Efficient Translesion Replication Past Oxaliplatin and Cisplatin GpG Adducts by Human DNA Polymerase η[†]

Alexandra Vaisman,[‡] 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, and Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565-0871, Japan

Received January 20, 2000; Revised Manuscript Received March 3, 2000

ABSTRACT: Platinum anticancer agents form bulky DNA adducts which are thought to exert their cytotoxic effect by blocking DNA replication. Translesion synthesis, one of the pathways of postreplication repair, is thought to account for some resistance to DNA damage and much of the mutagenicity of bulky DNA adducts in dividing cells. Oxaliplatin has been shown to be effective in cisplatin-resistant cell lines and less mutagenic than cisplatin in the Ames assay. We have shown that the eukaryotic DNA polymerases yeast pol ζ, human pol β, and human pol γ bypass oxaliplatin-GG adducts more efficiently than cisplatin-GG adducts. Human pol η, a product of the XPV gene, has been shown to catalyze efficient translesion synthesis past *cis*,*syn*-cyclobutane pyrimidine dimers. In the present study we compared translesion synthesis past different Pt-GG adducts by human pol η. Our data show that, similar to other eukaryotic DNA polymerases, pol η bypasses oxaliplatin-GG adducts more efficiently than cisplatin-GG adducts. However, pol η-catalyzed translesion replication past Pt-DNA adducts was more efficient and less accurate than that seen for previously tested polymerases. We show that the efficiency and fidelity of translesion replication past Pt-DNA adducts appear to be determined by both the structure of the adduct and the DNA polymerase active site.

cis-Diamminedichloroplatinum(II) (cisplatin)[1] is an effective chemotherapeutic agent which is widely used in the treatment of a variety of human tumors. However, intrinsic and acquired resistance represent major limitations to its usefulness. In addition, cisplatin has been classified as a probable human carcinogen (*1*). One strategy to overcome these limitations has been to design new platinum complexes that might form structurally unique adducts in DNA. The second-generation platinum drug (*trans-R,R*)-1,2-diaminocyclohexaneoxalatoplatinum(II) (oxaliplatin) has recently been approved for the treatment of colorectal cancer in Europe and is in phase III clinical trials in the United States. Cisplatin and oxaliplatin form the same types of adducts at the same sites of the DNA (primarily intrastrand GG and AG cross-links) (*2*, *3*). Thus, the Pt-DNA adducts formed by these complexes differ only in the carrier ligands which they retain upon binding to DNA. Cisplatin forms adducts with *cis*-diammine carrier ligands while oxaliplatin forms adducts with the (*trans-R,R*)-1,2-diaminocyclohexane (dach) carrier ligand. Pt-DNA adduct formation results in severe local distortions of the DNA double helix (*4*), which hinder DNA polymerase progression. This inhibition of DNA replication is believed to be a critical step in platinum drug cytotoxicity. However, Pt-DNA adducts are not an absolute block to DNA replication in vivo. We have shown that cells have the ability to replicate past Pt-DNA adducts. This replicative bypass is increased in drug-resistant cells and consistently discriminates between oxaliplatin and cisplatin adducts (*5*−*7*). These data suggest that some cellular DNA polymerases must be capable of replicating past oxaliplatin and cisplatin adducts with different efficiency.

The enzymes utilized for translesion synthesis past Pt-DNA adducts in vivo have not yet been identified. However, several DNA polymerases with varied structure, function, fidelity, and processivity have been examined in vitro for their ability to carry out translesion synthesis past Pt-DNA adducts. The replicative enzymes pol α, pol δ, and pol ϵ have been shown to be incapable of replicating platinated DNA in vitro (*8*−*10*) even in the presence of PCNA (*8*). On the other hand, viral polymerase HIV-1 RT, yeast DNA polymerase ζ, and human DNA polymerases β and γ are able to traverse through Pt-DNA adducts with an efficiency of 5−15% compared with their ability to replicate undamaged DNA (*8*, *11*, *12*). While HIV-1 RT did not discriminate between cisplatin and oxaliplatin adducts, pol β, pol ζ, and pol γ all bypassed oxaliplatin adducts better than cisplatin adducts. Two of these polymerases, pol β and pol ζ, may be involved in error-prone translesion synthesis in vivo. For example, cell culture studies suggest that pol β can contribute to mutagenic translesion synthesis past Pt-DNA adducts when overexpressed (*13*), and Srivastava et al. (*14*) have shown that pol β is overexpressed in some human tumors. Although no data implicating pol ζ in bypass of Pt-DNA adducts in vivo have been established, genetic evidence suggests that

[†] This research was supported by a research contract from Sanofi-Synthelabo Pharmaceuticals.

* Corresponding author. Phone: (919) 966-3286. Fax: (919) 966-2852. E-mail: Stephen_Chaney@med.unc.edu.

[‡] University of North Carolina.

[§] Osaka University.

