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Interfacial Peptide Inhibitors of HIV-1 Integrase Activity and Dimerization

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Abstract—Peptides derived from the interfacial region of dimeric HIV-1 integrase were evaluated as inhibitors of integrase's 3′-endonuclease activity. Three peptides were found to be moderately potent inhibitors with IC_{50} values in the low micromolar range. The mode of inhibition was probed through protein crosslinking experiments. Active interfacial peptides were found to inhibit crosslinking of the dimeric form of integrase. Interfacial peptides that were poor inhibitors had no effect on integrase crosslinking.

HIV integrase is an essential enzyme of the virus that is responsible for integration of viral DNA into the host genome.[1] The overall integration process occurs in three steps, in which the integrase enzyme catalyzes the first two: processing of the 3′ ends of the viral DNA to expose the conserved CA-$_{OH}$3′ sequence, followed by a strand transfer reaction in which the recessed 3′ end of the viral DNA is ligated to the 5′ end of the target DNA.[2–5] Structurally HIV-1 integrase is composed of three domains: a dimeric catalytic domain from residues 50–212[6] (Fig. 1a), a C-terminal DNA binding domain,[7] and an N-terminal domain composed of residues 1–47.[8] The essential nature of integrase in HIV replication has made it the focus of numerous inhibition efforts.[9,10] The dimeric nature of the catalytic core suggests that an inhibitory mechanism that disrupts the dimerization of the enzyme may be effective.[11,12] Herein, we disclose the effect of five peptides derived from the dimeric interface of HIV-1 integrase on enzymatic activity and dimerization.

There are five regions of secondary structure from the catalytic domain of HIV-1 integrase that are found in the dimeric interface: α1, α3, α5, α6 and β3 (Fig. 1b). The peptide sequences corresponding to these regions (Table 1) were prepared by a solid phase approach on the Rink resin[13] using an Fmoc-based synthetic strategy. The peptides were cleaved from the resin and purified to homogeneity by reverse-phase HPLC. Each peptide was characterized by mass spectrometry and amino acid analysis. Each peptide was evaluated for secondary structure by circular dichroism, and all peptides were found to adopt a random coil conformation in aqueous solution. Addition of 30% TFE was found

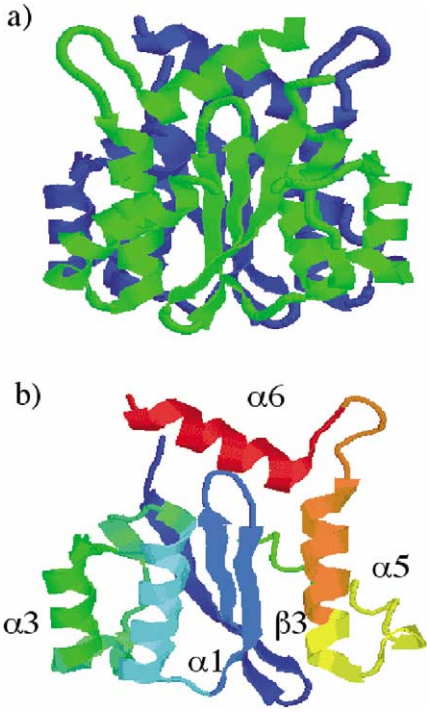

Figure 1. The catalytic domain of the HIV-1 integrase (a) dimer and (b) secondary structural elements at the dimerization interface.

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to promote a helical conformation for peptides α5 and α6 of 50 and 35%, respectively.

