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Monoclonal Antibodies against Human Immunodeficiency Virus Type 1 Integrase: Epitope Mapping and Differential Effects on Integrase Activities In Vitro

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Human immunodeficiency virus type 1 (HIV-1) integrase (IN) catalyzes the integration of viral DNA into the host chromosome, an essential step in retroviral replication. As a tool to study the structure and function of this enzyme, monoclonal antibodies (MAbs) against HIV-1 IN were produced. Epitope mapping demonstrated that the 17 MAbs obtained could be divided into seven different groups, and a selection of MAbs representing these groups were tested for their effect on in vitro activities of IN. Four groups of MAbs recognized epitopes within the region of amino acids (aa) 1 to 16, 17 to 38, or 42 to 55 in and around the conserved HHCC motif near the N terminus of IN. MAbs binding to these epitopes inhibited end processing and DNA joining and either stimulated or had little effect on disintegration and reintegration activities of IN. Two MAbs binding to epitopes within the region of aa 56 to 102 in the central core or aa 186 to 250 in the C-terminal half of the protein showed only minor effects on the in vitro activities of IN. Three MAbs which recognized an epitope within the region of aa 262 to 271 of HIV-1 IN cross-reacted with HIV-2 IN. MAbs binding to this epitope clearly inhibited end processing and DNA joining and stimulated or had little effect on disintegration. In contrast to the N-terminal-specific MAbs, these C-terminal-specific MAbs abolished reintegration activity of IN.

Integration of viral DNA into a chromosome of the host cell is an essential step in the retroviral life cycle (for reviews, see, e.g., references 4, 18, and 33). The process is catalyzed by the viral enzyme integrase (IN) and proceeds in three steps (5, 17). First, two nucleotides are removed from the 3′ ends of the viral DNA (3′-end processing). The recessed 3′ ends of the viral DNA are then joined to 5′ staggered sites in the target DNA in a concerted cleavage and ligation reaction (DNA joining). Integration is completed by repair of the short gaps flanking the viral DNA intermediate and subsequent joining of the 5′ ends of viral DNA to the target DNA.

The 3′-end-processing and DNA-joining reactions can be reproduced in vitro with purified IN and short DNA substrates that mimic the ends of viral DNA (7, 9, 20, 26). If presented with a branched DNA substrate that mimics the product of the DNA-joining reaction, purified IN in vitro also catalyzes an apparent reversal of the DNA-joining reaction, termed disintegration (11). The products of disintegration are substrates for a subsequent round of integration, and products resulting from this reintegration activity may also be observed (30).

Mutational analysis of IN from human immunodeficiency virus type 1 (HIV-1) has identified three separate functional domains within the enzyme. The N- and C-terminal domains are required for 3′-end processing and DNA joining (12, 25, 30, 32), whereas a central core domain lacking both these regions is sufficient for disintegration (8). The central core domain contains a highly conserved D,D(35)E motif, characterized by the three acidic residues Asp-64, Asp-116, and Glu-152, which are essential for all catalytic activities of HIV-1 IN (12, 15, 22). The precise roles of the N- and C-terminal regions of HIV-1 IN in the 3′-end-processing and DNA-joining reactions are not known, but the C-terminal region binds DNA nonspecifically (16, 24, 32, 34). The N-terminal domain contains a highly conserved HHCC motif (His-12, His-16, Cys-40, and Cys-43) that resembles the zinc finger motif found in many transcription factors (21), and it has been suggested that this domain may play a role in the assembly of an active multimeric form of IN (14).

In this study we report the production and characterization of a panel of monoclonal antibodies (MAbs) against HIV-1 IN that bind to different epitopes within the IN molecule. As a complement to mutational analysis, the antibodies were used to study the relationship between structure and function of IN by observing the effects of MAb binding on the various in vitro activities of the enzyme.

MATERIALS AND METHODS

IN proteins and peptides. Purified, bacterially expressed HIV-1(HXB2) IN for immunization and screening of hybridomas was kindly provided by S. Worland, Agouron Pharmaceuticals, San Diego, Calif. (1). HIV-1(NL4-3) IN used in enzymatic activity assays was expressed in *Escherichia coli* and purified according to the method of Vincent et al. (30).

TrpE-IN fusion proteins and TrpE were expressed from plasmids obtained from V. R. Prasad, Albert Einstein College of Medicine, New York, N.Y. (pKS7 and pATH2) (28), or N. Mueller-Lantzsch, Institut für Medizinische Mikrobiologie, Universitätsklinikum, Homburg/Saar, Germany (pAVI-1) (31). TrpE-IN deletion mutants containing the N-terminal part (amino acids [aa] 1 to 102), TrpE-IN(1-102); the central part, TrpE-IN(103-185); or the C-terminal part, TrpE-IN(186-288), of HIV-1 IN were expressed from the plasmids pAVI-1N, -1M, and -1C, respectively, also provided by N. Mueller-Lantzsch (31).

Purified N-terminal deletion mutants of HIV-1 IN, IN(17-288), and IN(39-288) and bacteria expressing the C-terminal deletion mutant IN(1-250) were obtained from A. Billich, Sandoz Research Institute, Vienna, Austria (25). A recombinant peptide, IN(1-55), containing the N-terminal 55 aa of HIV-1 IN was expressed from pRL470, provided by R. L. LaFemina, Merck Research Laboratories, West Point, Pa. (6).

