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Identification of a β³-Peptide HIV Fusion Inhibitor with Improved Potency in Live Cells

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Abstract

We recently reported a β^3-decapeptide, βWWI-1, that binds a validated gp41 model *in vitro* and inhibits gp41-mediated fusion in cell culture. Here we report six analogs of βWWI-1 containing a variety of non-natural side chains in place of the central tryptophan of the WWI-epitope. These analogs were compared on the basis of both gp41 affinity *in vitro* and fusion inibition in live, HIV-infected cells. One new β^3-peptide, βWXI-a, offers a significantly improved CC_{50}/EC_{50} ratio in the live cell assay.

Linear peptides derived from the C-terminus of HIV-1 gp41 (C-peptides) are potent HIV fusion inhibitors[1]. These molecules bind to the gp41 N-peptide region and inhibit an intramolecular protein-protein interaction that drives fusion of viral and host cell membranes[2–4]. Previous work has shown that the protein-protein interface consists of a highly conserved pocket on the N-peptide surface that is occupied by three C-peptide side chains: Trp-628, Trp-631 and Ile-635[3–5]. These three residues comprise the WWI epitope[3–5]. Simple[6–9] and constrained[10–13] α-peptides, aromatic foldamers[14], peptide-small molecule conjugates[15], and small molecules[16, 17] that bind this N-peptide surface pocket inhibit gp41-mediated cell fusion with IC_{50} values ranging from 250 pM for α-peptides to 5 μM for small molecules. We previously reported a set of β^3-decapeptides that present a WWI epitope on one face of a salt bridge[18–21] and macrodipole-stabilized[22] 14-helix[23, 24]. One of these molecules, βWWI-1, binds a validated gp41 model *in vitro* and inhibits gp41-mediated fusion in cell culture[25]. Past work by Chan and co-workers[6] demonstrated the importance of the three epitope residues, particularly the central tryptophan, in both gp41 affinity and viral infectivity. Here we report six analogs of βWWI-1 containing a variety of nonnatural side chains in place of the central tryptophan of the WWI-epitope. These analogs were compared on the basis of both gp41 affinity *in vitro* and fusion inibition in live, HIV-infected cells. One new β^3-peptide, βWXI-a, offers a significantly improved CC_{50}/EC_{50} ratio in the live cell assay.

We synthesized a small collection of β^3-decapeptides (βWXI-a—f) containing a variety of nonnatural side chains in place of the central tryptophan of the WWI-epitope (Figure 1). These nonnatural residues included those with both entended or alternative π-systems (βWXI-b,d)

and halogen-substituted aromatic rings (βWXI-a,c,e,f) to probe the steric and electronic requirements of the N-peptide surface pocket in the context of a β^3-peptide. βWWI-1, a previously described β-peptide HIV fusion inhibitor[25], was synthesized as a positive control.

Each β-peptide was labeled at the N-terminus with 6-(fluorescein-5(6)-carboxamido) hexanoic acid N-hydroxy-succinimidyl ester (Flu) and employed in a direct fluorescence polarization (FP) assay to determine its affinity for IQN17, a fusion protein containing 17-residues from the gp41 N-terminus joined to a 29 residue isoleucine zipper[10]. IQN17 exists as a stable trimer in solution[10] and effectively recreates the N-peptide surface pocket for C-peptide-like ligands. β-peptides βWXI-a-f^{Flu} bound IQN17 with equilibrium dissociation constants between 12.1 μM (βWXI-d) and 105.4 mM (βWXI-b) (Table 1, Figure 1A). With the exception of pyridyl-containing βWXI-b, all new β-peptides bound IQN17 about as well as βWWI-1 (K_D = 16.5 ± 0.6 μM). These results are significant if not surprising, given the loss off affinity that typically results from altering the central tryptophan residue[6, 25].

All seven β-peptides were evaluated for the ability to promote cell survival in an MTT colorimetric assay[26, 27]. In this method, MT-2 human T-cells are plated with varying concentrations of β-peptide inhibitor and cultured with wild-type HIV-1 IIIB[28–30]. After 5 days incubation, the number of live cells that remain is determined by addition of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). MTT is reduced in the mitochondria of live cells to formazan (λ_{max} = 595 nm) and quantified by UV. The EC_{50} values reported represent the β-peptide concentration required to achieve 50% survival of infected cells (Figure 2; Table 1).

