Biochemical and molecular modeling studies of human immunodeficiency virus type 1 reverse transcriptase (RT) have revealed that a structural element, the minor groove binding track (MGBT), is important for both replication frameshift fidelity and processivity. The MGBT interactions occur in the DNA minor groove from the second through sixth base pair from the primer 3'-terminus where the DNA undergoes a structural transition from A-like to B-form DNA. Alanine-scanning mutagenesis had previously demonstrated that Gly262 and Trp266 of the MGBT contributes important DNA interactions. To probe the molecular interactions occurring in this critical region, eight mutants of RT were studied in which alternate residues were substituted for Trp266. These enzymes were characterized in primer extension assays in which the template DNA was adducted at a single adenine by either R- or S-enantiomers of styrene oxide. These lesions failed to block DNA polymerization by wild-type RT, yet the Trp266 mutants and an alanine mutant of Gly262 terminated synthesis on styrene oxide adducted templates. Significantly, the sites of termination occurred primarily 1 and 3 bases following adduct bypass, when the lesion was positioned in the major groove of the template-primer stem. These results indicate that residue 266 serves as a “protein sensor” of altered minor groove interactions and identifies which base pair interactions are altered by these lesions. In addition, the major groove lesion must alter important structural transitions in the template-primer stem, such as minor groove widening, that allow RT access to the minor groove.

Rapid and efficient DNA polymerization requires stable interactions between the polymerase and the template-primer to facilitate highly processive DNA synthesis. When examined alone, the leading/lagging strand polymerases from human, Escherichia coli, and T4 bacteriophage are poorly processive, but become highly processive after binding accessory proteins (1, 2). Each of these replication machines also exhibits high fidelity. Although the presence of an editing 3′ → 5′ exonuclease activity contributes significantly to this low error rate, evidence supports a general correlation between polymerase processivity and fidelity (3).

Human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) is a heterodimer of p66 and p51 subunits and exhibits both polymerase and RNase H activities. However, RT does not have an associated 3′ → 5′ exonuclease function (4) and is only moderately processive (5). RT is one of the most error-prone DNA polymerases examined to date, averaging one error per 2 kilobase pairs replicated (4, 6). RT appears to lack a true processivity factor in vivo, although HIV nucleocapsid protein may operate in a manner similar to single-stranded binding protein to increase RT processivity through regions of DNA secondary structure (7). Since the rapid mutation rate of HIV is widely considered a major stumbling block in the development of therapies to combat acquired immunodeficiency syndrome, an understanding of how RT structure is correlated with function during DNA synthesis is of high priority.

Several crystal structures of RT have been reported, including the apoprotein (8), co-crystals with non-nucleoside inhibitors (9–11), co-crystal with DNA (12), and a ternary complex with bound DNA and dNTP (13). These structures have identified substrate binding and active sites for this multifunctional enzyme. Whereas the polymerase and RNase H domains are formed by the amino- and carboxyl-terminal regions of the p66 subunit, both the p66 and p51 subunits form an apparent DNA-binding cleft. The primary sequence that links the polymerase and RNase H domains has been termed the connection subdomain. The p51 subunit lacks a RNase H domain. One subdomain (residues 244–322; referred to as “thumb” using the hand analogy (see Ref. 9)) of the polymerase domain, which is primarily α-helical in character, is believed to be important in RT double-stranded nucleic acid binding. Indeed, comparisons of RT crystal structures with and without template-primer suggest that this subdomain may move as much as 30 Å (8, 14). Non-nucleoside inhibitor binding to RT has been shown to affect the mobility of this subdomain (10, 11), offering one explanation for how this class of drugs may inhibit enzyme catalysis. However, the specific protein contacts provided by

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1 The abbreviations used are: HIV-1, human immunodeficiency virus type 1; RT, reverse transcriptase; SO, styrene oxide; MGBT, minor groove binding track.
RT to bind template-primer, along with a detailed structural description of these interactions, remain elusive. Molecular modeling has been successfully applied to the RT/DNA crystal structure in an attempt to examine specific protein-DNA interactions since the resolution of the crystal structure was insufficient to define these interactions (15, 16). The refined structure of the Fab/DNA/RT complex (12) and the x-ray crystal structure of a covalently trapped DNA/dNTP/RT complex (13) confirm many of the predictions of this model.

