

## Identification of Sequential Motifs Relevant to Inhibitory Activity against HIV Integrase

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### Introduction

As the clinical treatment of AIDS or HIV-1-infected patients, “highly active anti-retroviral therapy (HAART)”, which uses a combination of two (or three) different agents that are usually chosen from two drug categories (reverse transcriptase inhibitors and protease inhibitors), has brought us a significant success. A fusion inhibitor, enfuvirtide (DP-178, T-20, Fuzeon, Trimeris & Roche), has also been clinically used. However, there still remain several serious problems even with the above chemotherapy, which involve the emergence of viral strains with considerable adverse effects, multi-drug resistance (MDR) and high costs. Thus, development of

novel drugs possessing action mechanisms different from the above inhibitors would be currently desirable. We have previously developed selective antagonists against the chemokine receptor CXCR4, which is identified as a co-receptor of an HIV entry. In this study, we focus on HIV integrase [1]. Effective compounds with inhibitory activity against integrase have been searched from peptide libraries, which are composed of fragments (overlapping peptides) of proteins derived from HIV-1 RNA sequence.

### Results and Discussion

Through screening by a strand transfer assay using purified HIV-1 integrase from a semi-random library of

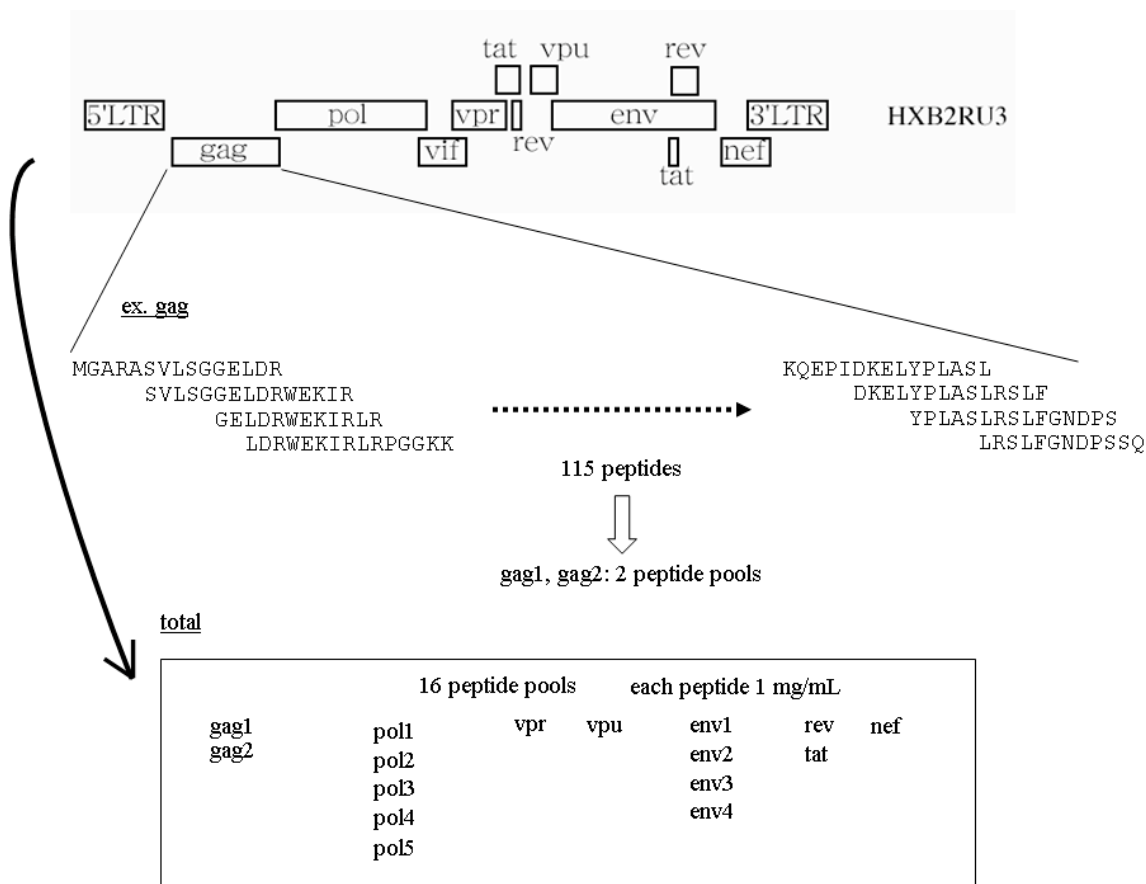


Fig. 1. Overlapping peptides from HIV-1 is a semi-random peptide library. As an example, gag forms 2 peptide pools, and as a whole 16 pools are prepared from HIV-1 gene products.

overlapping peptides spanning HIV-1 gene products Gag, Pol, Env, Vpu, Vpr, Rev and Tat (Fig. 1), we found hit

a copper resin. Cell lysates containing tri-Flag-tagged Vpr was incubated with the resin. The bound proteins were

