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Mutagenic replication of the major oxidative adenine lesion 7,8-dihydro-8-oxoadenine by human DNA polymerases

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Abstract

Reactive oxygen species attack DNA to produce 7,8-dihydro-8-oxoguanine (oxoG) and 7,8-dihydro-8-oxoadenine (oxoA) as major lesions. The structural basis for the mutagenicity of oxoG, which induces G to T mutations, is well understood. However, the structural basis for the mutagenic potential of oxoA, which induces A to C mutations, remains poorly understood. To gain insight into oxoA-induced mutagenesis, we conducted kinetic studies of human DNA polymerases β and η replicating across oxoA and structural studies of pol β incorporating dTTP/dGTP opposite oxoA. While pol η readily bypassed oxoA, it incorporated dGTP opposite oxoA with a catalytic specificity comparable to that of correct insertion, underscoring the promutagenic nature of the major oxidative adenine lesion. Pol η and pol β incorporated dGTP opposite oxoA ~170-fold and ~100-fold more efficiently than that opposite dA, respectively, indicating that the 8-oxo moiety greatly facilitated error-prone replication. Crystal structures of pol β showed that, when paired with an incoming dTTP, the templating oxoA adopted an *anti* conformation and formed Watson-Crick base pair. When paired with dGTP, oxoA adopted a *syn* conformation and formed a Hoogsteen base pair with Watson-Crick-like geometry, highlighting the dual-coding potential of oxoA. The templating oxoA was stabilized by Lys280-mediated stacking and hydrogen bonds. Overall, these results provide insight into the mutagenic potential and dual-coding nature of the major oxidative adenine lesion.

GRAPHICAL ABSTRACT

INTRODUCTION

The integrity of the human genome is persistently threatened by endogenous and exogenous DNA damaging agents. One type of DNA damaging agents is reactive oxygen species, which can be generated by ionizing radiation and endogenous free radicals. When reactive

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oxygen species damage DNA, they can produce a wide variety of genotoxic lesions, such as 7,8-dihydro-8-oxoguanine (oxoG) and 7,8-dihydro-8-oxoadenine (oxoA),¹ which are implicated in cancer development and aging (Figure 1).² The mutagenic properties of oxoG are well established both *in vitro* and *in vivo*.^{1,3} The mutagenicity of oxoG is caused by the lesion's ability to form a stable Hoogsteen base pair with adenine, which cannot occur in undamaged DNA. The templating oxoG in an *anti* conformation can form three Watson-Crick hydrogen bonds with an incoming dCTP. On the other hand, the templating oxoG in a *syn* conformation can form two Hoogsteen hydrogen bonds with an incoming dATP, giving rise to G to T transversion mutations.⁴⁻⁷ In the oxoG:dA base pair, the O6 and N7 of oxoG engage in hydrogen bonds with the N6 and N1 of dA, respectively. The base pair geometry of oxoG:dATP is essentially identical to that of a cognate dT:dATP Watson-Crick base pair (Figure 1B), thereby facilitating the misincorporation of dATP opposite the lesion by evading the geometric constraints imposed by the catalytic-site architecture of DNA polymerases.^{4,5}

Compared with oxoG, the mutagenic properties of oxoA remain poorly understood. OxoA, the major form of oxidized adenine lesions found in irradiated DNA,⁸ is present at levels one-third to one-half that of oxoG.^{9,10} OxoA is present in human cancerous tissues and squamous cell carcinoma of the larynx.^{11,12} This major oxidative adenine lesion is removed 3-fold faster than oxoG in human lymphoblast cells.⁹ Human thymine DNA glycosylase (TDG) and *E. coli* mismatch-specific uracil DNA glycosylase (MUG) can cleave oxoA from oxoA:T, oxoA:G and oxoA:C base pairs. 8-Oxoguanine DNA glycosylase (hOGG1) and endonuclease VIII-like protein 1 (NEIL1) can excise oxoA from oxoA:C base pairs *in vitro*.¹³⁻¹⁶ In addition, TDG removes thymine opposite oxoA, albeit with a lower efficiency, to form an abasic site opposite oxoA.^{13,17} While oxoG is readily bypassed by RNA polymerase II, oxoA significantly blocks the enzyme.¹⁸ The presence of oxoA inhibits the 3' to 5' exonuclease activity of WRN helicase.¹⁹ Prokaryotic DNA polymerases such as *Escherichia coli* DNA polymerase I and Taq DNA polymerase almost exclusively incorporate dTTP opposite oxoA.^{20,21} By contrast, mammalian DNA polymerases α and β frequently misincorporate dGTP opposite the lesion *in vitro*.²² OxoA is at least 10-fold less mutagenic than oxoG in bacterial cells.²³ In contrast, in mammalian cells, the same lesion induces mutation at a rate similar to that of oxoG.^{22,24} Despite these advances in the understanding of oxoA, the structural basis for the promutagenic replication across oxoA by mammalian DNA polymerases is unknown. A Drew-Dickerson dodecamer structure of the oxoA:dG base pair shows that oxoA adopts a *syn* conformation and forms a Hoogsteen base pair with guanine (Figure 1B).²⁵ While the C8 carbonyl oxygen of oxoG does not participate in the interbase hydrogen bonds of oxoG:dA, the C8 carbonyl oxygen of oxoA forms a hydrogen bond with N1 of guanine, thereby forming a wobble base pair conformation. A structure for DNA polymerase in complex with oxoA-containing DNA is not available, thereby limiting our understanding of the miscoding properties of oxoA. In addition, kinetic studies of translesion synthesis DNA polymerases catalyzing past oxoA have not been reported.

To gain insights into oxoA-induced mutagenesis, we chose the Y-family human DNA polymerase η (pol η) which plays an important role in translesion DNA synthesis. Pol η has been shown to proficiently bypass various DNA lesions including oxoG,²⁶ cisplatin-GG intrastrand cross-link adducts,^{27,28} and UV-induced thymine-thymine cyclobutane dimer,

^{29,30} O6-methylguanine,³¹ and N-methylformamido pyrimidine-dG.³² In particular, when oxoG is not removed prior to replication, translesion synthesis polymerases can significantly involve in oxoG bypass, where Pol η plays the major role in yeast.³³ Several biochemical studies show that Pol η efficiently and accurately catalyzes across oxoG.^{26,34–36} In contrast, yeast replicative DNA polymerase ϵ does not bypass oxoG at normal S-phase dNTP concentration and inefficiently and inaccurately bypasses the lesion at DNA damage-induced levels of dNTP concentration.³⁷ Bypass of oxoG by yeast Pol δ is somewhat problematic, as the enzyme incorporates nucleotides opposite oxoG ten-fold less efficiently and accurately than yeast Pol η does.^{33,38} The inefficient bypass of oxoG by replicative DNA polymerases might be caused by oxoG-induced change in the DNA backbone conformation³⁹ and oxoG's conformational flexibility. The conformational change of sugar-phosphate backbone induced by oxoG can interfere normal protein-DNA interactions and affects activities of various enzymes.⁴⁰ The presence of 8-oxo moiety destabilizes an *anti*-conformer of purines, thereby increasing the population of a *syn*-conformer. It is possible, like oxoG, the conformationally flexible oxoA could stall replicative DNA polymerases and be bypassed by translesion synthesis DNA polymerases such as Pol η .

