A rapid *in vitro* assay for HIV DNA integration

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ABSTRACT

Retroviruses synthesize a double stranded DNA copy of their RNA genome after infection of a permissive cell and subsequent integration of this DNA copy into the host genome is necessary for normal viral replication. Integration occurs by a specialized DNA recombination reaction, mediated by the viral IN protein. Because this reaction has no known cellular counterpart, it is a particularly attractive target in the search for specific inhibitors with low toxicity that may serve as therapeutic antiviral agents. We present a simple assay system that is suitable for screening potential inhibitors of HIV DNA integration. Only short oligonucleotides matching one end of HIV DNA and purified HIV IN protein are required as substrates. Furthermore, since each step of the assay can be carried out in the wells of microtiter plates, large numbers of reactions can be processed simultaneously.

INTRODUCTION

Each step of the HIV replication cycle presents a potential target for therapeutic intervention. Considerable progress has already been made in targeting several of these steps, notably reverse transcription (reviewed in ref.1). Parallel efforts to find specific inhibitors of other steps in the HIV replication cycle can be expected to lead to the development of new classes of therapeutic agents and facilitate the combined use of antiviral drugs with different target specificities.

A critical step in HIV replication is integration of the viral DNA, synthesized by reverse transcription within the cytoplasm of an infected cell, into the host genome (for recent reviews on retroviral DNA integration see refs. 2, 3, 4). Retroviral DNA integration involves a defined set of DNA cutting and joining reactions (depicted in Figure 1). Nucleolytic cleavage first removes two nucleotides from the 3′ ends of the double stranded viral DNA made by reverse transcription. In the subsequent DNA strand transfer reaction, joining of viral to target DNA is coupled with cleavage of the target DNA at the site of insertion; this reaction joins the 3′ ends of the viral DNA to the 5′ ends of a staggered break made in the target DNA. Removal of the two unpaired bases at the 5′ ends of the viral DNA and repair of the single strand gaps between the viral and host DNA completes the integration process.

Studies of several retroviruses have identified a region of the viral *pol* gene that encodes a product required for integration (reviewed in refs. 2, 3, 4). This product is the viral IN protein, which is cleaved from the carboxy terminus of a polyprotein precursor that also includes reverse transcriptase and the protease responsible for cleavage. Mutations in this region of the HIV *pol* gene abolish infectivity (5). The HIV IN protein is the only essential viral protein for both the nucleolytic cleavage (6, 7) and the DNA strand transfer (7, 8) reactions; the subsequent DNA repair step is presumably mediated by cellular enzymes.

The specialized DNA recombination reaction that mediates HIV DNA integration has no known cellular analogue and is therefore an attractive target in the search for specific inhibitors of HIV replication. Here, we report the development of a rapid HIV DNA integration assay that is suitable for large-scale screening of potential inhibitors.

MATERIALS AND METHODS

Oligonucleotide DNA substrates

Oligonucleotides were synthesized on an Applied Biosystems Model 380B DNA synthesizer. Biotin was incorporated at the 3′ end of oligonucleotides, where indicated, using a 3′-Amino Modifier C3-CPG column (Glen Research) and Biotin-XX-Ester (Glen Research) as recommended by the manufacturer. Oligonucleotides were purified by gel electrophoresis before use. Sequences: R84, 5′-AATGAAAGACCCCACCTGTA-Biotin; R85, 5′-TACAGGTGGGGTCTTTCATT; R89, 5′-ACTGCTA-GAGATTTTCCACA-Biotin; R90, 5′-TGTGGAAAATCTCTA-GCAGT. The HIV DNA substrate, which corresponds to the U5 end of HIV DNA, was made by labeling the 5′ end of R90 with ^{32}P and annealing with its complement, R89. Similarly, The MoMLV substrate, which corresponds to the U3 end of MoMLV DNA, was made by labeling the 5′ end of R85 with ^{32}P and annealing with its complement, R84. 100 ng of the oligonucleotide strand to be labeled with ^{32}P was phosphorylated by T4 polynucleotide kinase in the presence of 100 μCi of [γ-^{32}P] ATP (specific activity, 3000 Ci/mmol) in a reaction volume of 15 μl. EDTA was then added to a final concentration of 25 mM, and the polynucleotide kinase was inactivated by heating to 85°C for 15 min. NaCl was added to a final concentration of 0.1 M, together with 100 ng of the complementary unlabeled strand, in a total volume of 40 μl. The mixture was heated to 80°C, and the DNA was annealed by slow cooling. Unincorporated nucleotide was removed by passage through a Sephadex G-25 Quick Spin Column (Boehringer); this step is not essential. The oligonucleotide with biotin at its 3′ end