The EC_{50} values of β-peptides βWXI-a through f vary between 8.2 μM (βWXI-d) and > 250 μM (βWXI-b). With the exception of βWXI-b, which is inactive (EC_{50}> 250 μM), all of the new β-peptides (8.2 μM ≤ EC_{50} ≤ 19 μM) are more potent than βWWI-1 (EC_{50} = 56 μM) at promoting the survival of HIV-infected cells. Interestingly, two of the most potent new β-peptides (βWXI-c and f) share little structural similarity, with halogen substituents at *para*- and *ortho*- positions, respectively. βWXI-a and e, with EC_{50} = 18–19 μM, share a fluorine-containing substituent at the *meta* position of the phenyl side chain.

We also compared the new β-peptides in terms of cytotoxicity, determined as the viability of uninfected cells in the presence of inhibitor alone (Figure S1, Table 1). The CC_{50} values reported represent the β-peptide concentration required to inhibit MT-2 cell growth by 50%. CC_{50} values range from 31 μM (βWXI-f) to > 250 μM (βWXI-b), with a value of 100 μM for βWWI-1. Interestingly, although βWXI-d and f are characterized by the lowest EC_{50} values, each was cytotoxic at concentrations close to this value, with CC_{50}/EC_{50} ratios less than 4. Importantly, one new β-peptide, βWXI-a, exhibits an CC_{50}/EC_{50} ratio of 8, representing a significant improvement relative to βWXI-1 as well as βWXI-c-f.

The ability of βWXI-a to bind IQN17 and inhibit fusion in the MTT assay may be partially rationalized by a simple model in which the indole side chain of the central tryptophan is replaced by the central aromatic side chains of our β-peptides (Figure 3). A crystal structure of the gp41 fusion peptide solved by Sia *et. al.*[11] depicts the epitope-containing β-peptide C14linkmid bound to IQN17 and clearly shows association between the indole side chain and the N-peptide surface pocket. Substitution of the Trp indole ring of C14linkmid with the *m*-trifluoromethylphenyl side chain in βWXI-a suggests that the trifluoro-methylbenzene side chain is a reasonable structural mimic of the indole ring, whereas the 3-pyridyl side chain is not. Although βWXI-a is not as potent as Fuzeon in the MTT assay (EC_{50} = 37.5 nM), it has a significantly lower mass (1457 Da *vs.* 4492 for Fuzeon), and higher metabolic and proteolytic stablity[31–35]. Furthermore, due to the ability of the 14-helical scaffold to tolerate changes to

