Rational Development of β-Peptide Inhibitors of Human Cytomegalovirus Entry

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Human cytomegalovirus (HCMV) is a widespread and significant pathogen. At present, there is no HCMV vaccine, and the available drugs target only replication events. Thus, new therapeutic strategies are needed. HCMV fusion appears to require interactions of α-helical regions in viral surface glycoproteins gB and gH. Oligomers of β-amino acids (“β-peptides”) are attractive unnatural scaffolds for mimicry of specific protein surfaces, because β-peptides adopt predictable helical conformations and resist proteolysis. Here, we report the development of β-peptides designed to mimic the gB heptad repeat and block HCMV entry. The most potent β-peptide inhibits HCMV infection in a cell-based assay with an IC50 of ~30 μM. Consistent with our structure-based design strategy, inhibition is highly specific for HCMV relative to other related viruses. Mechanistic studies indicate that inhibitory β-peptides act by disrupting membrane fusion. Our findings raise the possibility that β-peptides may provide a general platform for development of a new class of antiviral agents and that inhibitory β-peptides will constitute new tools for elucidating viral entry mechanisms.

Current HCMV antiviral drugs primarily target DNA replication, and these drugs have widely recognized limitations as human therapeutic agents (e.g., toxicity). Because herpesviruses establish a lifelong latent infection, antiviral therapies aimed at prevention of virus entry represent an attractive alternative target for drug development. The anti-AIDS drug Fuzeon acts by blocking HIV entry (6), establishing the clinical viability of this therapeutic approach. However, differences between the HIV entry mechanism and the herpesvirus entry mechanism(s) indicate that the design strategy developed for HIV cannot be directly extrapolated to HCMV and related pathogens.

Herpesviruses employ multiple proteins to induce fusion between the viral envelope and the target cell membrane; in contrast, RNA viruses such as orthomyxoviruses, paramyxoviruses, filoviruses, and retroviruses use a single glycoprotein for membrane fusion (7). Reconstitution experiments from a number of laboratories suggest that initial recognition of target cells by herpesviruses involves a receptor-binding viral protein, which varies from virus to virus, and that fusion is then orchestrated by conserved glycoproteins gB and the gH/gL heterodimer (7, 8). Little is known at present about the molecular mechanism by which multiple herpesvirus glycoproteins induce membrane fusion. In contrast, considerable information is available on the molecular aspects of class I viral entry mechanisms (e.g., the HIV entry mechanism). Extensive structural analysis of class I fusion proteins has revealed conserved paradigms involving α-helical coiled-coil interactions, as in HIV protein gp41 (9). The α-helical coiled-coil is a very common mode of helix association in proteins, observed in both ternary structure (intramolecular) and quaternary structure (intermolecular) contexts (10). Protein segments competent for such interactions display a characteristic seven-residue pattern, the “heptad repeat,” in which hydrophobic side chains occur at the first and fourth positions. The entry machinery of viruses that employ type I fusion proteins relies upon both inter- and intramolecular coiled-coil interactions. These viral fusion proteins are typically organized into a homotrimer via associations mediated by a heptad repeat segment found near the membrane-spanning domain. A second heptad repeat is located distal to the membrane and near a hydrophobic sequence that inserts into the target cell membrane. When such fusion proteins are triggered, the heptad repeat segments fold back upon themselves forming a six-helix bundle. The resulting hairpin structure brings the viral and target cell membranes together. The free energy released upon formation of the six-helix bundle is thought to provide a driving force for membrane fusion (11). Synthetic peptides corresponding to the C-terminal heptad repeat segment of HIV fusion protein gp41 are very potent inhibitors of viral entry (6, 12); Fuzeon is a 36-residue residue segment of gp41 (13).

