JOURNAL OF VIROLOGY, Sept. 1995, p. 5631–5639 Vol. 69, No. 9
0022-538X/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Monoclonal Antibodies against Rous Sarcoma Virus Integrase Protein Exert Differential Effects on Integrase Function In Vitro

BARBARA MÜLLER, DIANE BIZUB-BENDER, MARK D. ANDRAKE, KATHRYN S. JONES,† AND ANNA MARIE SKALKA*

Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

Received 17 January 1995/Accepted 13 June 1995

We have prepared and characterized several monoclonal antibodies (MAbs) against the Rous sarcoma virus integrase protein (IN) with the aim of employing these specific reagents as tools for biochemical and biophysical studies. The interaction of IN with the purified MAbs and their Fab fragment derivatives was demonstrated by Western blot (immunoblot), enzyme-linked immunosorbent assay, and size exclusion chromatography. A series of truncated IN proteins was used to determine regions in the protein important for recognition by the antibodies. The MAbs described here recognize epitopes that lie within the catalytic core region of IN (amino acids 50 to 207) and are likely to be conformational. A detailed functional analysis was carried out by investigating the effects of Fab fragments as well as of intact MAbs on the activities of IN in vitro. These studies revealed differential effects which fall into three categories. (i) One of the antibodies completely neutralized the processing as well as the joining activity and also reduced the DNA binding capacity as determined by a nitrocellulose filter binding assay. On the other hand, this MAb did not abolish the cleavage-ligation reaction on a disintegration substrate and the nonspecific cleavage of DNA by IN. The cleavage pattern generated by the IN-MAb complex on various DNA substrates closely resembled that produced by mutant IN proteins which show a deficiency in multimerization. Preincubation of IN with substrate protected the enzyme from inhibition by this antibody. (ii) Two other antibodies showed a general inhibition of all IN activities tested. (iii) In contrast, a fourth MAb stimulated the in vitro joining activity of IN. Size exclusion chromatography demonstrated that IN-Fab complexes from representatives of the three categories of MAbs exhibit different stoichiometric compositions that suggest possible explanations for their contrasting effects and may provide clues to the relationship between the structure and function of IN.

The virus-encoded enzyme integrase (IN) plays a crucial role in the retroviral life cycle. After reverse transcription is completed, IN catalyzes the integration of the viral DNA into the genome of the host cell. This process involves the removal of (usually) a dinucleotide from each 3′ end of the linear viral DNA and the subsequent joining of the new 3′ hydroxyl ends to phosphate groups in the host DNA (for a recent review, see reference 22). Purified IN protein can carry out both reactions in vitro using model oligodeoxynucleotide substrates whose sequences match the ends of the viral LTR (4, 9, 19, 21, 23, 35).

A number of integrases from different retroviral sources have been purified and partially characterized. Some of these proteins have been expressed in large amounts in bacterial cells, making the enzyme accessible for detailed biochemical and biophysical analysis (19, 35–37). Various approaches have been applied to obtain information about structure-function relationships of retroviral INs. Migration of the protein in glycerol gradients, sedimentation analysis, size exclusion chromatography, and protein cross-linking experiments have revealed that IN is able to form oligomeric complexes in solution (1a, 11, 15, 18, 40). Kinetic studies indicate that an oligomer is the active form of RSV IN (18), a conclusion which is also implied in the case of HIV-1 IN by recent reports that IN proteins mutated in different regions can complement each other functionally (7, 11, 39). However, the relationship between IN multimerization and its various enzymatic activities has not been clearly determined.

Site-directed mutagenesis has been used to show that three invariant acidic amino acid residues, the signature components of the D,D(35)E motif, are crucial for IN function in vitro (12, 26, 38). Mutagenesis experiments have also demonstrated the importance of other highly conserved amino acid residues (10, 20, 25, 26, 38). Characterization of N- and C-terminally truncated forms of the protein led to the identification of a catalytic core domain which includes the D,D(35)E motif and retains the ability to catalyze phosphoryl transfer but is unable to carry out the specific processing and joining activities (5, 6, 17, 27). Deletion analysis has also been used in attempts to define regions in the protein that may be involved in binding DNA (13, 25, 30, 32, 43) and regions important for multimerization (1a, 11, 17, 19a). However, the interpretation of results from such studies is complicated by the possibility that removal of a part of the protein will influence or alter the folding of the remaining polypeptide chain.

As a complementary approach in investigating the structure and function of retroviral integrases, we have produced a series of MAbs for use as specific reagents to analyze the activities of the intact protein. In this report, we describe the preparation and characterization of five MAbs against RSV IN and their abilities to alter the enzymes' activities. We show that although all bind to the catalytic core domain of the enzyme, they display contrasting and differential effects on various IN functions in vitro.

* Corresponding author. Mailing address: Fox Chase Cancer Center, Institute for Cancer Research, 7701 Burholme Ave., Philadelphia, PA 19111. Phone: (215) 728-2490. Fax: (215) 728-2778.

† Present address: Retrovirus Research Center, Veterans Affairs Medical Center, Baltimore, MD 21201.

MATERIALS AND METHODS

Abbreviations. Abbreviations used in this work are as follows: aa, amino acid; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; GST, glutathione *S*-transferase; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; HIV-1, human immunodeficiency virus type 1; HPLC, high-performance liquid chromatography; LTR, long terminal repeat; MAb, monoclonal antibody; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; RSV, Rous sarcoma virus; and SDS, sodium dodecyl sulfate.

Proteins. RSV IN was expressed in *Escherichia coli*, purified as previously described (36), and used to immunize mice. Wild-type IN and mutated versions of the protein used in further experiments were purified by a modified procedure (27). The purified proteins were stored at −70°C in 50 mM HEPES (pH 8.2)–0.5 M NaCl–40% glycerol–1% thiodiglycol. IN concentrations are expressed as monomer units throughout this paper. Vectors used for production of truncated versions of RSV IN are described by Kulkosky et al. (27). Expression and purification of truncated IN proteins fused to GST will be described in detail elsewhere (1a). Briefly, IN sequences encoding the indicated amino acids were amplified by PCR and inserted into the expression vector pGEX2TK (Pharmacia). Fusion proteins, which carry the GST portion at the N terminus, were affinity purified with glutathione-agarose. Purified bacterially expressed HIV-1 IN was kindly provided by J. Kulkosky.

Oligodeoxynucleotide substrates. The oligodeoxynucleotide substrates used in the functional analyses were a blunt-ended duplex representing the last 18 bp of the U3 region of the RSV LTR, U3(18/18) (23), and a model processed duplex that lacks the two 3′-OH-terminal nucleotides from the minus strand, U3(16/18). The Y-intermediate (disintegration) substrate used for the cleavage-ligation reaction included four annealed DNA strands with the following sequences: 5′GACTACAGATAAGGAG3′, 5′CGTGAATCTC3′, 5′AATGTAGTC3′, and 5′GCTCCTTATCGAGATTCAC3′.

Oligodeoxynucleotides were synthesized by standard solid-phase methods and purified by reversed-phase HPLC. The concentrations were estimated from the optical density at 260 nm by using calculated molar extinction coefficients. Equimolar amounts of complementary strands in 25 mM Tris HCl, (pH 7.4)–50 mM NaCl were annealed by heating for 10 min at 80°C, followed by slow cooling to 4°C.

To obtain radioactively labelled substrates, prior to annealing one strand was labelled at the 5′ end with [γ^{32}P]ATP (ICN) and T4 polynucleotide kinase (New England Biolabs) according to standard procedures (31). Free [γ-^{32}P]ATP was removed by chromatography on DE-52 cellulose (Whatman) according to standard procedures. In the reaction mixtures, radiolabelled and unlabelled substrates were mixed at a ratio of approximately 1:1,000 to give a final concentration of 10 μM.

