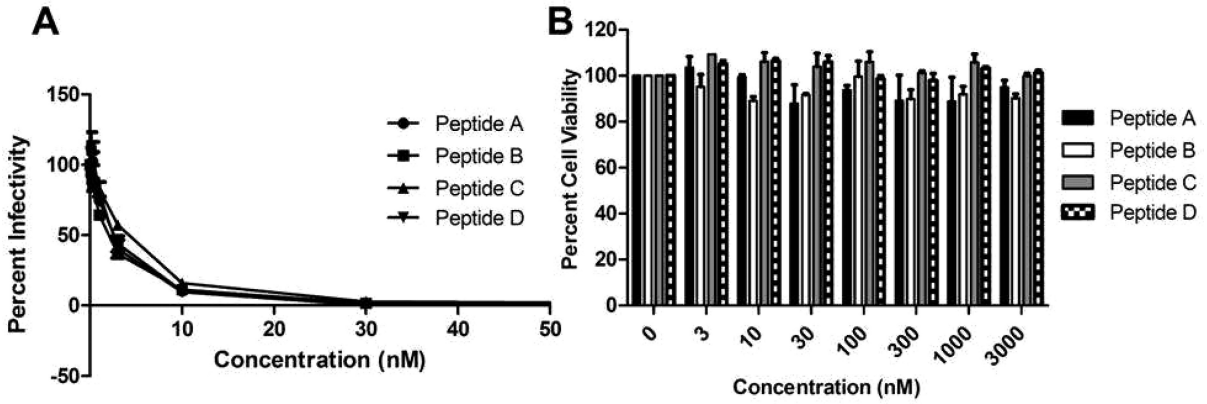

Figure 3. Comparison of covalent inhibitors and noncovalent inhibitors in virus entry and cytotoxicity. (A) Various concentrations of peptide inhibitors were added along with HXB2-enveloped virus onto plated TZM-bl cells. Viral entry levels were measured by luciferase assay at 2 days post-infection. (B) Plated TZM-bl cells were incubated with each peptide diluted in complete medium for 2 days. Cell viability in the presence of peptides was determined using a cell viability assay.

eliminate self-reactivity. This is a negative control for covalent peptide inhibition. The peptide B control has been shown to have similar inhibitory efficacy to native C34 in HIV cell−cell fusion[12] and viral entry (data not shown).

Reagents. The following reagents were obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: TZM-bl,[29−33] U87.CD4.CXCR4,[34] pNL4-3.HSA.R-E-[35] and pNL4-3.Luc.R-E-,[35,36] and pHXB2-env.[37] pCMV-VSV-G plasmid was a kind gift from Dr. Eric Freed (NCI-Frederick). 293T cells were obtained from ATCC (CRL-11268).

Cell Maintenance. 293T cells and TZM-bl cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen) supplemented with 10% heat inactivated FBS (Invitrogen) containing 100 μg/mL of streptomycin, 100 U/mL of penicillin, and 0.292 mg/mL of L-glutamine. U87.CD4.CXCR4 cells were maintained in DMEM supplemented with 15% FBS containing 1 μg/mL of puromycin, 300 μg/mL of G418, 100 μg/mL of streptomycin, 100 U/mL of penicillin, and 0.292 mg/mL of L-glutamine. All cell lines were incubated at 37 °C in a 5% CO_2 atmosphere.

Virus Preparation. To produce HIV-1 stock, pNL4-3.HSA.R-E- and pHXB2-env plasmids were transfected by a standard calcium phosphate transfection method (modified from the protocol of the lentiviral transfection[38]). Briefly, 8 × 10^6 293T cells per one 145 mm dish were seeded in the morning on the day of transfection, and 12 dishes total were used to make one viral stock. A total of 174 μg of pNL4-3.HSA.R-E- plasmid and 174 μg of pHXB2-env plasmid were diluted in distilled water. 2.5 M $CaCl_2$ was added, and then 2X HBSS buffer (pH 7.05) was added drop by drop. The DNA−Ca^{2+} mixture was incubated at room temperature for 30 min. The DNA−Ca^{2+} mixture was added to each plate and incubated overnight. The next morning, the medium was replaced with complete medium and further incubated for another 2 days.

At 2 days post-transfection, supernatant was harvested and filtered with a 0.45 μm pore size filter. The filtered supernatant was ultracentrifuged at 25 000 rpm, 4 °C, for 2 h using an L-90 ultracentrifuge (Beckman). Viral pellet was dried for 5 min and resuspended with a total of 600 μL of complete DMEM and stored at −80 °C until needed.