and ^{32}P at its 5′ end, used for testing the binding capacity of the avidin-coupled microtiter wells, was made by labeling R84 with [γ-^{32}P] ATP, as described above.

Preparation of avidin-coupled microtiter wells

Avidin (Pierce) was covalently coupled to CovaLink F8 Immunomodules (Nunc), supplied in the 96 well plate format. The coupling procedure was as follows. The bifunctional crosslinker Bis (sulfosuccinimidyl) suberate (Pierce) was dissolved in DMSO at a concentration of 10 mg/ml. This stock solution was then diluted to a working concentration of 125 μg/ml in 50% (V/V) DMSO, 50 mM carbonate buffer pH 9.6 (15 mM Na_2CO_3, 35 mM $NaHCO_3$, freshly made). 100 μl of this solution was added to each well. After incubation for 30 min at room temperature the solution was removed by aspiration and the wells were quickly washed twice with water. 100 μl of avidin solution (0.1 mg/ml in 50 mM carbonate buffer pH 9.6) was added and the wells were incubated overnight at room temperature. The unbound avidin was then removed by aspiration and the wells were washed twice with water. 200 μl of 0.15 M NaCl, 1% BSA, 0.05% NaN_3, in 10 mM sodium phosphate buffer pH 7.5, was added to the wells as a blocking agent. After incubation for 30 min at room temperature the wells were washed three times with 0.35 M NaCl, 0.05% Triton X-100, 0.05% NaN_3, in 10 mM sodium phosphate buffer pH 7.5. The plates are stable for at least four months when stored at 4°C.

HIV and MoMLV DNA integration reactions

HIV IN protein and MoMLV IN protein were expressed in Sf9 cells infected with a recombinant baculovirus that expresses HIV or MoMLV IN protein, and partially purified as previously described (7, 8, 9). HIV IN protein was also expressed in *E.coli* and purified by chromatography on butyl sepharose (6), followed by a differential precipitation step at low salt (A.Engelman and R.Craigie, unpublished). Purification of HIV IN protein by chromatography on butyl sepharose followed by heparin sepharose (6) yields a protein preparation that is equally active in the reactions described here. The specific activities of HIV IN protein expressed with the baculovirus and *E. coli* expression systems were indistinguishable. HIV and MoMLV DNA integration reactions were carried out essentially as previously described (7, 9). HIV DNA integration reactions (15 μl) contained 25 mM KCl, 50 mM potassium glutamate, 20 mM MOPS pH 7.2, 10 mM $MnCl_2$, 10% (w/v) glycerol, 10 mM 2-mercaptoethanol, 0.1 mg/ml BSA, 0.5 pmol HIV DNA substrate and approximately 10 pmol HIV IN protein. MoMLV DNA integration reactions (15 μl) contained 85 mM KCl, 25 mM potassium glutamate, 20 mM MOPS pH 7.2, 10 mM $MnCl_2$, 5% (w/v) glycerol, 10 mM DTT, 0.1 mg/ml BSA, 0.5 pmol MoMLV DNA substrate and approximately 1 pmol MoMLV IN protein. Reactions were carried out at 37°C for 1 hr. The effect of varying these reaction conditions on the efficiency of integration is shown in Figures 4 and 5. Potassium glutamate was included in reactions with HIV and MoMLV IN proteins partially purified from Sf9 cells only because it was carried over from the protein storage buffer. HIV IN protein purified from the *E. coli* source was stored in a buffer containing 1 M NaCl and reactions with this protein contained 50 mM NaCl instead of the mixture of potassium glutamate and KCl. When microtiter plates were used as reaction vessels, reaction volumes were scaled up to 45 μl so as to completely wet the bottom of the wells. Reaction efficiencies were indistinguishable regardless of whether reactions were carried out in conventional polystyrene microtiter wells, polyvinyl chloride microtiter wells, or polypropylene microcentrifuge tubes. Comparable amounts of IN protein to DNA substrate are required for an efficient reaction. We suspect that, like the mechanistically related Mu transposase (10), IN protein functions to complete only a single cycle of integration. This would be consistent with the biological role of the protein to integrate the single DNA copy of the viral genome synthesized per infecting virion.

