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Short cyclic peptides derived from the C-terminal sequence of α1-antitrypsin exhibit significant anti-HIV-1 activity

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ABSTRACT

Serpin A1 (α1-AT), the largest subgroup of serpins, presents in human plasma at high concentration and plays important regulatory roles in physiological and pathological processes. Accumulated evidence suggests that α1-AT may play a role in controlling HIV-1 infection. In this study, we designed and synthesized a set of short linear peptides derived from the C-terminal sequence of α1-AT. Since none of them showed significant anti-HIV-1activity, we proceeded to synthesize four short cyclic peptides having 7 amino acids, and we found that three of them exhibited significant anti-HIV-1 activity. One of these cyclic peptides, designated CPM, inhibited HIV-1 entry and infection at low μM level, indicating that these short cyclic peptides could serve as leads for the development of novel anti-HIV-1 therapeutics.

Acquired immune deficiency syndrome (AIDS) is a lethal disease caused by the human immunodeficiency virus (HIV). So far, 25 anti-HIV entities have been licensed by the US Food and Drug Administration (FDA) (http://www.fda.gov/oashi/aids/virals.html). However, drug resistance and adverse reactions during long-term medication make it essential to continue developing novel anti-HIV drugs with new mechanisms of action.

Serpins (SERine Proteinase INhibitors), which comprise 330∼400 amino acids and represent a superfamily of approximately 500 proteins, fold into a conserved structure consisting of three β-sheets and at least seven α-helices.[1] Serpin A1, also called α1-antitrypsin (α1-AT), is the largest subgroup of serpins. It presents in human plasma at high concentration and plays important regulatory roles in physiological and pathological processes.[2–5] Accumulated evidence suggests that α1-AT plays roles in the inhibition of the HIV-1 fusion with the target cells[6,7] and the suppression of HIV-1 infection.[8,9] Besides the whole protein, the peptides derived from α1-AT also exhibited potent anti-HIV-1 activity. For example, peptide A1-C26 that derives from the C-terminal 26-residue sequence α1-AT,[10,11] its analogue C105Y (CSIPPEVKFNKPFVYLI),[12] and VIRIP (VIRus-Inhibitory Peptide)[13] could all effectively inhibit HIV-1 infection. Although these results suggest the potential for further development as anti-HIV-1 therapeutics, such peptide drugs could not now be administered orally and would lack oral availability.

The carboxyl terminal fragment of al-AT (residues 370–374), FVFLM (Fig. 1), is the binding site of the serpin–enzyme complex (SEC) to its receptor.[10] Binding of FVFLM to the receptor could lead to proteinase inactivation, serving as 'hydrophobic core' for cellular uptake and nucleolar localization. A synthetic analogue of this pentapeptide, peptide 105C (FVYLI), was able to block the binding and internalization of al-AT-1261-trypsin complexes by HepG2 cells.[9]

We thus hypothesized that the peptides containing the sequences of FVFLM and FVYLI might have anti-HIV-1 activities. Based on this supposition, we synthesized two short linear peptides, FM5 (FVFLM) and PI6 (PFVYLI), as previously described.[14] Because these two peptides exhibited low solubility, we then

Figure 1. Bioactive peptides derived from the C-terminus of serpin A1. VIRIP corresponds to α1-AT residues 353–372. Peptide A1-C36 corresponds to residues 359–394. Peptide A1-C26 corresponds to residues 369–394. The FVFLM sequence (underlined) corresponds to the binding site of SEC to its receptor.[8]

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designed several analogous peptides by extending toward the N-termini of FM5, such as PM6 (PFVFLM), KM7 (KPFVFLM), and NM8 (NKPFVFLM), or by introducing polar amino acids in peptide sequences (Glu or Arg), including PE6 (PFVFLE), EM6 (EFVFLM), PEM6 (PEVFLM), and PR6 (PFVFLR) (Table 1). Their anti-HIV-1 activities were tested by a virus inhibition assay as previously described.[15] However, none of these peptides exhibited inhibitory activity on HIV-1_{IIIB} replication, even at concentrations as high as 100 μM (Table 1), suggesting that these short linear peptides might not maintain their active conformation.

