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Inhibition of Human Immunodeficiency Virus Type 1 Integrase by the Fab Fragment of a Specific Monoclonal Antibody Suggests that Different Multimerization States Are Required for Different Enzymatic Functions

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We have characterized a murine monoclonal antibody (MAb 35), which was raised against human immunodeficiency virus type 1 (HIV-1) integration protein (IN), and the corresponding Fab 35. Although MAb 35 does not inhibit HIV-1 IN, Fab 35 does. MAb 35 (and Fab 35) binds to an epitope in the C-terminal region of HIV-1 IN. Fab 35 inhibits 3′-end processing, strand transfer, and disintegration; however, DNA binding is not affected. The available data suggest that Fab 35 inhibits enzymatic activities of IN by interfering with the ability of IN to form multimers that are enzymatically active. This implies that the C-terminal region of HIV-1 IN participates in interactions that are essential for the multimerization of IN. Titration of the various IN-mediated enzymatic activities suggests that different degrees of multimerization are required for different activities of HIV-1 IN.

Integration is an obligatory step in the replication of retroviruses (including human immunodeficiency virus type 1 [HIV-1]), in which a linear double-stranded DNA copy of the viral genome is inserted into the chromosomal DNA of the host cell (4, 26, 51). It is generally believed that integration is required for the efficient transcription of viral DNA. Although limited viral gene expression was reported for some HIV-1 integrase (IN)-deficient mutants (44), integration appears to be required for the production of viral particles and for the establishment of a productive HIV-1 infection in human T-cell lines, cultured primary peripheral blood lymphocytes, and macrophages (20, 33, 53).

Integration is mediated by IN, a virus-encoded, virion-associated protein. IN is derived from the C terminus of the Gag-Pol polyprotein by proteolytic cleavage. Initially, it was believed that the only function of IN was to mediate the integration of viral DNA; however, the structure of viral particles produced by some IN-defective HIV-1 mutants is aberrant, which suggests that IN could play a role in virion assembly and maturation (20, 41).

The enzymatic activities of retroviral IN have been extensively studied in vitro. Reactions of this type can be performed either with preintegration complexes obtained from infected cells or by with purified recombinant IN and synthetic oligonucleotides that mimic the ends of viral DNA, which are the natural substrates for IN (5, 8, 10, 13, 16, 19, 22, 24, 25, 30–32, 40, 49). IN is an endonuclease that removes two nucleotides from the 3′ ends of linear viral DNA (4, 26, 51). This reaction creates recessed 3′ ends, which subsequently participate in a concerted strand transfer reaction that joins the 3′ termini of viral DNA to the 5′ ends of chromosomal DNA (4, 26, 51). In vivo, both ends of viral DNA are joined to the chromosomal DNA of the host cell. The distance between the sites where viral ends are inserted into the host genome is characteristic of the strain of retrovirus and varies between 4 and 7 bases (4, 26, 51). The IN-mediated joining reaction leaves small gaps at the host DNA-viral DNA junction that are repaired, presumably by host cell enzymes. In vitro, IN can catalyze the reverse reaction, disintegration, in which the product of the integration reaction is cleaved to produce the initial substrates of the strand transfer reaction (11). Integration is both specific (involving U3 and U5 terminal sequences of the viral long terminal repeats) and nonspecific (binding to a target DNA). All of the enzymatic activities require the presence of a divalent metal ion.

There appear to be three relatively independent domains in HIV-1 IN (9, 18). The N-terminal domain contains a zinc finger-like motif, HHCC (H-$X_{3–7}$-H-$X_{23–32}$-C-X_2-C) (7, 9, 29), which binds a metal ion and may be involved in the specific recognition of viral DNA ends. Mutations that alter the conservative Cys and His residues in this domain eliminate 3′ processing and strand transfer activities and reduce but do not eliminate disintegration activity. The central domain, which is relatively resistant to proteases, contains the highly conserved D,D_{35}-E region (D-$X_{39–58}$-D-X_{35}-E) (18, 19, 48), which forms the active site for all of the enzymatic activities of the protein. Relatively little is known about the functions of a C-terminal domain, which is less well conserved, varying in both length and sequence. However, this domain is important, since the deletion of the C terminus impairs IN functions (14, 18, 19). This segment appears to play a role in binding DNA (21, 35, 45, 54).

Crystal structures of the catalytic cores of HIV-1 (15) and Rous sarcoma virus (6) INs and a nuclear magnetic resonance spectroscopy-based structure of the C-terminal domain (34) have recently been reported. However, the structure of the complete molecule is not yet known. We have probed the

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functions of wild-type IN protein with monoclonal antibodies (MAbs) and Fab fragments derived from these MAbs. Here, we report the characterization and molecular cloning of a monoclonal antibody Fab fragment which binds to an epitope on the C terminus of HIV-1 IN protein in close proximity to the putative DNA-binding domain. Although the intact MAb does not affect the enzymatic activities of IN, the Fab fragment does inhibit HIV-1 IN, apparently by interfering with the ability of the protein to multimerize. Titration experiments suggest that different degrees of multimerization could be required for different enzymatic functions of HIV-1 IN.

MATERIALS AND METHODS

Bacterial strains. In this study, we used *Escherichia coli* BL21(DE3)pLysS (Novagen) [F^- *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm*(DE3) pLysS(Cm^r)], XL-1Blue (Stratagene) (*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac*[F′ *proAB lacI*^{q}*Z*ΔM15 Tn*10*(Tet^r)]^c), and DH5α (GIBCO BRL) [*supE44 lacU169* (ϕ80*lacZ*ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*].

Construction of plasmids. Plasmids were constructed by standard methods (39).

(i) IN expression plasmid. *E. coli* DH5α cells were used to construct and maintain the IN expression plasmid. The HIV-1 IN coding sequence was PCR amplified from a plasmid that contained the BH10 provirus (55) with the forward primer spanning the first IN codon (AAAACATATGTTTTAGATGGAATA GATAAGGCCCA) and the reverse primer spanning the termination codon of the *pol* gene (AAAAGGATCCCTTTTCCATGTTCTAATCCTCATCC). *Nde*I and *Bam*HI recognition sites were introduced into the 5′ ends of the forward and reverse primer, respectively. The PCR fragment was cloned as a *Nde*I-*Bam*HI insert into the vector pET15b (Novagen), creating plasmid pIN10, so that the N-terminal coding sequence of IN was fused to a 6×His sequence encoded by the vector.

(ii)Fab 35 expression plasmid. Sequences encoding the Fd fragment of the heavy and light chains of MAb 35 were cloned from hybridoma 35 cells by phage display (12, 52). Poly(A)$^+$ RNA was isolated from the hybridoma cells with the Micro-FastTrack mRNA isolation kit (Invitrogen). cDNAs corresponding to the Fd fragment of the heavy chain and the entire light (κ) chain were synthesized and amplified as follows. A 1-μg portion of the poly(A)$^+$ RNA was annealed with 1 μg of the oligo(dT)$_{18}$ primer, and the first DNA strand was synthesized with avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) at 42°C for 1 h. cDNAs for the Fd and κ fragments were then amplified with *Taq* polymerase (Perkin-Elmer Cetus) in two separate PCRs with the sets of oligonucleotide primers described previously (37). The forward primers correspond to sequences encoding the N-terminal segments of the heavy- and light-chain variable regions. The reverse primers for the Fd fragment were complementary to the CH1 and hinge junction regions of the γ1, γ2A, and γ2B H-chain isotypes, and the reverse primers for the κ fragment were complementary to the C terminus of the L(κ)-chain constant region. PCR products were gel purified and extended by jumping PCR (37) followed by PCR assembly of a Fab expression cassette encoding both Fd and κ chains. The fragment was cleaved with restriction endonucleases *Sfi*I and *Not*I and ligated to the phage display vector pFAB5c (37) (kindly provided by J. Engberg). Electroporation-competent *E. coli* XL1-Blue cells (Stratagene) were electroporated with the ligated DNA and plated on 2×YT agar (39) containing 2% glucose and ampicillin (100 μg/ml), yielding a library of Fab fragments containing 5×10^6 clones.

