



Mechanism of action of the HIV-1 integrase inhibitory peptide LEDGF 361–370

Zvi Hayouka^a, Aviad Levin^b, Michal Maes^a, Eran Hadas^d, Deborah E. Shalev^c, David J. Volsky^d, Abraham Loyter^b, Assaf Friedler^{a,*}

^a Institute of Chemistry, The Hebrew University of Jerusalem, Safra Campus, Givat Ram, Jerusalem 91904, Israel

^b Department of Biological Chemistry, The Hebrew University of Jerusalem, Safra Campus, Givat Ram, Jerusalem 91904, Israel

^c The Wolfson Centre for Applied Structural Biology, The Hebrew University of Jerusalem, Safra Campus, Givat Ram, Jerusalem 91904, Israel

^d Molecular Virology Division, Columbia University Medical Center, New York, NY 10019, USA

ARTICLE INFO

Article history:

Received 10 February 2010

Available online 18 February 2010

Keywords:

Alanine scan

HIV-1

Homology modeling

LEDGF/p75

Integrase

Peptides

NMR

Fluorescence anisotropy

ABSTRACT

The HIV-1 integrase protein (IN) mediates integration of the viral cDNA into the host genome and is a target for anti-HIV drugs. We have recently described a peptide derived from residues 361–370 of the IN cellular partner protein LEDGF/p75, which inhibited IN catalytic activity *in vitro* and HIV-1 replication in cells. Here we performed a comprehensive study of the LEDGF 361–370 mechanism of action *in vitro*, in cells and *in vivo*. Alanine scan, fluorescence anisotropy binding studies, homology modeling and NMR studies demonstrated that all residues in LEDGF 361–370 contribute to IN binding and inhibition. Kinetic studies in cells showed that LEDGF 361–370 specifically inhibited integration of viral cDNA. Thus, the full peptide was chosen for *in vivo* studies, in which it inhibited the production of HIV-1 RNA in mouse model. We conclude that the full LEDGF 361–370 peptide is a potent HIV-1 inhibitor and may be used for further development as an anti-HIV lead compound.

© 2010 Elsevier Inc. All rights reserved.

Introduction

The human immunodeficiency virus type 1 (HIV-1) integrase protein (IN) is a target for developing anti-HIV drugs. It catalyzes the integration of the viral cDNA into the host genome, which is a crucial step in the HIV-1 life cycle [1]. The IN inhibitors that are currently used in the clinic or at advanced stages of development are strand transfer inhibitors, which act by inhibiting DNA binding of IN [2]. Raltegravir (MK-0518) was the first IN inhibitor that was approved by the U.S. Food and Drug Administration (FDA) [3] for the treatment of HIV-1 as part of combination anti-retroviral therapy. To prevent the emergence of drug-resistant virus strains, there is a constant need to develop new drugs against known and new targets essential for virus replication.

LEDGF/p75, the cellular counterpart of IN that stimulates its activity, belongs to a family of hepatoma-derived growth factor (HDGF) related proteins (HRPs). Five mammalian HRPs are known:

Abbreviations: AIDS, acquired immunodeficiency syndrome; AA, amino acids; HIV, human immunodeficiency virus; LTR, long terminal repeat; IN, integrase; MAGI, multinuclear activation of a galactosidase indicator; LEDGF, lens epithelium derived growth factor; IBD, integrase binding domain

* Corresponding author. Fax: +972 2 6585345.

E-mail address: assaf@chem.huji.ac.il (A. Friedler).

HRP1, HRP2, HRP3, HRP4 and LEDGF [4]. The IN-binding domain (IBD) of LEDGF, which resides within the C-terminal region of the protein [4,5], is necessary although insufficient to stimulate IN activity *in vitro* [4]. HRP2 also has a functional IBD [4,6] similar to LEDGF. Purified HRP2 protein potently stimulated the *in vitro* integration activities of recombinant HIV-1 IN. HRP2 predominantly localizes to the nucleus [4,6] but does not appear to tether expressed IN to the chromatin [7].

We have previously designed several IN-inhibitory peptides based on rational design and combinatorial library screening [8–12]. Two of these peptides were derived from the IN-binding loops of LEDGF, and were designed based on the crystal structure of the complex between a dimer of the catalytic core of IN and the IBD of LEDGF [5]. The two LEDGF-derived peptides, LEDGF 361–370 and LEDGF 401–413, inhibited IN catalytic activities *in vitro* as well as HIV-1 replication in infected cells [10]. Here, we studied the LEDGF 361–370 mechanism of action using biophysical, biochemical and cellular assays. Alanine scanning and NMR studies showed that almost all residues in LEDGF 361–370 are involved in IN binding and inhibition. Thus, the full peptide was chosen for studies *in vivo*. Treating mice with LEDGF 361–370 reduced EcoHIV DNA [13–15] burdens in infected mice and significantly inhibited viral RNA synthesis *in vivo*. We conclude that the full LEDGF 361–370 peptide may be used for further development as an anti-HIV lead compound.

