Bioorganic & Medicinal Chemistry 18 (2010) 6771–6775

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Peptidic HIV integrase inhibitors derived from HIV gene products: Structure–activity relationship studies

Shintaro Suzuki [a], Kasthuraiah Maddali [b], Chie Hashimoto [a], Emiko Urano [c], Nami Ohashi [a], Tomohiro Tanaka [a], Taro Ozaki [a], Hiroshi Arai [a], Hiroshi Tsutsumi [a], Tetsuo Narumi [a], Wataru Nomura [a], Naoki Yamamoto [c,d], Yves Pommier [b], Jun A. Komano [c], Hirokazu Tamamura [a,*]

[a] Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan
[b] Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4255, USA
[c] AIDS Research Center, National Institute of Infectious Diseases, Shinjuku-ku, Tokyo 162-8640, Japan
[d] Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore

ARTICLE INFO

Article history:
Received 28 June 2010
Revised 17 July 2010
Accepted 20 July 2010
Available online 25 July 2010

Keywords:
HIV integrase
Inhibitory peptide
Glu-Lys pairs
Ala-scan

ABSTRACT

Structure–activity relationship studies were conducted on HIV integrase (IN) inhibitory peptides which were found by the screening of an overlapping peptide library derived from HIV-1 gene products. Since these peptides located in the second helix of Vpr are considered to have an α-helical conformation, Glu-Lys pairs were introduced into the *i* and *i* + 4 positions to increase the helicity of the lead compound possessing an octa-arginyl group. Ala-scan was also performed on the lead compound for the identification of the amino acid residues responsible for the inhibitory activity. The results indicated the importance of an α-helical structure for the expression of inhibitory activity, and presented a binding model of integrase and the lead compound.

1. Introduction

Highly active anti-retroviral therapy (HAART), which involves a combination of two or three agents from two categories, reverse transcriptase inhibitors and protease inhibitors, has brought us remarkable success in the clinical treatment of HIV-infected and AIDS patients.[1] However, it has been accompanied by serious clinical problems including the emergence of viral strains with multidrug resistance (MDR), considerable adverse effects and nonetheless high costs. As a result, new categories of anti-HIV agents operating with mechanisms of action different from those of the above inhibitors are sought. HIV-1 integrase (IN) is a critical enzyme for the stable infection of host cells since it catalyzes the insertion of viral DNA into the genome of host cells, by means of strand transfer and 3′-end processing reactions and thus it is an attractive target for the development of anti-HIV agents. Recently, the first IN inhibitor, raltegravir (Merck),[2] has appeared in a clinical setting. It is assumed that the activity of IN must be negatively regulated during the translocation of the viral DNA from the cytoplasm to the nucleus to prevent auto-integration. The virus, as well as the host cells, must encode mechanism(s) to prevent auto-integration since the regulation of IN activity is critical for the virus to infect cells.[3] By screening a library of overlapping peptides derived from HIV-1 SF2 gene products we have found three Vpr-derived peptides, **1**, **2** and **3**, which possess significant IN inhibitory activity, indicating that IN inhibitors exist in the viral pre-integration complex (PIC).[4] The above inhibitory peptides, **1**, **2** and **3**, are consecutive overlapping peptides (Fig. 1). Compounds **4** and **5** are 12- and 18-mers from the original Vpr-sequence with the addition of an octa-arginyl group[5] into the C-terminus for cell membrane permeability, respectively. Compounds **4** and **5** have IN inhibitory activity and anti-HIV activity. Here, we report structure–activity relationship studies on these lead compounds for the development of more potent IN inhibitors.

2. Results and discussion

To determine which lead compound is most suitable for further experiments, five peptides **6–10**, which were elongated by one amino acid starting with compound **4** and extended ultimately to **5**, were synthesized (Fig. 2). Judging by the 3′-end processing and strand transfer reactions in vitro,[6] these peptides **4–10** had similar inhibitory potencies (Table 1). As a result, we concluded that 12 amino acid residues derived from the original Vpr-sequence are of sufficient for IN inhibitory activity, and any peptide among **4–10** is a suitable lead.