Immunization and antibody production. (C3H/Fb × BALB/c)F_1 mice (Fox Chase Cancer Center Animal Facility) were immunized intraperitoneally with 7 μg of purified, bacterially produced RSV IN protein mixed 1:1 with Freund's complete adjuvant. One month later, the mice were reimmunized with 20 μg of IN mixed 1:1 with Freund's incomplete adjuvant.

The mice were rested for 5 months and then reinjected intravenously with 25 μg of IN. Four days later, spleen cells from two mice were separately fused with SP2/O myeloma cells (33) by using polyethylene glycol 1000 (16). Hybridoma cells were selected for growth in medium containing hypoxanthine-aminopterin-thymidine. Culture supernatants of selected cell lines were tested for the presence of anti-RSV IN MAbs by ELISA (see below) using partially purified viral IN as the capture antigen. Five hybridoma cell lines that were shown to produce high titers of anti-RSV IN antibodies were cloned in soft agar for further analyses; two were from one fusion (labelled sA1 and nA2), and three were from the second fusion (labelled iB1, iB2, and B3). Isotypes were determined with the Isotype Ab-STAT kit from Sang Stat Medical Corp. (Menlo Park, Calif.).

Purification of anti-RSV IN MAbs and Fab fragments. Ascites tumors were prepared from pristane-primed *scid* mice, and the MAbs were purified as described previously (3) with the Affi-Gel Protein A Maps II kit (Bio-Rad, Rockville Centre, N.Y.). Fab fragments were generated from MAbs by cleavage with papain and purified with the Avid Chrom F(ab) kit according to the manufacturer's specifications (UniSyn Technologies, Tustin, Calif.).

ELISA. Purified full-length and truncated RSV IN (50 to 100 ng per well, unfused or fused to GST) were applied to high-binding microtiter plates (Costar, Cambridge, Mass.). The five anti-RSV IN MAbs were tested for reactivity with these proteins by using an alkaline phosphatase-conjugated goat anti-mouse immunoglobulin G (heavy and light chains) antibody and *p*-nitrophenyl phosphate as described previously (3). The titer was defined as the MAb concentration (in nanograms per milliliter) which gives an optical density at 405 nm reading fivefold greater than the background value (no primary antibody) in the color reaction.

Biotinylation of MAbs for the use in competition experiments. Purified anti-RSV IN MAbs were biotinylated by coupling to sulfosuccinimidyl-13-(biotinamido)-hexanoate (Biotin-X-X-NHS; Calbiochem, San Diego, Calif.) as previously described (3). Streptavidin conjugated to alkaline phosphatase was used instead of a secondary antibody to test for reactivity of the biotinylated MAb to RSV IN in an ELISA. The titer was determined as described above. When an alkaline phosphatase-conjugated secondary antibody was used for detection, titers of the biotinylated MAbs were not significantly different from those of the unmodified MAbs.

Immunoblotting. Partially purified proteins were separated by electrophoresis in SDS–12.5% polyacrylamide gels. A gel in which identical samples were loaded was stained with Coomassie brilliant blue and subjected to densitometry to verify that similar amounts of IN protein (ca. 1 μg per lane) were present in the relevant bands. Proteins from the unstained gel were transferred to polyvinylidene fluoride membranes (Millipore, Bedford, Mass.) by using the Millipore semidry blotting system according to the manufacturer's specifications. The membrane was blocked for 30 min at room temperature in PBS (25 mM sodium phosphate, 150 mM NaCl, pH 7.4) containing 0.5% (vol/vol) Tween 20 and 5% (wt/vol) nonfat dry milk. The filters were incubated with optimized dilutions (10 to 100 μg/ml) of purified anti-RSV IN MAbs in PBS containing milk and Tween 20 for 1 h at room temperature. After incubation, the filters were washed twice for 7 min each in PBS plus Tween 20. The anti-RSV IN MAbs were detected by incubation of the membrane at room temperature for 1 h with donkey anti-mouse immunoglobulin G coupled to horseradish peroxidase (Amersham; diluted in PBS according to the manufacturer's specifications). After two washes with PBS plus Tween 20 followed by one wash with PBS, peroxidase activity on the membranes was detected with the enhanced chemiluminescence reagents from Amersham Corp. (Arlington Heights, Ill.). Membranes were exposed to Kodak XAR film.

Functional assays. For use in functional assays, the immunoglobulins were dialyzed against 50 mM Tris HCl (pH 7.4)–50 mM NaCl. Dilutions were made with the same buffer containing 0.5 mg of BSA per ml. RSV IN (35 μM) was mixed with 2 volumes of the appropriate immunoglobulin dilution and incubated on ice for 30 min. As a control, the MAbs were replaced by nuclease-free BSA (Boehringer, Mannheim, Germany) or a purified anti-Ras MAb (2) in the same buffer. The preincubated IN protein was tested for its activity in the indicated in vitro assays.

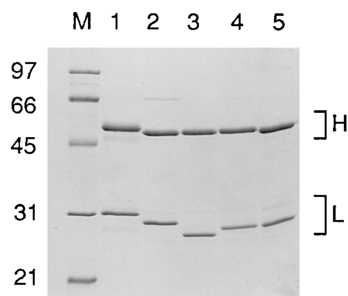
Standard conditions for processing, joining, and cleavage-ligation reactions were as follows: 10 μM of the appropriate ^{32}P-labelled oligodeoxynucleotide substrate and 1.1 μM IN in 50 mM Tris HCl (pH 8.0), 2 mM β-mercaptoethanol, 3 mM $MnCl_2$, 17 mM NaCl, and 1.6% glycerol (NaCl and glycerol from IN storage buffer). Reaction mixtures were incubated at 37°C (processing or joining) or 26°C (cleavage-ligation), and reactions were stopped by the addition of EDTA (final concentration, 25 mM). Products were quantitated either by separation of the reaction mixtures by electrophoresis in denaturing polyacrylamide gels (18% polyacrylamide, 7 M urea) and subsequent analysis using a Fuji BAS1000 phosphoimaging system or by using previously established solution assays (29).

For nitrocellulose filter binding assays, IN was incubated at the indicated concentrations with radioactively labelled substrate U3(16/18). Incubations were performed in a total volume of 20 to 50 μl of filter binding buffer (50 mM Tris HCl [pH 8.0], 25 mM NaCl, 5 mM $MgCl_2$, 0.1 mg of BSA per ml, 2 mM dithiothreitol) for 15 to 30 min on ice. Analysis of binding reactions on sequencing gels confirmed that under these conditions products of the joining reaction were not detectable after the incubation period. The samples were filtered through a membrane assembly consisting of nitrocellulose (BA85; Schleicher and Schuell) and DEAE-cellulose (DE81; Schleicher and Schuell) membranes with a slot blot apparatus (Minifold II; Schleicher and Schuell) according to a procedure described by Wang et al. (42). This was followed by two washes with 350 μl of filter binding buffer without BSA. Radioactivity retained on both filters was quantitated with a Fuji BAS1000 phosphoimaging system. Radioactivity bound to the nitrocellulose membrane gave a measure of the nucleoprotein complex, and that on the DEAE membrane provided the free DNA concentration. In the absence of IN or when IN was replaced by BSA, less than 1% of the radioactivity was retained on the nitrocellulose. This background value was determined in each experiment and subtracted from the values obtained when IN was added. Titration experiments were performed to establish concentrations at which approximately 30% of the DNA was found in a complex with IN (1 μM IN and 1 μM DNA).