Lopper and Compton (14) have identified a region in gB and a region in gH with the heptad repeat pattern characteristic of α-helical coiled-coils, and they speculated that coiled-coil interactions might play a role in the HCMV fusion mechanism. Peptides corresponding to these regions blocked HCMV entry and infection (14), although with low
potency. The inhibitory effects were observed for a variety of clinical and laboratory HCMV strains, but the HCMV-based peptides had no effect on infection by mouse CMV or herpes simplex virus (HSV), the glycoprotein heptad repeat sequences of which differ from those of HCMV. This selectivity suggests that the peptides act by forming specific contacts with proteins on the HCMV surface and thereby block coiled-coil interactions required for viral entry. The importance of heptad repeats in the gH protein was recently demonstrated also for HSV. Mirroring the HCMV findings, peptides encompassing a predicted coiled-coil sequence blocked HSV infection (15, 16), albeit weakly.

The identification of coiled-coil segments within herpesvirus proteins required for target cell entry raises the prospect that blocking coiled-coil formation could inhibit infection by these viruses. However, the poor inhibitory activity of peptides containing these coiled-coil sequences shows that the relatively straightforward design strategy underlying the development of Fuzozen as an anti-AIDS drug will not be successful for herpesviruses. We have therefore begun to explore an alternative and unprecedented strategy for disrupting coiled-coil interactions in the hope of identifying a viable approach for blocking herpesvirus entry. Our strategy employs “foldamers,” oligomers that are built up from unnatural subunits and that display discrete folding patterns (17–19). Coiled-coil formation and other protein-protein interactions bury large molecular surfaces, and such interactions have proven difficult to inhibit via small molecule-based approaches (20). The predictability of the shapes adopted by foldamers, and the ability to vary foldamer size in an incremental manner, make this family of molecules attractive for development of protein-protein interaction antagonists.

Here, we describe the development of β-amino acid oligomers (“β-peptides”) that block HCMV entry. We use a specific β-peptide secondary structure, the 12-helix (21) (defined by 12-membered ring C=O(i) → H-N(i+3) hydrogen bonds), to mimic putative α-helical segments within the HCMV gB glycoprotein (the α-helix is defined by C=O(i) → H-N(i+4) hydrogen bonds). High resolution structural data for 12-helical β-peptides (21, 22) suggest that this unnatural secondary structure is a good mimic of the α-helix in terms of overall shape. Three features of β-peptides make them attractive with regard to biomedical applications. First, their oligomeric nature makes them combinatorial design straightforward. Second, they can be engineered to display very high conformational stability (18, 22), which should minimize the entropic cost of binding to a target. Third, they are resistant to proteolytic degradation (23). The first feature is shared by conventional peptides, i.e. those composed of α-amino acids, but the other two features constitute advantages for β-peptides relative to conventional peptides. Helix-forming β-peptides have been shown to mimic the prokaryote-specific membrane disruption effects of α-helical host-defense peptides (24–26), and β-peptides with various folding propensities have been shown to interact with proteins or nucleic acids in biochemical assays (27–30). In all of these prior examples, the β-peptide is less active than the α-peptide to which it is compared. In contrast, we have identified a β-peptide inhibitor of HCMV entry that is more active than the gB-derived α-peptide we intend to mimic. Further, our findings constitute the first evidence for target-specific β-peptide activity in a cellular context.

**MATERIALS AND METHODS**

**β-Peptide Synthesis**—β-Peptides were prepared by standard manual Fmoc (N-(9-fluorenyl)methoxycarbonyl) solid-phase methods. (See supplemental material for details.)

