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Inhibition of Human Immunodeficiency Virus Integrase by Bis-Catechols

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The human immunodeficiency virus type 1 (HIV-1) integrase protein is required for the productive infection of T-lymphoid cells in culture (R. L. LaFemina, C. L. Schneider, H. L. Robbins, P. L. Callahan, K. LeGrow, E. Roth, W. A. Schleif, and E. A. Emini, J. Virol. 66:7414–7419, 1992). This observation suggests that chemical inhibitors of integrase may prevent the spread of HIV in infected individuals. In our search for such potential chemotherapeutic agents, we observed that β-conidendrol inhibits both the sequence-dependent and sequence-independent endonucleolytic activities of integrase with comparable potencies in vitro (50% inhibitory concentration, 500 nM). Structurally related compounds tested for their abilities to inhibit integrase generated a limited structure-activity analysis which demonstrated that potency is associated with the bis-catechol structure: two pairs of adjacent hydroxyls on separate benzene rings. β-Conidendrol did not inhibit several other endonucleases and/or phosphoryltransferases. Although β-conidendrol was not effective in preventing HIV-1 infection in cell culture, the in vitro data demonstrate that it is possible to identify selective agents targeted against this essential HIV-1 function.

Infection of replicating T cells by the human immunodeficiency virus type 1 (HIV-1) lentivirus results in the insertion of the double-stranded DNA product of reverse transcription into the host cell genome (for a review, see reference 17). Integration of the viral genome may be required for efficient transcription by the host cell (2). In addition, integration ensures that in the absence of a defined replication origin, the provirus is replicated and subsequently segregated into progeny cells (2). Integration requires both the sequence-specific and the coupled nonspecific, sequence-independent endonucleolytic and strand transfer activities of the virally encoded enzyme integrase (7, 8, 24, 33).

Integrase recognizes a defined sequence within both of the viral long terminal repeats (LTRs) and makes a specific, single-strand cleavage in the U5 LTR-positive strand and the U3 LTR-negative strand (3, 25, 31, 33). The resultant 3′-terminal dA hydroxyl is the donor substrate for subsequent joining to a 5′-phosphate that is produced by nonspecific processing of the host-cell target DNA substrate (3, 6, 16, 24). In vitro, the recombinant enzyme uses oligonucleotides as both the LTR-specific donor and nonspecific target substrates. Integrase specifically processes synthetic oligonucleotides representing the LTR termini by removing the terminal dinucleotide from the 3′ end of one strand. In vitro assays have established that the C and the A residues at positions 3 and 4 relative to the terminus of the processed LTR strand, respectively, contribute substantially to the specificities of the reactions (18, 19, 26, 33, 41). In a reaction that mimics the concerted nonspecific cleavage and joining activities of the enzyme, integrase randomly cleaves oligonucleotides for use as the target substrate and mediates a strand transfer reaction in which the two appropriately processed substrates are joined. Preprocessed LTR oligonucleotides which have a 3′ A hydroxyl and a two-nucleotide 5′ extension of the noncleaved strand are used to distinguish the effects on specific endonuclease activity from the effects on the nonspecific cleavage-strand transfer function (13, 26, 28).

Since integrase is essential for the spread of HIV-1 in cultured CD4-positive T-lymphoid cells (27), the enzyme is a potential target for antiviral chemotherapeutic intervention. In contrast to protease and reverse transcriptase, for which an extensive number of chemical inhibitors have been described (for a review, see reference 21), there is little information regarding selective inhibitors for any of the enzymatic reactions catalyzed by integrase. Previous studies have focused on inhibitors such as aurintricarboxylic acid (10), topoisomerase poisons (7b), and intercalators (1, 15) known to affect mechanistically related enzymes. In this report we describe and characterize the inhibition of HIV-1 integrase sequence-specific and sequence-independent hydrolysis and strand transfer activities by a unique structural class, bis-catechols. The most potent member in this class, β-conidendrol, is inactive against a variety of nucleic acid-processing enzymes at concentrations significantly greater than that required for inactivation of the integrase function. This is among the first reports to describe a selective inhibitor for this important antiviral target.

MATERIALS AND METHODS

Purification of recombinant HIV-1 integrase. Cloning and expression of the HIV-1 HXB2 integrase in *Escherichia coli* have been described previously (33). The procedure for purification of integrase was modified from the original method of Sherman and Fyfe (33) as reported previously (18). The cloning and expression of C43S integrase have been described previously, and this enzyme was as pure as the authentic enzyme (27).