Results

All residues in LEDGF 361–370 contribute to IN binding and inhibition

Based on the crystal structure of the IN–LEDGF IBD complex (PDB Code: 2b4j), we designed, synthesized and labeled a shorter derivative of LEDGF 361–370. This peptide, LEDGF 365–369 (for sequence, see Table 1), includes only the residues that are in close proximity to IN in the crystal structure, including residues I365 and D366 that are important for the interaction at the protein level [5,16] (Fig. 1A and B). Fluorescence anisotropy showed that LEDGF 365–369 bound IN 2-fold weaker than LEDGF 361–370, with $K_d = 10 \mu\text{M}$ (Fig. 2 and Table 1). Using a quantitative *in vitro* integration assay, LEDGF 365–369 was found to inhibit IN activity only by about 30%, while LEDGF 361–370 inhibited IN by about 80% at the same molar ratio and under the same experimental conditions (Fig. 3A).

To test the contribution of LEDGF-specific residues to the inhibitory activity of the peptide, we mutated its sequence based on the homologous sequences in HRP2. The full-length sequences of LEDGF and HRP2 have 29% identity and 49% similarity. The predicted IBD of HRP2 has 50% identity and 77% similarity to the IN-IBD. There is no structural data for the HRP2 protein. Therefore, the HRP2 IBD structure was predicted using homology modeling based on the LEDGF IBD structure (PDB Code: 2b4j) to design a LEDGF 361–370 homologous peptide derived from the HRP2 IBD. The HRP2 peptide, derived from residues 483–493 (Fig. 1C and Table 1), was synthesized according to this model. Fluorescence anisotropy showed that HRP2 483–493 bound IN with an affinity of around $8 \mu\text{M}$ (Fig. 2 and Table 1) and inhibited IN catalytic activity *in vitro* only by around 20% (Fig. 3A).

Alanine scan of LEDGF 361–370 was performed to further determine which precise residues participate in IN binding. Eleven peptides were synthesized based on the sequence of the LEDGF 361–370: in each, a single residue was substituted by alanine (for sequences, see Table 1). Fluorescence anisotropy binding studies showed that IN bound all the LEDGF 361–370 derived peptides with low micromolar affinity, similar to the parent LEDGF 361–370 peptide (Fig. 2 and Table 1). The peptides S362A, I365A and V370A showed weaker IN binding, by factors of 2–3 (Table 1 and Fig. 2). Since D366 is a key residue for IN binding at the protein level [5,16] and D369 is proximate in the sequence and may have a similar effect at the peptide level, a peptide in which both Asp residues were replaced by alanine was also synthesized. The D366/369A mutant bound IN 2-fold weaker than the WT LEDGF 361–370 (Fig. 2 and Table 1).

Changes in chemical shift of LEDGF 361–370 upon adding IN were followed by NMR to determine which residues participate in IN binding. Fig. 3B shows the overlay of the TOCSY spectra of LEDGF 361–370 with (red) and without (blue) IN. The deviations of backbone $\text{H}\alpha$ and HN proton chemical shifts upon IN binding are shown in Fig. 3C. The TOCSY spectra show clear deviations of chemical shift for L363, I365, L368 and D369, and lesser degrees of deviations for K364, D366 and N367. The HN of N361 was not detected possibly because its deviation from the free peptide chemical shift was sufficiently large to move it into the region of the water signal. The NMR results indicate that LEDGF 361–370 is unstructured in its free form due to the limited spread of the amide chemical shift region. The bound spectrum also had a small spread of chemical shift, indicating that the predominant species in solution is still unstructured.

Most of the alanine substituted peptides inhibited IN catalytic activity in the same manner as the parent LEDGF 361–370 peptide (Fig. 3A). This indicated that the substitutions had almost no effect on activity, and no single residue is solely responsible for the inhibitory activity of the peptide. These results are in agreement with the fluorescence anisotropy and NMR results. LEDGF 361–370 D366A and the double mutant LEDGF 361–370 D366/369A were less potent inhibitors compared to the other substituted peptides, indicating the importance of the Asp residues for IN inhibition (Fig. 3A).

LEDGF 361–370 inhibits HIV-1 replication in cells by inhibiting the integration step: a kinetic study

A kinetic study of HIV-1 inhibition in cells by LEDGF 361–370 was performed to further establish the viral replication cycle stage at which the peptide acts (Fig. 4A) [17]. Dextran sulfate, LEDGF 361–370, and AZT were added at different time points to HIV-1 infected Sup-T1 cells and the amount of viral p24 protein, which reflects efficiency of virus infection, was estimated at 48 h post-infection [18]. This experiment determines the time of addition during the viral life cycle at which the anti-HIV compound had an inhibitory effect. Inhibition is observed if the inhibitor is added before or during the stage at which it is active, but not if added after the stage at which it acts. Two reference compounds were dextran sulfate, a polyanion that interferes with the binding of the virus to the cell [19], and the nucleoside analog AZT that inhibits the reverse transcription process [20]. Dextran sulfate blocked virus infection only when added during the first 4 h post-infection (Fig. 4A), until virus–cell binding was finished, while AZT blocked infection when added within the

Table 1
IN binding and inhibition of IN catalytic activity by the LEDGF 361–370 derived peptides.

