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MUTAGENIC BYPASS OF THE BUTADIENE-DERIVED 2'-DEOXYURIDINE ADDUCTS BY POLYMERASES ETA AND ZETA

Priscilla H. Fernandes and R. Stephen Lloyd*

Center for Research on Occupational and Environmental Toxicology and the Department of Molecular and Medical Genetics, Portland, Oregon 97239

Abstract

Butadiene is a ubiquitous environmental chemical carcinogen, that when activated to its monoepoxide intermediate can react with the N3 position of cytosine, resulting in two stereoisomeric adducted bases that rapidly deaminate to N3 2'-deoxyuridine lesions. We have previously shown that replication of DNAs containing these adducts through mammalian cells resulted in ~97% mutagenicity, predominantly C to T transitions. Since replicative DNA polymerases were blocked by these lesions *in vitro*, translesional polymerases were assessed for their ability to bypass these adducts. While polymerases iota, kappa and zeta were significantly blocked one nucleotide prior to the lesion, pol eta incorporated nucleotides opposite the adducts with a preference for insertion of a G or A. Following polymerase dissociation and reassociation, pol eta was also able to extend primers with mispaired termini opposite the lesions, with extensions from the A and T mismatched primer termini being the most efficient. Pol zeta was also able to extend primers containing all mismatched nucleotides opposite the lesions, with the most efficient extension occurring off of the A mismatched primer.

Keywords

Butadiene; DNA adducts; butadiene-derived 2'-deoxyuridine adducts; translesion polymerases

1. INTRODUCTION

The initiation and progression of human neoplasias is a multifactorial process that encompasses the interplay of both genetic susceptibilities and environmental toxicant exposures. Key to establishing linkages between genotoxic exposures and increased cancer rates are large-scale epidemiological analyses. Such positive correlations have been made for 1,3-butadiene (BD) that is both a high volume industrial chemical used in the rubber industry and a common environmental contaminant [1,2]. The uptake of butadiene in humans occurs almost exclusively by inhalation and absorption through the respiratory tract, thus making individuals readily susceptible to its effects. In humans, the lympho/haematopoietic system is considered to be the target site for BD, since epidemiological studies indicate an increased risk of leukemia and lymphomas to occupational workers exposed to this chemical [3-6]. Although BD has been classified as a probable human carcinogen [7], the exact mechanisms underlying BD-mediated

*To whom correspondence should be addressed at Center for Research on Occupational and Environmental Toxicology, 3181 SW Sam Jackson Park Rd, Portland, OR 97239, Tel. 503 494-9957; Fax. 503 494-6831, Email: lloydst@ohsu.edu.

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mutagenesis and carcinogenesis are not well understood. Additional studies have established BD to be a multi-site rodent carcinogen, with mice being more sensitive than rats [8,9].

BD toxicity and carcinogenic potential are due to its metabolic activation to reactive epoxides [reviewed in [10]. BD is primarily oxidized to 1,2-epoxy-3-butene (EB) by cytochrome P450 2E1 and 2A6. EB can be further oxidized to 1,2,3,4-diepoxybutane (DEB). EB can also be hydrolyzed by epoxide hydrolase to 1,2-dihydroxy-3-butene that can be metabolized by cytochrome P450 to hydroxymethylvinylketone (HMK) [11]. 3,4-epoxy-1,2-butanediol (EBD) can be produced through partial hydrolysis of DEB and/or epoxidation of hydrolyzed EB. EB, DEB, EBD and HMK can react with nucleophilic sites in DNA to form their corresponding adducts [12-14].

Several BD-derived monoadducts have been characterized following treatment of DNA *in vitro*, as well as in DNA isolated from exposed organisms. Some of these include the N1, N², and N7 adducts of deoxyguanosine [15-18], the N1, N3, N⁶ adducts of adenine [19-22] and the N3 adducts of cytidine and thymidine [23,24]. Of particular interest are the N3 2' deoxyuridine (BD N3-dU) adducts. These adducts are formed when EB reacts with cytidine or calf thymus DNA to form the N3 deoxycytidine adducts which are unstable with a half life of less than 3 hours [24]. They undergo hydrolytic deamination to give rise to the BD N3-dU adducts (Fig. 1) that are highly stable and can remain persistent in DNA long enough to cause mutations [24].