Analysis of integrase reaction products on sequencing gels. The assay for specific 3′-end processing of oligonucleotide substrates representing the HIV LTR sequences was performed as described previously (33). The 5′ end of the U5 LTR-positive processed strand (5′-TGTGGAAAATCTCTAGCAGT) was labeled with T4 polynucleotide kinase (26). Equimolar amounts of the radiolabeled strand were annealed with unlabeled nonprocessed complement (5′-ACT GCTAGAGATTTTCCACA) to provide duplex substrates. Standard assays used 1 ng of radiolabeled substrate and 50 ng of integrase in a 20-μl reaction volume. The assays were unaffected by 5% dimethyl sulfoxide (DMSO); therefore, all compounds to be tested were dissolved in 50% DMSO and were diluted 10-fold into the reaction mixture (final concentration, 5% DMSO). The reaction prod-

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ucts were analyzed by electrophoresis on 20% sequencing gels; this was followed by autoradiography. Specific cleavage was demonstrated by removal of the terminal dinucleotide as determined by production of the 18-nucleotide cleavage product. The preprocessed duplex substrate (5′-TGTGGAAAATCTCTAGCA) was annealed to the same complement described above and served as a substrate for the non-sequence-specific functions of integrase (26).

Nuclease and phosphoryltransferase assays. The *Eco*RI assay used a 5′-end-labeled duplex 20-bp oligonucleotide containing an *Eco*RI site and *Eco*RI enzyme (Boehringer Mannheim) in buffer supplied by the manufacturer. Analyses of cleavage products were visualized by electrophoresis on 20% sequencing gels and then autoradiography. The HIV-1 reverse transcriptase (32), influenza virus transcriptase (35), and RNA polymerase II assays (35) were performed as described previously. The topoisomerase assays were performed as described previously (29, 30, 34, 36, 37). Compounds were tested in these additional assays according to the sensitivity and DMSO tolerance of each individual assay.

Microtiter strand transfer assay. We have previously described a microtiter strand transfer assay for integrase that is nonradioactive, more sensitive, and more amenable for the screening of large numbers of samples (18). The microtiter assay uses an immobilized 30-bp U5 donor substrate and a heterologous biotinylated 20-bp target sequence. Microtiter plates are coated with the U5 substrate oligonucleotide such that the amount of substrate immobilized per well is 1.25 pmol (final concentration, 12.5 nM in a 100-μl reaction mixture). The strand transfer assay was performed directly in the oligonucleotide-coated microtiter wells. Each reaction mixture of 100 μl included 20 mM Tris-HCl (pH 7.8), 25 mM NaCl, 3 mM $MnCl_2$, 5 mM β-mercaptoethanol, 50 μg of bovine serum albumin (BSA) per ml, and inhibitor in DMSO or DMSO alone. The LTR-specific cleavage reaction was initiated by the addition of 2 μl of integrase (final concentration, 40 nM). Since integrase is functional as an oligomer (12, 22, 38, 40) this value very likely represents an overestimate of the amount of enzyme present in the active oligomeric state (22). This is consistent with the observation that even when the amount of enzyme is in excess over the amount of substrate, the reaction is dependent on the amount of integrase, indicating that the active form of the enzyme is rate limiting in the reaction (1).

After 30 min at 37°C, 5 μl of the biotinylated target oligonucleotides was added. The strand transfer reaction was performed for an additional 15 min at 37°C. After extensive washing, the plates were blocked with 1% BSA in phosphate-buffered saline, and the strand transfer products were detected as described above. Plates were read at 405 nm with a microplate reader (Molecular Devices).

RESULTS

β-Conidendrol selectively inhibits HIV-1 integrase. To demonstrate inhibition of integrase-mediated sequence-specific processing, a 5′-end-labeled 20-bp oligonucleotide derived from the U5 LTR was used as a substrate in the reaction. Sequence-specific removal of the terminal dinucleotide from the 3′ end generates a smaller radiolabeled 18-nucleotide cleavage product detected following electrophoresis on sequencing gels (33). Compounds were screened randomly in the assay, which looked for inhibition of the production of the 18-nucleotide species. In this screen we identified a unique and apparently selective inhibitor of integrase, β-conidendrol.

As shown in Fig. 1A, in the presence of increasing concentrations of β-conidendrol, we observed dose-dependent inhibition of specific processing as evidenced by a decrease in the amount of the specific cleavage product. In addition, β-conidendrol inhibited the formation of the larger reaction products which result from strand transfer events in which two oligonucleotide substrates are joined. Since the specific cleavage product is the donor substrate for strand transfer, the parallel inhibition of strand transfer in the presence of β-conidendrol probably resulted from the enzyme's inability to produce appropriately processed donor substrates.