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TABLE 1. Characterization of anti-HIV-1 IN MAbs

MAb	Cross-reactivity with HIV-2 IN	MAb group	Epitope(s) (aa)
1C4	No	1	1–16
2C11	No	1	1–16
2E3	No	1	1–16
3E11	No	1	1–16
3F9	No	1	1–16
5F8	No	1	1–16
6G5	No	1	1–16
7B6	No	1	1–16
7C6	No	1	1–16
6C5	No	2a	17–38
8G4	No	2b	22–31 + 82–101
4D6	No	3	42–55
4F6	No	4	56–102
5D9	No	5	186–250
7C3	Yes	6	262–271
7F11	Yes	6	262–271
8E5	Yes	6	262–271

Twenty-eight synthetic peptides, IN(2-21), IN(12-31), IN(22-41), and so on to IN(282-288), covering the entire IN molecule except the N-terminal amino acid phenylalanine (3), were obtained from E. Björling and F. Chiodi, Karolinska Institute, Stockholm, Sweden.

Immunization and production of MAbs. Standard protocols were used for immunization of three female BALB/c mice with Freund's adjuvant (19). The mice were immunized three to five times with 7.5 to 15 μg of TrpE-IN in a bacterial lysate or 50 to 85 μg of purified IN. For one mouse the synthetic peptides IN(22-41), IN(32-51), IN(42-61), and IN(52-71) (10 μg of each) were included in the final booster dose. Spleen cells were fused with P3-X63-Ag8.653 or NSO mouse myeloma cells according to standard procedures (19). Hybridomas producing anti-IN antibodies were identified by screening culture supernatants against purified IN by enzyme-linked immunosorbent assay (ELISA). Positive cell lines were screened against synthetic peptides by ELISA and against TrpE-IN deletion mutants by Western blot (immunoblot). Selected hybridomas producing antibodies against different regions of IN were cloned by limiting dilution to obtain monoclonal cultures.

The MAbs obtained were isotyped with an isotyping kit from Zymed Laboratories Inc. (South San Francisco, Calif.). Before use in in vitro IN assays, the MAbs were purified from cell culture supernatants with a MAbTrapG column (Pharmacia LKB, Uppsala, Sweden) and subsequently passed over a PD-10 column (Pharmacia LKB) to change the buffer to 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), pH 7.5. The concentration of purified MAb was determined with the Bio-Rad (Richmond, Calif.) protein assay kit, and/or the optical density (OD) at 280 nm was determined with immunoglobulin G (IgG) (Sigma) as standard.

ELISA. MaxiSorp Immunoplates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with IN or deletion mutants of IN (50 to 200 ng of IN protein per well) or overnight at room temperature with synthetic IN peptides (1 μg per well). The coated wells were blocked and washed (10) and incubated with hybridoma culture supernatant (1 h, 37°C). Bound antibodies were detected after incubation with horseradish peroxidase-linked rabbit anti-mouse Ig (Dakopatts, Glostrup, Denmark) (1:1,000, 1 h, 37°C) with H_2O_2-*o*-phenylenediamine (Sigma) as substrate. The OD at 492 nm was determined.

Western blot analysis. Samples containing purified IN proteins (1 to 1.5 μg per well) or bacterial lysates containing IN or TrpE-IN proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 15% polyacrylamide gels (23) and transferred to nitrocellulose membranes (29). The membranes were blocked and washed (19) and incubated with hybridoma supernatant overnight, and this was followed by treatment with horseradish peroxidase-linked rabbit anti-mouse Ig (1:1,000; Dakopatts) for 1 h. Peroxidase activity was detected with 4-chloro-1-naphthol (Bio-Rad) as substrate.

Western blot analysis of MAbs with purified HIV-1(HXB2) or HIV-2(ROD) virions as antigen was performed with the HIV-1 (HIV blot 2.2; Diagnostic Biotechnology, Singapore, Singapore) or HIV-2 (New LAV Blot II; Diagnostics Pasteur, Marnes-La Coquette, France) Western blot kit. The HIV strips were incubated overnight with hybridoma supernatant and then with biotinylated rabbit anti-mouse Ig (1:1,000, 1 h; Dakopatts) and horseradish peroxidase-labelled streptavidin (1:1,000, 1 h; Boehringer Biochemica, Mannheim, Germany). Human HIV-1 or HIV-2 control sera (1:100) included in the kits were used as positive controls and were developed after incubation with horseradish peroxidase-labelled goat anti-human IgG (1:1,000, 1 h; Bio-Rad).

Determination of MAb effect on in vitro activities of HIV-1 IN. The effect of MAb binding on IN activity was assayed in the end-processing reaction with a standard integration substrate, in the DNA-joining reaction with a preprocessed substrate, and in the disintegration reaction with a Y-mer substrate. The oligonucleotide substrates were prepared as described previously (11, 14). The standard integration substrate consisted of two hybridized 21-mer oligonucleotides, 5′-ATGTGGAAAATCTCTAGCAGT-3′ (C220) and its complement (C120). The preprocessed substrate consisted of C120 annealed to an oligonucleotide identical to C220 except that the two 3′ residues were absent (B2-1). The Y-mer substrate consisted of the four oligonucleotides T1 (5′-CAGCAACGCAAGCT TG-3′), T3 (5′-GTCGACCTGCAGCCCAAGCTTGCGGTTCTG-3′), V1/T2 (5′-ATGTGGAAAATCTCTAGCAGGCTGCAGGTCGAC-3′), and C120. The substrates were labelled by treatment of C220, B2-1, or T1 with [γ-^{32}P]ATP and T4 polynucleotide kinase (14).