In addition to pol η , we chose human DNA polymerase β (pol β) as upregulated pol β has been implicated in bypass of various DNA lesions. Pol β is an X-family DNA polymerase that plays a pivotal role in base excision DNA repair (BER).⁴¹ In addition to its critical role in base excision repair, pol β has been implicated in the bypass of various nucleobase lesions such as oxoG and cisplatin-induced Pt-GG adducts.^{7,4243–45} Upregulation of pol β promotes a mutator phenotype and chromosomal instability and confer resistance to cisplatin chemotherapy.⁴⁶ Overexpression of pol β has been observed in many cancer tissues including ovarian, prostate, melanoma, colon, leukemia, and breast cancer cells. In particular, it has been shown that protein levels of pol β in melanoma, colon and breast cancers were ~10-, ~20- and ~290-fold higher, respectively, as compared with adjacent normal tissues.^{47,48} A 7-fold overexpression of pol β has been shown to promote a mutator phenotype and chromosomal instability.⁴⁹ Pol β can interfere with DNA replication *in vitro*.⁵⁰ Overexpression of pol β in CHO cells gives rise to a UV irradiation-resistant phenotype and hypermutagenicity,⁴⁸ suggesting that excess pol β can compete with pol η in bypass of cyclobutane pyrimidine dimer lesions. It has been hypothesized that upregulation of pol β can participate in lagging strand replication,⁵⁰ translesion DNA synthesis⁴⁶ and nucleotide excision repair.⁵¹ The enzyme is loaded into DNA replication forks in neurons in response to β -amyloid.⁵² Pol β has been shown to have a propensity to generate A to C transversions at certain template sites.^{53,54} One possible pathway to account for the A to C transversions is that upregulated pol β occasionally participates in translesion synthesis of oxoA, a common oxidative lesion.⁵⁵ Overexpression of pol β has been shown to greatly (~50-fold) increase the mutagenicity induced by oxidative stress.⁵⁶ The observed ~9-fold increase in the levels of oxoG and oxoA in breast tumor tissues compared with normal adjacent tissue^{57,58} together with the reported pol β upregulation in several tumor tissues (e.g., ~290-fold in breast cancer) suggests the possibility that in pol β -upregulated cells the enzyme could involve in bypass of oxoA to promote A to C mutations.

Herein, we report kinetic data for nucleotide incorporation opposite oxoA by human pol η and pol β and three crystal structures of pol β in complex with oxoA-containing DNA. These

human DNA polymerases-based studies provide new insights into the mutagenic potential and dual-coding nature of the major oxidative adenine lesion oxoA, which has been shown to preferentially induce A to C mutations in mammalian cells.

METHODS

Protein expression, crystallization and structure determination.

Pol β was expressed and purified from *E. coli* with minor modifications of the method described previously.⁵⁹ The ternary pol β complex with the templating oxoA was prepared and crystallized as described previously.^{60,61} To obtain the binary complex of pol β -DNA complex, pol β was incubated with a single-nucleotide gapped DNA containing a 16-mer template (5'-CCGAC[oxoA]GCGCATCAGC-3'), a complementary 10-mer upstream primer (5'-GCTGATGCGC-3'), and a 5-mer downstream primer (5'-pGTCGG-3'). Subsequently, a 10-fold molar excess of nonhydrolyzable dGMPNPP or dTMPNPP (Jena Bioscience) was added to the binary complex. Ternary pol β -DNA complex co-crystals with nonhydrolyzable dNTP analogs paired with templating oxoA were grown in a buffer solution containing 50 mM imidazole, pH 7.5, 14–23% PEG3400, and 350 mM sodium acetate. Crystals were cryoprotected in mother liquor supplemented with 12% ethylene glycol and were flash-frozen in liquid nitrogen. Diffraction data were collected at 100 K at the beamline 5.0.3 at the Advanced Light Source, Lawrence Berkeley National Laboratory. All diffraction data were processed using HKL 2000. Structures were solved by molecular replacement with a gapped binary complex structure (PDB ID 1BPX) and a ternary complex structure (PDB ID IBPY) as the search models.⁶⁰ The model was built using COOT and refined using CCP4.^{62,63} MolProbity was used to make Ramachandran plots.⁶⁴ All the crystallographic figures were generated using PyMOL.

Steady-state kinetics of single nucleotide incorporation opposite templating oxoA by DNA polymerases.

Steady-state kinetic parameters for insertion and extension opposite oxoA by pol β and pol η were measured with minor modification of protocols described previously.^{65,66,67} All the oligonucleotides DNAs for kinetic assays were synthesized by Midland Certified Reagent company (Midland, TX) and Integrated DNA Technologies (Coralville, IA). A single nucleotide gapped DNA was used for pol β kinetic studies and a recessed DNA was used for pol η kinetic studies.

To prepare DNA substrates for pol β -catalyzed nucleotide incorporation opposite oxoA, 34-mer template (5'-GTACCCGGGGATCCGTACG(oxoA)CGCATCAGCTGCAG-3'), 14-mer upstream primer (5'-FAM/CTGCAGCTGATGCG-3'), and 5'-phosphorylated 19-mer downstream primer (5'-phosphate/CGTACGGATCCCCGGGTAC-3') were annealed in hybridization buffer (10 mM Tris-HCl pH 7.0, 1 mM EDTA). DNA substrates for pol β -catalyzed extension of oxoA:dT and oxoA:dG were composed of 34-mer template (5'-GTACCCGGGGATCCGTACG(oxoA)CGCATCAGCTGCAG-3'), 15-mer upstream primer (5'-FAM/CTGCAGCTGATGCGN-3', N = T or G), and 5'-phosphorylated 18-mer downstream primer (5'-phosphate/GTACGGATCCCCGGGTAC-3'). Enzyme activities were determined using the reaction mixture (50 μ L) containing 50 mM Tris-HCl pH 7.0/9.0,

100 mM KCl, 5 mM MgCl₂, 80 nM single-nucleotide gapped DNA, and varying concentration of dNTP (e.g., for polβ-catalyzed insertion study: 0.19-25 μM dTTP, 0.78-100 μM dGTP, see SI Figures 1 and 2). To prevent end product inhibition and substrate depletion from interfere with accurate velocity measurement, the enzyme concentrations and reaction-time intervals were adjusted for every experiment (less than 20% insertion product formed). The reactions were initiated by the addition of the enzyme (1-5 nM) at 22 °C. After 1-2 min, the reactions were stopped with 20 μL of a gel-loading buffer (95% formamide with 20 mM EDTA, 45 mM Tris-borate, 0.1% bromophenol blue, 0.1% xylene cyanol). The reaction products were separated on 20% denaturing polyacrylamide gels. The product formation was quantified by analyzing gels using a Storm 860 Imager (Molecular Dynamics) and ImageQuant software. The k_{cat} and K_{m} were determined by fitting reaction rate over dCTP concentrations to Michaelis-Menten equation. Each experiment was repeated three times to measure the average of the kinetic results. The efficiency of nucleotide insertion was calculated as $k_{\text{cat}}/K_{\text{m}}$. The relative frequency of dNTP incorporation opposite oxoA was determined as $f = (k_{\text{cat}}/K_{\text{m}})_{[\text{dN:oxoA}]} / (k_{\text{cat}}/K_{\text{m}})_{[\text{dT:dA}]}$.