HIV-1 integrase with a double mutation (F185K, C280S) to improve solubility was expressed according to the procedure of Craigie and coworkers from the plasmid pINSD.His.Sol obtained from the NIH AIDS reagent program.[14] The integrase assay of Craigie and coworkers was used to monitor 3′-endonuclease activity with a ^{32}P-labeled, double stranded oligonucleotide 21-mer probe.[3] The effect of each of the five interfacial peptides was monitored by incubating the peptides with HIV-1 integrase for 30 min at 37 °C, followed by the addition of the radiolabeled probe. After 1.5 h, the mixture was analyzed by denaturing polyacrylamide gel electrophoresis to determine the extent of DNA cleavage. Without the addition of peptides, two main bands were observed on the gel corresponding to the original 21-mer and the 19-mer product (Fig. 2). The intensity of these bands was quantified using a phosphorimager. Additional bands were observed that have been observed by others, and may be attributed to an exonuclease activity for integrase.[15] In the presence of an increasing amount of a subset of the peptides, a decrease in the intensity of the 19-mer band was observed, with a relative increase in the intensity of the 21-mer band, pointing to inhibition of integrase activity. Peptides α1, α5, and α6 were found to be moderately potent inhibitors with IC_{50} values in the 2–3 μM range, whereas α3 and β3 demonstrated only low levels of inhibition at 1 mM (Table 1). The inhibition obtained for α1, α5, and α6 compares well with many of the examples of active site inhibitors of HIV-1 integrase.[9,10]

To assess the role of terminal residues, truncations were made to peptides α1 (α1s) and α6 (α6s). In the case of α1, removal of the N-terminal WP dipeptide (α1s) led to a 43-fold loss in potency. Similarly, truncating the N-terminus of α6 to a decapeptide (α6s) led to a 15-fold drop in activity. These results point to the essential nature of the N-terminal residues within these inhibitors.

The mode of inhibition was probed with protein crosslinking experiments to determine if the dimerization of HIV integrase was also inhibited with interfacial peptides. Treatment of HIV-1 integrase with an excess of the crosslinking reagent BS^3 (Pierce, 25 equiv) led to the appearance of a second band with SDS PAGE which is in accordance with the molecular weight of the crosslinked dimer (66 kD). Addition of increasing amounts of the peptide α1, however, led to a decrease in the intensity of this crosslinked band (Fig. 3a). Similar results were obtained with both α5 and α6, whereas agents that were not inhibitors, such as α3 and the less active truncated peptides α1s and α6s, had no effect on the crosslinking reaction with up to a 200-fold excess of peptide with respect to integrase (Fig. 3b).

These data may be compared to other known peptide inhibitors of HIV-1 integrase. The natural product peptides, integramides A and B, have been shown to inhibit HIV-1 integrase by a unknown mechanism (IC_{50} values of 17 and 10 μM, respectively).[16] Combinatorial approaches have been used to identify two peptides, a 12-mer and a hexapeptide, HCKFWW, that inhibited integrase with IC_{50} values in the low micromolar range.[17,18] To date the mechanism of inhibition with these peptides has not been ascertained. A 30-mer peptide derived from residues 147–175 of HIV-1 integrase was an inhibitor of integrase activity, although at concentrations in the millimolar range.[19] Interestingly, this peptide is not derived from the interfacial region of integrase, and the mode of inhibition has not been directly determined. Also interesting is the report of longer analogues of the α1 and α5 peptides in which 3′-processing was inhibited, with IC_{50} values of 250 μM and 11 μM, respectively.[12]

In conclusion, these data demonstrate that a subset of interfacial peptides derived from HIV-1 integrase may act as inhibitors of the integrase 3′-processing activity. The most potent inhibitors of integrase activity were

Table 1. Sequence and activity[a] of the interfacial peptides derived from HIV-1 integrase

	Sequence	IC_{50}[b]
β3	G^{82}YIEAEVI^{89}-$CONH_2$	>1 mM
α1	Q^{95}ETAYFLLKLAGRWP^{109}-$CONH_2$	3.5 μM
α3	S^{123}TTVKAASWWA^{133}-$CONH_2$	>1 mM
α5	H^{171}LKTAVQMAVFIHNFKR^{187}-$CONH_2$	3.0 μM
α6	A^{196}GERIVDIIATDIQ^{210}-$CONH_2$	2.0 μM
α1S	Q^{95}ETAYFLLKLAGR^{107}-$CONH_2$	150 μM
α6S	A^{196}GERIVDIIA^{206}-$CONH_2$	30 μM

[a]Inhibition of 3′-processing of the oligonucleotide 5′-ATAGACCCTGCTAGAGATTT-3′ with its double stranded complement by HIV-1 integrase.
[b]Values are ±10%.