Assay conditions were identical in all three assays. Purified MAb (0.25 to 16 pmol) was preincubated with 1.0 pmol of purified IN at 25°C for 1 h in a solution containing 30 mM HEPES (pH 7.5), 10 mM dithiothreitol, 6 to 45 mM NaCl, 0.2 to 1.5 mM CHAPS {3-[(cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate}, 0.05% Nonidet P-40, and 0.02 to 0.16 mM EDTA (total volume, 5 μl). ^{32}P-labelled substrate (0.05 pmol) was added; the reaction buffer was adjusted to 25 mM HEPES (pH 7.5), 10 mM $MnCl_2$, 10 mM dithiothreitol, 20 mM NaCl, 0.05% Nonidet P-40, 0.1 to 0.75 mM CHAPS, and 0.01 to 0.08 mM EDTA; and the reaction mixture (10 μl) was incubated at 37°C for 30 min. Reaction products were subjected to electrophoresis in a 15% polyacrylamide–8 M urea gel and quantitated with a Molecular Dynamics (Mountain View, Calif.) PhosphorImager.

RESULTS

Production of MAbs. Immunization and hybridoma selection were performed with the aim of obtaining MAbs recognizing different epitopes within the IN molecule. The parent hybridomas were screened against synthetic peptides and/or deletion mutants of IN to identify a selected set of hybridomas that produced antibodies against different regions of IN. From this selection, 17 hybridomas were successfully cloned and found to be stable producers of anti-IN MAbs (Table 1).

All the MAbs were found to be of the same isotype, IgG1 with a κ light chain. Western blot analysis demonstrated that all MAbs reacted specifically with purified, bacterially expressed IN and TrpE-IN in a bacterial lysate with no detectable cross-reactivity with TrpE or other bacterial proteins. With commercially available Western blot kits, it was shown that all the MAbs also specifically recognized IN from HIV-1(HXB2) virions, whereas only three MAbs reacted with IN from HIV-2(ROD) virions (Fig. 1 and Table 1).

Epitope mapping. To identify the epitopes recognized by the

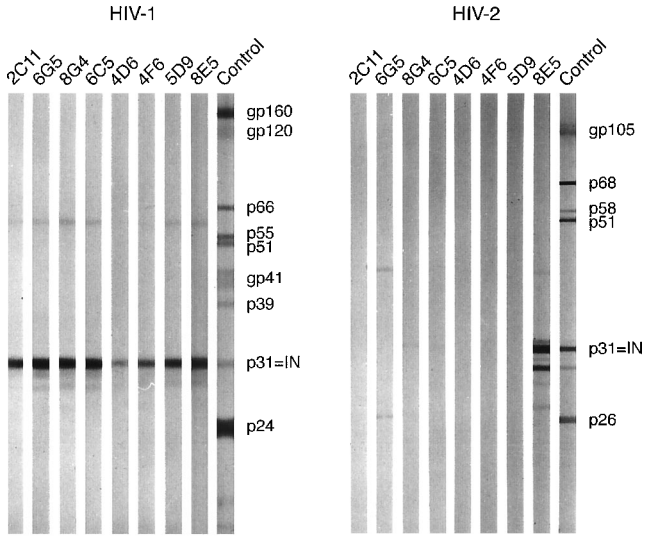

FIG. 1. Western blot analysis of HIV-1 and HIV-2 virions with selected anti-HIV-1 IN MAbs. The analysis was performed with commercially available HIV-1 and HIV-2 Western blot kits. The MAbs tested are indicated above each strip. Controls were incubated with human serum positive for HIV-1 or HIV-2.

different antibodies, several deletion mutants of IN and synthetic overlapping 20-mer oligopeptides covering the entire IN molecule (except the N-terminal phenylalanine) were used. Interactions between the MAbs and deletion mutants of IN were analyzed by Western blot and ELISA. The results are summarized in Fig. 2A. Interactions between the MAbs and the synthetic peptides were analyzed by ELISA, and results with six peptides that were recognized by some of the MAbs are summarized in Fig. 2B. The remaining 22 peptides (not included in Fig. 2B) were not recognized by any of the MAbs. According to reaction patterns with deletion mutants and overlapping peptides, the MAbs could be divided into at least six groups, as illustrated in Fig. 2.

The MAbs in group 1 (nine MAbs), group 4 (4F6), and group 5 (5D9) did not bind to any of the overlapping IN peptides. On the basis of reaction patterns with deletion mutants, these MAbs were found to bind within the region of aa 1 to 16 (group 1), 56 to 102 (group 4), or 186 to 250 (group 5).

The two MAbs in group 2 (6C5 and 8G4) gave identical reaction patterns with deletion mutants, binding only to deletion mutants that contained the region of aa 17 to 38. The two MAbs are, however, distinct, since 6C5 failed to recognize any of the synthetic peptides whereas 8G4 bound with high affinity to each of the two peptides IN(12-31) and IN(22-42) and with a lower affinity to the peptide IN(82-101) (Fig. 2B). The binding of 8G4 to the peptides IN(12-31) and IN(22-42) is consistent with an epitope located within the region of aa 17 to 38, but the additional, albeit lower, reactivity of this MAb with the peptide IN(82-101) makes it difficult to localize the epitope of this MAb unambigously.

