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Inhibiting HIV Fusion with a β-Peptide Foldamer

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Linear peptides derived from the HIV gp41 C-terminus (C-peptides), such as the 36-residue Fuzeon, are potent HIV fusion inhibitors.[1] These molecules bind to the N-peptide region of gp41 and act as dominant negative inhibitors of an intramolecular protein−protein interaction that powers fusion of the viral and host cell membranes.[2−4] The gp41 N-peptide region contains a surface pocket[3−5] that is less prone to mutation than other gp41 regions or HIV enzymes.[6] This pocket is occupied in the post-fusion state by three α-helical residues found near the gp41 C-terminus: Trp628, Trp631, and Ile635; together, these residues comprise the WWI epitope.[3−5] Simple[7,8] and constrained[9,10] α-peptides, aromatic foldamers,[11] peptide−small molecule conjugates,[12] and small molecules[13] that bind this pocket inhibit gp41-mediated fusion. Here, we describe a set of β^3-decapeptides, ***β*WWI-1−4**, in which the WWI epitope is presented on one face of a short 14-helix (Figure 1).[14] ***β*WWI-1−4** bind to a validated gp41 model *in vitro* and inhibit gp41-mediated fusion in cell culture. Our work suggests that β-peptide 14-helices, which are likely to be metabolically stable and protease resistant,[15−17] can function as *in vivo* inhibitors of intramolecular protein−protein interactions.[18]

We synthesized[19] four β^3-peptides (***β*WWI-1−4**) containing the WWI epitope in both possible orientations on each available face of a β^3-decapeptide[14] possessing significant 14-helix stability in aqueous solution due to electrostatic macrodipole stabilization[20] and side chain−side chain salt bridges.[21,22] We also prepared ***β*WAI-1** as a control, as previous work has documented the significant contribution of the central Trp631 to gp41 affinity and viral infectivity.[7] The circular dichroism spectra of ***β*WWI-1−4** and ***β*WAI-1** all display the expected minima at 214 nm (Figure 2A).[14,20,23] The spectra of ***β*WWI-1−4**, but not ***β*WAI-1**, also show a transition at 227 nm, which may result from distortions in the 14-helix or the presence of two tryptophan residues in close proximity.[24] Two-dimensional NMR spectroscopy in CD_3OH confirmed the presence of 14-helix structure in ***β*WWI-1**; NOESY spectra showed five of seven possible $C_\alpha(i) \rightarrow C_\beta(i+3)$ NOEs and three of six possible ($C_N(i) \rightarrow C_\beta(i+3)$ NOEs. No NOEs inconsistent with 14-helical structure were observed.[19]

Each β-peptide was fluorescently labeled[19] at the N-terminus and used in direct fluorescence polarization (FP) experiments to determine its affinity for the gp41 model IZN17.[25] IZN17, which exists as a stable trimer in solution,[25] contains 24 residues of an isoleucine zipper[26] fused in register to 17 residues from gp41 containing the pocket for the WWI epitope.[25] All four β-peptides, ***β*WWI-1−4**^{Flu}, bound IZN17 well, with equilibrium affinities of

	X_3	X_6	X_9	X_2	X_5	X_8
βWWI-1	I	W	W	V	V	V
βWWI-2	V	V	V	I	W	W
βWWI-3	V	V	V	W	W	I
βWWI-4	W	W	I	V	V	V
βWAI-1	I	A	W	V	V	V

Figure 1. Sequences of ***β*WWI-1−4** and ***β*WAI-1**. β^3-homoamino acids are identified by the single letter code used for the corresponding α-amino acid. O signifies ornithine.

