

Efficient and Error-Free Replication Past a Minor-Groove DNA Adduct by the Sequential Action of Human DNA Polymerases ι and κ

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DNA polymerase ι (Pol ι) is a member of the Y family of DNA polymerases, which promote replication through DNA lesions. The role of Pol ι in lesion bypass, however, has remained unclear. Pol ι is highly unusual in that it incorporates nucleotides opposite different template bases with very different efficiencies and fidelities. Since interactions of DNA polymerases with the DNA minor groove provide for the nearly equivalent efficiencies and fidelities of nucleotide incorporation opposite each of the four template bases, we considered the possibility that Pol ι differs from other DNA polymerases in not being as sensitive to distortions of the minor groove at the site of the incipient base pair and that this enables it to incorporate nucleotides opposite highly distorting minor-groove DNA adducts. To check the validity of this idea, we examined whether Pol ι could incorporate nucleotides opposite the γ -HOPdG adduct, which is formed from an initial reaction of acrolein with the N² of guanine. We show here that Pol ι incorporates a C opposite this adduct with nearly the same efficiency as it does opposite a nonadducted template G residue. The subsequent extension step, however, is performed by Pol κ , which efficiently extends from the C incorporated opposite the adduct. Based upon these observations, we suggest that an important biological role of Pol ι and Pol κ is to act sequentially to carry out the efficient and accurate bypass of highly distorting minor-groove DNA adducts of the purine bases.

Replicative DNA polymerases synthesize DNA with high fidelity, and because of their intolerance of geometric distortions in DNA, they are blocked by DNA lesions. The DNA polymerases belonging to the Y family, however, are low-fidelity enzymes, able to promote replication through DNA lesions. DNA polymerase η (Pol η) from both the yeast *Saccharomyces cerevisiae* and humans can efficiently and accurately replicate through a *cis-syn* thymine-thymine dimer (16, 19, 43, 45), and genetic studies in yeast and humans have also indicated a role for Pol η in the error-free replication through cyclobutane dimers formed at 5'-TC-3' and CC sites (39, 49). Consequently, mutational inactivation of Pol η in humans causes the variant form of xeroderma pigmentosum (15, 28), characterized by a greatly enhanced predisposition for sunlight-induced skin cancers. Pol η can also efficiently replicate through other DNA lesions, such as 8-oxoguanine and O⁶-methylguanine (11, 14). Pol η , however, is inhibited by the N²-guanine adducts of 1,3-butadiene or benzo[*a*]pyrene diol epoxide (30, 37).

In addition to Pol η , humans contain two other Y-family DNA polymerases, Pol ι and Pol κ . By contrast to Pol η , which promotes lesion bypass both by efficiently inserting the nucle-

otide opposite the lesion and by extending from the inserted nucleotide, Pol ι and Pol κ are apparently more specialized in their roles in lesion bypass (36). For example, while Pol ι can incorporate nucleotides opposite the 3' T of a (6-4) TT photoproduct or opposite an abasic site, it is unable to carry out the subsequent extension reaction (18). A role for Pol κ in the extension step has been suggested from its ability to proficiently extend from nucleotides opposite the 3' T of a TT dimer or from nucleotides incorporated opposite an O⁶-methylguanine; Pol κ , however, is very inefficient at incorporating nucleotides opposite both these DNA lesions (10, 42).

The evidence for the involvement of Pol ι and Pol κ in lesion bypass, however, remains rather limited, and it is unlikely that they make a major contribution to the replicative bypass of the above-mentioned DNA lesions, where they have been implicated to have a role at the nucleotide incorporation or the extension step of lesion bypass. For example, opposite an abasic site, we expect the replicative polymerase, Pol δ , to be much more effective at the nucleotide incorporation step than Pol ι (12), and at cyclobutane pyrimidine dimers, Pol η and not Pol κ would be the major contributor to their bypass.