**Cell Lines, Viruses, and Antibodies**—Normal human dermal fibroblasts (NHDFs) and NIH3T3 cells were cultured in Dulbecco’s modified Eagle’s medium, supplemented with 10% fetal bovine serum, 1% l-glutamine, and 1% penicillin-streptomycin. The AD169 strain of HCMV was propagated in NHDFs and purified as previously described (31). HCMV-GFP indicator virus encodes GFP regulated as an immediate early protein and was kindly provided by Deborah H. Spector (University of California, San Diego) (32). Murine CMV-EGFP (strain RVG102), with EGFP driven by an immediate early 1/3 promoter was a gift from A. Campbell (Eastern Virginia Medical College, Norfolk, VA); the virus was propagated in NIH3T3 fibroblasts. Herpes simplex virus (HSV-1(KOS)gL86), containing an *Escherichia coli lacZ* reporter gene, was a generous gift from Rebecca Montgomery (University of Wisconsin, Madison) (33); the virus was grown in 79BV4 cells. Vesicular stomatitis virus pseudotyped with G protein and containing a GFP marker (VSV-ΔG) was a kind gift from Yoshihiro Kawaoka (34) (University of Wisconsin, Madison) (34). Monoclonal antibody against the major tegument phosphoprotein pp65 was purchased from the Rumbaugh-Goodwin Institute for Cancer Research, Inc. Alexa Fluor® 488 goat anti-mouse secondary antibody was purchased from Molecular Probes (Eugene, OR). The 27-78 antibody against glycoprotein B (gB) was a kind gift from William Britt (35). The use of polyclonal 6824 antibody against glycoprotein H (gH) was previously described (36). The goat anti-mouse (horseradish peroxidase-linked) and goat anti-rabbit horseradish peroxidase secondary antibodies were purchased from Pierce.

**Viruses Entry Assay**—Lypophilized β-peptides were dissolved in filter-sterilized de-ionized H2O. The concentration of individual β-peptides and α-peptides was calculated based on absorbance (275 nm) measured with DU®530 spectrophotometer (Beckman, Fullerton, CA). Extinction coefficients were calculated based on information available on the Oregon Medical Laser center web site (omlc.ogi.edu/spectra/PhotochemCAD/html/alpha.html). A precipitate formed upon addition of some β-peptide stock solutions to cell culture medium while others (e.g. 19) did not lead to precipitate formation. Because only some inactive and active β-peptides displayed precipitation, we concluded that this phenomenon is not related to HCMV entry inhibition. Cells were grown in 12-well plates and infected with the indicated virus (multiplicity of infection of 0.5 plaque forming units/cell). Controls for HCMV-GFP and MCMV-EGFP entry were prepared by pretreating virions with heparin (30 μg/ml) as described previously (37). To inhibit VSV infection, cells were treated with 30 mM NH4Cl. For flow cytometric detection of GFP expression, cells were recovered by trypsinization and centrifugation. The samples were analyzed on a FACScan flow cytometer (BD Biosciences) with a standard filter set. The cells were gated for propidium iodide exclusion (live cells) and assayed for GFP content. The data were analyzed using FlowJo (version 6.1, Tree Star Inc., Ashland, OR). Inhibition data were normalized to percent control infection. Active β-peptides were synthesized independently several times; distinct samples displayed similar activities. For the HSV-1 entry assay, a confluent monolayer of NHDFs cells was grown in a 96-well plate and infected with HSV-1(KOS)gL86 as described above. At 6 h post infection, the cells were lysed in buffer (100 mM NaH2PO4, 10 mM KCl, 1 mM MgSO4, 0.1% Nonidet P-40). O-Nitrophenyl-β-D-galactopyranosidase was added to 2.3 mg/ml, and the mixture was incubated at 25 °C for 6 h. Then absorbance at 420 nm was measured using SpectraMax® 190 spectrophotometer (Molecular Devices, Sunnyvale, CA). The assay was set up in quadruplicate and performed three times. The peptide inhibition data were normalized to the level of control infection.

**pp65 Translocation Assay**—NHDF cells were grown on glass coverslips in 12-well plates as above. The HCMV was diluted with 100 μM
β-peptide in SF-Dulbecco’s modified Eagle’s medium and cooled to 4 °C. The treatment was then added to cooled cells, which were incubated at 4 °C for 90 min, assuring viral attachment but not entry. The cells were then transferred to 37 °C for 35 min. The cells were then fixed in 3% paraformaldehyde and immunostained for pp65 as described (14). Images were taken on the Nikon Eclipse TE2000-S with appropriate filters, using consistent exposure times.