* Corresponding author.
E-mail address: tamamura.mr@tmd.ac.jp (H. Tamamura).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.07.050

1 AGVEAIIRILQQLLF
2 IIRILQQLLFIHFRI
3 LQQLLFIHFRIGCQH
4 Ac-LQQLLFIHFRIG-RRRRRRRR-NH_2
5 Ac-EAIIRILQQLLFIHFRIG-RRRRRRRR-NH_2

Figure 1. Amino acid sequences of compounds **1**–**5**. Compounds **1**–**3** are consecutive overlapping peptides with free N-/C-terminus. These were found by the IN inhibitory screening of a peptide library derived from HIV-1 gene products. Compounds **4** and **5** are cell penetrative leads of IN inhibitors.

4 Ac-LQQLLFIHFRIG-RRRRRRRR-NH_2
6 Ac-ILQQLLFIHFRIG-RRRRRRRR-NH_2
7 Ac-RILQQLLFIHFRIG-RRRRRRRR-NH_2
8 Ac-IRILQQLLFIHFRIG-RRRRRRRR-NH_2
9 Ac-IIRILQQLLFIHFRIG-RRRRRRRR-NH_2
10 Ac-AIIRILQQLLFIHFRIG-RRRRRRRR-NH_2
5 Ac-EAIIRILQQLLFIHFRIG-RRRRRRRR-NH_2

Figure 2. Amino acid sequences of compounds **6**–**10**, which are elongated by one amino acid from compound **4** to **5**.

Table 1
IC_{50} values of compounds **4**–**10** toward the 3′-end processing and strand transfer reactions catalyzed by HIV-1 IN

Compound	IC_{50} (μM)	
	3′-End processing	Strand transfer
4	0.13 ± 0.02	0.06 ± 0.01
5	0.09 ± 0.01	0.04 ± 0.01
6	0.10 ± 0.01	0.07 ± 0.01
7	0.13 ± 0.02	0.11 ± 0.01
8	0.26 ± 0.04	0.11 ± 0.03
9	0.11 ± 0.01	0.07 ± 0.01
10	0.08 ± 0.01	0.05 ± 0.01

Structural analysis showed that the Vpr-derived peptides, **1**, **2** and **3**, are located in the second helix of Vpr and were thus considered to have an α-helical conformation.[7] Compound **5** was adopted as a lead for the development of compounds with an increase in α-helicity since a longer peptide is likely to form a more stable α-helical structure than a shorter one. Initially, Glu (E) and Lys (K) were introduced in pairs into compound **5** at the i and $i+4$ positions. In general, such disposition of Glu-Lys pairs at i and $i+4$ positions is considered to cause an increase in α-helicity due to formation of an ionic interaction of a β-carboxy group of Glu and an ε-amino group of Lys. Several analogs of **5** with Glu-Lys pairs were synthesized by Fmoc-solid phase peptide synthesis (Fig. 3). In the inhibitory assay against the 3′-end processing and strand transfer reactions catalyzed by HIV-1 IN in vitro, compounds **11** and **15** showed more potent inhibitory activities than **5** (Table 2). Substitution of Glu-Lys for His^{14}-Gly^{18} or Ile^{3}-Leu^{7} caused no decrease in IN inhibitory activity but a significant increase in activity, suggesting that Ile^{3}, Leu^{7}, His^{14} and Gly^{18} are not indispensable for activity. Substitution of Glu-Lys for Ala^{2}-Ile^{6} or Gln^{9}-Ile^{13} caused a slight decrease in IN inhibitory activity against the 3′-end processing and strand transfer reactions (compounds **12** and **13**), indicating that Ala^{2} and/or Ile^{6}, and Gln^{9} and/or Ile^{13} are partly required for activity. Substitution of Glu-Lys for Ile^{4}-Gln^{8} caused a 2–4-fold decrease in IN inhibitory activity against the 3′-end processing and strand transfer reactions (compound **14**), showing that Ile^{4} and/or Gln^{8} are essential for activity. Substitution of Glu-Lys for Leu^{11}-Phe^{15} caused an eightfold decrease in IN inhibitory activity against the 3′-end processing reaction and a 1.5-fold decrease in IN inhibitory activity against the strand transfer reaction (compound **16**), indicating that Leu^{11} and/or Phe^{15} are indispensable for activity, especially for inhibition against 3′-end processing. Compound **17** has two substitutions of Glu-Lys for His^{14}-Gly^{18} and for Ala^{2}-Ile^{6}, which are common to compounds **11** and **12**, respectively. A twofold decrease in both IN inhibitory activities of compound **17** is mostly due to the substitution for Ala^{2}-Ile^{6} common to **12**, although **17** is slightly less active than **12** in both IN inhibitory assays.