Studies from the Wilson and Kunkel laboratories have provided strong evidence that amino acid residues in α-helix H (αH, residues 255–268) (17, 18), but not α-helix I (αI, residues 278–286) (19), influence nucleic acid binding and frameshift fidelity of polymerase-competent enzyme. Most importantly, several residues of αH (i.e. Gln255, Gly262, and Trp266) are part of a motif referred to as the minor groove binding track (MGBT) (15). The MGBT is situated in the minor groove of the template-primer stem where DNA undergoes a 45° bend that accompanies the change from A-like to B-form DNA (20), and makes “nonspecific” sugar and base contacts two to six base pairs upstream of the 3′-terminus of the primer (15, 16). An earlier study analyzing the effects of site-defined styrene oxide (SO) DNA adducts on RT-mediated DNA synthesis suggested that this monocyclic aromatic hydrocarbon major groove adduct causes RT to terminate polymerization. Since these major groove lesions do not significantly alter the solution structure of DNA (21, 22), it was suggested that the adducts may restrict DNA bending in the template-primer stem and/or interfere with key RT-DNA contacts (possibly those supplied by MGBT residues) (23). In this report, we examine whether the major groove SO lesions induce structural alterations in the DNA minor groove that are monitored by residues known to interact in the minor groove in the vicinity of the DNA bend (15, 16). To monitor structural changes in the minor groove, mutants have been created at residues Gly262 and Trp266 that are known to affect DNA binding (16–18). Eight mutants at residue 266 have been analyzed and compared with previous work (16) to ascertain whether the influence of the SO lesions occurs in a systematic manner consistent with altered DNA interactions with this residue. By examining the replication profiles of wild-type RT and Gly262 and Trp266 mutant enzymes on SO-adducted templates, we have manipulated potential nucleic acid interactions at the protein surface as the position of the SO lesion changes as the primer is progressively extended. Analyses of our results further define the structural role of these residues, as well as confirm their sites of interaction during nucleic acid synthesis.

**EXPERIMENTAL PROCEDURES**

**Expression and Purification of Wild-type and Mutant Forms of HIV-1 RT**—Mutant and wild-type forms of HIV-1 RT were prepared as described previously (16, 17, 19).

**Preparation of SO Adducted Templates—Oligonucleotides (11-mers) containing site- and stereospecifically modified R- and S- enantiomers of SO at N6-adenine were synthesized as described previously (24) and were the gift of Drs. Thomas and Connie Harris (Vanderbilt University, Nashville, TN). The site of adduction was the third position within codon 11 of the human N-ras gene: GTGCGGGTGTG, where the underlined A was the site of adduction. These sequences, whether unadducted or adducted with R- or S-SO N6-adenine, were assembled into 63-mer oligonucleotides as illustrated in Fig. 1. Both the 11- and 32-mers were fully phosphorylated with T4 DNA kinase (New England Biolabs, Beverly, MA) and ATP. The 63-mer was assembled on a 46-mer scaffold that was complementary to part of the 63-mer DNA. Each of the three 5′-3′ ended oligonucleotides were added to a final concentration of 500 μM, and the final reaction mixture also contained 200 μM bovine serum albumin and 1 mM dithiothreitol. Reactions were initiated at 37 °C in a 10-μl volume by enzyme addition. Aliquots (2.5 μl) were removed and added to 5.0 μl of stop buffer (95% (v/v) formamide, 20 μl EDTA, 0.05% (w/v) bromophenol blue, 0.05% (w/v) xylene cyanol). DNA samples were separated by electrophoresis through 15% polyacrylamide, 8 M urea gels at 2000 V for 3 h. Further reaction details are indicated in the figure legends. The relative intensities of the termination sites were quantified using either a Molecular Dynamics PhosphorImager or STORM860 of autoradiographs. Termination probabilities at each site were calculated as the ratio of products at a site to the products at that site plus all greater length products. Percentages were calculated around a 10-nucleotide window around the adducted site. All data averaging and analysis of standard deviations represent the compilation of three or four independent experiments, except for data on the G262A mutant polymerase, in which only two data sets were collected.

