Mutations within the RNase H domain of human immunodeficiency virus type 1 reverse transcriptase abolish virus infectivity

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The C-terminal region of human immunodeficiency virus (HIV) reverse transcriptase (RT) contains the domain responsible for RNase H activity. To determine the importance of this RNase H domain, specific changes in the C-terminal region of a recombinant RT expressed in Escherichia coli were introduced by amino acid substitutions and specific deletions. The enzyme activities of purified wild-type and mutant RT/RNase H proteins, standardized for protein content, were compared by filter assays and thermal inactivation kinetics. A point mutation of His 539→Asn produced an enzyme with a marked thermolabile RNase H function (nine-fold increase in inactivation), whereas RT function was only marginally more labile than that of the wild-type (two-fold). A second mutation, His 539→Asp, impaired both enzyme activities to a similar degree (four- to five-fold). A C-terminal deletion of 19 amino acids (aa) (aa 540 to 558) and a C-terminal truncation of 21 aa (aa 540 to 560) reduced RT as well as RNase H activity. A 130 aa deletion enzyme exhibited no RNase H activity and insufficient RT activity to allow inactivation studies. Two mutants, the 19 aa deletion and His→Asn, were introduced into proviral HIV-1 DNA clones to determine whether changes in enzyme activity, particularly RNase H activity, affected virus infectivity. Both mutants were non-infectious, indicating that the C-terminal 19 to 21 amino acids and His 539 of the RT/RNase H protein are essential for HIV replication. These results are consistent with the assumption that RNase H is essential for the infectivity of HIV-1.

Introduction

Human immunodeficiency virus (HIV), the causative agent of AIDS, like all retroviruses possesses a reverse transcriptase (RT) enzyme which is carried into the cell within the virus particle and is responsible for transcribing the viral RNA genome into a dsDNA copy. All known RTs exhibit two catalytic activities: a DNA polymerase activity, which can extend the 3' end of a primer and copy either RNA or DNA templates as required to form the first and second strands of the viral DNA, and an RNase H activity which degrades RNA only when it is in the form of an RNA-DNA hybrid duplex (Moelling et al., 1971; Verma, 1975; Moelling, 1977). The RNase H activity can account for the degradation of the viral RNA, for second-strand primer formation and for primer removal.

A computer-aided comparison of the amino acid sequences of various RTs and those of other polymerases led to a proposal for the functional organization of the protein and assigned the RNA- and DNA-dependent DNA polymerase activity to the N terminus region and the RNase H function to the C terminus (Johnson et al., 1986). Analysis of HIV-1 RT, purified from virions, has confirmed that the RNase H domain lies at the C terminus (Hansen et al., 1988). RT from virions has been shown to be present as a 66/51K heterodimer with a common N terminus (Di Marzo Veronese et al., 1986); the 66K protein, p66, possesses both RT and RNase H function (Hansen et al., 1988) and the 51K protein, p51, exhibits low level RT function (Lori et al., 1988). In addition, a 15K protein, p15, with RNase H function has been purified from viral extracts and identified as the C-terminal RT domain using a specific monoclonal antibody (Hansen et al., 1988). The p15 RNase H is generated as a consequence of p66/51 heterodimer formation and indicates some independence of the RNase H domain (T. Schulze & K. Moelling, unpublished results). Mutational analysis of the conserved regions within the N terminus of recombinant RT confirmed that the polymerase domain lies within the N terminus (Larder et al., 1987a). Further mutational studies on recombinant RT support the above alignment of function but have indicated that both require proper folding of the entire molecule for generation of stable RT and RNase H functions (Hizi et al., 1989; Prasad & Goff, 1991 SGM
1989). Analysis of C-terminal truncated mutant enzymes of recombinant HIV RT indicated that truncations of greater than 21 amino acid (aa) residues lead to significant reductions in RT activity (Hizi et al., 1988; Tisdale et al., 1988) although removal of up to 130 aa (about 51K) did not totally abolish RT activity in a highly expressing construct (Tisdale et al., 1988). In our previous studies we observed that RT activity was diminished markedly when a 29 aa truncated mutant enzyme was made, whereas the 21 aa truncated mutant enzyme had significant RT activity but reduced RNase H activity (Tisdale et al., 1989). This region, aa 530 to 540, within the RT molecule therefore appeared important to both RT and RNase H activity and, in addition, forms part of the epitope recognized by monoclonal antibodies (Hansen et al., 1988; Tisdale et al., 1989), suggesting that truncations in this region possibly lead to changes in conformation of the enzyme. Sequence analysis of this region shows that only one residue, His 539, is highly conserved among retrovirus RTs and Escherichia coli RNase H (Johnson et al., 1986). We were therefore interested in preparing point mutations of this residue and determining their effect on RT and RNase H functions.

