Cyclic hexapeptide mimics designed to disrupt HIV-1 integrase interaction with key cell protein involved in viral import

Review Article

Efforts to fight HIV infection have included the development of drugs that disrupt the integration of proviral DNA into the host genome. Researchers based at Monash Institute of Pharmaceutical Sciences, Australia, have developed cyclic hexapeptides that disrupt the interaction between the viral integrase and a key protein involved in viral import, lens epithelium-derived growth factor (LEDGF/p75). The peptides closely mimicked the structure of the LEDGF binding domain but their low affinities indicate that more interactions must be included in order to achieve the potency needed for an effective drug.

HIV-1 integrase: a validated anti-HIV drug target

HIV-1 integrase is key to integrating the viral DNA into the host genome, including playing a role in transporting the HIV pre-integration complex (PIC) into the cell nucleus and guiding it to the host DNA. As a result, a number of drug compounds have been developed to bind to the active site of the integrase/DNA complex to inhibit integration in the fight against HIV. The multiple roles of integrase in genomic integration have also stimulated the development of compounds that act in other ways – for example through binding to alternative sites to evoke allosteric effects, or interference in interactions with other host-cell proteins. LEDGF/p75 is one such protein and is recognized by HIV-1 integrase to aid PIC import into the cell nucleus and targeting of the PIC to actively transcribed regions of DNA.

Cyclic peptide design and synthesis

The researchers explored the possibility of using small cyclic peptides to mimic the interaction between HIV-1 integrase dimer interface and a reverse-turn in residues 364–367 (KIDN) in the integrase-binding domain (IBD) of LEDGF. The increased peptide rigidity resulting from cyclization might also improve potency and stability. The challenge was to design functional peptides that were as short and rigid as possible. Reverse turns were therefore incorporated using dipeptide scaffolds placed external to the *i* → *i*+4 tetrapeptide (Figure 1, Table 1).

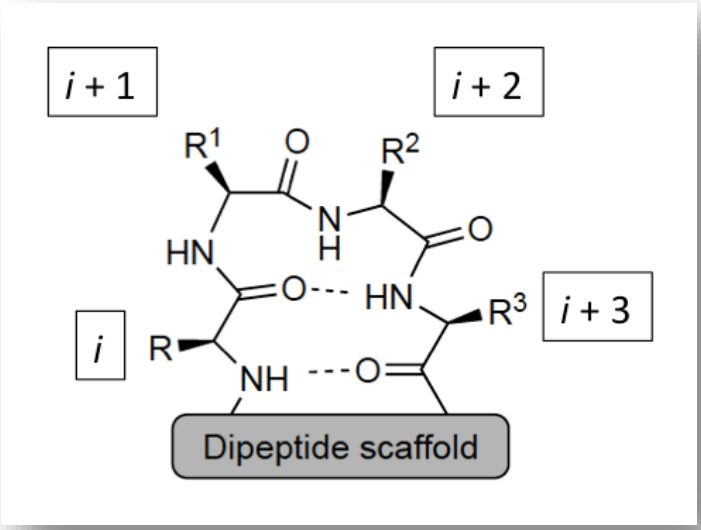

***Figure 1**. Cyclic peptides were based on a turn-promoting dipeptide scaffold comprising D–Xaa–L–Pro, where Xaa represents a naturally occurring amino acid, and the KIDN tetrapeptide. From Figure 2, Northfield et al, 2018.*

Table 1.** The peptides used in the study**.

Peptide	Peptide Structure	Structural characteristics
1	ox(Ac–CKIDNC–NH_2)	Cyclized KIDN tetrapeptide used terminal cysteines oxidized to form disulfide bridge.
2	cyclo(PKIDNp)	A backbone cyclized peptide using D-Pro–L-Pro scaffold at non-interface side of cyclic peptide.
3	cyclo(PKZDNv)	To fit into hydrophobic pocket, norleucine (Z) was incorporated into Ile position with D-Val–L-Pro scaffold.
4	cyclo(PKIDNG)	A glycine residue was incorporated to increase scaffold flexibility.