**RESULTS**

In order to assess the consequence(s) of mutations in the MGBT of RT on damaged DNA, the primer extension activity of each enzyme was measured on a 63-mer template containing either no adduct, or the R- or S-SO adduct coupled to the N6-position of a specific adenine (Fig. 1). The methods for the chemical synthesis of these unique lesions has been described elsewhere (23, 24). The solution structure of the R- and S-SO lesions within an oligodeoxynucleotide duplex revealed that the adducts, which lie in the major groove, are well accommodated by the DNA duplex and do not distort the local helix geometry (21, 22). However, the R- and S-SO stereoisomers differ in their spatial orientation; the R-SO N6-adenine adduct is directed toward the 5′-end of the template (21), whereas the S-SO adduct is directed toward the 3′-terminus of the template (22). As a result, they have nearly opposite orientations in duplex DNA, although the R- and S-SO adducts are chemically identical. The two adducts, therefore, may report protein-DNA interactions at different positions within the template-primer, even though they are attached to the same nucleotide in the template. Crystal structures of several DNA polymerases (25–28), including RT (12, 13), indicate that the DNA is A-like near the polymerase active site. Since DNA polymerases interact with duplex DNA through the DNA backbone and minor groove, these adducts allow us to assess whether there are structural perturbations translated to the minor groove and/or whether the local DNA structure is altered these lesions as RT deforms the DNA substrate during replication.

To simplify analysis of the experiments, primer extension reactions were conducted under conditions allowing single encounters between RT and the template-primer (i.e. most of the primer is left unextended). Consequently, the data could be analyzed in terms of termination probabilities, or the percentage of polymerase molecules that dissociate at each template base (29).

**R- and S-SO Lesions in a Template-Primer Construct Do Not Significantly Terminate DNA Synthesis by Wild-type RT**—In a previous report, we demonstrated that site-specific N6-adenine R- or S-SO lesions situated in a 63-mer template terminated RT extension of a 17-mer primer 3–5 bases after adduct bypass (23). However, extension past these adducts by wild-type RT on a 63-mer template primed with a 17-mer primer was essentially unhindered by the SO modification, based on limited termination observed in the vicinity of the lesion (Fig. 2). This apparent discrepancy is due to the different template-primers used in these studies. The local sequence context of the R- and S-SO lesions in this work (5′-GGCACGGTGTTG-3′, where the
underlined adenine was modified) and the previous study (5'-CGGACAAGAGC-3', where the SO adducts were localized to one of the three underlined adenines) are distinct. Single base changes in the nucleotides flanking the SO adduct can alter termination probability of RT on these substrates by as much as 4-fold (23). Thus, the difference in the local sequence contexts between the adducted template-primer constructs could readily explain the differences in adduct bypass.

Additionally, the 63-mer template shown in Fig. 1 would have a much longer single-stranded overhang than the template-primer substrate characterized previously (23) when RT encountered the adduct. It has been suggested that optimal RT binding to nucleic acid requires an overhang of 6 or 7 nucleotides (14, 30), yet the region 3–5 bases after adduct bypass where maximal RT termination was observed only provided a 3–6-nucleotide single-stranded overhang. Since that template-primer may not offer an “optimal” substrate for RT binding, the termination probabilities calculated at certain positions may have been amplified by the weak DNA interactions occurring near the end of the template. In contrast, the 63-mer template primed with a 17-mer primer studied here provides a 23-nucleotide overhang once the polymerase encounters the adduct. As a result, this template-primer construct is an optimal (i.e. fully stabilizing) substrate in the region analyzed. Since the termination probabilities in this study are analyzed relative to the unadducted template-primer, the reason for the apparent differences with the previous work does not affect our interpretations in this study.

G262A and W266A RT Are Less Processive and Exhibit More Adduct-directed Termination than Wild-type RT—Characterization of a “horizontal” scan of alanine mutants in RT implicated Gly262 and Trp266 as being important in RT binding to template-primer (17). Although the activity of both mutants was comparable to wild-type enzyme, the mutants displayed dissociation rate constants for template-primer that were severe orders of magnitude greater than wild-type RT. These mutants also exhibited a lower fidelity than wild-type enzyme for template-primer slippage initiation errors (18). Since these results strongly suggested that Gly262 and Trp266 influence RT interactions with template-primer, the behavior of each alanine mutant at these two residues on SO-adducted DNA was analyzed (Fig. 2). In contrast to the activity of wild-type RT (lanes 1–3) on the SO-adducted substrates, both the W266A (lane 6) and the G262A (lane 9) enzymes terminated DNA synthesis 1–3 bases after bypassing the lesion (Fig. 2). The findings that the dominant termination sites arose after synthesis past the adduct, as opposed to opposite or one base 3’ to the adduct, are consistent with earlier results (23). As observed earlier with M13 DNA, both G262A RT and W266A RT were poorly processive as compared with the wild-type enzyme (18).

