Efficiency of Extension of Mismatched Primer Termini across from Cisplatin and Oxaliplatin Adducts by Human DNA Polymerases β and η in Vitro[†]

Ekaterina Bassett,[‡,#] Alexandra Vaisman,[‡,§,#] Jody M. Havener,[‡] Chikahide Masutani,[||] Fumio Hanaoka,[||,⊥] and Stephen G. Chaney*,[‡]

Department of Biochemistry and Biophysics, Lineberger Comprehensive Cancer Center, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599-7260 USA, Graduate School of Frontier Biosciences, Osaka University and CREST Japan Science and Technology Corporation, 1-3 Yamada-oka, Osaka 565-0871, Japan, and RIKEN, Wako-shi, Saitama 351-0198, Japan

Received July 31, 2003; Revised Manuscript Received September 23, 2003

ABSTRACT: DNA polymerases β and η are among the few eukaryotic polymerases known to efficiently bypass cisplatin and oxaliplatin adducts in vitro. Our laboratory has previously established that both polymerases misincorporated dTTP with high frequency across from cisplatin- and oxaliplatin−GG adducts. This decrease in polymerase fidelity on platinum-damaged DNA could lead to in vivo mutations, if this base substitution were efficiently elongated. In this study, we performed a steady-state kinetic analysis of the steps required for fixation of dTTP misinsertion during translesion synthesis past cisplatin- and oxaliplatin−GG adducts by pol β and pol η. The efficiency of translesion synthesis by pol η past Pt−GG adducts was very similar to that observed for this polymerase when the template contains thymine−thymine dimers. This finding suggested that pol η could play a role in translesion synthesis past platinum−GG adducts in vivo. On the other hand, translesion synthesis past platinum−GG adducts by pol β was much less efficient. Translesion synthesis by pol η is likely to be predominantly error-free, since the probability of correct insertion and extension by pol η was 1000−2000-fold greater than the probability of incorrect insertion and extension. Our results also indicated that for pol η the frequency of misincorporation is the same across from the 3′G and the 5′G of the platinum−GG adducts for both cisplatin and oxaliplatin adducts. On the other hand, pol β is more likely to misinsert at the 3′G of the adducts and misinsertion occurs at higher frequency for oxaliplatin−GG than for cisplatin−GG adducts.

cis-Diamminedichloroplatinum(II) (cisplatin) is a widely used chemotherapeutic agent effective in a variety of testicular, ovarian, and head and neck cancers. However, intrinsic and acquired tumor resistance often limits clinical use of this drug. To overcome drug resistance, platinum agents with different carrier ligands have been developed. (*trans-R,R*)-1,2-Diaminocyclohexaneoxalatoplatinum(II) (oxaliplatin) is a third-generation platinum drug, which is effective in cisplatin-resistant tumors and has been recently approved for clinical use in the United States. Another potential problem in the therapeutic use of cisplatin is its mutagenicity (*1*). Cisplatin has been classified as a probable human carcinogen by The International Agency for Research on Cancer (IARC). While less is known about the mutagenicity of oxaliplatin, other platinum(II) complexes that form 1,2-diaminocyclohexane (DACH) adducts in DNA are less mutagenic than cisplatin in the Ames assay (*2*).

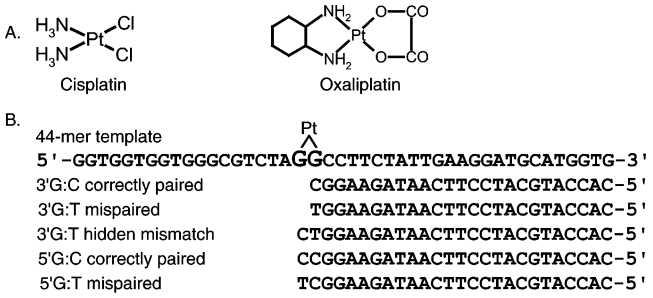

FIGURE 1: Structures of the platinum complexes and sequences of the oligonucleotide substrates with site-specific platinum adducts. (A) Chemical structures of cisplatin and oxaliplatin. (B) 44-mer template hybridized to the correctly paired or mispaired primers was used for steady-state kinetic analyses of correctly paired and mispaired primer extension. The GG site of platination is indicated. Platinated oligonucleotides were constructed and hybridized to the primers as described in Materials and Methods.

Cisplatin and oxaliplatin (Figure 1A) predominantly form intrastrand cross-links at GG and AG sequences on DNA (*3*, *4*). One of the postulated mechanisms for platinum mutagenicity is error-prone translesion synthesis past these cross-links. Previous studies from our laboratory indicated that translesion synthesis past platinum adducts occurs in vivo and is increased in cisplatin-resistant cell lines (*5*, *6*).

[†] This research was supported in part by Grant CA84480 from the National Institutes of Health (to S.G.C.), and in part by Grant P30ES10126 from the National Institute of Environmental Health Sciences (to S.G.C.).

* To whom correspondence should be addressed: Department of Biochemistry and Biophysics, CB 7260, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7260. Tel.: 919-966-3286; fax: 919-966-2852; e-mail: Stephen_Chaney@med.unc.edu.

[#] These authors have contributed equally to this work.

[‡] University of North Carolina.

[||] Osaka University.

[⊥] RIKEN.

[§] Present address: Section on DNA Replication, Repair, and Mutagenesis, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892-2725.

10.1021/bi035359p CCC: $25.00
Published on Web 11/01/2003

The polymerases responsible for bypass of platinum adducts in vivo have not yet been identified, but several polymerases have been tested for their ability to perform translesion synthesis past these lesions in vitro. DNA polymerases α, δ, and ϵ are completely blocked by cisplatin adducts, even in the presence of the accessory proteins proliferating cell nuclear antigen (PCNA)[1] and replication protein A (RPA) (*7*, *8*).

DNA polymerase η (pol η) is highly efficient in catalyzing complete translesion replication past platinum adducts in vitro (*9*, *10*). Pol η, encoded by the yeast *RAD30* and *hRAD30A* genes (*11*, *12*), is the primary polymerase for translesion synthesis past cis,syn cyclobutane thymine−thymine dimers caused by UV radiation (*11*, *12*). Deletion of the *RAD30* gene leads to the increased UV sensitivity and UV hypermutability (*13*). Mutations in the *hRAD30A* gene lead to xeroderma pigmentosum variant (XPV) syndrome (*12*, *14*), characterized by a high incidence of sunlight-induced skin cancers. The ability of pol η to perform translesion synthesis past various types of bulky DNA adducts has been vigorously examined. Pol η has been implicated in bypass of 7,8-dihydro-8-oxoguanine (8-oxoG) lesions (*15*), acrolein-derived γ-HOPdG adducts (*16*), and [6−4] TT photoproducts (*17*) in vivo. Because pol η is the most efficient polymerase to perform translesion synthesis past platinum adducts in vitro (*10*), it is considered an important candidate for this role in vivo.

Another enzyme that could be potentially involved in translesion synthesis past platinum adducts in vivo is pol β. This polymerase is capable of bypassing platinum adducts in vitro and has been shown to elongate the arrested products of DNA polymerases α, δ, and ϵ at platinum−DNA adducts (*8*, *18*). A high frequency of errors has been found in the vicinity of a cisplatin adduct in products of translesion synthesis by pol β in vitro (*8*, *19*), which led to the hypothesis that pol β may be involved in error-prone translesion synthesis past platinum adducts (*8*). It is not yet clear whether pol β is involved in this process in vivo at normal levels of expression, but several lines of evidence have indicated that pol β may be involved in error-prone translesion synthesis past platinum adducts when it is overexpressed. Mammalian cells overexpressing pol β are hypermutable to cisplatin (*20*, *21*), and several types of human tumors overexpress pol β (*22*). In addition, amplification of the pol β gene has been observed in human cancer cells resistant to cisplatin (*23*, *24*). All these lines of evidence indicate the need for investigation of the role for pol β in translesion synthesis past platinum adducts.

Other DNA polymerases have been tested for their ability to bypass platinum−DNA adducts in vitro. Pol λ, pol κ, and pol ι are unable to perform translesion synthesis past platinum adducts (*25*−*27*). Yeast pol ζ has been implicated in the extension of correctly matched and mismatched primers across from bulky adducts (*28*), and has demonstrated bypass activity on templates containing cisplatin and oxaliplatin lesions (*29*), but the efficiency of translesion synthesis past platinum adducts by pol ζ was significantly lower than that of either pol β or pol η. Our laboratory recently showed that pol μ bypasses cisplatin and oxaliplatin adducts in vitro (*30*), but this polymerase primarily generates frameshift errors during this process. The mutation spectra of cisplatin in vivo indicate that base substitutions are induced most frequently by this drug (*31*−*34*). Therefore, pol β and pol η are more likely than pol μ to participate in translesion synthesis past platinum adducts in vivo, and are the focus of the present study.