We have previously synthesized the BD N3-dU adducts and site-specifically constructed them into oligodeoxynucleotides and examined their mutagenic potential in mammalian cells [25]. Replication of vectors containing these adducts was found to be ~97% mutagenic, with the majority of the mutations being C to T and C to A mutations. Although these lesions could be bypassed in COS-7 cells, the identity of candidate polymerase(s) that carried out this synthesis was not evident, since these adducts were highly blocking to replicative polymerases δ and ϵ . Therefore in this study, we have examined the ability of several translesion DNA polymerases (human polymerases ι , κ , η and yeast polymerase (pol) ζ) to bypass these adducts.

2. MATERIALS AND METHODS

2.1. Materials

Human DNA polymerases ι , κ , η and yeast pol ζ were obtained from Enzymax (Lexington, KY). These polymerases are all full-length proteins that are tagged at their N-termini with the calmodulin-binding peptide (~5 kDa) [26,27]. They were purified to near homogeneity, with their respective activities on undamaged DNA as the template, being very similar to those in the literature that were purified using a His6-tag. The slight exonuclease contamination in the pol η preparation was only detectable in the absence of dNTPs or when the lesion completely blocked the polymerase. This nuclease activity was not detected when pol η was actively copying a DNA template, and therefore, this minor contaminating exonuclease apparently does not appreciably affect the translesion synthesis analyzed in our studies. Purified DNA polymerase ζ consists of the full-length Rev3 and Rev7. The Rev3-Rev7 protein complex was purified according to Guo et al. [28], with the activity and purity also being similar to those published [28].

COS-7 cells were purchased from American Type Culture Collection (Rockville, MD). Heparin sodium salt (H4784) was obtained from Sigma. A control undamaged oligodeoxynucleotide (32-mer) with a deoxycytosine (dC) in place of BD N3-dU, was purchased from Midland Reagent Co. (Midland, TX).

2.2 DNA substrates

In vitro polymerase reactions were carried out using a series of oligodeoxynucleotide primers. The primers used were –2 with a sequence 5'-GGCGATCATTGCTT-3', –1 with a sequence 5'-GGCGATCATTGCTTA-3' and 0 with a sequence 5'-GGCGATCATTGCTTAX-3', where X represents G, C, A or T. These primers were annealed to templates with the following sequence, 5'-ACCATGCCTGCAAGAAU*TAAGCAATGATCGCC-3', where U* represents the site of the BD N3-dU adducts or control dC. Primer oligodeoxynucleotides were first phosphorylated with T4 polynucleotide kinase using [γ - 32 P] ATP and purified using P-6 Bio-Spin columns supplied with Tris-HCl buffer (pH 7.4). The 32 P-labeled primers were then mixed with either adducted or non-adducted template oligodeoxynucleotides at a molar ratio of 1:4 in 25 mM Tris HCl buffer (pH 7.6), 50 mM NaCl, heated at 90°C for 3 min, and cooled to 25°C overnight. To confirm the completion of primer annealing, aliquots were assayed on a 7.5% native PAGE or digested with the restriction enzyme, DpnII that recognizes the restriction site GATC and the products were analyzed on a 20% denaturing polyacrylamide gel.

2.3 DNA polymerase assays

In vitro polymerization assays were carried out using two polymerase buffers: Pol A buffer containing 25 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, 5 mM dithiothreitol, 0.1mg/ml BSA, and 10% glycerol and Pol B buffer containing 25 mM Tris-HCl (pH 7.5), 10 mM NaCl, 5 mM MgCl₂, 5 mM dithiothreitol, 0.1mg/ml BSA, and 10% glycerol.

For the primer extension reactions catalyzed by polymerases ι , κ , and η , the reaction mixtures contained 5 nM –2 primer/template DNA in Pol A buffer and dNTPs at a final concentration of 10 μ M. Reactions were incubated for increasing time intervals (0, 0.5, 1, 2, 5, 10, 15 min) at 25°C with 10 nM pol ι , 5 nM pol κ or 5 nM pol η . The pol ζ reaction mixture (5 nM –2 primer/template DNA in Pol B buffer and dNTPs at a final concentration of 100 μ M) was incubated as described above with 14 nM polymerase.

