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Characterization of HIV-1 Integrase Interactions with Viral DNA

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ABSTRACT

HIV-1 integrase (integrase) catalyzes the insertion of viral DNA into human chromosomes and is a focus for development of anti-integrase inhibitors to combat HIV infection. Integrase catalyzes two steps, a DNA-end cleavage reaction (3'-processing), and a DNA-end joining reaction (strand transfer). Together, these steps result in viral DNA integration into the genome of the host cell, resulting in persistent infection. Better understanding of the mechanism of integration and interactions of the viral and host DNA with integrase are required for optimal inhibitor development. Recombinant integrase protein was purified and activity was evaluated in a biochemistry assay under bulk conditions to optimize 3'-processing and strand transfer activity. Integrase binding to DNA was then measured under these conditions, using quartz crystal microbalance. This technique will also be used to characterize the disruption of integrase binding to DNA by inhibitors. Finally, integrase/DNA complexes will be visualized by atomic force microscopy to explore their interaction. This study addresses gaps in knowledge of viral DNA and HIV-1 integrase interactions as well as the effects of inhibitors on DNA binding.

INTRODUCTION

Since its discovery in 1981, HIV/AIDS has become a world-wide epidemic. More than 25 million people have died and over 34 million people are currently infected (WHO Factsheet No. 360). Treatment includes a triple therapy "cocktail" of drugs. These drugs include a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) in combination with two nucleoside reverse transcriptase inhibitors (NRTI). Together, these drugs target all steps of the viral life cycle: reverse transcription, proteolytic maturation, integration, and fusion (Summa 2008). This has increased the median length of survival to eight years, up from one year of survival after diagnosis in 1987. However, this drug treatment program requires strict adherence and is not a cure for viral infection. Due to the rapidly mutating nature of the virus, drug resistance often occurs. Recently, an inhibitor drug targeting HIV-1 integrase was added to HIV/AIDS drug treatment, providing one more method to combat the virus. Further research of HIV-1 integrase and inhibitors targeting the enzyme will help create more effective treatment (Cocohoba 2008).

Human Immunodeficiency Virus-1 (HIV-1) integrase is the viral protein responsible for catalyzing the insertion of viral DNA into human chromosomes. Integrase binds both ends of the linear viral DNA as well as the host chromosomal DNA, resulting in integration. Once integration occurs, the human cell harbors the HIV genome, resulting in chronic infection. Development of efficient integrase inhibitors has been hampered by poor understanding of structure and function of the enzyme (Guiot 2006). The three-dimensional structure of the full-length protein has been difficult to resolve either in the presence or absence of a viral DNA substrate, limiting understanding of the interactions between HIV-1 integrase and DNA. This study will address gaps in knowledge of viral DNA and HIV-1 integrase interaction as well as the effects of inhibitors on DNA binding (Pommier 2005).

PROJECT GOALS

- Purify recombinant integrase protein
- Verify integrase activity by catalytic assay
- Measure interaction of integrase and DNA by quartz crystal microbalance
- Image interaction of integrase and DNA by atomic force microscopy