For VSV-G pseudotyped HIV production, pCMV-VSV-G plasmid and pNL4-3.Luc.R-E- plasmid were used as described above.

Virus Titration. The infectious titer of HIV-1 stock was determined by X-Gal staining based upon the titration method (adapted from ref 39). 8 × 10^4 TZM-bl cells per well were seeded in a 12-well plate with complete DMEM the day before infection. The following day, the medium was removed and replaced with diluted virus in a total volume of 200 μL in the presence of 20 μg/mL DEAE-dextran. The virus inoculated cells were incubated for 2 h at 37 °C, and 1 mL of complete DMEM was added, followed by a further incubation of 2 days.

The medium was then removed, and the cells were fixed with 2 mL of fixation buffer (1% formaldehyde, 0.2% glutaraldehyde, PBS) for 5−10 min at room temperature. The fixed cells were washed three times with PBS and incubated with 500 μL of X-Gal staining buffer (0.4 mg/mL X-Gal in 4 mM potassium ferrocyanide, 4 mM potassium ferricyanide, 2 mM magnesium chloride, PBS) for 24 h at 37 °C. Colonies of blue cells were counted under the microscope at a magnification of 100×, and infectious viral titers were quantified.[39]

Cytotoxicity Assay. A total of 10 000 TZM-bl receptor cells per well were plated to a 96-well plate. The following day, diluted peptides in complete DMEM were added to the plated cells and incubated further for 2 days at 37 °C under 5% CO_2 atmosphere. Cell viability was then measured using CellTiter-Blue Cell Viability Assay (Promega) according to the manufacturer's recommendations, and the plate was analyzed using a Spectra Max M5 Plate Reader (Molecular Devices).

Temperature-Arrested State (TAS) Prime-Wash Assays. A total of 10 000 TZM-bl receptor cells per well were plated to a white bottom 96-well plate the day before infection. Virus was added per well at a multiplicity of infection (MOI) of 0.3 with or without peptide inhibitors. Plates were centrifuged at 1000*g* at 4 °C for an hour (modified from ref 40) .

Virus-spinoculated TZM-bl cells were further incubated for 3 h at room temperature (21 °C). Cells were washed five times with 200 μL of D-PBS with ions (Invitrogen) for 1 h at room temperature. Complete medium was added to the infected cells, and the temperature was shifted to 37 °C. Viral entry levels were measured by a luciferase assay system (ONE-Glo, Promega) at 36−48 h post-infection according to manufacturer's protocol using the plate reader Spectra Max M5 (Molecular Devices). Experiments were performed in triplicate and normalized to the appropriate fusion signal in the absence of inhibitor. IC_{50} values were computed by

fitting a four-parameter nonlinear regression model with *R* statistical software.[41,42]

RESULTS

We have previously shown that covalent peptides permanently inhibit HIV envelope-mediated cell−cell fusion.[12] In order to show that covalent peptides with a maleimide moiety (Figures 1 and 2) inhibit cell-free virus entry as well, HIV entry was analyzed using a temperature-arrested state prime-wash assay.[43,44] The conformational changes that occur to the envelope complex of HIV leading to viral entry can be initiated and arrested at lowered temperature (21−25 °C). Fusion pore formation and entry of the core will not proceed until the temperature is raised to 37 °C. This phenomenon, referred to as the temperature arrested state, provides a window within which we can prove covalent entrapment of gp41 fusogenic intermediates.

Covalent Peptides Inhibit HIV Entry at the Same Level as Noncovalent Peptides and Are Not Toxic to Cells. To determine whether covalent peptides and noncovalent peptides have the same efficacy of inhibition despite the chemical changes that have been made, TZM-bl cells were infected with an MOI of 0.3 in the presence of each peptide at varying concentrations and HIV entry levels were measured at 48 h post-infection (hpi). As shown in Figure 3A, the covalent inhibitors (peptides A, C, and D) showed no difference when compared to noncovalent peptide (peptide B). Inhibitory concentration at 50% (IC_{50}) of each peptide was 2.11 ± 0.56 nM (peptide A), 1.77 ± 0.31 nM (peptide B), 3.36 ± 0.55 nM (peptide C), and 2.33 ± 0.37 nM (peptide D). These results indicate that covalent modification did not appreciably alter the binding step of peptides to the exposed gp41 regions. Next, cytotoxicity of the covalent peptide inhibitors was observed to see if covalent modification of peptides affects cell survival. Plated TZM-bl cells were incubated with each peptide diluted in complete medium for 2 days. Cell viability in the presence of peptides was determined using the CellTiter-Blue Cell Viability Assay kit (Figure 3B). Cell viability was not affected by any of the peptides even at the highest concentrations, up to 3 μM, suggesting covalent modification of peptide inhibitors does not affect cell viability and loss of cell viability does not account for any decrease in entry levels.