To generate phage particles displaying the Fab fragment, clones of the transformed cells were superinfected with a helper phage, VCSM13 (Stratagene), and grown overnight. Phage particle minipreps were prepared from culture supernatants by two rounds of polyethylene glycol precipitation followed by acetic acid precipitation as described previously (43). To select phage clones that displayed the functional Fab fragment, phage minipreps were tested for the ability to bind IN by enzyme-linked immunosorbent assay (ELISA). Wells of 96-well plates (Costar) were coated overnight at 4°C with IN diluted in phosphate-buffered saline (PBS; 20 μg/ml) and then blocked with 3% bovine serum albumin (BSA) in PBS. The wells were washed with PBS that contained 0.1% Tween 20 and filled with phage minipreps that were diluted twofold with PBS–3% BSA. Following incubation at room temperature for 2 h, the wells were washed, incubated with biotinylated sheep anti-M13 antibodies (5′-3′, Inc.), washed, and incubated with streptavidin conjugated with horseradish peroxidase (GIBCO BRL). Finally, the wells were washed, and the immune complexes were detected with *p*-nitrophenylphosphate (pNPP) (Kirkegaard & Perry).

To construct a plasmid expressing a soluble Fab 35 fragment, DNA from the IN-binding phagemid clone was cleaved with restriction endonuclease *Eag*I (this cleavage released a fragment containing a truncated gene III) (37) and the fragment containing the vector and Fab coding sequences was gel purified and recircularized by ligation. The resulting plasmid was designated pFAB35(8)s. In this plasmid, the C-terminal coding region of the L(κ) chain was fused to the 6×His sequence encoded by the vector.

Bacterial expression and purification of the HIV-1 IN and antibody 35 Fab fragment. (i) HIV-1 IN. Plasmid pIN10 was introduced into *E. coli* BL21(DE3)pLysS cells. IN expression was induced in the BL21(DE3)pLysS/pIN10 cells by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG). IN was detected in the lysates of induced cells by immunoblot with IN-specific MAbs.

IN protein carrying the N-terminal 6×His affinity tag was purified from induced BL21(DE3)pLysS/pIN10 cells as described previously (19, 40, 46) with modifications. The cells were resuspended in buffer A (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.5], 5 mM dithiothreitol, 1 mM EDTA, 0.5 mg of lysozyme per ml) and sonicated. The homogenate was subjected to centrifugation at 10,000 × *g* for 30 min, and the pellet was resuspended in buffer B (20 mM HEPES [pH 7.5], 100 mM NaCl, 1 mM β-mercaptoethanol). The suspension was incubated on ice for 15 min and subjected to centrifugation at 10,000 × *g* for 30 min. The pellet was resuspended in buffer C (20 mM HEPES [pH 7.5], 1 M NaCl, 1 mM β-mercaptoethanol, 10 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate [CHAPS]) and stirred at 4°C for 2 h. The sample was subjected to centrifugation at 85,000 × *g* for 1 h. The supernatant was loaded onto a Ni^{2+} nitrilotriacetic acid column (Qiagen) equilibrated with the buffer C. The column was washed with buffer C and then with buffer D (50 mM sodium phosphate [pH 6.0], 300 mM NaCl, 1 mM β-mercaptoethanol, 10 mM CHAPS). IN was eluted with a 0 to 500 mM imidazole gradient in buffer D. Peak fractions were pooled, yielding a protein preparation that was approximately 90% pure.

Pooled fractions from the Ni^{2+} NTA column were loaded onto a Sephacryl S-200 column (Pharmacia) equilibrated with 20 mM HEPES (pH 7.5)–1 M NaCl–1 mM EDTA–10 mM CHAPS. Fractions containing the majority of IN were pooled, diluted fivefold with buffer E (20 mM HEPES [pH 7.5], 1 mM EDTA, 10 mM CHAPS), and loaded onto a heparin-Sepharose column (Pharmacia, NJ) equilibrated with Buffer E containing 180 mM NaCl. The column was washed with equilibration buffer, and the IN was eluted by applying a 180 to 800 mM NaCl gradient in buffer E. Fractions containing IN were pooled, aliquoted, and stored frozen at −70°C. The final concentration of NaCl in the purified IN preparation was 570 mM. The purity of IN eluted from the heparin-Sepharose column approached 100%.

(ii) Soluble Fab 35. Plasmid pFAB35(8)s was introduced into *E. coli* XL-1Blue cells, and the expression of soluble Fab 35 was induced by adding IPTG. The Fab 35 fragment was purified from the periplasmic fraction of the induced XL-1Blue/pFAB35(8)s cells by immobilized-metal affinity chromatography (23, 28, 42) followed by ion-exchange chromatography. The periplasmic extract was obtained by mild osmotic shock (38). Briefly, cells were resuspended in 0.5 M sucrose–20 mM Tris (pH 8.0)–1 mM EDTA. An equal volume of the same buffer diluted fivefold with water was added, and the suspension was stirred at 4°C for 1 h. The suspension was subjected to centrifugation at 10,000 × *g* for 20 min, and the supernatant was dialyzed against loading buffer (0.5 M betaine-glycine, 40 mM sodium phosphate [pH 7.5]). The supernatant was subjected to centrifugation at 85,000 × *g* for 1 h and loaded onto a metal chelate Fast Flow Sepharose column (Pharmacia) that was charged with Zn^{2+} and equilibrated with loading buffer. The column was washed with loading buffer, and the Fab 35 was eluted with a 0 to 500 mM imidazole gradient in loading buffer. Fractions containing Fab 35 were pooled, dialyzed against buffer A (20 mM Tris [pH 8.0]) and loaded onto a fast performance liquid chromatography Mono-Q column (Pharmacia) equilibrated with buffer A. Fab 35 was then eluted with a 0 to 500 mM NaCl gradient in buffer A. Fractions containing Fab 35 were pooled, and the protein was concentrated by ultrafiltration on a MicroSep 10K Microconcentrator (Filtron Technology Corp.). The purity of the recombinant Fab 35 approached 100%, as determined by silver staining on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel. The specific IN-binding activity of the recombinant Fab 35 was assayed by ELISA on wells coated with IN, as well as by the ability of Fab 35 to bind IN on the immunoblots. In both assays, immune complexes formed by IN and Fab 35 were detected by alkaline phosphatase-conjugated goat anti-mouse immunoglobulin G (IgG; Gibco BRL).

IN enzymatic activity assays. All enzymatic assays were performed essentially as described previously (19) with minor modifications. To prepare a substrate for the 3′-end processing reaction, oligonucleotides AE117 and AE150 (19), whose sequences correspond to the terminal sequence from the U5 end of the HIV-1 long terminal repeat, were annealed, filled in with [α-^{32}P]TTP with Sequenase version 2.0 DNA polymerase (U.S. Biochemical Corp.) and separated from unincorporated nucleotides by quick-spin column chromatography on Sephadex G-25. The substrate for the strand transfer reaction was formed by annealing an unlabeled oligonucleotide AE117 with AE119 which was 5′-end labeled with T4 polynucleotide kinase. The substrate for the disintegration assay was prepared by self-annealing the 5′-end-labeled "dumbbell" oligonucleotide (11). Enzymatic assays were performed with 0.45 μg (13.5 pmol) of purified IN (1 μl of the IN preparation) and 1 pmol of the oligonucleotide substrate (specific activity, 1.23×10^6 cpm/pmol) in reaction buffer (20 mM HEPES [pH 7.5], 10 mM $MnCl_2$, 1 mM dithiothreitol, 0.05% Nonidet P-40) in a total volume of 20 μl. Reaction mixtures were incubated at 37°C for 1 h, and the reaction was stopped by adding an equal volume of 2× loading buffer (80% formamide, 0.01% bromphenol blue, 0.01% xylene cyanol). The mixtures were heated at 96°C and run on a 20% polyacrylamide–7 M urea gel in Tris-borate-EDTA (TBE) buffer. IN reaction products were detected by autoradiography of the wet gels at −70°C.

IN DNA-binding activity assay. UV cross-linking was used to determine DNA-binding activity in the presence of Fabs. The reaction volume was 20 μl. IN and the Fab were mixed in microcentrifuge tubes under reaction conditions identical to those used for the enzymatic assays and incubated on ice for 20 min. The oligonucleotide used as the disintegration substrate was added, and the mixtures were incubated on ice for 15 min. Following a 15-min incubation at room temperature, the mixtures were irradiated with 280 nm UV light, using a Transilluminator (Fotodyne). After an 8-min exposure at room temperature, an equal volume of 2× protein sample buffer was added to the mixtures. The reaction mixtures were heated at 100°C for 4 min and fractionated by SDS-PAGE (12% polyacrylamide). Cross-linked DNA-IN complexes were detected by autoradiography of the dried gels at −70°C.