In earlier studies we demonstrated that single amino acid changes in the N terminus of HIV-1 RT, which significantly reduced RT activity, also, when introduced into infectious clones, attenuated virus infectivity (Larder et al., 1989). In this study our aim was to establish the importance of the RNase H domain to virus replication by introducing specific C-terminal deletions and point mutations into infectious HIV-1 DNA.

**Methods**

**Preparation of RT/RNase H mutants.** Mutagenesis was carried out using the parental M13 clone mpRT4 which expresses an active 66K RT polypeptide following infection of E. coli (Larder et al., 1987b). Mutations were introduced using synthetic oligonucleotides as described (Kunkel et al., 1987) and confirmed by nucleotide sequencing (Sanger et al., 1977). Mutant polypeptides, produced in E. coli after induction with isopropyl β-D-thiogalactopyranoside (IPTG), were assessed for expression by PAGE and by Western blotting.

**Purification of recombinant HIV-1 RT mutants.** The C-terminal mutants of HIV-1 RT were prepared from fresh M13 phage stocks by infection of E. coli cells, strain TG1, with 10 p.f.u./cell in 100 ml 2 x TY medium (Maniatis et al., 1982). For expression of the mutant HIV-1 RT proteins, cultures were induced with IPTG (100 µg/ml) for 6 h and, for harvesting, the bacteria were concentrated by centrifugation (10000 r.p.m., 5 min, 4°C), lysed using lysozyme/NP40/NaCl buffer, sonicated and sedimented (40000 r.p.m., 1 h) (Hansen et al., 1987). The supernatant was diluted 20-fold with buffer PM (50 mM-Tris-HCl, pH 7.0, 1 mM-EDTA pH 8.0, 1 mM-DTT, 0.1% NP40 and 10% glycerol) and applied to a DEAE-cellulose column (1.5 x 5 cm). The flowthrough was applied to a phosphocellulose column (1.5 x 5 cm) and the column was washed (50 ml of PM buffer plus 50 mM-NaCl and 100 mM-NaCl, respectively and 25 ml PM plus 50 mM-NaCl) and eluted with PM plus 250 mM-NaCl and then with PM plus 400 mM-NaCl (five fractions, 5 ml each). Two fractions (10 ml total) were diluted 1:5 with PM and applied to a poly(U)-Sepharose column (Pharmacia; 1 x 1 cm). The column was washed with PM plus 50, 100 and 150 mM-NaCl and eluted with PM plus 250 and 400 mM-NaCl. Protein content as well as RT and RNase H activities were determined (Bradford, 1976) and enzyme-containing fractions were stored in 50% glycerol at -20°C. We have previously demonstrated that the RNase H recovered from the poly(U)-Sepharose column co-sediments with the p66 RT, is of viral origin and is free of other RNase H contaminants (Hansen et al., 1987, 1988).

**Thermal inactivation kinetics.** To compare the in vitro properties of the RT and RNase H activities of the mutants, the purified enzyme preparations were standardized by enzyme titrations in standard RT and RNase H assays. The enzyme concentrations varied from 0.1 to 1 ng/µl. Reaction mixtures containing 50 mM-Tris-HCl pH 8, 5 mM-MgCl2, 50 mM-KCl, 1 mM-DTT and either 1 µg poly(C) or oligo(dT)16, plus [32P]dCTP (0-1 nmol, 1 Mci/ml, 5 x 104 c.p.m..ml corresponds to 1 nmol [3H]TMP incorporation) in a 100 µl RT assay, or labelled [35S]UTP, specific activity 106 c.p.m./pmol, in the RNase H assay, were employed. Incubation was for 60 min and 15 min at 37°C, respectively. The amount of enzyme resulting in linear incorporation or degradation was determined by enzyme titrations in standard RT and RNase H assays.