For the MAbs in group 3 (4D6) and group 6 (7C3, 7F11 and 8E5), the results with the synthetic peptides were in agreement with the results obtained with deletion mutants. 4D6 recognized all deletion mutants containing aa 39 to 55 as well as the synthetic peptide IN(42-61), indicating that the outer limits of its epitope are aa 42 to 55. The MAbs in group 6 recognized all deletion mutants containing aa 251 to 288 and also recognized the peptides IN(252-271) and IN(262-281) with equal affinity, suggesting that the major part of the epitope of these MAbs is located in the region of aa 262 to 271.

Effects of MAbs on in vitro activities of IN. To study the effect of MAb binding on the in vitro activities of IN nine purified MAbs were selected to represent all the MAb groups identified in this study. IN was preincubated with each of these MAbs and assayed for end processing, DNA joining, and disintegration. In addition, reintegration (30) of the released viral DNA (observed as a ladder of bands between substrate and product) in the disintegration reaction was measured as a function of the amount of disintegration product produced in the reaction. All the purified MAb preparations except MAb 7C3 (MAb group 6) were free from contaminating nuclease activity, which would interfere with the assays, as determined by control reactions with the end-processing substrate in the absence of IN (data not shown). The small amount of contaminating nuclease activity present in the 7C3 preparation may have influenced the results at high MAb concentrations in some of the assays (see below).

A MAb specific for TrpE (27) was used as a control for the specificity of the MAb effects. As shown in Fig. 3 and 4, preincubation of IN with anti-TrpE did not significantly affect the activity of IN in any of the in vitro assays tested, even at concentrations equal to a 16-fold excess of MAb over IN (1,600 nM).

The effect of MAb binding on the end-processing and DNA-joining activities of IN is shown in Fig. 3. With the exception of 4F6 and 5D9, all the MAbs tested clearly inhibited both the end-processing and the DNA-joining reactions. MAbs belonging to groups 2a, 2b (6C5 and 8G4), and 6 (7C3 and 8E5) inhibited more than 85% of the IN activity when present in the reaction in a fourfold molar excess over IN (400 nM). At higher concentrations these MAbs inhibited the reactions almost completely. MAbs in group 1 (5F8 and 1C4) inhibited end processing and DNA joining less at low concentrations of MAb, but at higher concentrations ($\geq$800 nM) these MAbs also gave close to 100% inhibition. 4D6 (group 3) appeared to reach a plateau at about 75% inhibition of the end-processing and DNA-joining reactions at MAb concentrations higher than 200 nM (twofold molar excess of MAb over IN). Of the nine anti-IN MAbs tested, the two MAbs in groups 4 and 5, 4F6 and 5D9, had the smallest effects on the end-processing and DNA-joining activities of IN. Both of these MAbs affected the DNA-joining reaction less than they did the end-processing reaction.

The effect of MAb binding on disintegration and reintegration activities of IN is shown in Fig. 4. In contrast to the results with end processing and integration, none of the MAbs tested in this study inhibited the disintegration activity at concentrations up to 400 nM. Even at 1,600 nM, only three MAbs, 4D6, 4F6, and 5D9, from MAb groups 3, 4, and 5, respectively, significantly (about 40%) inhibited disintegration. Several MAbs, belonging to MAb groups 1 (1C4), 2b (8G4), 3 (4D6), and 6 (7C3 and 8E5), stimulated disintegration, with a peak in disintegration activity at MAb concentrations equal to or twofold higher than the IN concentration in the reaction (100 to 200 nM). About 40% of the substrate underwent disintegration in the absence of MAb under the reaction conditions used to obtain the results depicted in Fig. 4. Under reaction conditions under which the level of disintegration activity was lower, the stimulatory effect of MAb binding was even more pronounced (results not shown).

Of all the IN activities tested, reintegration activity most clearly differentiated between MAbs binding to different parts of the IN protein (Fig. 4). MAbs belonging to groups 1, 2a, and 2b, binding within the first 38 aa from the N terminus (5F8, 1C4, 6C5, and 8G4), either stimulated or had no significant effect on the reintegration activity. The two MAbs in groups 4 and 5, 4F6 and 5D9, also stimulated reintegration activity. In contrast, the only MAb in group 3 (4D6, epitope within aa 42 to 55) and the two MAbs in group 6 (7C3 and 8E5) binding to an epitope close to the C terminus (aa 262 to 271) significantly inhibited the reintegration activity. 4D6 resulted in only partial inhibition of reintegration, reaching a plateau of inhibition of about 50% at a MAb concentration of 400 nM. MAbs in group 6, however, gave close to complete inhibition of reintegration activity, as exemplified by MAb 8E5. The apparent increase in reintegration activity observed at higher concentrations of 7C3, the other MAb in group 6, is likely to be an artifact due to the presence of contaminating nuclease in the purified preparation of this MAb.

DISCUSSION

The MAbs described in this study were produced and selected with the aim of obtaining a panel of MAbs binding to a wide range of epitopes within the HIV-1 IN molecule. The epitopes on IN recognized by the MAbs were mapped by using both deletion mutants and overlapping synthetic peptides to identify a smaller set of MAbs that would be useful for investigating the relationship between structure and function of IN.