Separation of strand transfer products in avidin-coupled microtiter wells

After the integration reactions, mixtures were adjusted to 20 mM Tris pH 8, 0.4 M NaCl, 10 mM EDTA, 0.1 mg/ml sonicated calf thymus DNA in a final volume of 100 μl. The samples were added to avidin-coupled microtiter wells which were then gently agitated at room temperature for 1hr. Unbound DNA was removed by 3 washes (200 μl, 5 min each) with 30 mM NaOH, 0.2 M NaCl, 1 mM EDTA, followed by a quick wash with 10 M Tris pH 8, 1 mM EDTA. The length of the alkali wash can be decreased without adversely affecting the removal of unbound DNA; increasing the total time of this wash step to 1 hr decreased the recovery of bound DNA by about 10% (data not shown). Bound DNA was then liberated by addition of 100 ml formamide and gentle agitation at room temperature for 15 min.

The relative efficiencies of integration were assessed by directly determining the quantity of released ^{32}P with a liquid scintillation counter, or by autoradiography after transfer of the ^{32}P-labeled strand transfer products to DEAE membrane (Schleicher & Schuell). Quantitation of the ^{32}P-label transferred to the DEAE membrane, using a Molecular Dynamics

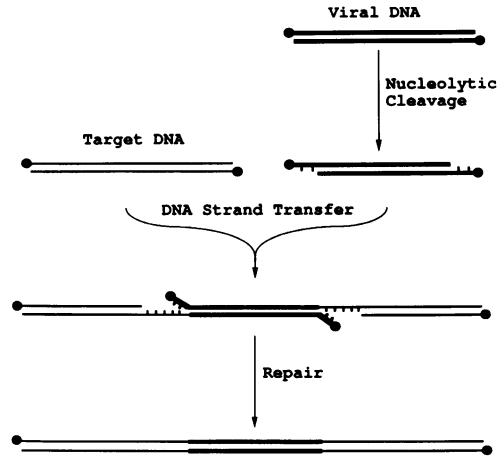

Fig. 1. Mechanism of retroviral DNA integration. A double stranded DNA copy of the viral genome is synthesized by reverse transcription (shown at top right; filled circles represent the 5′ ends of DNA strands). A nucleolytic cleavage step removes two nucleotides from the 3′ ends of the initially blunt ended viral DNA. A subsequent DNA strand transfer step joins the resulting recessed 3′ ends of the viral DNA to the 5′ ends of a staggered break made in the target DNA; the 3′ ends of the break in the target DNA remain unjoined at this stage. The unpaired bases at the 5′ ends of the viral DNA are then removed and gap repair completes the integration process. The viral IN protein carries out the nucleolytic cleavage and DNA strand transfer reactions; cellular enzymes are likely to be responsible for trimming the unpaired bases from the 5′ ends of the viral DNA and the gap repair step. Thick lines represent viral DNA and thinner lines the target DNA. Unpaired bases are shown as bars perpendicular to the DNA strands.

