# 8-OxodGTP incorporation by DNA polymerase β is modified by active site residue Asn279

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# 8-OxodGTP Incorporation by DNA Polymerase $\beta$ Is Modified by Active-Site Residue Asn279[†]

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ABSTRACT: To understand how the active site of a DNA polymerase might modulate the coding of 8-oxo-7,8-dihydrodeoxyguanine (8-oxodG), we performed steady-state kinetic analyses using wild-type DNA polymerase $\beta$ (pol $\beta$) and two active-site mutants. We compared the coding of these polymerases by calculating the ratio of efficiencies for incorporation of dATP and dCTP opposite 8-oxodG and for incorporation of 8-oxodGTP opposite dA and dC. For wild-type pol $\beta$, there is a 2:1 preference for incorporation of dCTP over dATP opposite 8-oxodG using a 5′-phosphorylated 4-base gap substrate. Mutation of either Asn279 or Arg283 to alanine has almost no effect on the ratio. 8-OxodGTP is preferentially incorporated opposite a template dA (24:1) by wild-type pol $\beta$; mutation of Asn279 to alanine results dramatic change whereby there is preferential incorporation of 8-oxodGTP opposite dC (14:1). This suggests that interactions of 8-oxodGTP with Asn279 in the polymerase active site may alter the conformation of 8-oxodGTP and therefore alter its misincorporation.

Oxidative damage of DNA may contribute to mutagenesis, carcinogenesis, and aging (*1*). A major product of DNA oxidative damage is 8-oxo-7,8-dihydrodeoxyguanine (8-oxodG)[1] (*2*, *3*). In reactions catalyzed by DNA polymerases, dATP is inserted opposite 8-oxodG at a frequency that depends on the polymerase involved (*4*). The repair polymerases, DNA polymerase I and DNA polymerase $\beta$ (pol $\beta$), incorporate dCTP preferentially over dATP (*4*). The replicative polymerases, DNA polymerase α, DNA polymerase $\delta$, and DNA polymerase III, preferentially incorporate dATP (*4*). Structural studies of the 8-oxodG·dC and 8-oxodG·dA base pairs indicate that 8-oxodG pairs with dC in the anti conformation but assumes the syn conformation when pairing with dA (Figure 1) (*5*−*8*). These observations lead us to hypothesize that the structure of the DNA polymerase active site could modulate the mutagenic potential of 8-oxodG, possibly by preferential binding of 8-oxodG in either the syn or the anti conformation.

We selected pol $\beta$, a simple polymerase with no exonuclease activity, to explore the relationship between polymerase active-site structure and 8-oxodG coding. The structure of pol $\beta$, in a ternary complex with either a standard primer-template substrate (*9*) or a 1-base gap substrate, has been determined crystallographically (*10*). In these structures, Asn279 and Arg283 are critical residues in the polymerase active site (*11*). Asn279 interacts with the base of the bound nucleoside triphosphate, ddCTP, in a way that is unbiased

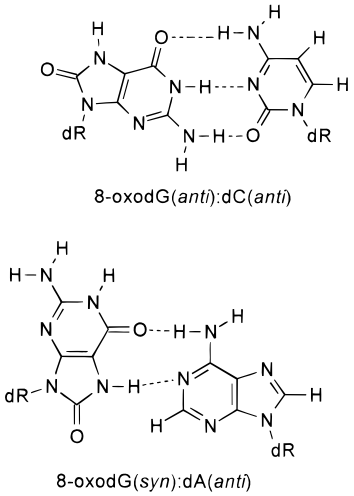


FIGURE 1: Base pairing for 8-oxodG opposite dC or dA.

such that interaction is possible with all four incoming nucleotides (*9*). Pre-steady-state experiments indicated that the N279A mutation results in a minor decrease in fidelity (4-fold) (*12*) while a gap-filling in vitro reversion assay showed an apparent increase in fidelity (*11*). The crystal structure shows that Arg283 is hydrogen bonded to the sugar−phosphate backbone and the N3 of the template base (guanine in the crystal structure) (*9*, *10*). Mutation of Arg283 to alanine decreases fidelity between 35- and 300-fold depending on the mismatch and catalytic efficiency by 5000-fold (*11*). The structure of the pol $\beta$ ternary complex shows close contacts surrounding the bound dNTP and the templating base with no room for large conformational changes after binding in the active site (*10*, *13*). In this paper, we present evidence that features in the DNA polymerase active site modulate the mutagenic potential of 8-oxodGTP, pos-

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[1] Abbreviations: dNTP, deoxynucleoside triphosphate; 8-oxodG, 7,8-dihydro-8-oxodeoxyguanine; pol $\beta$, DNA polymerase $\beta$; DTT, dithiothreitol.