1 5 10 15
5 Ac-EAIIRILQQLLFIHFRIG-RRRRRRRR-NH_2
11 Ac-EAIIRILQQLLFI*E*FRI*K*-RRRRRRRR-NH_2
12 Ac-E*E*IIR*K*LQQLLFIHFRIG-RRRRRRRR-NH_2
13 Ac-EAIIRILQ*E*LLF*K*HFRIG-RRRRRRRR-NH_2
14 Ac-EAI*E*RIL*K*QLLFIHFRIG-RRRRRRRR-NH_2
15 Ac-EA*E*IRI*K*QQLLFIHFRIG-RRRRRRRR-NH_2
16 Ac-EAIIRILQQL*E*FIH*K*RIG-RRRRRRRR-NH_2
17 Ac-E*E*IIR*K*LQQLLFI*E*FRI*K*-RRRRRRRR-NH_2

Figure 3. Amino acid sequences of compounds **11**–**17**, into which Glu-Lys pairs have been introduced.

Table 2
IC_{50} values of compounds **5** and **11**–**17** toward the 3′-end processing and strand transfer reactions catalyzed by HIV-1 IN

Compound	IC_{50} (μM)	
	3′-End processing	Strand transfer
5	0.09 ± 0.01	0.04 ± 0.01
11	0.05 ± 0.01	0.01 ± 0.001
12	0.12 ± 0.01	0.047 ± 0.01
13	0.14 ± 0.02	0.065 ± 0.01
14	0.23 ± 0.03	0.15 ± 0.002
15	0.04 ± 0.01	0.031 ± 0.01
16	0.71 ± 0.21	0.06 ± 0.004
17	0.18 ± 0.06	0.08 ± 0.02

Anti-HIV activity of these compounds was assessed by an MT-4 Luc system, in which MT-4 cells were stably transduced with the firefly luciferase expression cassette by a murine leukemia viral vector. MT-4 Luc cells constitutively express high levels of luciferase. HIV-1 infection significantly reduces luciferase expression due to the high susceptibility of MT-4 cells to HIV-1 infection. Protection of MT-4 Luc cells from HIV-1-induced cell death maintains the luciferase signals at high levels. In addition, the cytotoxicity of test compounds can be evaluated by a decrease of luciferase signals in these MT-4 Luc systems. The parent compound **5** showed significant anti-HIV activity at concentrations above 1.25 μM, as reported previously (Fig. 4).[4] Compound **15** showed a significant inhibitory effect against HIV-1 replication, and is thus comparable to compound **5**. Compounds **11**, **14** and **16** also displayed weak antiviral effects at concentrations of 2.5 and 5.0 μM and compounds **12**, **13** and **17** failed to show any significant anti-HIV activity. These results suggest that there is a positive correlation between IN inhibitory activity and anti-HIV activity of the compounds. None of these compounds showed significant cytotoxic effects at concentrations below 5.0 μM.

The structures of compounds **5** and **11**–**17** were assessed by CD spectroscopy. Because the aqueous solubility of these peptides is not high the peptides were dissolved in 0.1 M phosphate buffer, containing 50% MeOH at pH 5.6. The CD spectra suggest that the parent compound **5**, which has no Glu-Lys pair, forms a typical α-helical structure, and the other compounds, with the exception of **11** and **15**, form α-helical structures similarly (Fig. 5). The order of strength of α-helicity is **12**, **16** > **14** > **17** > **5** > **13**. Compounds **11** and **15** have no characteristic pattern, although IN inhibitory activities of both compounds are superior to that of the parent

