Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 9598–9602, October 1992
Biochemistry

Mutational analysis of the integrase protein of human immunodeficiency virus type 2

(retrovirus/integration)

DIK C. VAN GENT, ANTOINETTE A. M. OUDE GROENEGER, AND RONALD H. A. PLASTERK*

Netherlands Cancer Institute, Division of Molecular Biology, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Communicated by Martin Gellert, July 9, 1992

ABSTRACT **Purified integrase protein (IN) can nick linear viral DNA at a specific site near the ends and integrate nicked viral DNA into target DNA. We have made a series of 43 site-directed point mutants of human immunodeficiency virus type 2 IN and assayed purified mutant proteins for the following activities: site-specific cleavage of viral DNA (donor cut), integration (strand transfer), and disintegration. In general, the different activities were similarly affected by the mutations. We found three mutations that (almost) totally abolished IN function: Asp-64 → Val, Asp-116 → Ile, and Glu-152 → Leu, whereas 25 mutations did not affect IN function. A few mutations affected the different activities differentially. Near the amino terminus a zinc finger-like sequence motif His-Xaa$_3$-His-Xaa$_{20-30}$-Cys-Xaa$_2$-Cys is present in all retroviral IN proteins. Two mutations in this region (His-12 → Leu and Cys-40 → Ser) strongly inhibited donor cut but had less effect on strand transfer. The central region of IN is most highly conserved between retroviral INs. Three mutants in this region (Asn-117 → Ile, Asn-120 → Leu, and Lys-159 → Val) were inhibited in strand transfer but were inhibited less strongly in donor cut. Mutation of Asn-120 (to glycine, leucine, or glutamate) resulted in changes in integration-site preference, suggesting that Asn-120 is involved in interactions with target DNA. We did not find a mutant in which one activity was lost and the others were unaffected, supporting the notion that IN has only one active site for the catalysis of donor cut and strand transfer.**

An important step in the life cycle of all retroviruses is integration of the viral cDNA into the host-cell genome. Integration is essential for production of additional viral particles (1). The integration reaction has been studied in detail *in vitro* (2–5). After reverse transcription the integrase protein (IN; encoded by the 3′ one-third of the viral *pol* gene) binds to the linear, double-stranded viral DNA. IN removes a few (usually two) nucleotides from both ends of the viral cDNA by hydrolysis of the phosphodiester bond 3′ of a conserved CA dinucleotide (6–12). Subsequently the newly generated viral 3′-hydroxyl DNA ends are transferred to phosphates in the target DNA. In one concerted reaction the phosphodiester bond in the target DNA is broken, and a new one is generated between viral DNA and target DNA (13). Both ends are integrated in a coordinated fashion, in such a way that small single-stranded gaps are left between the viral DNA and host DNA. These gaps are then filled in, probably by cellular enzymes, resulting in a target-DNA duplication.

Recombinant IN can accomplish several reactions *in vitro*: IN mediates (*i*) site-specific cleavage of double-stranded DNA molecules that represent the blunt ends of the viral DNA (donor cut; refs. 6–8, 10, and 11), (*ii*) integration of one viral DNA end into another DNA molecule (strand transfer; refs. 6, 7, and 14), and (*iii*) the reverse reaction of integration, called disintegration (15). The substrate requirements for cleavage and integration have been determined: a conserved 5′-CA-3′ sequence near the end of the DNA is necessary, but not sufficient, for efficient cleavage and integration (16–18). Although the substrate requirements of IN are known in detail, it is not yet clear which parts of IN are required for the different steps in the integration reaction. Near the amino terminus of the 32-kDa IN, a conserved sequence motif is found: His-Xaa$_3$-His-Xaa$_{20-30}$-Cys-Xaa$_2$-Cys; this has been referred to as a putative zinc finger (19). There is some evidence that this region is involved in recognition of specific long terminal repeat sequences (20). The middle part of the protein (position 113–162) is most strongly conserved between retroviral INs and also shows homology to transposase proteins of bacterial insertion sequences (20, 21). Analysis of mutant INs revealed amino acids in this region that are essential for IN activity (22).

We describe here a series of 41 site-directed point mutants of human immunodeficiency virus type 2 (HIV-2) IN. Most mutants are equally affected in all three reactions (donor cut, strand transfer, and disintegration), suggesting that the 32-kDa IN carries out all these reactions with a single active site. In a few mutants the different activities were differentially affected, suggesting that the putative zinc-finger region near the amino terminus may be primarily required for donor cut and less for strand transfer and that some amino acids in the central region (Asn-117, Asn-120, and Lys-159) may primarily play a role in strand transfer. Three mutations (in Asp-64, Asp-116, and Glu-152) resulted in inactive proteins that could, however, still bind DNA; any of these might be involved in catalysis of donor cut and strand transfer.