Size exclusion chromatography of IN-Fab complexes. Size exclusion chromatography was performed using a Superdex 75HR 10/30 column (Pharmacia) on a Rainin HPLC system. For all experiments, a flow rate of 0.5 ml/min was used with a mobile phase of 20 mM HEPES (pH 7.0)–0.5 M NaCl–1% (vol/vol) glycerol. Purified IN and Fab fragments were mixed at equimolar ratios (final concentrations, 25 μM each) and incubated for 30 min on ice prior to injection on the column. Absorbance of the column eluate was monitored at both 280 and 220 nm. Fractions were taken from all peaks and analyzed by SDS-PAGE for the presence of the expected protein species. The column was calibrated with seven different globular proteins (1,350 to 150,000 Da). The log of the known molecular weights of these standard proteins was plotted versus the elution behavior (partition coefficient [K_{av}] or elution time), and linear regression was applied to calculate the apparent molecular weights of relevant peaks in the samples.

RESULTS

Production and characterization of anti-RSV IN MAbs. Mice were immunized with purified recombinant RSV IN, prior to fusion of spleen lymphocytes with mouse myeloma cells. Two separate fusions, each with a spleen from a single



lane	mAb	Isotype	Titer (ng/ml)
1	sA1	IgG2a, κ	3
2	nA2	IgG1, κ	3
3	iB1	IgG1, κ	3
4	iB2	IgG1, κ	3
5	B3	IgG1, κ	3

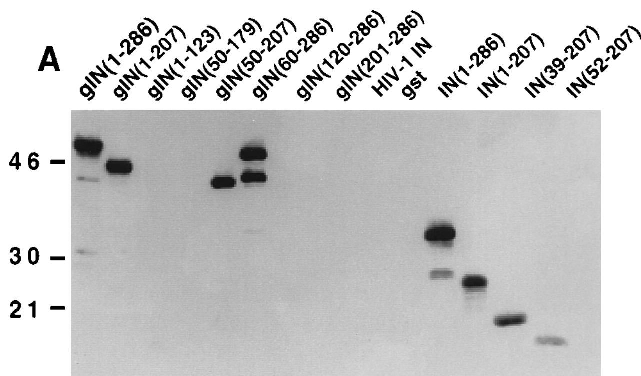
FIG. 1. Characterization of the purified MAbs. Antibodies were purified from ascites fluid and characterized as described in Materials and Methods. The upper part of the figure shows a reducing SDS-PAGE gel (12.5% polyacrylamide) stained with Coomassie brilliant blue. Two micrograms of each purified MAb was applied. H, heavy chains; L, light chains; M, molecular mass markers (molecular masses of marker proteins are shown at the left in kilodaltons). The table below shows the isotype and titer (concentration of purified antibody that gave an optical density at 405 nm reading fivefold greater than the background) for each antibody. IgG2a, κ, immunoglobulin G2a (κ).

immunized mouse, were performed. Five stable hybridoma cell lines that secreted high levels of anti-RSV IN antibodies were selected for cloning and further characterization. MAbs produced by the hybridoma clones were purified from ascites fluid, and their subclasses were determined. The purified MAbs were also tested for their binding to viral and recombinant RSV IN by titration ELISAs. All MAbs were found to bind to the protein with similar avidity (Fig. 1).

The interaction of the MAbs with a series of N- and C-terminally truncated versions of RSV IN was tested to determine which regions of the protein were important for recognition. The truncated IN proteins were expressed in *E. coli* either directly or, to facilitate their purification, as GST fusion proteins; all were recognized well in an immunoblot experiment using polyclonal rabbit antiserum raised against the full-length protein. Binding of the MAbs was tested by Western blot (immunoblot) and ELISA. Although all MAbs had very similar avidities as determined by ELISAs (Fig. 1), their reactivities in immunoblot experiments were different. The reactivity of MAb nA2 was much stronger than those of the other four MAbs; representative data with this reagent are shown in Fig. 2A. MAbs sA1 and B3 displayed extremely weak reactivity in Western blot experiments, suggesting that these MAbs bind to conformational epitopes that are not maintained well under the denaturing conditions of the immunoblot. Thus, the reactivity of these two MAbs with mutated proteins was determined solely by ELISA.

Results from the ELISAs are summarized in Fig. 2B. Although quantitative differences were seen, all five MAbs recognized the same subset of truncated proteins, each of which included a central IN domain spanning aa 50 to 207. When either N- or C-terminal deletions extended into that region, no interaction with the MAbs could be detected. We conclude that all five of these MAbs recognize epitopes within the central portion of IN and that these epitopes are probably not contained in a simple linear sequence.

In the context of the GST-IN fusion protein, both the N-terminal (aa 1 to 50) and the C-terminal portions of IN (aa 207 to 286) could be deleted without causing a detectable reduction of MAb binding in titration ELISAs or Western blot



B

	IN(1-286)	gIN(1-286)	gIN(1-236)	IN(1-207)	gIN(1-207)	gIN(1-173)	gIN(60-286)	gIN(120-286)	gIN(158-286)	gIN(201-286)	IN(39-207)	IN(52-207)	gIN(50-207)	gIN(178-236)	gIN(201-266)	gst
sA1	++++	++++	++++	++	++++	-	++++	-	-	-	-	-	++++	-	-	-
nA2	++++	++++	++++	++++	++++	-	++++	-	-	-	+	++	++++	-	-	-
iB1	++++	++++	++++	+	++++	-	++++	-	-	-	-	-	++++	-	-	-
iB2	++++	++++	++++	+	++++	-	++++	-	-	-	-	-	++++	-	-	-
B3	++++	++++	++++	++	++++	-	++++	-	-	-	-	-	++++	-	-	-

C-terminal deletions N-terminal deletions internal fragments

FIG. 2. Mapping epitopes of the MAbs. (A) Immunoblot of truncated RSV IN proteins. Purified proteins (1 μg each) were separated by SDS-PAGE, transferred to polyvinylidene fluoride membranes, and probed with antibody nA2 (10 μg/ml) as described in Materials and Methods. Positions of molecular mass markers are indicated at the left with the molecular masses given in kilodaltons. (B) Relative reactivity of the MAbs with truncated RSV IN proteins in titration ELISAs (see Materials and Methods). ++++, reactivity similar to that of the wild type; +++, reactivity up to 10-fold reduced; ++, reactivity up to 50-fold reduced; +, reactivity up to 250-fold reduced; −, no binding detectable at the concentrations tested; g, GST as the fusion partner.

assays [Fig. 2A, lane gIN(50-207)]. N- and C-terminal fragments (aa 1 to 123 and 201 to 286) fused to GST showed no interaction with the immunoglobulins [Fig. 2A, lanes gIN(1-123) and gIN(201-286)]. These results suggest that aa 1 to 50 and 207 to 286 do not directly contribute to the recognized epitopes. However, we found that similar deletions of N- and C-terminal sequences in the nonfused IN protein led to significant reductions in MAb binding in ELISAs and Western blot assays (Fig. 2). The most likely explanation for this difference is that the presence of an adjacent polypeptide stabilizes the conformation of complex epitopes within the core region and thereby facilitates MAb binding.

We also tested the ability of the MAbs to compete with each other for binding to RSV IN. This was done by measuring the binding of a biotinylated MAb to IN which had been preincubated with an unmodified MAb. The results showed that MAbs sA1, iB1, iB2, and B3 compete very efficiently with each other (Fig. 3, top). This is consistent with the conclusion that they all recognize epitopes within the same central region. Since further experiments revealed different effects on IN function and differences in complex formation were detected in biophysical studies (described below), we conclude that these MAbs do not compete for binding to the same epitope but either recognize overlapping epitopes or compete because of steric interferences. MAb nA2, which also recognizes the core region, does not compete with the other MAbs (Fig. 3, bottom).

Cross-reactivity of the MAbs with bacterially expressed HIV-1 IN was also tested. Western blot experiments indicated a very weak reaction with MAb iB2 and an even weaker reaction with MAbs nA2 and B3 (data not shown). This low level of cross-reactivity was undetectable in ELISAs and therefore was not quantitated.