For the single nucleotide incorporation reactions with pol η , the same conditions were used as that for the primer extension reactions with a 5nM –1primer/template DNA for an incubation time of 10 min at 25°C. For the extension assays using pol η , the reaction mixture contained 5 nM 0 primer/template DNA in Pol A buffer and dNTPs at a final concentration of 100 μ M and were incubated for increasing time intervals (0, 1, 3, 5, 7 min) at 25°C with 1 nM of the polymerase. These experiments were repeated in triplicate. The percentage of extension was quantitated as the (intensity of the bands from the +2 position)/(total intensity of the bands). The average rates of the extension were then determined from the slopes of the polymerization reactions. The fold difference was calculated as the ratio of (rate of extension for control)/(rate of extension for the adduct). Extension assays using yeast pol ζ were identical except reactions were carried out in Pol B buffer, using 10 nM polymerase.

For the heparin trapping experiments with pol η , the reaction mixture contained 5 nM –2 primer/template DNA or –1 primer/template DNA in Pol A buffer without MgCl₂ and with dNTPs at a final concentration of 10 μ M and was preincubated with 2.5 nM of the polymerase for 2 min at 25°C. The reaction was initiated by addition of MgCl₂ or MgCl₂ and heparin at a final concentration of 5 mM MgCl₂ and 100 μ g/ml heparin and incubated for 5 min at 25°C. To test the effectiveness of the trap, the polymerase was preincubated with MgCl₂ and heparin for 7 min at 25°C and then added to the reaction mixture containing the primer/template DNA and dNTPs at a final concentration of 10 μ M and incubated for another 5 min at 25°C.

In assays designed to test for a synergistic reaction between pol η and pol ζ , all reactions contained 5 nM –1 primer/template in Pol B buffer with dNTPs at a final concentration of 10 μ M and were incubated for 20 min at 25°C with 1 nM, 20 nM, respectively and a combined 1

and 20 nM of the two polymerases. Following termination of these reactions, (lanes 1 and 5), (lanes 2 and 6), (lanes 3 and 7), and (lanes 4 and 8) were loaded with 2.5 μ l aliquots from the control and adduct-containing reactions, respectively for pol η , pol ζ , the combination of the individual reactions of pol η and pol ζ , and the concomitant reaction of pol η and pol ζ , respectively. These experiments were performed in duplicate. The DNA products of the polymerization were quantitated by calculating the extension as the (intensity of the bands from the +1 position)/(total intensity of the bands) for the additive lanes 3 and 7 and the concomitant lanes 4 and 8. The extension for the concomitant lane was divided by the extension for the additive lane to give a ratio that signifies the synergistic effect.

All reactions were terminated by the addition of stop solution consisting of 95% (v/v) formamide, 20 mM EDTA, 0.02% (w/v) xylene cyanol, and 0.02% (w/v) bromophenol blue. Reaction products were resolved on a 15% denaturing gel containing 8 M urea and visualized by autoradiography. Quantitative analyses were performed using a PhosphorImager and Image-Quant software.

3. RESULTS

3.1 Survey of translesional polymerases on the BD N3-dU adducted template

Previously we have determined that the DNA replicative polymerases pol δ and ϵ were blocked one nucleotide prior to BD N3-dU adducts [25]. Since replication of plasmids containing these lesions through COS-7 cells yielded a high mutagenic frequency, we initiated an investigation to determine if any of the specific translesion polymerases (ι , κ , η and ζ) or a combination thereof could potentially bypass the BD N3-dU adducts. Since the BD-modified oligodeoxynucleotide represents a deamination product of an adducted cytidine, the control, unmodified oligodeoxynucleotide was designed to contain a cytidine at the analogous position in the DNA, instead of uridine, reflecting a more biologically relevant control.

In the case of pol ι and pol κ , while extension was observed with the control unadducted DNA, primer extension using a template containing the BD N3-dU adducts was very significantly blocked one nucleotide 3' of the lesion (Fig. 2A and Fig. 2B). In contrast, primer extension reactions using pol η , resulted in a significant incorporation of nucleotides opposite the adducts (Fig. 2C). Although a low percentage of full-length product was produced, the majority of the synthesis was blocked following incorporation of nucleotides opposite the lesion (0 position). These data suggest that pol η may be a good incorporator, but a relatively poor extender.