The contrast between the wild-type RT and the mutant enzymes, G262A and W266A is more clearly illustrated in Fig. 2B, where termination probability is plotted at each nucleotide position within a 10-nucleotide window around the adducted site. The position of the SO adduct is designated 0, and DNA synthesis is from left to right. Although the termination probabilities manifested by wild-type RT (upper panel) were nearly indiscernible of the SO lesion, the replication profiles of the G262A and W266A mutants were strongly influenced by one or both of the styrene adducts. For example, when W266A replicated DNA containing the S-SO adduct, significantly elevated termination was observed 1, 3, and 4 nucleotides after bypass of the lesion (sites designated +1, +3, and +4, respectively). This termination was severalfold greater for the template containing the S-SO adduct than for the unadducted template or the template containing the R-SO lesion. Similar results were observed with G262A in which the major termination sites were +1 and +2 with the S-SO template. Much like the W266A mutant, translesion synthesis past the S-SO adduct by G262A induced more termination than replication past the R-SO lesion.

"Vertical” Scan Mutants of Trp266 Differ Dramatically in Their Ability to Bypass R- and S-SO Adducts—Given the key role of Trp266 in RT-nucleic acid interactions several base pairs upstream of the polymerase active site, we examined whether this side chain can recognize changes in the DNA minor groove during replication past the SO adduct. To do this, we examined the ability of seven vertical scan mutants of residue 266, in

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2 It should be noted that weak RT binding near the end of SO-adducted templates does not alter the interpretation or conclusions of our previous work (23). The consequences of a lower DNA binding affinity in the vicinity of the adduct would be fully manifested in the control (i.e. unadducted template-primer).

3 A “vertical scan” describes the site-directed mutagenesis of a single protein residue to several alternate amino acids. In contrast, a “horizontal scan” represents mutagenesis of a series of adjacent protein residues to the same amino acid.
FIG. 2. In vitro replication of DNA-containing styrene oxide by wild-type RT, and alanine mutants of Gly

262
and Trp

266.

A, three 63-mer templates (125 fmol) were constructed that contained either no lesion (U) (lanes 1, 4, and 7), the R-SO lesion (lanes 2, 5, and 8), or the S-SO lesion (lanes 3, 6, and 9) seven bases downstream of the 3'-hydroxyl of a labeled 17-mer primer. DNA syntheses were carried out by the wild-type RT (10 fmol for 5 min) (lanes 1–3), W266A mutant (5 fmol for 5 min) (lanes 4–6), and G262A mutant (25 fmol for 2 min) (lanes 7–9). The arrow indicates the position of the site of the template lesion. B, termination probabilities were calculated for a 10-nucleotide window around the adduct site, in which incorporation opposite the adduct is the 0 position. Nucleotide incorporation opposite template nucleotides 5' to the lesion (i.e., after bypass) is positive (+) and 3' to the lesion is negative (−). Termination probability was calculated as outlined under "Experimental Procedures." Standard deviations were calculated for a minimum of three independent data sets, except for G262A, which was calculated for duplicate analyses. White open bar, unadducted template-primer DNA; solid bars, R-SO adducted template-primer DNA; stippled bar, S-SO adducted template-primer DNA.
FIG. 3. In vitro replication of R- and S-SO adducted templates by mutants of Trp<sup>266</sup>. A, conditions were as in Fig. 2. Unadducted templates (U) are in lanes 1, 4, 7, 10, 13, 16, 19, 22, and 25; R-SO-containing templates (R) are in lanes 2, 5, 8, 11, 14, 17, 20, 23, and 26; S-SO-containing templates (S) are in lanes 3, 6, 9, 12, 15, 18, 21, 24, and 27. The amount of polymerase and time of DNA synthesis were as follows: wild-type, 12.5 fmol for 4 min (lanes 1–3); W266F, 12 fmol for 3 min (lanes 4–6); W266Y, 3 fmol for 3 min (lanes 7–9); W266I, 12 fmol for 3 min (lanes 10–12); W266L, 12 fmol for 3 min (lanes 13–15); W266V, 12 fmol for 3 min (lanes 16–18); W266A, 12 fmol for 3 min (lanes 19–21); W266R, 15.5 fmol for 3 min (lanes 22–24); W266E, 3 fmol for 3 min (lanes 25–27). The arrow indicates the position of the site of the template lesion. B, termination probabilities were calculated for a 10-nucleotide window around the adduct site, in which incorporation opposite the adduct is the 0 position. Terminations opposite template nucleotides 5' to the lesion are positive and 3' to the lesion are negative. Termination probability was calculated as outlined under “Experimental Procedures.” All experiments are the compilation of three or four independent analyses, and the error bars represent standard deviation. White open bar, unadducted template-primer DNA; solid bars, R-SO adducted template-primer DNA; stippled bar, S-SO adducted template-primer DNA.
addition to W266A, to bypass the SO adducts. Alternate side-chain substitutions included two aromatic (W266Y, W266F), three aliphatic (W266I, W266L, W266V), and two charged side chains (W266R, W266E). These mutants have been extensively studied and demonstrated to alter RT-nucleic acid interactions in a manner that indicates that Trp266 contributes both van der Waals and hydrogen bonding interactions (16). We are therefore in a position to decipher whether translesional DNA synthesis also occurs in a manner consistent with altered interactions with residue 266.