**FIGURE 1.** A, structures of β-amino acids. B, flat projection of the α-helix (closed circles and bold letters) and 12-helix (open circles and italicized letters) and their superposition; circles indicate the positions of the α-carbons (closed circles) or α- and β-carbons (open circles), respectively. C, structures of selected first-generation compounds.

**FIGURE 2.** Characterization of first-generation β-peptides in a cell-based infectivity assay. HCMV-GFP was inoculated onto NHDF cells in the presence of β-peptides, and the infection was allowed to proceed for 2 h before unfused extracellular virions were inactivated with citrate buffer. A mock trial was included in each experiment, for which cells were subjected to assay procedures without exposure to virus or inhibitors. HCMV-GFP treated with heparin, a known inhibitor of HCMV entry, was used as a basis for comparison. The cells were analyzed by flow cytometry, assaying for propidium iodide (PI) exclusion and GFP content. The absence of PI staining indicated live cells, and the presence of GFP fluorescence signified a productive HCMV-GFP infection. The height of the gray bar on the chart signifies the percentage of live cells; the height of the black bar indicates the percentage of HCMV infection.

**TABLE 1**

| Peptide | $X_2$ | $X_7$ | $X_{12}$ | Inhibition (100 μM)a |
|---------|-------|-------|----------|----------------------|
| 3       | ββ'-Leu | ββ'-Phe | ββ'-Leu | 6.4                  |
| 4       | ββ'-Leu | ββ'-Phe | ββ'-Ile  | 7.5                  |
| 5       | ββ'-Leu | ββ'-Phe | ββ'-Phe  | 10.5                 |
| 6       | ββ'-Leu | ββ'-Phe | ββ'-1(Nap) | Toxic               |
| 7       | ββ'-Leu | ββ'-Phe | ββ'-2(Nap) | Toxic           |
| 8       | ββ'-Leu | ββ'-Phe | ββ'-Tyr  | 9.6                  |
| 9       | ββ'-Leu | ββ'-1(Nap) | ββ'-Val  | Toxic               |
| 10      | ββ'-Leu | ββ'-2(Nap) | ββ'-Val  | Toxic               |
| 11      | ββ'-Leu | ββ'-Tyr  | ββ'-Val  | 13.2                 |
| 12      | ββ'-Leu | ββ'-Leu  | ββ'-Val  | 20.2                 |
| 13      | ββ'-Phe | ββ'-Phe  | ββ'-Val  | 22.1                 |
| 14      | ββ'-2(Nap) | ββ'-Phe | ββ'-Val  | 52.2                 |
| 15      | ββ'-1(Nap) | ββ'-Phe | ββ'-Val  | 63.8                 |
| 16      | ββ'-Tyr  | ββ'-Phe  | ββ'-Val  | 5.9                  |
| 17      | ββ'-Ile  | ββ'-Phe  | ββ'-Val  | 6.8                  |
| 18      | ββ'-1(Nap) | ββ'-1(Nap) | ββ'-Val  | 77.9                 |
| 19      | ββ'-2(Nap) | ββ'-1(Nap) | ββ'-Val  | 93.4                 |
| 20      | ββ'-Trp  | ββ'-1(Nap) | ββ'-Val  | 73.2                 |
| 21      | ββ'-1(Nap) | ββ'-Trp  | ββ'-Val  | 29.7                 |
| 22      | ββ'-2(Nap) | ββ'-Trp  | ββ'-Val  | 25.5                 |
| 23      | ββ'-Trp  | ββ'-Trp  | ββ'-Val  | 26.1                 |

aData here are presented as percent inhibition. Data in other figures are presented as percent control infection.
RESULTS

