The ribonuclease H activity of the reverse transcriptases of human immunodeficiency viruses type 1 and type 2 is modulated by residue 294 of the small subunit

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ABSTRACT

Reverse transcriptases (RTs) exhibit DNA polymerase and ribonuclease H (RNase H) activities. The RTs of human immunodeficiency viruses type 1 and type 2 (HIV-1 and HIV-2) are composed of two subunits, both sharing the same N-terminus (which encompasses the DNA polymerase domain). The smaller subunit lacks the C-terminal segment of the larger one, which contains the RNase H domain. The DNA polymerase domain of RTs resembles a right hand linked to the RNase H domain by a connection subdomain. Despite the high homology between HIV-1 and HIV-2 RTs, the RNase H activity of the latter is substantially lower than that of HIV-1 RT. The thumb subdomain of the small subunit controls the level of RNase H activity. We show here that Gln294, located in this thumb, is responsible for this difference in activity. A HIV-2 RT mutant, where Gln294 in the small subunit was replaced by a proline (present in HIV-1 RT), has an activity almost 10-fold higher than that of the wild-type RT. A comparative in vitro study of the kinetic parameters of the RNase H activity suggests that residue 294 affects the $K_m$ rather than the $k_{cat}$ value, influencing the affinity for the RNA-DNA substrate.

INTRODUCTION

Reverse transcriptases (RTs) of retroviruses have two catalytic functions, the DNA polymerase activity (capable of copying both RNA and DNA templates) and the ribonuclease H (RNase H) activity, which hydrolyzes the RNA in DNA-RNA heteroduplexes (1,2). Due to its cardinal role in the replication cycle of retroviruses and the specificity of its biological activities, RT is a major target for drug treatment against human immunodeficiency viruses (HIVs) that cause human acquired immunodeficiency syndrome (AIDS) (3). Both HIV-1 RT and HIV-2 RT are heterodimers, consisting of two polypeptides (p66/p51 and p68/p54, respectively) (4,5). Of the two HIV RTs, the structure of HIV-1 RT has been resolved in a number of forms as well as in the presence of nucleic acids (6–9). Recently, the structure of HIV-2 RT has also become available (10). The large subunit in both RTs is composed of two domains, DNA polymerase and RNase H. The DNA polymerase domain resembles a right hand with fingers, palm and thumb subdomains, joined to the RNase H domain by a connection subdomain. The DNA polymerase active site is located within the palm subdomain of the p66 or p68 subunit and the RNase H domain is located at the C-terminus of this large subunit. The smaller RT subunit is a proteolytic cleavage product of the larger subunit (where the whole C-terminal segment is removed). Therefore, the p51 or p54 subunit lacks the RNase H domain. The DNA polymerase domain of this subunit has the same subdomains as p66 or p68 (designated fingers, palm, thumb and connection), but its relative folding in the heterodimers is different. Other polymerases also show an overall resemblance to a right hand structure with the active site located in the palm subdomain. The enzymes with this structure are: the DNA-dependent DNA polymerases of both polymerase β and the Klengow large fragment of both Escherichia coli polymerase I and Taq polymerase, the DNA-dependent RNA polymerase of T7, the RNA-dependent RNA polymerase of poliovirus and the RT of murine leukemia retrovirus (11–16).

The specific activities of the DNA polymerase function of purified recombinant RTs of HIV-1 and HIV-2 are similar. However, the RNase H specific activity of HIV-1 RT was shown in several studies to be about 10-fold higher than that of HIV-2 RT from both the Rod and D194 strains (17–20). We have constructed chimeric recombinant RTs derived from HIV-1 and HIV-2 sequences and shown that part of the DNA polymerase domain, rather than the RNase H domain per se, has a significant effect on the level of the RNase H activity (17–20). Moreover, we have recently presented evidence that the observed difference in the RNase H activity between the HIV-1 and HIV-2 RTs is quantitative rather than qualitative, since the pattern of RNA cleavage by the two RTs is indistinguishable. In addition, we have generated new chimeric HIV-1/HIV-2 RTs to define the boundaries of the
protein segment within the DNA polymerase domain which is responsible for the differences in the level of RNase H activity. The data presented imply that the thumb subdomain of the smaller RT subunit has a major role in determining the level of RNase H activity, probably through interactions with the RNase H catalytic domain of the larger subunit (20).