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sibly by altering the equilibrium conformation of the modified nucleoside triphosphate.

## EXPERIMENTAL PROCEDURES

*Materials.* [$\gamma$-$^{32}$P]ATP was obtained from Amersham Corp. Human DNA polymerase $\beta$'s (wild-type and mutant enzymes) were overexpressed and purified as described (*11*, *14*). T4 polynucleotide kinase was purchased from U. S. Biochemical Corp. 8-OxodGTP was purchased from Amersham Corp.

*Synthesis and Purification of Oligonucleotides.* Oligodeoxynucleotides were synthesized by solid-state methods using an automated DNA synthesizer. Published methods were used for the synthesis and purification of oligodeoxynucleotides containing 8-oxoguanine (*15*, *16*). Oligonucleotides were purified by electrophoresis on a 20% polyacrylamide gel in the presence of 7 M urea. DNA was eluted from gel slices passively and desalted using a SEP-PAK C18 cartridge (Waters). Oligonucleotides were quantified by $A_{260}$ using molar extinction coefficients; the sequences of oligonucleotides used for these studies are shown in Figure 2. The oligonucleotide primer was 5′-radiolabeled with T4 polynucleotide kinase and [$\gamma$-$^{32}$P]ATP according to the manufacturer's protocol. The downstream primer was 5′-phosphorylated using T4 polynucleotide kinase and ATP. Primer-template substrates were prepared by hybridizing 5′-radiolabeled primer to the complementary 29mer using a slight excess of template and downstream primer over primer (1.1:1.0) in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 100 mM NaCl.

*Primer Extension Reactions.* Polymerization reactions (10 $\mu$L) contained 100−200 nM primer/template and 0−2 mM dNTP in 20 mM Tris-HCl, pH 8, 10 mM $MgCl_2$, 2 mM dithiothreitol, 4.5 mM NaCl, 0.4% glycerol. Pol $\beta$ (final concentration: wild-type, 1−110 nM; N279A, 1−220 nM; and R283A, 7−1600 nM) was diluted in a solution containing 50 mM Tris-HCl, pH 7.4, 500 mM NaCl, 5 mM DTT, and 50% glycerol. Initially the polymerase and DNA substrate were incubated at 37 ± 1 °C, and the appropriate amount of dNTP was added to initiate the reaction (0.5−20 min). Reactions were quenched by addition of 10 $\mu$L of 95% formamide dye mixture (95% formamide, 10 mM EDTA, 0.001% xylene cyanol, 0.001% bromphenol blue) and heated at 100 °C for 3 min before aliquots (1.5 $\mu$L) were subjected to electrophoresis on a 20% denaturing (7 M urea) polyacrylamide gel. The amount of radioactivity in the product bands was quantified using a Molecular Dynamics PhosphorImager and ImageQuant software.

*Data Analysis*. Kinetic parameters were determined from primer extension reactions. Apparent values for the Michaelis−Menten constant [$K_{m(app)}$] and the $V_{max}$ for incorporation opposite dG and 8-oxodG were obtained by least-squares nonlinear regression to a rectangular hyperbola. $k_{cat}$ is calculated by dividing the $V_{max}$ by the enzyme concentration. Standard errors derived from the curve-fitting are reported. Apparent values for the specificity constant, $k_{cat}/K_{m(app)}$, for incorporation of 8-oxodGTP and dGTP were determined by linear regression of the Lineweaver−Burk reciprocal plot. This method was used because we were unable to use saturating concentrations of 8-oxodGTP. In this case, the value for the specificity constant is the reciprocal of the slope and can be determined, but the individual values for $k_{cat}$ and $K_{m(app)}$ cannot be determined with accuracy. Less than 20% of the primer is extended under the steady-state conditions used in our kinetic studies ensuring single-hit kinetics (*17*, *18*).