The linear peptides to be cyclized were prepared using standard Fmoc solid phase peptide synthesis using a PS3® peptide synthesizer. The linear form of peptide 1 was synthesized on Rink Amide resin and the linear Ac-CKIDNC-NH_2 was then oxidized in ammonium bicarbonate to form the cyclic disulfide. The linear precursors to the head-to-tail cyclic peptides (2–4) were synthesized on 2-chlorotrityl resin to enable cleavage of the peptides from the resin using mildly acidic conditions to retain side-chain protecting groups. The cyclisation conditions, 3 eq. of diphenylphosphorylazide (DPPA) and 4 eq. of DIPEA over 2-5 days at 4°C in DMF, were optimized after extensive exploration of alternative solvents, bases, cyclizing reagents and reaction temperatures. The cyclic peptides were purified to >95% purity by reversed phase HPLC.

LEDGF mimetics bind as native tetrapeptide and without scaffold interference

Studies using X-ray crystallography of the cyclic peptide/HIV-1 integrase core domain complex showed that the integrase core domain is dimeric with a pseudo two-fold symmetry. One copy of the cyclic peptide is bound at each of the equivalent LEDGF IBD binding sites at the integrase dimer surface. The presentation of the native KIDN tetrapeptide mimicked the structure of the native binding interaction, which was the main objective for these truncated LEDGF mimetics (see Figure 2). Reducing the mimetics to six residues conserved the key binding interactions of the KIDN tetrapeptide, partly due to the avoidance of conformational changes caused by scaffold binding to the enzyme.

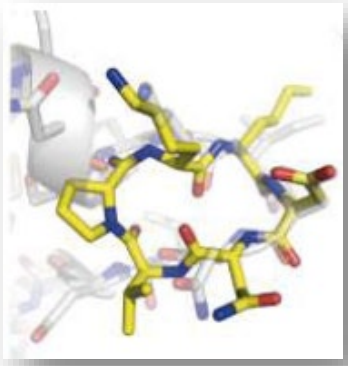

Figure 2. *Superimposition of the cyclic peptide cyclo(PKZDNv) over the LEDGF IBD crystal structure. From Figure 3, Northfield et al, 2018.*

Selecting appropriate scaffolds that do not contribute to binding is clearly important. Changing the linker residues from Gly to D-Pro or D-Val did not greatly affect the conformation. In contrast, the disulfide-linked cyclic peptide 1 has a unique buckled shape that affects peptide–integrase binding since the oxidized cysteine residues forming the disulfide bond scaffold hydrogen bond to the integrase. Comparison with the native integrase/IBD complex, however, showed that the KIDN motif of all the cyclic peptides was correctly presented and without interference from scaffold binding.

Low binding affinity indicates mimicry of the crystal bound pose is not enough

Using nuclear magnetic resonance- and surface plasmon resonance spectroscopy, the researchers found that the peptides bound to the protein with only millimolar affinity. When they applied a conformational search method they found that the favored solution conformations of the peptides differed from the crystal conformations, resulting in a significant free energy penalty associated with peptide binding. The low affinities of the cyclic mimetics could therefore be due to the resulting energetic barrier that the ligand must cross before binding.

Additional interactions are needed

The KIDN loop is a hotspot in the binding of LEDGF to HIV-1 integrase but these results indicate that generating high-affinity peptide ligands requires more than just close mimicry of the crystal bound pose. Peptide inhibitors with sufficient potency to disrupt this important protein-protein interaction in the viral life cycle must include additional interactions.

Reference

Cyclic hexapeptide mimics of the LEDGF integrase recognition loop in complex with HIV-1 integrase. Northfield SE, et al. ChemMedChem. 2018 Aug 10;13(15):1555-1565. doi: 10.1002/cmdc.201800129. Epub 2018 Jul 6.

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