Design, Synthesis, and Characterization of Cyclic Peptides Targeting HIV-1 Capsid

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Xinyong Cai[1], William M. McFadden[1], Angele Bruce[1], Zachary C. Lorson[1], Xin Wen[1], Huanchun Zhang[1], Karen A. Kirby[1], Monika Raj[1], Stefan G. Sarafianos[1]
[1]Emory University, Atlanta, GA 30322, United States

Introduction

Small molecules targeting the HIV-1 capsid (CA) have been developed to target the FG-binding pocket (FG-BP). Among them, Lenacapavir (LEN) is FDA-approved both for individuals with multidrug-resistant HIV-1 infection and for pre-exposure prophylaxis. Here, we introduce a novel class of CA inhibitors based on cyclic peptides (CyPeps). CyPeps offer the dual advantages of high oral bioavailability and structural versatility. They are rationally designed by mimicking host factors that naturally bind the CA FG-BP and thus incorporate an FG-binding motif for precise recognition.

Methods

The IC_{50} of FG-BP targeting CyPep cyFG5 was assessed against wild-type (WT) HIV-1 virus-like particles (VLPs) using an endogenous reverse transcription assay with NL4-3 ΔEnv VLPs. To elucidate the molecular basis of inhibition, we determined the first structure of native WT CA in complex with a cyclic peptide (cyFG5) by X-ray crystallography. This structure was compared to previously reported WT unliganded and PF74-bound, and Nup153 peptide-bound structures (PDB: 4XFX, 4XFZ). Site-directed mutagenesis was performed on CA residues that interact with cyFG5, and binding kinetics were quantified by biolayer interferometry (BLI) using disulfide-stabilized WT and mutant CA hexamers.

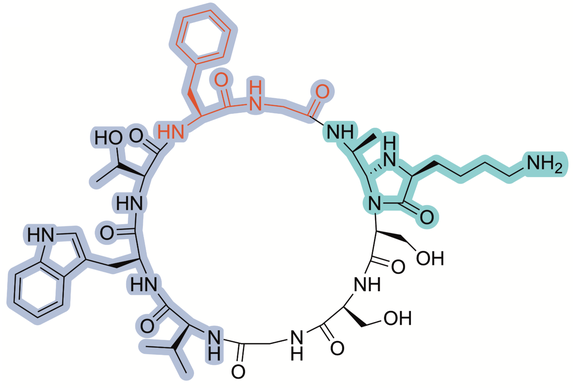


Figure 1. Structure of cyFG5: Chemical structure of cyFG5. In red is the FG dipeptic motif that mimics host factors. Highlighted in purple and blue are sequences adopted from peptide of Nup153, a nuclear pore protein known to bind CA at FGBP. In cyan is the site for peptide cyclization.

Host factor mimicking cyclic peptides bind HIV-1 capsid at the phenylalanine-glycine binding pocket

