

Peptide inhibitors of the HIV-1 integrase protein

Zvi Hayouka, Aviad Levin, Abraham Loyter and Assaf Friedler



Prof. Assaf Friedler was born in Haifa, Israel, in 1971. He did his undergraduate studies in chemistry at the Hebrew University of Jerusalem, and Ph.D studies in chemistry at the Hebrew University of Jerusalem under the supervision of Prof. Chaim Gilon, in peptide chemistry and medicinal chemistry. After receiving his PhD degree, in 2000, Prof. Friedler moved to Cambridge, UK, to do his post-doctoral research at the MRC centre for protein engineering in the lab of Prof. Sir Alan Fersht, in the field of biophysical studies of protein-protein interactions. The major achievement of the post-doc was development of peptides that refold and reactivate mutants of the tumor suppressor p53. Since 2004 Prof. Friedler runs his independent research group at the institute of chemistry in the Hebrew University of Jerusalem, Israel (<http://chem.ch.huji.ac.il/~assaf/>). Since October 2010 he serves as the head of the school of chemistry at the Hebrew university. His major research interests are using peptides to study protein-protein interactions in health and disease, and developing peptides as drugs that modulate these interactions. Specifically, studies are focused on biological systems related to AIDS and cancer, for example: (1) development of peptide inhibitors of the HIV-1 integrase protein; (2) mapping the interaction networks of apoptosis-related proteins; (3) modulating protein oligomerization using peptides. Prof. Friedler recently won the prestigious starting grant from the ERC (European Research Council) as well as the outstanding young scientist prize by the Israeli Chemical Society.



Zvi Hayouka earned his B.sc degree in chemistry and biology at Hebrew University of Jerusalem, at 2004. In 2005, Zvi performed his M.Sc. study in the laboratories of Prof. Tuvia Sheradsky, Dept. of Organic Chemistry, and Prof. Zvi Selinger, Dept. of Biological Chemistry, The Hebrew University of Jerusalem, on the topic of Restoring the GTPase activation of mutant protein Ras. In 2010, Zvi has completed his Ph.D studies at the Institute of Chemistry, the Hebrew University of Jerusalem, in the laboratory of Prof. Assaf Friedler in collaboration with Prof. Abraham Loyter from the Dept. of Biological Chemistry. His research topic was the development of HIV-1 Integrase inhibitors, as described in the current article. Zvi has been awarded several prizes during his Ph.D studies, including the Dimitris Chorafas prize for excellent PhD students, the Chorev Award for excellent PhD students from the Israeli Medicinal Chemistry Society, and the Levine-Jortner Award for excellent PhD students from the Israeli Chemical Society.

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ABSTRACT

The HIV-1 Integrase protein (IN) mediates the integration of the viral cDNA into the host genome and is an emerging target for anti-HIV drug design. Our research in the past few years focused on the development of peptide inhibitors of IN. We designed such inhibitors using two approaches: (1) Rational design based on protein-protein interactions of IN: Peptide sequences derived from the IN-binding sites of IN-binding proteins are already optimized by nature to bind IN and have the potential to inhibit it. We designed two peptides derived from the IN-binding loops of the cellular binding partner of IN, LEDGF/p75 (LEDGF 361-370 and LEDGF 401-413) and two peptides derived from the HIV-1 Rev protein, which we found to bind IN; (2) Selection of a peptide, termed IN-1, using combinatorial library screening. All five peptides bound IN with low micromolar affinity in a cooperative mechanism as indicated by Hill coefficients around 4. The peptides inhibited the DNA-binding of IN as well as its enzymatic activity *in vitro*. The five selected peptides shifted the IN oligomerization equilibrium from the dimer towards the tetramer. According to these findings, we have proposed a new approach for inhibiting proteins by “shiftides”: ligands that specifically bind an inactive oligomeric state of a protein and thus shift the oligomerization equilibrium of the protein towards it. The lead peptides penetrated cells and consequently blocked HIV-1 replication in infected cultured cells due to inhibition of integration. The most potent peptides *in vitro* and in cells were LEDGF 361-370 and Rev 13-23. LEDGF 361-370 significantly inhibited HIV-1 infection in mice model. We conclude that the five peptides, particularly LEDGF 361-370, are promising anti-HIV lead compounds for further study and development.