Of the 17 MAbs obtained in this study, 12 recognized four different epitopes included within the first 55 aa from the N terminus and 3 bound to an epitope in the region of aa 262 to 271 near the C terminus. Only one MAb, 4F6, recognized an

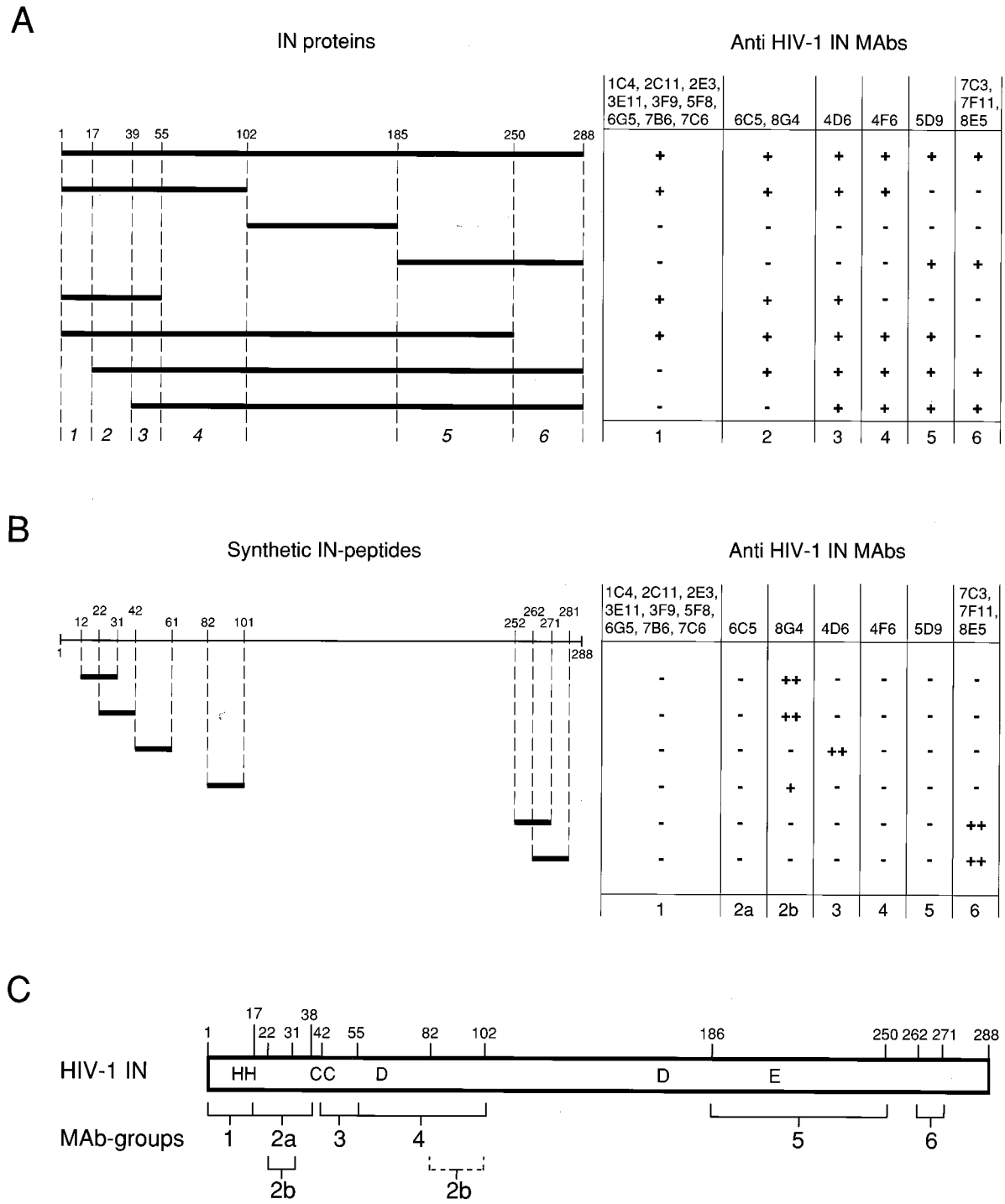

FIG. 2. Epitope mapping of anti-HIV-1 MAbs. (A) Epitope mapping with IN deletion mutants. The bars in the left part of the figure represent the IN proteins used, with the numbers at the top indicating the amino acid positions in HIV-1 IN. The full-length IN protein, aa 1 to 288, was tested both with and without a TrpE fusion partner. The deletion mutants (aa 1 to 102, 103 to 185, and 186 to 288) were tested as TrpE-IN fusion proteins, while the remaining mutants were nonfused proteins. All IN proteins were tested by both Western blot and ELISA. The MAbs were grouped according to their reaction patterns with the deletion mutants as shown in the right part of the figure, and the groups are numbered at the bottom right. Each MAb group recognizes an epitope within the region identified by the same number in italics in the bottom left part of the figure. (B) Epitope mapping with 20-mer synthetic peptides. Only peptides that gave a positive reaction with some of the MAbs are shown. The peptides were tested by ELISA, and the MAbs tested are indicated at the top right. Relative absorbance values are given as follows: ++, OD > 2.4; +, OD ≈ 1.5; −, OD < 0.5. Native IN gave an OD of >2.5 for all the MAbs. Controls without MAb gave an OD of <0.2 for all the peptides. As in panel A, the numbers at the bottom right correspond to MAb groups. The synthetic peptides allowed distinction between the two MAbs in group 2 (group 2a and 2b) and a more narrow definition of the epitopes of the MAbs in groups 3 and 6. (C) Schematic representation of binding regions for different MAb groups on a linear map of HIV-1 IN protein. The positions of the conserved amino acid residues of the HHCC motif and the D,D(35)E motif are shown. The numbers above the illustration of the IN protein indicate amino acid positions. The epitope regions recognized by the different MAb groups are indicated below the IN protein. The MAb in group 2b recognized synthetic peptides representing two separate regions of the IN protein. The broken lines used to delineate one of these regions indicate a region that was detected when mapping with peptides but was not confirmed when mapping with deletion mutants.