However, some reports[16–18] have demonstrated that cyclization is a promising strategy to enhance bioavailability of peptides. Specifically, with constrained conformations, the cyclic peptides possessed improved in vivo stability and better selectivity.[19–21] Therefore, we selected four linear peptides, including PM6, PE6, PR6, and PEM6, for synthesis of the cyclopeptides CPM, CPE, CPR, and CPEM, respectively (Table 2), using the thioester method[22] with some modifications. The inhibitory activities of the cyclic peptides were then determined using an HIV-1_{IIIB} replication assay, as described above. Surprisingly, three of the four cyclic peptides exhibited anti-HIV-1 activity. CPM, the most active peptide, showed an IC_{50} (50% inhibitory concentration) of 8.96 μM against HIV-1_{IIIB} replication (Table 2). All the four peptides exhibited low cytotoxicity with a CC_{50} (50% inhibitory concentration)[15] of >200 μM to MT-2 cells (Table 2).

To determine the mechanism of action, we tested the activity of cyclic peptides on HIV-1 envelope protein (Env)-mediated cell–cell fusion, as previously described[23] and the IC_{50} was calculated using the CalcuSyn software.[24] CPM showed an IC_{50} of 67.2 μM in the cell–cell fusion assay, which is reasonable, based on its anti-HIV-1 activity, as described above, and results of the pseudovirus replication assay, which is explained below (Table 3). Noticeably, CPM was about 5- to 6-fold more potent than CPE and CPR in inhibiting HIV-1_{IIIB} replication, while former was almost equally potent as latter, suggesting that CPM may target not only the HIV-1 fusion step, but also other step of HIV-1 replication.

To further determine whether a cyclic peptide could block HIV-1 entry, we constructed a pseudovirus expressing the envelop protein gp160 of the HIV-1 R5 virus SF162 and tested the inhibitory activity of peptide CPM on the single cycle infection of the pseudotyped HIV-1 as previously described.[25] The results showed that the cyclic peptide CPM significantly inhibited HIV-1 pseudovirus infection with an IC_{50} of 5.95 μM (Fig. 2), suggesting that these cyclic peptides inhibited HIV-1 infection by blocking virus entry, an outcome which is consistent with the mechanisms of action of the long linear peptides derived from α1-AT, such as VIRIP.[13] We will further modify the peptide sequences by introducing unnatural amino acids, such as D-amino acids, so that the short cyclic antiviral peptide can be orally administered.

In conclusion, a set of linear and cyclic peptides derived from the C-terminal sequence of α1-AT were designed and synthesized. A cyclic peptide CPM showed low μM inhibitory activity against HIV-1 infection. The mechanism study showed that this short cyclic peptide inhibited HIV-1 infection by blocking virus fusion with and entry into the target cells, suggesting that that CPM and other short cyclic peptides could serve as leads for the development of novel anti-HIV therapeutics.

Table 1
Inhibition of the designed peptides on HIV-1_{IIIB} replication

Peptides	Sequences	MW	IC_{50} (μM)
FM5	FVFLM	696.9	>100
PM6	PFVFLM	794.0	>100
KM7	KPFVFLM	922.2	>100
NM8	NKPFVFLM	1,036.3	>100
PI6	PFVYLI	792.0	>100
PE6	PFVFLE	791.9	>100
EM6	EFVFLM	826.0	>100
PEM6	PEVFLM	776.0	>100
PR6	PFVFLR	819.0	>100

Table 2
Inhibition of the cyclic peptides on HIV-1_{IIIB} replication

Peptide	Sequence	MW	IC_{50} (μM)	CC_{50}(μM)
CPM	Cyclo-(CPFVFLM)-SH	837.8	8.96 ± 2.23	>200
CPE	Cyclo-(CPFVFLE)-SH	835.6	55.84 ± 3.31	>200
CPR	Cyclo-(CPFVFLR)-SH	862.9	74.07 ± 22.84	>200
CPEM	Cyclo-(CPEVFLM)-SH	819.7	>100	>200

Table 3
Inhibition of the bioactive peptides on cell–cell fusion

Peptide	Sequence	IC_{50} (μM)
PM6	PFVFLM	>200
PE6	PFVFLE	>200
PR6	PFVFLR	>200
PEM6	PEVFLM	>200
CPM	Cyclo-(CPFVFLM)-SH	67.20 ± 4.05
CPE	Cyclo-(CPFVFLE)-SH	115.58 ± 6.28
CPR	Cyclo-(CPFVFLR)-SH	73.98 ± 7.44
CPEM	Cyclo-(CPEVFLM)-SH	>200