We therefore examined whether β-conidendrol could inhibit the processes involved in strand transfer independently of specific cleavage by using a preprocessed donor substrate (PWT) (26). This substrate consists of a U5 LTR lacking the terminal two nucleotides on the plus strand such that the 3′-terminal dA residue is accessible for strand transfer. As shown in Fig. 1B, β-conidendrol inhibited strand transfer of the preprocessed substrate (PWT) with a 50% inhibitory concentration (IC_{50}) indistinguishable from that shown for specific cleavage in Fig. 1A. However, disappearance of the 18 − N products in the presence of β-conidendrol suggested concomitant inhibition of nonspecific processing of potential target substrates. Therefore, the inhibition of strand transfer in both experiments could be explained by an effect on either of the enzyme's endonucleolytic activities. In these experiments, there was no apparent difference between the inhibition of sequence-specific processing and nonspecific hydrolysis and strand transfer.

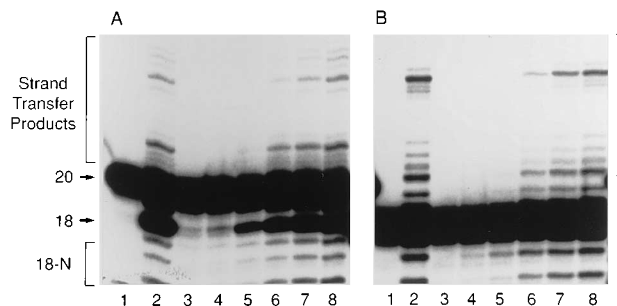

FIG. 1. Inhibition of HIV integrase by β-conidendrol. (A) The 20-nucleotide wild-type substrate was incubated in the absence or presence of integrase (IN) to produce the 18 nucleotide (18) specific cleavage product, nonspecific cleavage products (18 − N) and strand transfer products (greater than 20 nucleotides) as indicated. (B) Incubation of integrase with the 18-nucleotide preprocessed wild-type (PWT) substrate as described in the text. In both panels A and B β-conidendrol was tested in threefold serial dilutions from 152 to 0.60 μM (lanes 3 to 8). Lane 2 is the solvent control (10% DMSO); lane 1 contains no enzyme. The 20-nucleotide substrate and the 18-nucleotide product were excised from the gel and counted to determine the percent inhibition by β-conidendrol.

The inhibition of nonspecific processing by β-conidendrol was confirmed by using a mutated form of the enzyme in which a single amino acid substitution in the enzyme's putative zinc finger reduces both specific processing and strand transfer but does not affect the enzyme's ability to cleave DNA nonspecifically (27). As shown in Fig. 2, nonspecific processing by the mutant enzyme was as effectively inhibited by β-conidendrol as either nonspecific or specific processing by the wild-type enzyme. Since β-conidendrol is an effective inhibitor of both endonuclease activities, the apparent inhibition of strand transfer may be entirely a consequence of the compound's effect on cleavage, although we cannot eliminate the possibility that β-conidendrol affects both the cleavage and strand transfer activities of integrase.

Since the sequence-specific and sequence-independent endonuclease functions of the enzyme were affected to the same degree, it was important to demonstrate selectivity by investigating the effect of β-conidendrol on other endonucleases and/or phosphoryltransferases. As shown in Table 1, β-coniden-

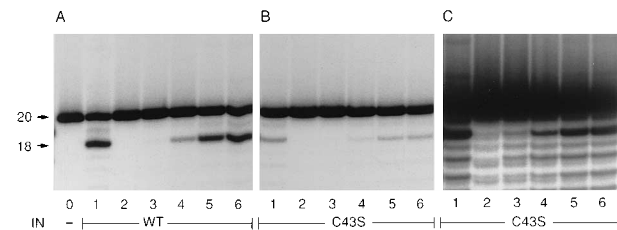

FIG. 2. Inhibition of nonspecific cleavage of HIV-1 integrase by β-conidendrol. The 20-nucleotide U5 LTR substrate was incubated in the presence of wild-type HIV-1 integrase (A) or in the presence of the C43S integrase mutant (B and C). Panel C is a darker exposure of panel B. The 18-nucleotide-specific cleavage product is noted. In each case, the levels of β-conidendrol used were fivefold dilutions from 7.5 to 0.012 μM (lanes 2 to 6). Lane 0, no enzyme control; lanes 1, solvent control (no β-conidendrol). Data were analyzed as described in the legend to Fig. 1.