To prepare DNA substrate for polη-catalyzed incorporation opposite oxoA, 34-mer template (5'-GTACCCGGGGATCCGTACG(oxoA)CGCATCAGCTGCAG-3') and 5'-FAM-labeled 14-mer primer (5'-FAM/CTGCAGCTGATGCG-3') were annealed in hybridization buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). For kinetic studies of polη-catalyzed extension across oxoA:T and oxoA:G, the 34-mer template and 5'-FAM-labeled 15-mer primer (5'-FAM/CTGCAGCTGATGCGN-3', N = T or G) in the same buffer conditions were used. Enzyme activities were determined using the reaction mixture containing 40 mM Tris-HCl pH 8.0, 60 mM KCl, 10 mM dithiothreitol, 250 μg/ml bovine serum albumin, 2.5 % glycerol, 5 mM MgCl₂, 100 nM primer/template DNA, and varying concentration of incoming dNTP (e.g., for polβ-catalyzed insertion study: 0.39-50 μM dTTP, 0.39-50 μM dGTP, see SI Figures 1 and 2). The insertion and extension reactions were initiated by the addition of polη (2-5 nM) at 22 °C. After 1 min, the reactions were stopped with 20 μL of a gel-loading buffer (95% formamide with 20 mM EDTA, 45 mM Tris-borate, 0.1% bromophenol blue, 0.1% xylene cyanol). The quenched reaction mixtures were separated by 20% polyacrylamide, 8M urea denaturing gel electrophoresis and analyzed as described above.

Nucleotide incorporation opposite oxoA in the presence of a mixture of dTTP and dGTP.

To assess whether *anti*- and *syn*-conformers of oxoA can compete during replication, we evaluated dTTP/dGTP incorporation opposite oxoA by using single-nucleotide gapped DNA and recessed DNA for polβ and polη, respectively. The single-nucleotide gapped DNA for polβ study was composed of 34-mer oxoA template (5'-GTACCCGGGGATCCGTACG[oxoA]CGCATCAGCTGCAG-3'), 14-mer upstream primer (5'-FAM/CTGCAGCTGATGCG-3'), and 19-mer downstream primer (5'-phosphate/CGTACGGATCCCCGGGTAC-3') in the annealing buffer. The recessed DNA for polη study contained the 34-mer oxoA template and 14-mer primer (5'-FAM/CTGCAGCTGATGCG-3') in the annealing buffer. Insertion reactions were initiated by adding polη (2 nM) or polβ (2 nM) to the reaction mixture containing 40 mM Tris-HCl pH 8.0, 60 mM KCl, 10 mM dithiothreitol, 250 μg/ml BSA, 2.5 % glycerol, 5 mM MgCl₂, 80

nM primer/template DNA, and 100 μ M of dTTP and dGTP. After incubation at 22°C for 1 min, the reactions were quenched with 20 μ L of the gel-loading buffer. The quenched reaction mixtures were separated by 20% denaturing polyacrylamide gels and the replication products were visualized on Storm Imager (SI Figure 3).

Primer extension across oxoA in the presence of excess pol β and a recessed DNA substrate.

To evaluate the impact of pol β upregulation on oxoA bypass, we used excess pol β and a recessed DNA containing 34-mer template (5'-GTACCCGGGGATCCGTACG[oxoA]CGCATCAGCTGCAG-3') and 5'-FAM-labeled 14-mer (5'-FAM/CTGCAGCTGATGCG-3') in hybridization buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). Pol β -catalyzed primer extension activities were determined using the reaction mixture (50 μ L) containing 50 mM Tris-HCl pH 7.0, 100 mM KCl, 5 mM MgCl₂, the recessed DNA substrate (80 nM), and 100 μ M dNTP mix. The primer extension reactions were initiated by adding varying concentration of pol β (1, 5, 50, and 200 nM) at 37 °C (SI Figure 4). After 1 h, the primer extension reactions were quenched by adding 20 μ L of the gel-loading buffer. The replication products were resolved on 20% denaturing polyacrylamide gels. The product formation was monitored using a Storm 860 Imager.

RESULTS

Bypass of oxoA by DNA polymerases is promutagenic.

To evaluate whether replication across oxoA by polymerases is promutagenic, we determined the kinetic parameters for pol β and pol η incorporating a nucleotide opposite a templating oxoA and dA (Table 1, Figure 2 and Figure S1A–B). Pol η inserted dTTP opposite oxoA with a relative efficiency of 0.62 (17.0 for dA vs. 10.5 for oxoA), indicating that pol η readily bypassed the oxoA lesion. The catalytic specificity (k_{cat}/K_m) of dGTP incorporation opposite oxoA was about one-half of that for dTTP insertion opposite oxoA (5.1 vs. 10.5), highlighting that pol η -mediated translesion synthesis of oxoA is highly promutagenic. In addition, incorporation of dGTP opposite oxoA was ~100-fold (4.8×10^{-1} vs. 4.7×10^{-3}) more efficient than that opposite dA, suggesting that the presence of the 8-oxo moiety greatly promoted mutagenic replication. Furthermore, pol η proficiently extended across both oxoA:dT and oxoA:dG base pairs with k_{cat}/K_m values of 7.5 and 6.7 (Table 1 and Figure S2 A–B), respectively, illustrating that the presence of oxoA did not induce a significant barrier to pol β -mediated catalysis. In the presence of dTTP and dGTP, pol η readily incorporated both nucleotides opposite templating oxoA (Figure S3A), suggesting that both *anti*- and *syn*-conformers of oxoA can compete during translesion DNA synthesis. These results suggest that the major oxidative adenine lesion, if not removed prior to replication, can promote mutagenic replication by pol η .

Pol β incorporated dTTP opposite oxoA only ~2.5-fold less efficiently (206.2 vs. 78.7) than opposite dA (Table 1 and Figure S1C), suggesting that an oxoA lesion at the templating position is not a strong impediment to DNA replication. While pol β misincorporated dGTP opposite dA with a relative efficiency of 1.9×10^{-4} , it incorporated dGTP opposite oxoA with a relative efficiency of 8.3×10^{-2} (Figure S1D), thereby increasing the misincorporation rate

by ~400-fold. In addition, the catalytic specificity of oxoA:dGTP was ~170-fold (0.0039 vs. 6.5) greater than that for dA:dGTP, suggesting that a templating oxoA greatly facilitates mutagenic replication by some DNA polymerases. To evaluate the effect of pH on pol β -catalyzed nucleotide incorporation, we determined the kinetic parameters for dGTP incorporation opposite dA and oxoA at pH 9.0. At the elevated pH, the catalytic specificity for dA:dGTP and oxoA:dGTP increased 10- and 7-fold, respectively.