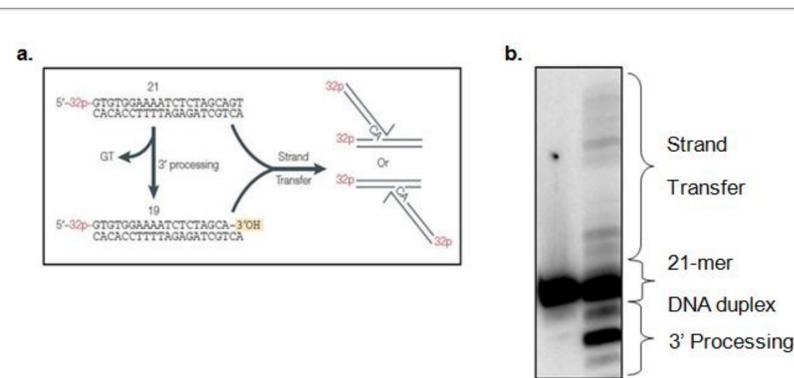


Figure 1. *In vitro* catalytic assay of HIV-1 Integrase. (a.) Diagram of an assay that represents the catalytic steps resulting in HIV-1 genome integration into host cell. The 21 base pair substrate DNA is derived from the end of HIV-1 viral DNA sequence. Integrase 3'-processing occurs when the enzyme recognizes and binds to a conserved nucleotide sequence (5'-CAGT-3') at the DNA end. Integrase cleaves the terminal dinucleotide (5'-GT-3'), leaving a 3'-OH group that can subsequently attack a phosphodiester bond in the target (host) DNA to achieve strand transfer. (b.) *In vitro* reaction products visualized by gel electrophoresis. DNA is ³²P-labeled on the 5' end of the cleaved strand. Reaction conditions are 20 mM Mops, pH 7.2, 5 mM NaCl, 14.3 mM β-mercaptoethanol, 7.5 mM MnCl₂, 1 mM integrase, and 20 nM 21-mer DNA duplex. Samples are incubated for 60 minutes at 37°C. Lane 1. No integrase. Lane 2. with 1 μM HIV-1 integrase. The 3'-P and strand transfer products are labeled.

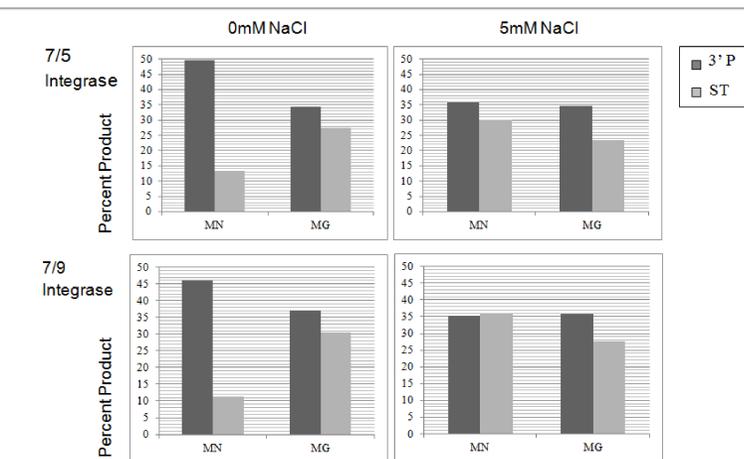


Figure 2. The effect of sodium chloride concentration on 3'-processing and strand transfer. Reaction conditions are 20 mM Mops, pH 7.2, 0 (left panels) or 5 (right panels) mM NaCl, 14.3 mM β-mercaptoethanol, 7.5 mM MnCl₂, 1 μM HIV-1 integrase, and 20 nM 21-mer duplex DNA. Two integrase preparations were compared: 7/5/12 (top panels) and 7/9/12 (bottom panels). 3'-processing (dark grey bars) and strand transfer (light grey bars) reaction products were quantified using ImageQuant software.

Results: Levels of 3' processing and strand transfer are consistent with previous studies. Manganese exhibits higher product percentages, although magnesium is the *in-vivo* metal cofactor.

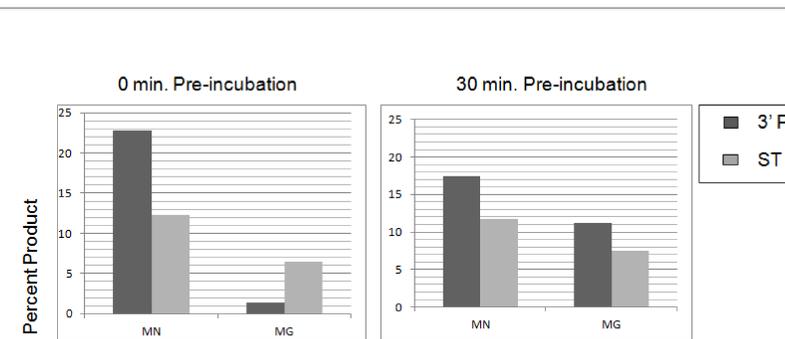


Figure 3. The effect of reaction component pre-incubation on 3'-processing and strand transfer. Reaction conditions were 20 mM Mops, pH 7.2, 5 mM NaCl, 14.3 mM β-mercaptoethanol, 7.5 mM MnCl₂, 1 μM integrase, and 20 nM 21-mer duplex DNA. This experiment tested the effect of pre-integration of DNA and HIV-1 integrase on formation of reaction products. Metal was added after the pre-incubation, to start the reaction. The left and the right panels show reaction products after 0 minutes of preincubation or 30 minutes of preincubation on ice, respectively. 3'-processing (dark grey bars) and strand transfer (light grey bars) reaction products were quantified using ImageQuant software.