TABLE 1. Specificity of β-conidendrol for enzymatic reactions associated with integrase

Reaction	IC_{50}
Integrase	
Specific cleavage[a]	0.50 μM
Nonspecific cleavage[a]	0.50 μM
Strand transfer[a]	0.50 μM
Strand transfer[b]	0.30 μM
Other	No inhibition[c]
*Eco*RI	150 μM
HIV RT[d]	200 mM
RNA polymerase II	10 μM
Influenza polymerase	10 μM
Topoisomerase I	300 μM
Topoisomerase II	300 μM

[a] As measured by polyacrylamide gel electrophoresis analysis.
[b] As measured in the microtiter strand transfer assay.
[c] β-Conidendrol was not inhibitory in these assays at the levels indicated. References for these assays are presented in Materials and Methods.
[d] RT, reverse transcriptase.

drol was specific for the enzymatic reactions associated with integrase. β-Conidendrol did not inhibit a variety of nucleic acid-processing enzymes, including the restriction enzyme *Eco*RI, HIV-1 reverse transcriptase, HeLa RNA polymerase II, mammalian topoisomerases I and II, and the influenza virus polymerase-endonuclease complex. The observation that β-conidendrol did not affect these assays suggests that this compound does not interact nonspecifically with nucleic acid.

β-Conidendrol was inhibitory in the integrase assay at submicromolar levels and, to the extent that it was tested in other assays, exhibited a high degree of selectivity. Therefore, we tested this compound in an HIV-1 spread assay to investigate potential antiviral activity. β-Conidendrol was neither antiviral nor cytotoxic in MT4 cells at levels of up to 100 μM (data not shown).

TABLE 2. Inhibition of integrase sequence-specific endonuclease activity

Compound[a]	IC_{50} (μM)[b]
β-Conidendrol (A)	0.50
α-Conidendrol (A)	0.50
L-739,633 (B)	7
α-Conidendrin (C)	>35
L-619,323 (D)	1.7
L-724,180 (E)	9.0
L-724,189 (F)	>35
Hematoxylin (G)	0.50
Brazelin (H)	35

[a] The letters in parentheses correspond to the structures presented in Fig. 3. β-Conidendrol differs from α-conidendrol at the asymmetric carbon.
[b] Inhibition of formation of the 18-nucleotide specific cleavage product.

The bis-catechol structure is required for activity. To determine the specific structural moiety required for inhibitory activity, a large number of analogs related to β-conidendrol were compared. All of the active compounds tested were similar with regard to their ability to affect both the sequence-specific and sequence-independent endonucleolytic activities of integrase (data not shown). A subset of these compounds is shown in Fig. 3, and the data for inhibition of integrase sequence-specific endonuclease activity are summarized in Table 2. The compound in Fig. 3A is conidendrol with the indicated asymmetric carbon. Both the α and β forms of conidendrol were equipotent inhibitors of the specific and nonspecific activities