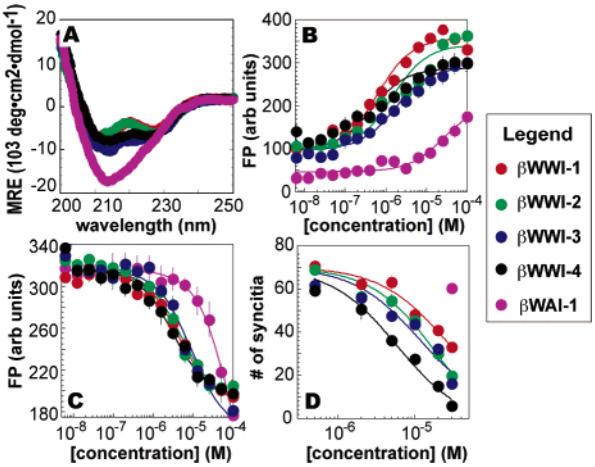

Figure 2. (A) CD spectra of ***β*WWI-1−4** and ***β*WAI-1** at 5 μM in PBC buffer. (B) Fluorescence polarization analysis of the binding of IZN17 and (C) the inhibition of **C14wt**^{Flu}•IZN17 complexation by ***β*WWI-1−4** and ***β*WAI-1**. (D) Inhibition of syncytia formation by ***β*WWI-1−4** and ***β*WAI-1**.[19]

0.75 ± 0.1, 1.0 ± 0.3, 2.4 ± 0.7, and 1.5 ± 0.4 μM, respectively (Figure 2B). Interestingly, in this case, IZN17 affinity is relatively insensitive to the orientation of the WWI epitope relative to either the 14-helix macrodipole or the salt-bridging face.[14] The affinity of ***β*WWI-1−4** for IZN17 is nearly identical to that of the highest affinity α-peptide of comparable size (K_d = 1.2 μM).[10] Also, ***β*WWI-1** binds IZN17 with significantly higher affinity than it binds carbonic anhydrase II ($K_d \geq 115$ μM) or calmodulin ($K_d > 100$ μM), two globular proteins that recognize hydrophobic and/or helical molecules.[19]

Two experiments were performed to investigate the binding mode of ***β*WWI-1−4**. First we performed competition fluorescence polarization experiments to assess whether ***β*WWI-1−4** competed with **C14wt**^{Flu} (suc-MTWMEWDREINNYTC^{Flu}), a fluorescent analogue of a gp41 ligand[10] that binds IZN17 with an affinity of 4.1 μM. ***β*WWI-1−4** competed well, with IC_{50} values of 4.0 ± 0.7, 4.6 ± 0.4, 13 ± 4.1, and 3.3 ± 1.4 μM, respectively (Figure

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2C). We also synthesized the **βWWI-1** analogue **βWAI-1** containing alanine in place of the central tryptophan of the WWI epitope. **βWAI-1**^{Flu} bound IZN17 with lower affinity ($K_d \geq 20\ \mu M$) than **βWWI-1** and **βWAI-1** and competed poorly with **C14wt**^{Flu} for IZN17 ($IC_{50} = 72.9 \pm 5.0\ \mu M$).[27] These data suggest that the affinity of **βWWI-1−4** for IZN17 results from interactions between the WWI epitope and the targeted IZN17 pocket.

βWWI-1−4 were then evaluated for their ability to inhibit gp41-mediated cell−cell fusion in an assay that accurately predicts potency in HIV infectivity assays.[9] HeLa cells that express CD4 and a *tat* inducible β-gal gene[28] were co-cultured in the presence of varying concentrations of β-peptides with HXB2 Env-expressing CHO cells[29] that express HIV-1 *env*, *tat*, and *rev*. Without inhibitors, these cells fuse and form syncytia that express β-galactosidase and can be detected with 5-bromo-4-chloro-3-indoyl-β-D-galactoside.[28] β-Peptides **βWWI-1−4** inhibited cell−cell fusion with EC_{50} values of 27 ± 2.5, 15 ± 1.6, 13 ± 1.9, and $5.3 \pm 0.5\ \mu M$, respectively, whereas **βWAI-1** was inactive (Figure 2D).[19] The EC_{50} values measured for **βWWI-1−4** are equal if not better than those measured for L-peptides,[10] cyclic D-peptides,[9] aromatic foldamers,[11] or small molecules.[13] Although less potent than Fuzeon ($IC_{50} = 0.11$ nM),[1] **βWWI-1−4** are one-third the size, likely metabolically stable,[15] and can be optimized combinatorially. These results suggest that molecules such as **βWWI-1−4** could represent leads toward inhibitors or antigens effective against HIV or other viruses, such as SARS,[30] Ebola, HRSV, and influenza,[31] that employ common fusion mechanisms.

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Supporting Information Available: β-peptide synthesis and binding and cell fusion assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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