Inhibition by Covalent Peptides on HIV Entry Is HIV Envelope Specific. We have shown previously in cell−cell fusion that inhibition by covalent peptides is not due to nonspecific covalent reaction by the maleimide group and also that inhibition by covalent peptide inhibitors can be targeted to a specific amino acid.[12] To identify whether covalent peptides inhibit HIV envelope-mediated entry specifically, entry of VSV-G pseudotyped HIV was tested (Figure 4). VSV-G pseudotyped virus was produced by transfection with pCMV-VSV-G plasmid and pNL4-3.Luc.R-E- plasmid. U87.CD4.CXCR4 cells were incubated with VSV-G pseudotyped virus at an MOI of 0.5 in the presence of each peptide for 2 days. The viral entry level was measured by luciferase assay. None of the peptides, either covalent or noncovalent, showed any dose-dependent inhibition up to 3 μM (Figure 4). The same experiment was performed in a different cell line, TZM-bl, in order to show whether there is any cell-type dependent inhibitory effect. None of the peptides blocked VSV-G pseudotyped virus entry (data not shown), suggesting covalent inhibition by modified peptides inhibits HIV entry specifically regardless of cell type up to very high concentration.

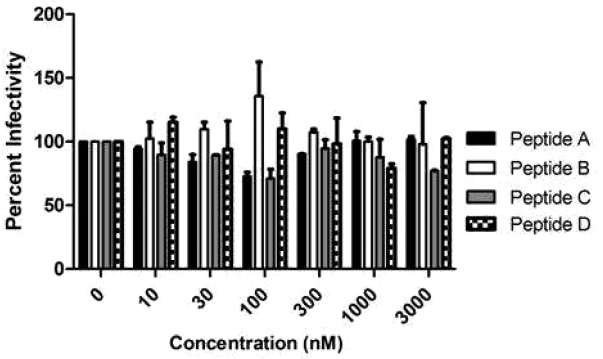

Figure 4. Covalent inhibitors have no effect on VSV-G pseudotyped virus up to high concentration. U87.CD4.CXCR4 cells were infected with VSV-G pseudotyped virus in the presence of each peptide for 2 days. The viral entry level was measured by luciferase assay.

In order to prove specificity of the peptide sequence, a nonspecific peptide, growth hormone releasing factor (GRF) Ac-YADAIFTQSYRKVLAQLSARKLLQDILSRK(AEEA-MPA)-CONH2, was also tested (data not shown). This peptide did not produce any effect on HIV entry levels, indicating that the inhibition that we measure with the C34-derived peptides is sequence specific for HIV gp41.

Covalent Peptides Permanently Inhibit HIV Entry. It was hypothesized that covalently reactive peptide inhibitors would bind to the exposed NHR of gp41 on the virus surface, form a covalent bond with Lys^{574}, and lead to permanent inhibition of HIV entry. We were able to test this hypothesis using a temperature-arrested state (TAS) prime-wash assay.[43,44] This methodology is based upon the fact that HIV envelope conformational changes occur at room temperature, 21−25 °C, but full fusion pore formation does not proceed until the temperature is shifted to 37 °C. Therefore, peptides can be added during or after the initiation of envelope conformational changes and exposure of the targeted region on gp41. Peptides that do not covalently react with the targeted amino acids can be washed away before shifting to 37 °C, at which point full fusion will proceed.

When the HIV entry level was measured at 48 h after the TAS prime-wash assay, a noncovalent inhibitor peptide B did not show any inhibition effect even as high as 1 μM with extensive washing (Figure 5A). However, the covalent inhibitors, peptides A, C, and D, showed significant, dose-dependent inhibition of HIV entry (Figure 5B). After extensive washing, the IC_{50} values for the covalent inhibitors were calculated to be 233.1 ± 48.9 nM for peptide A, 199.4 ± 70.2 nM for peptide C, and 127.3 ± 42.3 nM for peptide D. The maximum inhibition level achieved with the covalent peptides was 16% for peptide A, 9% for peptide C, and 11.5% for peptide D at a concentration of 1 μM. In comparison, the IC_{50} values for inhibition of cell−cell fusion with peptide A and peptide C were 465.1 ± 102.7 and 679.0 ± 77.5 nM, respectively.[12] These data indicate that modified peptides are able to react with gp41 on the virus surface covalently and are not dissociated even after extensive washing. It should be noted that all of the covalent peptide inhibitors surprisingly showed similar inhibition of viral entry despite the location of the reactive group along the C34 peptide sequence. Herein we