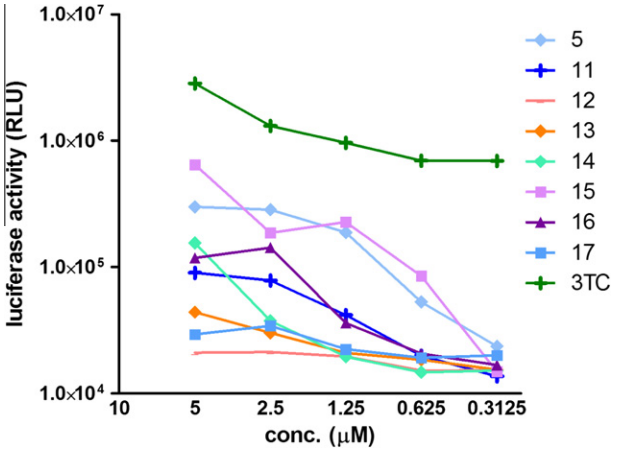

Figure 4. Luciferase signals in MT-4 Luc cells infected with HIV-1 in the presence of different concentrations of compounds **11**–**17**. Luciferase activity is expressed as relative luciferase units (RLU). 3TC is an HIV reverse transcriptase inhibitor, which was used as a positive control.

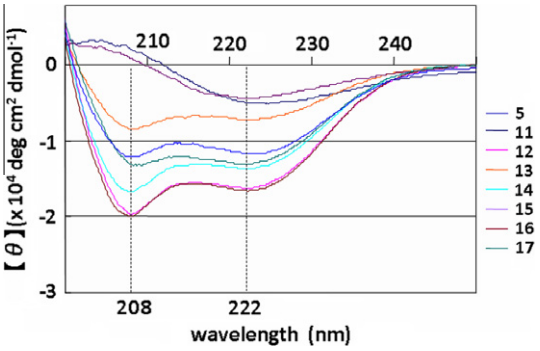

Figure 5. CD spectra of compounds **5** and **11**–**17** (5 μM) in 0.1 M phosphate buffer, pH 5.6 containing 50% MeOH at 25 °C.

compound **5**. Replacement of His[14]-Gly[18] and Ile[3]-Leu[7] by Glu-Lys in compounds **11** and **15**, respectively, caused a significant decrease in α-helicity, possibly due to formation of unfavorable salt bridges such as Glu[14]-Arg[16] and Glu[3]-Arg[5]. Introduction of a Glu-Lys pair into Gln[9]-Ile[13] in compound **13** caused a slight decrease in α-helicity, possibly due to interference in the formation of a salt bridge of Glu[1]-Arg[5] by that of Arg[5]-Glu[9]. In the other analogs, increases in α-helicity were observed to result from the introduction of Glu-Lys pairs as we had initially postulated. Overall, there is no positive correlation between IN inhibitory or anti-HIV activity and the degree of α-helicity of the compounds.

In order to identify the amino acid residues responsible for IN inhibitory and anti-HIV activities of these peptides, an Ala-scan of compound **4** was performed (Fig. 6). Compounds **18–22**, **25**, **27** and **29** showed IN inhibitory activities against the 3′-end processing and strand transfer reactions similar to those of **4** (Table 3). Ala-substitution for Leu[7], Gln[8], Gln[9], Leu[10], Leu[11], His[14], Arg[16] or Gly[18] did not cause any significant change in either of IN inhibitory activities, indicating that the replaced amino acids are not essential for IN inhibition. Ala-substitution for Phe[12], Ile[13], Phe[15] or Ile[17] gave compounds **23**, **24**, **26** and **28**, which were 2–4 times less active in both the IN inhibitory assays, suggesting that Phe[12], Ile[13], Phe[15] and Ile[17] are indispensable for IN inhibition. Assessment of anti-HIV activity in the MT-4 Luc system showed that all compounds **18**–**29** produced dose-dependent inhibition of HIV-1 replication, although they displayed cytotoxicity at 10 μM (**4**, **19–23**, **26** and **27**) or above 5 μM (**24** and **25**) (Fig. 7). Compounds **23** and **24**,

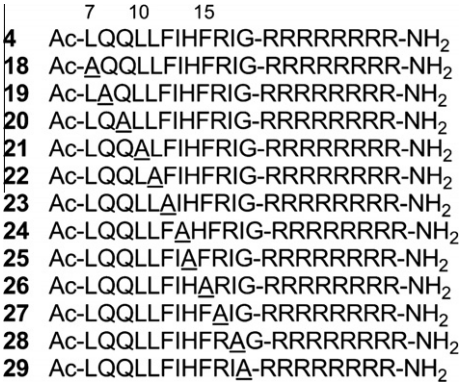

Figure 6. Amino acid sequences of compounds **18**–**29** based on an Ala-scan of compound **4**. Position numbers correspond to alignment with compound **5**.