MATERIALS AND METHODS

DNA Techniques. Standard DNA techniques were done as described (23). For site-directed mutagenesis the pMa/c system was used (24). The *Eco*RI–*Hin*dIII fragment of pRP279 (25), containing the HIV-2 IN gene under the transcriptional control of the bacteriophage T7 promoter, was cloned into vector pMc 5-8, resulting in plasmid pRP621. Heteroduplexes were made of single-stranded DNA of pRP621 and the *Eco*RI–*Hin*dIII vector fragment of pMa 5-8. To create single-site mutants we used oligonucleotides that introduced the desired triplet into the HIV-2 IN gene. In addition, a restriction enzyme recognition site was added for facilitated identification of mutants. The sequences of the oligonucleotides are available from the authors upon request.

Production, Purification, and Immobilization of HIV-2 IN. Isolation of HIV-2 IN has been described (25). We modified

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HIV, human immunodeficiency virus; IN, integrase protein. Mutants are named as follows: the first letter signifies the amino acid in the wild-type HIV-2 IN, followed by the position and the amino acid substituted for the wild-type amino acid.

*To whom reprint requests should be addressed.

this procedure for rapid isolation of small quantities of mutant proteins. IN was expressed in *Escherichia coli* BL21(DE3) (26), harboring the lysozyme-expressing plasmid pLysS. For a typical protein isolation IN was extracted from a pellet of a 50-ml culture, as described (25). The Dounce supernatant (containing IN) was mixed with 0.5 ml of a 50% (vol/vol) suspension of butyl-Sepharose 4B (Pharmacia) in Dounce buffer (20 mM Tris·HCl, pH 7.5/1 M NaCl/2 mM dithiothreitol/1 mM EDTA) and incubated for 1 hr. The beads were washed twice with Dounce buffer and once with Dounce buffer without dithiothreitol. Proteins were eluted with 0.7 ml of elution buffer (20 mM Tris·HCl, pH 7.5/150 mM NaCl/1 mM EDTA/0.5% Nonidet P-40), and additional protein was eluted with 0.7 ml of elution buffer with 100 mM NaCl instead of 150 mM. Both elution fractions were mixed, and NaCl was added to a final concentration of 1 M.

To the protein preparations ≈1 μl of a 50% suspension of thiopropyl-Sepharose 6B (Pharmacia) in Dounce buffer without dithiothreitol was added per pmol of IN. The mixture was stirred for at least 2 hr at 4°C. The beads were washed with Dounce buffer and twice with 20 mM Mops, pH 7.2/1 mM dithiothreitol and then resuspended as a 50% suspension in this buffer. The immobilized IN was stored at −70°C. Freezing causes a decrease in activity, but once frozen the protein remains stable (at least for a few months; data not shown).

Assays for IN Activity. The cleavage and integration assay was done as described (25). Immobilized IN was incubated for 1 hr at 30°C in a 10-μl reaction mixture containing 20 mM Mops (pH 7.2), 25 mM NaCl, 1 mM $MnCl_2$ (or 5 mM $MgCl_2$ when indicated), 3 mM dithiothreitol, 0.2 pmol of oligonucleotide substrate (double-stranded 28-mer HIV-2 U5 end; Fig. 1*A*), and 3 pmol of immobilized IN. The actual HIV-2 U5 DNA end probably has the sequence 5′-CAGGT-3′ instead of 5′-CAGG-3′ (27). However, we previously showed that the substrate we used is efficiently cleaved and integrated by HIV-2 IN (25).

The integration assay was done in the same buffer containing 0.2 pmol of recessed HIV-2 U5 end (Fig. 1*A*, substrate 2). After 15-min incubation at 30°C, 5 pmol of double-stranded HIV-2 U5 substrate (or, when indicated, of the randomly chosen oligonucleotide 5′-GCGTGTATGAATC-CGTCGAGAGCTACTA-3′ hybridized to its complementary strand) was added to provide additional target DNA for integration, and incubation was continued overnight at 30°C. Note that we cannot formally exclude that mutant INs that