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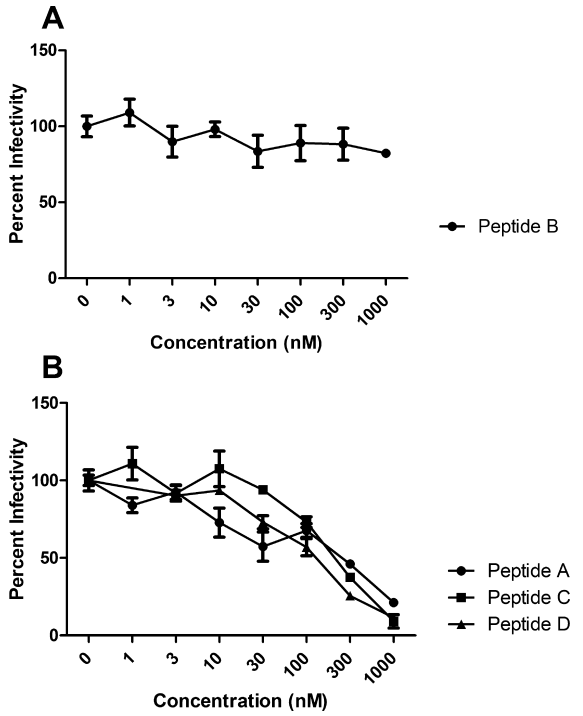

Figure 5. Permanent inhibition of HIV-1 entry by covalent peptides in a temperature-arrested state (TAS) prime-wash assay at MOI ~0.3. (A) Infectivity levels for noncovalent peptide B. (B) Infectivity levels for covalent peptides A, C, and D. HXB2-enveloped virus was spinoculated onto TZM-bl cells with various concentrations of peptides for 1 h at 4 °C. Cells were further incubated with virus for an additional 3 h at 21−22 °C. Unbound peptides and dissociated peptides were washed away over the course of 1 h at room temperature. Complete medium was added, and the temperature was shifted to 37 °C. HIV entry level was measured by luciferase assay at 2 days post-infection.

present strong evidence verifying that covalent peptides can be utilized to permanently trap the gp41 intermediate on the virus surface and, in so doing, inhibit HIV viral entry as well as cell−cell fusion.

DISCUSSION

In this study, we show that covalent peptide entry inhibitors permanently bind to a transient gp41 intermediate on the virus surface and inhibit viral entry into the host cell in an HIV-envelope specific manner. We had previously reported this effect in the case of cell−cell fusion.[12] In the host, HIV spreads not only by cell−cell fusion but also by newly produced virus so that both phenomena are physiologically relevant. Herein we provide strong evidence that a simple chemical modification on a peptide entry inhibitor can permanently trap the HIV gp41 intermediate on the virus surface as well.

The inhibitory efficacy of the covalent peptide inhibitors is similar between viral entry and cell−cell fusion with IC_{50} values for viral entry being slightly lower: ~127−133 nM as compared to ~465−679 nM, respectively. One goal of covalent entrapment of the gp41 intermediate is to elicit novel immune responses in the treated host. The covalent entrapment of virus envelope protein may also better prevent reinfection by newly produced virus particles and slow progression within the host. It is interesting to note that free-floating peptide seems to be able to out-compete the natural substrate (CHR), and this may be due to the other conformational restrictions that are placed on the metastable conformation of HIV gp41; i.e., gp120 association and proximity of the viral and cellular membranes. Covalent entrapment would constitute a type of suicide inhibition also referred to as mechanism-based inhibition. Mechanism-based inhibitors have been successfully utilized in HIV treatment in the case of enzymatic reactions like that of the inhibitor AZT with reverse transcriptase. Interestingly, a peptide derivative of HIV-1 $Pr55^{gag}$ was found to potently inhibit cleavage by viral protease in a mechanism-based way.[45] Mechanism-based inhibitors could also prove to be advantageous in microbicides, permanently disabling the viral entry machinery before contact is made with a host cell. It is also of interest to study if this type of suicide inhibition could lead to lowered resistance, a lower rate of the development of resistance, or different resistance mutations and these studies are ongoing.