Carrier ligands of the platinum drugs cause different extents of DNA distortion. Most DNA polymerases capable of bypassing platinum lesions are able to discriminate between cisplatin and oxaliplatin carrier ligands (*5*, *6*, *35*). Previous studies performed in our laboratory have shown that both pol β and pol η in their distributive modes catalyzed translesion synthesis past oxaliplatin adducts with higher efficiency than past cisplatin adducts in the AGG sequence context (*10*, *19*). These findings were counterintuitive, since the carrier ligand of oxaliplatin is much bulkier than that of cisplatin (Figure 1). In addition, pol β but not pol η catalyzed a higher frequency of misincorporation across from oxaliplatin adducts than cisplatin adducts, and the most frequently misinserted nucleotide by both polymerases was dTTP (*10*, *19*). To characterize the process of translesion synthesis past cisplatin and oxaliplatin adducts by pol β and pol η more quantitatively, we performed steady-state kinetic analyses of each insertion and extension step of translesion synthesis with correctly matched (G:C) and mismatched (G:T) primers ending opposite either the 3′ or the 5′ base of the platinum adducts.

MATERIALS AND METHODS

Construction of Platinum Adduct-Containing Templates. Primer-templates were assembled from synthetic oligonucleotides as described previously (*29*). Briefly, platination reactions were performed with the aquated derivatives of cisplatin (Sigma) and *trans-R,R*-dach(Cl_2)Pt, the biotransformation product of oxaliplatin (kindly provided by Dr. Paul Juniewicz, Sanofi-Synthelabo). The template sequence used in this study is shown in Figure 1B. The site-specifically platinated 44-mer was obtained by ligation of a purified 12-mer carrying a single platinum adduct within a *Stu*I restriction site, with a 14-mer on the 5′-end and an 18-mer on the 3′-end of the template, using a 35-mer as a scaffold. Following a 16 h ligation at 16 °C by T4 DNA ligase, the duplex was digested with *Stu*I to remove any unplatinated product, and the oligonucleotides were separated on a 12% denaturing polyacrylamide gel. The resulting 44-mers were gel-excised and purified using MicroSpin G-25 columns (Amersham Pharmacia Biotech). The absence of any unplatinated oligonucleotide in the final 44-mer carrying a single platinum adduct has also been confirmed by the inability of *Stu*I to digest the 44-mer hybridized to its complementary stand, and by the inability of *Stu*I to digest the products of translesion synthesis on platinated 44-mer templates by pol β. Previous studies have shown that for platinated templates prepared in this manner, greater that 95% of the adducts are located in the GG portion of the AGG sequence (*36*). DNA substrates for in vitro replication were prepared by hybridizing ^{32}P-end-labeled primers to the undamaged or platinated templates.

Steady-State Polymerization Kinetics. Recombinant human pol β (*37*) and pol η (*12*) were purified as described

[1] PCNA, proliferating cell nuclear antigen; RPA, replication protein A; pol η, DNA polymerase η; XPV, xeroderma pigmentosum variant; pol β, DNA polymerase β; RFC, replication factor C.

Table 1: Steady-State Kinetic Analysis of Extension of G:C and G:T Primer Termini by Pol β[a]

extension	Pt adduct	k_{cat}	K_m	k_{cat}/K_m	f_{rel}	$f_{mis\ ext}$
A. Extension from 3'G						
		min^{-1}	μM	$mM^{-1}\ min^{-1}$		
C	control	0.63 ± 0.06	3.2 ± 0.7	197 ± 5.2	1	-
-C↓	cisplatin	0.42 ± 0.03	95.0 ± 8.0	4.4 ± 0.6	2.2×10^{-2}	-
-GGA-	oxaliplatin	0.49 ± 0.03	96.0 ± 6.0	5.1 ± 0.6	2.6×10^{-2}	-
C	control	0.19 ± 0.024	26 ± 2	7.1 ± 0.307	1	3.6×10^{-2}
-**T**↓	cisplatin	0.06 ± 0.003	756 ± 53	0.08 ± 0.012	1.1×10^{-2}	1.8×10^{-2}
-GGA-	oxaliplatin	0.07 ± 0.009	731 ± 115	0.10 ± 0.003	1.4×10^{-2}	2.0×10^{-2}
B. Extension from 5'G						
T	control	0.68 ± 0.04	1.1 ± 0.1	600 ± 65	1	-
-CC↓	cisplatin	0.29 ± 0.02	1.7 ± 0.2	175 ± 17	2.9×10^{-1}	-
-GGA-	oxaliplatin	0.39 ± 0.04	3.4 ± 0.3	113 ± 16	1.9×10^{-1}	-
T	control	0.21 ± 0.08	7.5 ± 0.5	27.6 ± 14.9	1	4.6×10^{-2}
-**T**C↓	cisplatin	0.05 ± 0.01	38 ± 10	1.3 ± 0.5	4.7×10^{-2}	7.4×10^{-3}
-GGA-	oxaliplatin	0.09 ± 0.04	21 ± 3	4.1 ± 2.2	1.5×10^{-1}	3.6×10^{-2}
C. Extension of G:T mismatch from 5'G						
T	control	0.33 ± 0.060	513 ± 62	0.64 ± 0.056	1	1.1×10^{-3}
-C**T**↓	cisplatin	0.05 ± 0.006	767 ± 24	0.06 ± 0.006	9.4×10^{-2}	3.4×10^{-4}
-GGA-	oxaliplatin	0.06 ± 0.008	898 ± 57	0.07 ± 0.013	1.1×10^{-1}	6.2×10^{-4}

[a] Steady-state kinetic assays were performed using 5 fmol of pol β and 150 fmol of primer-templates. Incubation time was 5 min for undamaged DNA templates and 15 min for platinated DNA templates. dCTP concentrations ranged from 1 to 8 μM for undamaged template and from 32 to 1024 μM for the platinated templates to measure extension of the 3′G:C base pair. dCTP concentrations ranged from 10 to 160 μM for the undamaged template and from 500 to 8000 μM for the platinated templates to measure extension of the 3′G:T mispair when the next correct base pair was 5′G:C. dTTP concentrations ranged from 4 to 64 μM for the undamaged template and from 8 to 128 μM for the platinated templates to measure extension of the 5′G:C pair when the next correct base pair was A:T. To determine efficiency of extension of the 5′G:T mispair when next correct base pair is A:T, dTTP was used at 100−1600 μM concentrations for the undamaged template and 500−8000 μM for the platinated templates. To determine the efficiency of extension of the 3′T hidden mismatch when the next correct base pair was A:T, dTTP concentrations ranged from 0.5 to 10 μM on undamaged template and from 4 to 250 μM on platinated templates. Kinetic parameters (K_m and k_{cat}) and insertion efficiency ($f = k_{cat}/K_m$) for dCTP incorporation by pol β were determined using Hanes-Woolf plots (see Materials and Methods). The insertion efficiency (k_{cat}/K_m) was determined from each individual repeat and the individual values were averaged to obtain the values presented in this table. The relative insertion efficiency was determined as $f_{rel.} = f_{Pt}/f_{undamaged}$. The relative efficiency of mismatch extension ($f_{mis\ ext}$) was determined as the ratio of efficiencies of extension of mispaired relative to correctly paired primer termini on the same template. Data are means (± standard error) from five to eight different experiments using three independent template preparations.