The MAbs alter IN enzymatic activities. (i) Processing and joining of LTR sequences. The central domain of IN contains the catalytic site of the enzyme. This domain includes the three invariant acidic amino acid residues which characterize the D,D(35)E sequence motif and have been shown to be crucial for all IN functions in vitro (12, 26, 38). It was of interest therefore to determine whether these MAbs, all of which bind to the central region, had an effect on the enzymatic activities of IN. Purified IN catalyzes the removal of a dinucleotide from the 3′ end of model oligodeoxynucleotide substrates (Fig. 4A, left) and the joining of the newly processed ends into a target DNA molecule in vitro (Fig. 4B, left) (4, 9, 19, 23, 35). To investigate the effect of the MAbs on these and related activities of IN, the enzyme was preincubated with each of the purified immunoglobulins and subsequently tested for its activity as described in Materials and Methods. In control reactions, MAbs were replaced by BSA or a purified anti-Ras MAb (2) (Fig. 4, lanes 2). Preliminary experiments verified that the MAbs used in these experiments contained no detectable contaminating protease or nuclease activities under assay conditions described in the legend to Fig. 4.

The most dramatic effect was observed with MAb nA2, which was able to neutralize both in vitro processing and joining activities (Fig. 4A and B, lanes 4). However, although specific processing at the −2 position was blocked, this antibody did not abolish nonspecific cleavage of DNA by IN, as indicated by the appearance of faint bands at the −3 and −4 positions. This was confirmed by analyzing cleavage of oligodeoxynucleotide duplexes with a random sequence unrelated to the RSV LTR (not shown). In this case, the presence of MAb nA2 did not prevent DNA cleavage at positions within the duplex, although cleavage at the −1 and −2 positions was found to be reduced. The overall cleavage pattern observed with wild-type IN in the presence of MAb nA2 strongly resembled that produced by N- and C-terminally truncated IN proteins that contain the catalytic core domain [RSV IN(1-207), IN(39-207) and IN(52-207)] (27) (Fig. 4A, lanes 4 and 8; other data not shown).

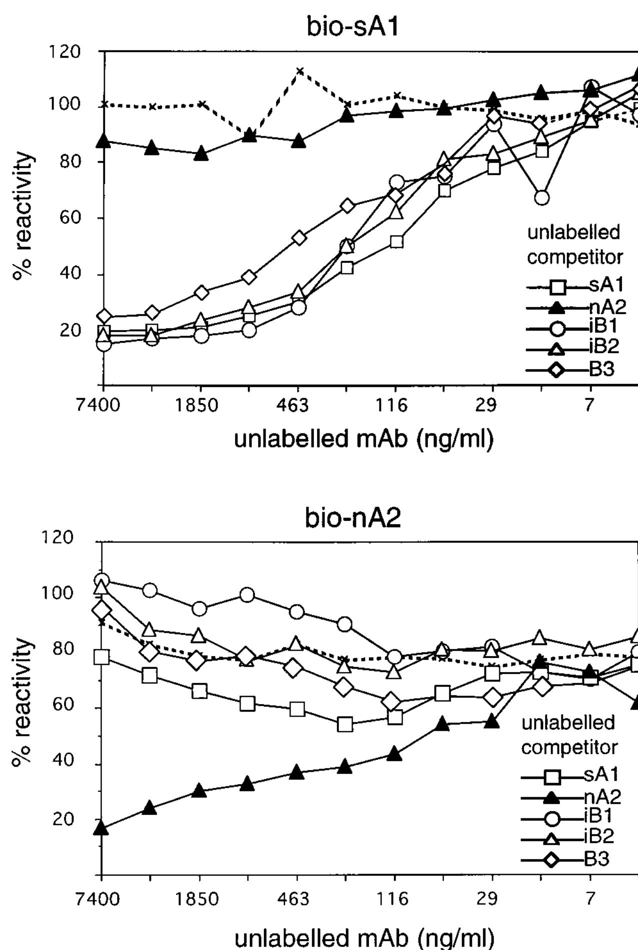


FIG. 3. Competition of the MAbs for binding to IN. Competition assays were performed as described elsewhere (3). Briefly, as in an ELISA, IN was preincubated with serial 1:1 dilutions of an unlabelled MAb (7 to 7,400 ng/ml) in wells of a 96-well microtiter plate. After washing the wells, a fixed concentration of biotinylated MAb (20 ng per well) was added. Binding of the biotinylated MAb was then detected with streptavidin conjugated to alkaline phosphatase. The figure shows competition of all MAbs with biotinylated sA1 (top) and biotinylated nA2 (bottom). The unlabelled competitors were MAbs sA1, nA2, iB1, iB2, and B3. The dotted line shows the reactivity of biotinylated MAb in the row of control wells (no unlabelled competitor added). Reactivities of the biotinylated MAb with the preincubated antigen were plotted as relative values. The reactivity measured without competing MAb in the first row of the plate was defined as 100%. Note that the values on the abscissa are not a linear concentration scale but represent sequential dilution steps.

MAbs iB1 and iB2 also inhibited the processing reaction (Fig. 4A, lanes 5 and 6, respectively), but not as strongly as nA2. Furthermore, in contrast to MAb nA2, MAbs iB1 and iB2 inhibited both specific processing at the −2 position and nonspecific DNA cleavage to a similar degree. MAb sA1 showed little effect on processing under these conditions, but a stimulatory effect on the joining activity was observed (Fig. 4A and B, lanes 3). To verify that these effects were due to specific interactions between the MAbs and IN, we performed analogous processing reactions using purified HIV-1 IN and its cognate oligodeoxynucleotide substrate. None of the anti-RSV IN MAbs had any effect on HIV-1 activities (data not shown).