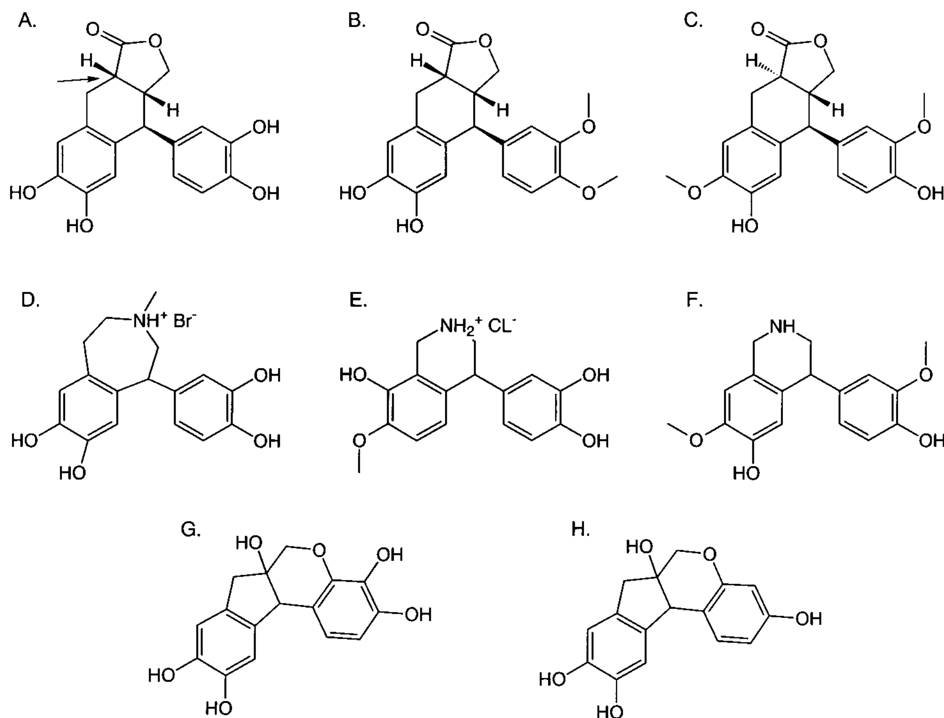

FIG. 3. Structures of β-conidendrol and related compounds described in Table 2. (A) β-conidendrol. The arrow denotes the asymmetric carbon. (B) L-739,633. (C) α-Conidendrin. (D) L-619,323. (E) L-724,180. (F) L-724,189. (G) Hematoxylin. (H) Brazelin.

of integrase (IC_{50}, 500 nM). In contrast, α-conidendrin (Fig. 3C), in which two of the distal hydroxyl groups are replaced by methoxy groups, was not inhibitory at concentrations as high as 35 μM. Substitution of a pair of proximal hydroxyl groups resulted in a compound of intermediate potency (Fig. 3B, L-739,633, 7 μM). These data suggested a correlation between potency and the number and/or arrangement of hydroxyl groups.

Two other structures, L-619,323 and hematoxylin, shown in Fig. 3D and G, respectively, displayed reasonable inhibitory activity against HIV integrase and were structurally analogous to β-conidendrol with regard to the number and arrangement of hydroxyl substituents. L-619,323 (Fig. 3D) was approximately threefold less active than either conidendrol (1.7 μM). Replacement of one hydroxyl group reduced the potency (Fig. 3E, L-724,180, 9 μM), while replacement of the two distal hydroxyl groups completely abolished the activity (Fig. 3F, L-724,189, >35 μM). Similarly, hematoxylin (Fig. 3G) had an IC_{50} comparable to that of β-conidendrol (0.50 μM), whereas brazelin (Fig. 3H), which is identical to hematoxylin except for the absence of one hydroxyl group, was about 100-fold less active. The relative potency of the compounds in each structural set demonstrates that inhibitory activity is associated with the specific spatial disposition of the hydroxyl groups. On the basis of these data and others not shown here, we determined that the active pharmacophore was a bis-catechol; that is, the most active compounds contain two pairs of adjacent hydroxyls on nonadjacent benzene rings.

DISCUSSION

Despite the observation that integrase plays a critical role in the life cycle of HIV (2, 27), to date compounds which specifically inhibit integrase in vitro have not been described. Although aurintricarboxylic acid was shown to inhibit integrase in vitro (10), this compound is an inhibitor of a variety of phosphoryltransferase enzymes including HIV-1 reverse transcriptase (9). Similarly, while suramin inhibits HIV-1 integrase in vitro, this compound also inhibits a diverse range of enzymatic activities (7a). Topoisomerase inhibitors (1, 7b, 10a, 15) are relatively ineffectual against HIV-1 integrase, suggesting that there are mechanistic differences between the two enzymes. Finally, intercalating agents and structures which bind DNA have been reported to inhibit integrase in vitro, but they are likely to have limited selectivity and, therefore, limited utility as therapeutic antiviral agents. We undertook the approach of random screening to look for structures which would be both more potent and selective. To our knowledge the bis-catechols are the first demonstration of a novel structural class of selective inhibitors for HIV-1 integrase. Although these compounds failed to exhibit antiviral activity in cell culture, the in vitro data demonstrate that it is possible to find low-molecular-weight agents which target specific functions of this essential enzyme.

Limited structure-activity analysis determined that hydroxyl substituents in the bis-catechol configuration are critical for the compound's inhibitory activity against integrase. Substitution of two distal hydroxyl groups more profoundly affected potency than substitution of a pair of adjacent hydroxyl groups. All of the active bis-catechols inhibited both the sequence-specific and the sequence-independent endonucleolytic activities of integrase. Inhibition of the general endonucleolytic activity of integrase was demonstrated by using a mutant enzyme, C43S, which has been described previously (27). This single amino acid substitution in the putative zinc finger of integrase severely abrogates specific processing activity but has no effect on the nonspecific endonucleolytic activity. When this substitution is introduced into an N-terminal 55-amino-acid peptide it abolishes the binding of zinc (5).