**Fig. 1. Details of mutagenesis of the RNase H domain of HIV-1 RT.** Mutations were introduced into infectious clones, attenuated virus infectivity (Larder et al., 1989). In this study our aim was to establish the importance of the RNase H domain to virus replication by introducing specific C-terminal deletions and point mutations into infectious HIV-1 DNA.
approximately 50% of hybrid hydrolysis was determined and used for further inactivation studies. The activities especially of the purified mutant enzymes, were similar initially but decreased during storage and were undetectable after about 6 weeks. Inactivation kinetics were measured immediately before storage and at biweekly intervals. Differences in protein content did not influence the inactivation kinetics. The inactivation temperature of 45 °C was predetermined to inactivate only slightly the wild-type (wt) enzyme within a reasonable period of time. Inactivation kinetics experiments were performed by setting up parallel tubes with RT and RNase H reactions from which either the [3H]TTP or the [3S]M13-RNA/DNA hybrid were omitted. They were added prior to the assays performed at 37 °C to determine the residual activities.

Preparation of mutant HIV-1 DNA clones. To prepare mutations in the infectious proviral HIV-1 clone HXB-2D (Fisher et al., 1985), the parental M13 clone mpRT1/H, which contains a 2.55 kb fragment from the pol gene of HXB-2D inserted into M13mp19, was used (Larder & Kemp, 1989). This clone expresses active protease and the p66/51 RT heterodimer in E. coli. Mutagenesis was carried out as described for mpRT4 and the presence of mutations was confirmed by sequencing. Mutations were transferred by cloning a 19 kb Ball fragment within the pol gene into the HIV-1 DNA clone (see Fig. 1). Mutant DNA clones were confirmed by restriction enzyme analysis (Larder & Kemp, 1989).

Transfection of cells with HIV-1 DNA clones. Three human lymphoid cell lines were used, MT-4 and MT-2 cells (Harada et al., 1985) and CEM cells (Foley et al., 1985). Cells 2.5 x 10^6 were transfected with 10 μg of DNA from each HIV-1 DNA clone by electroporation using a Bio-Rad Gene Pulser (Larder & Kemp, 1989). The cultures were maintained in RPMI 1640 medium containing 10% (v/v) foetal calf serum plus antibiotics, and fresh cells 5 x 10^6 were added after 48 h incubation. These cultures were observed daily for development of a c.p.e., and culture supernatants taken for p24 assays (Innotest; p24 antigens detection kit). Cells (approximately 2 x 10^6) and culture supernatant 0.5 ml were passed separately onto fresh cell cultures 5 x 10^6 9 days after transfection. These cultures were again monitored for c.p.e. and p24 production. RT assays were carried out on virus pellets (Beckman TL-100 ultracentrifuge, 40000 r.p.m., 10 min, 4 °C) disrupted with 0.5% Triton X-100, using 5 μM-poly(rA).oligo (dT)~ 0 as the template-primer and [3H]TTP (10 ktCi/ml) as described (Larder & Kemp, 1989).

Amplification and cloning of HIV RT from input DNA and infected cells. To confirm that the mutant DNA clones contained the inserted mutations the RT coding region from input DNA (used in transfection) was amplified by the polymerase chain reaction (PCR) and cloned into the M13 vector mp tac18-I (Larder & Kemp, 1989). Clones were sequenced by the dideoxynucleotide chain-termination procedure (Sanger et al., 1977). Total DNA was extracted from HIV-infected cells showing positive c.p.e. and amplified by PCR as described above to check the C-terminal sequence of RT from virus-infected cultures.

HIV recombination experiments. Recombination experiments were performed as described (Clavel et al., 1989; Srinivasan et al., 1989). HIV-1 DNA clones, including wt HXB-2D, were digested with Ball and the resulting Ball deleted fragment, 10.5 kb in size, was purified from gels (Maniatis et al., 1982). The wt 2.55 kb HindIII/EcoRI pol fragment from mpRT1/H was prepared similarly (Fig. 1). Approximately 5 μg of each of the two DNA fragments was cotransfected into MT2 cells; controls transfected with the Ball deleted fragment alone were included. Cells were observed daily for development of c.p.e. and supernatant from positive cultures was taken for infectivity assays by endpoint dilution in MT2 cells (Larder et al., 1990).