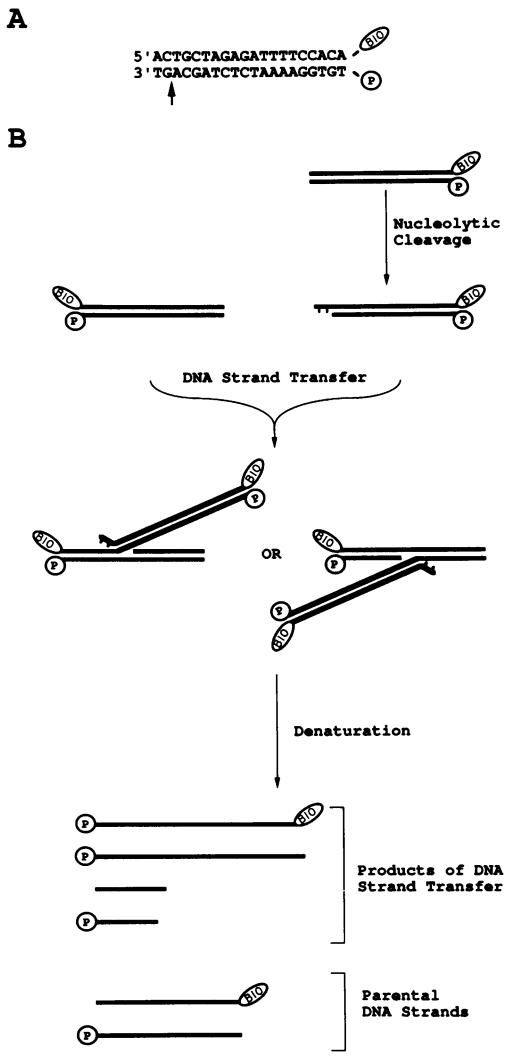

Fig. 2. HIV nucleolytic cleavage and DNA strand transfer reactions carried out with oligonucleotide DNA substrates and HIV IN protein. (A) The DNA substrate corresponds to the terminal 20 base pairs of the U5 end of HIV DNA, shown so that the U5 terminus is on the left. The top (minus) strand is labeled with biotin (BIO) at its 3' end and the bottom (plus) strand is labeled with ^{32}P (P) at its 5' end. In the presence of HIV IN protein, these duplex oligonucleotides serve as both the viral DNA substrate for the nucleolytic cleavage reaction and as the target DNA for the subsequent DNA strand transfer reaction. The arrow indicates the position of nucleolytic cleavage by HIV IN protein. (B) The cleavage reaction removes two bases from the 3' end of the bottom strand of the oligonucleotide substrate. In the subsequent DNA strand transfer reaction the resulting recessed 3' end is joined to the 5' end of a cut made in a strand of another duplex oligonucleotide that serves as the target DNA. Because there is no specific sequence requirement for DNA to act as a target, this reaction joins these recessed 3' DNA ends to many different nucleotide positions on both the top and bottom strands of the target DNA, generating ^{32}P-labeled DNA strands that are heterogeneous in length and longer than the original DNA substrate; shorter DNA strands of heterogeneous length are also produced because the 3' ends of the cut made in the target DNA remain unjoined. The longer DNA strand in the strand transfer product that results from utilization of the top strand as the target is labeled with biotin at its 3' end and ^{32}P at its 5' end. This DNA strand can be easily separated from other labeled DNA strands using avidin-coupled microtiter wells and quantitated by counting the ^{32}P label. The oligonucleotide integration assay accurately reproduces two critical steps of the HIV DNA integration reaction, specific cleavage of the viral DNA ends and the DNA strand transfer reaction. Characterization of the strand transfer products reveals that they are exactly as expected for the authentic HIV DNA integration reaction carried out by an oligonucleotide corresponding to a viral DNA end (7). A pair of such DNA strand transfers inserts a pair of viral DNA ends into a target DNA in the retroviral DNA integration reaction.

PhosphorImager, yielded relative integration efficiencies in agreement with those determined by scintillation counting before transfer. Strand transfer products, in formamide, were transferred to DEAE membrane using a Biorad BioDot apparatus.