Table 3
IC_{50} values of compounds **18–29** toward the 3′-end processing and strand transfer reactions catalyzed by HIV-1 IN

Compound	IC_{50} (μM)	
	3′-End processing	Strand transfer
4	0.11 ± 0.03	0.05 ± 0.01
18	0.12 ± 0.004	0.08 ± 0.01
19	0.13 ± 0.02	0.06 ± 0.01
20	0.10 ± 0.004	0.06 ± 0.01
21	0.12 ± 0.02	0.07 ± 0.01
22	0.13 ± 0.003	0.06 ± 0.01
23	0.34 ± 0.06	0.18 ± 0.03
24	0.33 ± 0.02	0.22 ± 0.01
25	0.13 ± 0.01	0.06 ± 0.01
26	0.25 ± 0.02	0.12 ± 0.01
27	0.11 ± 0.01	0.05 ± 0.01
28	0.20 ± 0.03	0.16 ± 0.02
29	0.09 ± 0.01	0.09 ± 0.01

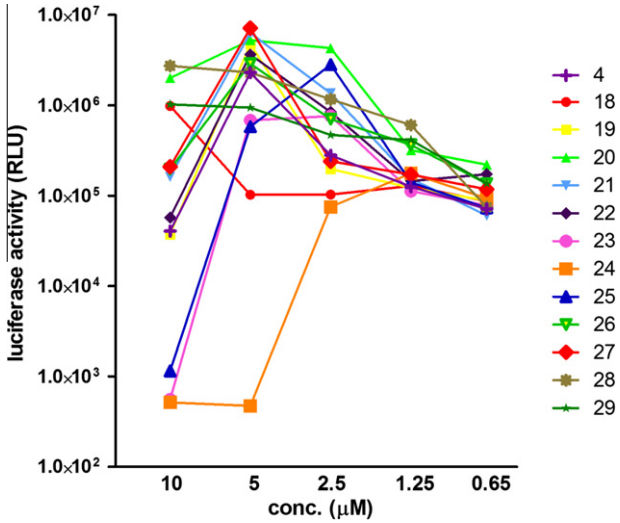

Figure 7. Luciferase signals in MT-4 Luc cells infected with HIV-1 in the presence of various concentrations of compounds **18**–**29**. Luciferase activity was valued as RLU.

with Ala-substitution for Phe[12] and Ile[13], respectively, showed weaker inhibitory activity than **4** at 5 μM. Consequently, Phe[12] and Ile[13] were deemed to be critical for activity, which is consistent with the IN inhibitory activity results. A control peptide isomer of **5** (Ac-QIFEHLAGIIQILRFLRI-R_8-NH_2) did not show anti-HIV activity at

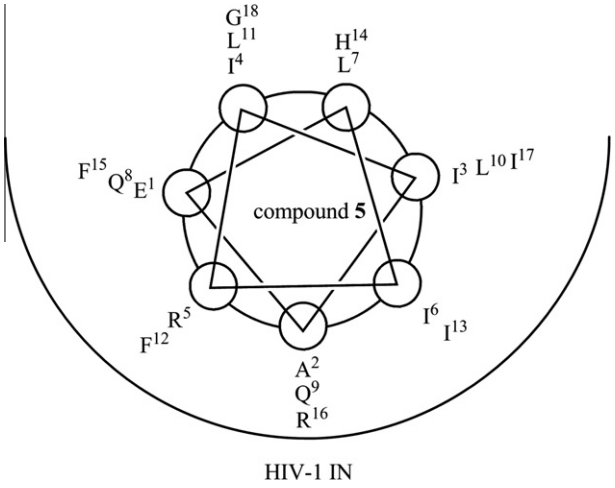

Figure 8. Brief presumed drawing of the binding model of HIV-1 IN and compound **5**.

concentrations below 10 μM, suggesting that the original Vpr-sequence, with the exceptions of Phe[12], Ile[13], Phe[15] and Ile[17], is critical for activity.