Peptide	Sequence	K_d^a (μM)	Hill coefficient	IN inhibition ^b (%)
LEDGF 361–370	WNSLKIDNLDV	5.1 ± 0.1	3.6 ± 0.3	81
N361A	WASLKIDNLDV	7.5 ± 0.2	3.0 ± 0.3	76
S362A	WNALKIDNLDV	11.2 ± 0.2	6.1 ± 0.6	79
L363A	WNSAKIDNLDV	5.3 ± 0.2	3.6 ± 0.3	71
K364A	WNSLAIDNLDV	5.3 ± 0.3	4.9 ± 0.7	68
I365A	WNSLKADNLDV	9.0 ± 0.8	5.0 ± 0.9	65
D366A	WNSLKIANLDV	5.5 ± 0.1	4.0 ± 0.4	58
N367A	WNSLKIDALDV	7.7 ± 0.1	5.1 ± 0.7	75
L368A	WNSLKIDNADV	6.0 ± 0.4	4.2 ± 0.4	75
D369A	WNSLKIDNLAV	7.0 ± 0.4	3.6 ± 0.3	73
V370A	WNSLKIDNLDA	14.0 ± 0.2	5.4 ± 0.6	68
D366/369A	WNSLKIANLAV	9.5 ± 0.2	5.3 ± 0.3	43
LEDGF 365–369	WIDNLD	10.0 ± 0.5	3.6 ± 0.2	30
HRP2 483–493	WKFALKVDSPDV	8.0 ± 0.9	4.4 ± 0.6	25

^a K_d values were determined using fluorescence anisotropy binding studies described in Fig. 2. IN ($60 \mu\text{M}$) was titrated into the fluorescein-labeled Ala scan peptides (100 nM). Data were fit to the Hill equation as described in [10].

^b The values are based on the data presented in Fig. 3.

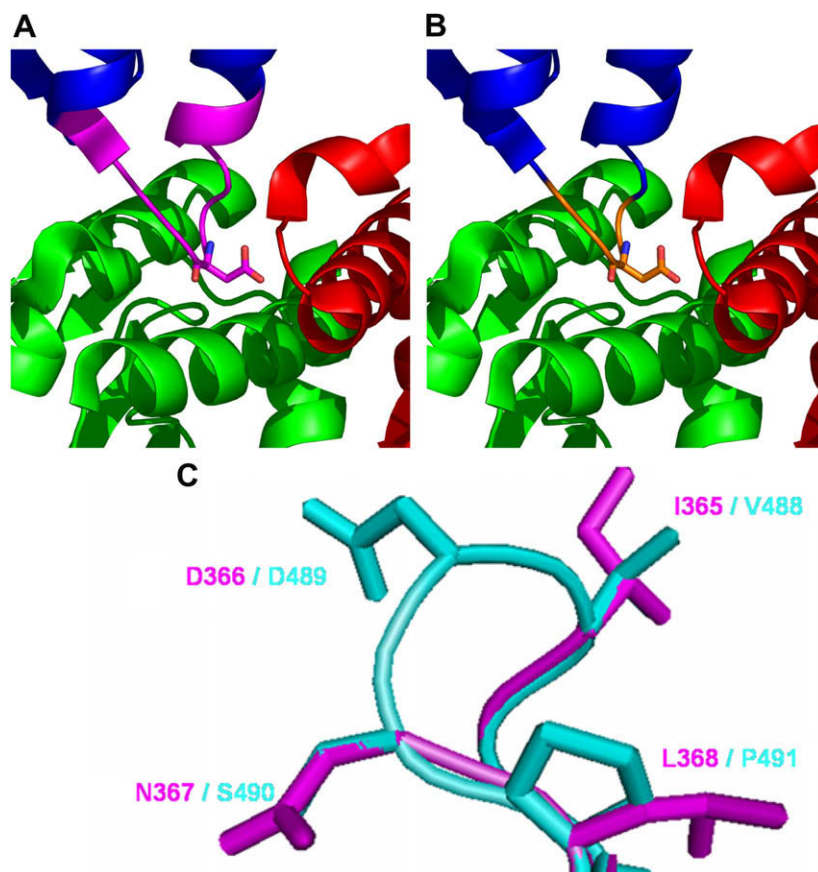


Fig. 1. LEDGF 361–370 derivatives design. Based on the crystal structure of the IN-LEDGF IBD complex (PDB Code: 2b4j), we designed a shorter LEDGF-derived peptide containing residues 365–369, which are the main residues that participate in IN binding. Shown is the structure of the LEDGF-IN complex [5], with the two IN monomers in red and green, and the LEDGF IBD in blue. (A) LEDGF 361–370 in magenta (B) LEDGF 365–369 in orange. (C) Designing a LEDGF 361–370 homolog derived from HRP2. The differences between LEDGF 361–370 (magenta) and its homolog HRP2 483–493 (cyan) (in sticks) based on the homology modeling of the predicted HRP2 IBD structure based on the crystal structure of IN-LEDGF IBD (PDB Code: 2b4j). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