Introduction

The virus that causes the acquired immunodeficiency syndrome (AIDS) was first identified in 1983 and later named human immunodeficiency virus type 1 (HIV-1)¹. Over the past 25 years, almost 60 million individuals have been infected with HIV-1 and nearly 25 million have died of AIDS. In 2008, approximately 33.4 million individuals were infected with HIV-1 worldwide, 2.5 million became newly infected and 2 million deaths occurred due to AIDS (WHO 2009 report). HIV-1/AIDS has become a major cause of death worldwide. In the last decade, there was a huge progress in the field of anti-HIV therapy, making AIDS in many cases a chronic disease rather than a lethal disease. Anti-HIV drugs block different stages of the viral life cycle that are crucial for viral replication¹. Currently approved anti-HIV drugs inhibit HIV-1 entry into cells and inhibit the viral enzymes reverse transcriptase (RT) and protease². The major problem with the currently used anti-HIV therapy is the high mutation rate that the virus undergoes, which results in the emergence of drug-resistant virus strains. To overcome this problem, it is important to identify new drug targets and to develop new approaches for the design of drugs against them. The HIV-1 integrase protein (IN) is such a novel target.

IN catalyzes the integration of the reverse-transcribed viral DNA into the host cell genome, which is an essential step in the viral replication cycle³. The IN-catalyzed integration proceeds in two steps⁴: (I) 3'-end processing, where IN removes a pGT dinucleotide from the 3' end of each strand of the linear viral DNA⁴. This step occurs in the cytoplasm and is carried out by two IN dimers that bind the viral cDNA at its two long terminal repeats (LTR) termini⁵; (II) Following nuclear import, the two LTR DNA-bound dimers approach each other in the presence of the cellular protein LEDGF/p75, form a tetramer and integration proceeds to the strand transfer step⁶⁻⁹. This step leads to integration of the viral cDNA into the target host genome (Figure 1). Structurally, IN is composed of three domains: an N-terminal zinc-binding domain (residues 1-50)¹⁰, a catalytic core domain (CCD; residues 51-212)¹¹ and a C-terminal DNA binding domain (residues 213-280)¹². A catalytic triad composed of residues D64, D116 and E152 in the CCD is responsible for the enzymatic activity of IN¹¹. IN has no mammalian homologues, and the discovery of

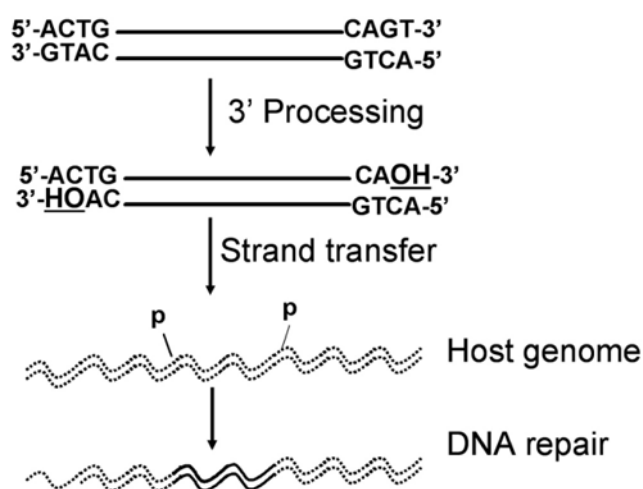


Figure 1: Mechanism of the integration reaction. The 3'-processing reaction occurs in the cytoplasm, whereas strand transfer takes place in the nucleus. The strand-transfer reaction is concerted: both viral DNA ends are inserted into the host chromosomal DNA at the same time. The DNA ends are probably bridged by host cellular factors⁴⁸.

effective IN inhibitors is a promising way for developing new, specific anti-retroviral drugs. Recently, raltegravir (Merck) was the first FDA approved IN inhibitor that came into the market¹³. Several more IN inhibitors are currently in advanced clinical trials, such as GS9137 (Gilead). The two advanced IN inhibitors are Diketo acids derivatives. The Diketo acids were first discovered from random screening and their potency and activity was significantly improved¹⁴. Recently the crystal structure of the Prototype Foamy Virus (PFV)-IN tetramer, which is homologous to the HIV-1 IN, was solved in the presence and absence of the two potent IN inhibitors MK-0518 and GS-9137¹⁵.