One important consideration is the disparity between the observed IC_{50} values with and without the washing step. The IC_{50} values are 2 orders of magnitude higher with washing, rising from low nanomolar without washing to ~127−679 nM with washing. This may be due to a technical issue such as placement of the reactive group, linker length, or the choice of reactive group. Placement of the reactive group has a substantial effect when peptides are studied in isolation; however, differences in IC_{50} in cell culture did not vary dramatically with reactive group placement, suggesting that there likely are variations in proximity during the conformational changes that take place. This is not surprising due to the dynamic nature of the process. It is intriguing to note that the efficacy of covalent modification in the case of synthesized peptides studied *in vitro* by coincubation and reverse phase chromatography was quite predictable based upon analysis of the six-helix bundle structure.[13−17] We found that placement of the covalent moiety directly at the salt bridge between Asp^{632} and Lys^{574} was much more effective at cross-linking the two peptides than at more distant locations in either direction (ref 12 and data not shown). Placement at the N-terminus of the peptide at Trp^{628} (as in peptide A) or near to the C-terminus at Lys^{655} (as in peptide D) demonstrated much less effective cross-linking. The effects of placement were, however, not as predictable, in the virus-cell context which is a better mimic of the physiological situation because the entire envelope complex, the receptors, and membranes are all in place. Indeed, we surprisingly saw very little difference in IC_{50} values with placement at the C-terminus versus near the N-terminus of the peptide. It is important to note that we do not yet have a full atomic level structural picture of either the intermediate state structure or the initial structure of HIV gp41 before contact with the target cell surface. Interestingly, our findings suggest the possibility that there are stages during the conformational changes that occur to HIV gp41 that mediate membrane fusion in which both the N- and C-termini of the C34 sequence are in proximity to Lys^{574}.

It might be possible to increase the potency of the covalent trap (lower the IC_{50}) by adding a longer linker between the peptide and the reactive group. It would be straightforward to add a PEG linker of varying length during the synthesis. However, specificity for reactivity with Lys^{574} might be reduced with longer linker, leading to a mixture of trapped intermediate structures. This would not necessarily be detrimental in the development of therapeutics or in the potential use as an

antigen as mixed populations could elicit a broader neutralizing antibody response. For mechanistic and potential structural studies, it would be advantageous to maintain homogeneity of the trapped intermediate structure.

It might also be beneficial to use a reactive group that reacts more strongly with primary amines than does maleimide, for instance a succinimidyl ester. However, a stronger reactive group can also lead to more rapid degradation during synthetic routes, storage, and reconstitution. Maleimide is a moderately reactive group which makes it a preferred choice for *in vitro* proof-of-concept initiatives including cell culture experiments. In planning drug development and *in vivo* studies, it might indeed be necessary to consider a less reactive group such as a simple peptide (amide) bond because maleimide or more labile reactive groups would be susceptible to rapid degradation due to reaction with cysteine-34 of serum albumin, free cysteine, or glutathione. A less reactive group would give the peptide the potential for greater stability before contact with HIV gp41. However, we do see quite good specificity for gp41 in cell culture medium until we get to very high concentrations.[12] Experimentation with a broader range of reactive moieties is clearly warranted.

Another interesting alternative to consider regarding the disparity in the observed IC_{50} values with and without the washing step is the possibility that the conformational changes that occur to the gp41 molecule mediating membrane fusion are more complex than currently understood and that there is an additional conformational change that leads to further exposure of the NHR after the shift to 37 °C. This is a very intriguing area of research, and further work is ongoing in our laboratories to better understand this process on a structural level.

In this work, we have shown that HIV entry inhibitor peptides modified with a covalently reactive group can be used to trap the gp41 intermediate on the surface of the virus, complementing earlier work we had reported in which we showed this effect in cell−cell fusion experiments. Covalent inhibitors are not only potential candidates for therapeutics and vaccine development but also useful tools for the study of the gp41 intermediate since they are able to permanently trap an intermediate conformation. This is an important advancement which will serve to uncover further details of the conformational changes in HIV envelope, specifically gp41, that allow the virus core to enter the cell and initiate HIV infection.

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ABBREVIATIONS

HIV, human immunodeficiency virus; gp41, glycoprotein 41; AEEA, aminoethoxyethoxyacetic acid; NHR, N-heptad repeat; CHR, C-heptad repeat; CCR5, C-C chemokine receptor type 5; CXCR4, C-X-C chemokine receptor type 4; MPER, membrane proximal external region; TM, transmembrane; 6HB, six-helix bundle; FBS, fetal bovine serum; DMEM, Dulbecco's Modified Eagle Medium; DEAE, diethylaminoethyl; VSV-G, vesicular stomatitis virus G-protein; MOI, multiplicity of infection; TAS, temperature-arrested state.

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