Results: Pre-incubation of 20nM 21-mer duplex DNA and 1 μM HIV-1 integrase had no substantial overall effect on 3' processing and strand transfer when manganese was used as a cofactor. An increase in 3' processing was seen when magnesium was the metal cofactor.

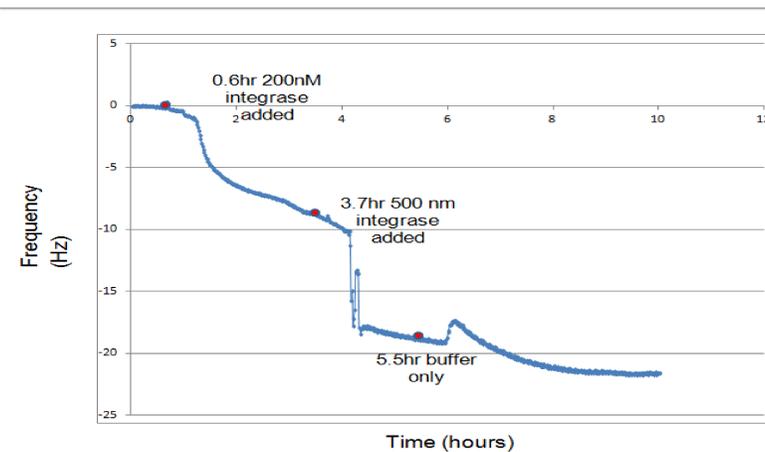


Figure 4. Integrase binding to DNA, as measured by quartz crystal microbalance. Quartz crystal microbalance sensors were functionalized with duplex DNA. Reaction components were flowed over sensors, and change in resonance frequency was measured. Reaction conditions were 20 mM Mops, pH 7.2, 5 mM NaCl, 7.5 mM MnCl₂, 200-500 nM HIV-1 integrase. 21-mer thiol duplex DNA was bound to the sensor surface, and the concentration is unknown.

Results: The drop in frequency with addition of integrase-containing buffer indicates integrase binding of integrase to DNA. We are currently replicating this result and will use this data to calculate a dissociation constant between integrase and DNA.

METHODS

Purification

HIV-1 integrase was expressed from a plasmid in an *Escherichia coli* expression system, and purified using chelating nickel chromatography. Protein was eluted with increasing concentrations of imidazole. Purified protein was visualized by SDS-poly acrylamide gel electrophoresis, and desired fractions were dialyzed into storage buffer overnight. Generally, 300 mg of protein was obtained from several rounds of purification. Purity was estimated at ~95% after SDS-Page gel electrophoresis of the purified protein product. Protein was aliquoted and stored at 80°C.

Catalytic assay

Integrase (1 μM) was incubated with DNA (20 nM 21base pair duplex substrate) substrates for 1h at 37°C. This high concentration of integrase was used to maintain the multimeric complex required for enzymatic activity. Reactions were quenched by the addition of an equal volume of gel loading dye containing formamide. Products were separated on 20% polyacrylamide denaturing sequencing gels. Gels were visualized using a Molecular Dynamics 445 SI phosphorimager. Densitometric analysis was performed using ImageQuant software.

QCM

Quartz Crystal Microbalance Q-Sense E4 gold sensors (Biolin Scientific, Inc.) were cleaned with UV/ozone, ethanol, and RNase-free water. Sensors were then functionalized by immersion of 5 μM solution thiolated DNA duplex (H6-C6-DNA duplex) reaction buffer for 2h. Unlinked DNA duplexes were removed from sensor by gentle washing with reaction buffer. Sensor was placed in a QCM module and equilibrated with reaction buffer at 10 μl/min. Reaction buffer was added containing HIV-1 integrase (200 nM, 500 nM, and 1 μM) at 10 μl/min until stabilization of frequency.

RESULTS

- HIV-1 assays exhibit 3' processing and strand transfer percentages consistent with previous studies
- Preliminary studies suggest an increase in binding activity with an increase in enzyme concentration.
- Further studies will visualize the DNA-enzyme complex using Atomic Force Microscopy to measure the binding affinity of HIV-1

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ACKNOWLEDGEMENTS

- Jeffers Memorial Trust
- VCU Undergraduate Research Opportunities Program