By contrast to most DNA polymerases, including Pol η and Pol κ , which exhibit nearly similar efficiencies and fidelities of nucleotide incorporation opposite each of the four template bases (17, 19, 44), Pol ι is unusual in that the efficiency and fidelity of nucleotide incorporation by this polymerase is dependent on the identity of the template base. Pol ι exhibits a higher efficiency of correct nucleotide incorporation opposite purine template bases than opposite pyrimidine templates (9,

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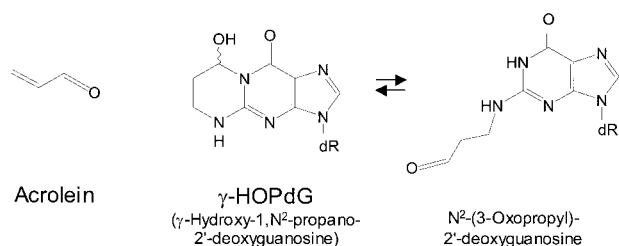


FIG. 1. Structure of acrolein and its N² deoxyguanosine adducts.

18, 40). Opposite template A, Pol κ incorporates nucleotides with a high efficiency and fidelity, misincorporating nucleotides with frequencies of 10^{-4} to 10^{-5} . Opposite template T, however, Pol κ incorporates nucleotides with a very low efficiency and fidelity, preferring to misincorporate a G opposite T ~ 10 times more efficiently than it incorporates the correct nucleotide, A.

Extensive interactions with the DNA minor groove provide classical DNA polymerases with the ability to incorporate the correct nucleotide opposite different template bases with nearly similar efficiencies, and therefore, any distortion of the DNA minor groove is inhibitory to synthesis by these polymerases (see reference 47 for a discussion and references). Because of the preference of Pol κ for incorporating nucleotides opposite template purines, we reasoned that Pol κ might not be as sensitive to DNA minor-groove distortions at the site of the incipient base pair and that this could allow Pol κ to incorporate nucleotides opposite minor-groove DNA adducts. Since the minor-groove N² group of guanine is highly reactive, able to conjugate with a variety of endogenously formed adducts, we sought an N²dG binding adduct that is a frequently formed product of inborn metabolism. Acrolein, an α,β -unsaturated aldehyde, is generated in vivo as the end product of lipid peroxidation and during metabolic oxidation of polyamines, and it is a ubiquitous environmental pollutant formed by the incomplete combustion of organic materials (3, 4, 26, 35). The reaction of acrolein with the N² of dG followed by ring closure at N-1 leads to the formation of the cyclic adduct γ -hydroxy-1,N²-propano-2'-deoxyguanosine (γ -HOPdG) (Fig. 1), and this adduct has been shown to be present in the DNAs of human and rodent tissues at comparatively high levels (2, 4, 32, 33). In the nucleoside and in single-stranded DNA, γ -HOPdG exists primarily in its ring-closed form (25, 34). However, nuclear magnetic resonance studies have shown that in duplex DNA, the exocyclic ring opens to form N²-(3-oxopropyl)-2'-deoxyguanosine when γ -HOPdG is paired with a C (Fig. 1). For this isomer, the adducted G participates in a normal Watson-Crick base pairing with C, and the N²-propyl chain stays in the minor groove pointing toward the solvent (5, 22, 24).

γ -HOPdG presents a strong block to synthesis by DNA polymerases (20). Although both yeast and human Pol η can weakly replicate through the γ -HOPdG adduct, DNA synthesis is inhibited right before the lesion and also opposite from it, and steady-state kinetic analyses have indicated that the efficiency of C incorporation opposite γ -HOPdG with yeast and human Pol η is ~ 200 and 100-fold lower, respectively, than C incorporation opposite an undamaged G. The inhibition at the

extension step was less severe, with incorporation being reduced ~ 10 - to 20-fold (29).

Here we have examined the ability of Pol κ and Pol κ to replicate through the γ -HOPdG adduct. Using steady-state kinetics, we showed that Pol κ is highly efficient at incorporating a C opposite this lesion, and in fact, the efficiency of C incorporation opposite the adduct is nearly the same as that opposite the nondamaged G residue. Furthermore, we found that Pol κ efficiently extends from a C paired with the γ -HOPdG adduct. From these observations, we conclude that Pol κ and Pol κ act sequentially to efficiently and accurately bypass the γ -HOPdG adduct, and we propose that an important biological function of Pol κ is to incorporate nucleotides opposite minor-groove DNA adducts of purines and that of Pol κ is to carry out the subsequent extension reaction.