In the current review we will present the development of IN - inhibitory peptides performed in our labs. The peptides were designed using two approaches: (1) Rational design based on protein-protein interactions of IN^{16, 17}; (2) Selection using combinatorial library screening by Yeast Two Hybrid System^{18,19}. We will focus on the rational design of peptidic IN inhibitors. The combinatorial screening approach is outside the scope of the current review. For more details about it see refs.^{18,19}.

The Rational Design strategy for developing peptidic IN inhibitors

For developing IN-inhibitory peptides, we combined structure-based design with quantitative biophysical studies of peptide-protein interactions, enzymatic assays and structural studies using NMR. We designed IN inhibitors based on protein-protein interactions of IN and searched for peptidic sequences derived from IN-binding proteins. This is because IN-binding proteins are already optimized by nature to bind IN and thus peptides derived from their IN-binding interfaces also have the potential to bind and inhibit its activity.

Using the rational design approach we developed four peptidic IN inhibitors: two peptides derived from the IN-binding loops of the cellular binding partner of IN, LEDGF/p75 (LEDGF 361-370 and LEDGF 401-413)^{16, 20}, and two peptides derived from the HIV-1 Rev protein, which we found to bind IN (Rev 13-23 and Rev 53-67)^{17, 21}. The sections below describe the inhibitors development in detail.

Example 1: designing inhibitors based on the cellular IN binding protein LEDGF/p75

LEDGF/p75 is the major cellular co-factor of IN, and it is essential for its activity by tethering it to the chromosomes²². LEDGF/p75 is a nuclear protein that binds the IN tetramer in the nucleus^{8,23}. Purified recombinant LEDGF/p75 protein stimulated IN catalytic function *in vitro*²⁴. The cellular functions of LEDGF/p75 remain largely uncharacterized, although initial reports have indicated a role for LEDGF/p75 in transcriptional regulation²⁵.

LEDGF/p75 is a member of the hepatoma-derived growth factor family, and it contains 530 residues and several functional domains. In accordance with its ability to interact with IN^{21, 22, 26}, an evolutionarily conserved integrase-binding domain (IBD) of ~80 amino acids (residues 347–429) was mapped to the LEDGF C-terminus. The crystal structure of the dimeric catalytic core domain of IN in complex with the IBD of LEDGF/p75 was solved in 2005²⁷. Two inter-helical loops extend to interact with the IN core catalytic domain dimer interface and one loop also interacts with the IN N-terminal domain²⁷. These loops served as basis for designing IN inhibitors. Two key features of IN that are complementary to and recognized by LEDGF/p75 IBD were observed according to the crystal structure:

(1) the specific backbone conformation of α 4/5 connector residues 168–171 and (2) a hydrophobic patch accommodating the side chains of LEDGF residues Ile-365, Phe-406, and Val-408²⁸. Based on the crystal structure, we designed and synthesized two peptides derived from the IN-binding loops of LEDGF/p75 LEDGF 361-370 and LEDGF 401-413 (Table 1).

Table 1: Sources and sequences of IN inhibitors described in the current review

Peptide	Sequence	Source
LEDGF 361-370	WNSLKIDNLDV	Rational design ^{20,16}
LEDGF 401-413	WKKIRRFKVSQVIM	Rational design ¹⁶
Rev 13-23	LKTVRLIKFLY	Rational design ^{21,17}
Rev 53-67	RSISGWILSTYLGRP	Rational design ¹⁷
IN1	WQCLTLTHRGFVLLTITVLR	Combinatorial screening ^{18,19}

Fluorescence anisotropy was used to determine the binding affinity of IN to the LEDGF/p75 derived peptides. IN bound LEDGF 361-370 with a K_d of 4 μ M and LEDGF 401-413 with a K_d of 12 μ M. IN binding to the peptides was strongly cooperative, with a Hill coefficient around 4 (Figure 2 and Table 2). IN binding to a fluorescein-labeled 36-base pairs double stranded viral LTR DNA was in agreement with the previous reports^{5, 29}, and had a K_d of 37 nM and a Hill coefficient of 2. Using fluorescence anisotropy competition experiments we found that the LEDGF derived peptides inhibited DNA binding of IN by 3- to 6- fold (Table 2)¹⁶.