The observation that β-conidendrol inhibited the nonspecific cleavage activity of the putative zinc finger mutant as well as the fact that it inhibited either the nonspecific or the specific endonucleolytic activities of the wild-type enzyme indicate that β-conidendrol does not inhibit integrase by interacting with the putative zinc finger. This mechanism of action has been proposed for a previously described inhibitor of integrase, dihydroxyanthraquinone (DHNQ) (15). Although it is not a biscatechol, DHNQ does contain two hydroxyl substituents which may be critical for its activity. It had been proposed that the phenolic hydroxyl substituents of DHNQ would be effective chelators of divalent cations (15). Because we have been unable to overcome the β-Conidendrol-mediated inhibition by adding extra zinc, manganese, or magnesium to the cleavage reaction (data not shown), inhibition by this compound is not likely due to chelation. β-Conidendrol did not inhibit a variety of nucleic acid-processing enzymes, and there is no evidence of inhibition by intercalation.

For integrase, the existence of distinct viral and target substrate-binding sites has been proposed previously (for example, see the model presented in reference 39). Given that β-Conidendrol inhibited both the sequence-specific and sequence-independent endonuclease activities of integrase, it is possible that the inhibitor binds to the enzyme at these two sites. Although the binding site for each substrate may be exclusive, it is possible that the second site (i.e., target) is affected by binding to the donor substrate. Further experimentation will be required to address this and to determine if the two sites are present on one or multiple molecules of integrase. Both genetic and biophysical studies have suggested that strand transfer requires a multimeric form of the enzyme (11, 12, 20, 22, 23, 38, 39).

β-Conidendrol seems to be selective for HIV-1 integrase in vitro, but we have not been able to demonstrate antiviral activity in cell culture. The reasons for this apparent lack of functional activity may either be trivial, such as instability or the lack of cell penetration, perhaps because of the polar nature of the compound, or may point to significant differences between the in vitro and in vivo reactions catalyzed by the enzyme. Since the process of integration in vivo is accomplished by a nucleoprotein complex which contains integrase and perhaps other viral components (4, 14) in a well-defined architecture that is determined during assembly of the viral core, it is conceivable that the reactions monitored in vitro with the recombinant enzyme supplied in *trans* fail to accurately recapitulate the integration process as it occurs during infection (7). This is at least in part true, because no laboratory to date has been able to efficiently reconstitute an integration reaction with recombinant HIV-1 integrase, wherein both viral LTR termini are concomitantly inserted into a target DNA substrate. It is hoped that the inhibitors of the in vitro reactions, such as the bis-catechols, will aid in the dissection of the preintegration complex to determine where these discrepancies lie and to evaluate which of the reactions catalyzed by this enzyme are most sensitive to inhibition during the HIV-1 life cycle.

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REFERENCES

1. **Billich, A., M. Schauer, S. Frank, B. Rosenwirth, and S. Billich.** 1992. HIV-1 integrase: high-level production and screening assay for the endonucleolytic activity. Antiviral Chem. Chemother. **3:**113–119.
2. **Brown, P. O.** 1990. Integration of retroviral DNA. Curr. Top. Microbiol. Immunol. **157:**19–48.
3. **Brown, P. O., B. Bowerman, H. E. Varmus, and J. M. Bishop.** 1989. Retroviral integration: structure of the initial covalent product and its precursor, and a role for the viral Integrase protein. Proc. Natl. Acad. Sci. USA **86:** 2525–2529.
4. **Bukrinsky, M. I., N. Sharova, T. L. McDonald, T. Pushkarskaya, W. G. Tarpley, and M. Stevenson.** 1993. Association of integrase, matrix, and reverse transcriptase antigens of human immunodeficiency virus type 1 with viral nucleic acids following acute infection. Proc. Natl. Acad. Sci. USA **90:**6125–6129.
5. **Burke, C. J., G. Sanyal, M. W. Bruner, J. A. Ryan, R. L. LaFemina, H. L. Robbins, A. S. Zeft, C. R. Middaugh, and M. C. Cordingley.** 1992. Structural implications of spectroscopic characterization of a putative zinc finger peptide from HIV-1 integrase. J. Biol. Chem. **267:**9639–9644.
6. **Bushman, F. D., and R. Craigie.** 1991. Activities of human immunodeficiency virus (HIV) integration protein *in vitro*: specific cleavage and integration of HIV DNA. Proc. Natl. Acad. Sci. USA **88:**1339–1343.
7. **Bushman, F. D., T. Fujiwara, and R. Craigie.** 1990. Retroviral DNA integration directed by HIV integration protein in vitro. Science **249:**1555–1558.