Primer extension by these mutants is shown in Fig. 3A. The termination pattern by the wild-type RT and two mutants with aromatic substitutions (W266F and W266Y) was similar, in that both mutants bypassed the SO adducts with termination probabilities comparable to the wild-type enzyme. Both W266F and W266Y, however, demonstrated a slight elevated termination probability at the +1 position after bypassing the S-SO lesion (i.e. the adduct is one base into the template-primer stem).

In contrast, synthesis by the aliphatic side-chain mutants, including W266A, revealed a marked increase in termination probability at numerous nucleotides beyond and including the SO-adducted base (Fig. 3B). The increases in adduct-dependent termination by these RT mutants were most prominent at the +1, +3, and sometimes +4 sites, when the adduct was positioned in the template-primer stem. In all cases, adduct-dependent increases in termination probability were more pronounced with the S-SO adduct than the R-SO adduct.

The W266R mutant bypassed the SO lesion with efficiencies comparable to that of some of the aliphatic side-chain mutants. Again, the +1 position dominated the spectrum of termination sites, and adduct-dependent increases were seen at +1, +3, and +4. Although the R-SO adduct did increase termination slightly at some sites (+1, +1, +1), the largest increases were caused by the S-SO lesion. By comparison, the W266E mutant exhibited such limited processivity under these conditions that most of the extended primers never reached the adducted site. However, increased termination probabilities were most evident at +3 for R-SO, and 0, +1, and +3 for S-SO.

The termination probability at a target site is greater for a polymerase with low processivity relative to a processive polymerase. The fraction of termination that is due to a DNA adduct cannot be accurately calculated unless a correction is made for the intrinsic processivity of the polymerase (31).
Without such a correction, a polymerase with low processivity may exhibit a higher termination probability at an adducted site than a polymerase with a higher processivity simply because termination is highly probable at that position, whether the adduct is present or not. To better appreciate the consequences of the adduct on termination, we replotted the data in Fig. 3B after dividing the adduct-dependent termination probability at each template nucleotide position by the termination probability at the corresponding position in the unadducted template. This analysis adjusts for the sequence-specific, adduct-independent termination on DNA, leaving the residual termination probability that results from the effect of the lesion on replicative bypass DNA synthesis. The results of these analyses are shown in Fig. 4 for the R-SO and S-SO templates (panels A and B, respectively). Although termination induced by R-SO lesions was consistently lower than that induced by S-SO lesions, the maximum R-SO-dependent terminations for W266A, W266I, and W226V were still quite large in some instances (e.g. more than 5-fold at position +3, Fig. 4A). Indeed, the +3 position was the site of maximum termination probability on the R-SO template for all six of the non-aromatic substitutions. Similar results were obtained after analysis of the S-SO-induced termination sites, except that the +1 position was the site of maximum termination probability on this template for all of the substitutions at residue 266 except for W266E. Given that the R- and S-SO adducts likely differ in their orientation on DNA and in their ability to terminate RT polymerization, the finding that both lesions induce termination after lesion bypass at similar sites along the DNA template supports the conclusion that these major groove lesions induce structural alterations that are monitored in the DNA minor groove by residue 266 upstream of the polymerase active site.

