Human Immunodeficiency Virus Type 2 Preintegration Complexes: Activities In Vitro and Response to Inhibitors

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Received 1 October 1996/Accepted 26 December 1996

We have established an assay for the function of preintegration complexes (PICs) of human immunodeficiency virus type 2 (HIV-2) to investigate the integration mechanism and to develop additional methods for screening candidate integration inhibitors. We partially purified HIV-2 PICs and found that they were competent to integrate viral cDNA into target DNA in vitro. Analysis of the structure of integration products on Southern blots revealed forms consistent with those expected for authentic integration products and circular forms containing one and two long terminal repeats. To determine whether in vitro products had the detailed structure expected of integration products formed in vivo, we recovered product molecules and analyzed junctions between viral DNA and target DNA. In the integration junctions of all nine molecules examined, we observed the 5-bp duplication of target sequence characteristic of integration in vivo. We investigated the possible role in integration of Vpx, a protein present in HIV-2 but not HIV-1 and known to be present in viral cores. Although association of Vpx with viral cDNA was detectable, our studies revealed no obvious role of Vpx in integration since the activities of PICs from Vpx[−] virions were indistinguishable from those of wild type. We have also investigated the use of HIV-2 PICs as tools to screen candidate HIV inhibitors. Assays with HIV-2 PICs, like assays with HIV-1 PICs, were less sensitive to many small molecule inhibitors than were reactions with purified integrase only. Comparing results of assays with PICs from HIV-1 and HIV-2 may be particularly useful, since inhibitors active against both may be more widely useful and less vulnerable to escape mutants.

Retroviruses encode a protein, named integrase, that mediates the DNA breaking and joining reactions involved in integration. The integration system acts on the directly repeated terminal sequences of the linear viral cDNA, the long terminal repeats (LTRs). For most retroviruses, integrase removes 2 nucleotides from the 3' end of each LTR, thereby exposing recessed 3' hydroxyl groups (Fig. 1A, part 1) (4, 5, 22, 30, 32, 35, 37a). However, human immunodeficiency virus type 2 (HIV-2) integrase removes three bases from one 3' end and two from the other 3' end (41). The recessed 3' ends of the viral DNA are joined to protruding 5' ends of breaks made on each strand of the target DNA (Fig. 1A, part 2) (4, 15, 27). Unpairing of the target DNA bases between the points of joining yields an intermediate that has single-stranded gaps at each host-viral DNA junction (Fig. 1A, part 3). Repair of the gaps, presumably by host proteins, produces a duplication of host sequence flanking the integrated provirus (Fig. 1A, part 4). The length of the duplication is characteristic of each type of retrovirus (5 bp for HIV-1 and -2).

Active preintegration complexes (PICs) have been isolated from cells infected with Moloney murine leukemia virus (MoMLV), HIV-1, or avian sarcoma leukosis virus and studied as a source of integration activity in vitro (3, 9, 11, 25). For the cases of HIV and MoMLV PICs, some of the viral cDNA has been shown to be modified as expected by removal of 3' bases (4, 15, 26a, 30). Reactions in vitro in which PICs are mixed with a naked target DNA yield a product in which the recessed 3' ends are joined to 5' ends in the target DNA (Fig. 1A, part 2) (4, 15). The viral 5' DNA ends are not connected to target DNA in these in vitro reactions.

Recombinant integrase proteins can carry out the covalent attachment of model viral DNA to target DNA (6, 7, 21). However, the products of such reactions predominantly yield a partial reaction in which only one viral DNA end is joined to target DNA, in contrast to the concerted joining of viral DNA ends catalyzed by PICs.

Here we report the establishment of PIC assays for HIV-2. HIV-1 and HIV-2 are closely related, and both cause human immunodeficiency. However, the two viruses differ in a variety of features. HXB2 (HIV-1) and ROD9 (HIV-2) are only 58% identical in nucleotide sequence (13, 18), and each is more closely related to a particular simian immunodeficiency virus than to the other HIV (37). Evidently, HIV-1 and HIV-2 represent independent jumps of simian viruses into humans. Furthermore, the Vpx protein is encoded by HIV-2 but not by HIV-1 (17). Although Vpx is a component of the viral core (23) and possibly the PIC, its function is not clear. To address these and other issues, we isolated HIV-2 PICs from infected cells and characterized their activities in vitro.