In the present investigation we have studied the precise amino acid residues located in the 79 residue long thumb subdomain which are responsible for the difference in the RNase H activity between HIV-1 and HIV-2 RTs (see Fig. 1). By constructing additional chimeric HIV-2/HIV-1 RT molecules in which shorter HIV-1 RT peptide fragments were incorporated into HIV-2 RT, we show that a single amino acid residue in the thumb of the smaller RT subunit of HIV-2 RT (residue 294) is responsible for the reported difference in the RNase H activity. At this position (located in the loop between helices αI and αJ in HIV-1 RT) there is a proline in HIV-1 RT and a glutamine in HIV-2 RT. Thus, a HIV-2 RT mutant in which this residue was replaced by proline in just the p54 subunit exhibits a RNase H activity (and, hence, a strand transfer activity) almost as high as wild-type HIV-1 RT. In addition, the steady-state kinetics of the RNase H activities of wild-type and chimeric HIV-1 and HIV-2 RTs were studied. It was found that there are substantial differences in the apparent $K_m$ values (with similar turnover numbers, $k_{cat}$ values). This indicates that HIV-1 RT (as well as the chimeric HIV-2/HIV-1 RTs with high RNase H activities) have an enhanced affinity for the RNA-DNA substrate relative to HIV-2 RT, with no significant differences in the rates of the RNase H catalytic process.

**MATERIALS AND METHODS**

**Plasmid construction for the expression of RTs**

All recombinant RTs in this study were expressed in *E. coli* with six-histidines tags and purified as previously described in detail (20). The plasmids, designated pHIV-1 RT and pHIV-2 RT (Rod), induce the expression of the wild-type heterodimeric RTs of the BH-10 isolate of HIV-1 and the Rod isolate of HIV-2, respectively (19,21). In order to generate the new chimeric HIV-2/HIV-1 RT used in this study, we have employed a cassette mutagenesis method similar to the one used for the mutagenesis of HIV-1 RT (22). We have constructed a *Bsp*MI cassette by introducing the *Bsp*MI restriction site into the HIV-2 RT coding region in the pUC12N HIV-2 RT expressing plasmid (in which the *Hind*III site downstream of the RT coding insert was replaced by a *Nco*I restriction site). The cassette, created by two PCR amplification steps, differs from the parental HIV-2 RT expressing plasmid in that 100 bp of the RT sequence (nucleotides 851–951) were deleted. The deleted segment codes for the residues from Gly285 to Tyr318 in the HIV-2 RT protein. It was replaced by a DNA segment containing a *Hind*III recognition site flanked by two *Bsp*MI sites, orientated in opposite directions. The synthetic DNA fragments to be inserted in the *Bsp*MI cassette contained the specific HIV-1 RT coding segments that were introduced into the backbone of the HIV-2 RT gene (as described specifically below). The oligomers were phosphorylated, hybridized and ligated into the linearized *Bsp*MI cassette-containing plasmid, as described (22). After ligation, the DNA was treated by *Hind*III to linearize plasmid DNA molecules that did not contain the inserts and, thus, to eliminate the background plasmids. After transforming *E. coli* DH5α, the DNA of the selected plasmids was sequenced for verification. The genes constructed for the expression of the chimeric RTs replaced the gene for the wild-type HIV-2 RT in the vector HIV-2 RT (Rod) (20). This vector induces the co-expression of both the p68 subunit of the RT and the protease of HIV-2. Therefore, the simultaneous synthesis in the bacteria of both recombinant proteins leads to the proteolytic processing of the p68 homodimers by the recombinant protease. This results in the expression of heterodimeric chimeric RTs with identical modifications in both subunits (designated T1–T8; see below). The subunit-selective mutant of HIV-2 RT, designated T10 (see below), was also expressed in bacteria from the plasmid constructed to co-express separately the two HIV-2 RT subunits (20), the large one as a wild-type protein and the small subunit as the Q294P mutant. In this system, each of the genes encoding the two protein subunits is under the control of an independent lacZ promoter, hence, the induction leads to the simultaneous synthesis of both subunits in the recombinant bacteria.