Preparation of the purified MAbs, Fab, and F(ab′)$_2$ fragments. (i) MAb purification. Ascitic fluid was delipidated by centrifugation at 6,000 × *g* for 15 min and diluted fivefold with buffer A (1.5 M glycine, 3 M NaCl [pH 8.9]). The MAbs were bound to a protein A-Sepharose 4 Fast Flow column (Pharmacia), and the column was washed with buffer A. The MAbs were eluted with 100 mM citric acid (pH 3.0) and neutralized with 1 M Tris-Cl (pH 8.0). The buffer was exchanged with 50 mM sodium acetate (pH 5.5), and then the MAb was concentrated with a stirred-cell concentrator with a YM 30,000-molecular-weight-cutoff cellulose acetate membrane (Amicon). Purified MAbs were aliquoted and stored at −80°C.

(ii) Fab fragment preparation. Purified MAbs were diluted to a concentration of 2 mg/ml with 50 mM sodium acetate (pH 5.5). Papain conjugated to agarose beads (Sigma) was added at 0.313 mg/mg of MAb in the presence of 50 mM cysteine and 1 mM EDTA. The reaction mixture was incubated at 37°C for 6 to 8 h in a shaking water bath. The beads containing the bound papain were removed by filtration through a 0.2-μm-pore-size cellulose acetate membrane (Millipore). The reaction mixture was diluted twofold with 1.5 M glycine–3 M NaCl (pH 8.9), and Fab fragments were separated from Fc fragments by binding the Fc fragments to a protein A-Sepharose 4 Fast Flow column. The flowthrough material was collected, and the buffer was exchanged with 50 mM sodium phosphate–100 mM NaCl (pH 6.0) with a Centricon-30 concentrator (Amicon).

(iii) F(ab′)$_2$ preparation. The buffer containing the MAbs was exchanged with 100 mM citric acid (pH 3.0), and the MAbs were diluted to 2 mg/ml. Pepsin conjugated to agarose beads (Sigma) was added at 0.1 mg/mg of MAb. The reaction was allowed to proceed for 1.5 h at 37°C in a shaking water bath and was terminated by removing the beads containing the bound pepsin by filtration through a 0.2-μm cellulose acetate membrane. The buffer was exchanged with 50 mM sodium phosphate–100 mM NaCl (pH 6.0) with a Centricon-30 concentrator.

IN assays in the presence of MAbs and their fragments. All MAbs and their fragments were added to the reaction mixtures as follows, unless specified otherwise. MAbs and F(ab′)$_2$ fragments were added at a MAb/IN molar ratio of 1:1. The Fab fragments were added at a Fab/IN ratio of 2:1. Anti-mouse and anti-rabbit IgGs were added at an IgG/Fab/IN ratio of 1:2:1 (or an IgG/IN ratio of 1:1 for control experiments). The reaction mixtures were prepared without the labeled oligonucleotide substrates and preincubated on ice for 30 min. Labeled substrates were added, and the reactions were then allowed to proceed at 37°C for 1 h. The reactions were stopped by adding equal volumes of 2× loading buffer, and the mixtures were run on a 20% polyacrylamide–7 M urea gel in TBE buffer as described above.

Epitope mapping. The IN epitope recognized by MAb 35 was determined with the NovaTope Epitope-Mapping System (Novagen). A DNA fragment containing the IN coding sequence was obtained by cleavage of pIN10 by restriction endonucleases *Nde*I and *Bam*HI. A mixture of short random DNA fragments (~120 bp long) was generated by digestion of the IN DNA fragment with DNase I. The peptide library was constructed by cloning these fragments into the pTOPE-1b(+) vector as recommended by the manufacturer. Expression of the gene 10-IN peptide fusion proteins was induced by transferring the library onto the nitrocellulose filters impregnated with IPTG. Filter replicas were incubated on NZY agar plates (39) at 37°C, and colonies were lysed and then probed with MAb 35. Positive clones were identified by incubating the filters with alkaline phosphatase-conjugated goat anti-mouse IgG and then with nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate (GIBCO BRL). Plasmid DNA was isolated from positive clones, and the sequence of the IN-derived inserts was determined by dideoxy termination sequencing with Sequenase version 2.0 DNA polymerase. The specificity of MAb 35 for the IN-derived peptides was confirmed by immunoblot analysis of the fusion proteins synthesized by the positive clones.

RESULTS

Fab fragments inhibit HIV-1 IN, the corresponding MAbs do not. We used oligonucleotide-based enzymatic assays to test a panel of five MAbs (clones 3, 4, 17, 35, and 44) specific for HIV-1 IN for the ability to inhibit IN. These antibodies were obtained from hybridomas generated following immunization of mice with a fragment of a recombinant HIV-1 IN protein (27). The antibodies recognized the C terminus of HIV-1 IN (data not shown). All antibodies were isolated from ascitic fluid by purification on a protein A-Separose column. In preliminary experiments, the antibodies were shown to bind the IN protein under the conditions of an enzymatic assay (in a buffer of low ionic strength) (data not shown). Purified antibodies were added to the strand transfer and disintegration reactions at an antibody/IN molar ratio of 1:1, and none of the MAbs inhibited these enzymatic activities. In fact, some of the MAbs consistently enhanced strand transfer activity when they were added at a molar excess over IN (data not shown). Fab fragments were prepared from the antibodies by papain cleavage. The Fc fragments were removed by chromatography on protein A-Sepharose. The MAbs and their Fab fragments were tested in parallel in strand transfer and disintegration reactions. MAbs were added to IN at a molar ratio of 1:1 (a ratio of antigen-binding sites to IN of 2:1). Fabs were added at twice this ratio (taking into account the difference in the number of an antigen-binding sites on MAbs and Fabs). The mixtures were incubated on ice for 30 min to allow the antibodies to bind IN. Oligonucleotide substrates were then added, and the reactions were allowed to proceed at 37°C. As shown in Fig. 1, neither the strand transfer nor the disintegration was inhibited by any of the MAbs (compare lanes 3, 5, 7, 9, and 11 with lanes 2). In contrast, Fab fragments of the antibodies completely abolished the strand transfer activity (Fig. 1A, compare lanes 4, 6, 8, 10, and 12 with lane 2). At a Fab/IN molar ratio of 2:1, Fabs 3 and 35 completely inhibited the disintegration activity of IN (Fig. 1B, compare lanes 4 and 10 with lane 2) whereas Fabs 4, 17, and 44 partially inhibited disintegration (Fig. 1B, compare lanes 6, 8, and 12 with lane 2).

A titration experiment was performed in which the inhibitory Fab 35 and MAb 35 were added to the reactions at different molar ratios relative to a constant concentration of IN (Fig. 2). Strand transfer activity was completely inhibited by Fab 35 at Fab 35/IN ratios of 2:1 and 1:1 (Fig. 2A, lanes 4 and 6). Partial inhibition was observed at a ratio of 1:2 (lane 8). No inhibition was observed at a ratio of 1:4 (lane 10). In contrast, these concentrations of Fab 35 only partially suppressed the disintegration reaction (compare lanes 4 and 6 [Fig. 2A] with lanes 6 and 8, [Fig. 2B]). A molar excess of Fab 35 (4:1) was required to abolish disintegration (Fig. 2, lane 4).

We then tested the panel of the Fabs in the 3′-end-processing assay, in which IN cleaves a dinucleotide (GpT) from a double-stranded oligonucleotide that mimics the U5 end of viral DNA. Fab fragments were preincubated with the enzyme on ice at a Fab/IN ratio of 2:1, the oligonucleotide substrate was added, and the reaction mixtures were incubated at 37°C. All of the Fabs dramatically inhibited the 3′-end-processing reaction (Fig. 3). None of the parental MAbs inhibited 3′-end processing (data not shown).

Thus, Fab fragments of several MAbs that recognize the C terminus of IN were able to differentially inhibit the enzymatic activities of HIV-1 IN. The inhibitory effects of the Fab fragments on the enzymatic activities of HIV-1 IN are summarized in Table 1.

