Differential Tolerance to DNA Polymerization by HIV-1 Reverse Transcriptase on N^6 Adenine C_{10}R and C_{10}S Benzo[a]pyrene-7,8-dihydrodiol 9,10-Epoxide-adducted Templates*

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To determine the effect of various stereoisomers of benzo[a]pyrene-7,8-dihydrodiol 9,10-epoxide (BPDE) on translesion bypass by human immunodeficiency virus-1 reverse transcriptase and its α-helix H mutants, six 33-mer templates were constructed bearing site- and stereospecific adducts. This in vitro model system was chosen to understand the structure-function relationships between the polymerase and damaged DNA during replication. Comparison of the replication pattern between wild type human immunodeficiency virus-1 reverse transcriptase and its mutants, using primers which were 3' to the lesion, revealed essentially similar patterns. While these primers terminated with all three of the C_{10}R and two of the C_{10}S BPDE-adducted templates failed to allow any synthesis, the S configuration at C_{10} is directed toward the 5' side of the modified strand. In contrast, the pyrenyl moiety of (+)-anti-trans- isomers is oriented toward the 3' side of the modified strand.

Furthermore, there are several reports to show that the chirality of adducted molecules plays a major role in influencing biological processes, such as exconucleolytic processing and DNA replication. Structural data on double-stranded oligonucleotides adducted with BPDE at N^2 of guanine revealed that the pyrenyl moiety of (+)-anti-trans-isomers is directed toward the 3' end of the modified strand. However, NMR studies on duplexed oligodeoxyribonucleotides containing these adducts on N^6 of adenine showed that the C_{10}R adducts were partially intercalated on the 5' side, and the C_{10}S adducts, although existing in more than one conformation, appear to be mainly intercalated from the 3' side.

Extensive in vitro studies have been reported on the replicative fate of DNAs damaged by bulky adducts. In particular, BPDE-adducted templates exhibit replication blockage opposite and/or one base 3' to the adduct, with translesion synthesis rarely occurring. In order to elucidate the molecular mechanisms by which polymerases could bypass a lesion site, and to gain an understanding of the structures within the polymerase that govern translesion synthesis, the study of enzymatic functions such as fidelity and regulation of DNA replication is critical. HIV-1 reverse transcriptase (HIV-1 RT) is a hypermutable enzyme that catalyzes the addition of approximately 20,000 nucleotides with extremely low fidelity during replication, resulting in mutations at a frequency of 1/1700 nucleotides inserted (35–37). Both the crystal structure and the co-crystal structure with duplex DNA are known and numerous site-specific mutants that were designed with structure-function aspects in mind are readily available (38–41). This facilitates the choice of HIV-1 RT and oligonucleotides containing site-specifically placed adducts are powerful tools for exploring how individual chemical lesions, formed in DNA, are converted into mutations, especially those in critical target classes such as cellular proto-oncogenes or tumor suppressors (11, 12). Activation of certain members of the ras proto-oncogene family (13–15) is a result of mutations predominantly in the codons for amino acid residues 12 or 61 (16). Previous data derived from a model prokaryotic system concerning the mutagenicity of six site- and stereospecific BPDE lesions placed on adenine N^6 position of N-ras codon 61 revealed a narrow range of mutation frequencies that were exclusively A → G transitions (17).

Benzo[a]pyrene is a ubiquitous by-product of incomplete combustion and is one of the most potent carcinogens known (1–4). In mammalian cells, it is metabolized by cytochrome P-450 and epoxide hydrolase to a variety of products, including the ultimate DNA-damaging agent BPDE1 (5–9). The carcinogenic and mutagenic effects of BPDE depend on covalent modification of DNA and subsequent interactions with various polymerases. There are a large number of potential adducts which arise by the cis and trans openings of the epoxide group by the exocyclic N^2, O^6, or N-7 positions of guanine or N^6 position of adenine or N^4 position of cytidine. Structure-activity studies in several polymeric aromatic hydrocarbon systems have shown that closely related positional or stereoisomers have dramatically different tumorogenicities (10). In this context, oligonucleotides containing site-specifically placed adducts are powerful tools for exploring how individual chemical lesions, formed in DNA, are converted into mutations, especially those in critical target classes such as cellular proto-oncogenes or tumor suppressors (11, 12). Activation of certain members of the ras proto-oncogene family (13–15) is a result of mutations predominantly in the codons for amino acid residues 12 or 61 (16). Previous data derived from a model prokaryotic system concerning the mutagenicity of six site- and stereospecific BPDE lesions placed on adenine N^6 position of N-ras codon 61 revealed a narrow range of mutation frequencies that were exclusively A → G transitions (17).