**Assays for the RNase H activity of the purified RTs**

All methods have been previously described in detail (18,20). The soluble assay was performed by monitoring the release of $[^3]$HAMP from the synthetic substrate $[^3]$Hpoly(rA)$_n$·poly(dT)$_m$. The second assay was performed by following the cleavage of 5′-$^32$P-end-labeled 267 nt RNA, synthesized in vitro from the pBLRA30 plasmid. This RNA, derived from the U5 and R regions of the HIV-1 genome LTR, was annealed to a 20 nt long synthetic oligonucleotide DNA. The RNase H cleavage products were electrophoretically resolved on a denaturating sequencing polyacrylamide gel and the sizes and amounts of the 5′-$^32$P-end-labeled RNA products were determined by densitometry of the autoradiograms. This assay was designated the PAGE assay (20).

**Strand transfer assay**

This assay was described in detail recently (20). A 5′-$^32$P-end-labeled 20 nt synthetic DNA was annealed to the same 267 nt RNA used in the PAGE RNase H assay (that serves as the primary RNA template). A second 144 nt RNA (synthesized from the pG89 plasmid) was used as the secondary or the acceptor RNA template. The products of the reactions in the presence of RTs and all four dNTPs were resolved on denaturating polyacrylamide gels and the pattern of the 5′-end-labeled DNA products was analyzed after autoradiography.

**RESULTS**

The rationale for constructing all chimeric HIV-2/HIV-1 RTs

The research presented in this communication was conducted as an outcome of the results presented earlier (20). Our specific aim was to identify the precise and minimal amino acid sequences in the thumb of the small RT subunit that have the highest impact on the level of the RNase H activity. The study was initiated with the previously described chimera,
designated E/E, in which the whole thumb subdomain in both subunits of heterodimeric HIV-1/HIV-2 RT (residues 244–322) was replaced by the comparable segment of HIV-1 RT (20; see also Fig 1). Then, we shortened the HIV-1 RT-derived sequences which were incorporated within HIV-2 RT in a sequential manner. Only chimeric RTs with high RNase H activity relative to wild-type HIV-2 RT were further modified to produce RT versions which contain less and less of the appropriate HIV-1 RT sequences (Fig. 1). All chimeras shown were designated T (thumb) with the proper number (see Table 1). Chimera T1 (with residues 285–318 of HIV-1 RT) led to the generation of the chimeras designated T2 and T3 (with residues 285–300 and 301–318 of HIV-1 RT, respectively). Since T2 had a high RNase H activity, its HIV-1 RT-derived sequence was further analyzed in chimeras T4 and T5. Chimera T5 (with residues 292–298 of HIV-1 RT) had a high RNase H activity, consequently, its HIV-1 RT-derived sequences were further analyzed in chimeras T6 and T7. Chimera T6, with only residues 294 and 295 from HIV-1 RT, had a high RNase H activity, therefore, we generated the two single mutants of HIV-2 RT, designated T8 and T9. The T8 mutant, in which Q294 was replaced by a proline in both subunits of HIV-2 RT, was the one with an elevated RNase H activity. Based on these findings and on the previous results that have emphasized the importance of the small RT subunit to the RNase H activity (20), the mutant designated T10 was generated. In this RT version, the Q294P mutation was incorporated into only the small subunit (with a wild-type large subunit). This HIV-2 RT mutant had a high RNase H activity.