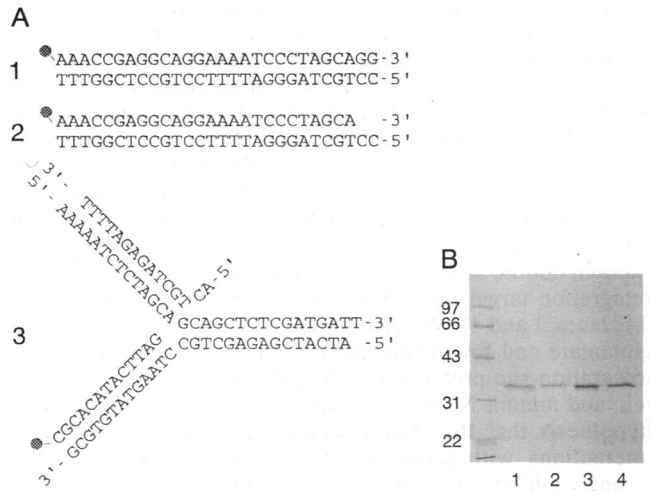

FIG. 1. (*A*) Substrates used in cleavage (substrate 1), integration (substrate 2), and disintegration assays (substrate 3). Position of the ^{32}P label is depicted by a dot. (*B*) SDS/PAGE analysis of wild-type HIV-2 IN (lane 1) and mutants D64V, D116I, and E152L (lanes 2–4). Positions of molecular weight markers (× 10^{-3}) are indicated at left.

are slower than wild type may reach the same level of strand-transfer products after this prolonged incubation; these we would then score as wild type for integration.

The disintegration assay (15) was done under the same conditions as the cleavage and integration assay. As DNA substrate 0.2 pmol of a Y-shaped oligonucleotide was used (Fig. 1*A*, substrate 3).

RESULTS

Purification and Properties of HIV-2 IN. HIV-2 IN was expressed in and purified from *E. coli*. We developed a quick, small-scale isolation procedure for HIV-2 IN (see *Materials and Methods*). The protein was immobilized on the solid-support thiopropyl-Sepharose. After immobilization, IN can still cleave viral DNA and mediate strand transfer and disintegration (Fig. 2 *A–C*, lane 2). Immobilized HIV-2 IN is active for 4–8 hr at 30°C, whereas soluble HIV-2 IN loses most of its activity within 1 hr (data not shown).

Generation and Purification of Mutant Proteins. The HIV-2 IN gene under control of the bacteriophage T7 promoter was cloned into phasmid vector pMc 5-8. By site-directed mutagenesis we made 41 mutants in which one triplet was changed. Most mutations were made in charged or polar amino acids that are conserved among retroviral INs because they are more likely to be involved in interactions with DNA than hydrophobic residues, which often have a more structural function in the protein.

All mutant proteins were isolated in the same way as the wild-type HIV-2 IN. Five mutants were not soluble in 1 M NaCl (H16L, C65V, E87L, F121V, and S123L; Table 1). We conclude that they are probably folding mutants; they were not further analyzed. Mutant S81A had a lower solubility in 1 M NaCl than wild-type HIV-2 IN. We, therefore, used 10 times more induced *E. coli* culture for isolation of this mutant protein. Mutants H12L, C40S, and C43L had a lower affinity for butyl-Sepharose than wild-type protein. However, we could obtain a sufficient amount of protein to do the assays. All other mutants had purification properties similar to wild-

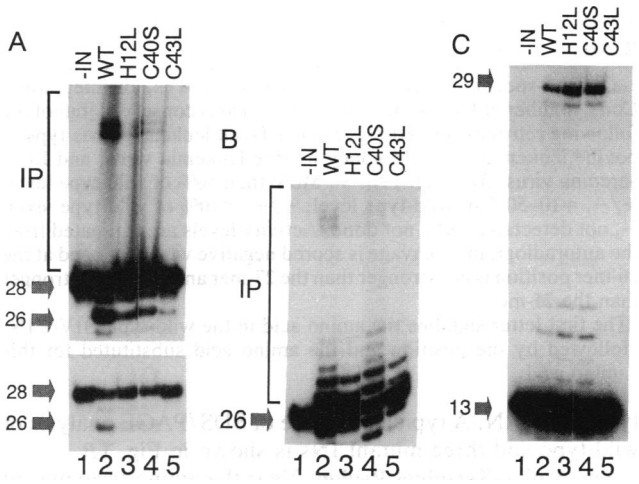

FIG. 2. Mutational analysis of the amino-terminal region. Cleavage (*A*), integration (*B*), and disintegration (*C*) assays of wild-type (WT) IN (lane 2) and mutants H12L, C40S, and C43L (lanes 3–5). (*A Lower*) Shorter exposure of *A Upper*; *B* shows integration of precleaved DNA in an overnight integration reaction with excess target DNA. (*A Upper*) Strand transfer that follows donor cut of a blunt-ended DNA in a 1-hr reaction. Position of 28-mer substrate and 26-mer cleavage product in *A*, 26-mer substrate in *B*, and 13-mer substrate and 29-mer disintegration product in *C* are indicated by arrows. IP, products resulting from integration of one oligonucleotide into another. Lane 1, without IN.