MATERIALS AND METHODS

Substrates. Nonadducted oligodeoxynucleotides were obtained from Midland Certified Reagent Co. (Midland, Tex.). Oligodeoxynucleotides containing a site-specific γ -HOPdG were synthesized as previously described (34) and have been extensively characterized (22–24). The -1 primer was a 21-mer oligodeoxynucleotide with the sequence 5'-AGCCC AAGCT TGGCG CGGAC T, and 0 primer-T and 0 primer-C were 21-mer oligodeoxynucleotides with the sequence 5'-GCCC AAGCT TGGCG CGGAC TN, where N is T or C, respectively. The template strands were 38-mer oligodeoxynucleotides with the sequence 5'-GCT AGCGA GTCCG CGCCA AGCTT GGGCT GCAGC AGGTC, where the underlined G is either a nondamaged G or a γ -HOPdG. Construction of template oligodeoxynucleotides was done according to the previously published procedure (20). The primer strands were ³²P 5'-end labeled with polynucleotide kinase (Roche Diagnostic Corporation) and [γ -³²P]ATP (Amersham Life Sciences). The ³²P-, 5'-end-labeled primer strands (100 nM) were annealed to the template strands (100 nM) in 50 mM Tris-HCl (pH 7.5) and 100 mM NaCl by heating to 90°C for 2 min and slowly cooling to room temperature over several hours. Solutions of dGTP, dATP, dTTP, and dCTP (0.1 M each) were purchased as the sodium salt, pH 8.3, from Roche and were stored at -80°C until use.

Purification of human Pol κ and Pol κ . GST-Pol κ and GST-Pol κ were expressed in *S. cerevisiae* strain BJ5464 carrying either plasmid pPOL114 or pPOL42, respectively, as described previously (9, 13, 17, 18). These glutathione S-transferase (GST) fusion proteins were purified as described before for Pol η (41, 46). Briefly, the GST fusion proteins were bound to a glutathione 4B Sepharose matrix (Amersham Pharmacia), washed, and removed from the matrix by treatment with PreScission protease (Amersham Pharmacia), which removes the GST protein from the enzyme, leaving full-length Pol κ or Pol κ fused to a seven-amino-acid N-terminal peptide.

DNA polymerase assays. DNA polymerase activity was measured in the presence of 25 mM Tris-HCl (pH 7.5), 10 mM NaCl, 5 mM MgCl₂, 5 mM dithiothreitol (DTT), and 0.1 mg of bovine serum albumin per ml. Either Pol κ , Pol κ , or both Pol κ and Pol κ (1 nM each) were incubated at 22°C with the DNA substrate (5 or 10 nM) and a 20 μM concentration of either dGTP, dATP, dTTP, dCTP, or all four deoxynucleoside triphosphates (dNTPs) for 30 min. Reactions were quenched by the addition of 10 volumes of formamide loading buffer, and the products were run on a 15% polyacrylamide sequencing gel containing 8 M urea.

Steady-state kinetics. The efficiency of nucleotide incorporation (k_{cat}/K_m) was measured under the conditions described above, except that the concentration of the dNTP was varied from 0 to 500 μM and the incubation time ranged from 2 to 10 min. The components of the quenched reactions were analyzed by separating the unreacted substrates and the products by 15% polyacrylamide gel electrophoresis and determining the gel band intensities with a PhosphorImager (Molecular Dynamics). The observed rate of nucleotide incorporation was graphed as a function of dNTP concentration, and the k_{cat} and K_m steady-state parameters were obtained from the best fit of the data to the Michaelis-Menten equation: $v_{\text{obs}} = k_{\text{cat}}[\text{E}][\text{dNTP}]/(K_m + [\text{dNTP}])$, where [E] is enzyme concentration.