The LEDGF derived peptides were tested for their ability to inhibit the catalytic activities of IN in a quantitative *in vitro* assay^{4, 17}. LEDGF 401-413 and LEDGF 361-370 strongly inhibited IN catalytic activity *in vitro* in concentration depended manner (Figure 3).

Fluorescein-labeled LEDGF 361-370 and LEDGF 401-413 penetrated cells. These peptides inhibited HIV-1 replication in infected lymphoid cells, demonstrated by their ability to reduce the amounts of the viral p24 released by these cells (Figure 4).

To reveal the mechanism of DNA-binding inhibition, we studied whether the peptides affect the oligomerization equilibrium of IN as was implied by the cooperative

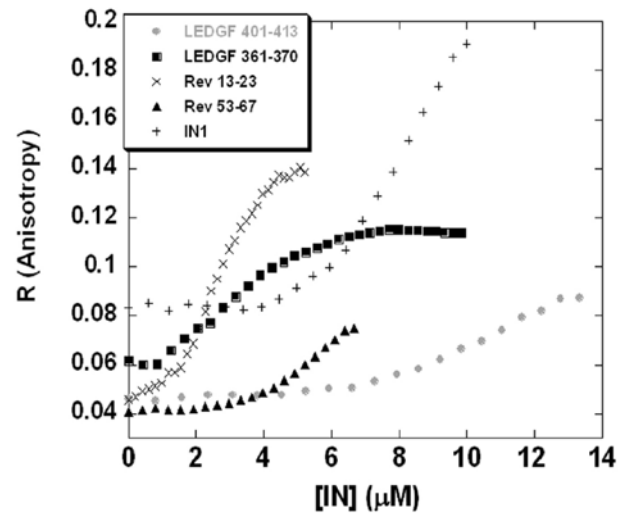


Figure 2: The five inhibitory peptides developed in our lab bind IN at the micromolar range. Shown are fluorescence anisotropy binding studies. IN was titrated into the fluorescein-labeled peptides (100 nM). Data were fit to the Hill equation for binding affinity and Hill coefficient see Table 2^{16-18, 21}.

binding mode of the peptides to IN. We used analytical gel filtration to study the effect of ligand binding on IN oligomerization equilibrium. Our findings indicated that IN was tetrameric in presence of the LEDGF peptides, and dimeric in the presence of LTR DNA, in agreement with our fluorescence anisotropy results. The oligomeric state of the truncated mutant IN 52-288 was not affected by binding peptides or LTR DNA, indicating that the effect is specific and that the N-terminus of IN is involved in the binding process or in the oligomerization process¹⁶.

Example 2: peptides based on the IN binding protein HIV-1 Rev

In our labs we found a novel interaction of IN with the HIV-1 Rev protein in HIV-1 infected cells¹⁷. Rev is a 116-AA viral auxiliary protein that mediates the nuclear export of partially-spliced or un-spliced viral RNA^{30, 31}. We showed that disruption of the Rev-IN complex by IN-derived peptides or infection by a Rev-deficient virus stimulated integration and resulted in large numbers of integration event/cell^{32, 33}. Based on our results we identified Rev as a novel regulator of integration that prevents multi-integration and thus prevents genomic

instability³²⁻³⁵. We performed peptide mapping using the HIV-1 Rev subtype B consensus sequence to identify the regions that mediate binding to IN. Five fluorescein-labeled peptides covering the full length of the Rev protein were synthesized and their interaction with IN was studied using fluorescence anisotropy¹⁷. Rev 1-30 and Rev 49-74 bound IN with K_d values at the low micromolar range, and a Hill coefficient of around 4, indicating binding of IN tetramer to the peptides. Peptides Rev 31-48, 74-93 and Rev 94-116 did not bind IN¹⁷. Based on screening an NIH Rev derived peptides library we selected the two Rev-derived inhibitory peptides Rev 9-23 (5993) and Rev 53-67 (6004) (Table 1) for further study. We synthesized two peptides: (1) one corresponding to 5993 (Rev 9-23, Table 1) but lacking its first four amino acids (DEEL) (Rev13-23, Table 1), in order to obtain a cell-permeable short peptide deficient of the negatively charged amino acids, and (2) one bearing the complete sequence of peptide 6004 (Rev 53-67, Table 1). Rev13-23 and Rev53-67 both bound IN at the low micromolar range with Hill coefficient of 4 (Figure 2, Table 2). These Rev derived peptides inhibited IN catalytic activity *in vitro* and blocked HIV-1 replication in cells¹⁷.

