Methods 47 (2009) 229–236

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Biochemical and biophysical analyses of concerted (U5/U3) integration

Duane P. Grandgenett *, Sibes Bera [1], Krishan K. Pandey [1], Ajaykumar C. Vora, Jacob Zahm, Sapna Sinha

Saint Louis University Health Sciences Center, Institute for Molecular Virology, Doisy Research Center, Rm 621, 1100 South Grand, St. Louis, MO 63104-1009, USA

ARTICLE INFO

Article history:
Accepted 4 November 2008
Available online 29 November 2008

Keywords:
Retrovirus concerted integration
Integrase
Preintegration complex
Reconstituted system
Synaptic complex

ABSTRACT

Retrovirus integrase (IN) integrates the viral linear DNA genome (~10 kb) into a host chromosome, a step which is essential for viral replication. Integration occurs via a nucleoprotein complex, termed the preintegration complex (PIC). This article focuses on the reconstitution of synaptic complexes from purified components whose molecular properties mirror those of the PIC, including the efficient concerted integration of two ends of linear viral DNA into target DNA. The methods described herein permit the biochemical and biophysical analyses of concerted integration. The methods enable (1) the study of interactions between purified recombinant IN and its viral DNA substrates at the molecular level; (2) the identification and characterization of nucleoprotein complexes involved in the human immunodeficiency virus type-1 (HIV-1) concerted integration pathway; (3) the determination of the multimeric state of IN within these complexes; (4) dissection of the interaction between HIV-1 IN and cellular proteins such as lens epithelium-derived growth factor (LEDGF/p75); (5) the examination of HIV-1 Class II and strand transfer inhibitor resistant IN mutants; (6) the mechanisms associated with strand transfer inhibitors directed against HIV-1 IN that have clinical relevance in the treatment of HIV-1/AIDS.

1. Introduction

Significant progress has augmented our understanding of the molecular processes involved in human immunodeficiency virus type-1 (HIV-1), gamma, and alpha retrovirus integration in vivo [1]. Integrase (IN) catalyzes two independent reactions in different cellular compartments [2,3]. Within the cytosolic preintegration complex (PIC), IN removes the terminal dinucleotides from the U5 and U3 blunt-ends synthesized during reverse transcription, exposing an active 3′ OH group adjacent to the conserved CA motif. Upon transport of the PIC into the nucleus, IN mediates the concerted insertion of the 3′ OH recessed DNA ends into a chromosome by a cutting and joining reaction known as strand transfer. With the emergence of resistant HIV-1 strains against highly active antiretroviral therapy (HAART), IN is an attractive alternative and new therapeutic target. Strand transfer inhibitors directed against IN effectively prevent HIV-1 integration at low nM concentrations in vivo and prevent the development of HIV-1/AIDS in infected individuals [4–6].

We and others have developed a reconstituted system to investigate concerted integration that uses purified recombinant HIV-1 IN [7–13], alpha retrovirus IN [14–17], or IN purified from avian myeloblastosis virus (AMV) particles [17–19]. IN efficiently mediates the concerted insertion of relatively large-size DNA substrates (0.5–4 kb) harboring U3 or U5 viral end sequences into a supercoiled plasmid as target DNA. The methods described in this article are useful in investigating the retrovirus concerted integration pathway in vitro. These methods have provided insights into (i) the biochemical mechanisms of HIV-1 integration and identification of novel nucleoprotein intermediates in the concerted integration pathway; (ii) the mechanisms by which strand transfer inhibitors block the catalytic activity of HIV-1 IN; (iii) the role of cellular co-factors in the HIV-1 integration process. Emphasis will be placed on biochemical and biophysical studies that help to understand the mechanistic basis of PIC function in HIV-1 infected cells and the mechanisms through which strand transfer inhibitors block this process.

2. Description of methods

2.1. Overview of the concerted integration assay

Efficient reconstitution of concerted retroviral DNA integration in vitro requires three macromolecular reaction components (IN, viral DNA substrate, and target DNA), all present in optimized solution conditions for IN catalysis. High quality purified IN appears to be a prerequisite for effective assembly of the HIV-1 synaptic complex (SC). Within the SC, IN non-covalently juxtaposes the two viral DNA ends (Fig. 1a and b) [12,20]. The SC is a transient intermediate in the concerted integration pathway (Fig. 1b) [12,20]. We purify bacterially expressed HIV-1 IN at relatively low protein concentrations at pH 6.8, a feature which seems to minimize severe aggrega-

* Corresponding author. Fax: +1 314 977 8798.
E-mail address: Grandgdp@slu.edu (D.P. Grandgenett).
[1] These authors contributed equally to this work.

