Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5199–5202

Inhibition of HIV-1 integrase activity by synthetic peptides derived from the HIV-1 HXB2 Pol region of the viral genome

Zahrah Zawahir and Nouri Neamati*

Department of Pharmaceutical Sciences, School of Pharmacy, University of Southern California, 1985 Zonal Avenue, Los Angeles, CA 90089, USA

Received 27 May 2006; accepted 5 July 2006
Available online 1 August 2006

Abstract—Peptides deriving from the HIV-1 HXB2 *Pol* gene sequence were evaluated for inhibitory activity against wild-type (WT) and mutant HIV-1 integrase (IN). The most potent peptide corresponding to a region on the reverse transcriptase (RT) subunit of the *Pol* polyprotein showed IC_{50} value of 5 and 2 μM for 3′-processing and strand transfer, respectively. These peptides, and their analogs, may potentially be used in the elucidation of structural and functional epitopes of IN involved in protein–protein and protein–small molecule interactions.

The *Pol* genomes of retroviruses encode three essential enzymes—protease (PR), reverse transcriptase (RT), and integrase (IN). The integration of reverse-transcribed human immunodeficiency virus (HIV) genome into host cell DNA is an essential step in viral replication. This process is catalyzed by IN in two steps.[1] After viral DNA assembly on IN, two nucleotides adjacent to a conserved CA dinucleotide are cleaved from each 3′-end of double-stranded viral cDNA (3′-processing). In the subsequent strand transfer step, IN nicks the chromosomal DNA in staggered positions and joins the 3′-end of the newly processed viral DNA into the 5′-end of the chromosomal DNA nicks. Host repair enzymes such as human Rad 18 are thought to complete the 5′-end joining reaction to produce the provirus.[2,3] These catalytic steps occur in the cytoplasm and the nucleus of infected cells, respectively. Integration and events prior to it are believed to occur as part of a pre-integration complex (PIC), in which IN, RT, and other essential viral and cellular proteins interact, in the cytosol (early PIC) and in the nucleus (late PIC).[4,5] To date, validated viral and cellular co-factors of IN such as RT have been shown to both stimulate integration in vitro and in vivo[6,7] as well as inhibit integration.[4,8–10] The cellular and viral proteins that interact with IN each constitute a promising new therapeutic target in combating the virus.[11] Elucidating the structural and catalytic nature of such interactions is thus important, especially given that no crystal structure exists of full-length HIV-1 IN.

There is an interest in the possibility of designing inhibitors of IN that target cellular protein–IN and viral protein–IN interactions.[12–14] Target-sequence based disruption of functional protein–protein interactions by IN inhibitory peptides or their derivatives should adversely affect productive viral activity. It has been suggested that conformational analysis of peptides will allow the development of peptidomimetic and other inhibitors against IN and that peptide fragments reproducing critical functional epitopes would disrupt protein–protein interactions and inhibit biological activity of the enzyme.[13] Data from our group suggest that synthetic peptides deriving from the IN amino acid sequence implicate residues critical to enzyme structure and function.[14] Peptides may also have potential in clinical settings, given the success of Enfuvirtide, an anti-HIV-1 peptide drug derived from the viral glycoprotein involved in host cell entry. Several peptide inhibitors of IN targeting catalytic activity as well as oligomerization, although far from entering actual drug development, have been reported.[12,15–18] These include synthetic peptides[12,19–21] from various regions of the IN enzyme as well as those deriving from natural products.[22,23]

Keywords: HIV-1 integrase; Protein–protein interaction; Reverse transcriptase; Peptide; Mutant.
* Corresponding author. Tel.: +1 323 442 2341; fax: +1 323 442 1390; e-mail: neamati@usc.edu

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.07.022

Recently, small soluble peptides from the viral activator Tat domain were shown to significantly inhibit viral replication.[17]

We have previously presented preliminary data from in vitro screening of a library of 20-mer synthetic peptides deriving from the HIV-1 HXB2 Pol genome, against IN catalytic activity.[24] Herein we report the evaluation of these peptides for inhibition of both 3′-processing and strand transfer. Of these, the most potent inhibitory peptides derive from the RT region of the Pol genome (Fig. 1), while those peptides deriving from the PR region were inactive. A separate publication from our group[14] details the inhibitory activity of shorter peptides from the IN region, as well as their derivatives. It should be noted that a study published last year produced results significantly different from ours for the same RT peptides.[25]