Results

Preparation and expression of RNase H domain mutants

Five RT mutants were prepared with mutations in the C-terminal RNase H domain using the wt construct mpRT4 which expresses high levels of the 66K polypeptide (Larder et al., 1987b). Two mutants were produced by site-directed mutagenesis of histidine at position 539 to asparatic acid or asparagine. Histidine 539 is highly conserved within the pol gene of retroviruses (Johnson et al., 1986). Two other mutants were produced by introducing premature termination codons at different positions within the RT gene; one mutant is truncated by 130 aa, designated mt−130 and the other is truncated by 21 aa, designated mt−21 (In previous studies these were designated CTRT1 and CTRT14, respectively.) A fifth mutant was constructed by a deletion of 19 aa (aa 540 to 558), designated mt−19, leaving the two C-terminal aa unchanged. Bacterial cultures infected with these mutants were grown up and induced by IPTG, lysed and subjected to SDS–PAGE and Western blot analysis using monoclonal antibodies against the RT. Fig. 2(a, b) shows the mutant polypeptides produced from total bacterial lysates on stained SDS–polyacrylamide gels and a Western blot, respective-
ly. All exhibit prominent bands in the region of p66, except mt – 130 which exhibits high level expression of a 50K polypeptide. All were recognized by an RT monoclonal antibody, 4/20, against the RT which has been described previously (Hansen et al., 1988). This result served for standardization of the material.

Comparative analysis of the thermal inactivation activities of purified mutant polypeptides

For detailed comparative analysis of enzyme functions, mutant polypeptides were purified from bacterial lysates by DEAE–cellulose and phosphocellulose chromatography, followed by poly(U)-Sepharose chromatography, a step which removes low Mr RNase H of E. coli origin (Hansen et al., 1988). The peak enzyme fractions were brought up to 50% glycerol and stored at –20 °C. The enzymic activities of the wt and mutant enzymes were tested immediately and at bi-weekly intervals to follow the reduction of activities during storage. Fig. 2(c) demonstrates the purified enzyme preparations by silver staining. RT and RNase H assays were performed with the peak fractions of each of the mutants and of the wt included as a control. In the first analysis, concentration dependence was established (Fig. 3a) and the amount of enzyme which was necessary to incorporate linear amounts of radioactivity into DNA in RT assays, or the amount necessary to hydrolyse approximately 50% of the RNA–DNA hybrid used in the RNase H assays, was determined. These enzyme activities were then further used for in vitro inactivation studies in order to determine the stability of the individual mutants. Inactivation kinetics were performed by setting up parallel tubes, preincubating them at 45 °C and removing individual fractions at the time points indicated. Enzymic activities were then determined by filter assays.

The results of these inactivation experiments are shown in Fig. 3(b). The same data are also presented as percentages by taking the initial enzyme activity as 100% (Fig. 3c). From these plots the relative rates of inactivation were determined at 50% activity and were shown to be independent of the time point when the analysis was performed. The data show that the RNase H activity of the wt polypeptide proved more stable than the wt RT activity, with a 50% reduction in activity seen at approximately 40 min and 20 min, respectively. A similar observation has been described previously for the RT polypeptides of avian retroviruses (Moelling & Friis, 1979). From Fig. 3(b, c) it is apparent that mutant His→Asn showed a marked difference in the relative thermostability of the two activities. Comparison of initial activities (Fig. 3b top left at 0 min) revealed that its RT activity was slightly higher than wt and that the rate of RT inactivation was marginally increased (twofold, Fig. 3c top left). The initial RNase H activity, however, showed a greater than 50% reduction (Fig. 3b top right at 0 min) and a ninefold greater inactivation rate (Fig. 3c top right). With the His→Asp mutant, the initial RNase H function was reduced similarly to mutant His→Asn (Fig. 3b top right) but, in addition, both its RT and RNase H exhibited similarly increased inactivation rates (four- to fivefold; Fig. 3c top left and right). With mt – 19 and mt – 21, both RT and RNase H functions showed greater thermal inactivation kinetics (two- to threefold) compared to wt (Fig. 3b, c, bottom left and right).

After the purification procedure applied here, mt – 130 did not exhibit sufficient RT activity for inactivation kinetic studies. We have shown previously that after immunoaffinity purification this mutant possesses 5% of the RT activity of the corresponding wt but no detectable RNase H (Tisdale et al., 1989).

Preparation of RT mutant DNA clones

We wished to look at the effect of mutations in the RNase H domain on virus infectivity. Therefore, we prepared mutant clones using the infectious HIV DNA clone HXB-2D and used these for transfection of different T cell lines. To produce these constructs we used a 1.9 kb BafI fragment which cleaves in the RT and the endonuclease. We therefore remade the same mutants in a large pol gene construct (mpRT1/H; Fig. 1) containing a 2.55 kb pol fragment including the protease RT and some of the endonuclease. The mutation His→Asn and mt – 19 (aa 540 to 558) were prepared in mpRT1/H by oligonucleotide mutagenesis and confirmed by sequencing; the clones were checked for RT activity in crude E. coli extracts. It was important to establish that the protease cleavage site between RT and the endonuclease had not been disrupted particularly by mt – 19. Since the parental construct gives low level expression of protease-cleaved p66/51 RT, Western blots were carried out on the mutants, confirming the presence of equimolar 66/51K (data not shown). Mutations were transferred to the HIV-1 DNA clone as described above, checked for correct orientation by restriction enzyme analysis and the presence of the correct mutation was reconfirmed by using PCR to reclone the RT gene into M13 phage DNA for sequencing.