β-Peptide Inhibitor Design and Evaluation—Our β-peptide design effort focused on mimicry of the heptad repeat region previously identified in HCMV gB. No high resolution structural data are available for gB; therefore, we relied upon an idealized α-helical model for the segment to be mimicked. We envisioned a 12-helical β-peptide inhibitor that would display along one face a set of side chains matching those thought to contribute to interhelical interactions of the gB protein, i.e. the nonpolar side chains in gB that have the characteristic coiled-coil spacing (Leu-679, Ile-682, Phe-686, Tyr-689, and Val-693) (14). Formation of the 12-helix requires α-amino acid residues with a five-membered ring constraint, such as trans-aminocyclopentane carboxylic acid and trans-3-aminopyrrolidine-4-carboxylic acid (Fig. 1A) (18, 38, 39).

We used a comparative α-helical/12 helical net analysis to design an initial set of compounds (Fig. 1B). The α-helical net is a flat projection of the α-helix that illustrates the spatial relationship among side chain attachment points along the peptide backbone in an α-helical conformation (40). Analysis of a heptad repeat sequence reveals a continuous stripe of nonpolar side chains along one side of the α-helix; these side chains occupy the first and fourth positions of each heptad repeat. The 12-helix has 2.5 residues per turn (18), and 12-helical net analysis suggests that a stripe of hydrophobic side chains would be created by repeating pentads in which the first and third residues bear nonpolar side chains. Such a 12-helix should display a hydrophobic surface mimicking that of an α-helical heptad repeat segment, which could lead to an inhibition of biomolecular processes that require coiled-coil interactions.

Overlaying the α-helical and 12-helical nets predicted that a β-peptide of 13 residues would mimic the heptad repeat segment of gB. This overlay identified side-chain positions within a 12-helical β-peptide that would most closely approximate the set of five key gB side chains as projected from an α-helix. There are two possible side-chain attachment points in a β-amino acid residue (β² versus β³; Fig. 1A), an element of variability that does not exist among α-peptides. The helical net over-

FIGURE 3. Characterization of selected second-generation β-peptides. A, three β-peptides (3, 15, and 19) in addition to 1 were further analyzed. B, dose-response experiments for β-peptides showed in A. The HCMV-GFP infection was performed in the presence of β-peptides as described. Inhibition data were normalized to control infection. Representative dose curves are shown. β-Peptide 15 and 19 showed dose-dependent inhibition of HCMV entry, with β-peptide 19 being the most potent. The IC₅₀ for 19 is 30 μM, with a 95% confidence interval of 22–40 μM (determined by data analysis with GraphPad Prism 4 software), based upon three independent measurements. C, comparison of active β-peptides to α-peptides. HCMV-GFP was incubated with 100 μM of each α- or β-peptide, and the samples were assayed for cell viability and infectivity. At a concentration of 100 μM, the α-peptides had no detectable activity, whereas 15 showed 60% inhibition and 19 blocked infection to the level of the heparin control.

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lay clearly predicted the sequence positions for side-chain installation along the β-peptide, but this overlay did not allow distinction among several alternative $\beta^1$/$\beta^3$ patterns. Empirical tests were used to resolve this issue.

We prepared an initial set of isomeric β-peptides that differed from one another in $\beta^1$ versus $\beta^3$ attachment of side chains intended to mimic Leu-679 and Tyr-689 (supplemental Fig. S6). Four of the side chains on these β-peptides match perfectly the corresponding gB side chains; synthetic constraints required the use of $\beta^3$-homoleucine rather than $\beta^3$-homoisoleucine at the position intended to mimic Ile-682 of gB. These compounds were evaluated for inhibition of HCMV entry in a cell-based infectivity assay. A single compound (1, Fig. 1C) that blocked HCMV infection was identified (Fig. 2). HCMV (multiplicity of infection = 0.5 plaque forming units/cell) incubated in the absence of inhibitors resulted in 60% total infected fibroblasts. In the presence of 500 $\mu$M 1, the proportion of infected cells was reduced to 20%. No evidence of toxicity could be detected at this high concentration of 1. More detailed analysis revealed an IC$_{50}$ of $\sim$300 $\mu$M for inhibition of HCMV infection by 1 (data not shown).