The assumption that compound **5** forms an α-helical structure when binding to HIV-1 IN suggests the binding model of IN and **5** shown in Figure 8, as **5** forms an α-helical structure in 50% aqueous MeOH solution. In this model, Phe[12], Ile[13], Phe[15] and Ile[17], which were identified by the Ala-scan experiment as critical residues, are located in the pocket of IN. His[14] and Gly[18], which can be replaced by Glu-Lys with an increase of activity in compound **11**, are located outside of the pocket of IN. Ile[3] and Leu[7] can also be replaced by Glu-Lys while retaining activity in compound **15**, and Leu[7] is located outside of the pocket, whereas Ile[3] is located in the edge of the pocket. Compounds **11** and **15** might form α-helical structures when binding to IN, although **11** or **15** does not show α-helicity in the CD spectrum. Thus, these compounds might retain IN inhibitory activity. This binding model is compatible with the results of structure–activity relationship studies involving Glu-Lys substitution and Ala-scan. The reason for decreases in IN inhibitory and anti-HIV activity of compounds **12** and **17**, which show increases of α-helicity, are possibly due to substitution of Glu-Lys for Ala[2] and Ile[6], which are located in the pocket of IN. The reason for a decrease in activity of compounds **14** and **16**, which show increased α-helicity, might be due to substitution of Lys for Gln[8] and Phe[15], respectively, which are located in the pocket of IN. The reason for decreases in IN inhibitory and anti-HIV activity of compound **13**, which also shows a decrease of α-helicity, are possibly due to substitution of Glu-Lys for Gln[9] and Ile[13], which are located in the pocket of IN.

3. Conclusion

In the present study, structure–activity relationship studies were performed on Vpr-derived peptides **4** and **5**, which had been previously identified as HIV-1 IN inhibitors.[4] The Glu-Lys substitution experiments and Ala-scan data suggest that several amino acid residues of **4** and **5** are indispensable for IN inhibitory and anti-HIV activities, and a binding model of IN and **5** were proposed. Furthermore, two novel compounds **11** and **15**, which contained Glu-Lys pairs and showed more potent IN inhibitory activities than compound **5**, were found. These data including the binding model should be useful for the development of potent HIV-1 IN inhibitors based on Vpr-peptides.

4. Experimental

4.1. Chemistry

All peptides were synthesized by the Fmoc-based solid-phase method. The synthetic peptides were purified by RP-HPLC and identified by ESI-TOF-MS. Fmoc-protected amino acids and reagents for peptide synthesis were purchased from Novabiochem, Kokusan Chemical Co., Ltd and Watanabe Chemical Industries, Ltd. Protected peptide resins were constructed on NovaSyn TGR resins (0.26 meq/g, 0.025 and 0.0125 mmol scales for Glu-Lys substitution and Ala-scan peptides, respectively). All peptides were synthesized by stepwise elongation techniques. Each cycle involves (i) deprotection of an Fmoc group with 20% (v/v) piperidine/DMF (10 mL) for 15 min and (ii) coupling with 5.0 equiv of Fmoc-protected amino acid, 5.0 equiv of diiopropylcarbodiimide (DIPCI) and 5.0 equiv of 1-hydroxybenzotriazole monohydrate ($HOBt{\cdot}H_2O$) in DMF (3 mL) for 90 min. N-Terminal α-amino groups of Glu-Lys substitution and Ala-scan peptides were acetylated with 100 equiv of acetic anhydride in DMF (10 mL). Cleavage from the resin and side chain deprotection were carried out by stirring for 1.5 h with *m*-cresol (0.25 mL), thioanisole (0.75 mL), 1,2-ethanedithiol (0.75 mL) and TFA (8.25 mL). After removal of the resins by filtration, the filtrate was concentrated under reduced pressure, the crude peptides were precipitated in cooled diethyl ether and purified by preparative RP-HPLC on a Cosmosil 5C18-AR II column (10 × 250 mm, Nacalai Tesque, Inc.) with a LaChrom Elite HTA system (Hitachi). The HPLC solvents employed were water containing 0.1% TFA (solvent A) and acetonitrile containing 0.1% TFA (solvent B). All peptides were purified using a linear gradient of solvents A and B over 30 min at a flow rate of 3 $cm^3\ min^{-1}$. The purified peptides were identified by ESI-TOF-MS (Brucker Daltonics micrOTOF-2focus) (shown in Table S1 in Supplementary data). All peptides were obtained after lyophilization as fluffy white powders of the TFA salts. The purities of these peptides were checked by analytical HPLC on a Cosmosil 5C18-ARII column (4.6 × 250 mm, Nacalai Tesque, Inc.) eluted with a linear gradient of solvents A and B at a flow rate of 1 $cm^3\ min^{-1}$, and eluted products were detected by UV at 220 nm (shown in Figs. S1–S3 in Supplementary data).