Generation of PICs. SupT1 target cells were infected by coculture with CEMX174 virus-producing cells at a ratio of 50 to 1. Aliquots were withdrawn each hour from 0 to 9 h and analyzed to determine the time of maximum cDNA production (Fig. 1B). Cells were washed, permeabilized with 0.025% digitonin, and centrifuged to yield a cell extract. Aliquots from each time point were incubated in the presence or absence of linear target DNA (Fig. 1B).

Southern analysis revealed a DNA molecule of the size expected for linear HIV-2 cDNA (10.3 kb; Fig. 1, "viral DNA"). This species was detectable at 4 h postinfection, and its intensity increased steadily over 9 h. In lanes with added target DNA, a species was detected migrating at the size expected for a linear molecule of 15.7 kb, the size expected of the covalent integration product (10.3-kb viral DNA plus 5.4-kb target DNA; Fig. 1, "IP"). Other DNA products were also seen in

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FIG. 1. HIV-2 cDNA synthesis and integration. (A) Inferred pathway of HIV-2 integration in vivo. HIV-2 cDNA is shown by thin lines, and the target DNA is shown with thick lines. Two or three nucleotides are removed from the viral cDNA 3' ends (part 1). The gapped intermediate is shown before and after unpairing (parts 2 and 3). The repaired product of these reactions is the integration product (IP). Solid circles represent DNA 5' ends. The target DNA bases duplicated as a result of integration are indicated by the vertical lines. (B) HIV-2 PICs were generated essentially as described for HIV-1 (11). HIV-2-producing cells, CEMX174 ROD9, were maintained as described in reference 23. CEMX174 ROD9 cells were seeded at 5×10^5 cells per ml in 7-ng/ml phorbol myristate acetate, incubated for 24 h, and cocultured with SupT1 cells at a ratio of 1 to 50. Samples were removed each hour for 9 h, and cell extracts were prepared. Aliquots were incubated in the absence ("—") or presence ("+" lanes) of 5- μ g/ml linear PhiX174 DNA. DNA was isolated from samples, separated by gel electrophoresis, transferred to a membrane, and detected with a radiolabeled DNA sequence complementary to the HIV-2 LTR. The *Kpn*I fragment from nucleotide 301 to nucleotide 978 was subcloned and used to generate a radiolabeled probe as described in reference 27. Hybridization and probing conditions followed the QuikHyb (Stratagene) specifications. Positions of viral cDNA and putative integration products (IP) are indicated at the right.

both the presence and the absence of target DNA (Fig. 1B, "circular forms"). Further characterization of reaction products is discussed below. On the basis of these data, we prepared extracts between 6.5 and 7.5 h after initiation of the coculture.

Restriction enzyme analysis of integration products. Products formed in vitro reactions with HIV-2 PICs were cleaved with restriction enzymes to investigate their structure (Fig. 2). Based on previous studies, four DNA species were expected to be present: linear viral cDNA, circular forms containing one LTR, circular forms containing two LTRs, and integration products (4, 12, 25). For the case of HIV-2, the expected restriction map of each species is shown in Fig. 2A. The sizes of some diagnostic restriction fragments are shown in Fig. 2B.

Products were cleaved with restriction enzymes, and DNAs were analyzed by Southern blotting using sequences complementary to the HIV-2 LTR as probe. Products of reactions lacking or containing target were incubated without enzyme (Fig. 2C, lanes 1 and 5), with *Eco*RI (lanes 2 and 6), with *Hind*III (lanes 3 and 7), or with *Nco*I (lanes 4 and 8).

Digestion of linear viral cDNA (10.3-kb band in lanes 1 and 5) with *Eco*RI yielded the expected fragment of 7.0 kb (lane 2; the expected 3.3-kb fragment [Fig. 2B] was too small to remain on the gel pictured). After digestion with *Hind*III (lane 3) or *Nco*I (lane 4), the 10.3-kb full-length genome was not detected, but the expected smaller bands were detected at 3.9 kb (*Hind*III) and 8.5 kb (*Nco*I). Some unintegrated viral cDNA is expected to be present even in reactions with added target DNA (lanes 5 to 8), and the restriction fragments expected from digestion of the unintegrated linear cDNA can be seen in these lanes.