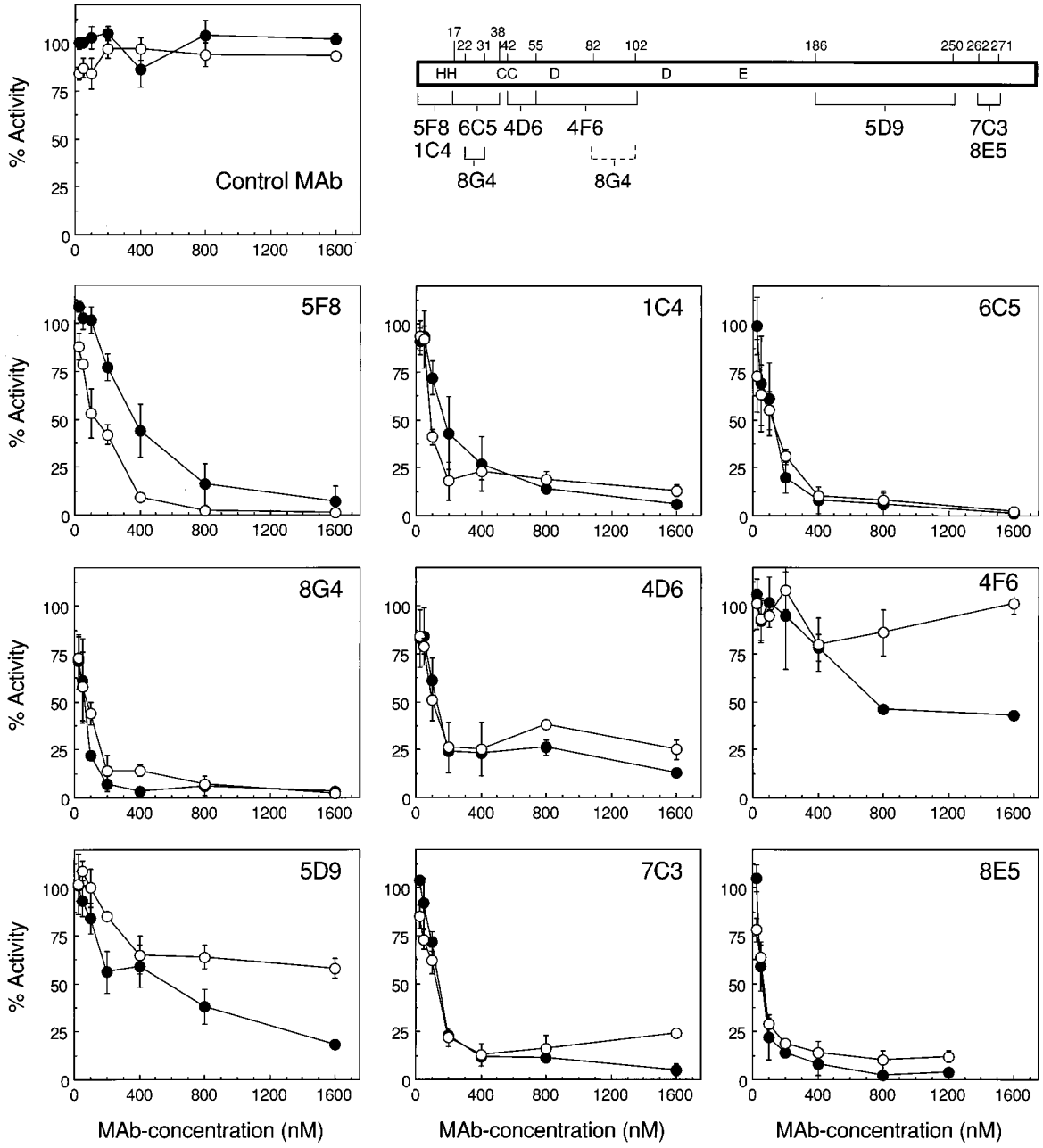

FIG. 3. Effect of selected anti HIV-1 IN MAbs on end-processing (solid circles) and DNA-joining (open circles) activities of IN. End-processing activity was assayed with the standard blunt-end DNA substrate, and DNA-joining activity was assayed with the preprocessed substrate. The MAbs tested are indicated in each panel. The epitopes recognized by these MAbs are illustrated at the top right. A MAb specific for the TrpE protein was tested as a control for the specificity of the MAb effects (Control MAb). The effect of MAb binding on IN activity was measured relative to a control reaction without MAb (100% activity). The curves are based on the averages of at least three independent measurements at each concentration, and the standard deviation bars are shown. The IN concentration in the reaction mixtures was 100 nM, and the MAbs were tested in concentrations ranging from 25 to 1,600 nM.

epitope (aa 56 to 102) within the central core domain of IN. A similar distribution of epitopes was found with a panel of anti-IN MAbs characterized by Bizub-Bender et al. (2), suggesting that the extreme ends of the IN polypeptide are the most immunogenic. Three MAbs in this study, all recognizing an epitope in the region of aa 262 to 271 of HIV-1(HXB2) IN, cross-reacted with IN from HIV-2(ROD). Comparison of the amino acid sequences revealed that the sequence of IN in the region of aa 262 to 271 was identical in the two species, which would explain this cross-reactivity. The amino acid sequences of all the other epitopes recognized by MAbs in this study were less conserved, with several amino acid differences between the two species of HIV IN that could account for the lack of cross-reactivity.