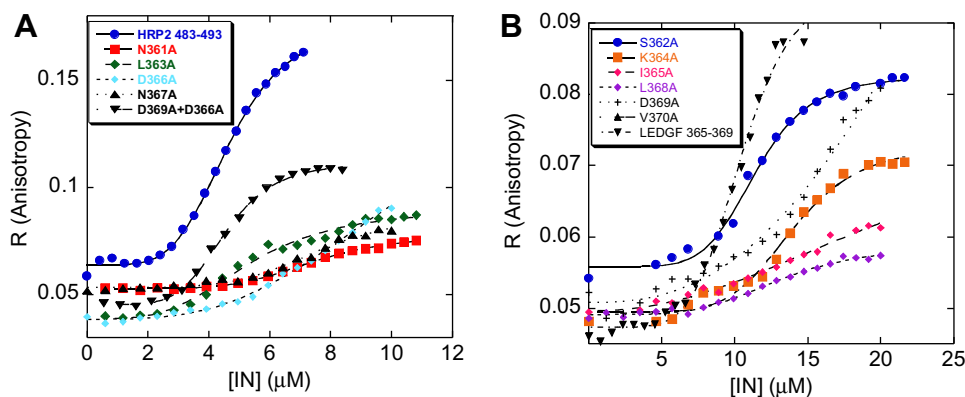


Fig. 2. Binding of the LEDGF 361–370 derived peptides to IN. Binding was determined using fluorescence anisotropy. IN (60 μM) was titrated into the fluorescein-labeled LEDGF 361–370 derived peptides (100 nM) as indicated in the figure. Data were fit to the Hill equation. Binding affinities and Hill coefficients are shown in Table 1.

first 10 h of infection, until the reverse transcription stage was completed (Fig. 4A). LEDGF 361–370 inhibited the integration step when added within the first 18 h after infection. When it was added after the integration period it did not affect HIV-1 replication. These results confirmed that LEDGF 361–370 inhibited HIV-1 replication in cells by specifically inhibiting the integration stage, as was proposed before [20].

LEDGF 361–370 inhibited HIV-1 infection in mice model

LEDGF 361–370 was tested for its potential antiretroviral activity *in vivo* using the model of infection of mice with chimeric HIV [13,14] (Fig. 4B and C). Mice were sacrificed and analyzed for virus gag DNA (Fig. 4B) and vif RNA (Fig. 4C) burdens in the spleen [21]. These results showed that prophylactic treatment of mice with

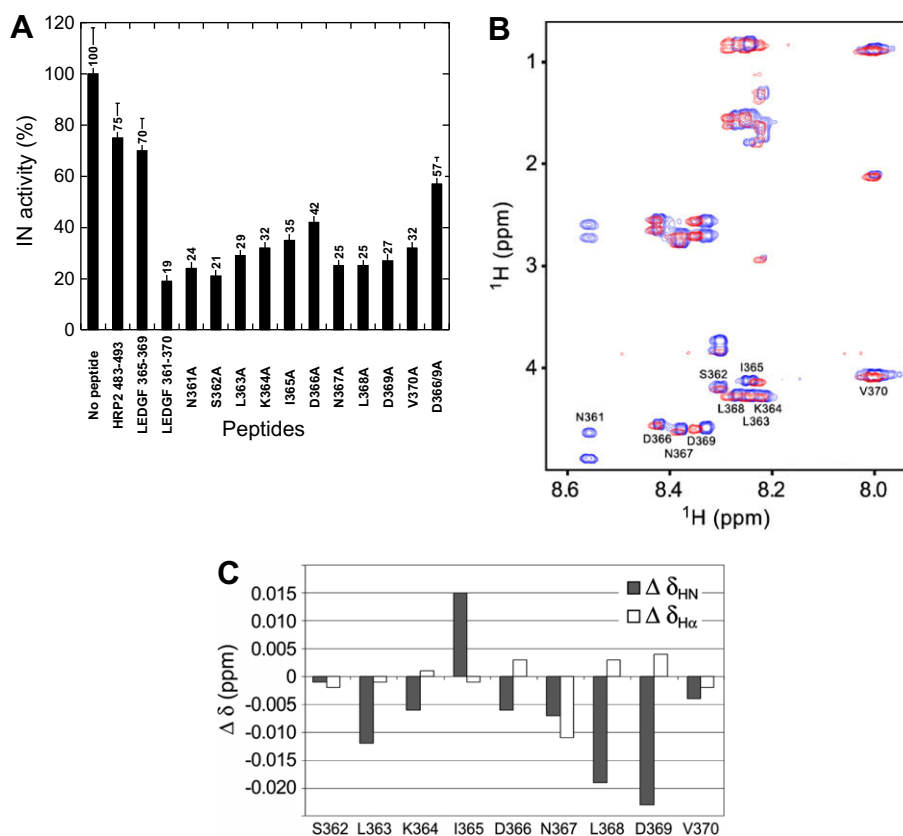


Fig. 3. Binding of LEDGF 361–370 to IN and the effect of its derived peptides on IN catalytic activity *in vitro*. (A) IN (390 nM) was incubated in the presence of the indicated peptides at 1:50 IN:peptide molar ratio and the overall integration process was monitored using the quantitative assay system. The results are summarized in Table 1. (B, C) Chemical shift deviations of the LEDGF 361–370 peptide upon IN binding—NMR studies: (B) overlay of the fingerprint region of the TOCSY spectra of LEDGF 361–370 with (red) and without (blue) IN. The spectra were assigned using TOCSY and NOESY spectra acquired under identical conditions. (C) The changes in the chemical shift of the LEDGF 361–370 amides and the alpha protons, calculated based on Fig. 3B. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