RESULTS AND DISCUSSION

A rapid *In Vitro* assay for HIV DNA integration

The assay system we present here is based on our previous demonstration that short oligonucleotides, matching the ends of HIV DNA, are efficient substrates for cleavage and subsequent DNA strand transfer in the presence of HIV IN protein (7). In this reaction, duplex oligonucleotides matching the U5 end of HIV DNA function as the viral DNA ends. Other molecules of this same DNA serve as the integration target. By incorporating biotin into the substrate DNA we have devised a rapid method for the separation and detection of DNA strand transfer products (Figure 2).

The reaction is depicted schematically in Figure 2B. The DNA strand that is cut by IN protein is shown labeled at its 5' end with ^{32}P. The cleavage reaction results in the shortening of this strand by two nucleotides to expose the terminal CA-3' end. In the subsequent DNA strand transfer reaction these 3' ends are joined to the 5' end of a cut made in a strand of another oligonucleotide duplex that serves as the target DNA. Upon denaturation (bottom) the strand transfer products include single DNA strands that are longer than the original DNA substrate; shorter DNA strands are also produced because the 3' end of the cut made in the target DNA remains unjoined after the strand transfer reaction. The strand transfer products are heterogeneous in length because many different nucleotide positions in the DNA substrate can be used as a target.

The products of DNA strand transfer can be visualized by autoradiography after separation from unreacted DNA substrate by denaturing gel electrophoresis (7, 9). However, this methodology is impracticable if very large numbers of reactions are to be processed. We have therefore devised an alternative strategy for separation and detection of reaction products. The DNA substrate is biotinylated on the 3' end of the DNA strand that is not cleaved by IN protein (Figure 2). The products of DNA strand transfer, but not unreacted substrate, therefore include single DNA strands with ^{32}P at their 5' end and biotin at their 3' end. These DNA strand transfer products are easily separated from other labeled DNA strands as follows. After reaction with

Table 1. Efficiency of binding and recovery of biotinylated oligonucleotides in avidin-coupled microtiter wells

Type of well	Applied oligonucleotide (ng/well)	Recovery (%)
Control (no avidin)	5	<0.0001
Avidin-coupled	5	72.6 ± 4.2
Avidin-coupled	10	69.9 ± 2.7
Avidin-coupled	25	57.4 ± 2.6
Avidin-coupled	50	32.7 ± 1.9

The 3'-biotinylated oligonucleotide R84 was labeled at its 5' end with ^{32}P and bound to avidin-coupled microtiter wells in the presence of various excess amounts of the same unlabeled oligonucleotide. The wells were washed with alkali and the bound oligonucleotide was eluted with formamide. Recovery is expressed as recovered percentage of input ^{32}P label; the values are the mean and standard deviation of ten repetitions. A time course of binding with 5 ng/well of oligonucleotide revealed that binding was complete after the 1 hr period allowed for binding (data not shown).

HIV IN protein, reaction mixtures are incubated in microtiter wells to which avidin has been covalently coupled. The bound DNA is then washed with an alkali solution to remove DNA strands that are retained by virtue of their hydrogen bonding to other strands that are bound through the avidin-biotin interaction; this alkali wash does not disrupt the avidin-biotin complex. The only labeled DNA strands remaining after this treatment are products of DNA strand transfer. The avidin-biotin coupling can then be dissociated by addition of formamide and integration is quantitated by either counting the ^{32}P label directly, or by autoradiography after transfer to DEAE membrane.

To assess the reproducibility of this detection method and the effective binding capacity of the microtiter wells for biotin, a test oligonucleotide mimicking the products of strand transfer was prepared by labeling a single stranded oligonucleotide at its 5′ end with ^{32}P and at its 3′ end with biotin. The test DNA was bound to the microtiter wells in the presence of various excess amounts of the same biotinylated oligonucleotide lacking the ^{32}P label, washed with alkali, and eluted with formamide exactly as for the authentic strand transfer products. The results (Table 1) demonstrate the high and reproducible recovery of the test oligonucleotide and the exceedingly low background due to an undetectable level of nonspecific binding in the absence of the avidin-biotin interaction.