**DISCUSSION**

In this report, we present evidence that residues Gly<sup>262</sup> and Trp<sup>266</sup> can act as “protein sensors” of structural perturbations in the DNA minor groove upstream of the polymerase active site. By studying altered forms of the enzyme (via amino acid substitutions) and the template-primer (by placing site-specific DNA modifications in the template), one can modulate protein-DNA interactions from the perspectives of the protein and the DNA. In this way, the binding interactions between RT and DNA can be tightly coupled and uncoupled, presenting a more detailed view of how the two macromolecules interact.

The results show that the predominant sites of R- and S-SO adduct-directed termination by the G262A and W266A mutants of RT occurred not opposite or immediately 3′ to the lesion, but instead, 1–3 bases after translesion DNA synthesis. This finding is in excellent agreement with the position of Trp<sup>266</sup> relative to the DNA as revealed in the model of the RT-DNA complex, where Trp<sup>266</sup> is nearest the +3 base pair (15, 16), and confirmed by x-ray crystal structures of RT-DNA com-
A normalized ranking of the consensus termination sites for the non-aromatic substitutions at residue 266 on SO-adducted templates

| Trp<sup>266</sup> mutant | R-SO Maximum change<sup>a</sup> | S-SO Maximum change<sup>a</sup> |
|--------------------------|-----------------------------|-----------------------------|
| Isoleucine               | +1, +3                      | 6.3                         | 0, +1                      | 13.8                        |
| Leucine                  | +1, +3                      | 3.1                         | +1, +3                     | 12.5                        |
| Valine                   | -2, +3                      | 7.7                         | -1, +1                     | 4.8                         |
| Alanine                  | +1, +3                      | 6.9                         | +1, +4                     | 7.7                         |
| Arginine                 | +1, +3                      | 2.4                         | +1, +1                     | 4.8                         |
| Glutamate                | +1, +3                      | 3.2                         | 0, +1                      | 7.6                         |

<sup>a</sup> Indicates the single largest -fold increase in normalized termination probability across the 10-nucleotide window. The underlined position refers to that position expressing the Maximum Change.

The two strongest relative termination sites induced by the SO lesion, normalized to wild-type enzyme, were tabulated for the 10-nucleotide window and are shown in Fig. 4. This normalized relative termination represents the ratio of the relative termination probabilities at each site for the mutant enzyme and the corresponding site for wild type. Positive numbers on the x-axis indicate the number of nucleotides inserted after lesion bypass, and 0 represents termination opposite the lesion. The consequence of this transformation is that any difference in sequence- or adduct-dependent termination by the RT mutants as compared to wild type is more clearly revealed since the calculation filters functional aspects of translesion bypass due to everything except for the contribution of the mutated residue itself. Negative values on the x-axis represent termination sites prior to the lesion reaching the polymerase active-site. The W266F and W266Y mutants were omitted because the activity of these mutant enzymes was very similar to the wild type RT.

In general, the R-SO lesion induced the most relative termination of DNA synthesis by RT when situated at the +3 position, whereas the S-SO lesion caused the most relative termination at the +1 position (Fig. 4 and Table I). This difference in the position of relative termination between the two adducts is consistent with the orientations of the R and S enantiomers in duplex DNA as determined by <sup>1</sup>H NMR. The styryl ring in R-SO points toward the 5’-end of the template (i.e. in the direction of polymerization) (21), but the styryl ring of S-SO points toward the 3’-end of the template (i.e. facing the oncoming polymerase) (22). Thus, a perturbation in the DNA that is triggered by an S-SO adduct would be expected to be detected by the polymerase before a similar distortion created by an R-SO lesion occupying the same position in the template.

Since significant termination occurs at the +1 as well as the +3 position, it is also possible that modification of Trp<sup>266</sup> may also result in “local” side-chain movements. In this context, the model structure (15, 16), as well as the published crystal structures of RT-DNA complexes (12, 13), indicate that there are van der Waals contacts between Trp<sup>266</sup> and Tyr<sup>252</sup>. Tyr<sup>252</sup> is part of the β12-β13 loop that is observed to interact with the DNA backbone between the first two nucleotides of the primer strand. This motif has termed the “primer grip” (20) and alteration of Trp<sup>266</sup> by mutagenesis has demonstrated the functional importance of this residue (32, 33). Thus, reducing the size of the side chain at residue 266 could result in a change in the position of Tyr<sup>252</sup> (i.e. primer grip) resulting in altered interactions near the primer 3’-terminus.