previously. Pol β was generously provided by Dr. Sam Wilson (NIEHS). The steady-state kinetic parameters V_{max} and K_m for dCTP and dTTP incorporation were determined by measuring chain elongation as a function of dNTP concentration (*38*, *39*). Primer-templates (150 fmol, expressed as primer-termini) were incubated at 37 °C in 5 μL reaction mixtures containing 50 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 2 mM dithiothreitol, 20 mM NaCl, 200 μg/mL bovine serum albumin, 2.5% glycerol, 5 fmol of pol β, and varying concentrations of deoxynucleotides as described in Table 1. Reactions with pol η were performed in 25 mM KH_2PO_4, pH 7.0, 5 mM $MgCl_2$, 5 mM dithiothreitol, 100 μg/mL bovine serum albumin, 10% glycerol, 7.5 fmol or 20 fmol of pol η, depending on the primer-template, and varying concentrations of deoxynucleotides as described in Table 3. Reactions were terminated by the addition of 0.7 vol of formamide-loading dye, and immediately transferred to ice. Products of reactions were resolved by denaturing polyacrylamide gel electrophoresis (containing 8 M urea and 16% acrylamide), and visualized and quantified using a Molecular Dynamics PhosphorImager and ImageQuant software. Initial studies were performed to demonstrate that time-dependent and deoxynucleotide concentration-dependent product accumulation was linear under the selected experimental conditions. Preliminary studies also demonstrated that less than 20% of the primers were extended under these conditions. Representative gels of dTTP incorporation by pol β and pol η on cisplatin- and oxaliplatin-damaged templates hybridized to the primer containing a hidden mismatch are presented in Figure 2. The velocities of dCTP and dTTP incorporation opposite the 5′G of the adducts and opposite the base immediately 5′ to the adduct were determined as described previously (*38*, *39*). V_{max} (the maximum reaction velocity) and K_m (dNTP concentration at which the reaction velocity is half-maximal) were determined using the Hanes-Woolf plot by method of linear least-squares fit (*39*). Apparent k_{cat} values were calculated based on the assumption that pol β and pol η were fully active. The k_{cat} values we report in this study were obtained by dividing V_{max} (in nM primer extended per min) by the enzyme concentration (nM). The efficiency of dNTP insertion by pol β and pol η was calculated as k_{cat}/K_m. To facilitate comparison of values for different platinum adducts, the relative insertion efficiency f_{rel} was calculated as f_{rel} = (k_{cat}/K_m for Pt)/(k_{cat}/K_m for undamaged DNA). To compare the values for mispaired and correctly paired primer termini on the same type of DNA templates, $f_{mis\ ext}$ was calculated as $f_{mis\ ext}$ = (k_{cat}/K_m for mispaired primer)/(k_{cat}/K_m for correctly paired primer on the same type of DNA template).

RESULTS

Extension of Correctly Paired and Mispaired Primer Termini across from Cisplatin and Oxaliplatin Adducts by pol β. While a single report on cisplatin-induced mutational spectra in mammalian cells indicates that mutations caused by platinum−GG adducts occur predominantly at the 3′ platinated G (*31*), three other studies report that substitution occurs at similar frequencies at the 3′ and the 5′ platinated

Table 2: Overall Efficiency of Insertion Opposite the 3′G and the 5′G and One Base Extension from the 5′G on Platinated Templates by Pol β Compared to that on Undamaged DNA[a]

	Pt adduct	f_{ins1}	f_{ins2}	f_{ext}	$f_{ins1} \times f_{ins2} \times f_{ext}$
A. Effect of platinum adducts on correct insertion and extension					
C → C → T → / -↓ -C↓ -CC↓ -CCT / -GGA- -GGA- -GGA- -GGA-	undamaged	1	1	1	1
	cisplatin	1.1×10^{-2}	2.2×10^{-2}	2.9×10^{-1}	7.0×10^{-5}
	oxaliplatin	2.2×10^{-2}	2.6×10^{-2}	1.9×10^{-1}	1.1×10^{-4}
B. Effect of platinum adducts on T misincorporation opposite 3'G and subsequent extension					
T → C → T → / -↓ -T↓ -TC↓ -TCT / -GGA- -GGA- -GGA- -GGA-	undamaged	1	1	1	1
	cisplatin	1.7×10^{-1}	1.1×10^{-2}	4.7×10^{-2}	8.8×10^{-5}
	oxaliplatin	5.6×10^{-1}	1.4×10^{-2}	1.5×10^{-1}	1.2×10^{-3}
C. Overall efficiency of T misincorporation opposite 3'G compared to correct insertion and extension					
	Pt adduct	f'_{ins1}	f'_{ins2}	f'_{ext}	$f'_{ins1} \times f'_{ins2} \times f'_{ext}$
T → C → T → / -↓ -T↓ -TC↓ -TCT / -GGA- -GGA- -GGA- -GGA-	undamaged	5.4×10^{-4}	3.6×10^{-2}	4.6×10^{-2}	8.9×10^{-7}
	cisplatin	8.4×10^{-3}	1.8×10^{-2}	7.4×10^{-3}	1.1×10^{-6}
	oxaliplatin	1.4×10^{-2}	2.0×10^{-2}	3.6×10^{-2}	1.0×10^{-5}

[a] The efficiencies for dCTP and dTTP incorporation opposite the 3′G were determined using the same DNA sequence and are taken from ref *19*; the efficiencies of extension of correctly paired and mismatched primer termini are taken from Table 1. f_{ins1} and f_{ins2} represent k_{cat}/K_m for dTTP or dCTP insertion opposite the platinated 3′G or 5′G as compared to the dCTP or dTTP incorporation opposite the 3′G or the 5′G on an undamaged DNA template. f_{ext} represents the ratio of k_{cat}/K_m for dTTP incorporation opposite the A (base 5′ to the GG site) on damaged compared to undamaged DNA template. $f_{ins1} \times f_{ins2} \times f_{ext}$ is the overall efficiency of incorporation opposite the 3′G and extension from the 3′G and the 5′G on platinated template relative to incorporation and extension on an undamaged DNA template. In panel C, f'_{ins1} represents k_{cat}/K_m for dTTP insertion opposite the 3′G as compared to the dCTP incorporation opposite the 3′G on the same DNA template. f'_{ins2} represents the ratio of k_{cat}/K_m for dCTP insertion opposite the 5′G on DNA templates with 3′G:T primer-template mispair compared to the same types of templates with correctly paired primer termini. f'_{ext} represents the ratio of efficiency of extension of the primer ending across from the 5′G in the context of the hidden mismatch compared to fully complementary primer-template. $f'_{ins1} \times f'_{ins2} \times f'_{ext}$ is the overall efficiency of dTTP misincorporation opposite the 3′G followed by dCTP incorporation opposite the 5′G and one base extension compared to sequential correct incorporation of three nucleotides opposite the GGA sites on the same type of DNA template.

sites (*32*−*34*). Therefore, we looked at steps required for dTTP misinsertion opposite both the 3′G and the 5′G of the platinum−GG adducts. There are three essential steps in translesion synthesis past a platinum−GG adduct, insertion of a dNTP opposite the 3′G, insertion of a dNTP opposite the 5′G, and extension from the 5′G. All three of these steps need to be analyzed to accurately estimate the relative frequency of misincorporation opposite platinum−GG adducts.

For pol β, we had previously shown that dTTP was the most frequently misinserted nucleotide opposite the 3′G (*19*). We had also shown that dTTP misinsertion was approximately 1800-fold less efficient than dCTP insertion on undamaged DNA and 72−118-fold less efficient than dCTP insertion on DNA containing platinum adducts (*19*). Overall, the presence of a platinum lesion opposite the 3′G on a primed single-stranded DNA template caused a V_{max}-mediated 15−25-fold increase in the misinsertion frequency of dTTP compared to the frequency of dTTP misinsertion opposite the same G on the undamaged DNA (*19*). This decrease in the pol β fidelity on damaged DNA could lead to platinum drug-induced mutations if this base substitution is successfully fixed during further elongation. To test the relative ability of pol β to extend mispaired termini from the 3′G on platinated and undamaged DNA templates, we first performed steady-state kinetic assays for dCTP insertion at the 5′G template site following either a correctly paired (G:C) or mispaired (G:T) primer 3′-terminus. These data are summarized in Table 1, which shows the effect of platinum adducts on primer extension compared to extension on undamaged DNA (f_{rel}) and the effect of G:T mismatches on primer extension compared to extension of correctly paired G:C primer termini on the same DNA template ($f_{mis\ ext}$). The presence of platinum adducts on DNA resulted in a 39−45-fold decreased efficiency (k_{cat}/K_m) of correctly paired 3′G:C primer extension (f_{rel}, Table 1A). As reported previously (*19*), this reduced dCTP incorporation efficiency was governed primarily by a significant increase in K_m, although some decrease in k_{cat} values was also observed. The presence of platinum adducts also caused a 71−88-fold decreased efficiency (k_{cat}/K_m) of mispaired 3′G:T primer extension (f_{rel}, Table 1A). This was also caused by both an increase in K_m and a decrease in k_{cat}. Overall, the presence of a mismatched 3′G:T primer termini decreased dCTP incorporation opposite the 5′G 28-fold on undamaged DNA and 51−55-fold on DNA containing platinum adducts ($f_{mis\ ext}$, Table 1A).

Next, we measured dTTP incorporation opposite the A on the 5′ side of the adduct on templates with fully complementary primer-templates and templates with 3′G:T mismatch (hidden mismatch). The results in Table 1B (f_{rel}) indicate that extension of this primer was inhibited 3−5-fold by the platinum adducts when no mismatch was present, and 7−21-fold when a mismatch was present opposite the 3′G. This inhibition seems to be governed to the same extent by a decrease in k_{cat} and an increase in K_m. The presence of a mismatch opposite the 3′G decreased the efficiency of extension from the 5′G by 22-fold on undamaged DNA and by 28−135-fold on DNA containing a platinum adduct ($f_{mis\ ext}$, Table 1B).