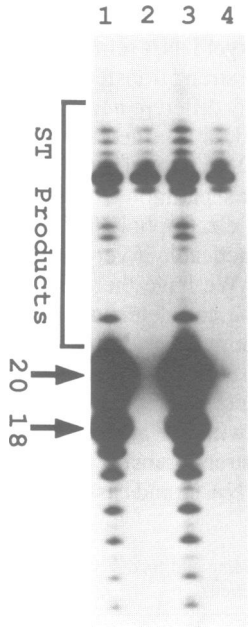

Fig. 3. Separation of HIV DNA strand transfer products in avidin-coupled microtiter wells. A cleavage and DNA strand transfer reaction was carried out with the HIV oligonucleotide DNA substrate and HIV IN protein. A portion of total DNA after reaction was added to 100 μl formamide, concentrated by ethanol precipitation in the presence of tRNA carrier, and electrophoresed in a denaturing polyacrylamide gel (lane 1). An equal portion of the DNA was bound to avidin-coupled microtiter wells and washed with alkali. The DNA that remained bound after this treatment was eluted with 100 μl formamide, concentrated by ethanol precipitation, and electrophoresed (lane 2). Ethanol precipitation of the DNA eluted from the microtiter well was necessary to concentrate it before loading the gel; the unfractionated DNA was treated in the same way as a control for any loss of DNA during this step. Lanes 3 and 4 show a duplicate of the experiment described for lanes 1 and 2. The migration positions of the labeled strand of the DNA substrate (20 mer), the nucleolytic cleavage product (18 mer), and the longer strand transfer products (ST Products) are indicated.

Figure 3 demonstrates the efficient separation of strand transfer products made by incubating the oligonucleotide HIV DNA substrate with HIV IN protein. Total DNA after the reaction (lane 1) and the DNA recovered from the avidin-coupled microtiter wells (lane 2) were electrophoresed in a denaturing polyacrylamide gel. Comparison of lane 1 and lane 2 confirms that strand transfer products, but not other labeled DNA species, are efficiently recovered. As expected, some species of strand transfer product are not recovered because the non-biotinylated strand of the substrate DNA may also be utilized as a target for strand transfer (see Figure 2).

Figure 4 exemplifies the rapid HIV DNA integration assay described above. Reactions were carried out under a variety of conditions as noted in the legend. ^{32}P-labeled strand transfer products were separated from unreacted labeled DNA strands by binding to avidin-coupled microtiter wells and washing with alkali. The eluted products were quantitated directly with a liquid scintillation counter or by autoradiography after binding to a DEAE membrane (Figure 4A). A portion of each reaction was also analyzed by electrophoresis without separation of substrate and products; the strand transfer products correspond to the heterogeneous sized fragments longer than the starting DNA substrate (Figure 4B). Comparison of Figures 4A and 4B confirms that the amount of ^{32}P label remaining after separating the strand transfer products in avidin-coupled microtiter wells accurately reflects the quantity of strand transfer product generated during the reactions.