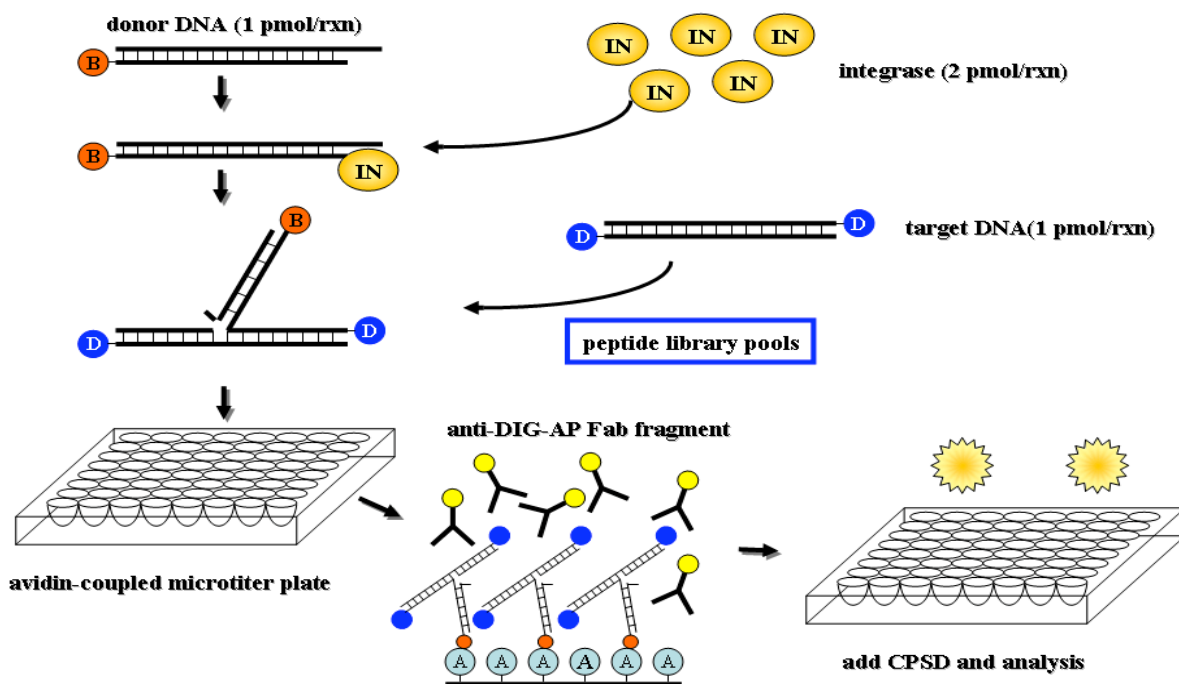


Fig. 2. Experimental procedure for screening by a strand transfer assay using purified HIV-1 integrase.

compounds with inhibitory activity against integrase (Fig. 2). These hit compounds have a certain sequential motif consisting six amino acid residues derived from Vpr. The most potent compound has a value of  $IC_{50}$  of 1.9  $\mu$ M, which is predicted to form an  $\alpha$ -helical structure in the context of the original protein Vpr.

To investigate whether these peptides bearing inhibitory activity against integrase suppress HIV-1 replication in cells, an octa-arginyl sequence was added at the C-terminus of original peptide sequences. Anti-HIV-1 activity was assessed based on the protection against HIV-induced cytopathogenicity in MT-4 Luc cells, which are transduced with luciferase in MT-4 cells, using HXB2 strains. As a result, the HIV-1 replication was inhibited at the level of  $IC_{50}$  values of approximately 5-10  $\mu$ M whereas the Vpr-derived peptides alone did not show significant inhibitory activity towards the HIV-1 replication. Thus, addition of an octa-arginyl sequence to the above original sequences caused inhibition of HIV replication in cells due to an increase in cell membrane permeability.

In the virological point of view, since Vpr and IN are present in the pre-integration complex (PIC), and given that the IN activity has to be regulated to prevent the auto-integration till nuclear entry, it is possible that Vpr associates with IN in the PIC to block the activity of IN. The PIC may uncoat Vpr upon nuclear entry such that IN become active to support integration of viral genome into the host chromosomal DNA.

To investigate whether integrase is able to be associated with Vpr, co-immunoprecipitation experiments were performed. Hexa-histidinyl tagged integrase was bound to

separated by the SDS-PAGE, followed by Western blotting. However, no Flag-tagged Vpr was detected. Thus, it proved that Vpr is not able to be associated with integrase. The implication from this experiment is that the peptides carrying the Vpr sequential motif bind to and inhibit integrase in vitro and possibly in vivo as well. However, these two whole proteins, Vpr and integrase, do not interact each other in physiological conditions. According to an NMR structure of Vpr, this sequential motif contributes to the helix-helix interaction of Vpr and thus does not seem to be exposed to interact with other proteins freely [2]. This finding is consistent with the results of the co-immunoprecipitation assay.

In summary, we found peptides containing a certain sequential motif derived from Vpr with integrase inhibitory activity. Addition of an octa-arginyl sequence to the above original sequences caused inhibition of HIV replication in cells. These data suggest that the Vpr-derived peptide motif could serve as a potent target to develop HIV-1 integrase inhibitors.

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#### References

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