To investigate the relationship between structure and function of IN, a panel of nine MAbs representing all the MAb groups identified in this study were tested for their effect on the end-processing, DNA-joining, disintegration, and reintegration activities of IN. Five of the MAbs tested, 5F8, 1C4, 6C5, 8G4, and 4D6, recognized four different epitopes (aa 1 to 16,

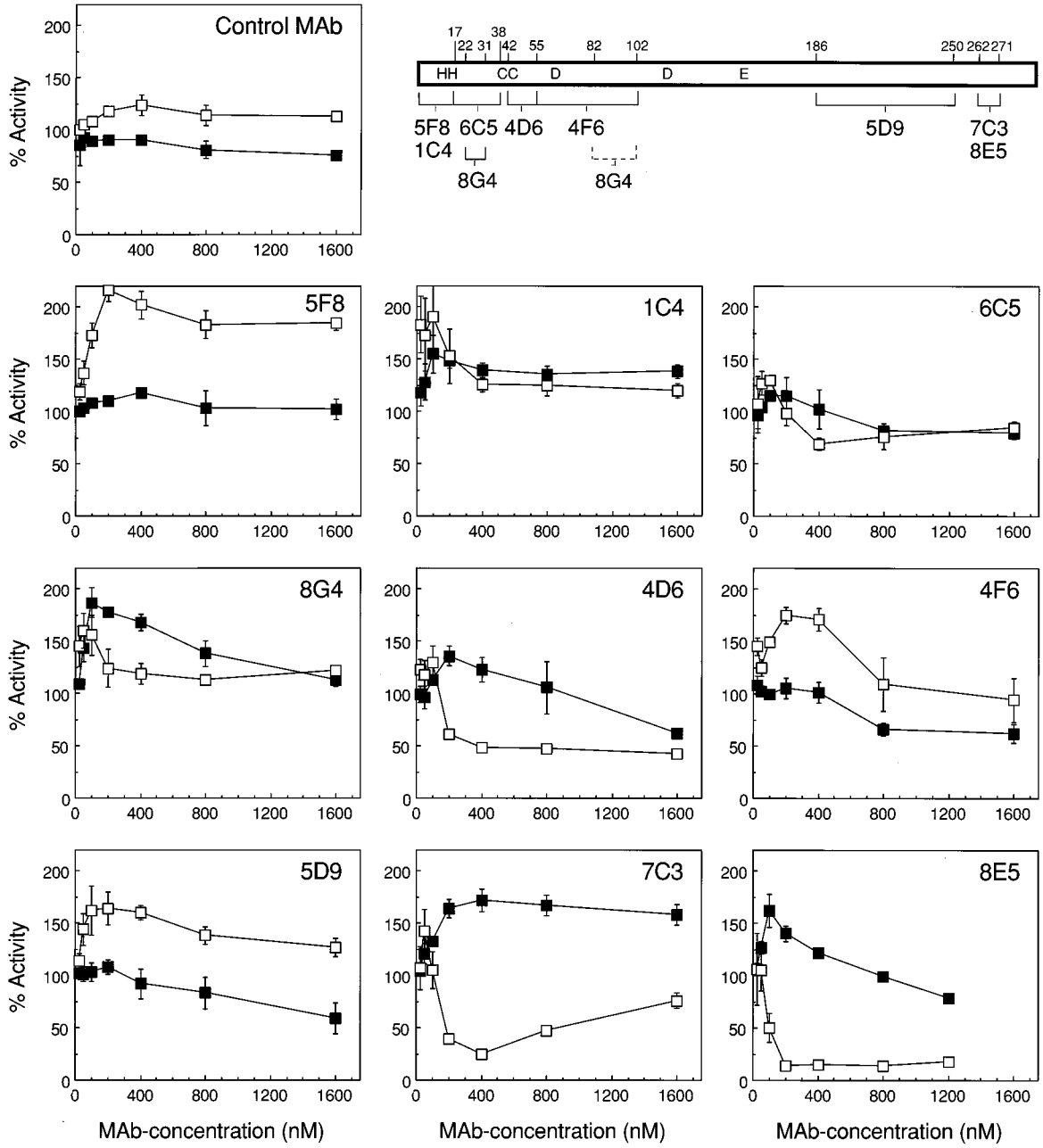

FIG. 4. Effect of selected anti-HIV-1 IN MAbs on disintegration (solid squares) and reintegration (open squares) activities of integrase. Disintegration and reintegration activities were determined with the Y-mer substrate. Reintegration activity was measured in the disintegration reaction by determining the amount of reintegration product (observed as a ladder of bands between disintegration substrate and product) as a function of the amount of disintegration product produced in the reaction. Other conditions and labelling are as described in the legend to Fig. 3.