Integration of HIV-2 cDNA into the target DNA should yield a circular product of 15.9 kb. Without digestion, a band was detected at 15.9 kb with a smear of material extending upward (lane 5). Evidently, some of the circular integration products were broken during the reaction or subsequent handling to yield the 15.9-kb band seen. Following digestion with *Eco*RI, a more prominent band was detected at 15.9 kb (lane 6). The 15.9-kb product was absent after digestion with *Hind*III or *Nco*I (lanes 7 and 8), and faster-migrating bands were present at either 11.6 or 15.1 kb. These higher-molecular-weight forms have the sizes expected for integration products. Detection of these products was dependent on the presence of target DNA in the integration reactions (compare lanes 5 to 8 with lanes 1 to 4).

DNAs of the sizes expected for one- and two-LTR circles were also detected. The production of these forms was inde-

pendent of target. These two species should differ in size by 900 bp, the length of a single LTR. Without digestion, these species are circular and therefore do not migrate in accordance with their linear length. Digestion of these forms with *Eco*RI should yield a 9.4-kb fragment from one-LTR circles and a 10.3-kb fragment from two-LTR circles (the same length as the linear viral DNA). In the absence of target and in the absence of digestion with *Eco*RI (Fig. 2C, lane 1), two forms that migrated above the viral cDNA were detected in low abundance. Following digestion with *Eco*RI, molecules of the expected sizes, 10.3 and 9.4 kb, were seen (lane 2). Digestion with *Hind*III yielded forms of 5.1 and 5.9 kb, while digestion with *Nco*I yielded species of 9.4 and 8.5 kb (Fig. 2, lanes 3 and 4), as expected. These forms were also present in lanes 5 to 8.

Reactions containing HIV-2 PICs yielded circular forms containing one and two LTRs in addition to the expected normal integration products. These structures have been described previously for other retroviruses (3, 12, 25). One-LTR circles are apparently formed by homologous recombination between LTRs or possibly as stalled reverse transcription intermediates (8, 19). Two-LTR circles form as a result of end-to-end ligation of the LTR tips (38, 42). A unit length circle can also be generated by integration of the viral cDNA into itself, yielding a circle with an inverted segment (12). Previous work has indicated that the one- and two-LTR circles are probably generated by host activities, since incubation of deproteinized linear HIV-1 cDNA with cell extracts leads to the formation of both (12). The inverted circle autointegration product is not formed with host extracts and is likely generated by integrase (12). The ratio of one-LTR circles to two-LTR circles is about 3:1 for reactions with HIV-2 PICs. For the case of HIV-1 PICs, the ratio was similar, though this was not the case for reactions with murine leukemia virus and avian leukosis virus PICs in vitro. SupT1 cells were used as target cells in the generation of both the HIV-1 and HIV-2 PICs studied (reference 11 and this work), so the similar abundance of the circular forms likely reflects the participation of SupT1 factors.

In many assays, the formation of one- and two-LTR circles was enhanced in the presence of target DNA (compare lanes 5 to 8 with lanes 1 to 4 in Fig. 2A). One possible explanation is that the added target DNA served as a trap for hypothetical factors bound to PICs that block circularization (24). Added target DNA may act as a competitor, binding the inhibitory factors and exposing the viral cDNA to the enzymes that carry out circularization.