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§ The abbreviations used are: BPDE, benzo[a]pyrene-7,8-dihydrodiol 9,10-epoxide; HIV-1 RT, HIV-1 reverse transcriptase; dNTPs, deoxyribonucleotide triphosphates; T-P, template-primer.

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its mutants as a unique system in which to monitor polymerase stalling as a diagnostic for adduct directed perturbations.

Preliminary primer extension studies using HIV-1 RT revealed that the C10R and C9S BPDE-adducted templates gave different termination sites, but none formed full-length products, except (+)-anti-trans-BPDE (20). Therefore, the present study was undertaken in order to understand if termination occurred due to pausing of the polymerase in the vicinity of the lesion or due to dissociation of the enzyme from the T-P at this point and subsequent inability to re-initiate synthesis. Furthermore, an attempt has been made to characterize HIV-1 RT and two mutants of a-helix H (G262A and W266A) by determining their polymerization behavior on site- and stereospecifically adducted BPDE templates. Because the amino acid residues 262 and 266 are in contact with the duplex region in the vicinity of the DNA minor groove, relatively close to the 3' terminus of the primer, these two mutants were utilized in the present study. Although these polycyclic aromatic hydrocarbon adducts are not likely to be encountered by HIV-1 RT in vivo, they serve as an ideal model for examining the effect of bulky adducts on DNA processing by polymerases.

MATERIALS AND METHODS

Enzymes, BPDE-adducted Oligodeoxynucleotides, and Radiolabeled Nucleotides—The p66/p51 heterodimeric form of HIV-1 RT was expressed in Escherichia coli from a plasmid containing the precise coding region from HXB2 and was a generous gift from Dr. S. H. Wilson (University of Texas Medical Branch, Galveston, TX) (42). The alkylation-substituted mutants forms of HIV-1 RT, G262A and W266A, were also gifts from Drs. S. H. Wilson and T. A. Kunkel (38). The T4 polynucleotide kinase and T4 DNA ligase were obtained from New England Biolabs Inc. (Beverly, MA). The six stereochemically defined BPDE adducts were constructed on adenine N6 at position two of N-32P-labeled [32P]ATP (3000 Ci/mmol) utilized for 5' end labeling of oligodeoxynucleotides was purchased from DuPont NEN.

Synthesis of Primers and Construction of BPDE-adducted 33-mer Templates and Their Subsequent Purification—Oligonucleotides of 17, 27, and 29 bases that were used as primers for DNA replication studies were synthesized on an automated Applied Biosystems 394 DNA/RNA synthesizer (Perkin-Elmer/Applied Biosystems Division, Foster City, CA), according to the manufacturer's protocol (Fig. 2). The six BPDE-adducted 11-mers were analyzed for their purity on 15% polyacrylamide sequencing gels (8.3M urea) by electrophoresis at 3000 V for 4 h and visualized by autoradiography using Hyperfilm MP (Amersham Corp.). Primer annealing was determined by separation of the annealed mixture on a 10% native polyacrylamide gel, and in each case, more than 99% of the primer existed as a T-P complex prior to the addition of the enzyme.

Quantitation of bands were performed by PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA). The percentage of partial and full-length products were determined in relation to the amount of unadducted primers.

T-P Depletion Assay and Binding Assay—The conditions utilized in these assays were similar to those described for the primer extension studies, except that there was an initial extension of either unadducted or adducted templates using an unlabeled 29-mer primer. After an initial incubation of 30 min at 37°C, unadducted templates annealed to labeled 29-mer primers were added in 5-fold excess, to serve as a hot chase, and to monitor for the presence of free enzyme. All four dNTPs (0.3 mM each) were added to the reaction mixture prior to addition of 40 fmol of enzyme. A similar experiment was designed to measure the initial binding of the polymerase to the various substrates. In these experiments each of the three templates was annealed with unlabeled 29-mer primer (5:1 ratio, respectively) and incubated with HIV-1 RT (10:1, primer:enzyme) for 20 min in the absence of dNTPs. Then a 5-fold excess of template, and 17-mer 32P-labeled primer, were added, and the reactions initiated by the addition of 0.33 mM dATP and 0.33 mM dGTP only. Aliquots were taken at 0, 0.5, 2, 10, and 20 min and analyzed as described previously.