In addition to the human translesion polymerases used above, we also wanted to investigate the potential of pol ζ to replicate past these lesions. However, due to a lack of availability of human pol ζ , experiments were conducted with yeast pol ζ . Thus, when the primer extension reactions were carried out with yeast pol ζ , significant blockage was seen 1 nucleotide prior to the site of the adducts (Fig. 2D). However, the limited amount of incorporation opposite the adducts also led to extension to full-length product, suggesting that pol ζ could extend a primer when the incorporation step opposite the lesion was already completed.

3.2 Single nucleotide incorporation assay with pol η

Based on the data presented in Fig. 2C, we determined the specificity of nucleotide incorporation by pol η opposite the BD N3-dU adducts. As seen in Fig. 3, pol η was able to incorporate all four nucleotides opposite the adducts with a preference of G and A. We had previously shown in a cellular mutagenesis assay [25] that A was the preferential nucleotide incorporated opposite the adducts. These data collectively suggest that pol η may be one of the translesion polymerases responsible for synthesis opposite the BD N3-dU adducts.

3.3 Primer extension reactions catalyzed by pol η past the BD N3-dU adducts annealed to different mismatched primers

Although pol η was a poor extender we tested its ability to extend a mismatched primer opposite the site of the lesion to determine if it is capable of extension once incorporation is complete. Fig. 4 and Table 1 show the kinetic analyses of extension past the adducts. In all four cases, analyses of the average rates of extension from three independent experiments revealed that there were only modest reductions in the rates of extension on the adduct-containing template relative to that of the corresponding control (Table 1). These data suggest that pol η can extend a mismatched terminus irrespective of whether there is a lesion or not. The fold difference between the correctly paired primer template and the 0-T/adducted template was the least, indicating that pol η preferentially extends T opposite the lesion (Fig. 4D), followed by extension of A opposite the lesion (Fig. 4C). Contrary to the primer extension experiments, these data suggest that pol η is a good extender following incorporation opposite the adducts.

3.4 Heparin trapping of pol η

The next step was to determine if the incorporation and extension past the BD N3-dU adducts could be catalyzed using single-hit kinetics or required multiple encounters with pol η . Trapping reactions of the polymerase were performed in which following polymerase binding, syntheses were carried out in the presence of heparin. Under these conditions, the -2 primer DNA (Substrate A) was extended by only 1 nucleotide (Fig. 5, lane 4), with no observation of insertion opposite the adducts. In the case of the -1 primer DNA (Substrate B), and in the presence of heparin, there was no incorporation or extension past the adducts (Fig. 5, lane 8). These data indicate that incorporation and extension by pol η past the BD N3-dU adducts requires more than one encounter by the polymerase.

3.5 Primer extension reactions catalyzed by pol ζ past the BD N3-dU adducts annealed to different mismatched primers

Although we demonstrated that pol ζ was very poor at incorporating nucleotides opposite the lesions (Fig. 2D), since pol ζ is known to be an extender of mismatched termini [29], its ability to catalyze extension of different mismatched 3' termini that were positioned opposite the BD N3-dU adducts was examined. Fig. 6 and Table 2 show the kinetic analyses of extension past the adducts. In all four cases, analyses of the average rates of extension from three independent experiments revealed that there were only modest reductions in the rates of extension of the primer hybridized to the adduct containing template relative to that of the corresponding control (Table 2). The fold difference between the correctly paired primer template and the 0-A/adducted template was the least indicating that pol ζ favors the extension of A opposite the lesion (Fig. 6C). In the case when T was placed opposite the adducts (0-T/adduct), two extension products were observed (Fig. 6D). This may be due to a slippage mechanism of the polymerase, whereby it translocates to the next templating nucleotide and forms a product that is one nucleotide shorter than the full-length product.

3.6 Synergistic effect between pol η and pol ζ in the bypass of the BD N3-dU adducts

Since pol η and pol ζ were identified as the most efficient enzymes to catalyze nucleotide incorporation opposite and extension beyond the BD N3-dU adducts, respectively, experiments were designed to test for a synergistic effect for both these polymerases in the bypass of these lesions. Accordingly, it was hypothesized that polymerization reactions using a simultaneous addition of pol η and pol ζ would result in the accumulation of significantly more full-length DNA products than if the pol η and pol ζ reactions were carried out separately. Fig. 7, lanes 1 and 5 show the DNA products of a pol η reaction using control and adduct-containing oligodeoxynucleotides, respectively, while lanes 2 and 6 show the same reactions, except using pol ζ . The DNAs in lanes 3 and 7 represent the additive polymerization reaction, in which