In summary, the maps in Fig. 2A are sufficient to describe all

FIG. 2. Characterization of integration products by cleavage with restriction enzymes. (A) Maps of four DNA species are shown: linear viral cDNA, one-LTR circles, two-LTR circles, and integration products. The positions of *Eco*RI (3216; numbering from the HIV-2 ROD9 sequence), *Hind*III (2015 and 6340), and *Nco*I (8526 and 9319) sites are indicated. LTR sequences are indicated by boxes, and the target *PhiX*174 DNA is drawn with a thick line. Maps are not shown to scale. (B) The expected sizes of some HIV-2 DNA fragments reactive with the LTR probe. The DNA species are indicated at the top, and the restriction enzymes are indicated to the left. Only fragments that are larger than 3.5 kb are shown, since only these fragments were analyzed in the Southern blot in panel C. (C) Southern analysis of integration products. Reactions were carried out in the absence (lanes 1 to 4) or presence (lanes 5 to 8) of 5- μ g/ml circular *PhiX*174 DNA. Products were incubated without enzyme (lanes 1 and 5), with *Eco*RI (lanes 2 and 6), with *Hind*III (lanes 3 and 7), or with *Nco*I (lanes 4 and 8). Products were detected by Southern blotting as described for Fig. 1. Positions of 3.5, 6, 10.3, and 15.7 kb are indicated at the left.

of the DNA forms seen by Southern analysis in restriction digests of reaction products.

Sequences of integration junctions. Analysis of the junctions between viral cDNA and target DNA provides a stringent test

of the authenticity of integration products. As a consequence of the integration mechanism, 5-bp duplications of host DNA are created at the junctions between HIV-2 DNA and target DNA in vivo (Fig. 1A). For integration in vitro, analysis of products generated by the MoMLV and HIV-1 systems indicates that the in vitro systems do not carry out the late DNA repair steps and that the final products are the gapped intermediates (Fig. 1A, part 3) (2, 15, 27).

To examine the structure of junctions between HIV-2 cDNA and target DNA created by integration, nine pairs of junctions were cloned and sequenced. Integration products were generated by incubating HIV-2 PICs with a 900-bp circular plasmid, pSupF, that contained a SupF selectable marker (Fig. 3A, step 1). DNA from integration reactions was digested with *Hind*III (Fig. 3A, step 2) and subjected to electrophoresis on an agarose gel. Integration products were not abundant enough to be visualized by ethidium bromide staining, so gel slices were excised in the molecular weight range expected for product DNAs (6.6 kb). For convenience in sequencing viral-host DNA junctions, DNA samples were amplified and cloned in bacteria. Gaps were repaired prior to amplification by treatment with DNA polymerase I and deoxynucleoside triphosphates (step 2). The repaired products were amplified with primers complementary to viral sequences at each LTR end, and then products were reamplified with nested viral primers (step 2, "outer" primers, then "inner" primers). Amplification extended from within the viral DNA sequences across the entire 900 bases of pSupF sequence and included both putative duplication sites (vertical lines). The amplification products were treated with Klenow fragment and dTTP to yield blunt DNA ends, with kinase and ATP to phosphorylate the ends, and with ligase to circularize the linear molecules (step 3). Ligation products were transformed into MC1061/P3 bacteria and selected for SupF activity (34). Plasmid DNA from the resulting colonies was isolated, and both viral-target DNA junctions in each clone were sequenced.

Analysis of nine junctions indicated that 5 bp of target DNA was duplicated in each integration event (Fig. 3B; duplicated sequences are in boldface). All viral sequences ended with the conserved 5'-CA-3' dinucleotide known to represent the point of cleavage by integrase in the LTR (38). All duplications were precisely 5 bp with no nucleotide substitutions. Each clone sequenced represented an integration event at a different point in the plasmid target. The observation that integration junctions formed in vitro match junctions formed in vivo supports the idea that similar DNA cleavage and joining reactions mediate both.

Fidelity of integration by HIV-2 PICs. For PICs of other viruses, sequence analysis of integration products has revealed exclusively the duplication characteristic of that virus. Altogether, 26 of 26 integration events characterized from retroviral PIC assays contain the expected duplications of target sequences (3, 9, 25). Evidently, these complexes carry out joining with a high degree of coordination between the points of joining at each end of the viral cDNA, as is characteristic of integration in vivo.

Although in vitro reactions with integrase purified from virions or from expression systems yield detectable products generated by coupled joining, diverse further duplications and single-ended products have also been found (4, 6, 14, 16, 21, 39, 40). These studies with purified integrase, while promising, do not yet mimic the full function of PICs efficiently.