RESULTS

Experimental Rationale—The interactions between BPDE-adducted templates and polymerases under in vitro conditions most often result in replication termination in the vicinity of the DNA base lesion. The goal of this study was to determine whether correlations exist between three properties of polymerase-DNA interactions: (i) the extension of a primer on the damaged template and the orientation of the adduct; (ii) the elongation of a primer on an adducted template and the binding of the polymerase to the T-P complex; and (iii) the ability to form full length products and the specific identity of amino acid residues in the polymerase.

In order to study the structure-function interactions between polymerases and damaged DNA during replication, HIV-1 RT was chosen because of its processive nature, low fidelity, and lack of 3′ → 5′ exonucleolytic activity (36, 37). Furthermore, the resolution of its crystal and co-crystal structures gives an insight into the various subunits and subdomains and their probable functions (44, 45). HIV-1 RT is a heterodimer with two subunits, p66 and p51, of which both the polymerase and RNase H activities are limited to the p66 subunit. Furthermore, p66 forms a nucleic acid binding cleft constituting the fingers, palm, and thumb subdomains (45). The amino acid residues from 257 to 266 are highly conserved and play an important role in catalytic cycling (41). The selection of the α-helix H of the thumb subdomain and play an important role in the movement of the T-P strand during polymerization. Both the α-helices (H and I) track along the DNA 4 to 5 nucleotides from the 3′ primer terminus, with the α-helix H in close contact with the primer strand (45). The selection of alanine substitution mutants, G262A and W266A, was pertinent because of their role in reduction of processivity and fidelity. Our expectation was that the results obtained using these mutants would shed light on how specific amino acid residues influence the replication fate of BPDE-adducted templates (Figs. 1 and 2). Although enzyme-substrate interactions occur in the minor groove of the DNA, perturbations can be translated as alterations in the major groove, because the two regions are coupled.

Primer Extension Analyses with HIV-1 RT, by Using −11, −1, and +1 Primers Individually on Unadducted and BPDE-adducted Templates—In order to study the factors that influence the replication of adducted DNAs, three primers were chosen that were 17, 27, and 29 bases in length with a common 5′ end (Fig. 2). These deoxyribonucleotides position their 3′-OH 11 and 1 base(s) upstream and 1 base beyond the adduct and...
Differential Extension by HIV-1 RT on BPDE-adducted Templates

**C10-S adducts**

![C10-S adduct](image)

**C10-R adducts**

![C10-R adduct](image)

**Fig. 1. Structures of the epoxy ring of stereochemically specific adenine N9 BPDE adducts placed at the second position of N-ras codon 61 within an 11-mer oligodeoxynucleotide.** The C10S adducts are demarcated from the C10R adducts.

are indicated by a, b, and c, respectively (Fig. 3). The T-P complexes were formed with unadducted DNA (panel 1), and each of the six site- and stereospecifically modified BPDE-adducted templates (panels 2–7), and primer extension studies were performed using HIV-1 RT. The C10S and C10R adducted DNA are grouped as B and C, respectively.

As shown in the control portion of this experiment (Fig. 3A), when any of the three primers were annealed to unadducted templates, the wild type HIV-1 RT was able to extend these primers to full-length 33-mers and also catalyze a 1 base, blunt-end addition with an efficiency greater than 50%. This non-template-directed extension can possibly be attributed to slippage of the newly synthesized primer strand and the polymerase. However, it seems less likely, because of the absence of homopolymeric or repeated sequences at the end of the single-stranded template (46).