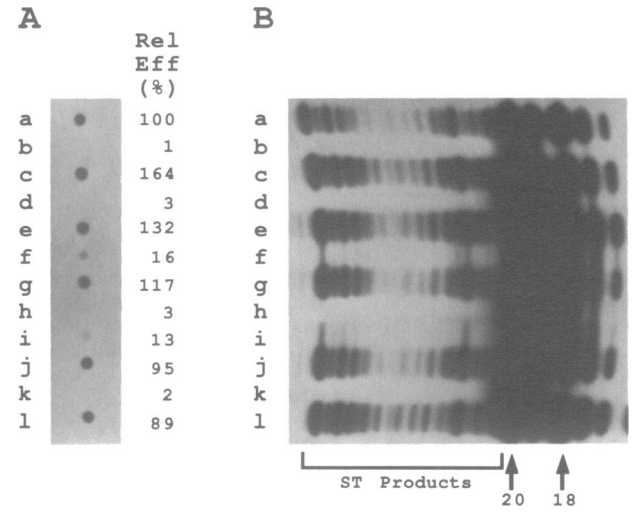

Fig. 4. HIV DNA integration assay. HIV integration assays were carried out under a variety of reaction conditions (a through l). (A) Relative efficiencies were determined by autoradiography after binding the recovered strand transfer products to DEAE membrane, or by directly counting the recovered ^{32}P label with a liquid scintillation counter. (B) A portion of each reaction was also electrophoresed in a 20% denaturing polyacrylamide gel; the direction of electrophoresis is left to right. The migration positions of the labeled strand of the DNA substrate (20 mer), the nucleolytic cleavage product (18 mer), and the longer strand transfer products (ST Products) are indicated. Reaction conditions differed from the 'standard' reaction (a) as follows: (b) corresponding protein fraction from Sf9 cells infected with wild-type baculovirus was substituted for HIV IN protein; (c) KCl was omitted from the reaction; (d) the KCl concentration was increased to 100 mM; (e) the pH was decreased to 6.2 (MES); (f) the pH was increased to 8.0 (Tris); (g) the reaction temperature was decreased to 30°C; (h) 10 μg/ml φX174 RF DNA was included in the reaction; (i) 10 μg/ml tRNA was included in the reaction; (j) 2-mercaptoethanol was omitted; (k) 1 mM EDTA was substituted for 10 mM $MnCl_2$; (l) purified HIV IN protein expressed in *E. coli* was substituted for HIV IN protein expressed with the baculovirus system.

Application of the assay to screening for inhibitors of HIV DNA integration

The assay described here is well suited as a primary screen for potential inhibitors of HIV DNA integration. Since each step of the assay can be carried out in the wells of microtiter plates, it is readily amenable to automation. Only a single protein factor, which can be easily purified from *E. coli* cells carrying the HIV IN gene on an expression vector, is required and the DNA substrate can also be made in large quantity; sufficient HIV IN protein for about 5000 assays can be purified from a 5 liter *E. coli* culture induced for expression of the protein (data not shown). The detection system need not be restricted to ^{32}P; fluorescent labels are among the more obvious alternative possibilities.

Although strongly inhibited by high concentrations of salt, the assay is not unduly sensitive to other reaction parameters that may be incidentally changed by addition of solutions containing potential inhibitory compounds (Figure 4, and unpublished data). In particular, DMSO, an alternative solvent for compounds that are not soluble at high concentration in aqueous solution, does not inhibit when present in reactions at concentrations up to 10%. The inhibition that is observed upon addition of an excess of ϕX174 RF DNA to the reaction (Figure 4, lane h), is primarily due to this DNA acting as a competitor for binding of HIV IN protein; RNA probably inhibits by the same mechanism. Much less inhibition is observed if the HIV IN protein is first bound to the oligonucleotide substrate before addition of competitor DNA (A.Engelman and R.Craigie, unpublished data), although some decrease in signal occurs because the ϕX174 RF DNA can also serve as a target for strand transfer.

A clinically useful inhibitor must be highly specific for the viral replication machinery and have a minimal impact on normal cellular processes. As a first step to exclude inhibitors that obviously lack the required specificity, their effect on a control reaction should be assayed in parallel; related cellular enzymes are a suitable control and parallel *in vitro* assays have facilitated the identification of specific inhibitors of HIV reverse transcriptase and protease (1). Unlike HIV protease and reverse transcriptase, which are related to essential cellular enzymes, other proteases and DNA polymerases, there is no known host analogue of the retroviral integration protein. *A priori* this makes integration a particularly attractive target in the search for specific inhibitors of the HIV replication cycle. However, some form of counterscreen is required to eliminate from consideration the many potential inhibitors that also inhibit a wide range of reactions involving DNA-protein interactions. For example, compounds that bind tightly to any DNA would be expected to inhibit HIV DNA integration, but would not be useful due to their lack of specificity. Many reactions involving interactions between protein and DNA could serve this purpose and there are no strong arguments favoring one particular reaction. However, for reasons of economy it would be attractive to screen for inhibitors of HIV DNA integration in parallel with screening for inhibitors of another potential target, for example, reverse transcriptase. A reverse transcriptase assay can even be set up using parallel methodology to the integration assay (unpublished data). Briefly, reverse transcripts are synthesized from a primer that is biotinylated at its 5′ end; labeled nucleotides incorporated into the primer are quantitated after separation from unincorporated nucleotides using avidin-coupled microtiter wells.