The 20-mer peptides tested against purified wild-type IN spanned the 1003 amino acid length of the Pol polyprotein, with 10-mer overlaps between sequential peptides. The most active peptide **56** (IC_{50} values of 5 μM for 3′ processing and 2.5 μM for strand transfer) derives from the RT connector region of the protein. Two other peptides, **34** and **53**, showing IC_{50} values in the micromolar range also derive from the RT palm and connector regions. All three are in close proximity to the active site of the enzyme (Table 1). Peptides **65** and **68** deriving from the RNase H region also exhibit inhibition of IN catalytic activity in the low micromolar range, although less potent than the previous three peptides. We also evaluated the five potent peptides against the IN (F185K/C280S) soluble mutant. While this mutant has been shown to be slightly more resistant as compared to wild-type to small molecule IN inhibitors, this is the first reported case of peptides showing similar inhibitory behavior (Table 1).

The full set of HIV-1 HXB2 Pol 20-mer peptides (1.0 mg, lyophilized) was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program Catalog (catalog number 4358). The first amino acid of the first peptide is recorded as amino acid 433 in the full-length genomic sequence of the *Pol* polyprotein in GenBank (Accession No. K03455). Serial dilutions of the peptides were performed in deionized water or DMSO. Original peptides and stock solutions

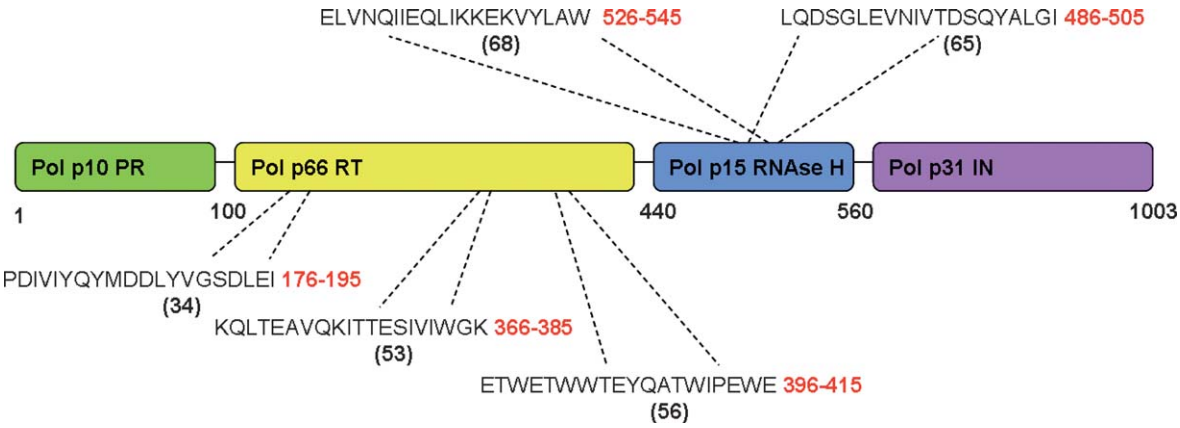

Figure 1. The five most potent peptides derive from different regions of the HIV-1 HXB2 Pol genome. Numbers adjacent to each peptide sequence indicate amino acid spans of the respective subunits on the Pol polypeptide (Numbering according to the HXB2 Numbering Engine nomenclature, Los Alamos National Laboratory). Numbers in parentheses indicate the number of the peptide (domains not drawn to scale).