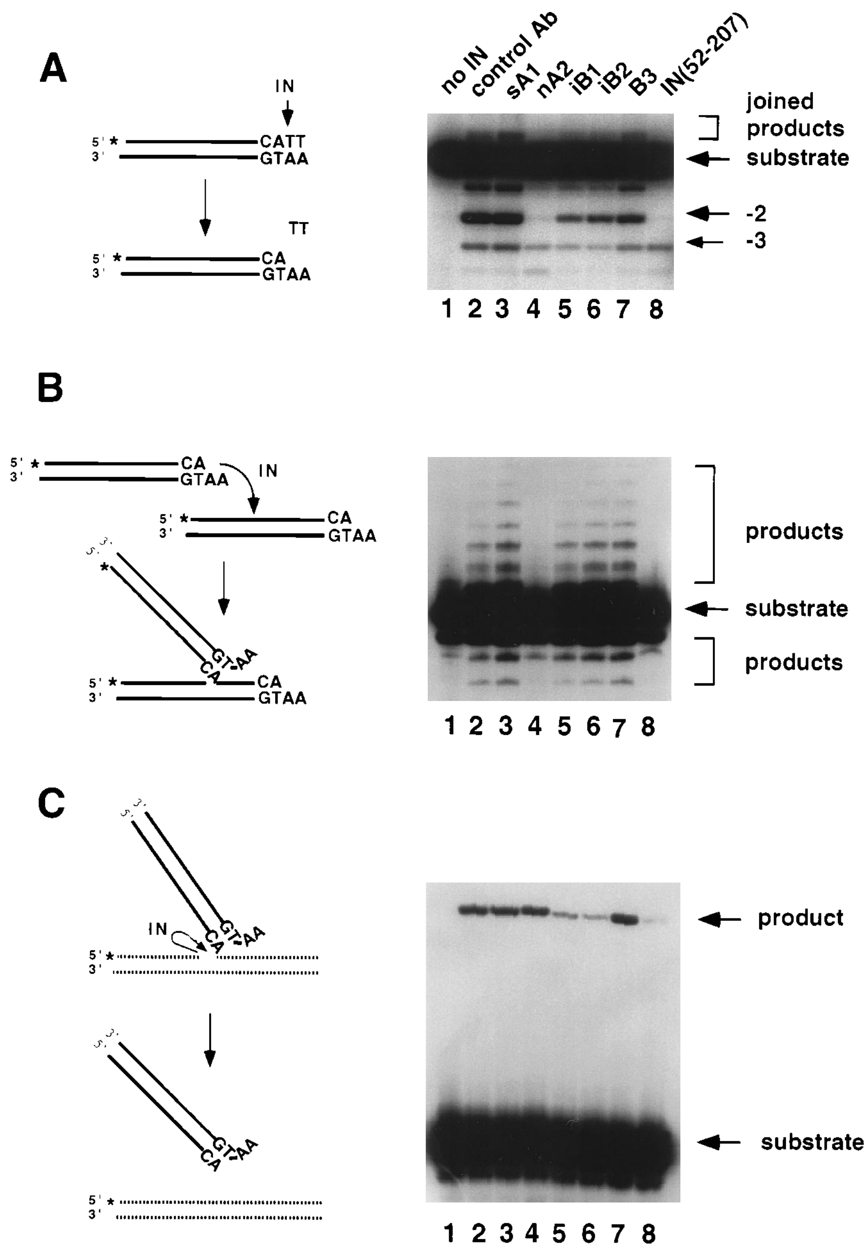


FIG. 4. Effects of the MAbs on in vitro enzymatic activities of RSV IN. Effects on (A) 3′ end processing of substrate U3(18/18); (B) joining of processed substrate U3(16/18); (C) cleavage-ligation on a model intermediate (disintegration) substrate that mimics covalently joined viral (solid line) and host (stippled line) sequences. To monitor reactions, one oligodeoxynucleotide in each substrate analog was labelled at the 5′ end with ^{32}P (indicated by the asterisk in the schematic representations). IN was preincubated with the MAbs at an equimolar ratio of IN monomer to MAb and subsequently analyzed as described in Materials and Methods. An anti-Ras antibody (2) was used in the control reactions. Final concentrations in the experiments shown here were 1.1 μM IN and 10 μM substrate. Reaction conditions were as follows: processing reaction, 37°C for 5 min (A); joining reaction, 37°C for 7.5 min (B); and cleavage-ligation, 26°C for 15 min (C). Reaction components were separated by denaturing PAGE (18% acrylamide, 7 M urea), and radioactivity was detected by exposing the gels to Kodak XAR film.

To analyze the functional effects of the MAbs on RSV IN in a more quantitative fashion, titration experiments were performed. IN was preincubated with various concentrations of MAbs and subsequently analyzed for in vitro processing and joining activities by previously established solution methods for the quantitation of reaction products (29). The results (Fig. 5) showed that MAb nA2 neutralized both activities at an approximately equimolar ratio of MAb to IN monomer. The inhibition of processing by MAb iB2 was less pronounced than that by nA2, with detectable activity remaining at the highest concentration of MAb iB2 tested. Some inhibition of the joining activity was also observed with this antibody. MAb sA1 had no significant effect on processing under these conditions but stimulated the joining activity about threefold. The titrations in Fig. 5 were performed using intact MAbs; similar results were obtained using Fab fragments, when corrected for the number of antigen binding sites per immunoglobulin molecule (not shown), indicating that the observed functional effects are not dependent on the bivalent nature of the reagent.

(ii) Cleavage-ligation reaction. Another in vitro reaction shown to be catalyzed by IN is a cleavage-ligation termed disintegration (8). The substrates for these reactions resemble

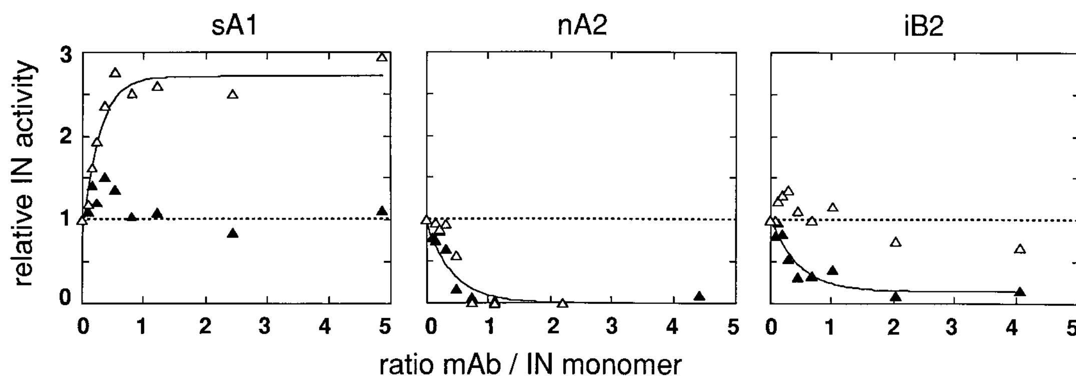


FIG. 5. Effect of increasing concentrations of MAbs on the processing and joining reactions. IN was preincubated with MAb sA1, nA2, or iB2 at the molar ratios indicated in the figure and subsequently analyzed for processing (filled triangles) or joining activity (open triangles) by previously established solution assays (29). Concentrations used in these experiments were 1.1 μM IN and 10 μM substrate, and reaction mixtures were incubated at 37°C for 5 min (processing) or 8 min (joining). Values represent averages from at least two independent measurements. The amount of product generated was normalized to the amount formed by IN which had been preincubated with BSA (dotted line).

the Y intermediate which results from the joining of a 3′ end of the viral DNA to a phosphate group within a target DNA molecule. Upon incubation with these substrates, IN mediates the release of the portion that represents the viral DNA end and repairs the nick in the target DNA duplex (see schematic representation in Fig. 4C, left). With HIV-1 IN, it has been demonstrated that the viral DNA sequences are not absolutely required for a detectable cleavage-ligation activity but that this portion of the model substrate can be single stranded or even be reduced to a single nucleotide (8, 34). It has been shown that RSV IN, like HIV IN, can carry out this cleavage-ligation reaction on a variety of substrates (6, 27, 28a). Experiments with mutant IN proteins have revealed that this activity is less sensitive to certain mutations or truncations which reduce or abolish the specific in vitro processing and joining activities (5, 6, 17, 27).

We tested the effects of the MAbs on the cleavage-ligation reaction using a small Y intermediate as a model substrate (Fig. 4C). Repair of the nick in the target DNA by IN generates a 19-mer oligodeoxynucleotide, the formation of which was monitored to follow the reaction. With the exception of MAb nA2 (lane 4), effects of the antibodies on cleavage-ligation paralleled those on processing. MAbs sA1 and B3 (lanes 2 and 3) did not have a notable effect; MAbs iB1 and iB2 were inhibitory (lanes 5 and 6). Titration experiments with increasing concentrations of MAbs showed that even a threefold molar excess of sA1 did not result in stimulation but rather inhibited the reaction somewhat (not shown). Surprisingly, MAb nA2, which in parallel experiments (Fig. 4A and B, lanes 4) had been found to block both specific processing and joining, inhibited cleavage-ligation only slightly.

(iii) DNA binding. Since the antibodies had displayed specific effects on various in vitro activities of IN, we were also interested in measuring their effects on the interaction of IN with DNA. It has been shown that purified IN binds to DNA in vitro. Nitrocellulose filter retention and UV cross-linking experiments have not revealed any DNA sequence specificity of this interaction; both RSV and HIV-1 IN bound DNA substrates resembling viral LTR ends and DNA with random sequences with similar efficiencies (10, 13, 25, 32). We measured DNA binding in a nitrocellulose filter retention assay using the U3(16/18) oligodeoxynucleotide duplex as a substrate. When IN was incubated with various concentrations of Fab fragments prior to the addition of DNA, neither Fab sA1 nor iB2 appeared to reduce DNA binding. In contrast, preincubation of IN with Fab nA2 resulted in a marked decrease in the amount of IN-DNA complexes detected (Fig. 6). Similar results were obtained with a single-stranded oligodeoxynucleotide with an unrelated sequence (not shown).