We were not able to detect significant misinsertion of dTTP opposite the 5′G of either cisplatin or oxaliplatin adducts in our previous studies (*40*). Therefore, pol β is not likely to be responsible for the mutations at this site. However, in the present study we asked whether it could be responsible for the mispair elongation produced by other DNA polymerase(s). The presence of platinum adducts on

Table 3: Steady-State Kinetic Analysis of Extension of G:C and G:T Primer Termini by Pol η [a]

extension	Pt adduct	k_{cat}	K_m	k_{cat}/K_m	f_{rel}	$f_{mis\ ext}$
A. Extension from 3'G						
		min^{-1}	μM	$mM^{-1}\ min^{-1}$		
C	control	1.80 ± 0.08	13.0 ± 0.9	142 ± 14	1	-
-C↓	cisplatin	0.70 ± 0.05	4.3 ± 0.2	160 ± 16	1	-
-GGA-	oxaliplatin	1.30 ± 0.08	7.9 ± 0.2	162 ± 8	1	-
C	control	0.10 ± 0.02	5.8 ±1.1	17.0 ± 1.0	1	1.2×10^{-1}
-**T**↓	cisplatin	0.12 ± 0.02	18.0 ± 2.4	7.0 ± 0.9	4.1×10^{-1}	4.4×10^{-2}
-GGA-	oxaliplatin	0.14 ± 0.01	18.0 ± 0.5	7.4 ± 0.9	4.4×10^{-1}	4.6×10^{-2}
B. Extension from 5'G						
T	control	0.42 ± 0.10	4.3 ± 2.1	122 ± 26	1	-
-CC↓	cisplatin	0.25 ± 0.11	9.5 ± 5.7	32 ± 5	2.6×10^{-1}	-
-GGA-	oxaliplatin	0.27 ± 0.06	7.3 ± 3.4	47 ± 15	3.9×10^{-1}	-
T	control	0.25 ± 0.03	4.3 ± 0.4	59 ± 3	1	4.8×10^{-1}
-**T**C↓	cisplatin	0.04 ± 0.01	25 ± 8	1.5 ± 0.7	2.5×10^{-2}	4.7×10^{-2}
-GGA-	oxaliplatin	0.14 ± 0.02	56 ± 6	2.4 ± 0.5	4.1×10^{-2}	5.1×10^{-2}
C. Extension of G:T mismatch from 5'G						
T	control	0.13 ± 0.02	159 ± 49	0.81 ± 0.29	1	6.6×10^{-3}
-C**T**↓	cisplatin	0.07 ± 0.01	195 ± 87	0.35 ± 0.15	4.3×10^{-1}	1.1×10^{-2}
-GGA-	oxaliplatin	0.06 ± 0.01	182 ± 41	0.32 ± 0.05	4.0×10^{-1}	6.8×10^{-3}

[a] Kinetic parameters (K_m and V_{max}) and insertion efficiencies for dCTP incorporation opposite the 5′G were determined using the same DNA sequence and are taken from ref *10*, and V_{max} was converted to k_{cat} as described in the Materials and Methods section. Steady-state kinetic assays were performed using 20 fmol of pol η and 150 fmol of primer-templates for extension of the 3′G:T base pairs, or 7.5 fmol of pol η and 150 fmol of primer-templates for extension of the 5′G:C, 5′G:T, and hidden mismatch base pairs. Incubation time was 5 min for undamaged DNA templates and 15 min for platinated DNA templates. dCTP concentrations ranged from 1 to 8 μM for the undamaged template and from 2 to 24 μM for the platinated templates to measure extension of the 3′G:T mispair when the next correct base pair was 5′G:C. dTTP concentrations ranged from 1 to 10 μM for the undamaged template and from 2 to 20 μM for the platinated templates to measure extension of the 5′G:C pair when the next correct base pair was A:T. To determine the efficiency of extension of the 5′G:T mispair when the next correct base pair was A:T, dTTP was used at 100−500 μM concentrations for the undamaged template and 100−1000 μM for the platinated templates. To determine the efficiency of extension of the 3′T hidden mismatch when the next correct base pair was A:T, dTTP concentrations ranged from 1 to 10 μM on undamaged template and from 4 to 80 μM on platinated templates. Kinetic parameters (K_m and k_{cat}) and insertion efficiency ($f = k_{cat}/K_m$) for dCTP incorporation by pol β were determined using Hanes-Woolf plots (see Materials and Methods). The insertion efficiency (k_{cat}/K_m) was determined from each individual repeat and the individual values were averaged to obtain the values presented in this table. The relative insertion efficiency was determined as $f_{rel} = f_{Pt}/f_{undamaged}$. The relative efficiency of mismatch extension ($f_{mis\ ext}$) was determined as the ratio of efficiencies of extension of mispaired relative to correctly paired primer termini on the same template. Data are means (± standard error) from five to eight different experiments using four independent template preparations.

DNA resulted in 3−5-fold decreased catalytic efficiency of correctly paired 5′G:C primer extension (f_{rel}, Table 1B), and in 9−11-fold decreased efficiency of mismatched 5′G:T primer extension (f_{rel}, Table 1C). This reduced dTTP incorporation efficiency was governed by both a significant decrease in k_{cat} and an increase in K_m. The presence of the 5′G:T mismatch decreased extension from the 5′G by approximately 1000-fold on undamaged DNA and 1600−2900-fold on DNA containing platinum adducts ($f_{mis\ ext}$, Table 1C). Once again, the extension of the G:T mismatch was significantly more efficient from the oxaliplatin adduct than from the cisplatin adduct. The strong inhibition of primer extension from the 5′G:T mismatch suggests that not only is the incorporation of T opposite the 5′G by pol β unlikely, but the probability of extension of the 5′G:T mismatch by this polymerase is also very low. Therefore, if pol β were involved in translesion synthesis past platinum−GG adducts it would not be expected to induce base substitution opposite the 5′G site.

The overall effect of the three dNTP insertion steps is summarized in Table 2. Table 2A evaluates the effect of platinum−GG adducts on correct insertion and extension relative to the error-free replication on undamaged template. These results indicate that dCTP incorporation was inhibited 31−91-fold opposite both the 3′G and 5′G, and extension from the 5′G was inhibited an additional 3−5-fold. Thus, the overall efficiency of chain elongation was inhibited by approximately 10^4-fold. When comparing oxaliplatin and cisplatin adducts, correct nucleotide incorporation was 2-fold more efficient for oxaliplatin opposite the 3′G, 1.2-fold more efficient for oxaliplatin opposite the 5′G, and 1.5-fold more efficient for cisplatin for extension from the 5′G to the 5′A. Thus, the overall efficiency of chain elongation was 1.6-fold greater for the oxaliplatin adducts than for the cisplatin adduct. These data are consistent with our previous data on pol β-catalyzed primer extension at low enzyme concentrations (*29*) where cisplatin adducts were somewhat more effective than oxaliplatin adducts at blocking translesion synthesis on single-stranded primed templates.

Table 2B shows the effect of platinum adducts on dTTP misinsertion opposite the 3′G, and extension of that mismatch from the 3′G and the 5′G. These data indicate that replication past the adduct with formation of the 3′G:T mismatch is approximately 14-fold more likely to occur with oxaliplatin adducts than with cisplatin adducts. Finally, Table 2C summarizes the overall likelihood of dTTP misinsertion and extension on undamaged and platinated templates. In Table 2C, dCTP incorporation opposite the 3′G on the same primer-templates was chosen as the standard, with relative efficiency of incorporation and extension values of 1. For the cisplatin adducts, the likelihood of dTTP misinsertion opposite the 3′G was 16-fold greater than for undamaged DNA but extension of the G:T mismatch was inhibited 2−6-fold at the subsequent two elongation steps, so that the overall likelihood of a T misinsertion and extension occurring for the 3′G was only slightly greater with the cisplatin adducts

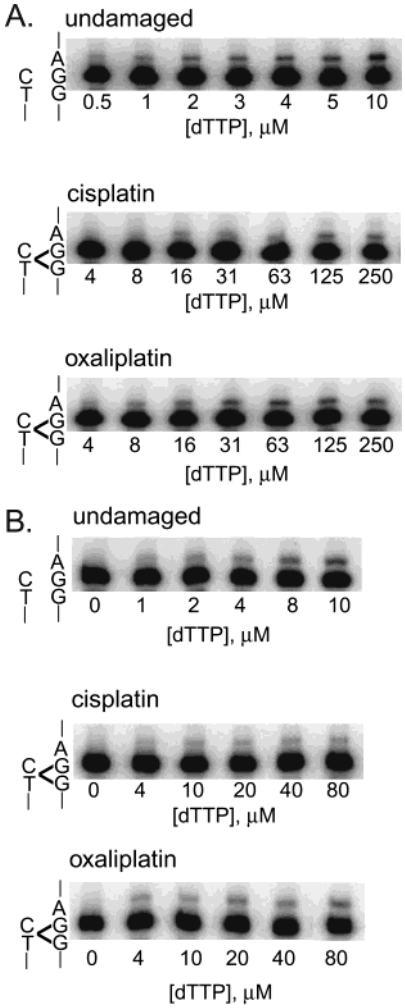

FIGURE 2: dTTP incorporation on DNA templates containing platinum−GG adducts and a hidden mismatch primer by pol β and pol η. (A) dTTP incorporation by pol β. (B) dTTP incorporation by pol η. The reactions were performed as described in the Materials and Methods, using 5 fmol of pol β and 150 fmol of primer-templates, and 7.5 fmol of pol η and 150 fmol of primer-templates. Incubation times were 5 min for undamaged DNA templates and 15 min for platinated DNA templates. The template sequence is shown to the left of each gel. Platinated guanines are indicated by brackets.