In vitro systems for integration of HIV DNA contained within nucleoprotein complexes isolated from HIV-infected cells have been developed (11, 12) and could serve as a possible secondary screen for compounds that pass the primary screen described here. Alternatively, secondary screening could consist of directly assaying for inhibition of HIV replication in cell culture.

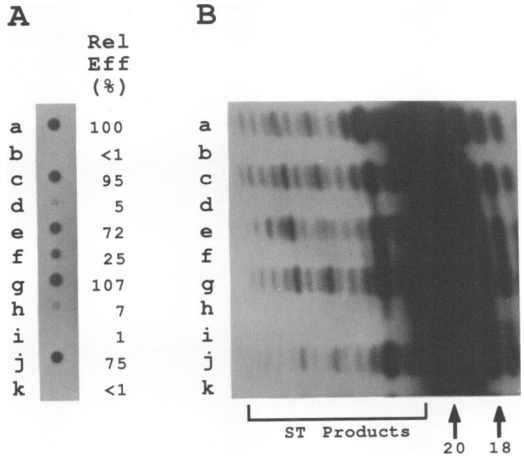

Fig. 5. MoMLV DNA integration assay. Reactions were carried out under a variety of conditions (a through k). Reaction conditions differed from the 'standard' reaction (a) as follows: (b) MoMLV IN protein was omitted; (c) the KCl concentration was decreased to 50 mM; (d) the KCl concentration was increased to 150 mM; (e) the pH was decreased to 6.2 (MES); (f) the pH was increased to 8.0 (Tris); (g) the reaction temperature was decreased to 30°C; (h) 10 μg/ml ϕX174 RF was included in the reaction; (i) 10 μg/ml tRNA was included in the reaction; (j) DTT was omitted from the reaction; (k) 1 mM EDTA was substituted for 10 mM $MnCl_2$. Other labeling is the same as in Fig. 4.

Application to other retroviruses

Assays for the DNA integration reaction of other retroviruses may be useful in searching for antiviral agents against viruses other than HIV. Certain core amino acid sequences are well conserved among retroviral integration proteins (13), and the integration proteins that have been studied biochemically are functionally very similar. In addition to HIV, the integration proteins of Moloney murine leukemia virus (MoMLV) and avian sarcoma-leukosis virus have now been shown to carry out the nucleolytic cleavage (9, 14, 15) and DNA strand transfer reactions (9, 16) *in vitro* with oligonucleotide DNA substrates matching the ends of their respective viral DNAs. By substituting the appropriate integration protein and DNA substrate combination the assay described here for HIV may be adapted for screening potential inhibitors of the DNA integration reaction of other retroviruses. Figure 5 shows an example of the integration assay carried out with MoMLV integration protein and oligonucleotide DNA substrate matching the ends of MoMLV DNA.

CONCLUSION

We have developed a rapid assay for HIV DNA integration that is suitable for screening potential inhibitors. This assay monitors both the specific nuclease activity of HIV IN protein on the ends of the viral DNA and the DNA strand transfer activity that joins the viral DNA to target sequence. The reproducibility and high signal to noise ratio make the assay both highly sensitive and quantitative. Oligonucleotides matching an end of HIV DNA and a single viral protein are the only substrates required. Since each

step can be carried out in the wells of microtiter plates it is well suited to automation for simultaneous processing of large numbers of assays.

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