RESULTS

Replication through γ -HOPdG by the sequential action of Pol κ and Pol κ . To examine the ability of Pol κ and Pol κ to

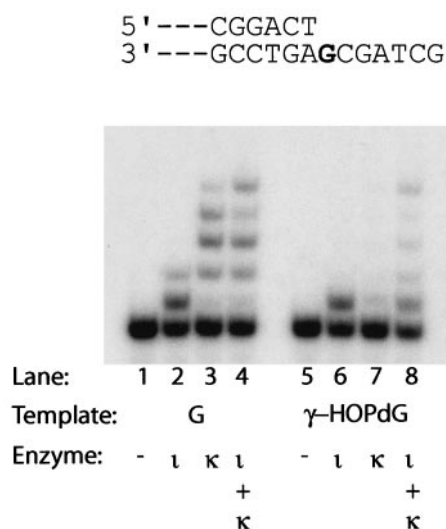


FIG. 2. Replicative bypass of the γ -HOPdG adduct by Pol ι and Pol κ . DNA substrate (5 nM) with either a template G or γ -HOPdG was incubated with all four dNTPs (20 μ M each) and 1 nM Pol ι (lanes 2 and 6), 1 nM Pol κ (lanes 3 and 7), or both Pol ι and Pol κ (lanes 4 and 8) for 30 min at 22°C. A bold G indicates the position of the undamaged G or γ -HOPdG adduct in the template.

bypass γ -HOPdG, we incubated 1 nM Pol ι and 1 nM Pol κ either individually or together with a 5 nM standing-start DNA substrate in which the 3' terminus of the primer is located one nucleotide before the γ -HOPdG adduct (-1 primer), and 20 μ M concentrations of each of the four dNTPs. Pol ι incorporates a nucleotide opposite the γ -HOPdG adduct nearly as well as opposite the nonadducted G (Fig. 2, compare lanes 2 and 6). By contrast, Pol κ is very poor at incorporating a nucleotide opposite the γ -HOPdG adduct, and consequently, it is unable to replicate through this DNA adduct (Fig. 2, compare lanes 3 and 7). Replication through the γ -HOPdG adduct, however, occurred when both Pol ι and Pol κ were combined (Fig. 2, compare lanes 4 and 8). These observations suggested that replication through the γ -HOPdG adduct can be achieved by the sequential action of Pol ι and Pol κ , where Pol ι incorporates a nucleotide opposite the adduct and Pol κ extends from this nucleotide.

Nucleotide incorporated by Pol ι opposite γ -HOPdG. To identify the nucleotide that is incorporated by Pol ι opposite γ -HOPdG, we performed single-nucleotide-incorporation experiments using the standing-start DNA substrate shown in Fig. 2. Pol ι (1 nM) was incubated with 10 nM of either the G or the γ -HOPdG DNA substrate in the presence of 20 μ M of one of the four dNTPs. As shown in Fig. 3A, opposite both the nonadducted G and the γ -HOPdG adduct, Pol ι incorporates the T and C nucleotides. To verify the inability of Pol κ to incorporate a nucleotide opposite γ -HOPdG, we examined whether Pol κ could incorporate any of the four nucleotides under conditions similar to those used for Pol ι . However, by contrast to the incorporation of a C opposite the nonadducted G template, we observed no significant incorporation of any of the nucleotides opposite the γ -HOPdG adduct by Pol κ (Fig. 3B).

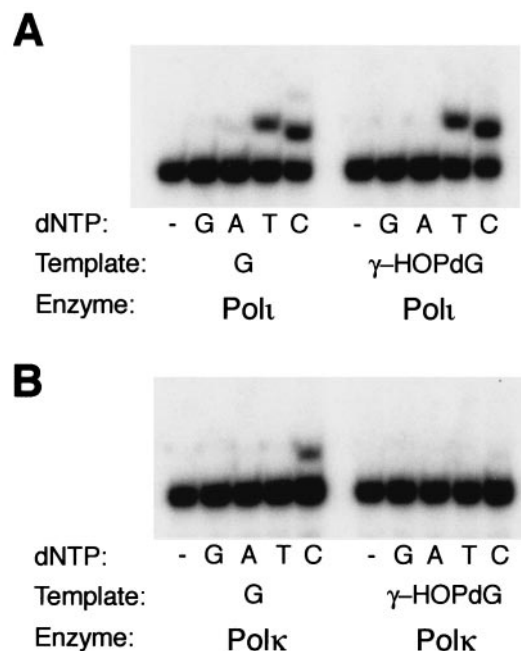


FIG. 3. Nucleotide incorporation opposite the γ -HOPdG adduct by Pol ι and Pol κ . DNA substrate (10 nM) with either a template G or γ -HOPdG was incubated with either 1 nM Pol ι (A) or Pol κ (B) and the indicated dNTP substrate (20 μ M) for 30 min at 22°C.