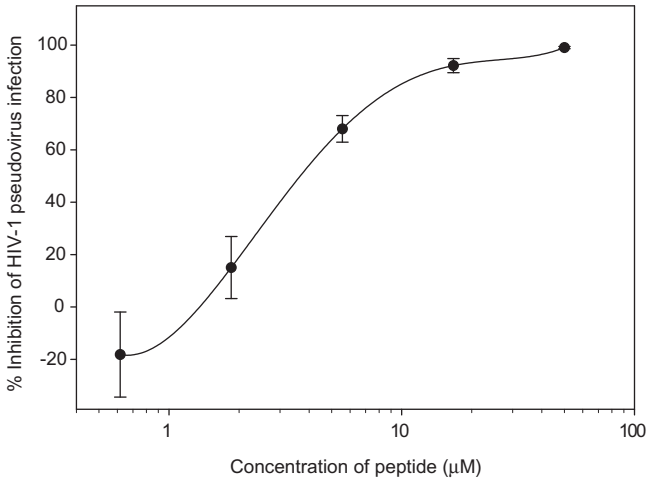

Figure 2. The inhibitory activity of CPM on HIV-1 pseudovirus infection assay. The peptide was tested in triplicate and the data presented as mean ± SD.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.02.037.

References and notes

1. Silverman, G. A.; Bird, P. I.; Carrell, R. W.; Church, F. C.; Coughlin, P. B.; Gettins, P. G.; Irving, J. A.; Lomas, D. A.; Luke, C. J.; Moyer, R. W.; Pemberton, P. A.; O'Donnell, E. R.; Salvesen, G. S.; Travis, J.; Whisstock, J. C. *J. Biol. Chem.* **2001**, *276*, 3329.
2. Lewis, E. C.; Mizrahi, M.; Toledano, M.; DeFelice, N.; Wright, J. L.; Churg, A.; Shapiro, L.; Dinarello, C. A. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 16236.