In contrast to the results obtained by using the unadducted DNAs, two of the C10S adducted templates ((+)syn-trans- and (−)anti-cis-) adducts caused the polymerase to terminate 1 base 3’ to the damaged site, using a 17-mer primer (panel 2, lane a, and panel 4, lane a, respectively), resulting in the accumulation of products that were 27 bases in length. Little to no extension was observed with the primer (panel 1, lane b, and panel 4, lane b, respectively). However, by using either of these primers, full-length products were obtained when an (−)anti-trans-BPDE-adducted template (C10S) was utilized for primer extension (panel 2, lanes a and b). Also in contrast, all adducts bearing the R configuration at the C10 position (group C) of the BPDE moiety (−anti-trans, (+)syn-trans, and (−)anti-cis) (panel 5, lanes a and b, and panel 6, lanes a and b, and panel 7, lanes a and b, respectively) did not promote termination 1 base prior to or opposite the lesion, but caused HIV-1 RT to form truncated products 1 base beyond the lesion, with generally negligible accumulation of full-length products. These reactions were performed under multiple hit conditions. However, observations under single hit conditions showed that both the C10R and C10S adducted templates formed truncated products that terminated one base prior to the lesion (data not shown).

The primer extension results obtained using the adducted templates with a 29-mer primer, which initiates synthesis 1 base beyond the lesion are presented in (lane c of panels 2–7). All the C10S BPDE-adducted templates ((+)anti-trans, (+)syn-trans, and (−)anti-cis) (panel 2, lane c, panel 3, lane c, and panel 4, lane c, respectively) allowed for the formation of full-length products when the lesion was located in the T-P stem. These fully extended products were formed at levels similar to the unadducted templates. In contrast, two of the three C10R adducted templates examined allowed negligible formation of full-length products. The (−)syn-trans-BPDE-adducted template (panel 6, lane c) was unique in that a prominent termination site 4 bases beyond the site of lesion was observed in addition to a significant amount of fully extended products.

In our subsequent experiments, similar observations were made when 73-mer unadducted and adducted DNAs were used as templates in which there is a 44-nucleotide single-stranded overhang (data not shown). These results indicate that the orientation of C10S and C10R BPDE adducts in the template strand has a major influence on protein-DNA interactions, independent of the length of the single-stranded template overhangs (data not shown). End effects are considered to play a role in nucleotide discrimination, especially in regard to the incorporation of deoxynucleotides. However, there are reports to suggest that the catalytic efficiency of nucleotide incorporation is independent of the length of the template overhang and that the ability to place a nucleotide opposite the 5’ end of the template is the same as it is to place a nucleotide at any other position (35).

**Steroselectivity of HIV-1 RT on TP Binding Dissociation and TP Depletion—**Analyses of the data presented in Fig. 3 revealed that the most significant contrast between a matched set of C10S and C10R BPDE adducts was for the (+) and (−)anti-trans-lesions, such that all primers could be readily utilized for the (+)anti-trans, while replication was blocked 1 base beyond the lesion for the (−)anti-trans-containing template (panels 2 and 5, respectively). These data raised further questions regarding the mechanism by which the (−)anti-trans-lesion blocked replication, while the (+)anti-trans-lesion allowed facile bypass. One possibility for this apparent distinction could be the inability of the polymerase to bind to the (−)anti-trans-BPDE-adducted template, once the primer had been extended 1 base beyond the lesion site. In order to determine whether there was differential binding and subsequent dissociation from a primer that is 1 base beyond the lesion, the
following experiment was designed and carried out (Fig. 4). Three T-P complexes were formed wherein the template (33-mer) was either unadducted or contained a (+)- or (−)-anti-trans-BPDE lesion and the primer utilized (29-mer) was unlabelled. The T-P ratio was 5:1 to ensure complete primer annealing. Limiting amounts of polymerase were added in the absence of dNTPs, such that the primer:polymerase ratio was 10:1. This mixture was allowed to equilibrate initially for 20 min and then a 5-fold excess of unadducted T (33-mer)-P (17-mer) complexes were added to each of the three samples, in which the primer was 32P-labeled. This incubation was carried out in the presence of dATP and dGTP alone, which allowed replication to occur exclusively on the labeled 17-mer primer strand, since the immediate upstream bases on its template were C, T, T, while the immediate downstream bases that would need to be incorporated on the unlabelled 29-mer primer were A, G, G. Thus, the rate of extension of the labeled primer would be a measure of the relative binding and dissociation from the original template primer. These results are shown in Fig. 4, B and C. In Fig. 4B, lanes 1–3 are the unadducted and (+)-anti-trans- and (−)-anti-trans-BPDE-containing complexes, respectively, and A–D represent increasing chase times of 0.5, 2, 10, and 20 min, respectively. Quantitative analyses of these data reveal that the enzyme dissociates more rapidly (approximately 2-fold) from the unadducted than from either of the adducted DNAs. Furthermore, these data strongly suggest that the binding of HIV-1 RT to the 29-mer primer on the C10R BPDE adduct containing DNA is comparable with that observed on the same primer complexed with the C10S BPDE-adducted template.