Table 1. Point mutants of HIV-2 IN

Mutant*	Cl	Int	Dis	Cons	Remarks
H12L	−/+	+/−	+	5	
H16L	ND	ND	ND	5	Insoluble
S39A	+	+	+	3	
C40S	−/+	+	+	5	
C43L	−/+	−/+	+/−	5	
K46L	+	+	+	1	
Q53L	+	+	+	1	
D64V	−	−	−	5	
C65V	ND	ND	ND	1	Insoluble
T66A	+	+	+	5	
S81A	+	+	+	5	Low solubility
E87L	ND	ND	ND	1	Insoluble
S93A	+	+	+	5	
K103Q	+	+	+	1	
R107L	+	+	+	4	
H114S	+	+	+	1	
T115V	+	+	+	5	
D116I	−	−/+	−	5	
N117I	+	+/−	+	4	Inactive with Mg^{2+}
N120L	+	+/−	+	2	Inactive with Mg^{2+}
F121V	ND	ND	ND	3	Insoluble
S123L	ND	ND	ND	5	Insoluble
K127T	+	+	+	2	
P142V	+	+	+	4	
Y143L	+	+	+	4	
N144V	+	+	+	4	
S147A	+	+	+	4	
Q148L	−/+	−/+	+	2	
E152L	−	−	−	5	
K159V	+	+/−	+	5	Inactive with Mg^{2+}
R166L	+	+	+	3	
K186Q	+	+	+	4	
R199S	+	+	+	1	
T206A	+	+	+	3	
K219N	+	+	+	1	
R228I	+/−	+/−	+	3	
K236S	+	+	+	3	
K244S	+	+	+	1	
K258L	+	+	+	1	
R263S	+	+	+	1	
R269I	+	+	+	3	

Cl, site-specific cleavage; Int, integration; Dis, disintegration; Cons, number of INs in which the amino acid is conserved [out of the following retroviruses: HIV-1, human T-cell leukemia virus type 1, bovine leukemia virus, Moloney murine leukemia virus, and Rous sarcoma virus (from ref. 19)]. +, More than 50% of wild-type level; +/−, ≈10–50% of wild-type level; −/+, <10% of wild-type level; −, not detectable; ND, not done. Activity levels are estimated from the autoradiogram. Cleavage is scored negative when the band at the 26-mer position is not stronger than the 27-mer and not much stronger than the 25-mer.

*The first letter signifies the amino acid in the wild-type HIV-2 IN, followed by the position and the amino acid substituted for this amino acid.

type HIV-2 IN. A typical example of SDS/PAGE analysis of wild-type and three mutant INs is shown in Fig. 1*B*.

The Amino-Terminal Region. Near the amino terminus all retroviral INs have a conserved motif His-Xaa_3-His-Xaa_{20-30}-Cys-Xaa_2-Cys. This motif somewhat resembles zinc fingers found in several DNA-binding proteins (19). Zinc fingers are often involved in specific DNA binding. However, IN does not have a much higher affinity for specific than for aspecific DNA (25). We constructed mutants in all four histidine and cysteine residues. Mutants H12L and C40S are inhibited in donor cut (Fig. 2*A*, lanes 3 and 4). However, the strand-transfer activity of C40S is close to wild type, and H12L is much less inhibited in strand transfer than in donor cut (Fig. 2*B*, lanes 3 and 4). Additionally, both mutants mediate disintegration at wild-type levels (Fig. 2*C*, lanes 3 and 4). These results show that His-12 and Cys-40 are important for donor cut but not so important for strand transfer and disintegration. Mutant C43L was inhibited in donor cut, strand transfer, and disintegration (Fig. 2 *A–C*, lane 5), suggesting that Cys-43 is important for all IN functions. We could not assay mutant H16L because this mutant was not soluble in 1 M NaCl; it probably has an altered structure. Mutations in two other amino acids, close to both cysteines (S39A and K46L) resulted in approximately wild-type levels of donor cut, strand transfer, and disintegration (Table 1). These results suggest that the putative zinc-finger region is not involved in catalysis of strand transfer but may play a role in the correct positioning on viral DNA ends for donor cut. The differences in purification properties and catalytic activities of mutants in these histidine and cysteine residues indicate that these residues do not have an equivalent role in a zinc-finger structure, and we consider it unlikely that, in fact, this is a real zinc-finger domain.