Table 1. A summary of the relative RNase H and DNA polymerase activities

<table>
<thead>
<tr>
<th>RT isoform</th>
<th>HIV-1-derived sequence (residues)</th>
<th>RNase H</th>
<th>Strand transfer</th>
<th>DNA polymerase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soluble</td>
<td>PAGE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-1 WT</td>
<td>NA</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>HIV-2 WT</td>
<td>285–318</td>
<td>6 ± 1</td>
<td>6 ± 2</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>T1</td>
<td>285–300</td>
<td>47 ± 6</td>
<td>50 ± 4</td>
<td>96 ± 10</td>
</tr>
<tr>
<td>T2</td>
<td>285–300</td>
<td>45 ± 3</td>
<td>58 ± 7</td>
<td>113 ± 12</td>
</tr>
<tr>
<td>T3</td>
<td>301–318</td>
<td>7 ± 1</td>
<td>9 ± 3</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>T4</td>
<td>286–288</td>
<td>4 ± 0</td>
<td>10 ± 2</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>T5</td>
<td>292–298</td>
<td>31 ± 5</td>
<td>39 ± 4</td>
<td>100 ± 12</td>
</tr>
<tr>
<td>T6</td>
<td>294–295</td>
<td>46 ± 4</td>
<td>37 ± 4</td>
<td>104 ± 9</td>
</tr>
<tr>
<td>T7</td>
<td>292–293, 298</td>
<td>6 ± 1</td>
<td>5 ± 1</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>T8</td>
<td>294</td>
<td>36 ± 4</td>
<td>55 ± 6</td>
<td>98 ± 8</td>
</tr>
<tr>
<td>T9</td>
<td>295</td>
<td>5 ± 1</td>
<td>6 ± 2</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>T10</td>
<td>294 (in p54 subunit only)</td>
<td>50 ± 7</td>
<td>57 ± 8</td>
<td>99 ± 11</td>
</tr>
</tbody>
</table>

The various RT versions and their designations are described in the text. All enzymatic activities were calculated relative to wild-type HIV-1 RT (with equal amounts of protein) and are expressed as a percentage of the corresponding activity of HIV-1 RT. The RNase H activity was assayed by the two methods described in Materials and Methods (18, 20). The ‘soluble’ assay used [3H]poly(rA)n•poly(dT)n as the substrate and the ‘PAGE’ assay is a quantitative evaluation of the activities as in the gel in Figure 2 (20). The quantitative strand transfer activity was calculated from the experiment described in Figure 3, as explained previously (20). The DNA polymerase activity was assayed using poly(rA)n•oligo(dT)12–18 as the template–primer and [3H]dTTP. All figures given in the table are the averages (± range) of three independent experiments performed in each assay. NA, not applicable.

Figure 1. Alignment of the sequences of the thumb subdomains from HIV-1 and HIV-2 RTs. The upper lines show the 79 residue long sequence of the BH10 strain of HIV-1 and the lower ones of the Rod strain of HIV-2. Asterisks mark identical residues. The boundaries of the different regions in the thumb subdomains are also indicated. This alignment was performed using the CLUSTAL W pairwise alignment.
All highly purified heterodimeric RT versions were assayed for their RNase H activities using the two methods described in detail previously (17,20) and briefly in Materials and Methods. Figure 2 shows the pattern of the RNA cleavage of the RNA-DNA heteroduplexes by the various RTs. We have already shown that the pattern generated by both wild-type HIV-1 and HIV-2 RTs, as well as by all previous chimeric RTs tested, were similar (20). Hence, it is not surprising that the same is also true for all the newly generated chimeric HIV-2/HIV-1 RTs. All RT versions perform two cleavages of the 5′-end-labeled RNA. The first cleavage produces a 47 nt long product. This means that the RNA is cut 17 nt away from the 3′-end of the DNA primer. The secondary cleavage generates 38 nt long RNA fragments (the −8 nt cleavage site). Therefore, as before, the differences seen are only quantitative rather than qualitative. The extent of RNA cleavage was quantified and the data is summarized in Table 1 (designated the PAGE assay). The results of the soluble assay with [3H]poly(rA)n·poly(dT)n as a substrate are also shown in Table 1 and are in close agreement with those of the PAGE assay.

The strand transfer activity of the chimeric RTs

During the process of reverse transcription of the retroviral RNA genome into double-stranded DNA there are two template switches or strand transfers (3). Both the DNA synthesis and RNase H functions of RT are simultaneously involved in this process. It was already shown that the specific DNA polymerase activities of wild-type HIV-1 RT and HIV-2 RT as well as the chimeric RTs studied are similar (17,20). Therefore, one would expect to see that the new chimeric RTs generated will also show similar polymerase activities (Table 1). Since strand transfer depends on both polymerase and RNase H activities, it is not surprising to find (as already shown earlier) that the transfer activity of the new chimeric RTs tested here are also determined primarily by the differences in the RNase H activity. The in vitro strand transfer assay described in Figure 3 was performed as shown in detail previously (20). The amount of the full-length 238 nt DNA synthesized, which represents the transfer activity, was quantified. As before, the data show that there is a very good correlation between the strand transfer activity and the RNase H activity of all RT versions tested (Table 1).
Table 2. Kinetic parameters for RNase H