While pol η extended both oxoA:dT and oxoA:dG (k_{cat}/K_m ; 7.5 and 6.7) without a significant decrease in the catalytic specificity compared to those for insertion (k_{cat}/K_m ; 10.5 and 5.1), pol β extended oxoA:dT and oxoA:dG base pairs with a drastic (>40-fold) reduction in the catalytic specificity compared to those for extension of undamaged DNA⁶⁶ (Table 1 and Figure S2C–D). Unlike pol η that extended both oxoA:dT and oxoA:dG with almost equal efficiency (k_{cat}/K_m ; 7.5 and 6.7), pol β extended the oxoA:dT base pair 4-fold more efficiently than the oxoA:dG base pair (k_{cat}/K_m ; 8.1 vs 2.1). In the presence of a mixture of dTTP and dGTP, pol β incorporated both nucleotides opposite templating oxoA with a preference for dTTP over dGTP (Figure S3B).

To assess the effect of upregulated pol β on the potential bypass of oxoA during non-BER pathways,^{50,51} we evaluated primer extension across oxoA using a recessed DNA substrate (34-mer oxoA template/14-mer primer), dNTPs, and varying pol β concentrations at 37 °C (Figure S4). While fully extended replication products were not observed with 1 nM pol β , they were observed at higher pol β concentrations, with the majority of the primer being fully extended with 50 nM pol β . The primer extension gel image (Figure S4) showed the presence of more than 25 bands, rather than 20 bands, suggesting that pol β -catalyzed bypass of oxoA in pol β -upregulated cells is promutagenic.

Templating oxoA exists as a mixture of *syn* and *anti* base conformers in the catalytic site of pol β .

To gain insights into the conformation of oxoA at the templating position, we solved a crystal structure of pol β bound to a single-nucleotide gapped DNA with a templating oxoA (Figure 3). The co-crystal structure was refined to 2.3 Å resolution (Table 2). The overall structure of the pol β -oxoA gapped binary complex was very similar to that of a published gapped binary complex (PDB ID: 1BPX).⁶⁰ The structure showed a 90° bend at the 5' side of the templating base and an open protein conformation, in which the minor groove-recognizing α -helix N was ~10 Å away from the catalytic site (Figure 3A). The electron density for oxoA indicated that the unpaired major oxidative adenine lesion existed as a mixture of *syn* and *anti* conformers (Figure 3D). The conformation of the templating oxoA was very similar to that of templating oxoG in the catalytic site of pol β .⁷ The template 5' and 3' of oxoA was distorted to prevent a potential steric clash between the O8 of oxoA and the O4' of the sugar moiety. This template distortion was previously observed in a single-nucleotide gapped pol β structure with a templating oxoG.⁷ The B-factor (~40-50) for the templating oxoA was higher than those for adjacent bases. The occupancies for *syn* and *anti* oxoA were 0.45 and 0.55, respectively. Overall, the pol β -oxoA binary complex structure indicated that, in the absence of an incoming nucleotide, the templating oxoA was flexible and in equilibrium between *syn* and *anti* conformers.

OxoA forms a Watson-Crick base pair with incoming dTTP.

To gain insights into the error-free bypass of oxoA by pol β , we determined a crystal structure of pol β in complex with a templating oxoA paired with a nonhydrolyzable dTMPNPP (hereafter dTTP*). This nonhydrolyzable nucleotide was used because it is isosteric to dTTP and its coordination to the active-site metal ions is essentially identical to that of dTTP. The pol β -oxoA:dTTP* ternary complex structure was refined to 2.0 Å resolution.

The pol β -oxoA:dTTP* ternary structure showed that the oxoA:dTTP base pair was well tolerated within the enzyme's catalytic site. The structure showed a closed protein conformation and a coplanar oxoA:dTTP* base pair conformation (Figure 4A and 4B). The overall structure of the pol β -oxoA:dTTP* complex was essentially identical to that of the published pol β -dA:dUTP* structure (PDB ID = 2FMS, RMSD = 0.339 Å).⁶⁸ The templating oxoA adopted an *anti* conformation and formed a Watson-Crick base pair with dTTP* (Figure 4B and 4C). The N6 and N1 of oxoA formed hydrogen bonds with the O4 and N3 of dTTP*, respectively. The λ angles (54.0° and 59.4°) and the C1'-C1' distance (10.5 Å) for the oxoA:dTTP* base pair were very similar to those of the dA:dUTP* base pair.

The conformation of the 5'-phosphodiester of oxoA was significantly different from those observed in the pol β ternary structure with the correct incoming nucleotide. To allow *anti*-oxoA at the templating position, the 5'-phosphodiester of the templating oxoA underwent a local conformational reorganization relative to the conformation of the 5'-phosphodiester of the templating dA in the published pol β -dA:dUTP* structure (Figure 4D and Figure 5B).⁶⁸ The phosphate-backbone reorganization of the templating oxoA prevented a steric clash with the O8 of the lesion. This minor structural perturbation may contribute to the slight decrease (~3-fold) in the insertion efficiency of dTTP opposite the templating oxoA relative to that opposite the templating dA (Table 1). The conformation of the 5'-phosphodiester of oxoA was indistinguishable from that of oxoG in the published pol β -oxoG:dCTP* structure (Figure 5C).⁷

The orientation of Lys280 in the oxoA:dTTP* structure was very different from those observed in the published pol β structures with oxoG:dCTP* and dA:dUTP* base pairs (Figure 5).^{7,68} In the published pol β -dA:dUTP* structure, Lys280 engages in a stacking interaction with the templating dA along the axis of the C4 and C5 of the purine and forms a hydrogen bond with the 5'-phosphate oxygen of the dA (Figure 5B).⁶⁸ A similar orientation of Lys280 has been observed in the pol β structure with a templating oxoG and incoming dCTP*. Lys280 moves toward the phosphate backbone to accommodate oxoG-induced reorganization of 5'-phosphodiester (Figure 5C).⁷ The conformation of the 5'-phosphodiester of *anti*-oxoA overlays well with that of *anti*-oxoG (Figure 5C), indicating that 8-oxoadenine and 8-oxoguanine have similar impacts on the 5'-phosphodiester conformation. In the pol β -oxoA:dTTP* structure, while Lys280 retained a stacking interaction with the templating base, it turned away from the 5' phosphate of the templating base and formed a hydrogen bond (2.9 Å) with Asn37. The hydrogen bond between Lys280 and Asn37 was lacking in other structures. The combined stacking and hydrogen bonding interactions mediated by Lys280 are likely to contribute to stabilizing the templating *anti*-oxoA.

Overall, the oxoA:dTTP* structure showed that, although a templating oxoA lesion induced minor conformational changes in protein (Lys280) and DNA (5' phosphate of oxoA), the *anti*-oxoA:dTTP base pair was readily accommodated within the nascent base-pair binding pocket of pol β , which was consistent with the kinetic data (Table 1).