Analysis of the role of Vpx protein. Vpx is found in the core of mature HIV-2 virions, suggesting that it is introduced into cells during infection (23), and thus could potentially be involved in integration. To investigate whether Vpx was asso-

FIG. 3. Identification of integration junction sequences. (A) Method for recovering integration junctions generated in vitro. Infected cell extract was incubated with circular pSupF DNA (34) as a target (step 1). Reaction products were purified and cleaved with *HindIII* (step 2). Integration products were not abundant enough to visualize with ethidium bromide. Therefore, the region of the gel expected to contain the *HindIII* fragment with integration junctions was excised based on its relative molecular size (6.6 kb). DNA from the gel slice was purified and treated with DNA polymerase 1 (New England Biolabs) and 250 nM deoxynucleoside triphosphates for 10 min at 30°C and heated to 70°C for 10 min (step 2). Reaction products were amplified by PCR across the viral-target DNA junctions with nested primers complementary to the terminal viral sequences (step 2): 121, 5' GCA ATT ATC TCT TCC TCC 3', and 122, 5' CCC TTC TTG CTT TGG GAA ACC 3', for 30 cycles. Ten percent of the PCR products was used for nested PCR with the following primers: 113, 5' CTG TAA AAC ATC CCT TCC A 3', and 115, 5' CGA GGC AGG AAA ATC CCT AGC A 3', for 30 cycles. The nested PCR products were treated with Klenow fragment and 500 μ M dTTP for 30 min at 37°C, with T4 polynucleotide kinase and 1 mM ATP, and

ciated with the viral cDNA, complexes were analyzed by immunoprecipitation. We incubated extracts containing active HIV-2 PICs with antisera raised against a variety of viral proteins and subsequently captured bound complexes on protein A-Sepharose beads. DNA was isolated from the immunoprecipitation supernatants and pellets, and samples were analyzed by PCR to determine whether viral cDNA was present (Fig. 4A).

DNA was prepared from the supernatants (lanes 1 to 4) and pellets (lanes 5 to 8) of immunoprecipitation reactions of infected cell lysates with anti-integrase (lanes 1 and 5), anticasid (lanes 2 and 6), anti-Vpx (lanes 3 and 7), or no antiserum (lanes 4 and 8). DNA fractions were amplified by PCR with primers complementary to HIV-2 *gag* coding sequences. Amplification products were subjected to electrophoresis on agarose gels and visualized with ethidium bromide. Precipitation with the anti-integrase antibody removed detectable viral cDNA from the supernatant (lane 1), but cDNA was detected in the pellet fraction (lane 5). For the case of the anticasid antibody, no precipitation was detected (compare lanes 2 and 6). Precipitation with the anti-Vpx antibody yielded a signal in both the supernatant and the pellet fractions, consistent with the idea that a portion of the Vpx protein is associated with the cDNA in our fractions. No precipitation of the viral cDNA was detected in the absence of antibody (lanes 4 and 8) or in the absence of beads (data not shown). Furthermore, no precipitation was seen in reactions containing antiserum against HIV-2 gp120 (envelope protein) or a preimmune serum (data not shown). Each of these antibodies was shown to be capable of immunoprecipitating its target protein (data not shown).

To investigate whether Vpx had any detectable influence on integration in vitro, we prepared PICs from a Vpx⁻ virus-producing cell line. The integration activity of these complexes was compared with that of wild-type complexes by Southern analysis (Fig. 4B). Wild-type HIV-2 PICs (lanes 1 and 2) and Vpx⁻ HIV-2 PICs (lanes 3 and 4) were incubated without target (lanes 1 and 3) and with target (lanes 2 and 4). Integration activities were similar in both sources of PICs (lanes 2 and 4).

In summary, although Vpx could be found in association with HIV-2 cDNA in our extracts, we detected no influence of this protein on integration in vitro.