Fab fragments do not impair the DNA-binding activity of the HIV-1 IN. Binding of viral DNA and host chromosomal DNA are critical steps in the IN-catalyzed reactions. The C-terminal domain of the HIV-1 IN is believed to contain a region that is involved in DNA binding (21, 32, 45, 54). It was possible that binding the Fab fragment to the C terminus of the IN would impair the ability of IN to bind DNA. Fab fragments were preincubated with IN, and a ^{32}P-labeled disintegration substrate was then added to the reaction mixture. The mixtures were incubated to allow DNA binding, the DNA-IN complexes were cross-linked by UV light, and the reaction mixture was

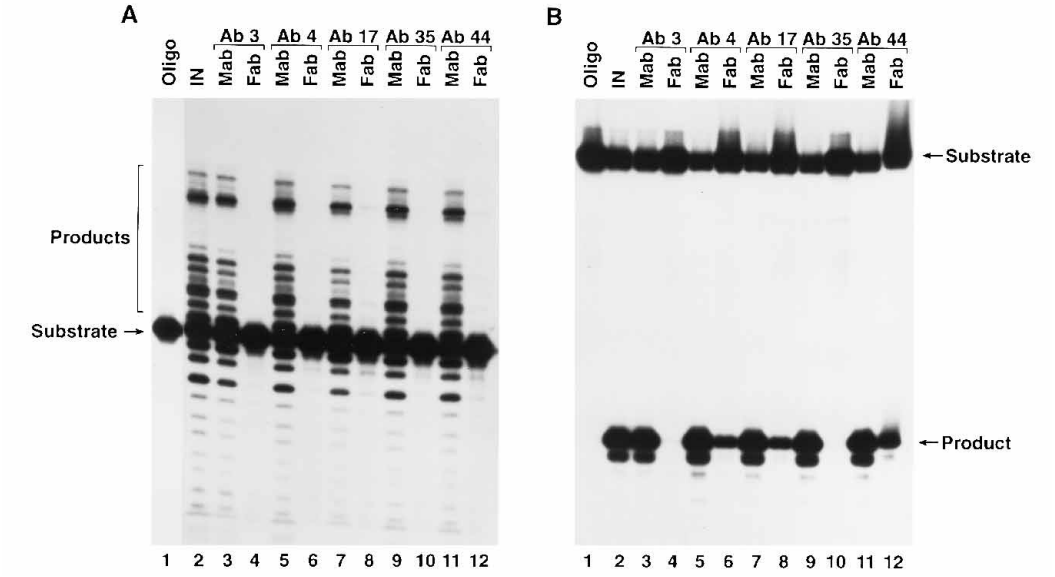

FIG. 1. Inhibition of strand transfer (A) and disintegration (B) reactions by Fabs. MAbs and Fabs were incubated with IN on ice at a molar ratio of an antigen-binding sites to IN of 2:1. Radioactively labeled oligonucleotide substrates were added, and the reaction mixtures were incubated at 37°C. Reaction products were resolved by PAGE (20% polyacrylamide) and detected by autoradiography (see Materials and Methods). In lanes 1, the oligonucleotide substrate was incubated in the reaction buffer without any added protein. In lanes 2, the oligonucleotide substrate was incubated with IN in the absence of MAb or Fab.

fractionated by SDS-PAGE (Fig. 4). A major cross-linked product was detected as a band of about 45 kDa, which corresponded to the expected molecular mass of the IN monomer (33 kDa) cross-linked to the 38-mer oligonucleotide substrate (12.3 kDa). Higher-molecular-mass complexes (~90 kDa) could represent IN dimers complexed to one or two substrate molecules. As can be seen in Fig. 4, none of the Fab fragments inhibited the formation of the predominant DNA-IN cross-linked product (~45 kDa) in this assay (compare lanes 3 to 7 with lane 2). However, Fabs 35 and 44 interfered with the

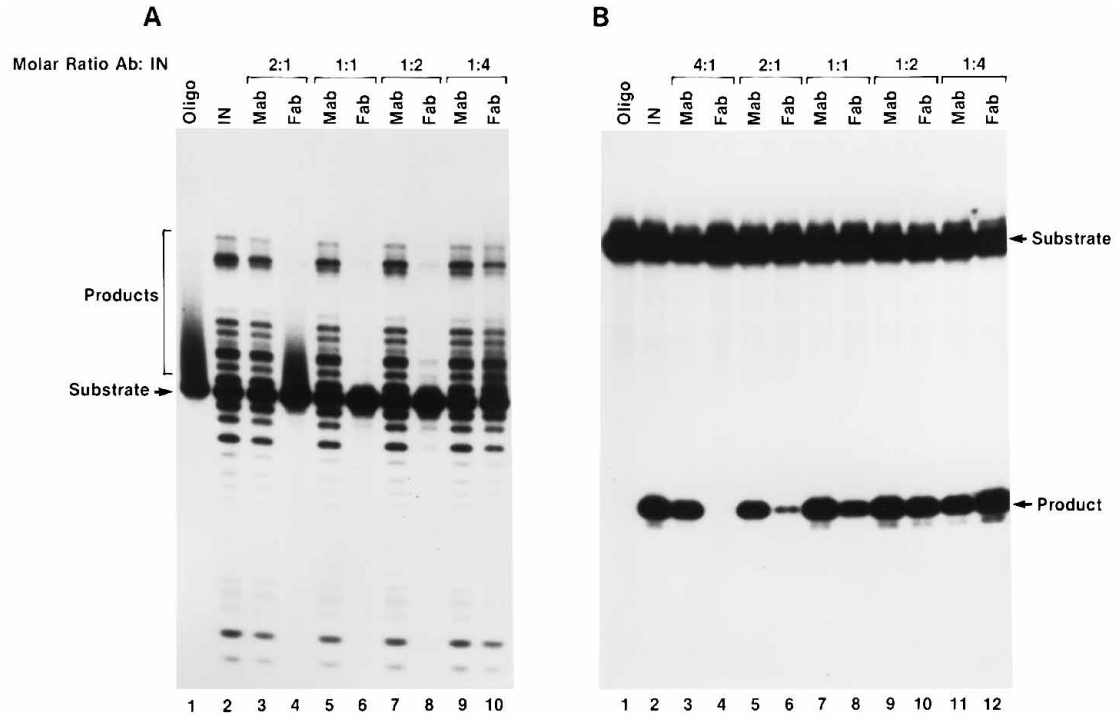

FIG. 2. Titration of MAb 35 and Fab 35 in strand transfer (A) and disintegration (B) reactions. MAb 35 and Fab 35 were incubated with IN on ice at different molar ratios to IN. Radioactively labeled oligonucleotide substrates were added, and the reactions were allowed to proceed at 37°C. Reaction products were analyzed as described in Materials and Methods. In lanes 1, the oligonucleotide substrate was incubated in the reaction buffer without any added protein. In lanes 2, the oligonucleotide substrate was incubated with IN in the absence of MAb or Fab.

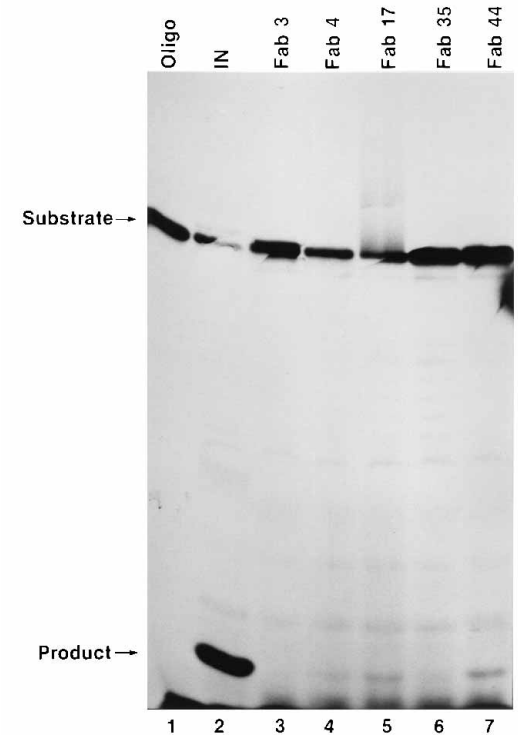

FIG. 3. Inhibition of 3′-end-processing reaction by Fabs. Fabs were incubated with IN on ice at a molar ratio of an antigen-binding sites to IN of 2:1. Radioactively labeled 3′-end-processing substrate was added, and the reaction mixtures were incubated at 37°C. The reaction products were resolved by PAGE (20% polyacrylamide) and detected by autoradiography. In lane 1, the oligonucleotide substrate was incubated in the reaction buffer without any added protein. In lane 2, the oligonucleotide substrate was incubated with IN in the absence of MAb or Fab.