than with undamaged DNA. For the oxaliplatin adduct, on the other hand, the likelihood of dTTP misinsertion opposite the 3′G was 26-fold greater than for undamaged DNA, and extension of the G:T mismatch was inhibited only 1.3−1.8-fold at the subsequent elongation steps. Thus, there is a 13-fold increase in the overall likelihood of a T misinsertion and extension for the 3′G with oxaliplatin-containing DNA than with undamaged DNA. These results indicate that replication past oxaliplatin adducts is significantly more error-prone than past cisplatin adducts (9−14-fold depending on whether misincorporation frequency is normalized to dCTP incorporation on undamaged DNA (Table 2B)) or damaged DNA (Table 2C). Therefore, if translesion synthesis past platinum−GG adducts were catalyzed by pol β, oxaliplatin adducts might be more mutagenic than cisplatin adducts.

Extension of Primer Termini across from Cisplatin and Oxaliplatin Adducts by pol η. Next, we looked at the steps required for dTTP misinsertion opposite both the 3′G and the 5′G of the platinum−GG adducts by pol η. We had previously shown that as with pol β, dTTP was the most frequently misinserted nucleotide opposite the 3′G by pol η (*10*). dTTP misinsertion was approximately 13-fold less efficient than dCTP insertion on undamaged DNA, and platinum−GG adducts did not have a significant effect on pol η fidelity opposite the 3′G (*10*). To test the relative ability of pol η to extend mispaired termini from the 3′G on platinated and undamaged DNA templates, we measured dCTP insertion at the 5′G following either a correctly paired (G:C) or mispaired (G:T) primer 3′-terminus. The presence of platinum adducts on DNA did not have a significant effect on the efficiency (k_{cat}/K_m) of correctly paired 3′G:C primer extension (f_{rel}, Table 3A). However, platinum adducts caused a 2-fold decreased efficiency (k_{cat}/K_m) of mismatched 3′G:T primer extension (f_{rel}, Table 3A). This bias occurred due to an increase in K_m. Overall, the presence of a 3′G:T mismatch decreased dCTP incorporation opposite the 5′G 8-fold on undamaged DNA and 22−23-fold on DNA containing platinum adducts ($f_{mis\ ext}$, Table 3A).

dTTP incorporation opposite the A on the 5′ side of the adduct was evaluated in the context of the hidden mismatch. The results in Table 3B (f_{rel}) indicate that primer extension was inhibited by the platinum adducts 3−4-fold on fully complementary primer-template, and 25−39-fold when a hidden mismatch was present opposite the 3′G. As in the case of pol β, this inhibition seems to be governed to the same extent by a decrease in k_{cat} and an increase in K_m. The presence of a mismatch opposite the 3′G decreased the efficiency of extension from the 5′G by 2-fold on undamaged DNA and by 20−21-fold on DNA carrying a platinum adduct ($f_{mis\ ext}$, Table 3B).

Finally, we evaluated misinsertion of dTTP opposite the 5′G of cisplatin and oxaliplatin adducts, and the extension from this template site. Previous studies from our laboratory indicated that only dTTP was incorporated to a significant extent across from the 5′G of the platinum adducts by pol η (*10*). Our previous studies showed that, as in the case of the 3′G, platinum−GG adducts did not have a significant effect on fidelity of pol η opposite the 5′G (*10*). In the present study, we asked whether pol η could be responsible for the mispair elongation from the 5′G of the adduct. The presence of platinum adducts on DNA resulted in 2−3-fold decreased efficiency of mismatched 5′G:T primer extension (f_{rel}, Table 3C). This reduced dTTP incorporation efficiency was due to both a decrease in k_{cat} and an increase in K_m. The presence of the 5′G:T mismatch decreased extension from the 5′G by 151-fold on undamaged DNA and 91−147-fold on DNA containing platinum adducts ($f_{mis\ ext}$, Table 3C). The extension of the 5′G:T mismatch was somewhat more efficient from the cisplatin adduct than from the oxaliplatin adduct, which is the opposite from our finding with pol β.

The overall efficiency of the three dNTP insertion steps is summarized in Table 4. In Table 4A, the effect of platinum−GG adducts on correct insertion and extension relative to error-free replication on undamaged template is evaluated. These results indicate that dCTP incorporation was not significantly inhibited by the platinum adducts opposite both the 3′G and 5′G, but the extension from the 5′G was inhibited 3−4-fold. Thus, the overall efficiency of chain elongation was inhibited 3−4-fold (Table 4A). which is approximately 3000-fold less than the inhibition of correct insertion and extension by platinum−GG adducts with pol

Table 4: Overall Efficiency of Insertion Opposite the 3′G and 5′G and One Base Extension from the 5′G on Platinated Templates by Pol η Compared to that on Undamaged DNA[a]

	Pt adduct	f_{ins1}	f_{ins2}	f_{ext}	$f_{ins1} \times f_{ins2} \times f_{ext}$
A. Effect of platinum adducts on correct insertion and extension					
C → C → T → -↓ -C↓ -CC↓ -CCT -GGA- -GGA- -GGA- -GGA-	undamaged	1	1	1	1
	cisplatin	9.0×10^{-1}	1	2.6×10^{-1}	2.3×10^{-1}
	oxaliplatin	1	1	3.9×10^{-1}	3.9×10^{-1}
B. Effect of platinum adducts on T misincorporation opposite 3'G or 5'G and subsequent extension					
T → C → T → -↓ -T↓ -TC↓ -TCT -GGA- -GGA- -GGA- -GGA-	undamaged	1	1	1	1
	cisplatin	1	4.1×10^{-1}	2.5×10^{-2}	1.0×10^{-2}
	oxaliplatin	1	4.4×10^{-1}	4.1×10^{-2}	1.8×10^{-2}
C → T → T → -↓ -C↓ -CT↓ -CTT -GGA- -GGA- -GGA- -GGA-	undamaged	1	1	1	1
	cisplatin	9.0×10^{-1}	8.5×10^{-1}	4.3×10^{-1}	3.3×10^{-1}
	oxaliplatin	1	1	4.0×10^{-1}	4.0×10^{-1}
C. Overall efficiency of T misincorporation opposite 3'G and 5'G compared to correct insertion and extension					
	Pt adduct	f'_{ins1}	f'_{ins2}	f'_{ext}	$f'_{ins1} \times f'_{ins2} \times f'_{ext}$
T → C → T → -↓ -T↓ -TC↓ -TCT -GGA- -GGA- -GGA- -GGA-	undamaged	7.9×10^{-2}	1.2×10^{-1}	4.8×10^{-1}	4.6×10^{-3}
	cisplatin	9.4×10^{-2}	4.4×10^{-2}	4.7×10^{-2}	1.9×10^{-4}
	oxaliplatin	7.9×10^{-2}	4.6×10^{-2}	5.1×10^{-2}	1.9×10^{-4}
C → T → T → -↓ -C↓ -CT↓ -CTT -GGA- -GGA- -GGA- -GGA-	undamaged	1	2.7×10^{-2}	6.6×10^{-3}	1.8×10^{-4}
	cisplatin	1	2.3×10^{-2}	1.1×10^{-2}	2.5×10^{-4}
	oxaliplatin	1	2.7×10^{-2}	6.8×10^{-3}	1.8×10^{-4}

[a] The efficiencies for dCTP and dTTP incorporation opposite the 3′G and the 5′G were determined using the same DNA sequence and are taken from ref *10*, and the efficiencies of extension of correctly paired and mismatched primer termini are taken from Table 3. f_{ins1} and f_{ins2} represent k_{cat}/K_m for dTTP or dCTP insertion opposite the platinated 3′G or 5′G as compared to the dCTP or dTTP incorporation opposite the 3′G or the 5′G on an undamaged DNA template. f_{ext} represents the ratio of k_{cat}/K_m for dTTP incorporation opposite the A (base 5′ to the GG site) on damaged compared to undamaged DNA template. $f_{ins1} \times f_{ins2} \times f_{ext}$ is the overall efficiency of incorporation opposite the 3′G and extension from the 3′G and the 5′G on platinated template relative to incorporation and extension on an undamaged DNA template. In panel C, f'_{ins1} represents k_{cat}/K_m for dTTP insertion opposite the 3′G as compared to the dCTP incorporation opposite the 3′G on the same DNA template. f'_{ins2} represents the ratio of k_{cat}/K_m for dCTP insertion opposite the 5′G on DNA templates with T:3′G primer-template mispair compared to templates with correctly paired primer termini. f'_{ext} represents the ratio of efficiency of extension of the primer ending across from the 5′G in the context of the hidden mismatch compared to fully complementary primer-template. $f'_{ins1} \times f'_{ins2} \times f'_{ext}$ is the overall efficiency of dTTP misincorporation opposite the 3′G followed by dCTP incorporation opposite the 5′G and one base extension compared to sequential correct incorporation of three nucleotides opposite the GGA sites on the same type of DNA template.