Steady-state kinetics of nucleotide incorporation opposite γ -HOPdG. Next, we quantified the efficiency of nucleotide incorporation opposite the γ -HOPdG adduct by Pol ι using steady-state kinetics. Pol ι (1 nM) was incubated with a 10 nM concentration of the DNA substrate containing undamaged G or γ -HOPdG in the template and various concentrations of one of the four dNTPs. The rate of nucleotide incorporation was graphed as a function of nucleotide concentration, and the k_{cat} and K_m parameters were obtained from the best fit of these data to the Michaelis-Menten equation (Fig. 4; Table 1). We detected no incorporation of dGTP or dATP opposite either template G or γ -HOPdG. Although Pol ι incorporates both dCTP and dTTP opposite template G, the incorporation of dCTP was about fivefold more efficient than that of dTTP (Fig. 4A and B; Table 1). Interestingly, the efficiency (k_{cat}/K_m) of dCTP incorporation opposite the γ -HOPdG adduct was only twofold lower than the efficiency of dCTP incorporation opposite the nonadducted G (Fig. 4A and C; Table 1). dTTP was also incorporated opposite γ -HOPdG, and the efficiencies of dTTP incorporation were the same opposite both the nonadducted and adducted template residues (Fig. 4B and D; Table 1).

Steady-state kinetics of extension of the primer terminus opposite from γ -HOPdG by Pol κ . Using steady-state kinetics, we examined the efficiency of extension by Pol κ from a C or a T nucleotide placed opposite the nonadducted G or the γ -HOPdG adduct. Pol κ (1 nM) was incubated with a 10 nM concentration of a DNA substrate containing a primer terminal T·G, C·G, T· γ -HOPdG, or C· γ -HOPdG base pair with various concentrations of dGTP, the nucleotide complementary to the first available template residue (Table 2). Interestingly, the efficiency of extension from the C· γ -HOPdG primer