Table 2: Binding affinities of the inhibitory peptides to IN and their effect on IN- DNA binding*

(a) IN binding to the inhibitory peptides

Peptide	K_d (μ M) of binding to IN	Hill coefficient
LEDGF/p75 361-370	3.7 ± 0.2	3.4 ± 0.2 ^{20,16}
LEDGF/p75 401-413	12 ± 0.6	4.5 ± 0.6 ¹⁶
Rev 13-23	2.8 ± 0.1	3.6 ± 0.5 ^{21,17}
Rev 53-67	6.9 ± 0.1	5.2 ± 0.9 ¹⁷
IN1	8.5 ± 0.1	4.5 ± 0.3 ^{18,19}

(b) IN binding to the viral LTR DNA in the presence of the peptides

DNA / Peptide	K_d of binding to IN (μ M)	Hill coefficient
FL'-LTR DNA only	0.034 ± 0.01	2.0 ± 0.3 ¹⁶
FL'- LTR DNA + LEDGF 361-370	0.099 ± 0.003	2.2 ± 0.1 ¹⁶
FL'- LTR DNA + LEDGF 401-413	0.20 ± 0.02	2.7 ± 0.4 ¹⁶
FL'-LTR DNA + Rev 13-23	0.320 ± 0.02	1.9 ± 0.1 ²¹
FL'-LTR DNA + Rev 53-67	0.300 ± 0.09	2.1 ± 0.2 ²¹

*Binding studies were carried out using fluorescence anisotropy, as described in the text. Affinities and Hill coefficients are taken from refs. 16-18, 21

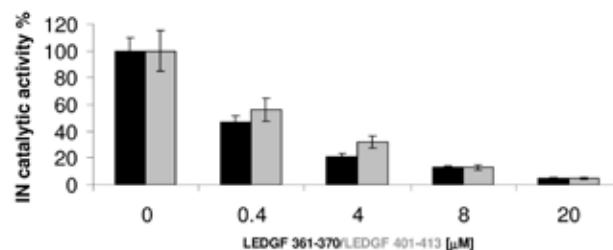


Figure 3: The peptides inhibit IN catalytic activities *in vitro*. IN was incubated with the designed peptides and the 3'-end processing and strand transfer enzymatic activities were monitored by ELISA based system a quantitative assay¹⁷.

We used fluorescence anisotropy to study whether the Rev derived peptides affect the DNA binding of IN. IN bound to a fluorescein-labeled 36-bp double stranded viral LTR DNA with K_d of 34 nM and a Hill coefficient of 2 (Table 2)^{16,21}. The Rev derived peptides significantly inhibited the binding of IN to the viral LTR DNA. The affinity of IN to the DNA was reduced 10-fold from 34 nM in the absence of the Rev peptides to 320 nM and 300 nM in presence of Rev 13-23 (1 μ M) and Rev 53-67 (1 μ M), respectively (Table 2)²¹.

The effect of the Rev derived peptides on the IN oligomeric state was tested using analytical gel filtration. IN was tetrameric in presence of the Rev derived peptides, but was dimeric in the presence of LTR DNA, indicating a shift in the oligomerization equilibrium in presence of the Rev derived peptides just like in the case of the LEDGF derived peptides^{17,21}.

Structure Activity Relationship Studies of the HIV-1 Integrase Inhibitory Peptide LEDGF 361-370

Of the five IN inhibitory peptides that were developed (two LEDGF/p75 derived peptides, two Rev derived peptides and the IN1 peptide selected from a combinatorial library), LEDGF 361-370 was the most potent according to our *in vitro* and *in vivo* assays results (See for example comparison between the ability of the peptides to inhibit HIV-1 replication in cells in Figure 4). In addition, an independent study showed that LEDGF