To further explore this stereospecific inhibition, a T-P depletion assay was designed wherein the unadducted, C10S ((−)-anti-trans) and C10R ((+)-anti-trans) adducted templates were individually annealed to an unlabeled 29-mer primer in the ratio of 5:1 (Fig. 5A). These T-P complexes were preincubated for 30 min with HIV-1 RT and all four dNTPs. The initial primer complex would allow for synthesis to occur, given that the nucleotide incorporation and subsequent extension were not hindered. Thus, following the formation of products, the enzyme would then be free in solution for further primer extension. The prediction was that the primers complexed with either the unadducted and C10S templates would be extended well, allowing the polymerase to be free to initiate the chase portion of the experiment, while the T-P complex with the C10R BPDE-adducted template would still have HIV-1 RT bound to it. This prediction was in concurrence with what was determined experimentally (Fig. 5, B and C). Furthermore, the rate of primer utilization was indistinguishable when either the unadducted or C10S BPDE-adducted DNAs served as templates, whereas the availability of HIV-1 RT for chain synthesis after preincubation with the labeled primer complexed with C10R BPDE-adducted template was 3-fold less.

Role of the α-Helix H Subdomain of HIV-1 RT in the Replication Fate of the C10R and C10S BPDE-Adducted Templates when the Lesion Is in the T-P Stem—In order to determine whether the minor groove binding α-helix H in HIV-1 RT plays a role in adduct-directed terminations, wild type enzyme was compared with two α-helix H mutants, G262A and W266A (Fig. 6). These residues have previously been shown to affect termination probabilities on unadducted templates (38). Panel 1 of Fig. 6 shows data for unadducted templates, while panels 2–7 show data for each of the six BPDE-containing DNAs. The lanes a–c refer to wild type, G262A, and W266A enzymes, respectively.

As shown in Fig. 6 and Table I, all of the C10S-configured BPDE-adducted templates were readily copied to full length, albeit at rates much reduced relative to unadducted templates, by the alanine substitution mutants of the α-helix H thumb subdomain of HIV-1 RT when primed with the +1 (29-mer) primer. However, the overall degree of extension of the primer on the three adducted templates and the unadducted template differed moderately with these enzymes. As tabulated in Table I, fully extended products with the C10S adducted templates and unadducted template, polymerized with the wild type HIV-1 RT, showed a range from 66 to 79%. With the G262A mutant, the values ranged from 25 to 83%, whereas with the W266A, the values covered a span from 5 to 79%. The (−)-anti-cis-BPDE-adducted template allowed the least extension, with each of the three enzymes utilized for polymerization. In all these reactions, the amount of degraded primers accounted for less than 1% of the total primer used per lane.

Two of the C10R BPDE-adducted templates ((−)-anti-trans and (+)-anti-cis), when copied individually by the two mutant enzymes (G262A and W266A), showed no polymerase extension at all. However, the (−)-syn-trans-BPDE-adducted template (also with C10R configuration), which yielded 24% full-length products when polymerized by HIV-1 RT, allowed some extension with G262A and W266A mutant enzymes. The
W266A mutant enzyme, lacking tryptophan, showed strong termination 3 bases 5' to the adducted site (Fig. 6).

DISCUSSION

Polycyclic aromatic hydrocarbon carcinogens such as BPDE are known to bind covalently to cellular nucleic acid, leading to mutations and alterations of gene expression that could result ultimately in tumorigenesis and carcinogenesis (47–52). Insight into the precise nature of the interactions between the different stereospecific BPDE-adducted templates and various polymerases has involved numerous in vitro studies, where generally, termination occurs at or one base prior to the site of the adduct (26, 27, 32–34). However, translesion synthesis with these bulky adducted templates has to date been observed with relatively few polymerases (20, 23). HIV-1 RT is one polymerase that was able to bypass the site of lesion.