OxoA forms a Hoogsteen base pair with dGTP with three interbase hydrogen bonds and Watson-Crick-like geometry in the catalytic site of pol β .

To gain insight into promutagenic replication across oxoA by DNA polymerases, we determined a co-crystal structure of pol β in complex with a templating oxoA paired with an incoming nonhydrolyzable dGMPNPP (hereafter dGTP*). The pol β -oxoA:dGTP* ternary complex structure was refined to a resolution of 2.0 Å.

The conformation of pol β in complex with oxoA:dGTP* was similar to a published pol β ternary structure with correct nucleotide incorporation (PDB ID 1BPY, RMSD = 0.459 Å) and the pol β -oxoA:dTTP* complex (RMSD = 0.225 Å), indicating that the oxoA:dGTP* base pair was well accommodated within the polymerase catalytic site (Figure 6). The protein underwent a large conformational change to assume a closed conformation, which is typically observed in pol β structures with correct insertions; a closed pol β conformation with a base pair mismatch has been also observed in a published pol β structures with the oxoG:dATP and oxodGTP:dA base pairs.^{7,69} The α -helix N moved ~10 Å toward the active site to sandwich the oxoA:dGTP* base pair between the primer terminus base pair and the α helix N (Figure 6A). OxoA and dGTP* formed a coplanar base pair conformation. In duplex DNA, an oxoA:dG base pair with two interbase hydrogen bonds and a wobble geometry was previously observed (Figure 1B).²⁵ In our pol β -oxoA:dGTP* ternary complex structure, the nascent *syn*-oxoA:*anti*-dGTP* base pair formed interbase hydrogen bonds with Watson-Crick-like geometry (Figures 6B and 6D). This suggests that the formation of oxoA:dGTP* with Watson-Crick-like geometry, which mimics a cognate dC:dGTP, is induced by pol β . The templating oxoA adopted a *syn* conformation and formed Hoogsteen base pair with dGTP* (Figure 6C). The exact nature of the oxoA:dGTP* base pair is very elusive. One possibility is that oxoA and dGTP are in front of each other without forming hydrogen bonds but just because of π -stacking interactions. Another possibility is that the enolate or rare enol tautomer of either oxoA or dGTP involves in the oxoA:dGTP base pairing (Figure 6F). Additional experiments such as NMR studies and melting point studies with bases lacking certain interactions would be required for accurate appreciation of the nature of oxoA:dGTP base pairing.

The Hoogsteen oxoA:dGTP base pair with Watson-Crick-like geometry fits snugly in the catalytic site of pol β , which partially explains the modest decrease (~12-fold) in insertion efficiency for dGTP:oxoA relative to that for dTTP:oxoA (Table 1); by contrast, the insertion efficiency for dGTP:dA decreased by ~5,300-fold relative to that for dTTP:dA. The base pair geometries, such as the C1'-C1' distance (10.6 Å) and the λ angles (51.7° and 52.5°) for *syn*-oxoA:dGTP*, were very similar to those of the correct base pair and *syn*-oxoG:dATP*. In addition, the base pair geometry of oxoA:dGTP was very similar to that of oxoA:dTTP, highlighting the dual-coding potential of oxoA.

The DNA conformation in the oxoA:dGTP* structure was essentially identical to that of the oxoA:dTTP* structure. The 5'-phosphodiester backbone of oxoA underwent conformational reorganization to accommodate *syn*-oxoA at the templating position (Figure 6E). The orientation and conformation of the 5'-phosphodiester of *syn*-oxoA were indistinguishable from those of *anti*-oxoA. Unlike the oxoA structures, in published pol β -oxoG structures, a significant conformational difference in the 5' phosphodiester of *syn*-oxoG and *anti*-oxoG has been observed.

The templating *syn*-oxoA was stabilized by multiple interactions. In addition to three interbase hydrogen bonds with dGTP, a hydrogen bond between Arg283 and the O8 of oxoA stabilized the *syn*-oxoA conformation at templating position (Figure 6B). The guanidine moiety of Arg283 was also hydrogen bonded to the O4' of the upstream template nucleotide.

The orientation and hydrogen bonding pattern of Lys280 in the oxoA:dGTP* structure were very different from those in the oxoA:dTTP* structure (Figure 7). As seen in the oxoA:dTTP* structure, the side chain of Lys280 in the oxoA:dGTP* structure was in van der Waals contact with *syn*-oxoA (Figure 7A). Although the conformation of the 5'-phosphodiester of *syn*-oxoA was very similar to that of *anti*-oxoA, the ϵ -NH₂ of Lys280 disengaged from the hydrogen bond with Asn37 and moved toward the phosphate backbone to a location ~6 Å from the position observed in the oxoA:dTTP structure (Figure 7B), forming a hydrogen bond with the 5'-phosphodiester oxygen of oxoA. The side chain of Lys280 formed a stacking interaction with *syn*-oxoA along the axis of the C8 and C2 of the purine. A similar stacking interaction has been observed in the pol β -oxoG:dATP* structure (Figure 7C),⁷ although in that structure Lys280 does not engage in hydrogen bond interaction with the phosphodiester of the templating base. While the orientation and conformation of Lys280 in the oxoA:dGTP* and the oxoG:dATP* structures are very similar, the 5'-phosphodiester conformation of the templating oxopurine is different (Figure 7C). In particular, the dihedral angles for C5'-O-P-O3' of oxoG and oxoA were -145.9 and -78.0, respectively. Thus, it appears that Lys280 is flexible and adjusts to maximize the stacking interaction with *syn*-oxoA.

Overall, the pol β -oxoA:dGTP* ternary structure indicates that the promutagenic oxoA:dGTP base pair is well accommodated within the nascent base pair binding pocket of the enzyme. These results provide the structural basis for the error-prone catalysis opposite oxoA by pol β *in vitro*.

DISCUSSION

Our kinetic and structural characterization of replication opposite an oxoA lesion by human DNA polymerases provides new insights into the miscoding properties of the major oxidative adenine lesion. The dGTP incorporation opposite oxoA by pol η and pol β is ~100-fold and ~170-fold more efficient than that opposite dA, respectively, suggesting that oxoA promotes mutagenic replication by mammalian DNA polymerases. A single-nucleotide gapped binary complex structure of pol β with templating oxoA shows a mixture of *syn* and *anti* conformers of the lesion, highlighting the flexibility of oxoA in the templating position.

The ternary complex structure of pol β inserting dTTP opposite oxoA displays Watson-Crick oxoA:dTTP base pair. When the templating oxoA is paired with an incoming dGTP, pol β is in a closed conformation and the nascent oxoA:dGTP adopts a Hoogsteen base pair with Watson-Crick-like geometry, indicating that the promutagenic oxoA:dGTP base pair is well tolerated within the active site architecture of pol β .