β (Table 2A). When comparing oxaliplatin and cisplatin adducts, correct nucleotide incorporation opposite the 3′G and the 5′G occurred with similar efficiency for both adducts, but extension from the 5′G to the 5′A was 1.5-fold more efficient for oxaliplatin than for cisplatin adduct. Thus, the overall efficiency of chain elongation was 1.5-fold greater for oxaliplatin adducts than for cisplatin adducts, which is consistent with our previous data demonstrating that cisplatin adducts were more effective than oxaliplatin adducts at blocking translesion synthesis on single-stranded primed templates (*10*).

Table 4B shows the effect of platinum adducts on dTTP misinsertion opposite the 3′G and the 5′G, and extension of the mismatches from the 3′G and the 5′G. These data indicate that G:T mismatches were extended slightly more efficiently when they occurred opposite oxaliplatin adducts than opposite cisplatin adducts, but the differences in insertion and extension efficiencies caused by these adducts were much smaller than those seen with pol β (Table 2B) and could be almost entirely explained by the greater efficiency of translesion synthesis past oxaliplatin adducts. Pol η-catalyzed bypass replication with formation of the 3′G:T mismatch is approximately 1.8-fold more likely to occur with oxaliplatin adducts than with cisplatin adducts, and replication with formation of the 5′G:T mismatch is 1.2-fold more likely to occur with oxaliplatin adducts. Finally, Table 4C summarizes the overall likelihood of dTTP misinsertion and extension. In Table 4C, dCTP incorporation opposite the 3′G on the same DNA templates was chosen as the standard, with relative efficiency of incorporation and extension values of 1. For both cisplatin and oxaliplatin adducts, the likelihood of dTTP misinsertion opposite the 3′G was similar to dTTP misinsertion on undamaged DNA, but the extension of the 3′G:T mismatch was inhibited 3−10-fold at the subsequent two elongation steps, so that the overall likelihood of a T misinsertion and extension occurring at the 3′G was inhibited 24-fold for both cisplatin and oxaliplatin adducts compared with undamaged DNA. In the case of the 5′G:T mismatch, dTTP misinsertion and extension was comparable for oxaliplatin-containing and undamaged DNA, but in the case of cisplatin-containing DNA, it was 1.4-fold more likely to occur. Finally, the overall efficiency of dTTP misinsertion and extension of the resulting 3′G:T mismatch was very similar for the 3′G and 5′G of both types of adducts. Taken together, our results indicate equal probability of pol η-dependent mutagenesis at the 3′G and 5′G of the platinum adducts. While the overall likelihood of dTTP misinsertions opposite G occurring on DNA containing oxaliplatin−GG adducts is not significantly different from that on undamaged DNA, the efficiency is slightly greater for cisplatin−GG adducts.

DISCUSSION

Previous studies have proposed that fidelity of translesion synthesis by pol η is primarily affected by the efficiency of chain extension of mismatched primer termini rather than by the efficiency of nucleotide insertion on DNA templates carrying various types of lesions, including platinum−GG

adducts (*9*, *41*). This inability to extend mismatched primer termini may contribute to prevention of mutation fixation during translesion synthesis by pol η (*9*). However, the ability of pol η to extend past the platinum lesions was examined only qualitatively in the previous study. Therefore, we performed a steady-state kinetic analysis of the three steps of translesion synthesis (insertion at the 3′G, insertion at the 5′G, and extension from the 5′G) past platinum−GG adducts by pol η, the most efficient enzyme to perform this function in vitro (*10*). We compared the efficiency of the insertion and extension steps of translesion synthesis past platinum−CG adducts catalyzed by pol η to the efficiency of these steps catalyzed by pol β, the second most likely enzyme to perform this function in vivo. We used templates containing cisplatin and oxaliplatin adducts in the AGG sequence context (the position of the adduct is underlined), since the AGG sequence context is a mutation hot spot for cisplatin adducts in vivo (*42*, *43*). An accurate comparison of translesion synthesis by these two polymerases was obtained by determining the catalytic efficiency (k_{cat}/K_m) of insertion of correct and incorrect dNTPs and extension of correctly paired and mispaired primer termini opposite the adduct under the conditions of template excess over the polymerase.

Correct Extension. First, we investigated whether pol η or pol β are capable of performing efficient extension of correctly matched primer termini opposite platinum−GG adducts. Damage-induced inhibition of pol η-catalyzed dCTP insertion opposite Pt−GG adducts was significantly less than that of pol β, while extension of correctly paired primers ending across from the adducts was inhibited to a comparable extent by both polymerases (Tables 2A and 4A). This explains why pol η is better than pol β in the overall catalysis of translesion synthesis past cisplatin and oxaliplatin lesions. The extent of translesion synthesis in this sequence context is determined primarily by the insertion steps for pol β and the extension step for pol η. Analogously, the preference for bypass of the oxaliplatin adduct by both polymerases is primarily determined by insertion opposite the 3′G for pol β and by extension from the 5′G by pol η . It has been shown that pol η-catalyzed extension past several other types of DNA adducts is also the limiting step of translesion synthesis (*9*). For example, extension of even correct primer termini across from [6−4] TT photoproducts by pol η is undetectable (*44*). Therefore, it has been proposed that another DNA polymerase, such as pol ζ or pol κ, may be responsible for completing the extension step from these adducts (*28*, *45*, *46*). However, in the case of platinum−GG adducts, the extension step of translesion synthesis is relatively efficient for both pol β and pol η, therefore translesion synthesis of platinum−GG adducts may not require a combined action of two polymerases in vivo.

The Effect of G:T Mismatches on Translesion Synthesis Past Cisplatin and Oxaliplatin Adducts. Error-prone bypass of some adducts appears to require two polymerases, one for dNTP misinsertion, and the second one for extension of the mismatched primer termini. For example, pol η is very inefficient at extending primer termini across from [6−4] photoproducts in vitro (*44*), and recent evidence in yeast indicates that pol η and pol ζ together contribute to error-prone bypass of these lesions (*17*). For pol η, the efficiency of extension of the 3′G:T mismatch for the next two steps is $2.1-2.3 \times 10^{-3}$ on platinum-damaged DNA relative to extension of fully complementary primers on undamaged DNA ($f_{mis\ ext}$ Table 3A × $f_{mis\ ext}$ Table 3B). Similarly, the efficiency of extension of the adduct-containing 5′G:T mismatch by pol η is $0.7-1.1 \times 10^{-2}$ ($f_{mis\ ext}$, Table 3C). Other polymerases would need to have greater mismatch extension abilities to be considered candidates for fixing potential mutations caused by pol η. Pol β cannot be considered such a candidate because its efficiency of extension of the 3′G:T mismatch is $1.3-7.2 \times 10^{-4}$ on platinum-damaged DNA ($f_{mis\ ext}$ Table 1A × $f_{mis\ ext}$ Table 1B), and its efficiency of extension of the 5′G:T mismatch is $3.4-6.2 \times 10^{-4}$ on platinated DNA ($f_{mis\ ext}$, Table 3C). Pol ζ and pol κ have been suggested for the role of mismatch extender for other adducts (*28*, *45*, *46*), but their ability to extend mismatches from platinum−GG adducts has not yet been tested. Pol μ preferentially causes frameshift errors in the vicinity of platinum adducts (*30*). Its ability to extend mismatched primer termini is also not known. In addition to catalyzing more efficient extension of mismatched primer termini than pol β, pol η is also more likely than pol β to misinsert a T opposite both the 3′G and the 5′G on platinum-containing DNA templates (Tables 2A and 4A). The overall probability of damage-induced mutations is higher for pol η-catalyzed translesion synthesis ($1.8-2.5 \times 10^{-4}$) than for pol β-catalyzed translesion synthesis ($1-10 \times 10^{-6}$) (Tables 2C and 4C). However, given the fact that a translesion polymerase is only required to insert two nucleotides opposite the adduct and perhaps extend those nucleotides by a single base, an overall error rate of 10^{-4} may be sufficient for accurate bypass of the lesion.