Transfection of cells with DNA from mutant HIV clones

Cells were transfected with DNA from wt or mutant HIV clones (– 19 and His→Asn) and production of virus
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Fig. 3. (a) Concentration dependence of mutant and wt enzyme activity. Purification of the wt and two mutant enzymes was performed in parallel using DEAE-cellulose, phosphocellulose and subsequent poly(U)-Sepharose chromatography. The amount of enzyme was standardized by protein content and titrated for activity. The amount of enzyme within the linear incorporation range was used as input for the inactivation kinetics of the RT (see b). The amount of enzyme required for hydrolysis of 50% of the hybrid (with mutants > 30%) was determined and used as input for the RNase H inactivation analysis. Only one of the two purified wt preparations is shown. (b) Thermal inactivation kinetics. Parallel tubes were incubated at 45 °C for the indicated periods of time and processed for determination of RT and RNase H activities. The initial activities, especially of the purified mutant enzymes, decreased during storage. (c) The results shown in (b) were replotted as percentages to allow comparison of inactivation rates (time required for 50% inactivation). In each graph, ○, wt enzyme; [], mutant His 539→Asp; ■, mt-21; Δ, His 539→Asn; ▲, mt-19.

was monitored by p24 supernatant levels (Fig. 4). The levels of p24 detected after 48 h are a measure of the virus produced from the input DNA, i.e. from the first cycle only which is not dependent on a functional RT. At 48 h p24 levels were similar in wt- and mutant-transfected cell supernatant but varied in different cell lines, with highest levels being obtained in MT2 cells indicating that these cells transfected most efficiently. As a measure for virus production, RT assays were performed on detergent-disrupted virus sedimented from supernatants harvested at 48 h from MT2 cells. Both mutant and wt virus gave comparable RT activity relative to p24 levels, His 539→Asn (10375 c.p.m.), mt-19 (9150 c.p.m.), wt (13915 c.p.m.) compared to a background of 2750 c.p.m. At this time point, the virion-associated RT activities exhibited by the wt and mutants did not show significant differences in stability which could have been due to protection of the enzymes by encapsidation.

To determine the infectivity of virus produced, fresh cells were added at 48 h post-infection. With wt virus, c.p.e. was observed 4 to 5 days after transfection into MT2 and MT4 cells and 6 to 7 days after transfection into CEM cells. With the mutants, no definite c.p.e. was observed suggesting the absence of infectious mutant virus. Similarly, with p24 assays high levels of p24 were detected after 5 days in all cell lines with HXB-2D controls, whereas no increase in p24 levels was detected for either mutant. Further passage of cells or supernatant from mutant cultures after 9 days incubation to fresh cell cultures did not yield infectious virus. From these data it
was concluded that both mutant HIV-1 DNA clones were non-infectious in a variety of T cell lines.

In one experiment in which mutant His 539→Asn was introduced into MT2 cells, p24 levels were seen to rise slowly over 13 days. When this culture was passed onto fresh cells, p24 levels increased at a rate comparable to controls. Cell pellets from these cultures were taken, DNA was extracted and RT sequences were amplified by PCR and cloned into M13 for sequencing. The sequence obtained was found to be wt His 539, suggesting that reversion had occurred during replication of the input DNA giving rise to further replication of wt virus (data not shown).