The present pol η kinetic studies showed that the efficiency of dGTP incorporation opposite oxoA increased ~100-fold relative to that of dGTP:dA, highlighting the promutagenic nature of the oxoA lesion. Published pol η kinetic studies show a 136-fold increase in dATP incorporation efficiency opposite oxoG relative to that of dATP:dG,²⁶ indicating that the oxoA:dGTP insertion efficiency of pol η is comparable to the oxoG:dATP insertion efficiency. The efficiency of dGTP insertion opposite oxoA relative to that of dTTP:oxoA is 0.48, which is similar to the reported efficiency (0.28) of dATP insertion opposite oxoG relative to that of dCTP:oxoG.²⁶ Published pol β kinetic studies have shown that insertion efficiencies for oxoG:dCTP and oxoG:dATP are ~10-fold and ~20-fold lower, respectively, than that for dG:dCTP.⁷⁰ The insertion efficiencies for oxoA:dTTP and oxoA:dGTP are decreased ~3-fold and ~32-fold, respectively, relative to that for dA:dTTP, indicating that the oxoA:dGTP misinsertion efficiency of pol β is comparable to the oxoG:dATP misinsertion efficiency. Templating strand distortion 5' and 3' to oxoG and oxoA could contribute to the decrease in insertion efficiency. The insertion efficiency of oxoA:dGTP is ~170-fold greater than that of dA:dGTP, underscoring the fact that oxoA lesions greatly promote inaccurate replication. In addition, the replication fidelity for oxoA [$(k_{\text{cat}}/K_m)_{\text{dTTP}}/(k_{\text{cat}}/K_m)_{\text{dGTP}}$] decreased by ~400-fold compared to that for dA [$(k_{\text{cat}}/K_m)_{\text{dTTP}}/(k_{\text{cat}}/K_m)_{\text{dGTP}}$],⁶⁶ underscoring the miscoding potential of oxoA.

Compared with the oxoG lesion, oxoA has been given much less attention in the literature. This is in part because the first reports on the mutagenic properties of oxoA showed highly accurate replication past oxoA by prokaryotic DNA polymerases^{20,21} and weak mutagenicity in *E. coli*.²³ The kinetic and structural results reported here suggest that mammalian DNA polymerases-mediated replication of the major oxidative adenine lesion is highly error-prone, which should warrant more attention on the promutagenic nature of oxoA in mammalian cells. While oxoA is at least an order of magnitude less mutagenic than oxoG in bacterial cells,²³ it is considerably mutagenic in mammalian cells, preferentially inducing A to C transversion mutations.^{22,24} Pol β has been shown to incorporate dGTP opposite oxoA.⁷¹ In addition, pol α inserts dGTP opposite the lesion albeit less efficiently.⁷¹ This is contrast with bacterial DNA polymerases that replicate across oxoA in a highly error-free manner.^{20,21} In the case of mammalian DNA polymerase α , dGTP incorporation opposite oxoA is only ~10-fold less efficient than dTTP:oxoA, suggesting that replication past oxoA by mammalian DNA polymerases is error-prone.⁷¹ The efficient and error-prone bypass of oxoA by human translesion synthesis DNA polymerase η suggests that replication across oxoA by mammalian translesion synthesis DNA polymerases is promutagenic. The frequent incorporation of dGTP opposite oxoA by human pol β , suggests that in pol β -upregulated cells the bypass of oxoA lesions by pol β could occur in an error-prone manner.

DNA polymerases increases replication fidelity by using geometric selection process, where they discriminate base pairs with Watson-Crick geometry against those with non-Watson-

Crick geometry in the replicating base pair site.^{72,73} The observation of oxoA:dGTP with Watson-Crick-like geometry in the catalytic site of pol β , which uses a geometric selection mechanism, suggests that the mutagenic oxoA:dGTP base pair is well tolerated within the active site of some DNA polymerases. While pol β lacks intrinsic proofreading exonuclease activity, it deters misincorporation by various mechanisms.⁴¹ In particular, pol β undergoes an open-to-closed conformational change during correct nucleotide insertion, whereas it takes on an open conformation in the presence of a nascent mismatched base pair (e.g., G:T).^{41,66,74} In addition, during correct insertion, pol β engages in minor groove interactions with the primer terminus base pair, templating base, and incoming nucleotide. Pol β is sensitive to geometric distortion induced by mismatches, deterring the formation of a closed protein conformation for base pairs with non-Watson-Crick geometry. For example, pol β structures with A:C, G:T, and O6MeG:T mismatches show an open protein conformation and a staggered base pair conformation in the presence of Mg²⁺ ion.^{61,66} The use of mutagenic Mn²⁺ in place of Mg²⁺ promotes the formation of Watson-Crick-like G:T and O6MeG:T base pairs and induces an open-to-closed conformational change of pol β . The mutagenic oxoG:dATP mismatch evades the geometric constraints of the enzyme by adopting a Hoogsteen base pair with Watson-Crick-like geometry.^{4,5,7,75,76} Likewise, the oxoA:dGTP mismatch eludes the geometric selection mechanism of pol β by forming a Hoogsteen base pair with Watson-Crick-like geometry, suggesting that the promutagenic oxoA:dGTP base pair can be readily accommodated within the active site of some DNA polymerases.

While conformations of oxoG:A in duplex DNA and nascent base pair binding pockets are essentially the same, those of oxoA:G are very different, highlighting that oxoA:G conformation is more readily influenced by environment than oxoG:A conformation. OxoG:A forms a Hoogsteen base pair with Watson-Crick-like geometry regardless of the sites. By contrast, oxoA:G forms a Hoogsteen base pair with a wobble geometry in duplex DNA²⁵ and Hoogsteen base pair with Watson-Crick-like geometry in the nascent base pair binding pocket of pol β . While the major tautomers of *syn*-oxoG and *anti*-dATP (the keto form of oxoG and the amino form of dATP) can form a Hoogsteen pair with Watson-Crick-like geometry, those of *syn*-oxoA and *anti*-dGTP cannot. To produce oxoA:dGTP with three hydrogen bonds and Watson-Crick-like geometry, either oxoA or dGTP must undergo ionization or tautomerization, which could occur within the nascent base pair binding pocket of pol β . Recent NMR and kinetic studies show that at neutral pH, dG:dT misincorporation by DNA polymerases such as pol α and pol β predominantly (>99%) involves the enol tautomer of dG or dT, which forms mainly via exchange from wobble dG:dT mispair.⁷⁷ On the other hand, at pH 8.4 and above, the keto tautomeric dG and the anionic dT⁻ primarily contribute to the mispairing.⁷⁷ It would be of interest to evaluate whether the Watson-Crick-like oxoA:dGTP base pair observed in the pol β active site involves an anionic or tautomeric intermediate of oxoA or dGTP.