Table 1. IC_{50} (μM) values of peptides against wild-type and mutant IN

Peptide	Peptide sequence	Activity	WT[a]	SM[a]	C130S	C130A
34	PDIVIYQYMDDLYVGSDLEI	3′-Processing	6 ± 1	28 ± 2	41 ± 2	20
		Strand transfer	10 ± 1	23 ± 2	2 ± 1	5
53	KQLTEAVQKITTESIVIWGK	3′-Processing	7 ± 1	51 ± 7	29 ± 1	15
		Strand transfer	4 ± 1	31 ± 7	2 ± 1	10
56	ETWETWWTEYWQATWIPEWE	3′-Processing	6 ± 1	13 ± 2	126 ± 3	25
		Strand transfer	2 ± 1	9 ± 2	27 ± 1	5
65	LQDSGLEVNIVTDSQYALGI	3′-Processing	11 ± 1	>167	18 ± 3	>167
		Strand transfer	2 ± 1	36 ± 14	4 ± 1	20
64	ELVNQIIEQLIKKEKVYLAW	3′-Processing	15 ± 2	113 ± 11	136 ± 5	45
		Strand transfer	14 ± 4	83 ± 5	7 ± 1	15

[a] Abbreviations: WT, wild-type IN; SM, soluble mutant.

were stored at −20 °C. Dilutions were stored at −4 °C. IN inhibition studies were performed with purified recombinant wild-type, soluble mutant (F182K, C280S), or C130S/A mutants IN and a 21-mer oligonucleotide substrate using established protocol.[26] Percent inhibition (% *I*) was calculated using the following equation: $100 \times [1 - (D - C)/(N - C)]$ where *D*, *C*, and *N* are the fractions of 21-mer substrate converted to 19-mer (3′-processing products) or strand transfer products for IN plus peptide, DNA plus IN, and DNA alone, respectively. IC_{50} values were determined by plotting peptide concentration against percent inhibition on a logarithmic scale.

A representative IN catalysis assay of the peptides from Table 1 is shown in Figure 2. Peptides **34**, **53**, and **56** all derived from the RT-RNase H region of the polyprotein. Previous reports have shown that RT and IN physically interact with and inhibit each other's catalytic activity.[8,27] The interaction between these two proteins is also a requirement for their respective catalytic activities.[9,28] IN has been shown to bind to two discontinuous regions on RT, the fingers-palm region (amino acids 1–242) and carboxy-terminus of the connection region of RT. The RT binding region on IN has been mapped to the carboxy-terminal region from amino acid residues 210 to 288.[9] Deletion analysis on RT has revealed two IN binding domains: the fingers-palm domain and the carboxy-terminal domain of the connection subdomain.[9] It is interesting to note that the two most active peptides, **53** and **56**, both derived from the RT connection domain. Peptide **56** contains six tryptophan residues that possibly contribute to its potency in inhibiting IN. Tryptophan-rich peptides have been known to possess IN inhibitory activity, as evidenced by indolicidin, a natural antimicrobial peptide, and its analogs.[22] It is thought that this amino acid intercalates into DNA and exerts inhibitory activity by sterically disrupting IN–DNA binding. It has been shown that a C130S mutant of IN is not able to interact with RT, possibly due to changes in the protein recognition interface at the C-terminal IN domain.[29] We evaluated the peptides derived from RT against the catalytic activity of this cysteine mutant with interesting results that show a marked selectivity for strand transfer (Table 1). These observations confirm important structural observations from our group with regard to the C130S mutant. Finally, Schiff-base assays in which inhibition of IN crosslinking with DNA may be observed showed that these peptides do not disrupt IN–DNA binding (data not shown).

In conclusion, the subset of active peptides derived from HIV-1 RT sequence we have described in this paper could potentially serve as novel probes toward finding protein 'hotspots' involved in protein–protein interactions, and thus contribute toward the understanding of IN interactions with other cellular and viral proteins.[30] We have already used peptides derived from IN, and their derivatives, to define potential areas of catalytic importance within IN.[14] Because such interactions are known to be essential for successful integration of viral