LEDGF 361–370 reduced viral DNA synthesis by about 50% two days after infection (Fig. 4B). The reduction did not reach statistical significance when analyzed by a two-tailed Student's *t*-test ($p = 0.075$) but was significant in a less stringent single-tailed *t*-test ($p = 0.037$). In contrast, LEDGF 361–370 reduced *de novo* synthesis of the single-spliced viral vif mRNA by about 80% (Fig. 4C) and this reduction was highly significant by all statistical measures including both single- and two-tailed *t*-tests ($p = 0.001$ and $p = 0.002$, respectively). We conclude that in the model of acute infection of mice with chimeric HIV, the LEDGF 361–370 peptide significantly inhibits *de novo* synthesis of viral RNA *in vivo*. Thus, LEDGF 361–370 may serve as a lead compound as an anti-HIV-1 inhibitor for further studies.

Discussion

Using a combination of alanine scan, fluorescence anisotropy, NMR and IN enzymatic assay, we show that the full LEDGF 361–370 sequence is required for IN binding and inhibition. The truncated analog, LEDGF 365–369, was a significantly weaker IN inhibitor than the parent peptide. Although the LEDGF–IN complex structure shows that residues 365–369 are important for IN binding at the protein level, they are not sufficient for inhibition at the peptide level. This may be due to missing interactions that stabilize its bioactive conformation. HRP2 483–493, the LEDGF 361–370 homolog, also showed very low inhibition of IN activity. This may be explained by the following differences between the peptide sequences: LEDGF I365 vs. HRP2 V488, LEDGF N367 vs. HRP2 S490,

and LEDGF L368 vs. HRP2 P491. The proline residue in the HRP2 peptide may reduce its conformational flexibility and prevent it from achieving the bioactive conformation required for IN inhibition.

LEDGF D366 is central in the interaction between the LEDGF and IN proteins [5]. When D366 was replaced by alanine, the peptide still bound IN and inhibited its activity by 60%. In the LEDGF sequence there are two pairs of consecutive hydrophobic and acidic residues: I365, D366 (ID) and L368, D369 (LD). While the ID motif is important for protein binding and activity, it is possible that the LD motif may participate in peptide binding. In HRP2 483–493, the leucine residue from the LD motif was replaced by proline, which may explain the loss of activity. The NMR results show that the residues whose chemical shifts significantly changed upon binding IN were I365, L368 and D369. The chemical shift changes for D366 were weaker. This supports the involvement of the LD motif in IN binding.

The alanine scan results showed no specific residue that is primarily responsible for the interaction of the peptide with IN. This was supported by the NMR chemical shift deviations that showed that the chemical environment of most of the residues changed upon binding IN. Other cases of alanine scans that implied that no single major amino acid is crucial for binding or activity were also reported for statherin [22] and aspartate transcarbamoylase [23]. Both studies showed that an alanine scan of a peptide, or even of a full-length enzyme, may suggest that no single amino acid is crucial for binding and activity. These findings are in line with the results obtained here.