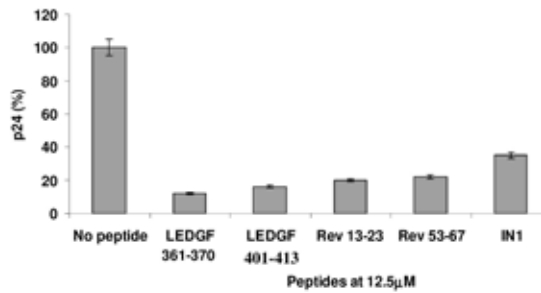


Figure 4: The designed peptides inhibit HIV-1 replication in infected cell culture. H9 T-lymphoid cells were incubated with the indicated peptides and the total amount of the released virus was estimated based on the p24 viral capsid protein content^{16-18,21}.

361-370 competes with the full length LEDGF/p75 on IN binding with $K_i = 4.6 \mu\text{M}$ ³⁶. Thus, LEDGF 361-370 was selected by us as a lead compound for further studies.

Alanine scan of LEDGF 361-370 was performed to determine which precise residues participate in IN binding²⁰. Eleven peptides were synthesized based on the LEDGF 361-370 sequence (for sequences see²⁰). 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 peptide²⁰. Since D366 was shown to be an important residue for IN binding at the protein level^{27,28}, and D369 is proximate in the sequence and may have a similar effect at the peptide level, a peptide in which both D366 and D369 residues were replaced by alanine was also synthesized. The D366/369A mutant bound IN 2-fold weaker than the wild type LEDGF 361-370²⁰. NMR studies showed that almost all of LEDGF 361-370 residues contribute to IN binding, in agreement with the alanine scan results,²⁰.

The effect of the alanine substituted derivatives of LEDGF 361-370 on the IN catalytic activity *in vitro* was determined using the quantitative integration assay. Most of the alanine substituted peptides inhibited IN catalytic activity in the same manner as the parent LEDGF 361-370 peptide²⁰. 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 inhibition²⁰.

The fluorescence anisotropy, NMR and *in vitro* activity results indicate that all residues in the parent LEDGF 361-370 sequence contribute to IN binding and inhibition. Hence, the full LEDGF 361-370 sequence is required for further studies and development.

LEDGF 361-370 and Rev 13-23 inhibited HIV-1 infection in mice model

LEDGF 361-370 and Rev 13-23 were studied for their antiretroviral activity *in vivo* using the model of infection of mice with chimeric HIV^{37,38} (Figure 5). Mice were analyzed for normalized vif RNA (Figure 5) burdens in the spleen. LEDGF 361-370 reduced *de novo* synthesis of the single-spliced viral vif mRNA by about 80%, while Rev 13-23 had a modest activity (Figure 5)²⁰. In summary LEDGF 361-370 peptide significantly inhibited *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.

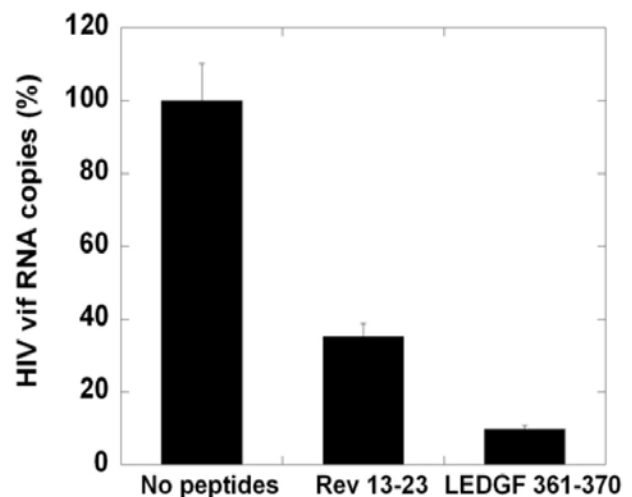


Figure 5: LEDGF 361-370 and Rev 13-23 inhibited HIV formation of HIV-1 Vif RNA in mice model. Mice were pretreated with 40 mg/kg/day of LEDGF 361-370, Rev 13-23 or vehicle for 2.5 days. Mice were sacrificed and analyzed for the normalized vif RNA²⁰.