7a. **Carteau, S., J. F. Mouscadet, H. Goulaouic, F. Subra, and C. Auclair.** 1993. Inhibitory effect of the polyanionic drug suramin on the in vitro HIV DNA integration reaction. Arch. Biochem. Biophys. **305:**606–610.

7b. **Carteau, S., J. F. Mouscadet, H. Goulaouic, F. Subra, and C. Auclair.** 1993. Effect of topoisomerase inhibitors on the in vitro HIV DNA integration reaction. Biochem. Biophys. Res. Commun. **192:**1409–1414.

8. **Craigie, R., T. Fujiwara, and F. Bushman.** 1990. The IN protein of Moloney murine leukemia virus processes the viral DNA ends and accomplishes their integration in vitro. Cell **62:**829–837.
9. **Cushman, M., S. Kanamathareddy, E. DeClercq, D. Schols, M. Goldman, and J. A. Bowen.** 1991. Synthesis and anti-HIV activities of low molecular weight aurintricarboxylic acid fragments and related compounds. J. Med. Chem. **34:**337–342.
10. **Cushman, M., and P. Sherman.** 1992. Inhibition of HIV-1 integration protein by aurintricarboxylic acid monomers, monomer analogs, and polymer fractions. Biochem. Biophys. Res. Commun. **185:**85–90.

10a. **D'Arpa, P., and L. F. Liu.** 1989. Topoisomerase-targeting antitumor drugs. Biochim. Biophys. Acta **989:**163–177.