aliquots of the individual pol η and pol ζ reactions (lanes 1 and 2) and (lanes 5 and 6) were physically combined following the termination of the reaction for the control and adduct-containing samples, respectively. Lanes 4 and 8 represent polymerization when both pol η and pol ζ were added simultaneously to the control and adduct-containing oligodeoxynucleotides, respectively. These experiments were performed in duplicate and the average ratio of concomitant addition effect / additive effect was determined. This ratio was found to be 1.18 for the control DNA and 2.7 for the adduct-containing DNA, thus indicating that there was a modest synergy in the production of full-length DNA products on the adduct-containing substrate when the two polymerases were simultaneously added. In all lanes containing pol η incorporating nucleotides opposite the lesions (lanes 5, 7, and 8), a doublet of bands was readily apparent, at that site. These data indicate that at least two different nucleotides had been incorporated which could be resolved under the electrophoretic conditions used. These data are consistent with the results from the single nucleotide incorporation assays for pol η .

4. DISCUSSION

Data presented within this and our previous study [25] reveal that the BD N3-dU adducts pose significant blocks to the progression of several DNA polymerases. However, this investigation has revealed that among all the polymerases tested to date (δ , ϵ , ι , κ , η and ζ), only pol η can incorporate nucleotides (primarily purines) opposite these lesions. Due to modest size and design of our deoxynucleotide substrates, we were not able to test what role PCNA might play in modulating the processivity of pol η in these reactions. Previous investigations have clearly demonstrated functional modulations of pol η through physical interaction with PCNA [30]. Thus, we consider it probable that *in vivo* replication bypass of and extension beyond BD N3-dU adducts could be facilitated by a pol η / monoubiquitinated PCNA complex [31,32]. This hypothesis can be further supported by our findings that pol η can extend all mismatched primers combinations opposite the BD N3-dU adducts (Fig 4 and Table 1). These data reveal that given sufficient time and stabilization of pol η with a mismatched primer/template, extension could occur. However, under our *in vitro* experimental conditions, heparin-trapping experiments revealed that multiple binding and dissociation events occurred prior to effective extension. The presence of PCNA could significantly alleviate this stuttering of the polymerase.

We further postulate that if a pol η / PCNA complex dissociates prior to extending a nucleotide opposite the BD N3-dU adducts, pol ζ may be capable of catalyzing these extensions. Like pol η , pol ζ activity is also stimulated by PCNA [33,34]. However, we are unaware of any data that suggest that pol η and pol ζ co-localize at stalled replication forks, even though both can complex with PCNA and both can associate with REV1 [35-37]. Although co-localization of pol η and ζ have not been reported, we find it interesting that concomitant addition of pol η and pol ζ to reactions using the BD N3-dU primer/template showed modest synergy in the bypass and extension to full-length products. These data open the possibility that certain replication blocking lesions could require previously unreported combinations of polymerases in translesion synthesis, similar to that previously described for two polymerase two-step mechanism of translesion synthesis [38-40]. In such a proposal, the replicative polymerases δ or ϵ first encounter the BD N3-dU adducts and would be blocked [25]. Recruitment of a pol η /PCNA complex could catalyze incorporation opposite and extend beyond the adduct. Such a role for pol η would further expand the scope of lesions that it can bypass, that currently includes cyclobutane pyrimidine dimers [41,42], O⁶-methyl guanine [43] and 7,8-dihydro-8-oxoguanine [44]. If pol η were unable to extend beyond the lesion, we consider it possible that pol ζ could efficiently extend all mismatched primers.

The data presented herein on the bypass of the BD N3-dU adducts agrees well with the types of mutations observed following replication past these lesions in COS-7 cells that were mainly C to T (53%) and C to A (32.5%) mutations, with a lower number of C to G (11%) mutations

[25]. These data suggest that primers with an A incorporated opposite the adducts were well extended during replication in cells, and are in accordance with the ability of pol η and pol ζ to extend the A mismatched primer efficiently. Thus overall, these data implicate pol η alone or possibly the combined effect of pol η and pol ζ on the BD N3-dU adducts to be responsible for the induction of C to T mutations in mammalian cells. This hypothesis will be tested by analyzing for changes in mutation spectra when these adducted DNAs are analyzed in wild-type versus pol η -deficient, XPV cells.

