Characterization of HIV-1 Integrase Reactions with Viral DNA

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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 host 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 worldwide epidemic. More than 25 million people have died and over 34 million people are currently infected (WHO Fact Sheet No. 369). Treatment includes a triple therapy “cocktail” of drugs. These drugs include an 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 HIV 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 tool to combat the virus. Further research of HIV-1 integrase and inhibitors targeting the enzyme will help create more effective treatments (Coccob 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. Limited 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

- Study HIV-1 integrase as a target for drug development
- 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

RESULTS