Our earlier in vitro studies with this polymerase involved six site- and stereospecific BPDE-adducted templates, each being annealed to a 17-mer primer located 11 bases downstream of the adducted base. Although some translesion synthesis by HIV-1 RT was observed past the 5'-oriented C_{10R} BPDE adducts, using −11 primers, no full-length products were formed. Furthermore, the 3'-oriented C_{10S} BPDE-adducted templates, in general, inhibited polymerization beyond the adduct, and this was unaltered by increased dNTP concentrations (20, 53).

In the current study, although −1 primers were designed in an attempt to force polymerization beyond the site of lesion, there was no change in the pattern of products produced. In contrast, the utilization of +1 primers led to full-length products with
the C10S BPDE-adducted templates, suggesting the influence of orientation on primer extension, given that the adduct extends nearly two bases in the direction of the tilt.

This differential extension of C10R and C10S BPDE-adducted templates initially suggested a differential affinity of polymerase binding. However, based on binding/dissociation assays (Fig. 4C), our data showed clearly that both of these sets of adducted templates have similar binding affinities and dissociation rates that are at least 3-fold slower than the unadducted template. As a corollary, this disparity in primer extension is likely to be attributed to some hindrance for nucleotide incorporation and/or for subsequent elongation. There are reports to show that, if there are no significant differences in polymerase binding affinities, then one can infer that reduced extension efficiencies are caused by an inherent difficulty that the enzyme has in extending a mismatch or a damaged site (54). The hydrophobic properties of the bulky adducts could be one of the contributing factors that stabilize binding.

The 3′ orientation of the C10S BPDE adducts on adenine rationalizes the formation of truncated products prior to the lesion, on utilizing either a 17- or 27-mer primer, given that the directionality of the polymerase movement is from 5′ → 3′. However, the replication pattern on the (+)-anti-trans-BPDE-adducted template with HIV-1 RT allowed for facile bypass and accumulation of full-length products. Given that there is a wide range over which the adduct is positioned, whether it is a 5′ or 3′ orientation relative to the template, it is possible that this lesion is oriented with a minimal distortion and inhibitory angle, thus offering very little interference or hindrance to the polymerase movement along its substrate. The same reasoning can be applied to the 5′-oriented (−)-syn-trans-BPDE-adducted template, which unlike the other C10R adducted templates examined, allows for the formation of full-length products on utilizing a 29-mer. A correlation between the spatial conformation...
tion and orientation of the whole polycyclic aromatic hydrocarbon moiety may be important. There are preferred conformations of the hydroxyl groups on C7 and C8 in each isomer (\(\text{anti-trans-}, \text{syn-trans-}, \text{and } \text{anti-cis-}\)) which may be axial or equatorial or some intermediate states such as pseudoaxial or pseudoequatorial (22). These differences in the conformation of the tetrahydrobenzo ring may possibly be described as contributing to the inhibitory angle. Thus, our results suggest that not only is the stereochemistry at the attachment point of the adduct (C10) significant but also the stereochemistry within the aralkyl ring. For example, in nucleosides with \(\text{anti-trans-}\) BPDE adducts, this ring adopts a half-chair conformation with the hydroxyl groups at C7 and C8 pseudoequatorial, whereas in the corresponding nucleoside, the aralkyl ring prefers a half-chair with pseudoaxial oxygen substituents at C7 and C8 (21). Each stereoisomer will perturb the DNA structure differently depending upon the orientation of the hydroxyl group, e.g. the angle between the adduct and the helix axis and the extent of intercalation may vary significantly with both adduct and sequence. Our studies show clearly that polymerase activity, at least in the case of HIV-1 RT, is highly sensitive to such perturbation.