The formation of minor groove interactions may facilitate the ionization or tautomerization of mismatched base pairs. Base ionization and tautomerization are influenced by various factors including base modification, pH, and metal ions.⁷⁸ With respect to base modification, halogenated uracil derivatives (e.g., 5-bromouracil, 5-fluorouracil) greatly favor base pairing with guanine at higher pH, indicating the involvement of an ionized base pair.^{79,80} In

addition, methylation of the N7 of dG lowers the pK_a of N7 by 2 and thus facilitates the ionization or tautomerization of the N1-H of dG.⁸¹ This induces a Watson-Crick-like N7-methylG:dT base pair and a shifted N7-methylG:dA base pair.⁸² Furthermore, N7 platination of dG alters the base pairing properties of dG by reducing the pK_a of the N1-H of dG.⁸³ In addition to base modification, minor groove contact by DNA polymerases could promote the ionization and tautomerization of bases. Recent studies of pol β in complex with the dG:dTTP base pair show that the hydrogen bond between the O2 of dTTP and Asn279 induces Watson-Crick-like geometry.⁸⁴ In the case of pol β in complex with oxoA:dGTP, the hydrogen bond between the N3 of the dGTP and Asn279 or between the O8 of oxoA and Arg283 could promote the formation of the enol tautomer of dGTP or oxoA, respectively. This could facilitate the formation of an oxoA:dGTP base pair with Watson-Crick-like geometry. Minor groove interactions by Arg283 of pol β are required to form *syn*-oxoG:*anti*-dATP with Watson-Crick-like geometry in the nascent base pair binding pocket of the enzyme.⁸⁵ Arg283Lys pol β induces the *anti*-oxoG:dATP base pair conformation, which resembles an *anti*-dG:*anti*-dATP mismatched structure.⁸⁵

Differences in the microenvironments of the nascent base pair binding pockets of DNA polymerases may produce mismatches with varying base pair conformations. In the presence of Mn^{2+} , the catalytic site of pol β induces Watson-Crick-like G:T but not a Watson-Crick-like A:C base pairing,⁶⁶ suggesting that the enzyme's active site architecture stimulates the formation of the enolate or enol tautomer of G:T but not the imino tautomer of A:C. The Watson-Crick-like G:T mismatch has been observed in the nascent base pair binding pocket of the X-family DNA polymerase pol λ but not in those of the B- and Y-family DNA polymerases.⁸⁶ By contrast, the A-family *Bacillus Sterarothermophilus* DNA polymerase I large fragment induces Watson-Crick-like A:C mismatches in the presence of active site Mn^{2+} . This is likely due to stabilization of the rare imino tautomer of A:C by the enzyme's active site.⁸⁷

In summary, our study provides insights into the mutagenic potential and dual-coding nature of the major oxidative adenine lesion oxoA, which has been paid much less attention than the oxoG lesion. In contrast to the accurate bypass of oxoA by prokaryotic DNA polymerases, replication past the same lesion by human DNA polymerases is highly promutagenic, incorporating dGTP opposite oxoA ~100-fold more efficiently than dGTP:dA insertion. This is consistent with the reported mutagenicity of oxoA in human cell lines. If oxoA is not removed by TDG, mutagenic replication would be greatly promoted. OxoA-mediated A to C transversion mutations may be ascribed to the flexibility of the templating oxoA, which can readily form a Hoogsteen base pair with incoming dGTP in the nascent base pair binding pockets of DNA polymerases. The greatly enhanced misincorporation frequency and the dual-coding properties of oxoA suggest that oxoA is comparably mutagenic to oxoG. Since pol β exhibits several characteristics of high fidelity DNA polymerases, it would be of interest to evaluate whether high fidelity DNA polymerases induce a Hoogsteen *syn*-oxoA:*anti*-dGTP base pair with Watson-Crick-like geometry and bypass oxoA in an error-prone manner.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Base pairing properties of 7,8-dihydro-8-oxoguanine (oxoG) and 7,8-dihydro-8-oxoadenine (oxoA) in duplex DNA. (A) *Syn* and *anti* conformers of oxoA. (B) Hoogsteen base pairing of *syn*-oxoG:*anti*-A and *syn*-oxoA:*anti*-G can promote G to T and A to C transversion mutations, respectively. In duplex DNA, *syn*-oxoG:*anti*-A adopts a Watson-Crick-like geometry, whereas *syn*-oxoA:*anti*-G adopts a wobble geometry.

Figure 2.

Nucleotide incorporation opposite templating oxoA by pol β and pol η . Single nucleotide gapped DNA (for pol β) or primer-template DNA (for pol η) was mixed with increasing concentrations of polymerase and the reactions were initiated by addition of dGTP or dTTP. A 5'-FAM-labeled primer was used for the single nucleotide incorporation study. Each reaction was conducted for 2 minutes at 22 °C and the quenched samples were separated on 20% denaturing polyacrylamide gels.

Figure 3.

Binary complex structure of polβ bound to a single nucleotide gapped DNA with templating oxoA. (A) Overall structure of the gapped binary complex structure. Protein is in an open conformation. The templating oxoA is shown in black. Template strand is shown in yellow and upstream- and downstream primers are shown in orange. The α -helix N is shown in red. (B) Close-up view of the active site of the polβ binary structure. (C) DNA sequence used for crystallography. The 5' end of the downstream primer was phosphorylated. (D) Conformation of oxoA at the templating position. A $2F_o - F_c$ electron density map contoured at 1σ around the templating oxoA in the gapped binary complex is shown. Templating OxoA is in equilibrium between *syn*- and *anti*-conformers.

Figure 4.

Ternary structure of polβ in complex with templating oxoA and an incoming nonhydrolyzable dTTP*. (A) Overall structure of the polβ-oxoA:dTTP* ternary complex. OxoA is shown in black and incoming dTTP* is shown in cyan. (B) Close-up view of the active site of the polβ-oxoA:dTTP* ternary complex. Protein is in a closed conformation and oxoA:dTTP* forms a Watson-Crick base pair. Hydrogen bonds are indicated by dotted lines. (C) Base pairing properties of *anti*-oxoA and dTTP* in the active site of polβ. A $2F_o - F_c$ electron density map contoured at 1σ around oxoA:dTTP* is shown. (d) Conformational differences between *anti*-oxoA (multiple colored, from polβ-oxoA:dTTP* structure) and *anti*-dA (white, from published polβ-dA:dUTP* structure)⁶⁸ in the templating position.

Figure 5.

Conformations of Lys280 in the presence of *anti*-dA and *anti*-oxopurines. (A) Interaction of Lys280 with *anti*-oxoA and Asn37 in the polβ-oxoA:dTTP complex structure. Lys280 stacks with *anti*-oxoA and is hydrogen bonded to Asn37. (B) Superposition of the polβ-oxoA:dTTP structure and published polβ-dA:dUTP* structure (PDB ID 2FMS).⁶⁸ The polβ-dA:dUTP* structure is in black and the polβ-oxoA:dTTP* structure is multicolored. The Lys280 in the polβ-dA:dUTP* structure stacks with dA and the ε-NH₂ of Lys280 forms a hydrogen bond with the 5'-phosphate backbone oxygen. The distance between the ε-NH₂ moieties of Lys280 in the polβ-oxoA:dTTP* and polβ-dA:dUTP structures is indicated. Note differences in the conformations of Lys280 and the 5'-phosphodiester. (C) Superposition of the polβ-oxoA:dTTP structure and published polβ-*anti*-oxoG:dCTP* structure (PDB ID 3RJI).⁷ Interaction of Lys280 with *anti*-oxoG nucleotide that is paired with incoming dCTP. Unlike in the polβ-oxoA:dTTP* structure, Lys280 stacks with oxoG and engages in hydrogen bond interactions with the 5'-phosphate oxygen of the oxoG lesion.