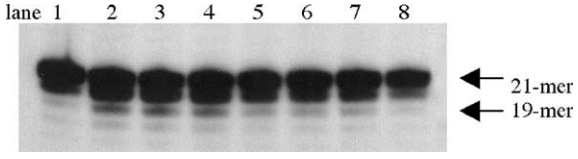

Figure 2. A representative denaturing polyacrylamide gel of the ^{32}P-labeled DNA 21-mer substrate (5 nM, lane 1) upon treatment with HIV-1 integrase (420 nM, lane 2), or HIV-1 integrase incubated with the peptide αα5 (1 μM, lane 3), (2 μM, lane 4), (4 μM, lane 5), (6 μM, lane 6), (8 μM, lane 7), and (10 μM, lane 8).

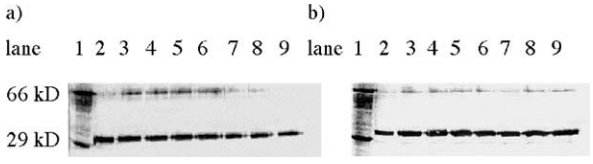

Figure 3. Representative SDS PAGE of the crosslinking reaction of HIV-1 integrase (1.2 μM) with BS^3 (25 equiv): molecular weight markers (lane 1), HIV-1 integrase (lane 2), HIV-1 integrase treated with BS^3 (lane 3), and integrase treated with BS^3 and either (a) the peptide α1 (4.7 μM, lane 4), (9.3 μM, lane 5), (19 μM, lane 6), (23 μM, lane 7), (28 μM, lane 8), (37 μM, lane 9), (b) the peptide α1S (48 μM, lane 4), (233 μM, lane 5), the peptide α3 (48 μM, lane 6), (233 μM, lane 7), or the peptide α6S (48 μM, lane 8), (233 μM, lane 9).

also able to disrupt the crosslinking of integrase dimer, whereas peptides with little or no inhibitory potency had no effect on crosslinking. These results suggest that interfacial peptides α1, α5, and α6 have the ability to block dimerization of HIV integrase. Further experiments are underway to improve the potency of these inhibitors using crosslinking strategies that have been developed for HIV protease.[11]

HIV-1 Integrase 3′-DNA Processing Assay

The mutant HIV-1 integrase, F185K/C280S, (420 nM final concentration, 20 μL total volume) was incubated with varying amount of inhibitor at 37 °C for 0.5 h in a pH 7.5 buffer containing 1 mM HEPES, 1 mM $MnCl_2$, 50 mM NaCl, 50 μM EDTA, 10% glycerol, 0.1 mg/mL BSA, 10 mM β-mercaptoethanol, 10% DMSO, and 25 mM MOPS.[3] The DNA cleavage reaction was carried out by treating the ^{32}P-labeled DNA substrate (5 nM) with the above mixture at 37 °C for 1.5 h. The reaction products were analyzed by electrophoresis using 20% denaturing polyacrylamide gels. The intensity of the bands was quantitated using a Phosphor Imager (Molecular Dynamics), and per cent inhibition was determined using the equation:

$$\%\text{Inhibition} = (1 - \frac{19'\text{mer} - 19^{100}\text{mer}}{19^{0'}\text{mer} - 19^{100}\text{mer}})^{*}100\%$$

where: 19′mer = the intensity of 19mer band with inhibitor, normalized for differences in loading; $19^{0'}$mer = the intensity of 19mer band without inhibitor; 19^{100}mer = the intensity of 19mer band without IN.

HIV-1 Integrase Crosslinking Assay

The mutant HIV-1 integrase, F185K/C280S, (1.2 μM final concentration) was incubated with varying amount of inhibitor at 37 °C for 1 h in a pH 7.5 buffer containing 25 mM HEPES, 1 M NaCl, 1mM DTT, and 1 mM EDTA. The reaction mixtures were treated with the crosslinking agent BS^3 (25 equiv, Pierce) for 30 min at 37 °C. The reaction mixtures were denatured and analyzed by SDS PAGE on 12% polyacrylamide gels.

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