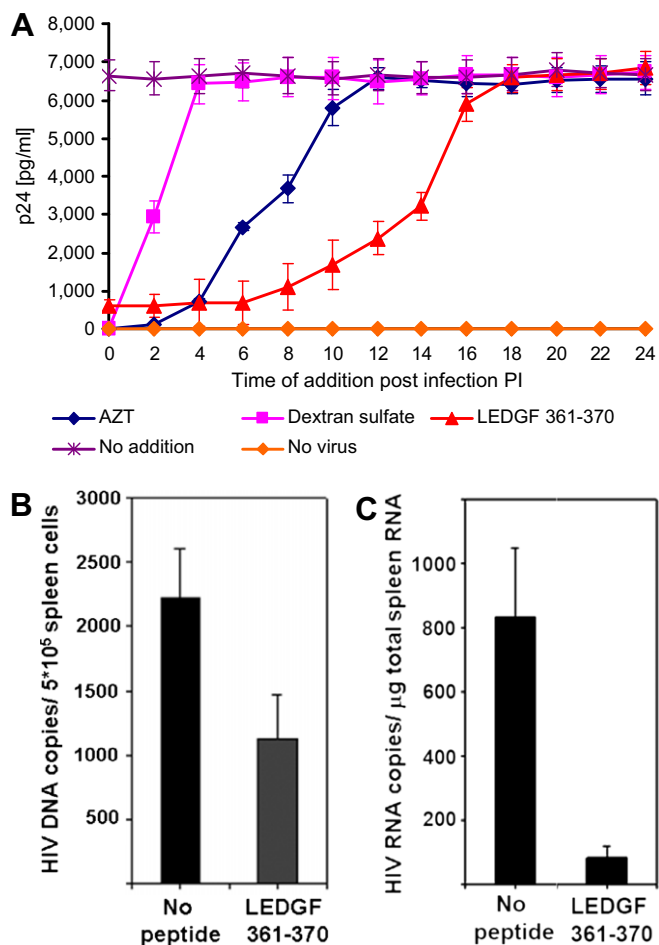


Fig. 4. LEDGF 361–370 inhibits the integration step in HIV-1 infected cells and in mice model. (A) Influence of the time of addition on p24 production: kinetic study. Sup-T1 cells were infected with HIV-1 and the indicated inhibitors were added at different time points PI. Viral p24 was determined at 48 h PI. No addition (purple X); no virus (orange diamond); dextran sulfate 20 μ M (magenta square); AZT 2 μ M (blue diamond); LEDGF 361–370 12.5 μ M (red triangle). (B, C) LEDGF 361–370 inhibited formation of HIV-1 vif RNA in mice model. Mice were pretreated with 40 mg/kg/day of LEDGF 361–370 or vehicle for 2.5 days, followed by inoculation with EcoHIV/NDK and continuation of treatment for additional 2 days. Mice were then sacrificed and analyzed for (B) virus gag DNA and (C) vif RNA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Our kinetic studies showed that LEDGF 361–370 specifically inhibited the integration step and has a profile of an IN inhibitor. This is in agreement with our previous results using other techniques [20]. *De novo* synthesis of HIV RNA, but not DNA, was inhibited by LEDGF 361–370 during acute infection of mice with EcoHIV. This is consistent with the proposed action of this peptide as a post-reverse transcription inhibitor of HIV replication. EcoHIV reproduces all HIV-1 functions *in vivo* except gp120/41 mediated receptor binding and fusion, and the infection [13,14]. The observed reduction but insignificant inhibition of viral DNA burdens in LEDGF 361–370 treated mice is consistent with this proposal, and it also indicates that LEDGF 361–370 does not block virus entry into the cells or other pre-RT steps of virus replication *in vivo* as was shown [10]. On the other hand, LEDGF 361–370 was highly effective in blocking *de novo* synthesis of viral RNA *in vivo*. The extent of this inhibition was higher than that of DNA synthesis (50% vs. 80%), indicating an effect on both the first cycle of virus infection *in vivo* and on subsequent virus spread. Since viral RNA synthesis occurs after proviral DNA integration, this result is

consistent with LEDGF 361–370 acting during integration or transcription of viral RNA.

In summary, we show that a rationally designed LEDGF-derived peptide that inhibits IN catalytic activity is also active in a mouse model. We conclude that LEDGF 361–370 has a potential as an anti-HIV drug lead.

Experimental

Peptide synthesis, labeling and purification. Peptides were synthesized on an Applied Biosystems (ABI) 433A peptide synthesizer and purified as described in [10].

Sequence alignment and homology modeling. The 3D model of human HRP2 IBD was built using homology modeling program SWISSMODEL [24] based on high-resolution crystal structure of homolog protein LEDGF IBD (PDB Code: 2b4j).

Protein expression and purification. Full-length IN 1–288 was expressed and purified as described in [10].

Fluorescence anisotropy. Measurements were performed using a PerkinElmer LS-55 luminescence spectrofluorimeter equipped with a Hamilton Microlab 500 dispenser as described in [10].

Quantitative estimation of IN catalytic activity *in vitro*. The IN enzymatic activity was determined by a quantitative assay as described in [9,25].

NMR measurements. A 1 mM solution of LEDGF 361–370 in 20 mM phosphate buffer, 100 mM sodium chloride, 0.02 wt% sodium azide with 10% v/v D₂O was prepared from the lyophilized powder. The solution had an apparent pH of 6.87. The NMR experiments were performed on a Bruker Avance 600 MHz DMX spectrometer operating at the proton frequency of 600.13 MHz using a 5 mm selective probe equipped with a self-shielded xyz-gradient coil. The water resonance was suppressed using a 3–9–19 pulse sequence with gradients for TOCSY [26,27] and NOESY [28,29] experiments. The transmitter frequency was set on the HDO signal that was calibrated at 4.868 ppm. Two-dimensional homonuclear spectra were acquired in the phase-sensitive mode with 4 K complex data points in t_2 and 512 t_1 increments. The spectral width was 12 ppm and the relaxation delays were set to 1.5 s. Data was acquired at 288.0 \pm 0.1 K. TOCSY spectra were recorded using the MLEV-17 pulse scheme for the spin lock at mixing periods of 100 ms. The NOESY experiments were collected with a mixing time of 100 ms. Spectra were processed and analyzed with the XWINNMR software package (Bruker Analytische Messtechnik GmbH) and SPARKY (provided by T.D. Goddard and D.G. Kneller, SPARKY 3, University of California, San Francisco). Resonance assignment was done according to the sequential assignment methodology developed by Wuthrich [30] based on the TOCSY and NOESY spectra measured under identical experimental conditions.

Mammalian cultured cells. Monolayer adherent HeLa, HEK293T, LC5-RIC, HeLa MAGI cells (TZM-bl) and the T-lymphocyte cell lines Sup-T1 and H9 were grown and treated as described in [10].

Viruses. Wild-type HIV-1 was generated by transfection of HEK293T cells with pSVC21 plasmid containing the full-length HIV-1HXB2 viral DNA. Wild-type and Δ env/VSV-G viruses as described in [9,17]. Cultured lymphocytes (1×10^5) were treated and infected as described in [9,17].