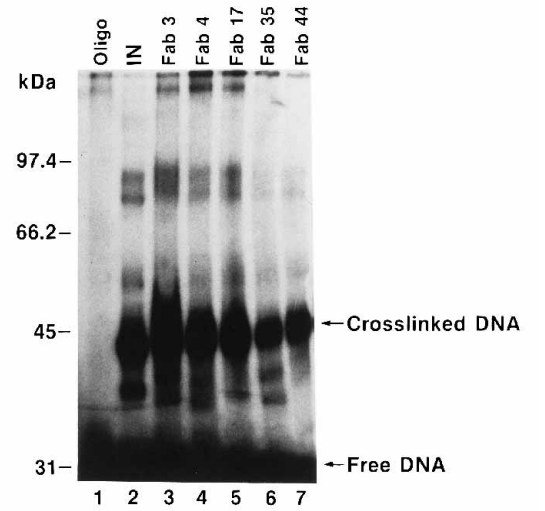

FIG. 4. UV cross-linking assay. Fabs were incubated with IN on ice at a molar ratio of 2:1. Radiolabeled disintegration substrate was added, and the reaction mixtures were irradiated with UV light at 280 nm. Cross-linked DNA-IN complexes were fractionated by SDS-PAGE (12% polyacrylamide) and detected by autoradiography. In lane 1, the substrate was incubated and irradiated without any added protein. In lane 2, the substrate was incubated and irradiated in the presence of IN and in the absence of Fabs.

formation of products with molecular masses greater than 45 kDa.

In the UV cross-linking assay (Fig. 4), Fab fragments were present in twofold molar excess relative to IN. To ensure that the DNA was bound to an IN-Fab complex rather than to free IN, we performed a titration experiment in which Fab 35 was incubated with IN at increasing Fab 35/IN molar ratios, radiolabeled disintegration substrate was added, and the mixture was incubated to allow DNA binding. The reaction mixtures were then irradiated with UV light and the cross-linking products were resolved by SDS-PAGE as described above. Aliquots of the same reaction mixtures were incubated at 37°C to measure the disintegration activity. IN retained the ability to bind DNA even in the presence of a 10-fold molar excess of Fab 35, whereas the disintegration activity was completely inhibited (data not shown). Furthermore, by using anti-mouse IgG, we were able to immunoprecipitate DNA that was cross-linked to IN in the presence of Fab 35, demonstrating that Fab 35 was present in the complexes with IN and DNA (data not shown).

TABLE 1. Inhibitory effects of the Fab fragments on enzymatic activities of HIV-1 IN

Activity	Inhibitory effect[a] of Fab:				
	3	4	17	35	44
3′-End processing	+++	+++	++	+++	++
Strand transfer	+++	+++	+++	+++	+++
Disintegration	+++	+	+	++	+
DNA binding	−	−	−	−	−

[a] +++, complete inhibition of activity; −, no effect. The table reflects the results obtained at a Fab/IN ratio of 2:1.

Epitope mapping of MAb 35. All of the MAbs in our panel apparently recognized a continuous epitope(s) of IN, since they reacted strongly with denatured IN on the immunoblots. To determine the binding site of MAb 35, DNA fragments were prepared from the IN coding sequence by DNase I digestion and an IN-specific peptide library was constructed by fusing these fragments with the 3′ end of a T7 phage gene 10 in a pTOPE-1b(+) plasmid vector. Three clones that expressed gene 10-IN peptide fusion proteins were detected from a library of 2×10^3 clones by probing the library with MAb 35. A consensus sequence, KAKIIRDYGK (IN amino acid residues 264 to 273) was established by aligning the sequences of three peptides. This segment is in the C-terminal portion of IN at the end of the putative DNA-binding domain. The binding specificity of MAb 35 was confirmed by detection of the gene 10-epitope 35 fusion proteins by MAb 35 in an immunoblot. Although the other antibodies in our panel were specific for the C terminus, none of the other antibodies reacted with the epitope recognized by MAb 35 (data not shown).

Inhibition of IN enzymatic activities is mediated by a specific Fab-IN interaction. If the specific binding of the Fab 35 to the cognate epitope on the IN molecule was responsible for the inhibition of the enzyme activities, a peptide that contained the Fab 35 epitope should rescue the activity by competing with IN for binding to the Fab fragment. Oligopeptides SP-805 and SP-718 were synthesized. SP-805 (VVPRRKAKIIRDY GKQMAGD) corresponds to IN amino acid residues 259 to 278 and contains the MAb 35 epitope. SP-718 (IDKAQDE HEKYHSNWRAM) corresponds to IN amino acid residues 5 to 22 and contains an N-terminal immunodominant epitope (47). To determine whether SP-805 could compete with IN for binding Fab 35, increasing amounts of SP-805 were mixed with Fab 35 at different peptide/Fab molar ratios. IN (Fab 35/IN molar ratio, 3:1) was then added, and the mixtures were further

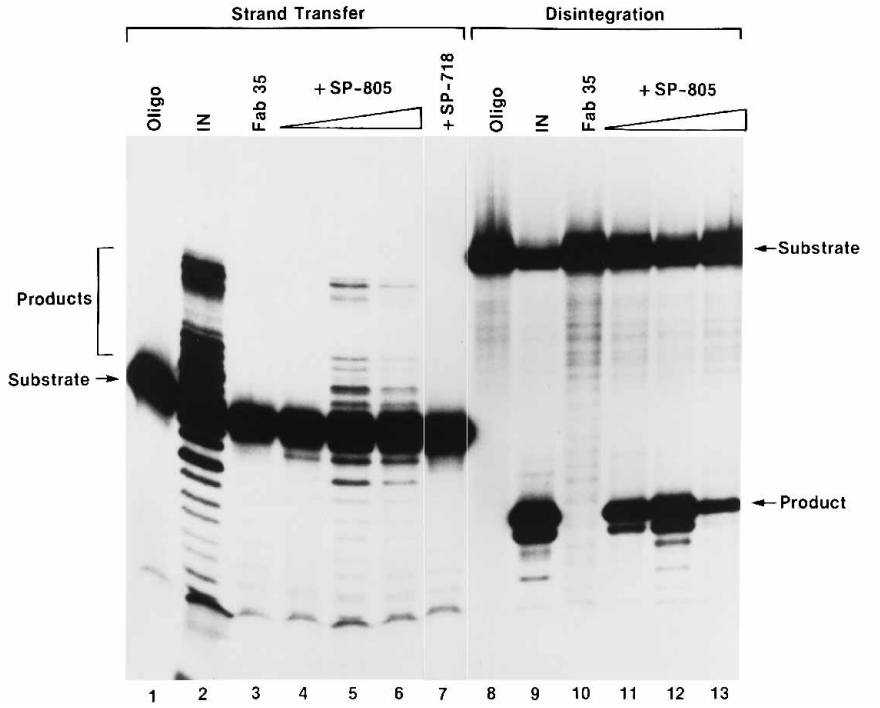

FIG. 5. Rescue of IN enzymatic functions by a specific competitor peptide. Oligopeptide SP-805 (VVPRRKAKIIRDYGKQMAGD) containing the MAb 35 epitope was mixed with Fab 35 at SP-805/Fab 35 molar ratios of 0.5:1 (lanes 4 and 11), 1:1 (lanes 5 and 12), and 2:1 (lanes 6 and 13). In separate reactions, an N-terminal epitope-specific oligopeptide, SP-718 (IDKAQDEHEKYHSNWRAM), was mixed with Fab 35 at an SP-718/Fab 35 molar ratio of 1:1 (lane 7). IN was added at a Fab 35/IN molar ratio of 3:1, and the mixtures were incubated on ice for 30 min to allow IN-Fab interaction. Radiolabeled oligonucleotide substrates were added, and the reactions were allowed to proceed under standard conditions. Reaction products were resolved by PAGE (20% polyacrylamide) and detected by autoradiography as described in Materials and Methods. Lanes: 1 and 8, oligonucleotide substrate alone; 2 and 9, substrate plus IN; 3 and 10, substrate plus IN plus Fab 35.