DNA protects from inhibition by MAb nA2. To investigate whether the presence of substrate affected the inhibition of IN by MAbs, we performed experiments in which the enzyme was incubated with the processing substrate either before or after an inhibitory MAb (nA2, iB1, or iB2) was added. Both steps were followed by a 15-min incubation on ice. The processing reaction was then started by the addition of Mn^{2+}. We found that the inhibitory effect of MAb nA2 was markedly reduced when DNA was added first, even though the subsequent addition of MAb was followed by an equilibration period (Fig. 7). The presence of a divalent cation during the preincubation with DNA was not required. A similar protection from the effects of MAb nA2 was also observed in the nitrocellulose filter binding experiments. When DNA was added first, the addition of this antibody did not result in a decrease in the amount of DNA bound to protein (not shown).

Stoichiometry of IN-Fab complexes. In order to confirm IN-Fab interactions and to analyze the composition of IN-Fab

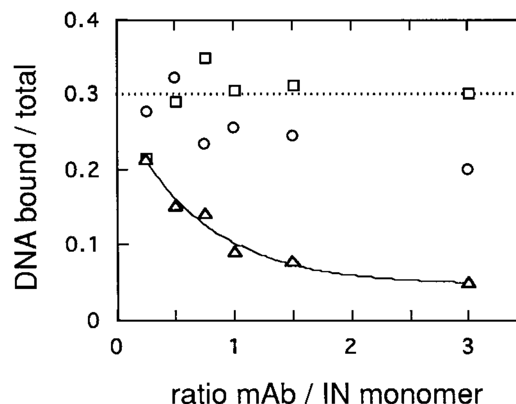


FIG. 6. Effects of MAbs sA1, nA2, and iB2 on DNA binding to IN. IN was preincubated with increasing concentrations of the MAbs prior to addition of radiolabelled DNA. DNA bound to IN was subsequently quantitated in a nitrocellulose filter binding assay as described in Materials and Methods [1 μM IN, 1 μM U3(16/18)]. In the absence of antibody, approximately 30% of the substrate was found bound to IN (represented by the dotted line). The figure shows mean values from two independent experiments. Squares, sA1; triangles, nA2; circles, iB2.

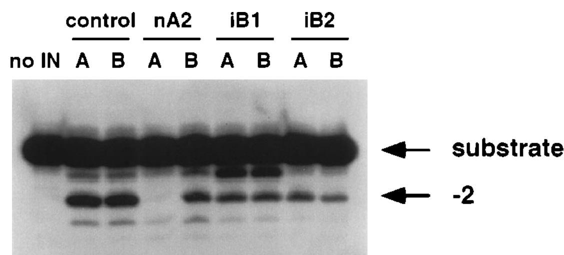


FIG. 7. Effect of preincubation of IN with substrate on the inhibitory effects of MAbs. Integrase (3.3 μM) was preincubated with a 4-fold excess of MAb (lanes A) or a 20-fold excess of substrate (lanes B) for 15 min on ice. Subsequently, the substrate (lanes A) or MAb (lanes B) was added, and preincubation was continued for another 15 min on ice. Reaction buffer and water were added, and reactions were started by the addition of $MnCl_2$. Final concentrations in all samples were 1 μM IN, 20 μM U3(18/18) substrate, and 4 μM MAb. Reaction mixtures were incubated for 8 min at 37°C, reactions were stopped by addition of loading buffer, and mixtures were analyzed on denaturing polyacrylamide gels. In the control reaction, BSA was used instead of the MAb.

complexes, we performed size exclusion chromatography on complexes formed after mixing equimolar amounts of both proteins. Figure 8 shows typical chromatograms obtained from analyses of Fab sA1, nA2, and iB2 complexes with IN. The elution time for each Fab fragment chromatographed by itself was determined (chromatograms 1, 3, and 5), and the calculated apparent molecular masses are in the expected range for native Fab fragments (40 to 50 kDa). Native IN alone elutes slightly faster, with an apparent molecular mass of 57 kDa, corresponding to a dimer (chromatogram 7). The various IN-Fab complexes displayed different elution behaviors. The complex of Fab fragment sA1 with IN elutes at a position consistent with a composition of one Fab fragment bound to an IN dimer (1:2 complex; see chromatogram 2). Preincubation of IN with up to a fivefold molar excess of Fab sA1 did not produce an additional increase in the apparent molecular weight of the complex or an increase in amount of Fab in the complex fraction (not shown). The nA2 Fab fragment appeared to form both 1:1 and 2:2 complexes (chromatogram 4), and the iB2 Fab fragment formed predominantly a 2:2 complex with IN (chromatogram 6). Fractions were taken from each of the peaks, and the presence of each component in the complex, as well as the ratio of the components, was confirmed by SDS-PAGE analysis (data not shown). Given that equimolar amounts of IN and Fab fragments were mixed in each case, the presence of a significant amount of unbound Fab sA1 detected in chromatogram 2 (but not in samples containing Fab nA2 and iB2; chromatograms 4 and 6, respectively) agrees with the stoichiometric assignment of one Fab sA1 to one IN dimer in the complex.

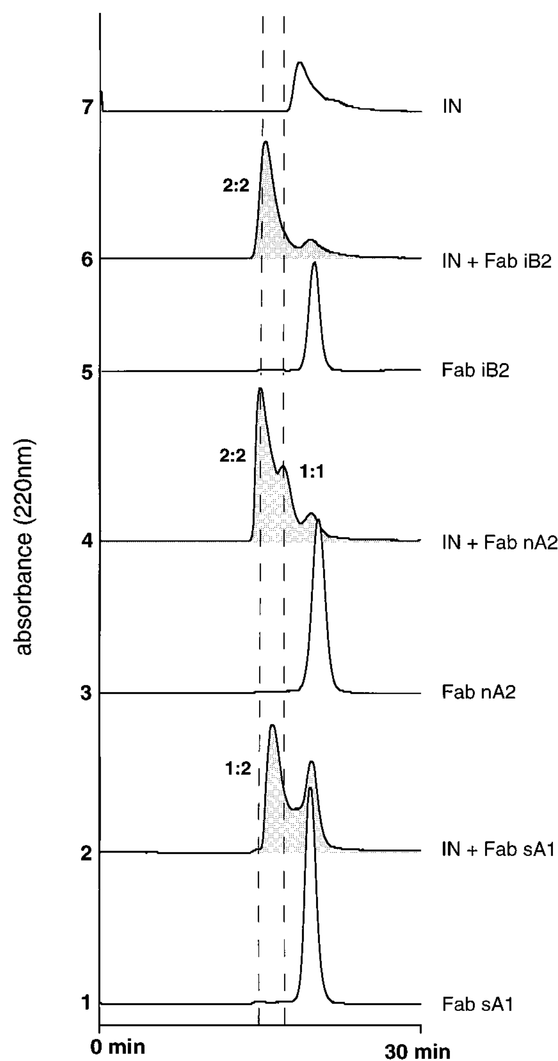


FIG. 8. Analysis of IN-Fab complexes by size exclusion chromatography. RSV IN was preincubated with the indicated Fab fragments at equimolar ratios (final concentration, 25 μM each) prior to separation by size exclusion chromatography as described in Materials and Methods. Chromatograms 1, 3, and 5 show the elution of the respective Fab fragments alone; chromatogram 7 shows the elution behavior of IN alone. Chromatograms 2, 4, and 6 show the elution of complexes formed between Fab fragments and IN. The stoichiometric composition, as deduced from apparent molecular weight and confirmed by SDS-PAGE analysis, is noted for each complex peak. The vertical dashed lines mark the elution time of the 1:1 and 2:2 complexes of IN-Fab nA2 for reference in the other Fab-IN complex elution profiles.