Substitutions of Trp<sup>266</sup> with an aromatic side chain (W266F, W266Y) were not particularly deleterious to RT function. This is not surprising, given the conserved nature of the mutation. However, Trp<sup>266</sup> substitutions by aliphatic side chains both decreased enzyme processivity and increased adduct-directed termination. These findings are consistent with the strong correlation observed between buried apolar surface area, DNA binding affinity, and frameshift fidelity (16). In addition, molecular modeling of the charged alternate side chains at residue 266 (glutamate and arginine) indicate that these side chains can form hydrogen bonds with surrounding residues. In the case of the glutamate substitution, the carboxylate side chain is within hydrogen bonding distance to the Gn<sup>269</sup> side-chain amide, which is also part of the MGBT. Alanine substitution for this residue (Q269A) diminishes the DNA binding properties of this mutant enzyme (15). Repulsive charge-charge interactions between the carboxylate of the glutamate side chain and the negatively charged phosphates in the DNA backbone may also contribute to the inability of this mutant to polymerize efficiently when the lesion, particularly S-SO, is positioned in the template-primer stem. Moreover, there is a significant loss of van der Waals interactions upon the glutamate substitution for Trp<sup>266</sup> (16). In contrast, arginine, like tyrosine, has the ability to form a hydrogen bond. In the model structure, one of the nitrogens of the guanidinium group is within hydrogen bonding distance to N<sup>9</sup> of a template purine. The model is supported by the data comparing adduct-directed termination by the W266R and W266E RT mutants. The W266R mutant was perhaps the least detrimental, aside from the conservative aromatic side-chain substitutions, to adduct bypass, whereas the W266E mutation was by far the most detrimental due to the combined effects of a loss in processivity and enhanced termination.

It is important to note that even the wild-type enzyme shows a small increase in termination at both the +1 and +3 positions after bypassing the R-SO lesion. However, the non-aromatic side-chain substitutions at residue 266 caused a disproportionate increase in relative termination at +1 and +3. In other words, when the termination probabilities (Fig. 3) are corrected for the intrinsic processivity of each mutant (Fig. 4) and then compared with the relative termination probability exhibited by wild-type enzyme, termination at the +1 and +3 positions are increased significantly for each mutant (Table I). Indeed, the +3 position, without exception, exhibits the greatest normalized relative termination probability for templates with the R-SO lesion. Thus, the vertical scan at Trp<sup>266</sup> results in RT mutants that consistently terminate synthesis at the +1 and +3 sites, even though the normalized relative termination probability through most other sites within the 10 nucleotide window was largely unaffected. A similar conclusion can be drawn from the S-SO normalized relative termination probabilities (Table I).

A priori, it is perhaps surprising that the adenine N<sup>9</sup>-SO adducts direct RT termination. The SO lesions used in this study are known to lie in the major groove without causing a significant perturbation to the local DNA structure (21, 22). In contrast, DNA binding by RT is predominantly through the DNA minor groove and RT is unable to bypass R- or S-SO lesions situated in the DNA minor groove (34). However, it is important to note that the sites of termination (positions +1 and +3 in the DNA template) correspond to the major groove adduct being positioned in the template-primer stem in the region of the RT-induced DNA bend. Although the structural significance of the DNA bend during enzyme catalysis is unclear, one possibility is that the MGBT residues displace the spine of water molecules along the minor groove, resulting in a widening of the minor groove and narrowing of the major groove. This would favor a structural transition in the DNA to be more A-like. However, if a monocyclic adduct, such as SO, is “sandwiched” in the major groove, groove narrowing may be restricted. Thus, one explanation for the adduct-induced termination of DNA synthesis by the Trp<sup>266</sup> mutants is that the SO lesion causes the DNA to become less flexible and more resistant to structural transitions, such as groove widening or narrowing. In this context, it has been demonstrated that RT has low processivity on rigid DNA (35, 36). This adduct-imposed
rigidity in the DNA helix, coupled with weak mutant RT DNA binding, should result in greater termination by the mutant polymerases, whereas the wild-type RT, capable of much stronger binding to DNA, does not terminate synthesis at efficiently when the adduct is positioned near Gly262 or Trp266.

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Vertical-scanning Mutagenesis of a Critical Tryptophan in the "Minor Groove Binding Track" of HIV-1 Reverse Transcriptase: MAJOR GROOVE DNA ADDUCTS IDENTIFY SPECIFIC PROTEIN INTERACTIONS IN THE MINOR GROOVE

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