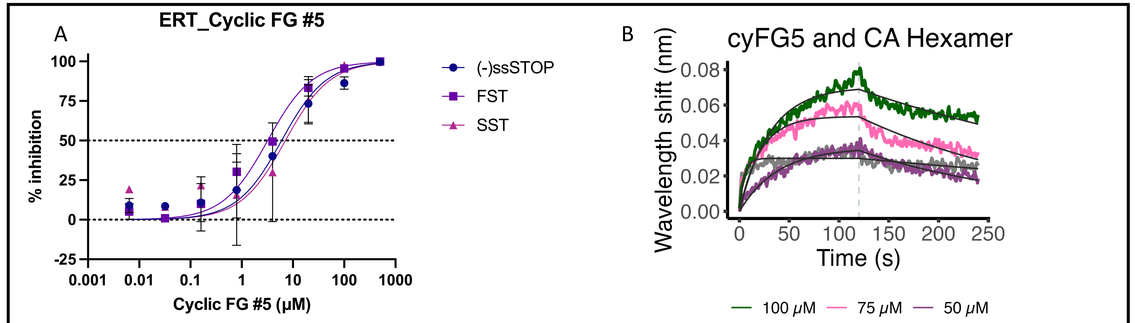


Figure 2. ERT assay and BLI show μM potency against HIV: A) ERT assay against WT HIV-1 VLPs. Percent inhibition shown on the y-axis and cyclic peptide concentration shown on the x-axis. Primers are designed to target three events during reverse transcription: minus strand strong stop (-ssSTOP) for early reverse transcription steps, first strand transfer (FST) for early-intermediate reverse transcription steps, and second strand transfer (SST) for late reverse transcription steps. IC_{50}: 6.0μM for ssSTOP, 3.2μM for FST, and 7.1μM for SST. B) Bio-layer interferometry (BLI) experiment of crosslinked CA hexamers and cyclic peptides. Global K_D = 7.97 μM, k_{on} = 4.20x10^2 M^{-1} s^{-1}, k_{off} = 3.12x10^{-2} s^{-1}.

Results

cyFG5 exhibited micromolar IC_{50} and K_D against/to purified WT VLPs and stabilized CA hexamers, respectively. Structural analysis revealed that cyFG5 establishes unique interactions with the C-terminal region of the adjacent CA monomer, distinct from existing ligands. Key contacts included residues S41, R132, N139, and R173. Alanine or serine substitutions were introduced at the identified residues in disulfide-stabilized CA hexamers. BLI measurements demonstrated reduced or abolished binding for mutants compared to WT, confirming the critical role of the C-terminal interactions.

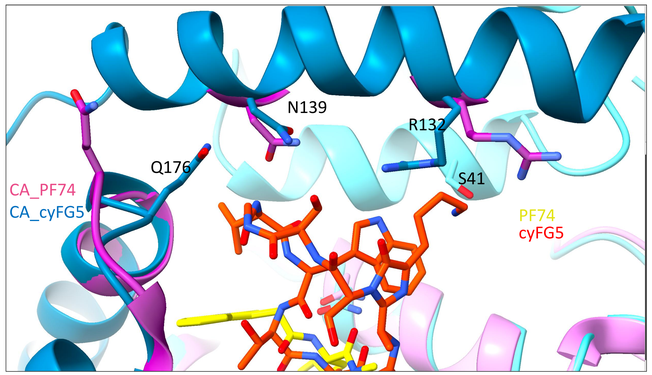


Figure 3. 2.3Å X-ray crystal structure of full-length CA in complex with cyFG5: Modeled cyFG5 shown as red sticks and CA in blue. The structure is superposed to published structure of CA in complex with PF74 with PF74 shown as yellow sticks and CA in purple. Residues S41, R132, N139, and R173 are observed to exhibit conformational difference upon cyFG5 binding. R132 is pushed away from the pocket towards the opposite side, away from the pocket. Q176 are shifted into the pocket by cyFG5 for ionic interactions.

	K_D (μM)	k_{on} (1/M s)	k_{off} (10^{-3}/s)
S41A	NB	NB	NB
R132A	NB	NB	NB
N139S	NB	NB	NB
M66I	18283.67	1.08	52.54
K70H	2.13	1304	2.78
N74D	62.91	248.45	7.70
WT	7.97	420.43	3.12

Table 1. Site-directed mutagenesis studies of cyFG5 with CA using BLI: Mutant disulfide stabilized CA hexamers were purified for BLI binding study. S41A, R132A, and N139S mutants completely lost binding to cyFG5, proving the significance of the interactions between the residues and cyFG5. Clinically relevant FGBP resistant mutations were also tested. Both M66I and N74D reduced binding but did not abolish interaction. Both k_{on} and k_{off} were affected dramatically by the two mutations. On the other hand, K70H increased affinity of cyFG5. This is mostly due to the increase in k_{on} where it can bind to CA hexamers faster, compared to WT. *NB: No-binding*.

Conclusion

Cyclic peptides represent a promising new class of CA-targeting antivirals for HIV-1 infection. We present cyFG5 as a prototype that binds the FGBP of CA. Site-directed mutagenesis corroborated the structural model, validating cyFG5's novel binding mechanism. These findings highlight the potential of structure-guided design to enhance the affinity and antiviral activity of cyclic peptides, establishing cyFG5 as a foundation for next-generation HIV-1 therapeutics.

We developed a new type of HIV-1 targeting antiviral. By revealing mechanistic details of our lead cyclic peptide, the study opens the door to designing next-generation treatments that could complement or improve current long-acting antiretroviral therapy.

Acknowledgement

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