Overall these investigations provide suggestive data for complex models for polymerase action and potential switching and co-operativity in the bypass of lesions resulting from environmental toxicant exposure and endogenously produced DNA lesions.

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ABBREVIATIONS

BD, 1,3-Butadiene; EB, 1,2-epoxy-3-butene; DEB, 1,2,3,4-diepoxybutane; HMK, hydroxymethylvinylketone; EBD, 3,4-epoxy-1,2-butanediol; BD N3-dU, N3 2' deoxyuridine; pol, polymerase; dC, deoxycytosine.

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Fig.1.

Formation of the BD N3-dU adducts. BD is metabolically activated to EB that can react with cytidine to form the N3 cytidine adducts. These adducts are unstable and undergo hydrolytic deamination to give rise to the stable BD N3-dU adducts.

Fig.2.

Primer extension reactions catalyzed by polymerases ι , κ , η or ζ on the BD N3-dU adducted template. Control dC or BD N3-dU adducted 32-mer DNA templates were annealed to a -2 primer. Time course experiments (at time intervals 0, 0.5, 1, 2, 5, 10 and 15 min) were carried out with the DNA substrates (5 nM) at 25°C in the presence of all four dNTPs (10 μ M in the case of ι , κ , η and 100 μ M in the case of pol ζ) with the indicated concentration of polymerases ι , κ , η or ζ . The positions of the 14-nucleotide primers and the 32-nucleotide full-length products are indicated. U* indicates the position of the modified U on the template.

Fig. 3.

Single-nucleotide incorporation by pol η on the BD N3-dU adducted template. Control dC or BD N3-dU adducted 32-mer DNA templates were annealed to a -1 primer. The DNA substrates (5 nM) were incubated at 25°C for 10 min with 0.5 nM pol η in the presence of 10 μ M of each of the four dNTPs. The position of the 15-nucleotide primer is indicated. U* indicates the position of the modified U on the template.

Fig.4.

Primer extension reactions catalyzed by pol η past the BD N3-dU adducts annealed to different mismatched primers. X in the DNA sequence corresponds to G, C, A or T. (A) Control dC or BD N3-dU 32-mer DNA templates were annealed to a 0 primer with G opposite the control dC or BD N3-dU adducts. (B) Control dC or BD N3-dU 32-mer DNA templates were annealed to a 0 primer with C opposite the control dC or BD N3-dU adducts. (C) Control dC or BD N3-dU 32-mer DNA templates were annealed to a 0 primer with A opposite the control dC or BD N3-dU adducts. (D) Control dC or BD N3-dU 32-mer DNA templates were annealed to a 0 primer with T opposite the control dC or BD N3-dU adducts. The DNA substrates (5nM) were incubated at 25°C for time intervals of 0, 1, 3, 5, and 7 min with 1 nM of pol η in the presence of 100 μ M dNTPs. The positions of the 0-nucleotide primers with their corresponding mismatches are indicated.

Substrate A = 5'GGCGATCATTGCTT3'
 3'CCGCTAGTAACGAATU*AAGAACGTCCGTACCA5'

Substrate B = 5'GGCGATCATTGCTTA3'
 3'CCGCTAGTAACGAATU*AAGAACGTCCGTACCA5'

Fig.5.

Heparin trapping of pol η . BD N3-dU adducted templates were annealed to a -2 (Substrate A) or -1 primer (Substrate B). Lanes 1 and 5 represent DNA substrates (5 nM) A and B respectively. Lanes 2 and 6 represent reactions in which pol η is preincubated with heparin and MgCl_2 for 7 min at 25°C and then added to the DNA substrates (5 nM) in the presence of 10 μM dNTPs. Lanes 3 and 7 represent reactions in which DNA substrates (5 nM) were incubated for 5 min at 25°C with 2.5 nM of pol η in the presence of 10 μM dNTPs and 5 mM MgCl_2 . Lanes 4 and 8 represent reactions in which DNA substrates (5 nM) were incubated for 5 min at 25°C with 2.5 nM of pol η in the presence of 10 μM dNTPs, 5 mM MgCl_2 and 100 $\mu\text{g/ml}$ heparin. The positions of the 14-nucleotide and 15-nucleotide primers and the 32-nucleotide full-length products are indicated. U* indicates the position of the modified U on the template

Fig.6.