If either pol η or pol β is involved in translesion synthesis past platinum−GG adducts, these data allow one to make some predictions about the mutation spectrum that is likely to be produced. For example, for pol η the overall probability of dTTP misinsertion and extension is approximately the same for the 5′G and the 3′G, while for pol β T misinsertion is only seen with the 3′G (*10*). In addition, pol η induces G:T mispairs with virtually the same probability for oxaliplatin and cisplatin adducts, yet the probability of G:T misinsertion is 9-fold greater for oxaliplatin than cisplatin adducts when translesion synthesis is performed by pol β (Tables 2C and 4C).

Predictability of Polymerase Function in Vivo Based in its Ability to Bypass Various Types of Adducts in Vitro. In vitro assays of dNTP misinsertion and extension are widely interpreted as predicting the likelihood of that polymerase playing a role in either error-prone or error-free translesion synthesis in vivo. Therefore, we attempted to analyze the predictability of polymerase function in vivo based on their efficiency in bypassing various types of adducts in vitro. Table 5 summarizes the in vitro catalytic properties of translesion synthesis past various types of adducts by pol η, and the in vivo findings for the corresponding adducts. In this table, only the in vitro studies that characterized dNTP insertion and extension steps of translesion synthesis using steady-state kinetics are included. Pol η is the most efficient polymerase for bypass of UV-induced cis,syn thymine-thymine dimers in vitro (*11*, *12*). Primer extension kinetic studies in vitro indicate that pol η is not significantly inhibited by the T−T dimers during the insertion and the extension steps of translesion synthesis (*47*−*49*). Thus, it is not surprising that pol η is also the essential polymerase for

Table 5: Overall Efficiency of Insertion and Extension by pol η on Templates Damaged with Various Types of Lesions Compared to that on Undamaged DNA[a]

organism	adduct	insertion at the 3′ base (f_{rel1})	insertion at the 5′ base (f_{rel2})	extension from the 5′ base (f_{ext})	overall	in vivo translesion synthesis
yeast	8-oxo-G	1 (*15*)	N/A	1 (*15*)	1	error-free (*15*)
human	cyclobutane TT dimer	1 (*47*)	1 (*47*)	0.6 (*48*)	0.6	error-free (*50*)
human	oxaliplatin	1 (Table 4A)	1 (Table 4A)	0.4 (Table 4A)	0.4	unknown
human	cisplatin	0.9 (Table 4A)	1 (Table 4A)	0.3 (Table 4A)	0.3	unknown
human	cyclobutane TT dimer	0.6 (*49*)	0.4 (*49*)	0.6 (*49*)	0.1	error-free (*50*)
yeast	[6−4]TT[b]	0.01 (*44*)	0.4 (*44*)	ND	0.004	error-prone (two-step with pol ζ) (*17*)
human	(+)-*trans*-*anti*-BPDE-*N*2-dG[c]	0.01 (*51*)	N/A	0.01(*51*)	0.0001	not essential for either error-prone or error-free (*52*−*54*)

[a] f_{rel1} and f_{rel2} represent k_{cat}/K_m or V_{max}/K_m for correct dNTP insertion (unless otherwise noted) opposite the damaged 3′ or 5′ base of the adduct as compared to the correct incorporation opposite the 3′ or the 5′ base on an undamaged DNA template. f_{ext} represents the ratio of k_{cat}/K_m or V_{max}/K_m for dNTP incorporation opposite the base 5′ to the lesion site on damaged compared to undamaged DNA template with the same type of primer as on undamaged DNA. $f_{rel1} \times f_{rel2} \times f_{ext}$ is the overall efficiency of incorporation opposite the adduct and extension from the adduct on damaged template relative to incorporation and extension of the correct dNTP on an undamaged DNA template. References from which the values are taken are indicated in parentheses next to each value. ND, not determined. [b] Yeast pol η preferentially incorporates dGTP opposite the 3′T, and yeast pol ζ efficiently extends the 3′G:T mismatch. Incorporation of dGTP by yeast pol η and extension of the 3′G:T mismatch by yeast pol ζ is shown. [c] Human pol η preferentially incorporates dATP opposite this adduct. Incorporation of dATP and extension of G:A mismatch by pol η are shown. Because no data on extension of correctly paired G:C primer terminus were presented in this study, the overall efficiency of translesion synthesis past this adduct in likely to be overestimated. Mismatch extension is thought to be performed by pol κ in the cells.

accurate bypass of thymine−thymine dimers in vivo (*50*). Similarly, pol η preferentially misinserts dGTP opposite the 3′T of the [6−4] TT photoproduct while pol ζ efficiently extends this type of mismatch (*44*), and recent studies suggest that both pol η and pol ζ play a role in mutagenic two-step bypass of 6−4 photoproducts in yeast (*17*). As yet another example, efficient and accurate bypass of the 7,8-dihydro-8-oxoguanine (8-oxoG) lesion by pol η in vitro is in agreement with error-free bypass of these lesions by pol η in yeast (*15*).

At the other end of the spectrum, pol η performs very inefficient bypass of (+) *trans*-*anti*-BPDE-*N*2-dG adducts, the major adducts induced by benzo[*a*]pyrene (*51*). In agreement with these studies, it has been reported that the response of XPV cells and normal cells to benzo[*a*]pyrene-diol-epoxide-I is identical when measured by post-replication repair activity and transformation to anchorage independence (*52*, *54*). The results presented in Table 5 suggest that if the overall efficiency of translesion synthesis by pol η is 0.0001 or less in vitro ((+) *trans*-*anti*-BPDE-*N*2-dG, Table 5), then pol η is probably not involved in bypass of these adducts in vivo. However, an overall efficiency of translesion synthesis as low as 0.004 ([6−4] TT, Table 5, pol η in combination with pol ζ) may be sufficient for bypass of these adducts in vivo. When one compares the effect of platinum adducts and cis,syn thymine−thymine dimers on correct insertion and extension by pol η, it is evident that the overall efficiency of translesion synthesis past these types of adducts is very similar (0.1−0.6 for thymine−thymine dimers (Table 5) and 0.3−0.4 for cisplatin and oxaliplatin adducts (Table 4A)). On the basis of this comparison, it is tempting to hypothesize that pol η is likely to play a role in translesion synthesis past platinum adducts in vivo. However, there are several other factors that may also be important for determining the efficiency of translesion synthesis past adducts in vivo. One is the presence of the accessory proteins such as PCNA, RFC, and RPA in the replication complex, which may influence the efficiency and the specificity of the adduct bypass. Such protein−protein interactions are absent in steady-state kinetic primer extension studies. The in vivo situation may also be influenced by the polymerase redundancy. Several polymerases may potentially compete for the same lesion. Finally, it is not clear whether translesion polymerases are present in the replication complex during replication of undamaged DNA or whether they have to be recruited to the lesion after the stalling of the replication fork using a signaling molecule.

Less is known about the function of pol β as a translesion polymerase. One recent study indicates that pol β is capable of catalyzing primarily dATP insertion opposite the 3′T of cis,syn thymine-thymine dimers and [6−4] photoproducts, but only with low efficiencies of 1.3×10^{-4} and 2.9×10^{-4}, respectively (*55*). These data could indicate a secondary biological role for pol β in bypass of UV-induced lesions. Other studies have shown that pol β is capable of catalyzing error-prone in vitro translesion synthesis past 7,8-dihydro-8-oxoadenine (*56*). It is not known whether pol β participates in translesion synthesis past cisplatin adducts at normal levels of expression in cells, however mammalian cells overexpressing pol β demonstrate higher cisplatin-induced mutation frequencies than cells with normal levels of pol β expression (*20*, *21*). Our study shows that pol β is less efficient than pol η in bypassing platinum adducts, therefore we have hypothesized that pol β may play a secondary role in translesion synthesis past platinum adducts in vivo. Experiments to elucidate the roles of pol β and pol η in translesion synthesis past cisplatin adducts in vivo are currently in progress in our laboratory.

ACKNOWLEDGMENT

We thank Dr. S. Wilson (NIEHS) for providing pol β. We would also like to thank Dr. Paul Juniewicz (Sanofi-Synthelabo) for providing us with the *trans*-R,R-dach(Cl_2)-Pt. We are grateful to Drs. Marila Cordeiro-Stone and Dale Ramsden for critical reading of the manuscript.

REFERENCES

1. Greene, M. H. (1992) *J. Nat. Cancer Inst. 84*, 306−312.
2. Leopold, W. R., Batzinger, R. P., Miller, E. C., Miller, J. A., and Earhardt, R. H. (1981) *Cancer Res. 41*, 4368−4377.
3. Eastman, A. (1987) *Pharmacol. Ther. 34*, 155−166.