incubated to allow the enzyme-Fab interaction. Finally, the DNA substrates were added, and the reactions were allowed to proceed under standard conditions. As can be seen in Fig. 5, Fab 35 completely abolished strand transfer and disintegration (lanes 3 and 10). The addition of SP-805 at a peptide/Fab molar ratio of 0.5:1 did not rescue the strand transfer activity (compare lane 4 with lane 2) but did rescue a substantial fraction of the disintegration activity (compare lane 11 with lane 9). Increasing the concentration of SP-805 to a peptide/Fab molar ratio of 1:1 restored a detectable amount of strand transfer activity (compare lane 5 with lane 2) and completely rescued the disintegration reaction (compare lane 12 with lane 9). Interestingly, the increase in SP-805 concentration to a peptide/Fab molar ratio of 2:1 did not further enhance IN activities. In fact, some decrease in the amounts of the reaction products was seen (compare lane 6 with lane 5, and compare lane 13 with lane 12). As expected, addition of the nonspecific control peptide SP-718 as a competitor rescued neither strand transfer (lane 7) nor the disintegration activity (data not shown). The effect of adding peptide SP-805 to a strand transfer assay mixture was repeated with peptide/Fab molar ratios ranging from 0.2:1 to 2:1. No strand transfer activity was detected at molar ratios of 0.2:1 to 0.5:1, and only trace activity was observed at ratios of 0.6:1 to 0.9:1. A sharp increase in the strand transfer activity was observed at a ratio of 1:1 (data not shown). No rescue was observed in the presence of the nonspecific control competitor peptide SP-718 at the same concentration. On the other hand, the Fab 35-specific peptide SP-805 failed to rescue strand transfer activity inhibited by Fabs 3 and 4 at peptide concentrations that were sufficient to rescue Fab 35-inhibited strand transfer reaction (data not shown).

Titration of the specific competitor peptide SP-805 in the 3′-end-processing assay showed that this activity could be rescued by a substantially lower concentration of the competitor peptide than could strand transfer activity (data not shown). As in the case of the strand transfer experiment, no rescue by the nonspecific control peptide SP-718 was observed. The Fab 35-specific peptide SP-805 did not relieve the inhibition of 3′-end processing caused by Fabs 3 and 4.

We tested the effect of the SP-805 and SP-718 peptides in the absence of Fab 35 on the IN-catalyzed strand transfer and disintegration reactions. IN was mixed with the peptides at peptide/IN molar ratios of 6:1, 3:1, and 0.5:1. Neither the strand transfer nor the disintegration reaction was affected by the inclusion of either specific or nonspecific peptides, even in the presence of molar excess of each of the peptides (data not shown), indicating that the rescue of Fab 35-inhibited reactions was a result of specific Fab 35-peptide interaction.

The minimal IN concentration required for strand transfer is different from the amount required for 3′-end processing and disintegration. The peptide titration data suggest that the minimal concentration of enzyme needed for the strand transfer, 3′-end-processing, and disintegration reactions are different. To test this conjecture, we performed 3′-end-processing, strand transfer, and disintegration reactions with serial twofold dilutions of IN in the absence of Fab. At IN concentrations of 1.36 and 0.68 μM, all three activities were observed (Fig. 6, compare lanes 2 and 3 with lanes 1). However, further dilution of the enzyme to 0.34 μM completely abolished the strand transfer activity whereas 3′-end-processing and disintegration activities declined in a concentration-dependent manner but were still detectable at IN concentrations as low as 0.0425 μM (lanes 4 to 7).

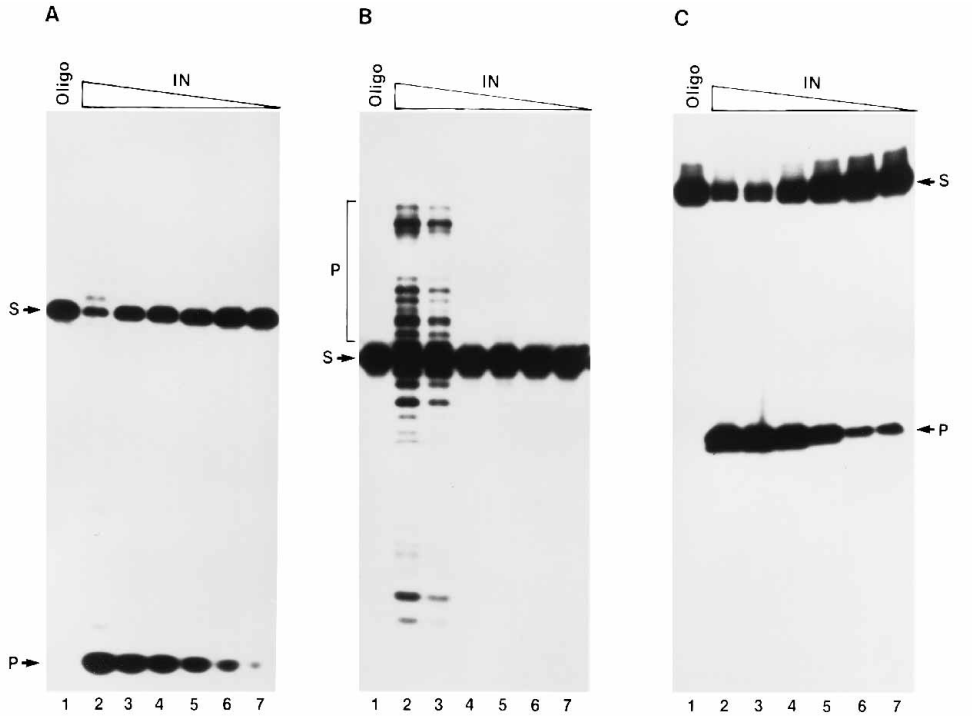

FIG. 6. Concentration dependence of 3′-end processing, strand transfer, and disintegration activities. Serial twofold dilutions of IN were made in 1× reaction buffer. Aliquots of each dilution were incubated with radioactively labeled oligonucleotide substrate under standard conditions. Reaction products were resolved by PAGE (20% polyacrylamide) and detected by autoradiography as described in Materials and Methods. (A) 3′-End-processing reactions. (B) Strand transfer reactions. (C) Disintegration reactions. Lanes: 1, oligonucleotide substrates alone; 2, IN (1.36 μM); 3, IN (0.68 μM); 4, IN (0.34 μM); 5, IN (0.17 μM); 6, IN (0.085 μM); 7, IN (0.0425 μM). Symbols: S, substrate; P, product.

A similar experiment was performed by Engelman and Craigie (19), who obtained slightly different results. They reported that disintegration activity was quite stable even at low enzyme concentrations; however, in their experiments, the joining reaction and 3′-end processing responded relatively similarly to enzyme dilution. There are modest differences in the protocols; however, we cannot account in any simple way for the differences between their results and ours.

The ability to inhibit IN activity depends on the antibody valence. As can be seen from Fig. 1, intact MAbs possessing two antigen-binding sites did not inhibit IN whereas monovalent Fab fragments generated from the same antibodies were strong inhibitors. This suggests that the ability of the antibody-IN interaction to affect IN activities might be dependent on the number of antigen-binding sites on the antibody (or the antibody fragment). To test this hypothesis, a secondary anti-mouse antibody was added to the inhibitory Fab fragment to bring two Fab fragments together, recreating a bivalent complex. A purified polyclonal rabbit anti-mouse IgG was added to Fab 35, the resulting complex was mixed with IN, and then the DNA substrate was added. As shown in Fig. 7, the presence of the anti-mouse IgG completely restored both the strand transfer (Fig. 7A, compare lane 5 with lane 4) and disintegration (Fig. 7C, compare lane 5 with lane 4) activities inhibited by Fab 35. The effect of the anti-mouse IgG was concentration dependent; it decreased with the decrease of the anti-mouse IgG/Fab 35 molar ratio (Fig. 7A and C, lanes 5 to 10). As expected, no rescue of the enzyme activities was observed upon addition of goat anti-rabbit IgG, which is unable to bind mouse Fab fragments (compare Fig. 7B, lane 1, with Fig. 7A, lane 5, and Fig. 8B, lanes 6 and 7). Neither anti-mouse nor anti-rabbit IgG affected the activity of IN (compare Fig. 7B, lanes 2 and 3, with Fig. 7A, lane 2, and compare Fig. 8B, lanes 8 and 9 with lane 2). This suggested that the effect of the anti-mouse IgG was specific and was mediated by binding of the secondary antibody to Fab 35 and the reconstitution of the bivalent complex.