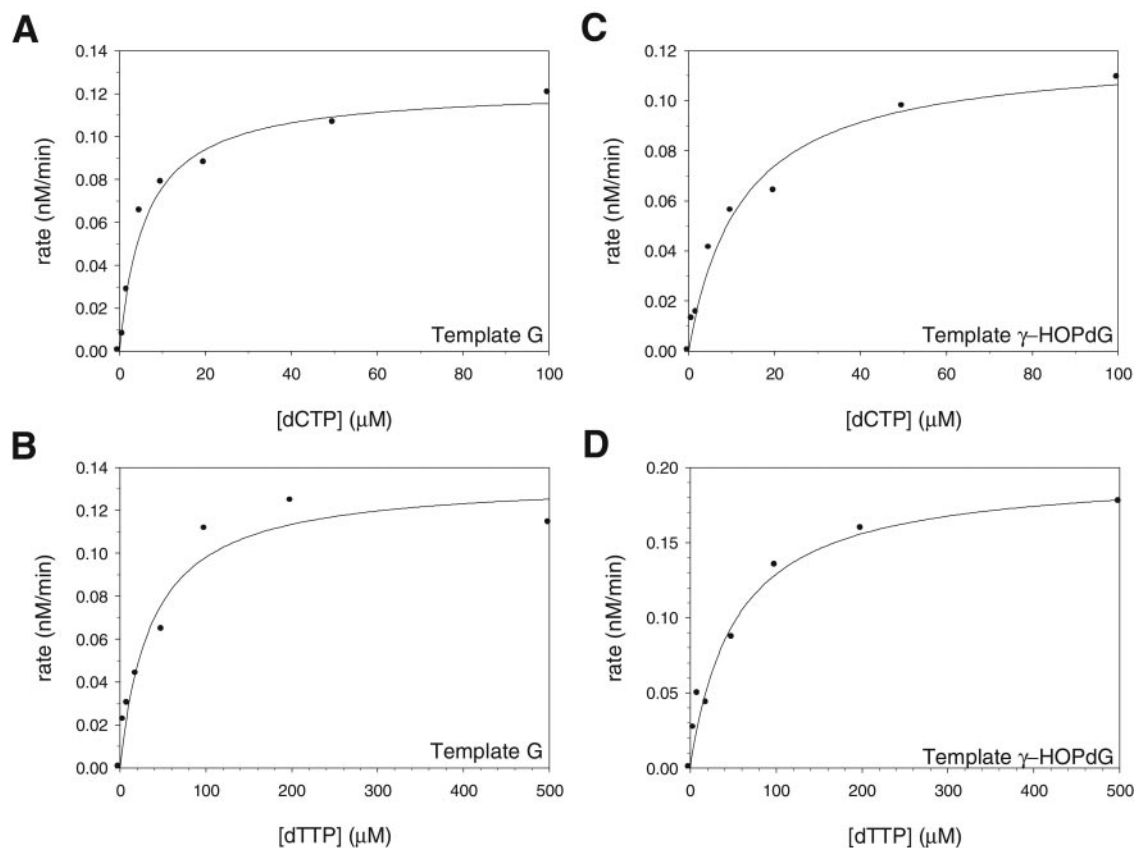


FIG. 4. Steady-state kinetics of nucleotide incorporation opposite the γ -HOPdG adduct by Pol κ . Steady-state rates of C incorporation opposite a template G (A), T incorporation opposite a template G (B), C incorporation opposite a template γ -HOPdG (C), and T incorporation opposite a template γ -HOPdG (D) were graphed as a function of dNTP concentration. The k_{cat} and K_m parameters were obtained from the best fit of the data to the Michaelis-Menten equation and are listed in Table 1.

terminal pair was about threefold higher than the efficiency of extending from the nonadducted C · G base-pair (Fig. 5; Table 2). By contrast to the efficient extension from a C opposite γ -HOPdG, we observed no significant extension from a T opposite γ -HOPdG (Table 2).

DISCUSSION

We show here that Pol κ efficiently incorporates nucleotides opposite γ -HOPdG, a predominant adduct formed from the

reaction of acrolein with the N² of guanine in DNA. DNA synthesis by Pol κ is highly error prone and opposite undamaged G, Pol κ incorporates a T with an efficiency that is only about fivefold lower than that of C incorporation. Remarkably, opposite the γ -HOPdG adduct, Pol κ incorporates the C and T nucleotides with nearly the same efficiency and fidelity as opposite the undamaged G template. Pol κ , however, is unable to carry out the subsequent extension reaction. Pol κ , on the other hand, does not incorporate nucleotides opposite γ -HOPdG but can perform the extension reaction. Interestingly, Pol κ

TABLE 1. Steady-state kinetic parameters for nucleotide incorporation by Pol κ opposite undamaged template G and γ -HOPdG

Template	dNTP	k_{cat} (min ⁻¹)	K_m (μ M)	k_{cat}/K_m^a	f_{inc}^b	Relative efficiency ^c
G	dGTP	ND ^d	ND	$<2 \times 10^{-4}$		
	dATP	ND	ND	$<2 \times 10^{-4}$		
	dTTP	0.13 ± 0.01	37 ± 10	3.5×10^{-3}	0.18	
	dCTP	0.12 ± 0.006	6.1 ± 1.1	2×10^{-2}	1.0	
γ -HOPdG	dGTP	ND	ND	$<2 \times 10^{-4}$		
	dATP	ND	ND	$<2 \times 10^{-4}$		
	dTTP	0.20 ± 0.01	53 ± 10	3.8×10^{-3}	0.38	0.2
	dCTP	0.12 ± 0.006	12 ± 2	1×10^{-2}	1.0	0.5

^a When no incorporation was detected, k_{cat}/K_m was below the detection limit for the assay, which is $2 \times 10^{-4} \mu\text{M}^{-1} \text{min}^{-1}$.

^b $f_{inc} = (k_{cat}/K_m)_{incorrect} / (k_{cat}/K_m)_{correct}$.

^c Efficiency of nucleotide incorporation opposite γ -HOPdG relative to the efficiency of C incorporation opposite undamaged G.

^d ND, no incorporation detected.