[1] Abbreviations: cisplatin, *cis*-diamminedichloroplatinum(II); oxaliplatin, (*trans-R,R*)-1,2-diaminocyclohexaneoxalatoplatinum(II); dach, (*trans-R,R*)-1,2-diaminocyclohexane; HIV-1 RT, human immunodeficiency virus 1 reverse transcriptase; XPV, xeroderma pigmentosum variant.

10.1021/bi000130k CCC: $19.00
Published on Web 03/30/2000

this polymerase is involved in error-prone translesion synthesis past UV-induced DNA adducts (*15*).

Recently, a new family of DNA polymerases that catalyze very efficient translesion synthesis past bulky DNA adducts has been discovered. This family is generally referred to as UmuC/DinB/Rev1/Rad30 on the basis of the genes originally shown to encode for enzymes with polymerase activity (reviewed in refs *16*−*19*). Those translesion polymerases which have been characterized are distributive and lack proofreading activity (*16*−*19*). They are thought to substitute for the stalled replication complex at the site of the lesion. After addition of one or two nucleotides these strictly distributive enzymes would then dissociate from the template, thus allowing reassociation of the highly processive replication complex and completion of genome replication. Since most cells appear to contain more than one member of this family, the specific polymerase involved in replicative bypass is likely to depend on the structure of the lesion containing DNA. The accuracy of translesion synthesis is likely to be determined by both the fidelity of DNA polymerase utilized and the type of DNA damage encountered.

With the discovery of this new family of translesion polymerases, it is now of interest to determine the lesion-replicating specificity of these enzymes and the mechanism by which the cell selects which DNA polymerase(s) to use for replicating damaged DNA. Eukaryotic DNA polymerase η is encoded by the yeast RAD30 and human XPV genes and has been shown to perform robust translesion synthesis past UV-induced *cis*,*syn*-cyclobutane thymine dimers (*20*, *21*). Defects in the gene expressing this protein result in increased UV sensitivity and UV-induced hypermutability in yeast cells (*22*, *23*) and in the autosomal recessive disease xeroderma pigmentosum variant (XPV) in humans (*21*, *24*, *25*). XPV is characterized by a high incidence of UV-induced skin cancers due to a decreased ability to elongate nascent DNA strands on UV-irradiated DNA in an error-free manner. Although genetic evidence suggests that pol η catalyzes a relatively error-free bypass of UV damage in vivo, it may represent an error-prone mechanism of bypass for other lesions (*22*). Pol η is able to bypass cisplatin-DNA adducts, but with lower efficiency than *cis*,*syn*-cyclobutane thymine dimers (data not shown). The present study was designed to evaluate whether pol η has the same specificity for translesion replication past cisplatin and oxaliplatin adducts as other eukaryotic DNA polymerases and to determine the fidelity of this translesion replication.

MATERIALS AND METHODS

Materials. Human recombinant pol η was purified as described previously (*21*). The structures of the platinum complexes used in these studies are shown in Figure 1A. Cisplatin was obtained from Sigma and Pt(dach)Cl_2 was provided by Dr. S. D. Wyrick (UNC). The template sequences used in present study are shown in Figure 1B. The primer-templates were constructed from synthetic oligonucleotides as described previously (*11*). Briefly, 12-mer oligonucleotides containing a single platinum adduct within a *Stu*I restriction site were ligated with a 14-mer and 18-mer using a 35-mer as a scaffold. After ligation, templates were restricted by *Stu*I to ensure the absence of any unplatinated oligonucleotides. *Stu*I cleavage was also used

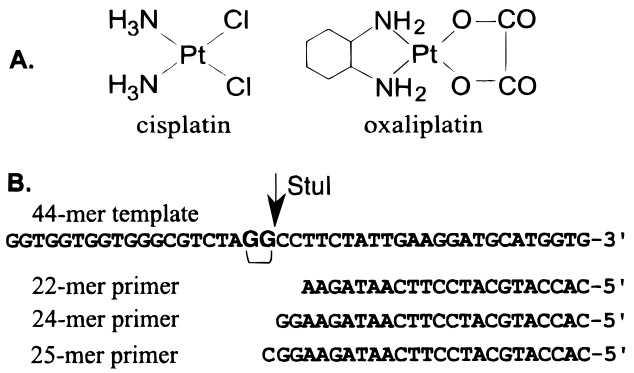

FIGURE 1: Structures and sequences of oligonucleotide substrates with site-specific platinum adducts. (A) Chemical structures of the platinum complexes. (B) The 44-mer templates hybridized to 22-mer primers were used in the primer extension assay. The 44-mer templates annealed with 24- and 25-mer primers were utilized in the kinetic studies and in misincorporation assays in the presence of individual nucleotides. The template GG site for platination is indicated. The site-specifically modified oligonucleotides were constructed and hybridized to the primers as described in Materials and Methods.

following all primer extension assays described below, and only *Stu*I-resistant elongation products were quantitated as translesion synthesis. DNA substrates were prepared by hybridizing ^{32}P-labeled primers to the undamaged or platinated templates.