the epitope face, it may be possible to identify β^3-peptides with further improved activity and decreased toxicity through combinatorial optimization[36, 37].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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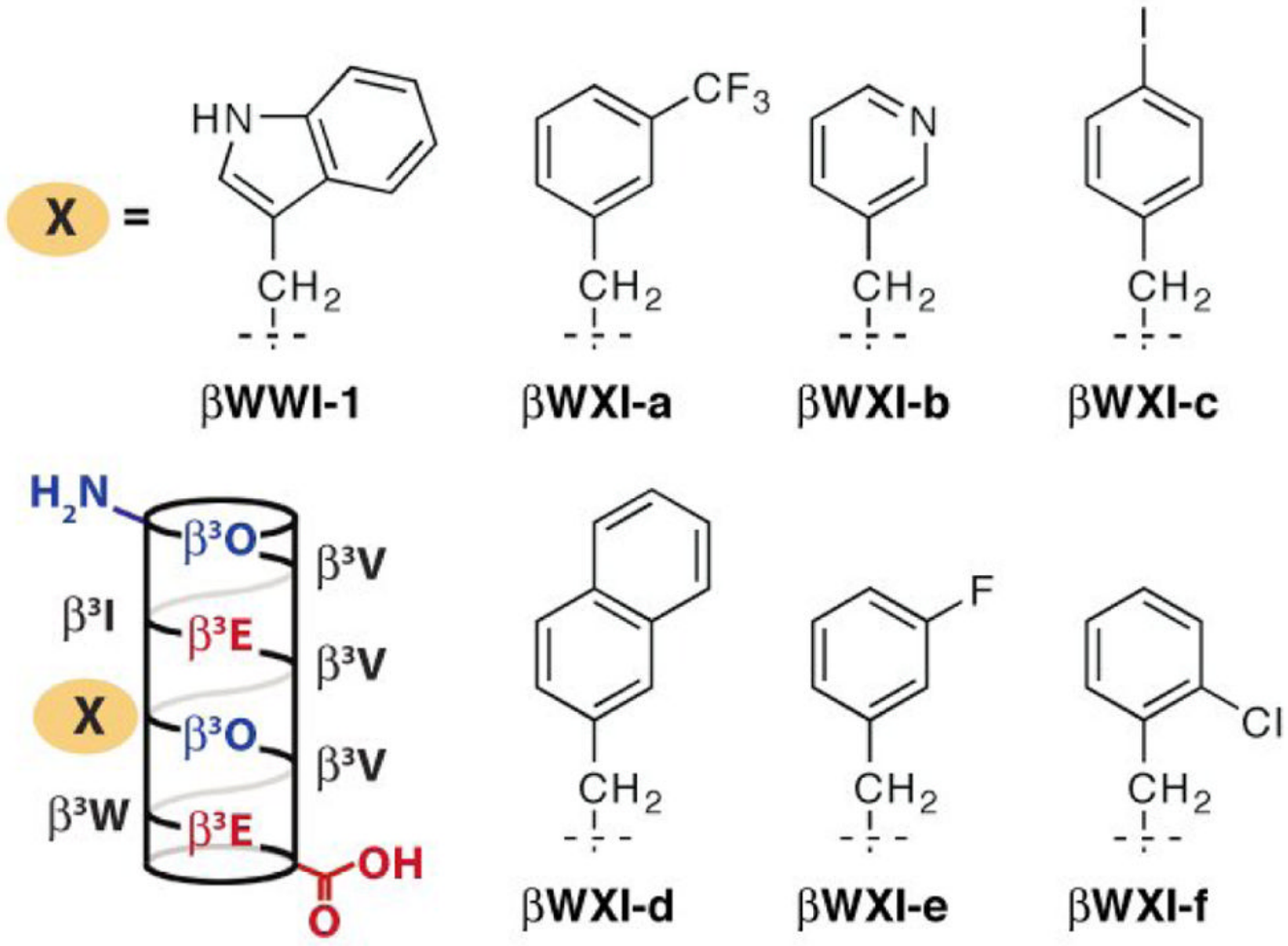

Figure 1.
Helical net representations of βWWI-1[25] and βWXI-a—f. β^3-homoamino acids are identified by the single letter code used for the corresponding α-amino acid. O represents ornithine.