Although $F(ab')_2$ derivatives of the Igs lack the Fc portion of the molecule, the two Fab fragments are connected by a disulfide bridge. If it is the monovalent and bivalent nature of the Fabs and their corresponding MAbs that distinguishes their ability (or inability) to inhibit IN, $F(ab')_2$ 35 should not be able to inhibit IN-catalyzed reactions. $F(ab')_2$ 35 was obtained from MAb 35 by pepsin digestion. It did not inhibit either strand transfer or disintegration, even when added in molar excess over IN (Fig. 8, compare lanes 2, 4, and 5). Thus, HIV-1 IN remains enzymatically active when bound to the bivalent antibody [complete MAb, $F(ab')_2$ fragment, or the secondary IgG-Fab 35 complex) and loses the activity upon binding to the monovalent Fab 35.

Cloning and expression of Fab 35. If it is the purified Fab 35 that is directly responsible for inhibiting IN, a cloned version of Fab 35 should be an equally effective inhibitor. cDNAs encoding the Fd fragment of the heavy chain and the complete light chain of Fab 35 were cloned from hybridoma 35 by the phage display technique. cDNAs for both Fd and light (κ) chains were synthesized and amplified by reverse transcription-PCR with a poly(A)$^+$ RNA isolated from hybridoma 35 cells. A bicistronic operon encoding the Fab fragment was assembled by PCR and cloned into a pFAB5c phage display vector (37). Phage particles were rescued from 50 phagemid clones by coinfection with a helper phage and were tested for their ability to recognize immobilized HIV-1 IN by phage ELISA. Four positive clones were found, and their binding specificity was confirmed by the inability of the phage particles to bind unre-

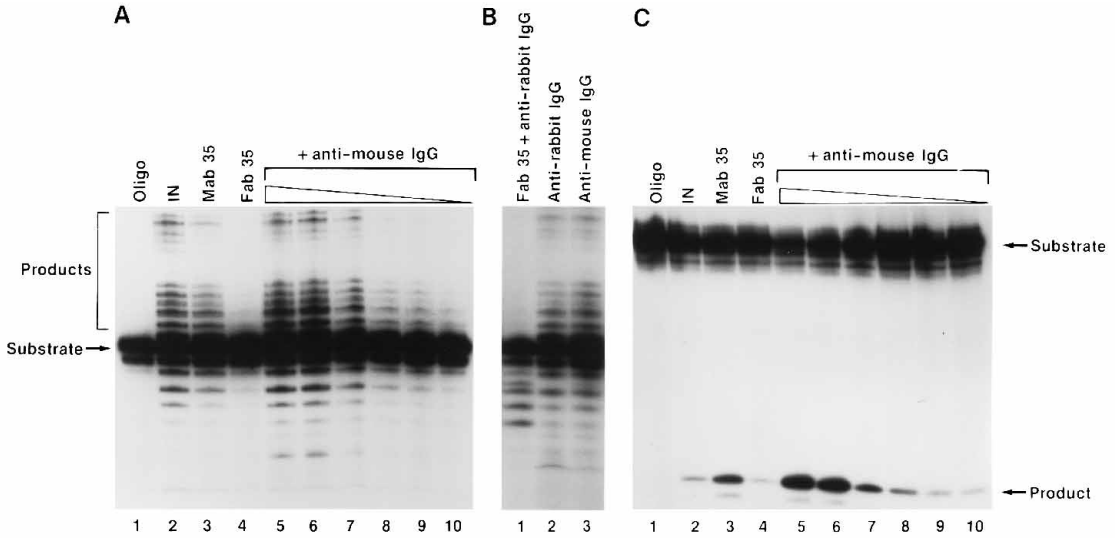

FIG. 7. Rescue of strand transfer and disintegration activities by anti-mouse secondary antibody. (A) Strand transfer reaction. Purified polyclonal anti-mouse IgG was mixed with Fab 35 at anti-mouse IgG/Fab35 molar ratios of 2:1 (lane 5), 1:1 (lane 6), 0.5:1 (lane 7), 0.25:1 (lane 8), 0.125:1 (lane 9), and 0.0625:1 (lane 10). IN was added at a Fab 35/IN molar ratio of 2:1, and the reaction mixtures were incubated on ice for 30 min. Radiolabeled strand transfer substrate was added, and the reaction mixtures were incubated under standard conditions. (B) Effects of a nonspecific anti-rabbit IgG in combination with Fab 35, anti-rabbit IgG alone, and anti-mouse IgG alone on a strand transfer reaction. Purified anti-rabbit IgG was added to Fab 35 at an IgG/Fab35 molar ratio of 1:2 (lane 1). IN was added at a Fab 35/IN molar ratio of 2:1, and the reaction mixtures were incubated on ice for 30 min. In separate reactions, IN was mixed with purified anti-rabbit and anti-mouse IgGs at an IgG/IN molar ratio of 1:1, in the absence of Fab 35 (lanes 2 and 3), and the reaction mixtures were incubated on ice for 30 min. Radiolabeled strand transfer substrate was added, and the reaction mixtures were incubated under standard conditions. (C) Disintegration reaction. Purified polyclonal anti-mouse IgG was mixed with Fab 35 at a anti-mouse IgG/Fab 35 molar ratios of 2:1 (lane 5), 1:1 (lane 6), 0.5:1 (lane 7), 0.25:1 (lane 8), 0.125:1 (lane 9), and 0.0625:1 (lane 10). IN was added at a Fab 35/IN molar ratio of 2:1, and the reaction mixtures were incubated on ice for 30 min. Radiolabeled disintegration substrate was added, and the reaction mixtures were incubated under standard conditions. Reaction products were resolved by PAGE (20% polyacrylamide) and detected by autoradiography. In lanes 1 of panels A and C, the oligonucleotide substrate was incubated in the reaction buffer without any added protein. In lanes 2 of panels A and C, the oligonucleotide substrate was incubated with IN in the absence of MAb or Fab.

lated antigens (BSA, lysozyme, and HIV-1 reverse transcriptase) in ELISA (data not shown). A pFab35(8)s plasmid that expressed a soluble version of Fab 35 with a 6× His tag at the C terminus was prepared. Purified Fab 35 bound HIV-1 IN in ELISA and in immunoblot assays (data not shown). The recombinant Fab 35 was able to inhibit IN strand transfer and disintegration activities as efficiently as was Fab 35 derived from MAb 35 (data not shown).

DISCUSSION

A panel of five MAbs was tested in the 3′-end-processing, strand transfer, and disintegration assays. Although the intact MAbs did not inhibit any IN enzymatic activities, the Fab fragments derived from the MAbs were strong and specific inhibitors of strand transfer. Two of the Fabs were potent inhibitors of the disintegration reaction; the other three Fabs inhibited this reaction to a lesser extent. All of the Fab fragments inhibited 3′-end-processing activity.

What is the mechanism by which these Fab fragments inhibit HIV-1 IN? Binding of Fab fragment to the IN could cause conformational changes in the IN molecule, rendering IN inactive. Since the binding site of Fab 35 is located near the putative DNA-binding region, Fab 35 could inhibit IN functions by interfering with the binding of IN to the DNA substrate. However, the antibodies from which inhibitory Fabs were obtained did not inhibit IN-catalyzed reactions. Furthermore, the ability of HIV-1 IN to bind DNA was not affected by the inhibitory Fabs. This agrees with results reported by Woerner and Marcus-Sekura (54), who found that a MAb recognizing an HIV-1 IN C-terminal epitope did not interfere with the binding of a DNA by HIV-1 IN in a Southwestern (DNA-protein) blot assay. These data suggest that neither a Fab-induced conformational change in the IN structure nor interference with substrate binding is a likely mechanisms of the inhibition.