Figure 6.

Ternary structure of pol β in complex with templating oxoA and incoming nonhydrolyzable dGTP*. (A) Overall structure of the pol β -oxoA:dGTP* ternary complex. (B) Close-up view of the active site of the pol β -oxoA:dGTP* ternary complex. Protein is in a closed conformation and oxoA:dGTP* forms a coplanar base pair. (C) Base pairing properties of oxoA:dGTP* in the catalytic site of pol β . Templating oxoA and dGTP* form three hydrogen bonds with Watson-Crick-like geometry. A $2F_o - F_c$ electron density map contoured at 1σ around the oxoA:dGTP* base pair is shown. (D) Comparison of conformations of

templating *syn*-oxoA and templating *anti*-dA (PDB ID 3LK9).⁶⁸ (E) Superposition of the *syn*-oxoA:dGTP* base pair with *anti*-oxoA:dTTP* base pair. (F) Speculative Watson-Crick-like oxoA:dGTP* base pair involving an enolate intermediate or a rare enol tautomer.

Figure 7.

Conformation of Lys280 in the presence of *syn*-8-oxopurines. (A) Interaction of Lys280 with *syn*-oxoA nucleotide paired with dGTP. Lys280 forms a favorable stacking interaction with *syn*-oxoA and a hydrogen bond with the 5'-phosphate of oxoA. (B) Superposition of the polβ-*syn*-oxoA:dGTP* (multicolored) and polβ-*anti*-oxoA:dTTP* (black) structures. The 5'-phosphodiester conformation of the oxoA nucleotide is very similar, whereas Lys280 conformation is different. The distance between the ε-NH₂ moieties of Lys280 is indicated. (C) Superposition of the polβ-*syn*-oxoA:dGTP (multiple colored) and published polβ-*syn*-oxoG:dATP (colored black, PDB ID 3RJF)⁷ structures. Unlike in the polβ-*syn*-oxoA:dGTP structure, Lys280 in the polβ-*syn*-oxoG:dATP structure is not hydrogen bonded to the 5'-phosphate oxygen. Note the conformational difference between the 5'-phosphodiester of *syn*-oxoA and *syn*-oxoG.

Table 1.

Kinetic parameters for nucleotide incorporation opposite oxoA and dA by pol η and pol β .

template:dNTP	pH	K_m (μ M)	k_{cat} ($10^{-3}s^{-1}$)	k_{cat}/K_m ($10^{-3}s^{-1}\mu M^{-1}$)	f^a	replication fidelity
polη						
dA:dTTP	8.0	5.35 ± 0.23	90.9 ± 5.8	17.0	1	
dA:dGTP	8.0	76.29 ± 4.79	6.30 ± 0.5	0.08	4.7×10^{-3}	212
oxoA:dTTP	8.0	3.56 ± 0.33	37.3 ± 2.3	10.5	1	
oxoA:dGTP	8.0	4.87 ± 0.31	24.8 ± 1.3	5.1	4.8×10^{-1}	2.1
dG:dCTP	8.0	4.81 ± 0.24	32.2 ± 2.4	6.7		
(oxoA:G extension)						
dG:dCTP	8.0	6.04 ± 0.50	45.4 ± 0.4	7.5		
(oxoA:T extension)						
polβ						
dA:dTTP	7.0	0.85 ± 0.10	175.3 ± 4.7	206.2	1	
dA:dGTP	7.0	107.4 ± 13.43	4.12 ± 0.32	0.039	1.9×10^{-4}	5287
dA:dGTP	9.0	20.12 ± 1.25	8.31 ± 1.58	0.40	1.9×10^{-3}	522
oxoA:dTTP	7.0	2.46 ± 0.18	193.6 ± 11.7	78.7	1	
oxoA:dGTP	7.0	13.01 ± 0.81	84.2 ± 3.5	6.5	8.3×10^{-2}	12
oxoA:dGTP	9.0	3.66 ± 0.34	168.0 ± 6.5	45.9	5.8×10^{-1}	1.7
dG:dCTP	7.0	4.38 ± 0.25	35.5 ± 0.7	2.1		
(oxoA:G extension)						
dG:dCTP	7.0	9.75 ± 0.69	20.0 ± 0.6	8.1		
(oxoA:T extension)						

^aRelative efficiency: $(k_{cat}/K_m)[dNTP:dA]/(k_{cat}/K_m)[dTTP:dA]$ or $(k_{cat}/K_m)[dNTP:oxoA]/(k_{cat}/K_m)[dTTP:oxoA]$. Kinetic parameters shown with standard deviations are averages of 3 to 5 independent determinations. A 5'-FAM labeled recessed DNA was used for pol η kinetic studies and a single-nucleotide gapped DNA was used for pol β kinetic studies.

Table 2.

Data Collection and Refinement Statistics.

PDB CODE	oxoA gapped (6E3R)	oxoA:dTTP (6E3V)	oxoA:dGTP (6E3W)
Data Collection			
space group	$P2_1$	$P2_1$	$P2_1$
Cell Constants			
a (Å)	54.572	50.134	50.588
b	79.048	80.381	79.594
c	54.966	55.674	55.718
α (°)	90.00	90.00	90.00
β	105.75	107.41	107.54
γ	90.00	90.00	90.00
resolution (Å) ^a	20-2.28 (2.32-2.28)	20-1.99 (1.96-1.99)	20-2.02 (2.05-2.02)
R_{merge}^b (%)	0.074 (0.430)	0.067 (0.371)	0.067 (0.370)
$\langle I/\sigma \rangle$	19.2 (2.29)	24.5 (2.56)	19.7 (3.00)
completeness (%)	99.1 (96.6)	100.0 (99.9)	100.0 (99.9)
redundancy	4.0 (3.9)	4.6 (4.4)	4.6 (3.9)
Refinement			
$R_{\text{work}}^c/R_{\text{free}}^d$ (%)	20.1/26.3	19.5/23.9	19.3/24.5
unique reflections	19876	29733	26914
Mean B Factor (Å ²)			
protein	39.98	30.14	25.03
ligand	38.27	18.92	12.97
solvent	38.23	31.85	25.51
Ramachandran Plot			
most favored (%)	95.6	98.5	98.5
add. allowed (%)	4.4	1.5	1.5
RMSD			
bond lengths (Å)	0.005	0.005	0.008
bond angles (degree)	1.006	1.195	1.298

^aValues in parentheses are for the highest resolution shell.^b $R_{\text{merge}} = \sum |I - \langle I \rangle| / \sum I$ where I is the integrated intensity of a given reflection.^c $R_{\text{work}} = \sum |F(\text{obs}) - F(\text{calc})| / \sum F(\text{obs})$.^d $R_{\text{free}} = \sum |F(\text{obs}) - F(\text{calc})| / \sum F(\text{obs})$, calculated using 5% of the data.