Inhibition of PIC activity by small molecules. HIV-2 PIC assays were also characterized for use in screening candidate integration inhibitors. A previous study indicated that HIV-1 PIC assays in vitro more closely mimic integration in vivo than do assays with purified HIV-1 integrase protein and as such are particularly useful in identifying potential integrase inhibitors

with ligase and 2 mM ATP. Reaction products were transformed into MC1061/P3 cells and selected for ampicillin and tetracycline resistance, which in this strain is conferred by SupF activity. DNA from candidate bacterial colonies was sequenced in two separate reactions with primer 123, 5' TGG AAG GGA TGT TTT ACA G 3', or 124, 5' GCT AGG GAT TTT CCC GCC GCC TCG 3', which is complementary to 113 or 115, respectively (step 3). Sequence reaction mixtures were prepared as described by the manufacturer (U.S. Biochemicals) with the manganese buffer. General methods for DNA manipulation were as described in reference 26. LTRs are indicated by boxes, target DNA is indicated by thick lines, and *HindIII* restriction sites are indicated by H3. Viral primers and the direction of polymerization are indicated by arrows. Repaired gaps in target DNA are indicated by vertical lines. (B) Integration site junctions. The sequence of the HIV-2 DNA plus strand from the 5' TG to the 3' CA is represented at the top for reference. The 7 nucleotides of target sequence flanking each insertion site is shown. Sequences that were duplicated in a single copy of target DNA are shown in boldface. Target DNA is shown at the top in thick lines; viral DNA is shown in a thin line. The sequence of each integration site was confirmed to lie within pSupF.

FIG. 4. The effect of Vpx on integration. (A) Proteins associated with HIV-2 viral cDNA. HIV-2 viral cDNA was immunoprecipitated and amplified by PCR to identify component proteins. Cell extracts were diluted twofold with buffer K with 1% Triton X-100 and incubated with 10 μ l of polyclonal HIV-1 integrase antiserum (6) (lanes 1 and 5), 10 μ l of monoclonal HIV-2 capsid antiserum (lanes 2 and 6; Advanced Biotechnologies), 10 μ l of monoclonal Vpx antiserum (lanes 3 and 7) (23), or without antiserum (lanes 4 and 8) for 2 h at 4°C. Protein A-Sepharose beads were added to 2%, and samples were incubated for 2 h and separated into unbound (lanes 1 to 4) and bound (lanes 5 to 8) fractions by centrifugation at 400 $\times$ g for 15 s. DNA was prepared from samples and amplified by PCR (30 cycles) with the following primers: 101, 5' GGC CCA AGT TCC GCA GGG GCT GAC ACC 3', and MH 102, 5' CCT CAT GTA GTG GTA TGG AAA AGT AAG CAT CCC C 3'. Amplification products were visualized on agarose gels with ethidium bromide. The primers amplified a 900-bp fragment from a positive control plasmid. Lane numbers are indicated below, and a 900-bp marker is indicated at the left. (B) Vpx does not affect integration activity of PICs. Reactions were carried out with HIV-2 PICs derived from wild-type virions (lanes 1 and 2) or derived from Vpx⁻ virions (lanes 3 and 4). An HIV-2-producing cell line was generated by transfection of 293T cells with a vpx mutant (20) and subsequent infection of CEMX174 cells (23, 29). Reactions were carried out in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of exogenous DNA. Products were prepared and detected as described for Fig. 1. Viral cDNA and integration products (IP) are indicated at right.

(10). Parallel use of HIV-2 and HIV-1 PIC assays in screening inhibitors may be useful in identifying compounds of the widest use and those least likely to be evaded by escape mutants.

HIV-2 PICs were partially purified by precipitation in buffer with lower concentrations of salt. Pellets were resuspended, incubated with or without drug, and then incubated with linear target DNA. Integration activity was monitored on Southern blots (Fig. 5). The intensity of each band was quantitated on a PhosphorImager, and the drug concentration sufficient for 50% inhibition (IC₅₀) was determined (Table 1). Inhibition of