The mechanism of action of the peptidic IN inhibitors: the shiftides approach

The inhibitory peptides described above bound preferentially to the IN tetramer and stabilized it as was shown by fluorescence anisotropy, analytical gel filtration and crosslinking experiments^{16, 18, 21}. We have termed such peptides as “shiftides”, since by stabilizing the IN tetramer they shift the IN oligomerization equilibrium towards it¹⁶. We proposed the shiftide concept, which utilizes peptides to modulate protein activity by specifically binding to an active/inactive oligomeric state of the target protein, resulting in shift of the oligomerization equilibrium and activation/inhibition of the protein respectively (Figure 6). The shiftides act in a similar manner to the allosteric model³⁹, according to which ligand binding can shift this equilibrium towards R- or T- states, as in the case of hemoglobin⁴⁰⁻⁴³: The shiftides add an additional dimension to the allosteric inhibitors, since they modulate the equilibrium between various oligomeric states, and not within a given oligomer. Shiftides open new directions in the field of oligomerization inhibitors, and are advantageous over conventional dimerization inhibitors^{44, 45} or ligands that covalently attach several monomers together⁴⁶. There are intrinsic problems with competitive dimerization inhibitors, because small molecules cannot usually supply enough binding energy for the large interfaces to be targeted, and the full-length protein will bind tighter than a peptide derived from it⁴⁷. The shiftide approach targets oligomerization by binding at a different site of the protein, in an allosteric mode. This overcomes the drawbacks of targeting a protein-protein interaction interface and presents a new way to modulate oligomerization in a non-competitive allosteric mechanism.

According to our proposed model¹⁶, in the case of IN the inhibitory peptides shift the oligomerization equilibrium of IN in the cytoplasm from a dimer, which binds the unprocessed LTR DNA and catalyses the 3'-end processing, to a tetramer that is probably unable to bind the unprocessed DNA and catalyze this reaction. Thus, the viral DNA substrate is not ready for strand transfer, preventing the integration. Moreover, since the IN tetramer is also unable to bind directly to the processed DNA as shown by cross linking experiments⁸, shifting the oligomeric state of IN towards a tetramer inhibits the strand transfer of a processed DNA template. The inhibitory peptides inhibit both integration steps, making

them advantageous over strand transfer inhibitors, which inhibit only the second integration step. In the case of IN1, the inhibitory peptide that was selected by combinatorial screening, we showed that the full length peptide inhibited IN activity *in vitro* and in cells and shifted IN oligomerization towards the tetramer. Each of the two halves of the parent IN1 peptide bound the IN dimer. We suggested that each half may bind another dimer and together they induce tetramerization¹⁹.

It is still not clear to us how the peptidic IN inhibitors induce IN tetramerization, and whether this activity is sufficient and/or necessary for inhibiting IN. To elucidate the structural basis for the shiftide activity, it is necessary to reveal the peptide binding sites in the IN protein at its dimeric and tetrameric forms using detailed structural analysis such as NMR or X-ray crystallography. Other projects in our lab are currently applying the shiftides approach for other biological systems

Conclusions

In this review we demonstrated the design of IN peptidic inhibitors using two approaches: (1) Rational design based on protein-protein interactions of IN; (2) Combinatorial library screening. All five peptides we developed bound IN with low micromolar affinity in a cooperative mechanism as indicated by Hill coefficients around 4. The peptides inhibited IN enzymatic activity *in vitro* and in cells and HIV-1 replication in cells and in mouse model. The five selected peptides shifted the IN oligomerization equilibrium from the dimer towards the tetramer. According to these findings, we have proposed a new approach for inhibiting proteins by “shiftides”:

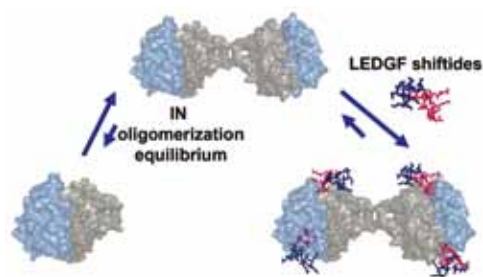


Figure 6: Mechanism of action of shiftides: Peptides or small molecules that shift the oligomerization equilibrium of proteins. Shown is the example of the IN protein and the LEDGF/p75 - derived peptides.

ligands that specifically bind an inactive oligomeric state of a protein and shift the oligomerization equilibrium of the protein towards it (Figure 6).

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