Since a major difference between the Fab fragments and the parental MAbs is the number of antigen-binding sites, we believe that inhibitory Fab fragments bind regions of HIV-1 IN molecules that are involved in the formation of oligomers, disrupting the multimeric structure essential for enzymatic activity (17). This would also explain the enhancement of activity seen in the presence of the MAbs, which could promote the appropriate oligomerization or stabilize enzymatically active IN oligomers. For Fab 35, two lines of evidence suggest that Fabs inhibit HIV-1 IN by interfering with oligomerization: (i) neither bivalent parental MAb nor the $F(ab')_2$ 35 (a bivalent derivative of the antibody, which lacks Fc fragment) negatively affected IN-catalyzed reactions, and (ii) strand transfer and disintegration reactions that were inhibited by Fab could be rescued by the addition of anti-mouse IgG. Since the anti-mouse antibody binds two Fab fragments, it could bring IN monomers together and restore the activity of IN by promoting oligomerization and/or by stabilizing an oligomeric complex. Thus, it appears possible that the Fab 35 fragment inhibits the enzyme by interfering with oligomerization of IN subunits and that the C-terminal segment of HIV-1 IN molecule participates in protein-protein interactions that are essential for the formation of enzymatically active oligomers. The IN of avian sarcoma virus is related in sequence, function, and structure to HIV-1 IN. Analysis of the determinants of multimerization of avian sarcoma virus IN demonstrated that deleting the C terminus reduced the capacity for multimerization; moreover, an isolated C-terminal fragment of avian sarcoma virus IN was capable of self-association (1).

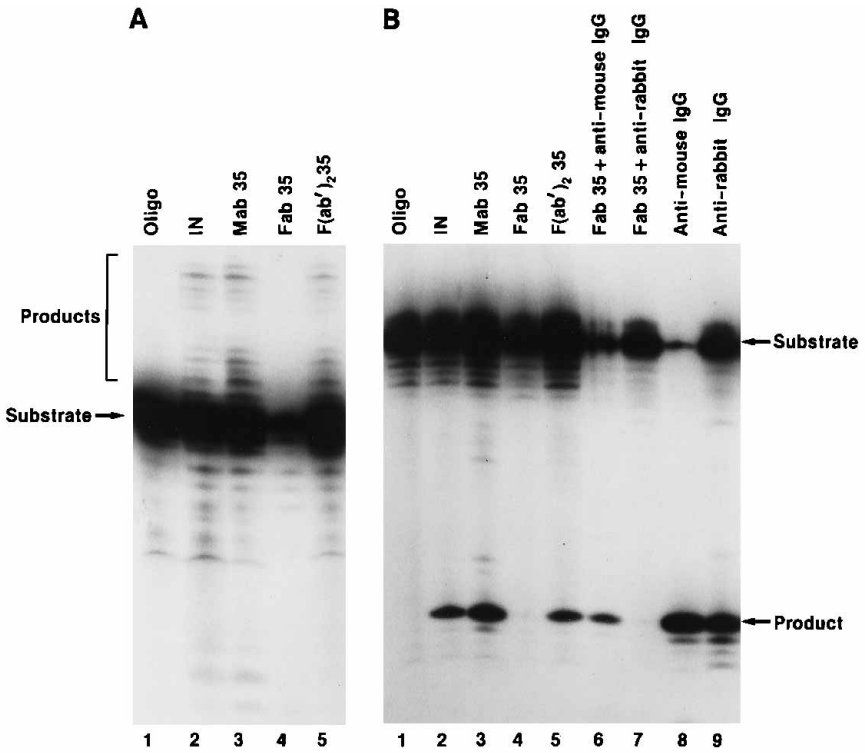

FIG. 8. Effect of $F(ab')_2$ 35 on strand transfer and disintegration activities. (A) Strand transfer reaction. MAb 35, FAb 35, and $F(ab')_2$ 35 were mixed with IN in separate reactions at an antibody/IN molar ratio of 2:1 (lanes 3 to 5), and the reaction mixtures were incubated on ice for 30 min. Radioactively labeled strand transfer substrate was added, and the reaction mixtures were incubated under standard conditions. (B) Disintegration reaction. MAb 35, Fab 35, and $F(ab')_2$ 35 were mixed with IN in separate reactions at an antibody/IN molar ratio of 2:1 (lanes 3 to 5), and the reaction mixtures were incubated on ice for 30 min. In a separate set of reactions, Fab 35 was mixed with anti-mouse and anti-rabbit IgGs at an IgG/Fab 35 molar ratio of 1:2 (lanes 6 and 7). IN was added at a Fab 35/IN molar ratio of 2:1, and the reaction mixtures were incubated on ice for 30 min. In another set of reactions, anti-mouse and anti-rabbit IgGs were mixed with IN at an IgG/IN molar ratio of 1:1 in the absence of Fab 35 (lanes 8 and 9). Radioactively labeled disintegration substrate was added, and the reaction mixtures were incubated under standard conditions. Reaction products were resolved by PAGE (20% polyacrylamide) and detected by autoradiography. In lanes 1, the oligonucleotide substrate was incubated in the reaction buffer without any added protein. In lanes 2, the oligonucleotide substrate was incubated with IN in the absence of MAb or Fab.

The inhibition of the enzymatic activities of HIV-1 IN by Fab 35 is similar to what has been reported for C-terminal deletion mutants (14). Deletions ranging in size from 22 to 32 residues have been reported to inhibit strand transfer activity; the effect of such deletions on 3′-end processing is more controversial; however, it is possible that the residual cleavage activity claimed in some reports is due to contamination by *E. coli* exonuclease (14, 18, 19).

It is clear that the C terminus of HIV-1 IN is important in the viral life cycle. Modifying a molecular clone of HIV-1 to remove 54 amino acids from the C terminus of IN results in the production of a virus that is replication defective; however, this deletion appears to have an effect on virion maturation in addition to whatever effects there are on the enzymatic activity of IN (20).

It is generally believed that the multimerization of IN subunits is an essential step in the formation of an enzymatically active complex with the DNA substrates. However, the number of IN subunits in the complex remains unknown. The following observations suggest that different enzymatic activities of HIV-1 IN could require different degrees of multimerization. (i) Relative to other enzymatic activities of IN, a significantly higher concentration of Fab 35 was required to completely inhibit disintegration than to abolish strand transfer activity. (ii) A much lower concentration of a specific competitor peptide was necessary to rescue disintegration reactions inhibited by Fab 35. A concentration of peptide sufficient to rescue the disintegration reaction was able to effect only a partial rescue of the 3′-end-processing reaction. However, at this concentration, the strand transfer activity was still completely inhibited. (iii) The effects of varying the IN concentration on the forward (strand transfer) and reverse (disintegration) reactions (in the absence of inhibitory Fab) are different. Since a decrease in the concentration of a multimeric protein can promote the dissociation of a multimer, it seems reasonable to suggest that the binding of Fab 35 to the C terminus of HIV-1 IN lowers the concentration of free enzyme below the level required to form the active oligomer. This concentration of enzyme necessary for the strand transfer activity appears to be different from that needed for the disintegration activity, suggesting that these activities might require different degrees of IN multimerization, a suggestion that has been made by others (19).

Retrovirus integration could be an attractive target for the development of antiretrovirus therapy. Integration is a critical step in viral replication; in addition, the integration reaction does not appear to have a counterpart in the growth and differentiation of normal cells. We have characterized and cloned a MAb Fab fragment which is able to inhibit 3′-end-processing and strand transfer activities of HIV-1 IN that are known to be essential steps in the in vivo integration process. Recently, a single-chain antibody derived from a human MAb that recognizes the CD4-binding region of HIV-1 envelope protein has been expressed intracellularly in eukaryotic cells (36). The antibody inhibited processing of the envelope precursor and syncytium formation, and it substantially reduced the infectivity of the HIV-1 particles produced by antibody-

expressing cells. This, together with the recent demonstration of successful expression and targeting of active antibody fragments to specific subcellular compartments (2, 3, 50), raises the possibility that the intracellular expression of Fab fragment that interferes with essential viral functions (for example, integration) will provide a basis for the development of an antiretrovirus gene therapy.

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