FIG. 5. Inhibition of HIV-2 PICs by small molecules. For inhibitor screens, 0.1 ml of PICs in crude cell extracts was partially purified as described elsewhere (10). In brief, crude cell extract was clarified with a 2,000 $\times$ g spin for 7 min at room temperature; supernatants were diluted 1:1 with buffer K lacking KCl, incubated for 5 min at room temperature, and spun at 2,000 $\times$ g for 10 min at room temperature. Pellets were suspended in BK T (buffer K with Tris [pH 8.0] instead of HEPES and 30% glycerol) at volumes equivalent to the starting material. Reaction mixtures with HIV-2 PICs contained no target DNA (lanes 1) or linear PhiX174 DNA (lanes 2 to 11), in the absence of drug (lanes 1 and 2) or in the presence of a drug (lanes 3 to 11). Drug stocks were added 1:50 to partially purified lysates and incubated for 10 min at room temperature before addition of target DNA. The concentrations of drug in the reaction mixtures are indicated above the lanes. Products were detected as described for Fig. 1. (A) AG (lanes 3 to 5), AUN (lanes 6 to 8), and SUR (lanes 9 to 11). (B) ALZ (lanes 3 to 5), QLZ (lanes 6 to 8), and PUR (lanes 9 to 11). Viral cDNA and integration products are indicated to the left. Signals from product molecules were quantitated with a PhosphorImager.

TABLE 1. IC₅₀ measurements of integration inhibitors^a

Compound	Purified HIV-1 integrase	HIV-1 PICs	HIV-2 PICs
AG		>100	>100
AUN	0.50	21	>100
SUR	0.14	96	>100
QTN	1.30	>100	>100
ALZ	7	18	18
QLZ	4	6	4
PUR	7	13	12

^a The drug concentration that yields IC₅₀ is indicated for the seven compounds. Inhibition of purified HIV-1 integrase protein (column 1) was determined in the terminal cleavage assay (7, 21, 35). Inhibition of HIV-1 PIC activity is indicated in column 2 (10), and inhibition of HIV-2 PIC activity is indicated in column 3. The data shown in columns 1 and 2 are taken from reference 10. Each value in column 3 represents the mean from at least six experiments. Integration activity was scored as the percentage of material in the integration product band divided by integration product plus viral cDNA. QTN, quercetagenin.

HIV-2 PICs by previously described inhibitors of purified HIV-1 integrase protein was examined as shown in Fig. 5A. Lane 1 displays the signal in the absence of drug or target DNA; lane 2 displays the signal in the presence of target. Acid green 25 (AG; lanes 3 to 5), aurintricarboxylic acid (AUN; lanes 6 to 8), suramin (SUR; lanes 9 to 11), and quercetagenin (not shown) all failed to inhibit HIV-2 PICs. For Fig. 5B, compounds previously shown to inhibit HIV-1 PICs as well as purified HIV-1 integrase were examined. The polyhydroxylated anthraquinones, quinalizarin (QLZ; lanes 6 to 8), purpurin (PUR; lanes 9 to 11), and alizarin (ALZ; lanes 3 to 5), all displayed inhibition (Table 1).

Assays comparing inhibition of PICs from HIV-1 with that of PICs from HIV-2 may help identify compounds that are less vulnerable to escape mutants. The reverse transcriptase (RT) inhibitor nevirapine provides a potentially instructive example. Nevirapine was found to be active against HIV-1 RT (IC₅₀, 70 nM) but not against HIV-2 RT (IC₅₀, >>250 μ M) (1). Nevirapine-resistant mutants of HIV-1 were found to arise quickly after initiation of drug treatment in patients (31). In retrospect,

the fact that HIV-2 RT was resistant could have been interpreted as an indication that the RT of HIV-1 could easily mutate to a resistant form more similar to HIV-2 RT. Although escape mutants can certainly arise against drugs effective against both HIV-1 and HIV-2, for the case of integrase inhibitors, it seems useful to combine assays of HIV-2 PICs with assays of HIV-1 PICs to identify compounds that are potentially less prone to evasion by escape mutants.

We are grateful to Vineet KewalRamani and Michael Emerman for their generosity with reagents. We thank John Kappes for the gift of the vpx provirus. We offer hearty thanks to Chris Farnet and Michael Miller for assistance and many useful suggestions, Sandrine Carreau and Bei-Bei Wang for discussions, and Leslie Barden and Verna Stitt for excellent technical support. The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases: 174xCEM (from Peter Cresswell), SupT1 (from James Hoxie), and anti-HIV-2 gp120 envelope antisera (from SmithKline Beecham Pharmaceuticals) (28, 33, 36).

This work was supported by the California Universitywide AIDS Research Program (M.S.T.H.) and by NIH grant R01AI34786-0104. F.D.B. is a Scholar of the Leukemia Society of America.

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