4. Woynarowski, J. M., Chapman, W. G., Napier, C., Herzig, M. C. S., and Juniewicz, P. (1998) *Mol. Pharmacol. 54*, 770−777.
5. Vaisman, A., Varchenko, M., Umar, A., Kunkel, T. A., Risinger, J. I., Barrett, J. C., Hamilton, T. C., and Chaney, S. G. (1998) *Cancer Res. 58*, 3579−3585.
6. Gibbons, G. R., Kaufmann, W. K., and Chaney, S. G. (1991) *Carcinogenesis 12*, 2253−2257.
7. Villani, G., Hubscher, U., and Butour, J.-L. (1988) *Nucleic Acid Res. 16*, 4407−4417.
8. Hoffmann, J. S., Pillaire, M. J., Garcia-Estefania, D., Lapalu, S., and Villani, G. (1996) *J. Biol. Chem. 271*, 15386−15392.
9. Masutani, C., Kusumoto, R., Iwai, S., and Hanaoka, F. (2000) *EMBO J. 19*, 3100−3109.
10. Vaisman, A., Masutani, C., Hanaoka, F., and Chaney, S. G. (2000) *Biochemistry 39*, 4575−4580.
11. Johnson, R. E., Prakash, S., and Prakash, L. (1999) *J. Biol. Chem. 274*, 15975−15977.
12. Masutani, C., Kusumoto, R., Yamada, A., Dohmae, N., Yokoi, M., Yuasa, M., Araki, M., Iwai, S., Takio, K., and Hanaoka, F. (1999) *Nature 399*, 700−704.
13. Roush, A. A., Suarez, M., Friedberg, E. C., Radman, M., and Siede, W. (1998) *Mol. Gen. Genet. 257*, 686−692.
14. Johnson, R. E., Kondratick, C. M., Prakash, S., and Prakash, L. (1999) *Science 285*, 263−265.
15. Haracska, L., Yu, S. L., Johnson, R. E., Prakash, L., and Prakash, S. (2000) *Nature Genet. 25*, 458−461.
16. Minko, I. G., Washington, M. T., Kanuri, M., Prakash, L., Prakash, S., and Lloyd, R. S. (2003) *J. Biol. Chem. 278*, 784−790.
17. Bresson, A., and Fuchs, R. P. (2002) *EMBO J. 21*, 3881−3887.
18. Hoffmann, J.-S., Pillaire, M.-J., Maga, G., Podust, V., Hubscher, U., and Villani, G. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 5356−5360.
19. Vaisman, A., and Chaney, S. G. (2000) *J. Biol. Chem. 275*, 13017−13025.
20. Canitrot, Y., Cazaux, C., Frechet, M., Bouayadi, K., Lesca, C., Salles, B., and Hoffmann, J.-S. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 12586−12590.
21. Canitrot, Y., Frechet, M., Servant, L., Cazaux, C., and Hoffmann, J. B. (1999) *FASEB J. 13*, 1107−1111.
22. Srivastava, D. K., Husain, I., Arteaga, C. L., and Wilson, S. H. (1999) *Carcinogenesis 20*, 1049−1054.
23. Scanlon, K. J., Kashani-Sabet, M., and Miyachi, H. (1989) *Cancer Invest. 7*, 563−569.
24. Kashani-Sabet, M., Lu, Y., Leong, L., Haedicke, K., and Scanlon, K. J. (1990) *Eur. J. Cancer 26*, 383−390.
25. Maga, G., Villani, G., Ramadan, K., Shevelev, I., Tanguy, L. G., Blanco, L., Blanca, G., Spadari, S., and Hubscher, U. (2002) *J. Biol. Chem. 277*, 48434−48440.
26. Ohashi, E., Ogi, T., Kusumoto, R., Iwai, S., Masutani, C., Hanaoka, F., and Ohmori, H. (2000) *Genes Dev. 14*, 1589−1594.
27. McDonald, J. P., Tissier, A., Frank, E. G., Iwai, S., Hanaoka, F., and Woodgate, R. (2001) *Philos. Trans. R. Soc. London B 356*, 53−60.
28. Tissier, A., Frank, E. G., McDonald, J. P., Iwai, S., Hanaoka, F., and Woodgate, R. (2000) *EMBO J. 19*, 5259−5266.
29. Vaisman, A., Lim, S. E., Patrick, S. M., Copeland, W. C., Hinkle, D. C., Turchi, J. J., and Chaney, S. G. (1999) *Biochemistry 38*, 11026−11039.
30. Havener, J. M., McElhinny, S. A., Bassett, E., Gauger, M., Ramsden, D. A., and Chaney, S. G. (2003) *Biochemistry 42*, 1777−1788.
31. Pillaire, M. J., Margot, A., Villani, G., Sarasin, A., Defais, M., and Gentil, A. (1994) *Nucleic Acids Res. 22*, 2519−2524.
32. de Boer, J. G., and Glickman, B. W. (1989) *Carcinogenesis 10*, 1363−1367.
33. Mis, J. R. A., and Kunz, B. A. (1990) *Carcinogenesis 11*, 633−638.
34. Louro, H., Silva, M. J., and Boavida, M. G. (2002) *Environ. Mol. Mutagen. 40*, 283−291.
35. Mamenta, E. L., Poma, E. E., Kaufmann, W. K., Delmastro, D. A., Grady, H. L., and Chaney, S. G. (1994) *Cancer Res. 54*, 3500−3505.
36. Caradonna, J. P., and Lippard, S. J. (1982) *J. Am. Chem. Soc. 104*, 5793−5795.
37. Beard, W. A., and Wilson, S. H. (1995) *Methods Enzymol. 262*, 98−107.
38. Boosalis, M. S., Petruska, J., and Goodman, M. F. (1987) *J. Biol. Chem. 262*, 14689−14696.
39. Creighton, S., Bloom, L. B., and Goodman, M. F. (1995) *Methods Enzymol. 262*, 232−256.
40. Vaisman, A., Warren, M. W., and Chaney, S. G. (2001) *J. Biol. Chem. 276*, 18999−19005.
41. Washington, M. T., Johnson, R. E., Prakash, S., and Prakash, L. (2001) *J. Biol. Chem. 276*, 2263−2266.
42. Burnouf, D., Daune, M., and Fuchs, R. P. P. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 3758−3762.
43. Bubley, G. J., Ashburner, B. P., and Teicher, B. A. (1991) *Mol. Carcinog. 4*, 397−406.
44. Johnson, R. E., Haracska, L., Prakash, S., and Prakash, L. (2001) *Mol. Cell. Biol. 21*, 3558−3563.
45. Johnson, R. E., Washington, M. T., Haracska, L., Prakash, S., and Prakash, L. (2000) *Nature 406*, 1015−1019.
46. Washington, M. T., Johnson, R. E., Prakash, L., and Prakash, S. (2002) *Proc. Natl. Acad. Sci. U.S.A. 99*, 1910−1914.
47. Johnson, R. E., Washington, M. T., Prakash, S., and Prakash, L. (2000) *J. Biol. Chem 275*, 7447−7450.
48. Washington, M. T., Johnson, R. E., Prakash, L., and Prakash, S. (2001) *Proc. Natl. Acad. Sci. U.S.A. 98*, 8355−8360.
49. Kusumoto, R., Masutani, C., Iwai, S., and Hanaoka, F. (2002) *Biochemistry 41*, 6090−6099.
50. Maher, V. M., Ouellette, L. M., Curren, R. D., and McCormick, J. J. (1976) *Nature 261*, 593−595.
51. Zhang, Y., Wu, X., Guo, D., Rechkoblit, O., Geacintov, N. E., and Wang, Z. (2002) *Mutat. Res. 510*, 23−35.
52. Cordeiro-Stone, M., Boyer, J. C., Smith, B. A., and Kaufmann, W. K. (1986) *Carcinogenesis 7*, 1783−1786.
53. Boyer, J. C., Kaufmann, W. K., Brylawski, B. P., and Cordeiro-Stone, M. (1990) *Cancer Res. 50*, 2593−2598.
54. Boyer, J. C., Kaufmann, W. K., and Cordeiro-Stone, M. (1991) *Cancer Res. 51*, 2960−2964.
55. Servant, L., Cazaux, C., Bieth, A., Iwai, S., Hanaoka, F., and Hoffmann, J. S. (2002) *J. Biol. Chem. 277*, 50046−50053.
56. Kamiya, H., Miura, H., Murata-Kamiya, N., Ishikawa, H., Sakaguchi, T., Inoue, H., Sasaki, T., Masutani, C., Hanaoka, F., Nishimura, S., and. (1995) *Nucleic Acids Res. 23*, 2893−2899.

BI035359P