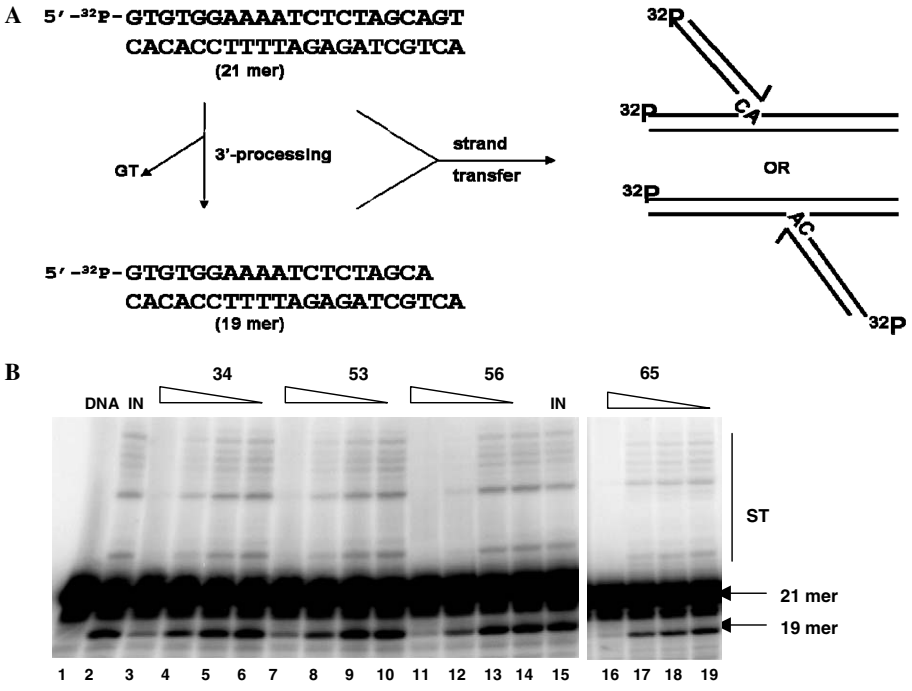

Figure 2. Representative gel showing inhibition of purified IN by peptide #34, 53, 56, and 65. (A) Schematic of IN activity in vitro: a 21-mer oligonucleotide corresponding to the U5 LTR 5′-end-labeled with ^{32}P is reacted with purified IN. The first step, 3′-processing involves nucleolytic cleavage of two bases from the 3′-end resulting in a 19-mer oligonucleotide. The 3′-ends are subsequently covalently joined at several sites to another identical oligonucleotide that serves as the target DNA. This reaction is known as strand transfer, and the products formed migrate slower than the original substrate. (B) Lane 1, DMA alone; lanes 2 and 15, IN alone; lanes 3–14, IN, DNA, and respective peptides at decreasing concentrations of 18, 6, 2, and 0.6 μM. ST, strand transfer.

cDNA into host DNA, an understanding of protein–protein and protein–DNA interactions will contribute to the development of effective inhibitors to IN catalytic activity if successful disruption of the interaction can be initiated.[31,32] Chemical derivatization or synthetic analogs of these peptides may also function as potent IN inhibitors by causing conformational changes in the protein that prevent productive integration. Although little is known about the inhibition mechanism of these peptides, additional information from affinity or binding studies with IN may be utilized to gain more insight into the three-dimensional structure of the protein.