Primer Extension Assays. Primer extension assays were performed using 22/44 primer-templates (Figure 1) as described previously (*11*). Primer-template (200 fmol, expressed as primer termini) was incubated with pol η at 37 °C in 10 μL reactions containing 500 μM dNTPs. Reaction times and enzyme concentrations are indicated in the figure legends. Reaction conditions for primer extension by pol η were as described previously (*21*). Reactions were terminated by heating at 80 °C for 10 min. Each reaction mixture was split into two aliquots, one of which was incubated with the *Stu*I restriction enzyme. Cleavage by the *Stu*I enzyme revealed that >95% of the product synthesized on platinated templates was resistant to restriction digestion, while product synthesized on undamaged templates was completely restricted. Reaction products were resolved by denaturing polyacrylamide gel electrophoresis and analyzed using a Molecular Dynamics PhosphorImager and ImageQuant software as described previously (*11*).

The extent of translesion synthesis on damaged templates was calculated as follows:

$$\%\ \text{translesion synthesis} = \frac{\text{bypass product following } \textit{Stu}\text{I restriction}}{\text{total primer termini}} \times 100 \quad (1)$$

where bypass product equals chain elongation past the platinum adduct (bands longer than the 26-mer). Total primer termini are defined as the sum of elongated and unelongated primers.

The efficiency of translesion synthesis relative to synthesis on undamaged DNA templates was calculated as follows:

$$\text{relative translesion synthesis (\%)} = \frac{\text{translesion synthesis on damaged DNA}}{\text{synthesis on undamaged DNA}} \times 100 \quad (2)$$

where translesion synthesis on damaged DNA is calculated by eq 1 and synthesis on undamaged DNA equals the sum of chain elongation products longer than the 26-mer, calculated as a percent of total primer termini.

Steady-State Polymerization Kinetics. Steady-state kinetic parameters K_m and V_{max} for dCTP incorporation were measured in standing start reactions as described previously (*26*) using 44-mer templates hybridized to 24- or 25-mer primers (Figure 1). Prior to kinetic studies, experiments were performed to determine the enzyme concentrations, dNTP concentrations, and times for which product accumulation was linear as a function of time. Less than 20% of the primers were extended as required for steady-state single hit conditions. DNA substrates (400 fmol) were replicated at 37 °C in 4 μL reaction mixtures containing 20 fmol of pol η and 1−40 μM dCTP for 2 min. V_{max} and K_m were determined from a Hanes−Woolf plot by linear least-squares fit as described in Creighton et al. (*26*). The efficiency of nucleotide insertion by pol η (f) was calculated as V_{max}/K_m. To facilitate comparison of values for different platinum adducts, the relative insertion efficiency f_{rel} was calculated as $f_{rel} = f_{Pt}/f_{control}$.

Misincorporation Assays. To measure nucleotide misinsertion opposite Pt-GG adducts, 24- and 25-mer primers were hybridized with control and damaged 44-mer templates (Figure 1). These DNA substrates (80 fmol) were incubated with 500 μM dNTP individually for 5 min using 2 ng (25 fM) of pol η in 5 μL of the total reaction mixture. Reactions were terminated, and products were analyzed as described above.

The kinetics of dTTP misincorporation opposite platinated and undamaged GG sites using 24- and 25-mer primers hybridized with control and damaged 44-mer templates (Figure 1) were determined in reactions similar to ones described in the previous section. The dTTP misinsertion efficiency (f_{mis}) was determined as the ratio of dTTP to dCTP insertion efficiencies.

RESULTS AND DISCUSSION

DNA polymerase η is capable of translesion synthesis past a number of bulky DNA adducts, including UV-induced cyclobutane pyrimidine dimers (*20*, *21*). The present study was designed to answer two questions: what is the specificity of translesion synthesis by human pol η for cisplatin versus oxaliplatin adducts and what is the accuracy of this bypass? The 1,2-d(GpG) intrastrand DNA adducts are the most abundant adducts formed in vitro and in vivo by both cisplatin and oxaliplatin. These adducts are also of particular interest since they correlate with the clinical efficacy of the drugs. Therefore, 44-mer oligonucleotides containing site-specifically placed cisplatin- and oxaliplatin-GG adducts (Figure 1) were used to examine the ability of pol η to catalyze translesion synthesis past Pt-DNA lesions in vitro. These templates are identical to the ones used in our previous studies with eukaryotic DNA polymerases β, ζ, and γ (*11*). Therefore, translesion replication by pol η can be considered in comparison to translesion replication by other eukaryotic DNA polymerases, particularly by pol β, which is the most efficient among the eukaryotic polymerases previously tested in its ability to bypass platinum adducts in vitro.