4.2. Expression and purification of F185K/C280S HIV-1 integrase from *Escherichia coli*

Plasmid encoding IN1–288/F185K/C280S was expressed in *Escherichia coli* strain C41. The solubility of the mutant protein was examined in a crude cell lysate, as follows. Cells were grown in 1 L of culture medium containing 100 μg/mL of ampicillin at 37 °C until the optical density of the culture at 600 nm was between 0.4 and 0.9. Protein expression was induced by the addition of isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 0.1 mM. After 2 h, the cells were collected by centrifugation at 6000 rpm for 30 min. After removal of the supernatant, the cells were resuspended in HED buffer (20 mM HEPES, pH 7.5, 1 mM EDTA, 1 mM DTT) with 0.5 mg/mL lysozyme and stored on ice for 30 min. The cells were sonicated until the solution exhibited minimal viscosity then it was centrifuged at 15,000 rpm for 30 min. After removal of the supernatant, the pellet was dissolved in TNM buffer (20 mM Tris/HCl, pH 8.0, 1 M NaCl, 2 mM 2-mercaptoethanol) with 5 mM imidazole and stored on ice for 30 min. The cells were then centrifuged at 15,000 rpm for 30 min and the supernatant was collected. The supernatant was then filtered through 0.45 μm filter cartridge and applied to a HisTrap column at 1 mL/min flow rate. After loading, the column was washed with 10 volume of TNM buffer with 5 mM imidazole. Protein was eluted with a linear gradient of 500 mM imidazole, containing TNM buf-

fer. Fractions containing IN were pooled and checked with SDS–PAGE.

4.3. CD spectroscopy of peptides with Glu-Lys substitution

CD measurements were performed on a JASCO J720 spectropolarimeter equipped with thermo-regulator (JASCO Corp., Ltd), using 5 μM of peptides dissolved in 0.1 M phosphate buffer, pH 5.6 containing 50% MeOH. UV spectra were recorded at 25 °C in a quartz cell 1.0 mm path length, a time constant of 1 s, and a 100 nm/min scanning speed with 0.1 nm resolution.

4.4. Integrase assays

Expression and purification of the recombinant IN in *E. coli* were performed as previously reported with addition of 10% glycerol to all buffers. Oigonucleotide substrates were prepared as described.[6] Integrase reactions were performed in 10 μL with 400 nM of recombinant IN, 20 nM of 5′-end [^{32}P]-labeled oligonucleotide substrate and inhibitors at various concentrations. Solutions of 10% DMSO without inhibitors were used as controls. Reaction mixtures were incubated at 37 °C (60 min) in buffer containing 50 mM MOPS, pH 7.2, 7.5 mM $MgCl_2$, and 14.3 mM 2-mercaptoethanol. Reactions were stopped by addition of 10 μL of loading dye (10 mM EDTA, 98% deionized formamide, 0.025% xylene cyanol and 0.025% bromophenol blue). Reactions were then subjected to electrophoresis in 20% polyacrylamide–7 M urea gels. Gels were dried and reaction products were visualized and quantitated with a Typhoon 8600 (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Densitometric analyses were performed using ImageQuant from Molecular Dynamics Inc. The concentrations at which enzyme activity was reduced by 50% (IC_{50}) were determined using 'Prism' software (GraphPad Software, San Diego, CA) for nonlinear regression to fit dose–response data to logistic curve models.

4.5. Replication assays (MT-4 luciferase assays)

MT-4 luciferase cells (1×10^3 cells) grown in 96-well plates were infected with HIV-1_{HXB2} in the presence of various concentrations of peptides. At 6–7 days post-infection, cells were lysed and the luciferase activities were measured using the Steady-Glo assay kit (Promega), according to the manufacturer's protocol. Chemiluminescence was detected with a Veritas luminometer (Promega).