<table>
<thead>
<tr>
<th>RT isoform</th>
<th>$K_m$ (μM)</th>
<th>$k_{cat}$ (s$^{-1}$)</th>
<th>$k_{cat}/K_m$ (s$^{-1}$ M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 wt</td>
<td>5.4</td>
<td>$5.4 \times 10^{-2}$</td>
<td>$100 \times 10^2$</td>
</tr>
<tr>
<td>HIV-2 wt</td>
<td>49.9</td>
<td>$4.1 \times 10^{-2}$</td>
<td>$8 \times 10^2$</td>
</tr>
<tr>
<td>T2</td>
<td>9.9</td>
<td>$2.7 \times 10^{-2}$</td>
<td>$28 \times 10^2$</td>
</tr>
<tr>
<td>T10</td>
<td>9.7</td>
<td>$4.2 \times 10^{-2}$</td>
<td>$42 \times 10^2$</td>
</tr>
</tbody>
</table>

Constant amounts of each highly purified HIV RT version were incubated with increasing amounts of $[^{3}H]$poly(rA)$_n$-poly(dT)$_n$ substrate, ranging from 2.5 to 20 μM (expressed in nucleotide concentrations, with specific radioactivities of ~600 to 75 d.p.m./pmol AMP for these concentrations, respectively). The incubations were performed for 10 min at 37°C under the standard soluble RNase H assay (18,20). It was found that the release of the RNase H hydrolysis products over time was always linear for HIV-2 RT long (Fig. 1) and in the previous study (20) that these enzymes were as efficient in the strand transfer reaction as the wild-type HIV-1 RT (20). The same phenomenon is apparent in the present study for all RT isoforms with a high RNase H activity (i.e. T1, T2, T5, T6, T8 and T10; see Table 1). However, we have also observed both in the present study (for the same isoforms outlined) and in the previous study (20) that these enzymes were as efficient in the strand transfer reaction as the wild-type HIV-1 RT (Table 1). It was already argued that this phenomenon is not unexpected, because the strand transfer reaction is probably substantially slower than the RNase H reaction. Consequently, small differences in the RNase H activity should not significantly affect the strand transfer reaction. Only when the RNase H activity is reduced to a level below which RNA cleavage is rate limiting will strand transfer be considerably affected. Nevertheless, it is still possible that factors other than the RNase H function per se may have minor effects on the level of the strand transfer activity (20).

The three-dimensional structures of both HIV-1 RT and HIV-2 RT show that proline 294 is located at the end of a loop between α-helices designated α6 and α7 (6–10,24,25). Since prolines are known to terminate α-helices, it might be possible that a glutamine at this position will allow residue 294 to make a strong interaction with the p68 RNase H subunit (20). Additional factors have been shown to affect RNase H activity, including the length of the small subunit (p54 or p58), the heteroduplex substrate nucleotide composition and/or the presence of Mg$^{2+}$ or Mn$^{2+}$ ions (23). While variations of these factors have been shown to either enhance or impair RNase H activity, the RNase H activity of HIV-2 RT was always significantly lower than that of HIV-1. In the study presented here, we have precisely identified which amino acids in the 79 residue long thumb domain (Fig. 1) are responsible for the observed differences in the RNase H activity of HIV-2 RT. We show a substantial enhancement of RNase H activity of HIV-2 RT by modifying a single residue in the small subunit thumb subdomain.

The results of the present study can be summarized as follows. First, residue 294, located in the thumb of the small RT subunit, is solely responsible for the quantitative differences observed between HIV-1 RT and HIV-2 RT. It was shown earlier that most of the chimeric HIV-2/HIV-1 RT versions (which show an enhanced RNase H activity) possess only about half of the specific RNase H activity of the wild-type HIV-1 RT (20). The same phenomenon is apparent in the present study for all RT isoforms with a high RNase H activity (i.e. T1, T2, T5, T6, T8 and T10; see Table 1). However, we have also observed both in the present study (for the same isoforms outlined) and in the previous study (20) that these enzymes were as efficient in the strand transfer reaction as the wild-type HIV-1 RT (Table 1). It was already argued that this phenomenon is not unexpected, because the strand transfer reaction is probably substantially slower than the RNase H reaction. Consequently, small differences in the RNase H activity should not significantly affect the strand transfer reaction. Only when the RNase H activity is reduced to a level below which RNA cleavage is rate limiting will strand transfer be considerably affected. Nevertheless, it is still possible that factors other than the RNase H function per se may have minor effects on the level of the strand transfer activity (20).