DISCUSSION

In this report, we have described the production and characterization of MAbs against RSV IN. Antisera against RSV IN peptides have been described previously (1, 14). Our laboratory has reported the characterization of a panel of MAbs against HIV IN (3), and the properties of some immunoreactive patients' sera which recognize HIV-1 IN have also been described previously (41). However, MAbs which specifically alter the enzymatic activities of IN have not yet been reported.

The five antibodies described here all recognize what appear to be conformational epitopes within the catalytic core region of IN (aa 50 to 207). Although mapping experiments revealed that all of the MAbs interacted with the same subset of truncated IN proteins (Fig. 2), it is unlikely that they recognize the same or even similar epitopes. The observation that Fab fragments of three of the MAbs form distinct complexes with IN and the finding that the antibodies exert contrasting effects on various IN activities in vitro indicate that the epitopes recognized are distinct. This is further supported by results from immunoblot and ELISA experiments using a series of full-length IN proteins with single substitutions of highly conserved amino acid residues (F for W-61 [W61F], T66A, S85G, I118A, D121E, F126A, I140A, L163A, and K164A) which reveal distinct differences in binding capacities of the five MAbs to these proteins (28b). For example, the binding of iB1 and iB2 was greatly decreased by a conservative mutation at the active site (D121E), consistent with their capacity to inhibit enzymatic activity. MAb sA1 also exhibited decreased reactivity against

the D121E protein but was even more affected by changes predicted to have indirect effects on protein structure (e.g., S85G and F126A) (3a). On the other hand, binding of MAb nA2 was only mildly affected by any of the substitutions.

Attempts to use phage display techniques (24) to determine the epitope for several of the MAbs revealed no unique sequences that appeared anywhere in the contiguous sequence of the core domain. This is not unexpected if individual amino acids from separate regions of the core domain contribute to these epitopes. These results together with the similarities in reactivity with the series of truncated proteins seem likely to reflect the requirement of a minimal core domain for proper folding of various epitopes within this core region.

Selective neutralization by MAb nA2. Among the five antibodies tested, MAb nA2 showed the strongest effects on activities of IN. It blocked both specific processing and joining activities and inhibited DNA binding. All of the MAbs described here bound to the catalytic core region of IN, but only MAb nA2 showed this inhibition pattern. Thus, the inhibitory effects observed cannot be explained simply by steric hindrance due to the binding of a large immunoglobulin molecule to the catalytic core. Biophysical studies also revealed a difference between the complexes formed with Fabs from nA2 and those from the other MAbs analyzed. In contrast to Fab fragments from sA1 and iB2, Fab nA2 was found in 1:1 complexes with full-length IN monomers. The properties of the wild-type IN-nA2 complexes described here show similarities with mutant IN proteins that are deficient in multimerization, i.e., the single-amino-acid substitution mutant RSV IN S85G and the catalytic core-containing deletion mutants IN(1-207), IN(39-207), and IN(52-207) (1a). In all of these cases, nonspecific endonucleolytic and cleavage-ligation activities are retained, while the specific processing and joining activities are severely diminished or undetectable (Fig. 4A) (27). Kinetic studies have indicated that the minimal unit of IN able to catalyze specific processing and joining in vitro is a dimer (18). Thus, one might hypothesize that MAb nA2 inhibits these reactions by interfering with dimerization. However, this simple hypothesis is not supported by the size exclusion data. Binding of Fab nA2 did not prevent IN dimerization; a substantial amount of the 2:2 complex was detected at a stoichiometric ratio of Fab to IN which gave complete inhibition of processing and joining activities.

Our current working hypothesis is that nA2 alters IN function by inducing a conformational change within the monomer. The observations that MAb nA2 did not affect the binding of any of the other MAbs to IN (Fig. 3) and that it was not able to block the cleavage-ligation activity (Fig. 4C) make it seem unlikely that a drastic conformational change is induced in the catalytic core region. However, more subtle changes such as a shifting of structural domains relative to each other could produce differential effects in IN activities, as well as alterations in the organization of multimers. We show here that inhibitory effects of nA2 are weakened if the DNA substrate is added first (Fig. 7). Substrate binding might prevent the proposed conformational change by stabilizing an active conformation or by inducing the formation of an altered structure and thus protect IN from the inhibitory effects of MAb nA2.

Selective stimulation by MAb sA1. In contrast to nA2, MAb sA1 did not inhibit any in vitro activity tested. On the contrary, it stimulated the joining activity about threefold. Only one sA1 Fab fragment appeared to bind per IN dimer. This suggests that the Fab fragments bind near the dimer interface and touch amino acids from both monomers or that only one can bind because of steric interference at the interface. Alternatively, the sA1 epitope may be accessible, or in the proper conformation, on only one IN subunit in the dimer. The finding that subfragments of IN could be bound by this Fab fragment as monomers in a 1:1 complex (data not shown) supports either of the last two hypotheses. Since iB2 and nA2 Fab fragments were both able to form 2:2 complexes with IN, their respective epitopes must be accessible on both subunits in the IN dimer.

The stimulatory effect of sA1 on the joining activity of the wild-type protein could be explained by a stabilization of IN multimers. Unfortunately, this question cannot be addressed directly, since assay systems that allow the determination of on and off rates of IN multimeric complexes have not yet been established. In preliminary experiments using size exclusion chromatography, binding of Fab sA1 was not found to enhance multimerization of an isolated catalytic core domain, IN(52-207).

In summary, we have shown that the various activities of IN can be differentially affected by the binding of specific MAbs to the central catalytic core domain. We have also observed that the Fab fragments form complexes with IN of different stoichiometric compositions. These antibodies should be useful for further structural and functional studies of the integrase both in vitro and in vivo.

ACKNOWLEDGMENTS

We express our sincere gratitude to several laboratory members: G. Merkel for providing purified RSV IN and several derivative proteins used in these studies, J. Kulkosky for purified HIV-1 IN, and R. Katz for helpful suggestions and critical review of the manuscript. C. Seeger also provided helpful comments on the manuscript. We would also thank the Fox Chase Cancer Center Hybridoma Facility and the Laboratory Animal Facility for excellent technical assistance and advice.

This work was supported by National Institutes of Health grants CA-47486 and CA-06927, a grant for infectious disease research from Bristol-Myers Squibb Foundation, and also an appropriation from the Commonwealth of Pennsylvania. B.M. is supported by a fellowship from the German AIDS-Stipendienprogramm. M.D.A. was supported, in part, by PHS fellowships 5F32 AI08642 and T32 CA09035-17.

REFERENCES

1. **Alexander, F., J. Leis, D. A. Soltis, R. M. Crowl, W. Danho, M. S. Poonian, Y.-C. E. Pan, and A. M. Skalka.** 1987. Proteolytic processing of avian sarcoma and leukosis viruses *pol-endo* recombinant proteins reveals another *pol* gene domain. J. Virol. **61:**534–542.

1a. **Andrake, M. D., and A. M. Skalka.** Multimerization determinants reside in both the catalytic core and C-terminus of ASV integrase. Submitted for publication.

2. **Bizub, D., E. Fischberg-Bender, E. P. Heimer, A. Felix, and A. M. Skalka.** 1989. Detection of transforming *ras* proteins containing leucine at position 61 by a new mouse monoclonal antibody, *ras*(53-69)Leu61. Cancer Res. **49:**6425–6431.

3. **Bizub Bender, D., J. Kulkosky, and A. M. Skalka.** 1994. Monoclonal antibodies against HIV-1 integrase: clues to molecular structure. AIDS Res. Hum. Retroviruses **10:**1105–1115.

3a. **Bujacz, G., M. Jaskólski, J. Alexandratos, A. Wlodawer, G. Merkel, R. A. Katz, and A. M. Skalka.** High resolution structure of the catalytic domain of avian sarcoma virus integrase. Submitted for publication.