An additional factor in considering termination probabilities of HIV-1 RT is the perturbing influence of the adduct on the configuration of the DNA when placed in the 4-base window of the T\(\text{P}\) duplex that is accessible to chemical cleavage (55). Prominent termination sites were visualized 4 bases 5\(\text{9}\) to the lesion, thus emphasizing the distant affects (Fig. 6). Although the intercalation of BPDE could effect only the immediate base 5\(\text{9}\) to the adducted site, it is possible that the bulky lesion prevents the duplex from unwinding 3–5 bases upstream and consequently blocks replication. It is possible that the pertur-
bation of the T-P is far greater if a lesion is in the minor groove (Adenine-N7-BPDE lesions are semi-intercalated from the major groove), even if the size of the bulky adduct is much smaller than BPDE, given that the damaged site is in closer proximity to the thumb subdomain. Observations along these lines have been made in our laboratory with guanine N2 styrene oxide lesions (56).

Studies with alanine-scanning mutants of α-helix H of HIV-1 RT have shown that one face of the core of this helix interacts with newly synthesized DNA 3–6 base pairs from the catalytic site, where the DNA is bent at about 45° (Fig. 7). Mutations within this region decrease polymerase fidelity and processivity relative to the wild type enzyme (41). Our study with the G262A and W266A mutants of α-helix H has shed light on their role in association with the adducted T-P at a distance (Fig. 7). As shown in Fig. 6, although all the C10S BPDE-adducted templates allowed the formation of full-length products, the degree of extension varied between enzymes. With special reference to the (∈-anti-cis-BPDE-adducted template, prominent stop sites were seen at positions 2 and 4 bases 5’ to the adduct on replicating with G262A, whereas with W266A, the truncated products mainly accumulated at a position 2 bases 5’ to the lesion, with minimal formation of full-length products. Similarly, the unique C10R (∈-syn-trans-BPDE-adducted template, which allowed formation of some fully extended molecules, did not show significant amounts of full-length products, when mutant enzymes were employed. W266A in particular exhibited strong termination sites 3 bases 5’ to the site of lesion. Although N3-adenine lesions are in the major groove, and the α-helix H interacts through the minor groove, it was still not surprising that the G262A and W266A mutants affected the polymerization pattern. The interactions with the minor groove are significantly hydrophobic, and therefore, the removal of tryptophan in W266A is suggestive of leaving a gap in the vicinity of the enzyme-T-P contact. This in turn could lead to the destabilization of the T-P complex, subsequently resulting in the fraying of DNA and dissociation of the enzyme (57). Our findings are also supported by the fact that G262A and W266A mutants are far less productive than HIV-1 RT (38). Furthermore, our data also suggest that aromatic amino acid groups prevent destabilization of DNA associated with the polymerase. As shown in Fig. 6 and Table I, the extension of the (∈-anti-cis-BPDE-adducted T-P complex is only half as efficient with the W266A mutant as compared with the G262A mutant. This indicates that lack of tryptophan inhibits polymerization. Furthermore, although alanine is also a hydrophobic residue, it has a considerably smaller side chain replacing the bulker side chain of tryptophan. This possibly suggests that the formation of a gap in the T-P complex by substituting alanine for tryptophan creates a less stable enzyme-substrate complex. These results highlight the importance of maintaining not only a hydrophobic environment in the minor groove of DNA, wherein the HIV-1 RT and substrate interact, but one with the right geometry.

Thus overall, for adduct bypass to occur, the polymerase must overcome several barriers present by the specific lesion. First, the adduct in the single-stranded template must allow the polymerase to synthesize up to the lesion and then assume a catalytic geometry to incorporate a nucleotide opposite the damaged base. Second, the polymerase must be able to translocate and incorporate a nucleoside 1 base beyond the adduct (38). Third, the damaged DNA must be able to translocate through the thumb and palm subdomains without causing major pausing and termination. All these factors could be altered in the presence of modified residues in the α-helix H.

In summary, our data show that differential in vitro replication of C10S and C10R BPDE-dA-adducted templates is not only controlled by the stereochemistry of the adduct at the C10 position, but also by the C7 and C8 positions in the aralkyl ring. Furthermore, our studies on RT and two of its mutants, with BPDE-damaged templates, provide evidence for protein-nucleic acid interactions in the minor groove that are manifested catalytically at a distance. Finally, primer extension on C10R versus C10S BPDE-adducted templates are independent of their binding affinities to the polymerase, even though the catalytic site is engaged with the 3’-hydroxyl terminus of the primer.

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