DISCUSSION

The similarities between the biochemical and molecular properties of the RTs of HIV-1 and HIV-2 have been described in a series of studies (17,18,20,22). It was documented that there is an outstanding difference in the levels of the RNase H activity of these two highly related RTs, which was recently attributed to the thumb subdomain of the small protein subunit, which was assumed to be in close contact with the RNase H domain located in the large RT subunit (20). Additional factors have been shown to affect RNase H activity, including the length of the small subunit (p54 or p58), the heteroduplex substrate nucleotide composition and/or the presence of Mg$^{2+}$ or Mn$^{2+}$ ions (23). While variations of these factors have been shown to either enhance or impair RNase H activity, the RNase H activity of HIV-2 RT was always significantly lower than that of HIV-1. In the study presented here, we have precisely identified which amino acids in the 79 residue long thumb domain (Fig. 1) are responsible for the observed differences in the RNase H activity of HIV-2 RT. We show a substantial enhancement of RNase H activity of HIV-2 RT by modifying a single residue in the small subunit thumb subdomain.

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The second conclusion that can be drawn from the present study is that the apparent dissimilarity observed in vitro between the levels of the RNase H activity of the two wild-type HIV RTs might result mostly from the substantial difference in the apparent $K_m$ values. This means that residue 294 probably affects the affinity of the RT for the RNA-DNA heteroduplex substrate. A proline in position 294 of the small subunit of the mutated HIV-2 RT induces an almost 10-fold increase in this affinity relative to the wild-type RT (where a glutamine is located at this position). On the other hand, the rates of RNA hydrolysis per se (as deduced from the apparent $k_{cat}$ values) are quite similar in both cases; thus, residue 294 does not contribute significantly to the observed differences in the rates of RNA hydrolysis but affects the affinity of the enzyme for its RNase H substrate.
of p54 is located more than 14 Å away from the closest RNase subunit is, nonetheless, structurally surprising, since the P294 residue of the HIV-2 RT enzyme due to the Q294P mutation in the p54 domain (Fig. 4). The marked increase in the RNase H activity (Accelrys).

The newly determined three-dimensional structure of HIV-2 RT was solved in the absence of RNA or DNA substrates (10) and a conformational change in the p68 thumb subdomain and also shortens the distance between the entire p54 thumb subdomain and the bound RNA–DNA heteroduplex (as based on the 1HYS crystal structures; 25). The existence of additional contacts of Q294 of the small subunit with the RNase H domain might hinder this movement that could be required for substrate binding. The presence of a proline at position 294 in HIV-2 RT that induces an increase in the RNase H activity. Similarly, we will see which residues other than glutamine at position 294 of HIV-1 RT might affect this activity.

A comparison of a large number of isolates of HIV-2 as well as of the highly related simian immunodeficiency virus (SIV) indicates that the Q294 of the RT is highly conserved (http://www.ncbi.nlm.nih.gov). Thus, Q294 was present in all 25 isolates of HIV-2 tested. In the case of SIV, Q294 was present in 24 out of 35 isolates analyzed; glutamic acid was found in five isolates and valine, alanine, leucine or threonine in all the rest. Interestingly, prolines were not detected at this position in any of the 60 HIV-2 and SIV isolates analyzed. This strongly suggests that glutamine at position 294 is required in almost all cases for the optimal viability of the virions. It also implies that a higher RNase H activity might be disadvantageous in the context of the life cycle of HIV-2 and SIV. We are now studying how mutations of Q294 in HIV-2 RT affect the life cycle of virions.

In conclusion, residue 294 plays an important role in affecting the affinity of the RT for its RNA-DNA RNase H substrate. We hope that by understanding the factors that induce the attenuation of the affinity of the RNase H domain of HIV RTs for its substrate it might be possible to develop novel means to block this activity.

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