The Central Region. The most strongly conserved region in retroviral INs is the middle part, approximately from position 113 to 162. This region also shows homology with INs of retrotransposons and transposases of bacterial insertion sequences (20, 21). The most strongly conserved amino acids are Asp-116 and Glu-152. The amino acids around these two acidic residues and the spacing between them are also highly conserved.

Mutant D116I does not cleave or disintegrate and mediates strand transfer at a very low level (Fig. 3 *A–C*, lane 4). Mutation of Glu-152 results in a protein that is inactive (E152L in Fig. 3 *A–C*, lane 8). Mutant Q148L is partially inhibited (Fig. 3 *A–C*, lane 7). However, not all conserved amino acids in this region appear essential for donor cut, strand transfer, and disintegration *in vitro*: mutant T115V is approximately as active as wild-type HIV-2 IN (Fig. 3 *A–C*, lane 3). INs containing a mutation in an amino acid located between the most conserved regions around Asp-116 and Glu-152 (K127T, P142V, Y143L, N144V, and S147A) show wild-type levels of activity (Table 1). In all these mutants the different activities were similarly affected.

We found three mutants in which one activity was more affected than the other: N117I, N120L, and K159V mediate the donor cut at nearly wild-type levels (Fig. 3*A*, lanes 5, 6, and 9), whereas strand transfer is clearly inhibited in the 1-hr cleavage and integration reaction. However, in the overnight integration reaction with precleaved viral DNA the amount of integration products in the reaction with these mutants is only slightly less than with wild-type IN (Fig. 3*B*, lanes 5, 6, and 9). A possible explanation for this phenomenon is that the amino acids Asn-117, Asn-120, and Lys-159 might be involved in interactions with the target DNA. Interestingly, in addition to its reduced strand-transfer efficiency, mutant N120L has a somewhat altered integration-site preference (compare Fig. 4*A*, lanes 1 and 2). This mutant also shows a difference in the pattern of integration products, when a random double-stranded 28-mer oligonucleotide was used as integration target instead of HIV-2 U5 ends (compare Fig. 4*A*, lanes 3 and 4). We also mutated Asn-120 to glycine and glutamate and found that both mutant proteins showed an integration-site preference different from the wild-type protein and mutant N120L (Fig. 4*B*). These results support the hypothesis that the region around Asn-120 is involved in interactions with target DNA. Mutant Q148L, which is strongly inhibited in both donor-cut and strand transfer, also seems to have a somewhat different integration-site preference (more +2 than +1 product in Fig. 3*B*, lane 7).

The different activities of some transposases [such as bacteriophage Mu transposase (28)] are differentially affected by different divalent cations. Therefore, all cleavage reac-

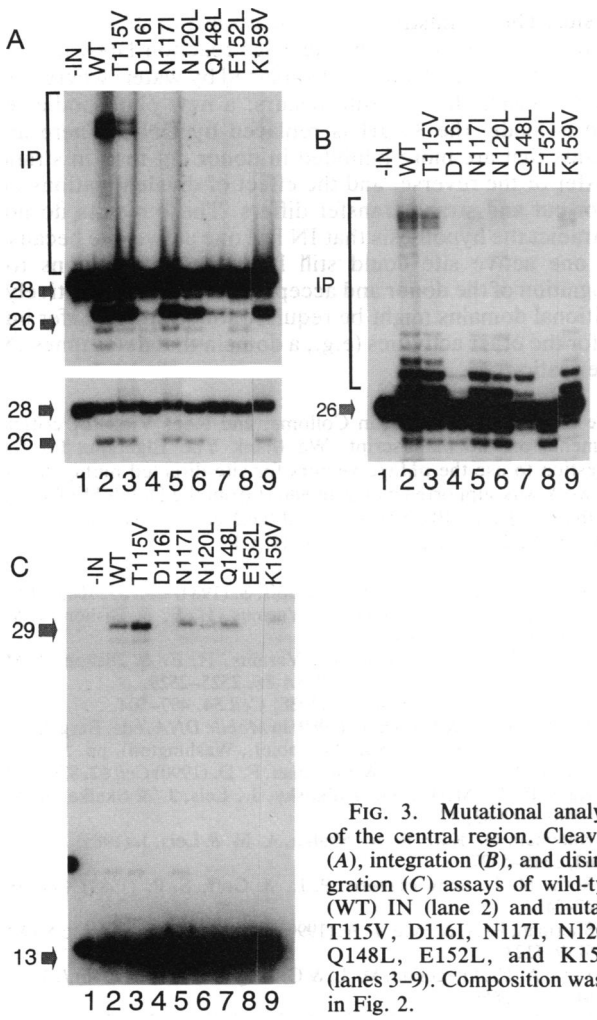

FIG. 3. Mutational analysis of the central region. Cleavage (*A*), integration (*B*), and disintegration (*C*) assays of wild-type (WT) IN (lane 2) and mutants T115V, D116I, N117I, N120L, Q148L, E152L, and K159V (lanes 3–9). Composition was as in Fig. 2.