11. **Drelich, M., M. Haenggi, and J. Mous.** 1993. Conserved residues Pro-109 and Asp-116 are required for interaction of the human immunodeficiency virus type 1 integrase protein with its viral DNA substrate. J. Virol. **67:**5041–5044.
12. **Engelman, A., F. D. Bushman, and R. Craigie.** 1993. Identification of discrete functional domains of HIV-1 integrase and their organization within an active multimeric complex. EMBO J. **12:**3269–3275.
13. **Engelman, A., and R. Craigie.** 1992. Identification of conserved amino acid residues critical for human immunodeficiency virus type 1 integrase function in vitro. J. Virol. **66:**6361–6369.
14. **Farnet, C. M., and W. A. Haseltine.** 1991. Determination of viral proteins present in the human immunodeficiency virus type 1 preintegration complex. J. Virol. **65:**1910–1915.
15. **Fesen, M. R., K. W. Kohn, F. Leteurtre, and Y. Pommier.** 1993. Inhibitors of human immunodeficiency virus integrase. Proc. Natl. Acad. Sci. USA **90:** 2399–2403.
16. **Fujiwara, T., and K. Mizuuchi.** 1988. Retroviral DNA integration: structure of an integration intermediate. Cell **54:**497–504.
17. **Goff, S. P.** 1992. Genetics of retroviral integration. Annu. Rev. Genet. **26:** 527–544.
18. **Hazuda, D. J., J. C. Hastings, A. L. Wolfe, and E. A. Emini.** 1994. A novel assay for the DNA strand-transfer reaction of HIV-1 integrase. Nucleic Acids Res. **22:**1121–1122.
19. **Hazuda, D. J., and R. L. LaFemina.** Unpublished data.
20. **Hazuda, D. J., A. L. Wolfe, J. C. Hastings, H. L. Robbins, P. L. Graham, R. L. LaFemina, and E. A. Emini.** 1994. Viral long terminal repeat substrate binding characteristics of the human immunodeficiency virus type 1 integrase. J. Biol. Chem. **269:**3999–4004.
21. **Huff, J. R.** 1991. HIV protease: a novel chemotherapeutic target for AIDS. J. Med. Chem. **34:**2305–2314.
22. **Jones, K. S., J. Coleman, G. W. Merkel, T. M. Laue, and A. M. Skalka.** 1992. Retroviral integrase functions as a multimer and can turn over catalytically. J. Biol. Chem. **267:**16037–16040.
23. **Kalpana, G. V., and S. P. Goff.** 1993. Genetic analysis of homomeric interactions of human immunodeficiency virus type 1 integrase using the yeast two-hybrid system. Proc. Natl. Acad. Sci. USA **90:**10593–10597.
24. **Katz, R. A., G. Merkel, J. Kulkosky, J. Leis, and A. M. Skalka.** 1990. The avian retroviral IN protein is both necessary and sufficient for integrative recombination in vitro. Cell **63:**87–95.
25. **Katzman, M., R. A. Katz, A. M. Skalka, and J. Leis.** 1989. The avian retroviral integration protein cleaves the terminal sequences of linear viral DNA at the in vivo sites of integration. J. Virol. **63:**5319–5327.
26. **LaFemina, R. L., P. L. Callahan, and M. G. Cordingley.** 1991. Substrate specificity of recombinant human immunodeficiency virus integrase protein. J. Virol. **65:**5624–5630.
27. **LaFemina, R. L., C. L. Schneider, H. L. Robbins, P. L. Callahan, K. LeGrow, E. Roth, W. A. Schleif, and E. A. Emini.** 1992. Requirement of active human immunodeficiency virus type 1 integrase enzyme for productive infection of human T-lymphoid cells. J. Virol. **66:**7414–7419.
28. **Leavitt, A. D., R. B. Rose, and H. E. Varmus.** 1992. Both substrate and target oligonucleotide sequences affect in vitro integration mediated by human immunodeficiency virus type 1 integrase protein produced in *Saccharomyces cerevisiae*. J. Virol. **66:**2359–2368.
29. **Liu, L. F., T. C. Rowe, L. Yang, K. M. Tewey, and G. L. Chen.** 1983. Cleavage of DNA by mammalian DNA topoisomerase II. J. Biol. Chem. **258:**15365–15370.
30. **Muller, M. T., J. R. Spitzner, J. A. DiDonato, V. B. Mehta, K. Tsutsui, and K. Tsutsui.** 1988. Single-strand DNA cleavages by eukaryotic topoisomerase II. Biochemistry **27:**8369–8379.
31. **Roth, M. M., P. L. Schwartzberg, and S. P. Goff.** 1989. Structure of the termini of DNA intermediates in the integration of retroviral DNA: dependence on IN function and terminal DNA sequence. Cell **58:**47–54.
32. **Sardana, V. V., E. A. Emini, L. Gotlib, D. J. Graham, D. W. Lineberger, W. J. Long, A. J. Schlabach, J. A. Wolfgang, and J. H. Condra.** 1992. Functional analysis of HIV-1 reverse transcriptase amino acids involved in resistance to multiple nonnucleoside inhibitors. J. Biol. Chem. **267:**17526–17530.
33. **Sherman, P. A., and J. A. Fyfe.** 1990. Human immunodeficiency virus integration protein expressed in *Escherichia coli* possesses selective DNA cleaving activity. Proc. Natl. Acad. Sci. USA **87:**5119–5123.
34. **Spitzner, J. R., and M. T. Muller.** 1988. A consensus sequence for cleavage by vertebrate DNA topoisomerase II. Nucleic Acids Res. **16:**5533–5556.
35. **Tomassini, J., M. E. Davies, M. E. Armstrong, J. Hastings, D. Hazuda, J. Lewis, W. McClements, A. Wolfe, H. Selnick, J. Baldwin, M. Bourgeois, G. Ponticello, E. Radilowski, G. Smith, and A. Tebben.** 1994. Inhibition of the cap-dependent endonuclease of influenza virus by 4-substituted 2,4-dioxobutanoic acid compounds. Antimicrob. Agents Chemother. **38:**2827–2837.
36. **Trask, D. K., J. A. DiDonato, and M. T. Muller.** 1984. Rapid detection and isolation of covalent DNA/protein complexes: application to topoisomerase I and II. EMBO J. **3:**671–676.
37. **Trask, D. K., and M. T. Muller.** Biochemical characterization of topoisomerase I purified from avian erythrocytes. Nucleic Acids Res. **11:**2779–2800.
38. **van Gent, D. C., C. Vink, A. A. M. Oude Groeneger, and R. H. A. Plasterk.** 1993. Complementation between HIV integrase proteins mutated in different domains. EMBO J. **12:**3261–3267.
39. **Vincent, K. A., V. Ellison, S. A. Chow, and P. O. Brown.** 1993. Characterization of human immunodeficiency virus type 1 integrase expressed in *Escherichia coli* and analysis of variants with amino-terminal mutations. J. Virol. **67:**425–437.
40. **Vink, C., A. A. M. Oude Groeneger, and R. H. A. Plasterk.** Identification of the catalytic and DNA-binding region of the human immunodeficiency virus type I integrase protein. Nucleic Acids Res. **21:**3373–3377.
41. **Vink, C., D. C. van Gent, Y. Elgersma, and R. H. A. Plasterk.** 1991. Human immunodeficiency virus integrase protein requires a subterminal position of its viral DNA recognition sequence for efficient cleavage. J. Virol. **65:**4636–4644.