4. **Bushman, F. D., and R. Craigie.** 1991. Activities of the human immunodeficiency virus (HIV) integration protein *in vitro*: specific cleavage and integration of HIV DNA. Proc. Natl. Acad. Sci. USA **86:**2525–2529.

5. **Bushman, F. D., A. Engelman, I. Palmer, P. Wingfield, and R. Craigie.** 1993. Domains of the integrase protein of human immunodeficiency virus type 1 responsible for polynucleotidyl transfer and zinc binding. Proc. Natl. Acad. Sci. USA **90:**3428–3432.

6. **Bushman, F. D., and B. Wang.** 1994. Rous sarcoma virus integrase protein: mapping functions for catalysis and substrate binding. J. Virol. **68:**2215–2223.

7. **Chow, S. A., and P. O. Brown.** 1994. Juxtaposition of two viral DNA ends in bimolecular disintegration reaction mediated by multimers of human immunodeficiency virus type 1 or murine leukemia virus integrase. J. Virol. **68:** 7869–7878.

8. **Chow, S. A., K. A. Vincent, V. Ellison, and P. O. Brown.** 1992. Reversal of integration and DNA splicing mediated by integrase of human immunodeficiency virus. Science **255:**723–726.

9. **Craigie, R., T. Fujiwara, and F. D. 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.

10. **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.
11. **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.
12. **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.
13. **Engelman, A., A. B. Hickman, and R. Craigie.** 1994. The core and carboxyl-terminal domain of the integrase protein of human immunodeficiency virus type 1 each contribute to nonspecific DNA binding. J. Virol. **68:**5911–5912.
14. **Grandgenett, D. P., R. J. Knaus, and P. J. Hippenmeyer.** 1983. Antibodies against a synthetic peptide of the avian retrovirus pp32 protein and the β DNA polymerase subunit. Virology **130:**257–262.
15. **Grandgenett, D. P., A. C. Vora, and R. D. Schiff.** 1978. A 32,000-dalton nucleic acid binding protein from avian retrovirus cores possesses DNA endonuclease activity. Virology **89:**119–132.
16. **Harlow, E., and D. Lane (ed.).** 1989. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Hickman, A. B., I. Palmer, A. Engelman, R. Craigie, and P. Wingfield.** 1994. Biophysical and enzymatic properties of the catalytic domain of HIV-1 integrase. J. Biol. Chem. **269:**29279–29287.
18. **Jones, K. S., J. Coleman, G. W. Merkel, T. Laue, and A. M. Skalka.** 1992. Retroviral integrase functions as a multimer and can turn over catalytically. J. Biol. Chem. **267:**16037–16040.
19. **Jonsson, C. B., G. A. Donacella, and M. J. Roth.** 1993. Characterization of the forward and reverse integration reactions of the Moloney murine leukemia virus integrase protein purified from *Escherichia coli*. J. Biol. Chem. **268:**1462–1469.

19a. **Kalpana, G. V., and S. P. Goff.** 1993. Genetic-analysis of homomeric interactions of human-immunodeficiency-virus type-1 integrase using the yeast 2-hybrid system. Proc. Natl. Acad. Sci. USA **90:**10593–10597.

20. **Katz, R. A., J. P. G. Mack, G. Merkel, J. Kulkosky, G. Zheng, J. Leis, and A. M. Skalka.** 1992. Requirement of a conserved serine in both processing and joining activities of retroviral integrase. Proc. Natl. Acad. Sci. USA **89:**6741–6745.
21. **Katz, R. A., G. Merkel, J. Kulkosky, Z. Ge, 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.
22. **Katz, R. A., and A. M. Skalka.** 1994. The retroviral enzymes. Annu. Rev. Biochem. **63:**133–173.
23. **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.
24. **Kay, B. K., N. B. Adey, Y. S. He, J. P. Manfredi, A. H. Mataragnon, and D. M. Fowlkes.** 1993. An M13 phage library displaying random 38-amino-acid peptides as a source of novel sequences with affinity to selected targets. Gene **128:**59–65.
25. **Khan, E., J. P. G. Mack, R. A. Katz, J. Kulkosky, and A. M. Skalka.** 1991. Retroviral integrase domains: DNA binding and the recognition of LTR sequences. Nucleic Acids Res. **19:**851–860.
26. **Kulkosky, J., K. S. Jones, R. A. Katz, J. P. G. Mack, and A. M. Skalka.** 1992. Residues critical for retroviral integrative recombination in a region that is highly conserved among retroviral/retrotransposon integrases and bacterial insertion sequence transposases. Mol. Cell. Biol. **12:**2331–2338.
27. **Kulkosky, J., R. A. Katz, G. W. Merkel, and A. M. Skalka.** 1995. Activities and substrate specificity of the evolutionary conserved central domain of retroviral integrase. Virology **206:**448–456.
28. **Leavitt, A. D., L. Shive, and H. E. Varmus.** 1993. Site-directed mutagenesis of HIV-1 integrase demonstrates differential effects on integrase functions *in vitro*. J. Biol. Chem. **268:**2113–2119.

28a. **Müller, B.** Unpublished data.

28b. **Müller, B., and D. Bizub-Bender.** Unpublished data.

29. **Müller, B., K. S. Jones, G. W. Merkel, and A. M. Skalka.** 1993. Rapid solution assays for retroviral integration reactions and their use in kinetic analyses of wild-type and mutant Rous sarcoma virus integrases. Proc. Natl. Acad. Sci. USA **90:**11633–11637.
30. **Mumm, S. R., and D. P. Grandgenett.** 1991. Defining nucleic acid binding properties of avian retrovirus integrase by deletion analysis. J. Virol. **65:** 1160–1167.
31. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
32. **Schauer, M., and A. Billich.** 1992. The N-terminal region of HIV-1 integrase is required for integration activity, but not for DNA binding. Biochem. Biophys. Res. Commun. **185:**874–880.
33. **Schulman, M., C. D. Wilde, and G. Köhler.** 1978. A better cell line for making hybridoma secreting specific antibodies. Nature (London) **276:**269.
34. **Sherman, P. A., M. L. Dickson, and J. A. Fyfe.** 1992. Human immunodeficiency virus type 1 integration protein: DNA sequence requirements for cleaving and joining reactions. J. Virol. **66:**3593–3601.
35. **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.
36. **Terry, R., D. Soltis, M. Katzman, D. Cobrinik, J. Leis, and A. M. Skalka.** 1988. Properties of avian sarcoma-leukosis virus pp32-related *pol*-endonucleases produced in *Escherichia coli*. J. Virol. **62:**2358–2365.
37. **van Gent, D. C., Y. Elgersma, M. W. J. Bolk, C. Vink, and R. H. A. Plasterk.** 1991. DNA binding properties of the integrase proteins of human immunodeficiency viruses type 1 and 2. Nucleic Acids Res. **19:**3821–3827.
38. **van Gent, D. C., A. A. M. Oude Groeneger, and R. H. A. Plasterk.** 1992. Mutational analysis of the integrase protein of human immunodeficiency virus type 2. Proc. Natl. Acad. Sci. USA **89:**9589–9592.
39. **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.
40. **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.
41. **Vinga-Martins, C., T. Schneider, A. Werno, W. Roenspeck, G. Pauli, and N. Mueller-Lantzsch.** 1992. Mapping of immunodominant epitopes of the HIV-1 and HIV-2 integrase proteins by recombinant proteins and synthetic peptides. AIDS Res. Hum. Retroviruses **8:**1301–1310.
42. **Wang, I., K. L. Chao, W. Bujalowski, and T. M. Lohman.** 1992. DNA induced dimerization of the *Escherichia coli* rep helicase. J. Biol. Chem. **267:**7596–7610.
43. **Woerner, A. M., and C. J. Marcus-Sekura.** 1993. Characterization of a DNA binding domain in the C-terminus of HIV-1 integrase by deletion mutagenesis. Nucleic Acids Res. **21:**3507–3511.