tions were also assayed with Mg^{2+} as divalent cation instead of Mn^{2+}. Wild-type HIV-2 IN cleaves slightly less efficiently with Mg^{2+} than with Mn^{2+}, and strand transfer is not carried out with Mg^{2+} (data not shown). Like wild-type HIV-2 IN, most mutants showed a slightly lower cleavage efficiency with Mg^{2+} than with Mn^{2+} as divalent cation. However, mutants N117I, N120L, and K159V mediate donor cut much less efficiently with Mg^{2+} than Mn^{2+} (data not shown).

Other HIV-2 IN Mutants. We also made mutations in the less conserved parts of the HIV-2 IN gene. Between the putative zinc finger and the central region we found one mutant, D64V, that was inactive in all assays (Fig. 5 *A–C*, lane 3). Other mutations in this region resulted in wild-type levels of donor cut, strand transfer, and disintegration (Table 1).

Mutant S81A was less soluble in 1 M NaCl, suggesting that a (large) proportion of the protein might be incorrectly folded or unstable. However, the small amount of protein that could be purified was as active as wild-type HIV-2 IN (Fig. 5*A*, lane 4). In the overnight integration reaction the level of strand transfer was somewhat lower than wild type (Fig. 5*B*, lane 4), suggesting that this mutant protein is less stable than wild-type HIV-2 IN. In addition, the pattern of integration products differs slightly. This difference might be the result of a small distortion in the protein structure. Alternatively, Ser-81 might play a role in recognition of target DNA. Ser-81 is certainly not a residue that plays an essential role in donor cut or strand transfer.

The carboxyl-terminal region is much less conserved between retroviral INs than the rest of the protein. Most mutants that contain a single-site mutation in this region are

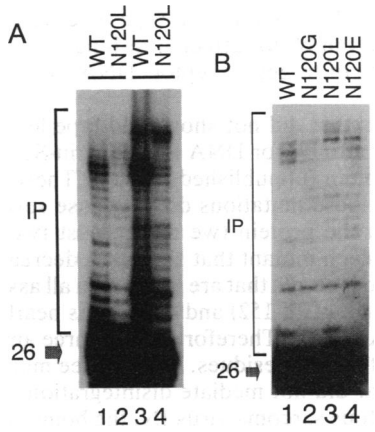

FIG. 4. Integration-site preference of mutants in Asn-120. (*A*) Integration mediated by mutant N120L. The integration assay was done with HIV-2 U5 oligonucleotides (lanes 1 and 2) or another double-stranded 28-mer oligonucleotide (lanes 3 and 4) as integration target. Integrations were done with wild-type IN (lanes 1 and 3) or mutant N120L (lanes 2 and 4). (*B*) Integrations mediated by wild-type (WT) HIV-2 IN (lane 1) and mutants N120G, N120L, and N120E (lanes 2–4). HIV-2 U5 oligonucleotides were used as integration targets.

as active as wild-type HIV-2 IN (for example, mutant K236S in Fig. 5 *A–C*, lane 7; see Table 1). We found one mutation in this region that reduces donor cut and strand transfer: R228I (Fig. 5 *A* and *B*, lane 6).

DISCUSSION

We developed a quick isolation method for HIV-2 IN and used this to screen a large panel of mutants. The method includes immobilization of (mutant) proteins on a solid support. We found that, in a qualitative sense, donor cut, strand transfer, and disintegration are carried out in the same way by immobilized and soluble IN. However, the higher stability of immobilized IN enabled us to make the integration assay more sensitive by prolonged incubation. As a result of stabilization by immobilization, mutations that cause a lower protein stability might be scored as wild type in our assays, whereas they might be considered less active or inactive when assayed in solution. Not much is known about the position of IN in the viral-core particle *in vivo*, but it is probably not free in solution. Therefore, our assay using immobilized IN may reflect the *in vivo* properties of the protein at least as well as assays with IN in solution. In any