TABLE 2. Steady-state kinetic parameters of extension from primer termini paired with a G or γ -HOPdG by Pol κ

Base pair at primer terminus ^a	k_{cat} (min ⁻¹)	K_m (μ M)	k_{cat}/K_m ^b	Relative efficiency ^c
T · G	ND ^d	ND	$<2 \times 10^{-3}$	
C · G	0.051 ± 0.0024	0.60 ± 0.10	0.084	
T · γ -HOPdG	ND	ND	$<2 \times 10^{-3}$	
C · γ -HOPdG	0.037 ± 0.0024	0.13 ± 0.042	0.28	3.33

^a Primer terminal base pairs are given in the form primer · template; incorporation was measured by using the next correct nucleotide, dGTP.

^b When no incorporation was detected, k_{cat}/K_m was below the detection limit for the assay, which is $2 \times 10^{-3} \mu\text{M}^{-1} \text{min}^{-1}$.

^c Efficiency of extension from a C · γ -HOPdG primer terminal base pair relative to the efficiency of extension from a C · G primer terminal base pair.

^d ND, no incorporation detected.

extends from the C opposite γ -HOPdG about threefold more efficiently than the extension from C opposite undamaged G. Pol κ , however, is highly inefficient at extending from a T inserted opposite γ -HOPdG by Pol ι . These observations suggest that efficient and error-free bypass of the γ -HOPdG adduct could occur by the sequential action of Pol ι and Pol κ , in which following a C incorporation by Pol ι , Pol κ performs the subsequent extension step. Even though Pol ι misincorporates a T opposite this adduct fairly frequently, since Pol κ does not catalyze the extension from this nucleotide, the resultant mispaired primer terminus would be accessible to removal by proofreading exonucleases.

In experiments in which γ -HOPdG was site-specifically incorporated into a simian virus 40 origin-based double-stranded vector, in both HeLa cells and XP-V cells, this adduct was found to be only marginally miscoding ($\leq 1\%$ base substitutions) (48). With a single-stranded shuttle vector, the incidence of base substitutions was only slightly higher in XP-V cells than in normal human cells (29). These observations indicated that synthesis across γ -HOPdG is quite accurate in human cells and that Pol η plays a minor, if any, role in γ -HOPdG bypass. This conclusion is in accord with the biochemical studies indicating that γ -HOPdG is a block to human Pol η at both the nucleotide incorporation and extension steps and that it is more apt to misincorporate nucleotides opposite γ -HOPdG than opposite an undamaged G (29). Based upon the findings presented

here, we suggest that error-free replication through the γ -HOPdG adduct in human cells could be achieved by the sequential action of Pol ι and Pol κ .

An important feature shared by DNA polymerases is that they interact with their DNA substrates principally through the DNA minor groove. Hydrogen bonding interactions between specific Arg, His, Asn, Gln, and Lys hydrogen bonding donors in the protein and the N3 hydrogen bonding acceptor of purine bases and the O² hydrogen bonding acceptor of pyrimidine bases in the DNA minor groove are observed in X-ray crystal structures of various DNA polymerases—bacteriophage T7 DNA polymerase (6), *Bacillus stearothermophilus* DNA polymerase I (21), *Thermus aquaticus* DNA polymerase I (27), and bacteriophage RB69 DNA polymerase (8). In addition, the importance of these polymerase-DNA minor-groove interactions has been shown in studies using DNA base analogs lacking the N3 or O² minor-groove hydrogen-bonding acceptors (31, 38). Presumably the disruption of these functionally important interactions by steric clashes with template minor-groove DNA lesions is responsible for the inability of classical DNA polymerases to incorporate nucleotides opposite such lesions. The proficient ability of Pol ι to incorporate nucleotides opposite γ -HOPdG suggests that this polymerase is refractory to distortions conferred upon the DNA minor groove by this adduct and that this may arise because Pol ι does not functionally interact with the DNA minor groove of the incip-