Effect of Pt-GG Adducts on DNA Replication by Pol η. Figure 2 shows the time course and enzyme concentration dependence for elongation of primers on undamaged DNA templates or templates containing a single platinum adduct. The positions of the primer (*22*), full-length reaction product (*44*), and platinum adducts are indicated. Distinct bands corresponding to every nucleotide in the sequence were clearly visible, consistent with the distributive nature of pol η (Figure 2A,C). The distribution of the products of primer elongation on platinated templates by pol η was different from that seen with other DNA polymerases. For eukaryotic pol α, δ, and ϵ, DNA synthesis was blocked immediately prior to the adduct (*8*, *9*). For pol β, pol γ, and pol ζ, DNA synthesis was inhibited at three major sites: the site immediately preceding the platinum adduct and the sites opposite both the 3′- and 5′Gs of the platinum adduct (*11*). The pause site just prior to the adduct is thought to indicate an inhibition of nucleotide incorporation opposite the 3′G of the platinum adduct. Similarly, the pause sites opposite the 3′- and 5′Gs are thought to represent inhibition of nucleotide incorporation opposite the 5′G of the platinum adduct and inhibition of elongation from the 5′G, respectively. Similar pause sites have been seen for all bacterial, bacteriophage, and viral DNA polymerases capable of translesion replication past Pt-GG adducts (*9*, *11*, *12*, *27*, *28*). In contrast, primer extension on damaged templates by pol η produced only one strong pause site opposite the 3′G of both cisplatin- and oxaliplatin-GG adducts. The pause sites just prior to the Pt-GG adduct and opposite the 5′G of the adduct were very weak, which suggests that pol η-catalyzed nucleotide incorporation opposite the template 3′G and primer elongation from the 5′G site are not significantly affected by the Pt-GG adduct.

To characterize nucleotide incorporation opposite platinated sites more quantitatively, kinetic experiments were performed on undamaged templates and templates with cisplatin and oxaliplatin adducts. Steady-state kinetic assays of dCTP incorporation opposite the 3′G and 5′G were performed using standing start reaction conditions (*26*). The kinetic parameters for dCTP insertion opposite the 3′- and 5′G using control and damaged DNA substrates are presented in Table 1. As anticipated from the primer extension studies, neither the V_{max} nor the K_m for dCTP incorporation opposite the 3′G was significantly affected by the presence of a Pt-GG adduct. Thus, the catalytic efficiency (V_{max}/K_m) for dCTP incorporation opposite the 3′G of cisplatin- and oxaliplatin-GG adducts was very similar to that seen at the same position with undamaged DNA. Interpretation of the kinetic parameters for dCTP incorporation opposite the 5′G is more complex. Both the V_{max} and the K_m for dCTP incorporation were significantly decreased by the presence of a Pt-GG adduct. Thus, at low concentrations of dCTP the overall efficiency (V_{max}/K_m) for dCTP incorporation opposite the 5′G would appear to be unaffected by the presence of a Pt-GG adduct. However, the K_m for dCTP incorporation is 27−53-fold less than the nuclear dCTP concentration during the S phase (*29*) and 63−125-fold less than the dCTP concentration used for the primer extension experiments (Figure 2). Therefore, under both physiological conditions and the conditions used for the primer extension assay, dCTP is present at saturating concentrations. Thus, the extent of dCTP incorporation opposite the 5′G of Pt-GG adducts is likely to be determined primarily by the effect of the adducts on the V_{max} of the reaction. This interpretation will need to be