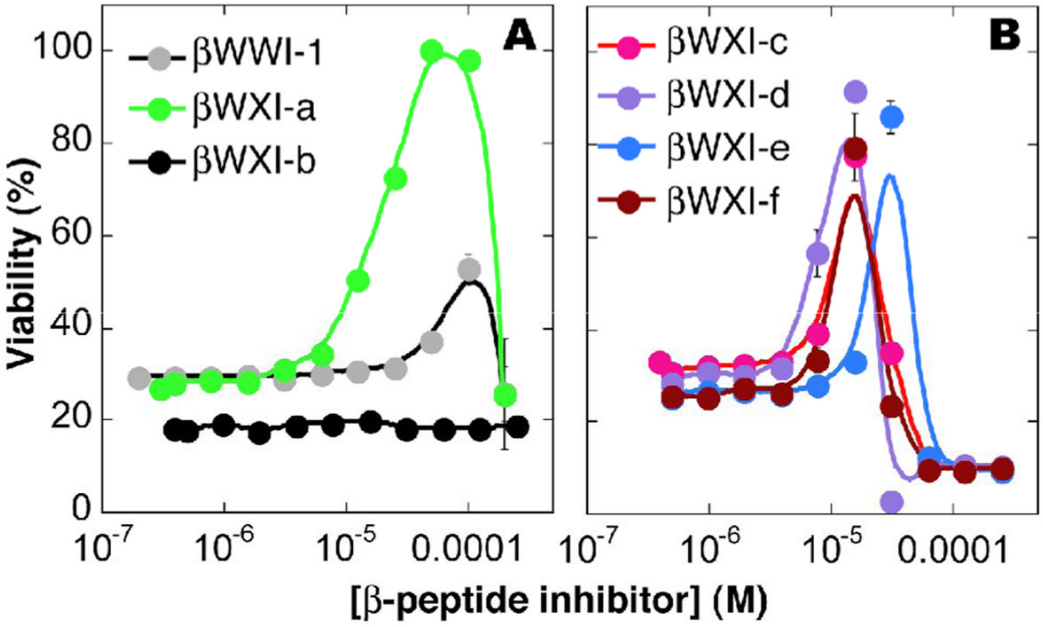

Figure 2.

Plots illustrating survival of HIV-infected MT-2 cells in the presence of the indicated β-peptide. EC_{50} values reported represent the β-peptide concentration required to achieve 50% survival of infected cells; CC_{50} values represent the concentration required to achieve 50% survival of uninfected cells. Viability was measured with an MTT colorimetric assay[26, 27] as described in the text.

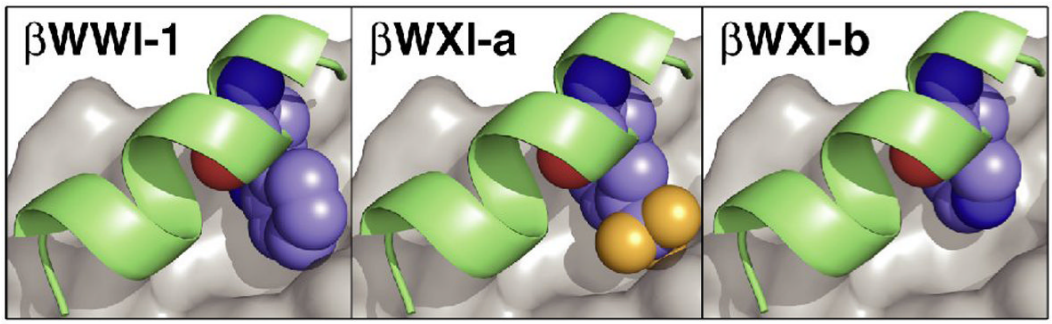

Figure 3.
Models representing the interface between the N-peptide surface pocket (grey) and the central epitope residue of βWWI-1, βWXI-a and βWXI-b. Models were constructed using the programs Spartan (Wavefunction, Inc.) and PyMOL (DeLano Scientific, LLC) and the high-resolution structure[10] 1gzl of the α-peptide C14linkmid bound to IQN17.

Table 1

Binding affinity and MTT assay results for peptides **βWWI-1** and **βWXI-a-f**.

Peptide	K_D[a] (μM)	EC_{50}[b] (μM)	CC_{50}[c] (μM)	Selectivity (CC_{50}/EC_{50})
βWWI-1	16.5 ± 0.6	56 ± 5.9	100 ± 19.6	1.8
βWXI-a	10.2 ± 0.3	19 ± 1.7	150 ± 3.3	7.9
βWXI-b	104.5 ± 8.2	> 250	> 250	N/A[d]
βWXI-c	14.1 ± 2.3	8.9 ± 1.3	23 ± 4.6	2.6
βWXI-d	12.2 ± 0.9	8.2 ± 5.0	23 ± 5.9	2.8
βWXI-e	15.7 ± 1.3	18 ± 3.7	50 ± 4.5	2.8
βWXI-f	13.3 ± 1.4	8.8 ± 7.4	31 ± 9.1	3.5

[a] For 50% binding of IQN17; binding curves were measured in triplicate.

[b] For 50% protection in MT-2 cells; antiviral curves used triplicate samples at each concentration.

[c] For 50% inhibition of MT-2 cell growth; toxicity curves also used triplicate samples.

[d] Not active.