Control experiments were conducted to test the structural hypothesis underlying our β-peptide design. Replacement of large nonpolar side chains with a methyl group, by substituting $\beta^2$- or $\beta^3$-homolaeanine at those positions, led to a substantial reduction of anti-HCMV activity. For example, no inhibition of HCMV infection was detected for β-peptide 2 (Fig. 2); this finding suggests that the hydrophobic side chains of 1 are critical for activity. We also investigated a sequence isomer of 1 (1-scr), in which the residues are scrambled. In the 12-helical conformation, 1-scr does not display the five side chains in a manner that mimics the putative α-helical display of gB. β-Peptide 1-scr proved to be highly toxic toward fibroblasts, in contrast to 1, which precluded the examination of 1-scr as a potential negative control compound. The origin of this toxicity is unclear; experiments with human red blood cells (supplemental Fig. S7) indicate that 1-scr does not simply disrupt cell membranes.

A set of twenty-one second-generation β-peptides was prepared, including fifteen compounds with a single $\beta^2$-residue change relative to 1 and six compounds with two $\beta^2$-residue changes (Table 1). Several of these new compounds were significantly more active than 1. The trends indicate that large, aromatic chains at position 2 or at both positions 2 and 7 enhance fusion inhibition, whereas, curiously, placement of such side chains at position 7 alone or position 12 alone leads to fibroblast toxicity. Three of these β-peptides were selected for further analysis (Fig. 3A). Dose-response experiments demonstrated that the most active β-peptide inhibitor, 19, had an IC$_{50}$ of $\sim$30 $\mu$M in the infectivity assay, a 10-fold improvement over the activity of 1 (Fig. 3B). At 100 $\mu$M, β-peptide 19 allows only $\sim$10% infection; in stark contrast, the α-peptide segments derived from gB are inactive at 100 $\mu$M (Fig. 3C).

Specificity of β-Peptide Inhibitors—Our β-peptide design is aimed at mimicry of the heptad repeat segment of the HCMV gB protein, a region that is invariant in over 50 clinical and laboratory strains of HCMV. The corresponding segments within glycoproteins of other herpesviruses, on the other hand, have different heptad repeat sequences (14). We tested our design hypothesis by assessing the ability of β-peptides 1, 3, 15, and 19 to block infection by two other herpesviruses, including the closely related MCMV and a more distantly related herpesvirus, HSV. The β-peptides active against HCMV display no detectable inhibitory activity against these viruses (Fig. 4). In addition, we observed no activity against an unrelated virus, VSV (supplemental Fig. S8). The high viral selectivity of the β-peptides suggests that inhibition of HCMV entry results from a very specific interaction between these foldamers and a component of the HCMV particle and supports the feasibility of a structurally tailored design strategy to achieve viral specificity.

β-Peptide Inhibitors Target Membrane Fusion—The infectivity assays used in the experiments described above measure immediate early gene expression; IE proteins are the first viral proteins expressed in infected cells. Inhibition of viral gene expression could reflect interference at a variety of points in the virus life cycle such as inhibition of IE gene transcription or translation. We performed a virion content delivery assay to test whether our β-peptides act at the viral entry stage, as they have been designed to do. Immediately upon membrane fusion, the phosphoprotein-rich tegument layer of the virus is released into the cytoplasm of the target cell. The pp65 protein, highly abundant in the virion tegument, diffuses rapidly to the nucleus after membrane fusion. Thus, nuclear localization of pp65 can be used to assess membrane fusion activity and rule out alternative mechanisms of β-peptide action. As in the infectivity assays, exposure of fibroblasts to soluble heparin serves as a positive control for viral entry inhibition: this treatment eliminates pp65 accumulation in the nucleus (Fig. 5). Similarly, the most potent β-peptide inhibitor, 19, blocked nuclear localization of pp65, whereas inactive β-peptides 1 and 3 had no effect on pp65 uptake. This observation indicates that the active β-peptides inhibit HCMV infection at the level of virus–cell membrane fusion.
DISCUSSION