17 to 38, 22 to 31, and 42 to 55) that at least partly overlap with the conserved HHCC domain (aa 12 to 43). With the possible exception of 4D6, all these MAbs completely inhibited end processing and DNA joining and either stimulated or had little effect on the disintegration and reintegration activities of IN. 4D6 showed a similar profile but with less-than-complete inhibition of end processing and DNA joining and, more significantly, 50% inhibition of reintegration. These results are in agreement with results obtained from mutational analysis of the HHCC region of IN. Deletion of between 23 and 50 aa from the N terminus (32) or introduction of point mutations in the conserved histidine or cysteine residues in this region (15, 30) completely abolished both end-processing and DNA-joining activities, whereas disintegration and reintegration activities were maintained at or close to wild-type levels. Mutations in or binding of MAbs to the HHCC domain thus appears to destroy the ability of this domain to perform a function that is required for end-processing and DNA-joining activities but is dispensable for disintegration and reintegration.

It has been suggested that the HHCC domain is required for assembly of an active multimeric form of IN, by virtue of protein-protein interactions between the HHCC domain in

one IN promoter and an *N*-ethylmaleimide-sensitive site in another (14). This HHCC domain-dependent multimerization is proposed to serve to extend the enzyme's binding sites for viral DNA, thus ensuring the formation of a stable IN-viral DNA complex which is required for the end-processing and DNA-joining reactions to occur (13). Since disintegration and reintegration are less dependent upon an active HHCC domain, this model would imply that the HHCC-dependent multimerization is not required for these activities. Our results with the MAbs that bind to the HHCC domain are compatible with the model of HHCC-dependent multimerization, but other possible explanations for the observed effects cannot be excluded at this time.

Two of the MAbs tested in this study, 7C3 and 8E5, bound to an epitope in the region of aa 262 to 271, near the C-terminal end of IN. These MAbs completely inhibited end processing and integration, stimulated or had little effect on disintegration, and in contrast to MAbs binding to the HHCC domain, nearly completely inhibited reintegration. The differences observed between the effects of 7C3 and 8E5, especially on disintegration and reintegration activities at higher MAb concentrations, can be explained by the presence of small amounts of contaminating nuclease in the purified 7C3 preparation. The results with these two MAbs are in agreement with results from deletion analysis of the C-terminal region of IN (32). Deletion of aa 271 to 288 was shown to have little effect on in vitro IN activities, whereas removal of aa 257 to 288 led to complete loss of end-processing, DNA-joining, and reintegration activities and a reduction (but not complete loss) of disintegration activity. A triple mutation in the amino acids R-262, R-263, and K-264 has further been shown to abolish end-processing and DNA-joining activities of IN (24). The region of aa 262 to 271 thus appears to be essential for end processing, integration, and reintegration but dispensable for disintegration.

The C-terminal region of IN contains a nonspecific, metal-independent DNA-binding domain within the region of aa 210 to 270 (16, 32, 34), and the three amino acids R-262, R-263, and K-264 have been shown to be essential for DNA binding (24). Engelman et al. (16) have presented evidence that in addition to containing this dominant DNA-binding domain, the core domain of IN contains an additional nonspecific but metal-dependent DNA-binding domain which binds only to the branched disintegration substrate (Y-mer). Taken together, these results may explain our results with the MAbs 7C3 and 8E5, which bind within the region of aa 262 to 271. If the region between aa 262 and 271 is required for binding of the linear DNA substrates mimicking the viral DNA ends but is dispensable for binding of the branched disintegration substrate, binding of MAbs to this region would lead to the observed complete inhibition of end processing, integration, and reintegration, whereas disintegration would be much less affected. If product release is rate limiting in disintegration, the increased release of the linear viral DNA product might lead to the stimulation of disintegration that is observed at lower concentrations of MAbs.

The last two MAbs tested in this study, 4F6 and 5D9, bound to epitopes within the regions of aa 56 to 102 and 186 to 250, respectively. With the possible exception of their effect on reintegration, both these MAbs had small effects on in vitro IN activities. The epitopes of these MAbs are defined within relatively wide limits and may be located in regions that have no specific function in integration or disintegration.

In conclusion, analyses of binding of MAbs to epitopes in the HHCC domain and the C-terminal DNA-binding domain of IN have given results that are in agreement with results from mutational analysis of the protein, supporting the assumption that the effects of mutations in these regions are not due to global conformational changes in other parts of the IN protein. The results obtained with MAbs binding to the HHCC domain and the C-terminal DNA-binding domain are compatible with a model in which their binding to these domains leads to inhibition of multimerization of integrase or inhibition of binding of linear viral DNA substrates, respectively. There are, however, many other possible explanations for the observed effects that cannot be excluded at this time. It should also be noted that binding of a MAb to a specific epitope could block a larger region around the epitope and possibly interfere with the function of other parts of the protein. It has, e.g., been shown that MAbs binding to epitopes within the N-terminal domain of IN can block the binding of MAbs to epitopes within the C-terminal region (2). The observation that the MAbs binding to the HHCC domain had an effect on reintegration activity different from that of MAbs binding near the C terminus suggests that if such an effect is important it still reflects distinct specialized functions of different regions of the IN molecule.

The MAbs presented in this study may prove to be valuable reagents for further studies of HIV-1 IN. In addition to their use for detection of IN in vitro by ELISA or Western blot, preliminary results indicate that many of the MAbs may be useful for detection of IN in infected or transfected cells. A subset of the MAbs form complexes with IN that are more soluble than IN alone (unpublished results) and may be used to facilitate crystallization of HIV-1 IN. Finally, MAbs binding to different regions of the IN molecule resulted in differential effects on various in vitro activities of the enzyme, and they may thus prove useful for further studies of the relationship between structure and function of this important viral enzyme.

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