Time-of-addition assay (the time at which various components were added to virus infected cultured cells). Sup-T1 cells were infected with wild-type HIV-1 at a MOI of 2, and the test compounds 20 μ M dextran sulfate, 2 μ M AZT, 12.5 μ M LEDGF 361–370, were added at different time points after infection (every 2 h for 24 h). Viral p24 production was determined at 48 h post-infection [18].

Evaluation of antiretroviral activity of LEDGF 361–370 *in vivo*. Peptides were tested for prophylaxis of HIV infection in mice

essentially as described in [14]. Animal infections were conducted with EcoHIV/NDK which was constructed, propagated, and tested as described in [13]. All animal studies were conducted with the approval of the St. Luke's–Roosevelt Institutional Animal Care and Use Committee. Groups of five adult 129 \times 1/SvJ mice were injected subcutaneously every 12 h for a total of 96 h with either 0.5 ml 2% DMSO in saline (vehicle) or 400 μ g (40 mg/kg/day) of LEDGF 361–370 in 0.5 ml of vehicle, respectively [31]. The animals were euthanized, spleens were removed, and a fragment of the spleen was placed in RNALater (Qiagen, Valencia, CA) for RNA extraction. DNA and RNA were isolated and burdens of viral gag DNA and single-spliced vif RNA were quantified by real-time PCR (QPCR) [14]. Statistical significance of differences among groups of treated and control mice were established by Student's *t*-test (Excel statistical package).

Acknowledgments

This study was supported by a starting grant from the European Research Council (ERC) (to A.F.) and by Grants DA017618 and NS054580 from the National Institutes of Health, the US Public Health Service (to D.J.V.). We would like to thank G. Bentsman and D. Kim for technical help in animal experiments.