3. Koulmanda, M.; Bhasin, M.; Hoffman, L.; Fan, Z.; Qipo, A.; Shi, H.; Bonner-Weir, S.; Putheti, P.; Degauque, N.; Libermann, T. A. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 16242.
4. Nita, I. M.; Serapinas, D.; Janciauskiene, S. M. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 1165.
5. Normandin, K.; Peant, B.; Page, C. L.; Ladurantaye, M.; Ouellet, V.; Tonin, P. N.; Provencher, D. M.; Mes-Masson, A. M. *Clin. Exp. Metastasis* **2010**, *27*, 55.
6. Hattori, T.; Koito, A.; Takatsuki, K.; Kido, H.; Katunuma, N. N. *FEBS Lett.* **1989**, *248*, 48.
7. Anderson, E. D.; Thomas, L.; Hayflick, J. S.; Thomas, G. *J. Biol. Chem.* **1993**, *268*, 24887.
8. Shapiro, L.; Pott, G. B.; Ralston, A. H. *FASEB J.* **2001**, *15*, 115.
9. Cordelier, P.; Strayer, D. S. *Biochim. Biophys. Acta.* **2003**, *1638*, 197.
10. Joslin, G.; Fallon, R. J.; Bullock, J.; Adams, S. P.; Perlmutter, D. H. *J. Biol. Chem.* **1991**, *266*, 11282.
11. Congote, L. F. *Biochem. Biophys. Res. Commun.* **2006**, *343*, 617.
12. Rhee, M.; Davis, P. *J. Biol. Chem.* **2006**, *281*, 1233.
13. Munch, J.; Standker, L.; Adermann, K.; Schulz, A.; Schindler, M.; Chinnadurai, R.; Pohlmann, S.; Chaipan, C.; Biet, T.; Peters, T.; Bernd, M.; Wilhelm, D.; Lu, H.; Jing, W.; Jiang, S.; Forssmann, W. G.; Kirchhoff, F. *Cell* **2007**, *129*, 263.
14. Shi, W.; Qi, Z.; Pan, C.; Xue, N; Debnath A. K.; Qie, J.; Jiang, S.; Liu, K.; Biochemical and Biophysical Research Communications 2008, 374, 767. These peptides were synthesized with an Fmoc strategy using Rink Amide Resin. Their N- and C-termini were acetylated and amidated, respectively. All the peptides were purified by reverse phase high performance liquid chromatography to >95% homogeneity. The molecular weight of the peptides was confirmed by MALDI-TOF-MS (Autoflex III, Bruker Daltonics).
15. Jiang S.; Lu H.; Liu S.; Zhao Q.; He Y.; Debnath A. K. Antimicrob. Agents Chemother. 2004, 48, 4349. For measuring the inhibitory activity of the peptides on infection of HIV-1 IIIB infection, 1 x 104 MT-2 cells were infected with 100 TCID50 HIV-1IIIB in the presence or absence of the peptides at graded concentrations. On the fourth day post-infection, the culture supernatants were collected for detection of HIV-1 gag antigen p24 antigen using an enzyme-linked immunosorbent assay (ELISA). For determining the cytotoxicity of the peptides, 100 μl of a peptide at a graded concentration was added to equal volumes of MT-2 cells (5×105/ml) in a well of 96-well plate. After incubation at 37 °C for 4 days, XTT (1 mg/ml; 50 μl/well; Poly-Sciences, Inc., Warrington, PA) was added. Four hours later, the soluble intracellular formazan was quantitated colorimetrically at 450 nm with a reference at 650 nm. The 50% cytotoxicity concentrations (CC_{50}) was calculated by using the software Calcusyn.
16. Camarero, J. A.; Muir, T. W. *J. Am. Che. Soc.* **1999**, *121*, 5597.
17. Yokoyama, F.; Suzuki, N.; Haruki, M.; Nishi, N.; Oishi, S.; Fujii, N.; Utani, A.; Kleinman, H. K.; Nomizu, M. *Biochemistry* **2004**, *43*, 13590.
18. Perlikowska, R.; Do-Rego, J. C.; Cravezic, A.; Fichna, J.; Wyrebska, A.; Toth, G.; Janecka, A. *Peptides* **2010**, *31*, 339.
19. Perlmutter, D. H.; Joslin, G.; Nelson, P.; Schasteen, C.; Adams, S. P.; Fallon, R. J. *J. Biol. Chem.* **1990**, *265*, 16713.
20. Camerino, M. A.; Kong, D.; Chalmers, D. K.; Thompson, P. E. *Int. J. Pept. Res. Ther.* **2008**, *14*, 323.
21. Sia, S. K.; Carr, P. A.; Cochran, A. G.; Malashkevich, V. N.; Kim, P. S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 14664.
22. Zhang L.; Tam J. P. J. Am. Chem. Soc. 1997, 119, 2363. The thioester peptides were synthesized as described by Zhang et al with the following modifications: (1) The Trityl(Trt) was used as protecting group to protect the thiol group of HSCH2CH2COOH and then attached it to onto the resin by N, N'-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBt). (2) The Trt protecting group was removed using 10%TIS/TFA and then the C-terminal amino acid was coupled to the thiol group to get thioester by 2-(1*H*-benzotriazol-1)-yl-1,1,3,3-tetramethyluronium hexafluoro-phosphate(HBTU)/diisopropylethylamine(DIEA). The lyophilized linear thiolester peptide was dissolved in 50% acetonitrile/H_2O containing 0.1 M Na_2HPO_4 at the final concentration of 2 mg/mL, followed by addition of 3 equiv TCEP and adjustment of the pH to 7.0. The reaction was monitored by HPLC. The cyclic peptides were then purified by reverse phase HPLC to >95% homogeneity. The molecular weight of the peptides was confirmed by MALDI-TOF-MS (Autoflex III, Bruker Daltonics).
23. Jiang S.; Lin K.; Strick N.; Neurath A. R. Nature 1993, 365, 113. For testing the inhibitory activity of the peptides on HIV-1-mediated cell-to-cell fusion, the chronically HIV-1IIIB-infected H9 (H9/HIV-1IIIB) cells were labeled with Calcein-Am (Molecular Probes, Inc., Eugene, OR). After washes, the fluorescence-labeled H9/HIV-1IIIB cells were incubated with MT-2 cells at 1:5 ratio at 37°C for 2 h in the absence or presence of the peptides at a graded concentration. The percentage of fused cells was counted under a fluorescence microscope (Zeiss, Germany) and the percentage of inhibition of cell fusion by the peptides was calculated as previously described.[14]
24. Chou T. C.; Hayball, M. P. 1991. CalcuSyn: Windows software for dose effect analysis. BIOSOFT, Ferguson, MO 63135, USA.
25. He Y.; Liu S.; Li J.; Lu H.; Qi Z.; Liu Z.; Debnath A. K.; Jiang S. J. Virol. 2008, 82, 11129. For determining the inhibitory activity of the peptides on infection by a pseudovirus expressing the envelop protein gp160 of the HIV-1 R5 virus SF162, U87-CCR5 cells (1×104/well) were seeded in 96-well plates and grown overnight. A peptide at indicated concentration was incubated with pseudotyped particles For construction and expression of a pseudovirus expressing the envelop protein gp160 of the HIV-1 R5 virus SF162, (1 ng p24/well) for 30 min at 37 °C. Subsequently, the virus-compound mixture was transferred to the cells and incubated for an additional 48 h. Cells were washed with PBS and lysed with the lysing reagent included in the luciferase kit (Promega, Madison, WI). Aliquots of cell lysates were transferred to luminometer plates, followed by the addition of luciferase substrate. The luciferase activity was measured in a microplate luminometer (Genios Pro, Tecan).