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Alternative nucleophilic substrates for the endonuclease activities of human immunodeficiency virus type 1 integrase

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ABSTRACT

Retroviral integrase can use water or some small alcohols as the attacking nucleophile to nick DNA. To characterize the range of compounds that human immunodeficiency virus type 1 integrase can accommodate for its endonuclease activities, we tested 45 potential electron donors (having varied size and number or spacing of nucleophilic groups) as substrates during site-specific nicking at viral DNA ends and during nonspecific nicking reactions. We found that integrase used 22 of the 45 compounds to nick DNA, but not all active compounds were used for both activities. In particular, 13 compounds were used for site-specific and nonspecific nicking, 5 only for site-specific nicking, and 4 only for nonspecific nicking; 23 other compounds were not used for either activity. Thus, integrase can accommodate a large number of nucleophilic substrates but has selective requirements for its different activities, underscoring its dynamic properties and providing new information for modeling and understanding integrase.

Introduction

Retroviral integrases are responsible for inserting a DNA copy of the retroviral genome into cellular DNA, a recombination event that makes retrovirus infections permanent and ultimately leads to immunodeficiency and other diseases (Brown, 1997). Integrase catalyzes two endonuclease reactions in vivo: sequence-specific nicking after conserved CA bases near the 3′ ends of unintegrated viral DNA to remove the terminal two nucleotides (the processing reaction) and sequence-independent insertion of the processed viral DNA into cellular DNA (the joining or strand-transfer reaction) (Craigie, 2001). Importantly, these activities can be modeled and studied in vitro (Craigie et al., 1990; Katz et al., 1990; Katzman et al., 1989). Integrase also exhibits two other in vitro endonuclease activities, disintegration (which is a reversal of the joining reaction) and nonspecific alcoholysis, that have facilitated study of the mechanism of this enzyme (Chow et al., 1992; Katzman and Sudol, 1996).

In all four reactions, integrase catalyzes one-step transesterifications in which the nucleophilic oxygen of an OH group nicks a DNA phosphodiester bond and joins to the 5′ phosphate on the 3′ side of the nick (Engelman et al., 1991; Katzman et al., 1991; Skinner et al., 2001; Vink et al., 1991). It has been known for some time that integrase can use various nucleophilic donor molecules to nick DNA. During processing, which has been called a site-specific alcoholysis reaction (Vink et al., 1991), the attacking OH group can be provided by water (Vink et al., 1991), certain alcohols (e.g., glycerol, ethylene glycol, serine, or threonine) (Katzman et al., 1991; Vink et al., 1991), or even the terminal 3′–OH at the viral DNA end (Engelman et al., 1991). Thus, the terminal nucleotides can be removed as a linear dinucleotide when water is the nucleophile, bound to an alcohol, or circularized (Fig. 1B). These products can be distinguished on gels if the radioactive label (the asterisk in Fig. 1B) is near the 3′ end of the DNA rather than at the 5′ end. Similarly, integrase was shown to use water or four different alcohols for nonspecific DNA nicking (Fig. 1A), and this activity – which resembles strand transfer in that almost any site in target DNA can be nicked – was named nonspecific alcoholysis (Katzman and Sudol, 1996). In contrast to the variety of nucleophiles used for these two activities, the

Abbreviations: HIV, Human immunodeficiency virus; ED, 1,2-ethanediol; DMSO, Dimethyl sulfoxide; MW, Molecular weight; IN, Integrase

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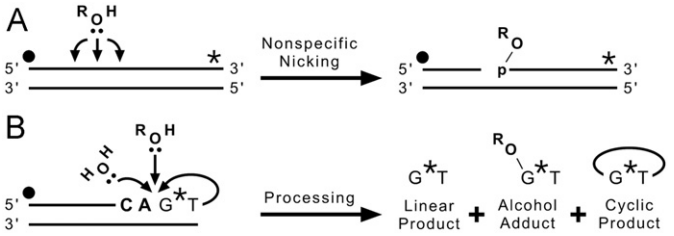

Fig. 1. Integrase assays for detecting the use of alternative nucleophiles to nick DNA. Oligodeoxynucleotides are depicted as straight lines, curved arrows indicate sites of nicking, and two dots represent donor electrons of nucleophilic oxygens. Because integrase attaches the attacking nucleophile to the 5′ phosphate (p in panel A) on the 3′ side of a nick, the nucleophile can be identified if one DNA strand is labeled with ^{32}P (the asterisk) between the final 2 nucleotides near the 3′ end (in contrast to other integrase assays that place the label at the 5′ end, as indicated by the circle). (A) Nonspecific nicking (the 3 arrows indicate that various DNA sites may be nicked). Labeled products of alcoholysis migrate on gels as a function of the attached R group, whereas products of hydrolysis (when ROH is a water molecule) comigrate on gels with oligonucleotides. (B) Specific processing after the conserved CA nucleotides (in boldface) near the ends of retroviral DNA. Depending on whether water (HOH), an alcohol (ROH), or the viral DNA 3′–OH end (the OH is not shown) acts as the nucleophile for nicking, the terminal nucleotides (GT in the case of HIV-1) are removed either as a linear dinucleotide, bound to an alcohol, or circularized, respectively.

strand transfer and disintegration activities use a specific 3′–OH end of DNA for nicking (Skinner et al., 2001).