1046-2023/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymeth.2008.11.002

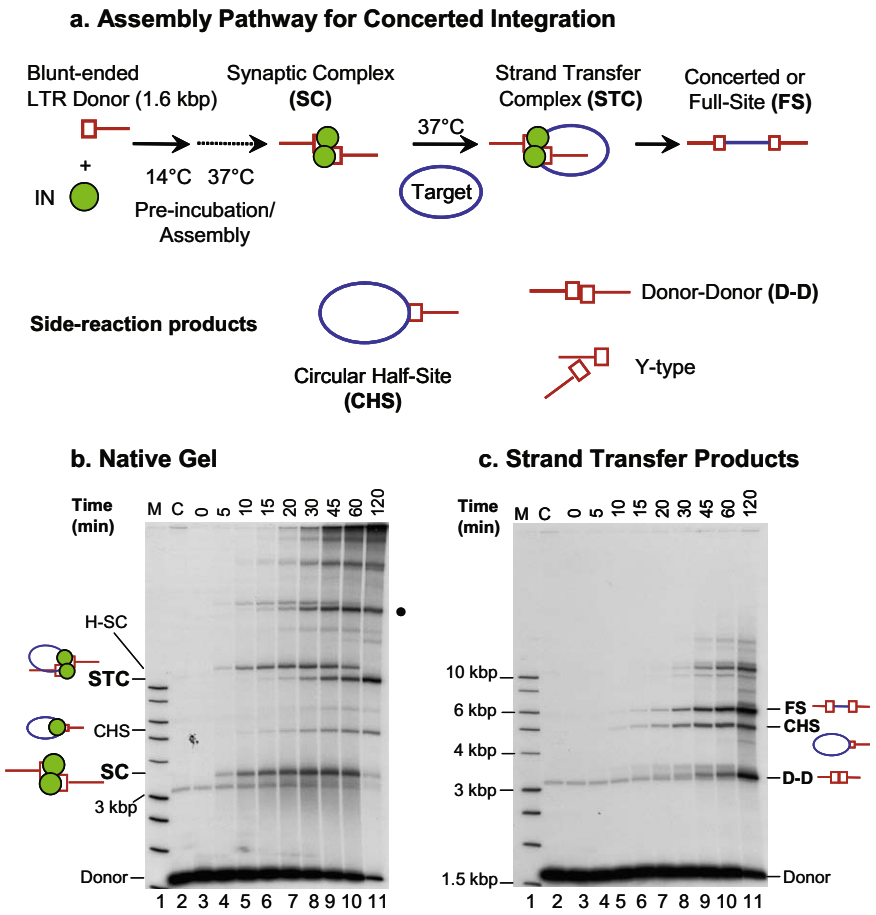

Fig. 1. Concerted retroviral DNA integration. (a) Schematic for HIV-1 SC and STC assembly, which results in concerted or full-site (FS) integration product formation. The reaction, depicted with a 1.6 kb blunt-ended HIV-1 U5-containing substrate, is applicable to a variety of linear viral DNA substrates (see Fig. 2 for additional HIV-1 examples). Side-reaction products (CHS, D–D, and Y-type) are also formed in the reaction mixture. (b) A typical time course experiment revealing the differential kinetics of SC and STC formation. IN (20 nM) was assembled with the 1.6 kb U5 blunt-ended donor DNA substrate (0.5 nM) at 14 °C, and further incubated with supercoiled target DNA at 37 °C for various times. The transient SC, which is identified here on a 0.7% native agarose gel, appears at ~5 min (lane 4), reaches near maximum between 30 min (lane 8) and 45 min (lane 9), and nearly disappears by 120 min (lane 11). The H-SC, presumably formed by SC multimers, follows similar kinetics. Upon binding target DNA, the SC is converted to the STC, with maximum quantities of STC detectable at ~120 min. The various IN–DNA complexes are marked on the left. Lane 1 contains a ^{32}P-labeled 1 kb ladder (marked M) and lane 2 is without IN (marked C). (c) Strand transfer products obtained after deproteinization of the same samples shown in panel b. The quantities of FS product parallel the quantities of STC. Other labeling is the same as in panel b.

tion of IN in-solution [9]. The final concentration of purified IN is ~2.5–3 μM. The supercoiled target as well as supercoiled donor DNA plasmids that contain viral long terminal repeat (LTR) sequences, are purified by velocity sedimentation on sucrose gradients to remove small-sized DNA contaminants. Linear viral DNA substrates (~0.5–4 kb) are obtained by restriction digestion of LTR-containing donor DNA plasmids, followed by one of several conventional purification techniques (see Section 3.2.1 below). Using the above purified components, the reconstitution system led to the identification of the transient SC [12] and strand transfer complex (STC) [11] in the HIV-1 concerted integration pathway (Fig. 1a and b).

Assembly of the HIV-1 SC and subsequent concerted integration into target DNA is depicted in Fig. 1a. A pre-assembly step where IN and the linear blunt-ended viral DNA substrate are incubated together at 14 °C for 15 min, followed by incubation at 37 °C, results in the assembly of the transient SC (Fig. 1b). As revealed by native agarose gel electrophoresis, maximum accumulation of the SC occurs at ~30–45 min (Fig. 1b, lanes 8 and 9), which is followed by its subsequent disappearance by ~120 min [12,20]. IN processes the viral 3′ OH ends gradually, between 5 and 60 min during the incubation at 37 °C [12] (data not shown). Simultaneously, the accumulating SC associates with supercoiled target DNA, resulting in the concerted insertion of the two 3′ OH recessed ends, producing the STC [11,12]. The STC accumulates gradually and its formation is essentially saturated by 120 min (Fig. 1b, lane 11). Higher-order forms of the SC (H-SC) appear on the native gel and may be due to looping of DNA by IN [21,22]. The STC also forms a higher-order complex on native gels (Fig. 1b, dark circle on right). The SC and H-SC are also formed in the absence of target (data not shown).

The STC consists of the concerted or full-site (FS) integration product [11] in association with IN. Using HIV-1 IN mutant W235F, it has been shown that a tetrameric form of IN is present in the SC when it is produced in the presence of a strand transfer inhibitor [11]. The FS integration product, along with other side-reaction products, including the circular half-site (CHS) and donor–donor (D–D) products, are visualized by deproteinization of the samples prior to agarose gel electrophoresis [7–11] (Fig. 1c). Following isolation of the concerted integration product, the size of the host-site duplication that results from DNA repair of the staggered insertion of the viral DNA ends into target DNA can be determined. Individual recombinant DNA molecules are genetically selected in bacteria, followed by DNA sequencing [9,13,23]. The methods and experimental approaches used to investigate HIV-1 concerted integration in vitro are outlined below.