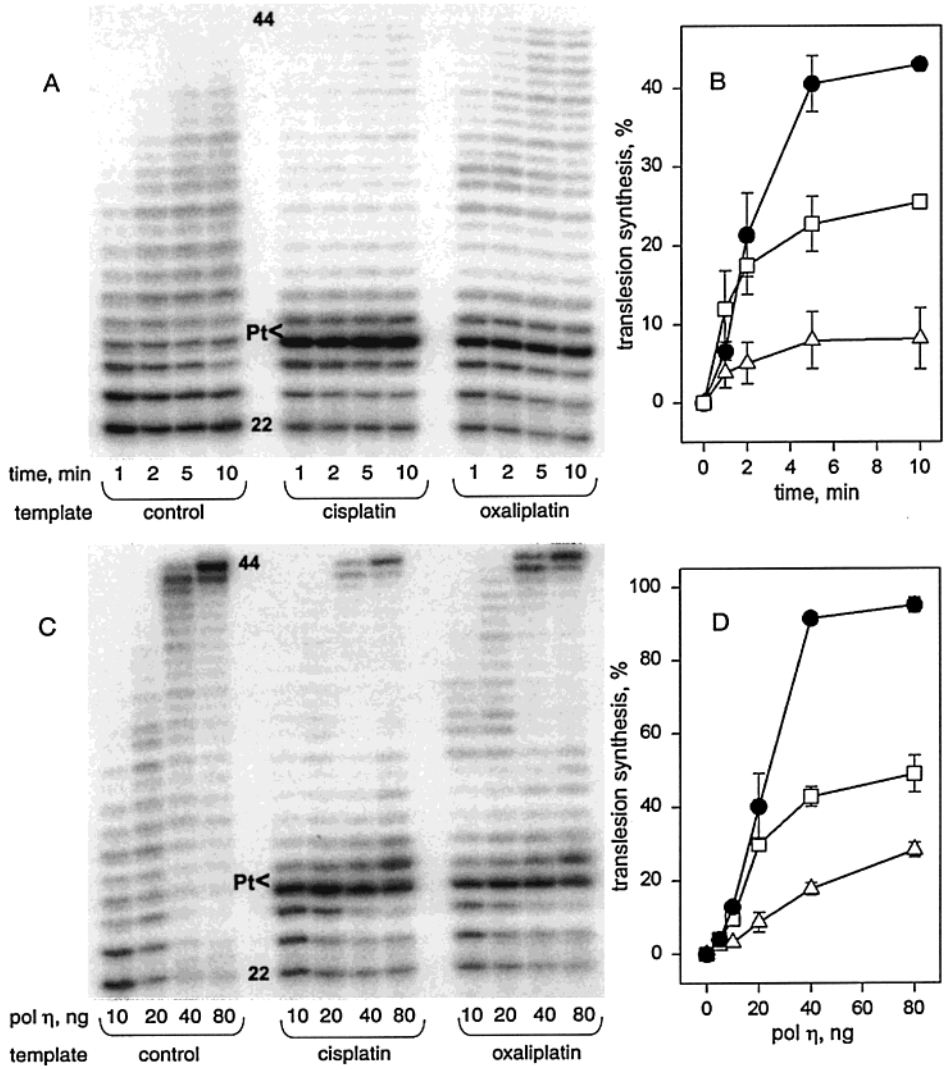

FIGURE 2: Primer extension activity of pol η. Time-course experiments (A, B) were conducted using 20 ng of pol η and 80 fmol of DNA primer-templates. Enzyme concentration dependence experiments (C, D) were carried out using 80 fmol of DNA primer-templates for 10 min. The primer-templates for these experiments consisted of the 22-mer primers hybridized to the 44-mer templates (Figure 1). The extent of translesion synthesis past cisplatin (△) and oxaliplatin (□) adducts (B, D) was calculated as a percent of total primer termini by eq 1 as described in Materials and Methods. Synthesis on control (undamaged) DNA (●) is shown for comparison and equals the sum of chain elongation products longer than the 26-mer, calculated as a percent of total primer termini. Data are the mean (±standard error) from four experiments.

Table 1: Steady-State Kinetic Analysis of dCTP Incorporation Opposite Pt-GG Adducts[a]

target	Pt adduct	V_{max}, min^{-1}	K_m, μM	V_{max}/K_m, mM^{-1} min^{-1}	f_{rel}
3′G	control	0.013 ± 0.001	6.7 ± 0.4	1.9 ± 0.2	
	cisplatin	0.015 ± 0.002	9.4 ± 2.2	1.7 ± 0.3	0.9
	oxaliplatin	0.015 ± 0.001	7.4 ± 1.1	1.9 ± 0.3	1.0
5′G	control	0.023 ± 0.001	13 ± 0.9	2.0 ± 0.2	
	cisplatin	0.0086 ± 0.0006	4.3 ± 0.2	2.0 ± 0.2	1.0
	oxaliplatin	0.016 ± 0.001	7.9 ± 0.2	2.0 ± 0.1	1.0

[a] Standing start kinetic assays were performed using 5 fmol of pol η and 400 fmol of primer-templates. The incubation time was 2 min. dCTP concentrations ranged from 1 to 40 μM. Kinetic parameters (K_m and V_{max}) and insertion efficiency ($f = V_{max}/K_m$) for dCTP incorporation by pol η were determined using Hanes−Woolf plots (see Materials and Methods). The relative insertion efficiency was determined as $f_{rel} = f_{Pt}/f_{control}$. The data are the means (±standard error) from three to five experiments with two different template preparations.

verified with pre-steady-state kinetic analysis. However, the V_{max} for dCTP incorporation opposite the 5′G is less for cisplatin- and oxaliplatin-GG adducts than for undamaged DNA (Table 1), which is consistent with the increased incorporation opposite the 3′G seen in the primer extension studies (Figure 2). Furthermore, the differences in V_{max} for dCTP incorporation opposite the 5′G are consistent with both the extent and specificity (oxaliplatin versus cisplatin) observed in the primer extension reactions (see below).