References

- [1] M.P. Sherman, W.C. Greene, Slipping through the door: HIV entry into the nucleus, *Microbes Infect.* 4 (2002) 67–73.
- [2] S.A. Merschman, P.T. Vallano, L.A. Wenning, B.K. Matuszewski, E.J. Woolf, Determination of the HIV integrase inhibitor, MK-0518 (raltegravir), in human plasma using 96-well liquid–liquid extraction and HPLC–MS/MS, *J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci.* 857 (2007) 15–24.
- [3] M. Lataillade, M.J. Kozal, The hunt for HIV-1 integrase inhibitors, *AIDS Patient Care STDS* 20 (2006) 489–501.
- [4] P. Cherepanov, E. Devroe, P.A. Silver, A. Engelman, Identification of an evolutionarily conserved domain in human lens epithelium-derived growth factor/transcriptional co-activator p75 (LEDGF/p75) that binds HIV-1 integrase, *J. Biol. Chem.* 279 (2004) 48883–48892.
- [5] P. Cherepanov, A.L. Ambrosio, S. Rahman, T. Ellenberger, A. Engelman, Structural basis for the recognition between HIV-1 integrase and transcriptional coactivator p75, *Proc. Natl. Acad. Sci. USA* 102 (2005) 17308–17313.
- [6] M. Vanegas, M. Llano, S. Delgado, D. Thompson, M. Peretz, E. Poeschla, Identification of the LEDGF/p75 HIV-1 integrase-interaction domain and NLS reveals NLS-independent chromatin tethering, *J. Cell Sci.* 118 (2005) 1733–1743.
- [7] S. Rahman, R. Lu, N. Vandegraaff, P. Cherepanov, A. Engelman, Structure-based mutagenesis of the integrase-LEDGF/p75 interface uncouples a strict correlation between in vitro protein binding and HIV-1 fitness, *Virology* 357 (2007) 79–90.
- [8] M. Maes, A. Levin, Z. Hayouka, D.E. Shalev, A. Loyter, A. Friedler, Peptide inhibitors of HIV-1 integrase: from mechanistic studies to improved lead compounds, *Bioorg. Med. Chem.* 17 (2009) 7635–7642.
- [9] J. Rosenbluh, Z. Hayouka, S. Loya, A. Levin, A. Armon-Omer, E. Britan, A. Hizi, M. Kotler, A. Friedler, A. Loyter, Interaction between HIV-1 Rev and integrase proteins: a basis for the development of anti-HIV peptides, *J. Biol. Chem.* 282 (2007) 15743–15753.
- [10] Z. Hayouka, J. Rosenbluh, A. Levin, S. Loya, M. Lebendiker, D. Veprintsev, M. Kotler, A. Hizi, A. Loyter, A. Friedler, Inhibiting HIV-1 integrase by shifting its oligomerization equilibrium, *Proc. Natl. Acad. Sci. USA* 104 (2007) 8316–8321.
- [11] Z. Hayouka, J. Rosenbluh, A. Levin, M. Maes, A. Loyter, A. Friedler, Peptides derived from HIV-1 Rev inhibit HIV-1 integrase in a shiftide mechanism, *Biopolymers* 90 (2008) 481–487.
- [12] A. Armon-Omer, A. Levin, Z. Hayouka, K. Butz, F. Hoppe-Seyler, S. Loya, A. Hizi, A. Friedler, A. Loyter, Correlation between shiftide activity and HIV-1 integrase inhibition by a peptide selected from a combinatorial library, *J. Mol. Biol.* 376 (2008) 971–982.
- [13] M.J. Potash, W. Chao, G. Bentsman, N. Paris, M. Saini, J. Nitkiewicz, P. Belem, L. Sharer, A.I. Brooks, D.J. Volsky, A mouse model for study of systemic HIV-1 infection, antiviral immune responses, and neuroinvasiveness, *Proc. Natl. Acad. Sci. USA* 102 (2005) 3760–3765.
- [14] E. Hadas, A. Borjabad, W. Chao, M. Saini, K. Ichiyama, M.J. Potash, D.J. Volsky, Testing antiretroviral drug efficacy in conventional mice infected with chimeric HIV-1, *AIDS* 21 (2007) 905–909.
- [15] M. Saini, E. Hadas, D.J. Volsky, M.J. Potash, Vaccine-induced protection from infection of mice by chimeric human immunodeficiency virus type 1, *EcoHIV/NL4-3*, *Vaccine* 25 (2007) 8660–8663.
- [16] P. Cherepanov, Z.Y. Sun, S. Rahman, G. Maertens, G. Wagner, A. Engelman, Solution structure of the HIV-1 integrase-binding domain in LEDGF/p75, *Nat. Struct. Mol. Biol.* 12 (2005) 526–532.
- [17] A. Levin, Z. Hayouka, M. Helfer, R. Brack-Werner, A. Friedler, A. Loyter, Peptides derived from HIV-1 integrase that bind Rev stimulate viral genome integration, *PLoS One* 4 (2009) e4155.
- [18] C. Pannecouque, W. Pluymers, B. Van Maele, V. Tetz, P. Cherepanov, E. De Clercq, M. Witvrouw, Z. Debyser, New class of HIV integrase inhibitors that block viral replication in cell culture, *Curr. Biol.* 12 (2002) 1169–1177.
- [19] M. Baba, R. Pauwels, J. Balzarini, J. Arnout, J. Desmyter, E. De Clercq, Mechanism of inhibitory effect of dextran sulfate and heparin on replication of human immunodeficiency virus in vitro, *Proc. Natl. Acad. Sci. USA* 85 (1988) 6132–6136.
- [20] M. Rigour, J.M. Lanchy, S.F. Le Grice, B. Ehresmann, C. Ehresmann, R. Marquet, Inhibition of the initiation of HIV-1 reverse transcription by 3'-azido-3'-deoxythymidine. Comparison with elongation, *J. Biol. Chem.* 275 (2000) 26944–26951.
- [21] J.A. Levy, Pathogenesis of human immunodeficiency virus infection, *Microbiol. Rev.* 57 (1993) 183–289.
- [22] S. Sekine, K. Kataoka, M. Tanaka, H. Nagata, T. Kawakami, K. Akaji, S. Aimoto, S. Shizukuishi, Active domains of salivary statherin on apatitic surfaces for binding to *Fusobacterium nucleatum* cells, *Microbiology* 150 (2004) 2373–2379.
- [23] N.J. Dembowski, E.R. Kantrowitz, The use of alanine scanning mutagenesis to determine the role of the N-terminus of the regulatory chain in the heterotropic mechanism of *Escherichia coli* aspartate transcarbamoylase, *Protein Eng.* 7 (1994) 673–679.
- [24] M.S. Baig, N. Manickam, Homology modeling and docking studies of *Comamonas testosteroni* B-356 biphenyl-2,3-dioxygenase involved in degradation of polychlorinated biphenyls, *Int. J. Biol. Macromol.* 46 (2010) 47–53.
- [25] A. Engelman, K. Mizuuchi, R. Craigie, HIV-1 DNA integration: mechanism of viral DNA cleavage and DNA strand transfer, *Cell* 67 (1991) 1211–1221.
- [26] M. Pionto, V. Saudek, V. Sklenar, Gradient-tailored excitation for single-quantum NMR spectroscopy of aqueous solutions, *J. Biomol. NMR* 2 (1992) 661–665.
- [27] A. Bax, D. Davis, MLEV-17 based two-dimensional homonuclear magnetization transfer spectroscopy, *J. Magn. Reson.* 65 (1985) 355–360.
- [28] J. Jeener, B.H. Meier, P. Bachmann, R.R. Ernst, Investigation of exchange processes by 2-dimensional NMR-spectroscopy, *J. Chem. Phys.* 71 (1979) 4546–4553.
- [29] R. Wagner, S. Berger, Gradient-selected NOESY—a fourfold reduction of the measurement time for the NOESY experiment, *J. Magn. Reson. A* 123 (1996) 119–121.
- [30] K. Wuthrich, *NMR of Proteins and Nucleic Acids*, John Wiley & Sons, New York, 1986.
- [31] C. Goffinet, I. Allespach, O.T. Keppler, HIV-susceptible transgenic rats allow rapid preclinical testing of antiviral compounds targeting virus entry or reverse transcription, *Proc. Natl. Acad. Sci. USA* 104 (2007) 1015–1020.