Primer extension reactions catalyzed by pol ζ past the BD N3-dU adducts annealed to different mismatched primers. X in the DNA sequence corresponds to G, C, A or T. (A) Control dC or BD N3-dU 32-mer DNA templates were annealed to a 0 primer with G opposite the control dC or BD N3-dU adducts. (B) Control dC or BD N3-dU 32-mer DNA templates were annealed to a 0 primer with C opposite the control dC or BD N3-dU adducts. (C) Control dC or BD N3-dU 32-mer DNA templates were annealed to a 0 primer with A opposite the control dC or BD N3-dU adducts. (D) Control dC or BD N3-dU 32-mer DNA templates were annealed to a 0 primer with T opposite the control dC or BD N3-dU adducts. The DNA substrates (5nM) were incubated at 25°C for time intervals of 0, 1, 3, 5, and 7 min with 10 nM of pol ζ in the presence of 100 μ M dNTPs. The positions of the 0-nucleotide primers with their corresponding mismatches are indicated.

Fig.7.

Synergistic effect of pol η and pol ζ on the BD N3-dU adducted template. Control dC or BD N3-dU adducted templates were annealed to a -2 primer. Lanes 1 and 5 represent 2.5 μ l of the DNA substrates (5 nM) that were incubated at 25°C for 20 min with 1 nM of pol η in the presence of 10 μ M dNTPs. Lanes 2 and 6 represent 2.5 μ l of the DNA substrates (5 nM) that were incubated at 25°C for 20 min with 20 nM of pol ζ in the presence of 10 μ M dNTPs. Lanes 3 and 7 indicate 2.5 μ l aliquots from the individual reactions of pol η (lanes 1 and 5) and pol ζ (lanes 2 and 6) after termination of those reactions. Lanes 4 and 8 represent 5 μ l of the DNA substrates (5 nM) that were incubated at 25°C for 20 min with 1 nM of pol η and 20 nM of pol ζ . The positions of the 15-nucleotide primers and the 32-nucleotide full-length products are indicated. U* indicates the position of the modified U on the template.

Table 1Extension of the BD N3-dU containing templates annealed to mismatched primers by pol η

Primer nucleotide opposite control dC or BD N3-dU	Average rate of extension (slope) ^a	Fold differential rates of extension of control vs. mismatched primers ^b	Fold differential rates of extension of G:control dC vs. mismatched primers ^c
G:control dC	5.5 $\pm$ 0.2	4.0	4.0
G:BD N3-dU	1.4 $\pm$ 0.2		
C:control dC	0.5 $\pm$ 0.1	0.5	5.5
C:BD N3-dU	1.0 $\pm$ 0.1		
A:control dC	1.4 $\pm$ 0.2	0.6	2.4
A:BD N3-dU	2.3 $\pm$ 0.2		
T:control dC	1.9 $\pm$ 0.2	0.4	1.3
T:BD N3-dU	4.4 $\pm$ 0.9		

^a Percentage of primer extended/time^b rate of extension of primer nucleotide opposite control dC/rate of extension of mismatched primer opposite BD N3-dU^c rate of extension of G opposite control dC/rate of extension of mismatched primer opposite BD N3-dU

Table 2Extension of the BD N3-dU containing templates annealed to mismatched primers by pol ζ

Primer nucleotide opposite control dC or BD N3-dU	Average rate of extension (slope) ^a	Fold differential rates of extension of control vs. mismatched primers ^b	Fold differential rates of extension of G:control dC vs. mismatched primers ^c
G:control dC	5.5±1.3	4.2	4.1
G:BD N3-dU	1.3±0.7		
C:control dC	1.3±0.8	1.4	6.1
C:BD N3-dU	0.9±0.2		
A:control dC	3.0±0.7	1.6	2.9
A:BD N3-dU	1.9±0.4		
T:control dC	4.3±0.4	2.9	3.7
T:BD N3-dU	1.5±0.4		

^a Percentage of primer extended/time^b rate of extension of primer nucleotide opposite control dC/rate of extension of mismatched primer opposite BD N3-dU^c rate of extension of G opposite control dC/rate of extension of mismatched primer opposite