Acknowledgments

N.O. and T.T. are supported by JSPS research fellowships for young scientists. This work was supported by Mitsui Life Social Welfare Foundation, Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, the Health and Labour Sciences Research Grants from Japanese Ministry of Health, Labor, and Welfare, and by the NIH Intramural Program, Center for Cancer Research, US National Cancer Institute.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.07.050.

References and notes

1. Mitsuya, H.; Erickson, J. In *Textbook of AIDS Medicine*; Merigan, T C., Bartlett, J. G., Bolognesi, D., Eds.; Williams & Wilkins: Baltimore, 1999; pp 751–780.
2. (a) Cahn, P.; Sued, O. *Lancet* **2007**, *369*, 1235; (b) Grinsztejn, B.; Nguyen, B.-Y.; Katlama, C.; Gatell, J. M.; Lazzarin, A.; Vittecoq, D.; Gonzalez, C. J.; Chen, J.; Harvey, C. M.; Isaacs, R. D. *Lancet* **2007**, *369*, 1261.
3. (a) Farnet, C. M.; Bushman, F. D. *Cell* **1997**, *88*, 483; (b) Chen, H.; Engelman, A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15270; (c) Gleenberg, I. O.; Herschhorn, A.; Hizi, A. *J. Mol. Biol.* **2007**, *369*, 1230; (d) Gleenberg, I. O.; Avidan, O.; Goldgur, Y.; Herschhorn, A.; Hizi, A. *J. Biol. Chem.* **2005**, *280*, 21987; (e) Hehl, E. A.; Joshi, P.; Kalpana, G. V.; Prasad, V. R. *J. Virol.* **2004**, *78*, 5056; (f) Tasara, T.; Maga, G.; Hottiger, M. O.; Hubscher, U. *FEBS Lett.* **2001**, *507*, 39; (g) Gleenberg, I. O.; Herschhorn, A.; Goldgur, Y.; Hizi, A. *Arch. Biochem. Biophys.* **2007**, *458*, 202.
4. Suzuki, S.; Urano, E.; Hashimoto, C.; Tsutsumi, H.; Nakahara, T.; Tanaka, T.; Nakanishi, Y.; Maddali, K.; Han, Y.; Hamatake, M.; Miyauchi, K.; Pommier, Y.; Beutler, J. A.; Sugiura, W.; Fuji, H.; Hoshino, T.; Itotani, K.; Nomura, W.; Narumi, T.; Yamamoto, N.; Komano, J. A.; Tamamura, H. *J. Med. Chem.* **2010**, *53*, 5356.
5. Suzuki, T.; Futaki, S.; Niwa, M.; Tanaka, S.; Ueda, K.; Sugiura, Y. *J. Biol. Chem.* **2002**, *277*, 2437.
6. (a) Yan, H.; Mizutani, T. C.; Nomura, N.; Tanaka, T.; Kitamura, Y.; Miura, H.; Nishizawa, M.; Tatsumi, M.; Yamamoto, N.; Sugiura, W. *Antivir. Chem. Chemother.* **2005**, *16*, 363; (b) Marchand, C.; Zhang, X.; Pais, G. C. G.; Cowansage, K.; Neamati, N.; Burke, T. R., Jr.; Pommier, Y. *J. Biol. Chem.* **2002**, *277*, 12596; (c) Semenova, E. A.; Johnson, A. A.; Marchand, C.; Davis, D. A.; Tarchoan, R.; Pommier, Y. *Mol. Pharmacol* **2006**, *69*, 1454; (d) Leh, H.; Brodin, P.; Bischerour, J.; Deprez, E.; Tauc, P.; Brochon, J. C.; LeCam, E.; Coulaud, D.; Auclair, C.; Mouscadet, J. F. *Biochemistry* **2000**, *39*, 9285; (e) Marchand, C.; Neamati, N.; Pommier, Y. In Vitro Human Immunodeficiency Virus Type 1 Integrase Assays. In *Methods in Enzymology (Drug-Nucleic Acid Interactions)*; Chaires, J. B., Waring, M. J., Eds.; Elsevier: Amsterdam, 2001; Vol. 340, pp 624–633.
7. Morellet, N.; Bouaziz, S.; Petitjean, P.; Roques, B. P. *J. Mol. Biol.* **2003**, *327*, 215.