Extent of Primer Elongation on Platinated Templates. We have previously shown that the extent of translesion synthesis past Pt-GG adducts (calculated by eq 1) was significantly greater for pol β than for pol γ and pol ζ (*11*). However, the extent of translesion synthesis relative to synthesis on an undamaged template (calculated by eq 2) was similar for all three enzymes. The extent of pol η-catalyzed translesion synthesis past the cisplatin-GG and oxaliplatin-GG adducts (calculated by eq 1; see Materials and Methods) is shown in Figure 2B,D. For pol η the maximum extent of translesion synthesis past platinum adducts was similar to that observed for pol β under similar reaction conditions (i.e., identical primer-template and incubation times, similar ratios of

enzyme to template) (*11*). However, translesion synthesis relative to synthesis on an undamaged template (calculated by eq 2) was significantly higher for pol η (15−35% for cisplatin adducts and 50−75% for oxaliplatin adducts) than for pol β, pol γ, and pol ζ [4−9% for cisplatin adducts and 7−15% for oxaliplatin adducts (*11*)]. The extent of translesion synthesis by pol η relative to synthesis on an undamaged template was consistent with the differences in the V_{max} for dCTP incorporation opposite the 5′G on damaged and undamaged DNA (Table 1). The $V_{max(Pt)}/V_{max(control)} \times 100$ for dCTP incorporation opposite the 5′G was 37% for the cisplatin-GG adduct and 70% for the oxaliplatin-GG adduct. Thus, pol η appears to be much more efficient at catalyzing translesion synthesis through Pt-GG adducts than any of the eukaryotic polymerases previously tested.

Effect of Carrier Ligand on the Extent of Translesion Synthesis. At all reaction conditions tested, cisplatin adducts were about 2−3.5-fold more effective than oxaliplatin adducts at blocking pol η-catalyzed DNA synthesis (Figure 2). This specificity for translesion synthesis past cisplatin- and oxaliplatin-DNA adducts is similar to the specificity we have reported recently for other eukaryotic DNA polymerases from three different DNA polymerase families, i.e., pol β, pol ζ, and pol γ (*11*). These data support our hypothesis that the carrier ligand specificity for translesion synthesis past Pt-DNA adducts may be determined in part by distinct structural characteristics of Pt-DNA adducts with different carrier ligands (*11*). In agreement with the primer extension experiments, the $V_{max(oxaliplatin)}/V_{max(cisplatin)}$ for dCTP incorporation opposite the 5′G (Table 1) was 1.9. However, there was no significant difference in either the V_{max} or K_m for nucleotide incorporation across from the 3′G of cisplatin- and oxaliplatin-GG adducts. Therefore, the increase in translesion synthesis past oxaliplatin adducts compared to cisplatin adducts is determined almost entirely by differences in V_{max} for dCTP incorporation opposite the 5′G of the adducts. In contrast, differences in translesion synthesis past cisplatin- and oxaliplatin-GG adducts for pol β were determined primarily by differences in the K_m for dCTP incorporation opposite the 3′G (*30*). Thus, it is clear that the conformation of the adducts is strongly influenced by their interaction with the enzyme active sites.

Fidelity of Pol η on Undamaged and Platinated Templates. Translesion synthesis might be mutagenic or nonmutagenic depending on whether the correct or incorrect nucleotide is incorporated opposite the lesion sites. To obtain qualitative information about misincorporation opposite the Pt-GG adducts, primer extension assays were performed in the presence of 500 μM nucleotide individually. These experiments were done with undamaged and platinated 44-mer templates hybridized to 24- and 25-mer primers (Figure 1) which ended just prior to the template 3′- and 5′G sites, respectively. The correct dCTP was preferentially incorporated opposite both the 3′- and 5′Gs (Figure 3). However, a significant degree of misincorporation was observed with both undamaged and damaged templates. The tendency to misincorporate different nucleotides across from the 3′G was dTTP ≫ dATP ≈ dGTP. The overall tendency for misincorporation opposite the 5′G was less than opposite the 3′G, and the pattern of misincorporation was dTTP ≫ dATP (Figure 3). No detectable misincorporation of dGTP was observed opposite the 5′G. The pattern of misincorporation

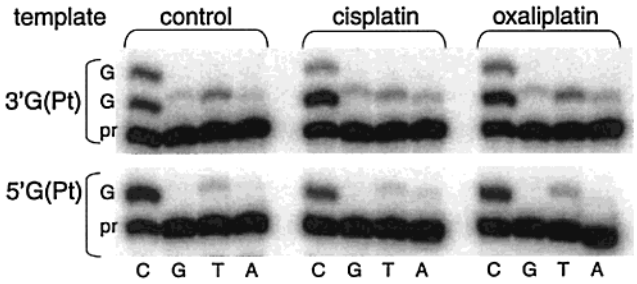

FIGURE 3: Specificity of nucleotide incorporation by pol η across from GG sites on control (undamaged) and platinated DNA templates. Nucleotide incorporation studies were performed for 5 min using 80 fmol of DNA template, 2 ng of pol η, and individual dNTPs present at a 500 μM concentration. (C, incubation with dCTP; G, incubation with dGTP; T, incubation with dTTP; A, incubation with dATP.) The template sequence is indicated on the left (pr = primer). Nucleotide incorporation opposite the 3′G was performed with the 24-mer primers hybridized to the 44-mer templates, and nucleotide incorporation opposite the 5′G was performed using the 25-mer primers hybridized to the 44-mer templates (see Figure 1 for primer and template sequences).