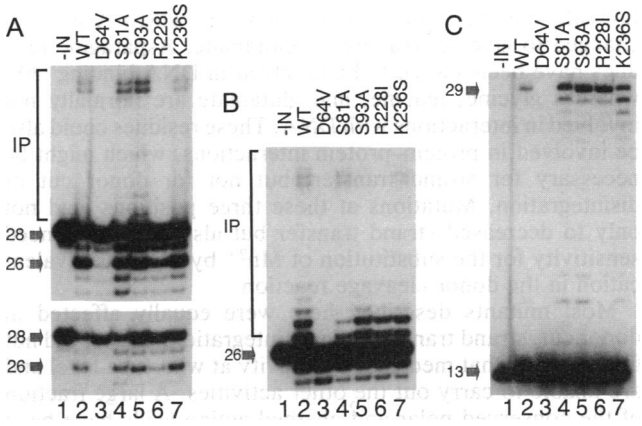

FIG. 5. Mutational analysis of other regions of HIV-2 IN. Cleavage (*A*), integration (*B*), and disintegration (*C*) assays of wild-type (WT) IN (lane 2) and mutants D64V, S81A, S93A, R228I, and K236S (lanes 3–7). Composition was as in Fig. 2.

case, mutants that have a wild-type level of activity in our assays are certainly not affected in binding of viral (donor) DNA, binding of target (acceptor) DNA, or catalytic potential.

All mutants that did not show wild-type levels of activity had a similar affinity for DNA and heparin-Sepharose as the wild-type protein (unpublished results). These observations indicate that these mutations do not cause severe structural alterations in the protein (we did at least two independent isolations of each mutant that showed a decreased activity). We found two mutants that are inactive in all assays (in amino acids Asp-64 and Glu-152) and one that is nearly inactive (in amino acid Asp-116). Therefore, these three amino acids are candidate active-site residues. These three mutants were the only ones that did not mediate disintegration.

Ser-85 of Rous sarcoma virus IN, the homolog of Ser-81 in HIV IN, has been proposed to be an active-site residue (29). Our results show that mutant S81A, although it is less soluble and probably less stable than wild-type HIV-2 IN, can cleave, integrate, and disintegrate. Therefore, this residue is probably more important for correct folding of the protein than for catalytic properties.

Another class of mutants is partially inhibited in donor cut and strand transfer. The amino acids specified by these mutants might be part of the active site. However, it is also possible that they inhibit IN action by a small distortion of the protein structure.

More informative mutants are the ones that are more inhibited in donor cut than in strand transfer (H12L and C40S) or *vice versa* (N117I, N120L, and K159V). Both mutants that are more inhibited in donor cut than in strand transfer are part of a conserved motif His-Xaa_3-His-Xaa_{20-30}-Cys-Xaa_2-Cys near the amino terminus. This motif resembles zinc fingers that have been found in transcription factors. Although HIV IN does not have a much higher binding affinity for specific than for random DNA (16, 25), it is tempting to speculate that this amino-terminal region is involved in the correct binding and positioning of the viral DNA end. For avian sarcoma leukosis virus IN it has also been shown that mutation of one of the conserved histidine residues results in a decrease in donor cut (20). Perhaps donor cut has different substrate requirements than strand transfer and disintegration. It has already been shown that a Y-shaped oligonucleotide of random sequence is a substrate in the disintegration reaction, so this reaction may depend less on recognition of the viral sequence (15).

Amino acids Asn-117, Asn-120, and Lys-159 play a role in strand transfer. One explanation for this observation is that these residues are involved in interactions with the target DNA. This hypothesis is supported by the changed integration-site specificity of mutant proteins in which Asn-120 is changed to glycine, leucine, or glutamate. Asparagine residues have been shown to be involved in DNA binding (30), whereas glycine, leucine, and glutamate are normally not involved in interactions with DNA. These residues could also be involved in protein–protein interactions, which might be necessary for strand transfer, but not for donor cut or disintegration. Mutations at these three positions lead not only to decreased strand transfer but also to an enhanced sensitivity for the substitution of Mn^{2+} by Mg^{2+} as divalent cation in the donor cleavage reaction.

Most mutants described here were equally affected in donor cut, strand transfer, and disintegration, and we did not find mutants that mediate one activity at wild-type level and are unable to carry out the other activities. A large fraction of the conserved polar and charged amino acids have been mutated. These results suggest that there is only one active site that can accomplish both hydrolysis of the specific phosphodiester bond 3′ of the conserved CA-3′ and the trans-esterification reaction that results in the actual strand transfer. The chemistries of the cleavage and strand-transfer reactions are similar: in the cleavage reaction DNA (in this case, the two 3′ nucleotides) is replaced by water, whereas in strand transfer the opposite occurs: a new phosphodiester bond is made, the 3′-OH is replaced by DNA. There are mutants that are more inhibited in donor cut than in strand transfer or the reverse, and the effect of divalent cations on donor cut and strand transfer differs. These results do not contradict the hypothesis that IN has one active site because this one active site could still have separate regions for recognition of the donor and acceptor DNA or, alternatively, additional domains might be required for strand transfer but not for the other activities (e.g., a domain that determines IN dimerization).