Our results suggest that β-peptides inhibit HCMV entry into target cells by interacting with viral fusion machinery. We propose that this inhibitory effect arises from the adoption by the β-peptides of a folded conformation, the 12-helix, which generates a specific side-chain arrangement that allows recognition of at least one target protein. Two-dimensional NMR data (supplemental Fig. S9) for 1 indicate a substantial 12-helical propensity. Our working hypothesis to explain HCMV fusion inhibition is based on the assumption that entry requires the gB protein, on the virion surface, to be initially triggered to adopt a fusion-active conformation by interaction with cellular receptors. We further assume that the heptad repeat segment of gB is exposed in the fusion-active conformation and that this segment must associate with the heptad repeat segments of other fusion-active gB protein molecules and/or with the heptad repeat segment of gH in order for fusion of the viral envelope with the cell membrane to proceed. We propose that the β-peptide binds to the heptad repeat segment of gB or gH in the fusion-active conformation, blocking homo- and/or hetero-protein-protein associations required for fusion. Because no structural information is yet available for gB or other HCMV glycoproteins, the β-peptide inhibitors we have developed may be useful research tools for further elucidation of the fusion mechanism.

FIGURE 5. β-Peptide inhibitors target HCMV membrane fusion. HCMV was pre-bound to NHDF cells at 4 °C in the presence of 100 μM of the indicated β-peptide for 2 h. At 4 °C, HCMV will bind to cells but not enter or fuse. The cultures were subsequently shifted to 37 °C and allowed to progress for 35 min to allow fusion and content delivery. pp65 was detected by immunohistochemistry as described. Representative images were taken at similar exposures. A, the control infection images show pp65 presence in the nucleus (nuclei indicated by DAPI stain). Heparin pretreatment of HCMV results in lack of pp65 delivery to cells. B, β-peptide 3 (100 μM), shown previously to be ineffective against HCMV, does not affect pp65 uptake. In contrast, potent β-peptides 19 shows a dramatic effect on pp65 uptake into cells.
The apparent success of our structure-based design strategy raises the prospect that \( \beta \)-peptides and other foldamers (17, 19, 41) will provide a general and powerful platform for the development of new antiviral agents. Evaluation of a relatively small number of 13-residue \( \beta \)-peptides allowed us to identify HCMV entry inhibitors that are substantially more potent than much longer gB-derived \( \alpha \)-peptides. The \( \beta \)-peptides have the further advantage, relative to \( \alpha \)-peptide inhibitors, of resistance to proteolytic degradation (23). Now that we have provided evidence for the feasibility of foldamer-based inhibition of HCMV entry, it will be important to explore additional 12-helical \( \beta \)-peptides and gB heptad repeat mimics based on alternative helical foldamer backbones (41) in an effort to achieve enhanced potency.

The significance of our foldamer-based approach for generating inhibitors of HCMV fusion is highlighted by the very poor inhibitory activity observed for \( \alpha \)-peptides derived from HCMV proteins gB and gH. In stark contrast, gp41-derived peptides show sufficient potency in blocking HIV entry to allow direct clinical application (Fuzeon). The inadequacy of \( \alpha \)-peptide inhibitors of HCMV and HSV entry suggests that a more sophisticated strategy will be required for development of fusion inhibitors effective against herpesviruses and other refractory pathogenic viruses. The ready application of combinatorial synthesis methods to \( \beta \)-peptides (42) and other foldamers should facilitate the discovery of new antiviral agents.

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