To date, the list of nucleophiles that integrase has been shown to use for nicking DNA includes water; 1,2-ethanediol (ethylene glycol); 1,2-propanediol (propylene glycol); 1,3-propanediol; 1,2,3-propanetriol (glycerol); serine; threonine; 3′ ends of DNA, and even 5′ ends of DNA (Diamond and Bushman, 2006; Engelman et al., 1991; Katzman et al., 1991; Katzman and Sudol, 1996; Vink et al., 1991). We reasoned that a better understanding of the range of nucleophilic compounds that integrase can accommodate as substrates for its endonuclease activities would reflect the configuration of its active site and provide insights into the structure of the enzyme and perhaps the chemistry of its catalytic mechanism. Thus, to begin to define the range of nucleophiles that can be used by integrase, we tested 45 carefully selected nucleophilic compounds (i.e., potential electron donors that varied in size, number of nucleophilic groups, or spacing between groups) as substrates for human immunodeficiency virus type 1 (HIV-1) integrase during site-specific nicking at the ends of viral DNA and during nonspecific DNA nicking reactions.

Results

Strategy to detect usage of nucleophilic compounds by integrase

We prepared double-stranded oligonucleotide substrates that were internally labeled with ^{32}P between the final two nucleotides at the 3′ end of one strand (Fig. 1), as described in Materials and methods. As is common with these substrates (Skinner et al., 2001), the labeled oligonucleotides undergo a degree of spontaneous degradation (likely due in part to radiolysis from the internal label), which conveniently provides a ladder of oligonucleotide markers on autoradiograms of denaturing gels (e.g., Fig. 2, lane 1), as confirmed by comparison to markers created by the action of the nonspecific nuclease DNase I (data not shown). Thus, the ability of integrase to use a compound as a nucleophile to nick DNA is indicated by the detection of an integrase-dependent radioactive band that does not migrate at the position of oligonucleotides but migrates at a novel position that is a function of the size of the compound, reflecting joining of the compound to the 5′ phosphate of the fragment of DNA that is released from the 3′ side of the nick (Fig. 1).

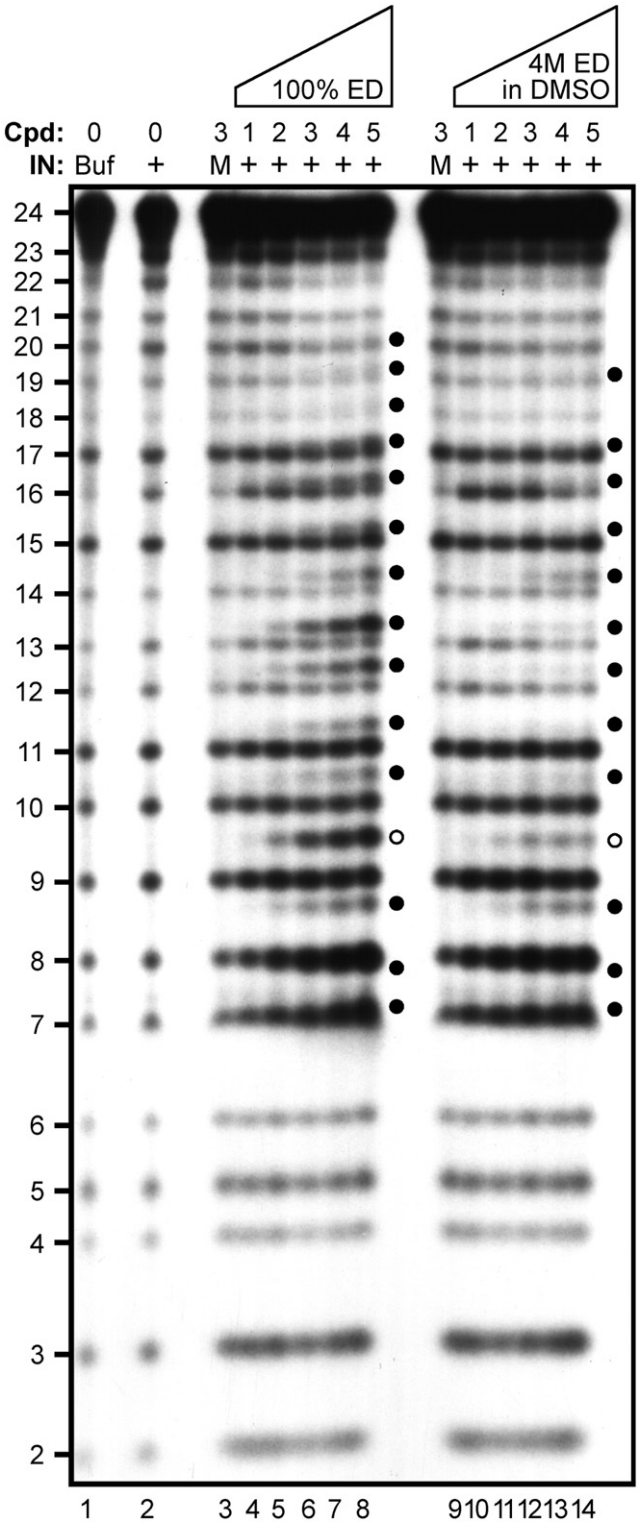

Fig. 2. Nonspecific nicking assay. Double-stranded 24-mers of nonspecific sequence were labeled near the 3′ end of one strand and used as substrates for reactions as described in Materials and methods. The integrase (IN) or control used for each reaction is indicated above the lanes as protein buffer (Buf), glycerol-free wild-type HIV-1 integrase (+), or an inactive integrase mutant (M). The volume (in μl) of nucleophilic compounds (Cpd) added to each 10-μl reaction also is shown: none for lanes 1 and 2; 1–5 μl of undiluted 1,2-ethanediol (100% ED) for lanes 3–8; and 1–5 μl of a 4 M solution of ED in DMSO for lanes 9–14. An autoradiogram from a 20% polyacrylamide gel in which the bromophenol blue dye had migrated 28 cm is shown, with nucleotide sizes indicated at the left. Circles after lanes 8 and 14 mark alcohol-dependent novel bands evident on the original autoradiograms, with the open circle between the 9 and 10 positions highlighting a particularly prominent product.