Table 2: Steady-State Kinetic Analysis of dTTP Insertion Opposite Pt-GG Adducts[a]

target	Pt adduct	V_{max}, min^{-1}	K_m, μM	V_{max}/K_m, mM^{-1} min^{-1}	f_{mis}
3′G	control	0.0054 ± 0.0001	36 ± 2	0.15 ± 0.01	0.079
	cisplatin	0.0056 ± 0.0004	36 ± 2	0.16 ± 0.01	0.094
	oxaliplatin	0.0053 ± 0.0002	36 ± 1	0.15 ± 0.01	0.079
5′G	control	0.0043 ± 0.0003	79 ± 2	0.054 ± 0.002	0.027
	cisplatin	0.0042 ± 0.0008	92 ± 1	0.046 ± 0.001	0.023
	oxaliplatin	0.0052 ± 0.0005	95 ± 5	0.055 ± 0.001	0.027

[a] Standing start kinetic assays were performed using 20 fmol of pol η and 400 fmol of primer-templates. The incubation time was 2 min. dTTP concentrations ranged from 20 to 160 μM. Kinetic parameters (K_m and V_{max}) and insertion efficiency ($f = V_{max}/K_m$) for nucleotide insertion by pol η were determined using Hanes−Woolf plots (see Materials and Methods). The misinsertion efficiency (f_{mis}) was determined as the ratio of incorrect (dTTP) to correct (dCTP) insertion efficiencies. The data are means (±standard error) from three different experiments using two independent template preparations.

opposite the template GG sites observed with pol η was different from that seen at the same sequence with other eukaryotic DNA polymerases. For pol β some misincorporation of dTTP was detected opposite the 3′G. However, no other nucleotides were misinserted by pol β opposite the 3′G, and no misincorporation opposite the 5′G site was detected even at 5 mM dNTP concentrations (*30*). For pol ζ, dGTP was the nucleotide most frequently misincorporated opposite the 3′G, while for pol γ it was dATP (data not shown). Misincorporation opposite the 5′G has not been determined for pol ζ and pol γ. These data suggest that Pt-DNA adducts can induce different mutations depending on which polymerase(s) is (are) utilized in translesion synthesis.

To characterize misincorporation by pol η more quantitatively, the kinetic parameters of dTTP incorporation opposite the 3′- and 5′Gs were determined (Table 2). Misincorporation of dGTP and dATP was relatively minor and was not quantitated. On undamaged DNA the efficiency (V_{max}/K_m) of dTTP incorporation was 13−37-fold less than the efficiency of dCTP incorporation, due to both a decrease in V_{max} and an increase in K_m. The presence of a Pt-GG adduct on the DNA template did not significantly affect the V_{max}, K_m, or overall efficiency (V_{max}/K_m) of dTTP incorpora-

tion opposite either the 3′- or 5′G. With undamaged DNA, the overall frequency of dTTP misinsertion (f_{mis}) by pol η was 8×10^{-2} opposite the 3′G and 2.7×10^{-2} opposite the 5′G (Table 2). This is comparable to the misinsertion frequency recently reported for yeast pol η (*31*) and much greater than the frequency of dTTP misinsertion seen with pol β using the same DNA template. With pol β the frequency of dTTP misinsertion opposite the 3′G was 5.4×10^{-4}, and dTTP misincorporation opposite the 5′G was too low to be reliably quantitated (*30*). Thus, in this particular sequence context pol η appears to be approximately 150 times more error prone than pol β on an undamaged DNA template.

In contrast to their lack of effect on the fidelity of pol η, platinum adducts caused a 15−25-fold increase in the dTTP misinsertion frequency opposite the 3′G by pol β (*30*). However, even in the presence of Pt-GG adducts, the frequency of dTTP misincorporation opposite the 3′G was 5−10 times greater for pol η than for pol β. These results suggest that translesion replication in vivo by pol η could be more mutagenic than by pol β. However, it is possible that translesion replication in vivo requires the presence of accessory factors which might change polymerase fidelity. In addition, the present study did not address other types of polymerase errors. For example, with pol β the frequency of frame-shift mutations appears to be greater than the frequency of base substitution mutations (*32*). Therefore, it remains to be determined whether pol β or pol η will be more error prone during translesion replication past Pt-GG adducts in vivo.