Recombination experiments to rescue defective proviral HIV clones

HIV like other retroviruses has been shown to recombine readily either using full-length proviral clones (Clavel et al., 1989) or mixtures of subgenomic fragments (Srinivasan et al., 1989). Since both the His 539→Asn and mt-19 clones gave rise to replication-defective virus it was considered valuable to determine whether infectious virus could be rescued from these mutants using DNA fragments containing the RT sequence. MT2 cells were cotransfected with a 2-55 kb pol gene fragment from mpRT1/H (Fig. 1) with the Ball fragment deletions of the proviral HIV clones and cells were observed for development of c.p.e. In all three cotransfections (His 539→Asn, mt-19, HXB-2D) c.p.e. was first observed after 9 days in a few cells, indicating that recombination had taken place. It developed into a strong c.p.e. by 13 days due to subsequent growth of the virus; the infectivity titres were for HXB2-D 10^3.7 TCID_{50}/ml, for His 539→Asn 10^{3.95} TCID_{50}/ml and for mt-19 10^{2.95} TCID_{50}/ml. The delay in development of the c.p.e. compared to that in transfection with intact HIV-1 DNA clones reflects the low rate of recombination, reported as 1 to 2% for a double crossover (Srinivasan et al., 1989). Transfections with Ball deletion fragments alone gave no c.p.e. Rescue of both defective HIV clones by RT sequences further confirms that the mutations introduced into the RT sequence had given rise to defective virus.

Discussion

In this study point mutations and C-terminal deletions were prepared within the RNase H domain of HIV-1 RT in an attempt to produce mutant RNase H enzymes. Previous studies with HIV-1 RT using deletion and insertion mutagenesis suggested that it may be difficult to separate the RT and RNase H functions (Hizi et al., 1989; Prasad & Golf, 1989) although this has been achieved relatively easily with murine leukaemia virus (Tanese & Golf, 1988). It appeared therefore that the two enzymic functions of HIV-1 RT were extremely sensitive to changes in enzyme folding and thus it would be most pertinent when comparing enzymic activities of mutant polypeptides to look at thermal stability. In addition, we chose to make an extremely conservative change of one highly conserved amino acid, His 539, within the RNase H domain. In space-filling terms, the most conservative change was His→Asn and this proved sufficient to render the RNase H activity significantly more thermolabile than that of RT. Interestingly, the less conservative change from His→Asp, including a change from negative to positive charge, significantly affected both RT and RNase H functions and resulted in the loss of the RTMAb 8 epitope (unpublished observation). This was not seen with His→Asn, possibly suggesting that a conformational change had occurred. This latter mutant further confirms the difficulty of separating the two functions. While this work was in progress, Schatz and coworkers reported a similar significant selective reduction in RNase H function by mutating His 539→Phe (Schatz et al., 1989). In addition, they mutated Glu 478→Gln which resulted in loss of RNase H function. However, in their study mutants were not analysed for thermal RT stability and therefore changes similar to those seen with the His 539 mutants described here may have been missed. It would obviously be interesting to know whether other highly conserved C-terminal residues within the RNase H domain also affect RT stability.

Further analysis was made using C-terminal deletions and truncations because previous studies had indicated
that it may be possible to separate RT and RNase H functions using short truncations of no greater than 21 aa (Tisdale et al., 1989). However, in these studies mt - 19 and mt - 21 clearly showed reductions in both RT and RNase H activities with the latter function the more stable as seen with wild-type enzyme. Mt - 19 and mt - 21 leave His 539 intact which may explain why the RNase H activity of these mutants is not as severely affected as is those with the His 539 point mutants. This difference in observations from previous studies (Tisdale et al., 1988) may have resulted from the different purification strategy and lower enzyme levels used in this study. Similarly, with mt - 130 no RT activity was detected after the purification procedure applied in this study, although previously we have shown that this mutant possesses 5% of wt RT activity but no RNase H activity (Tisdale et al., 1988). Mt - 130 removes more than the RNase H domain, which is composed of 120 aa. An active RNase H can be cleaved off by the HIV-1 protease in vitro, presumably by cleavage next to Phe 440, as demonstrated by mimicking this site using the synthetic peptide AETFYVE (T. Schulze & K. Moelling, unpublished results).

To evaluate the ability of these mutant enzymes to support the replication of HIV in T cell lines, HIV-1 DNA clones were constructed containing the same mutations in the RT protein. This analysis revealed, not surprisingly, that mt - 19 was non-infectious, indicating that the last 21 aa of HIV-1 RT are required for correct folding and stability of the RT enzyme during virus replication. More interestingly, the highly conservative change His 539→Asn, which preferentially reduced RNase H function, completely abolished virus infectivity. Although it is not possible to exclude the possibility that the minor reduction in RT stability seen in vitro may significantly affect RT function in the cell, this result suggests that the reduction in RNase H function is critical to HIV replication. This supports the earlier observation of Repaske and coworkers using a murine leukaemia virus that a functional RNase H is required for anti-HIV chemotherapy (Moelling et al., 1989).

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References


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