We thank Piet Borst, Sean Colloms, and Kees Vink for critical comments on the manuscript. We thank Ype Elgersma for the suggestion to use the pMa/c vectors for site-directed mutagenesis. This work was supported by Grant 88033 from the Dutch Ministry of Health (RGO) and a PIONIER grant of the Netherlands Organization for Scientific Research (NWO) to R.H.A.P.

1. Schwartzberg, P., Colicelli, J. & Goff, S. (1984) *Cell* **37**, 1043–1052.
2. Brown, P. O., Bowerman, B., Varmus, H. E. & Bishop, J. M. (1987) *Cell* **49**, 347–356.
3. Brown, P. O., Bowerman, B., Varmus, H. E. & Bishop, J. M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2525–2529.
4. Fujiwara, T. & Mizuuchi, K. (1988) *Cell* **54**, 497–504.
5. Varmus, H. E. & Brown, P. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington), pp. 53–108.
6. Craigie, R., Fujiwara, T. & Bushman, F. D. (1990) *Cell* **62**, 829–837.
7. Katz, R. A., Merkel, G., Kulkosky, J., Leis, J. & Skalka, A. M. (1990) *Cell* **63**, 87–95.
8. Katzman, M., Katz, R. A., Skalka, A. M. & Leis, J. (1989) *J. Virol.* **63**, 5319–5327.
9. Roth, M. J., Schwartzberg, P. L. & Goff, S. P. (1989) *Cell* **58**, 47–54.
10. Sherman, P. A. & Fyfe, J. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5119–5123.
11. Vora, A. C., Fitzgerald, M. L. & Grandgenett, D. P. (1990) *J. Virol.* **64**, 5656–5659.
12. Vink, C., Yeheskiely, E., Van der Marel, G. A., Van Boom, J. H. & Plasterk, R. H. A. (1991) *Nucleic Acids Res.* **19**, 6691–6698.
13. Engelman, A., Mizuuchi, K. & Craigie, R. (1991) *Cell* **67**, 1211–1221.
14. Bushman, F. D. & Craigie, R. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1339–1343.
15. Chow, S. A., Vincent, K. A., Ellison, V. & Brown, P. O. (1992) *Science* **255**, 723–726.
16. LaFemina, R. L., Callahan, P. L. & Cordingley, M. G. (1991) *J. Virol.* **65**, 5624–5630.
17. Leavitt, A. D., Rose, R. B. & Varmus, H. E. (1992) *J. Virol.* **66**, 2359–2368.
18. Vink, C., Van Gent, D. C., Elgersma, Y. & Plasterk, R. H. A. (1991) *J. Virol.* **65**, 4636–4644.
19. Johnson, M. S., McClure, M. A., Feng, D. F., Gray, J. & Doolittle, R. F. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7648–7652.
20. Khan, E., Mack, J. P. G., Katz, R. A., Kulkosky, J. & Skalka, A. M. (1991) *Nucleic Acids Res.* **19**, 851–860.
21. Fayet, O., Ramond, P., Polard, P., Prère, M. F. & Chandler, M. (1990) *Mol. Microbiol.* **4**, 1771–1777.
22. Drelich, M., Wilhelm, R. & Mous, J. (1992) *Virology* **188**, 459–468.
23. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
24. Stanssens, P., Opsomer, C., McKeown, Y., Kramer, W., Zabeau, M. & Fritz, H. J. (1989) *Nucleic Acids Res.* **17**, 4441–4454.
25. Van Gent, D. C., Elgersma, Y., Bolk, M. W. J., Vink, C. & Plasterk, R. H. A. (1991) *Nucleic Acids Res.* **19**, 3821–3827.
26. Rosenberg, A. H., Lade, B. N., Chui, C., Lin, S., Dunn, J. J. & Studier, F. W. (1987) *Gene* **56**, 125–135.
27. Whitcomb, J. M. & Hughes, S. H. (1991) *J. Virol.* **65**, 3906–3910.
28. Baker, T. A., Mizuuchi, M. & Mizuuchi, K. (1991) *Cell* **65**, 1003–1013.
29. Katz, R. A., Mack, J. P. G., Merkel, G., Kulkosky, J., Ge, Z., Leis, J. & Skalka, A. M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 6741–6745.
30. Wolberger, C., Vershon, A. K., Liu, G., Johnson, A. D. & Pabo, C. O. (1991) *Cell* **67**, 517–528.