Summary. Our data show that pol η catalyzes translesion synthesis past Pt-GG adducts with greater efficiency and lower fidelity than other eukaryotic polymerases that have been characterized to date. These data implicate human pol η in error-prone translesion replication past Pt-DNA adducts. Experiments to compare the cytotoxicity and mutagenicity of platinum complexes in pol η proficient and deficient (XPV) cell lines are currently in progress. Pol η is similar to DNA polymerases β, ζ, and γ in that it bypasses oxaliplatin-GG adducts more readily than cisplatin-GG adducts. This suggests that the conformation of cisplatin- and oxaliplatin-GG adducts is different and that these conformational differences have a similar influence on the ability of distantly related DNA polymerases to perform translesion synthesis. However, pol η is very different from other eukaryotic DNA polymerases tested in terms of the sites of replication termination (3′G vs 5′G), the fidelity of translesion synthesis, and the specificity of misincorporation (i.e., which dNTPs are misincorporated). These data suggest that the conformation of templates containing cisplatin- and oxaliplatin-GG adducts is significantly different in the active site of pol η than in the active sites of the DNA polymerases that have been studied previously.

ACKNOWLEDGMENT

We thank Dr. S. D. Wyrick (UNC) for providing us with the Pt(dach)Cl_2. We are indebted to Dr. J. T. Reardon, Dr. M. Cordeiro-Stone, and Dr. P. E. Juniewicz for critical reading of the manuscript. We also thank Dr. K. Bebenek and Dr. T. A. Kunkel for their help in optimizing the conditions for the steady-state kinetic assay with pol η.

REFERENCES

1. Greene, M. H. (1992) *J. Natl. Cancer Inst. 84*, 306−312.
2. Eastman, A. (1987) *Pharmacol. Ther. 34*, 155−166.
3. Woynarowski, J. M., Chapman, W. G., Napier, C., Herzig, M. C. S., and Juniewicz, P. (1998) *Mol. Pharmacol. 54*, 770−777.
4. Gelasco, A., and Lippard, S. J. (1998) *Biochemistry 37*, 9230−9239.
5. Gibbons, G. R., Kaufmann, W. K., and Chaney, S. G. (1991) *Carcinogenesis 12*, 2253−2257.
6. 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.
7. 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.
8. 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.
9. Villani, G., Hubscher, U., and Butour, J.-L. (1988) *Nucleic Acids Res. 16*, 4407−4417.
10. Huang, L., Turchi, J. J., Wahl, A. F., and Bambara, R. A. (1993) *Biochemistry 32*, 841−848.
11. 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.
12. Hoffmann, J. S., Pillaire, M. J., Garcia-Estefania, D., Lapalu, S., and Villani, G. (1996) *J. Biol. Chem. 271*, 15386-15392.
13. 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.
14. Srivastava, D. K., Husain, I., Arteaga, C. L., and Wilson, S. H. (1999) *Carcinogenesis 20*, 1049−1054.
15. Gibbs, P. E. M., Mcgregor, W. G., Maher, V. M., Nisson, P., and Lawrence, C. W. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 6876−6880.
16. Bridges, B. A. (1999) *Curr. Biol. 9*, R475−R477.
17. Woodgate, R. (1999) *Genes Dev. 13*, 2191−2195.
18. Friedberg, E. C., and Gerlach, V. L. (1999) *Cell 98*, 413−416.
19. Johnson, R. E., Washington, M. T., Prakash, S., and Prakash, L. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 12224−12226.
20. Johnson, R. E., Prakash, S., and Prakash, L. (1999) *Science 283*, 1001−1004.
21. 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.
22. Roush, A. A., Suarez, M., Friedberg, E. C., Radman, M., and Siede, W. (1998) *Mol. Gen. Genet. 257*, 686−692.
23. Johnson, R. E., Prakash, S., and Prakash, L. (1999) *J. Biol. Chem. 274*, 15975−15977.
24. Johnson, R. E., Kondratick, C. M., Prakash, S., and Prakash, L. (1999) *Science 285*, 263−265.
25. Masutani, C., Araki, M., Yamada, A., Kusumoto, R., Nogimori, T., Maekawa, T., Iwai, S., and Hanaoka, F. (1999) *EMBO J. 18*, 3491−3501.
26. Creighton, S., Bloom, L. B., and Goodman, M. F. (1995) *Methods Enzymol. 262*, 232−256.
27. Comess, K. M., Burstyn, J. N., Essigmann, J. M., and Lippard, S. J. (1992) *Biochemistry 31*, 3975−3990.
28. Suo, Z., Lippard, S. J., and Johnson, K. A. (1999) *Biochemistry 38*, 715−726.
29. Leeds, J. M., Slabaugh, M. B., and Mathews, C. K. (1985) *Mol. Cell. Biol. 5*, 3443−3450.
30. Vaisman, A., and Chaney, S. G. (2000) *J. Biol. Chem.* (in press).
31. Washington, M. T., Johnson, R. E., Prakash, S., and Prakash, L. (1999) *J. Biol. Chem. 274*, 36835−36838.
32. Osheroff, W. P., Jung, H. K., Beard, W. A., Wilson, S. H., and Kunkel, T. A. (1999) *J. Biol. Chem. 274*, 3642−3650.

BI000130K