References and notes

1. Esposito, D.; Craigie, R. *Adv. Virus Res.* **1999**, *52*, 319.
2. Mulder, L. C.; Chakrabarti, L. A.; Muesing, M. A. *J. Biol. Chem.* **2002**, *277*, 27489.
3. Yoder, K. E.; Bushman, F. D. *J. Virol.* **2000**, *74*, 11191.
4. Van Maele, B.; Debyser, Z. *AIDS Rev.* **2005**, *7*, 26.
5. Miller, M. D.; Farnet, C. M.; Bushman, F. D. *J. Virol.* **1997**, *71*, 5382.
6. Maertens, G.; Cherepanov, P.; Pluymers, W.; Busschots, K.; De Clercq, E.; Debyser, Z.; Engelborghs, Y. *J. Biol. Chem.* **2003**, *278*, 33528.
7. Van Maele, B.; Busschots, K.; Vandekerckhove, L.; Christ, F.; Debyser, Z. *Trends Biochem. Sci.* **2006**, *31*, 98.
8. Oz, I.; Avidan, O.; Hizi, A. *Biochem. J.* **2002**, *361*(Pt. 3), 557.
9. Hehl, E. A.; Joshi, P.; Kalpana, G. V.; Prasad, V. R. *J. Virol.* **2004**, *78*, 5056.
10. Tasara, T.; Maga, G.; Hottiger, M. O.; Hubscher, U. *FEBS Lett.* **2001**, *507*, 39.
11. Debyser, Z.; Cherepanov, P.; Van Maele, B.; De Clercq, E.; Witvrouw, M. *Antivir. Chem. Chemother.* **2002**, *13*, 1.
12. Desjobert, C.; de Soultrait, V. R.; Faure, A.; Parissi, V.; Litvak, S.; Tarrago-Litvak, L.; Fournier, M. *Biochemistry* **2004**, *43*, 13097.
13. Maroun, R. G.; Krebs, D.; Roshani, M.; Porumb, H.; Auclair, C.; Troalen, F.; Fermandjian, S. *Eur. J. Biochem.* **1999**, *260*, 145.
14. Li, H.-Y.; Zawahir, Z.; Song, L.-D.; Long, Y.-Q.; Neamati, N. *J. Med. Chem.* **2006**, *49*, 4477.
15. de Soultrait, V. R.; Desjobert, C.; Tarrago-Litvak, L. *Curr. Med. Chem.* **2003**, *10*, 1765.
16. Puras Lutzke, R. A.; Eppens, N. A.; Weber, P. A.; Houghten, R. A.; Plasterk, R. H. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 11456.
17. Agbottah, E.; Zhang, N.; Dadgar, S.; Pumfery, A.; Wade, J. D.; Zeng, C.; Kashanchi, F. *Virology* **2006**, *345*, 373.
18. Congote, L. F. *Biochem. Biophys. Res. Commun.* **2006**, *343*, 617.
19. Sourgen, F.; Maroun, R. G.; Frere, V.; Bouziane, M.; Auclair, C.; Troalen, F.; Fermandjian, S. *Eur. J. Biochem.* **1996**, *240*, 765.
20. Zhao, L.; O'Reilly, M. K.; Shultz, M. D.; Chmielewski, J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1175.
21. Maroun, R. G.; Gayet, S.; Benleulmi, M. S.; Porumb, H.; Zargarian, L.; Merad, H.; Leh, H.; Mouscadet, J. F.; Troalen, F.; Fermandjian, S. *Biochemistry* **2001**, *40*, 13840.
22. Krajewski, K.; Long, Y. Q.; Marchand, C.; Pommier, Y.; Roller, P. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3203.
23. Singh, S. B.; Herath, K.; Guan, Z.; Zink, D. L.; Dombrowski, A. W.; Polishook, J. D.; Silverman, K. C.; Lingham, R. B.; Felock, P. J.; Hazuda, D. J. *Org. Lett.* **2002**, *4*, 1431.
24. Zawahir, Z.; Sanchez, T.; Neamati, N. In *Inhibition of Integrase Activity by Peptides Derived from the HIV-1 HXB2 Pol Region of the Viral Genome*, The Eleventh West Coast Retrovirus Meeting, Palm Springs, CA, October 7–9, 2004; Palm Springs, CA, 2004; p 55.
25. Oz-Gleenberg, I.; Avidan, O.; Goldgur, Y.; Herschhorn, A.; Hizi, A. *J. Biol. Chem.* **2005,** *280*, 21987.
26. Mazumder, A., Neamati, N., Sundar, S., Owen, J., Pommier, Y. In *Antiviral Methods and Protocols*; Kinchington, D., Schinazi, R., Eds.; Humana: Totowa, 1999; Vol. 24, pp 327–338.
27. Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J. A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M. D. *Science* **2000**, *287*, 646.
28. Chow, S. A.; Vincent, K. A.; Ellison, V.; Brown, P. O. *Science* **1992**, *255*, 723.
29. Zhu, K.; Dobard, C.; Chow, S. A. *J. Virol.* **2004**, *78*, 5045.
30. Arkin, M. R.; Wells, J. A. *Nat. Rev. Drug Discov.* **2004**, *3*, 301.
31. Dayam, R.; Neamati, N. *Curr. Pharm. Des.* **2003**, *9*, 1789.
32. Dayam, R.; Deng, J.; Neamati, N. *Med. Res. Rev.* **2006**, *26*, 271.