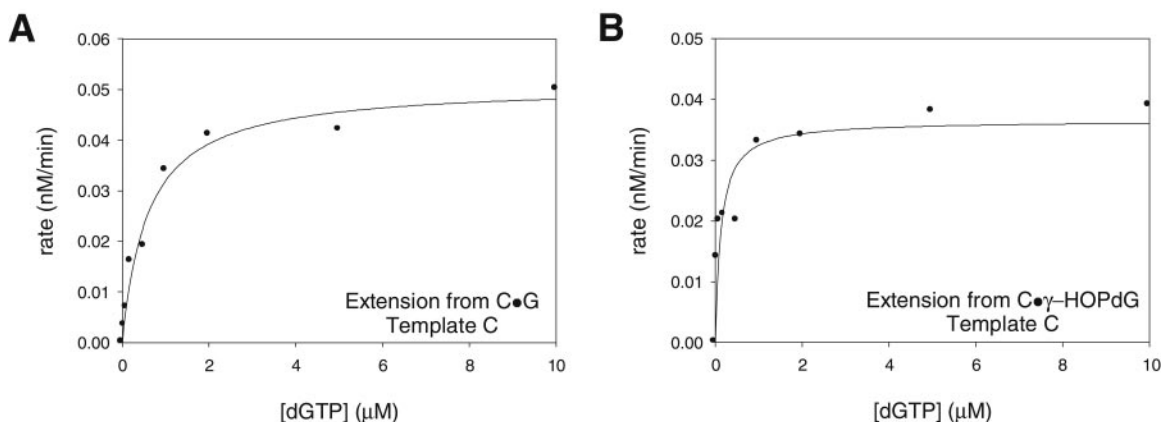


FIG. 5. Steady-state kinetics of extension from the nucleotide opposite the γ -HOPdG adduct by Pol κ . Steady-state rates of dGTP incorporation opposite a template C following a primer terminal C · G base pair (A) or a primer terminal C · γ -HOPdG base pair (B) were graphed as a function of dGTP concentration. The k_{cat} and K_m parameters were obtained from the best fit to the Michaelis-Menten equation and are listed in Table 2. In the C · G and C · γ -HOPdG primer terminal base pairs, a C is placed opposite the undamaged G or the γ -HOPdG adduct in the template, respectively.

ient base pair. The inability of Pol ι to extend from the nucleotide incorporated from opposite γ -HOPdG suggests, however, that this polymerase is sensitive to distortions conferred by this adduct at the primer terminus. Pol κ , on the other hand, is inhibited at incorporating a nucleotide opposite the γ -HOPdG adduct but is proficient at extending the C \cdot γ -HOPdG primer terminus. Pol ι and Pol κ thus differ remarkably in their response to this minor-groove DNA adduct.

Because of the high reactivity of the N² group of guanine, a variety of DNA adducts would form at this minor-groove position, which include the propano adducts and malondialdehyde-derived adducts. The propano adducts are formed from α,β -unsaturated aldehydes or enals, such as acrolein, crotonaldehyde, and *trans*-4-hydroxy-2-nonenal (3, 4). Lipid peroxidation, which becomes quite significant when cells are under oxidative stress, exposed to xenobiotics, or subjected to bacterial and viral infections, produces enals of various chain lengths ranging from acrolein to *trans*-4-hydroxy-2-nonenal as secondary products, as well as malondialdehyde (1, 7). These adducts are present in the DNAs of human and rodent tissues at relatively high levels (2, 4, 32, 33). Our finding that Pol ι is not inhibited when γ -HOPdG is the templating residue in the incipient base pair and that Pol κ is not inhibited when γ -HOPdG is present in the template strand at the primer terminus leads us to propose that one major role of Pol ι and Pol κ is to act sequentially at the nucleotide incorporation and extension steps, respectively, in the bypass of a wide range of minor-groove adducts of guanine.

DNA polymerases incorporate nucleotides opposite each of the four template bases with nearly equivalent efficiencies and fidelities, Pol ι being an exception to this rule. Since polymerase interactions with the DNA minor groove provide for the nearly equivalent efficiencies of nucleotide incorporation opposite different template bases, the presumed inability of Pol ι to functionally interact with the DNA minor groove of the incipient base pair might account for the unusual nucleotide incorporation specificities of this enzyme. Thus, we suggest that while the active site of Pol ι has become specialized for incorporating nucleotides opposite the highly distorting minor-groove adducts of purine bases, such as γ -HOPdG, one consequence of this is that Pol ι has lost the ability to efficiently and accurately incorporate nucleotides opposite pyrimidine bases.

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