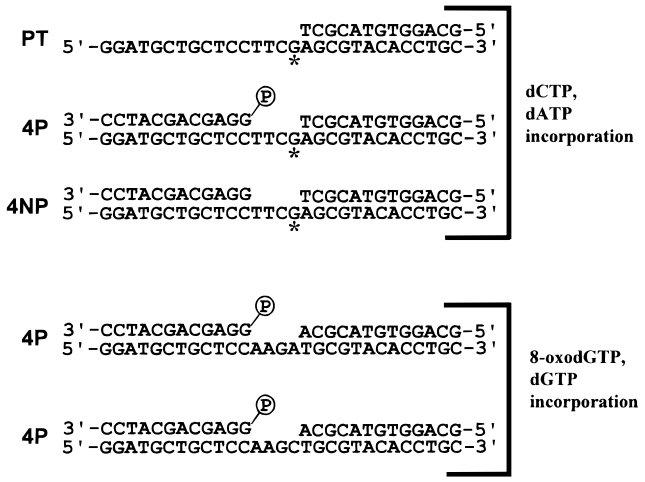


FIGURE 2: Structures and sequences of DNA substrates. Synthetic oligonucleotides were hybridized as described under Experimental Procedures to form either a standard primer-template (PT) with the now downstream primer, or 4-base gap templates (4P, 4NP). The presence of a phosphate group in the gap margin is indicated by the circled P. G* indicates the position of dG or 8-oxodG in the template.

## RESULTS

*Effect of Substrate Structure on Incorporation by Wild-Type Pol $\beta$.* Recent reports indicate that the efficiency and fidelity of polymerization may be affected by the structure of the DNA substrate, namely, the presence and size of a gap (*19*−*21*). In initial experiments to determine if substrate structure influences incorporation opposite 8-oxodG, we found that the substrate structure (Figure 2) had a dramatic effect on the efficiency [$k_{cat}/K_{m(app)dCTP}$] of incorporation opposite 8-oxodG; the incorporation efficiency varied 2 orders of magnitude over the three different substrates tested (Figure 2). In these experiments, the substrate yielding the highest efficiency of incorporation opposite both dG and 8-oxodG was the 4-base gap substrate with a 5′-phosphate (Figure 2; 4P). The standard primer-template (Figure 2; PT) substrate was the least efficient of the three. Both 4-base gap substrates (Figure 2; 4P and 4NP) are less efficient when 8-oxodG replaces dG. Because the 4-base gap substrate with a 5′-phosphate (Figure 2; 4P) had the highest efficiency, it was used for the subsequent studies.

*Effect of Active-Site Mutations on Incorporation Opposite 8-OxodG.* To determine which residues of the pol $\beta$ active site are important for the differentiation of dCTP and dATP incorporation opposite 8-oxodG, we performed a steady-state kinetic analysis using two active-site mutants of pol $\beta$ (N279A, R283A). Pol $\beta$ structural studies show that Arg283 contacts the template base and that Asn279 interacts with the base of the incoming dNTP (*9*). Kinetic parameters [$k_{cat}$ and $K_{m(app)dNTP}$] and incorporation efficiency [$k_{cat}/K_{m(app)dNTP}$] opposite 8-oxodG or dG were measured using a 4-base gap oligonucleotide substrate with a 5′-phosphate group in the gap (Figure 2; 4P). Using this substrate with a template dG, we measured a $K_{m(app)}$ for dCTP of less than 1 $\mu$M and a $k_{cat}$

of 27 $min^{-1}$ for wild-type pol $\beta$ (Table 1). The N279A mutation results in a 500-fold decrease in catalytic efficiency and the R283A mutation a 2.5 $\times$ $10^5$-fold decrease in catalytic efficiency on the unmodified template (Table 1). The effect of N279A results from a 80-fold increase in $K_{m(app)}$ and a 6-fold decrease in $k_{cat}$; the decreased efficiency of R283A results from a $10^3$-fold increase in $K_{m(app)}$ and a 180-fold decrease in $k_{cat}$ (Table 1).

Misincorporation of dATP opposite dG by wild-type pol $\beta$ had a 300-fold higher $K_{m(app)}$ and a 1400-fold decrease in $k_{cat}$ compared to incorporation of dCTP. The N279A mutation had only a small effect on the incorporation efficiency [$k_{cat}/K_{m(app)}$] for misincorporation of dATP opposite dG, resulting in a no change in fidelity compared to wild-type pol $\beta$ (Table 1). Incorporation of dATP opposite dG by the R283A polymerase compared to incorporation of dCTP had a 5-fold lower $K_{m(app)}$ and a 300-fold decrease in $k_{cat}$ (compared to 1400-fold for wild-type pol $\beta$). This results in an overall decrease in fidelity of greater than 6000-fold. Table 2 lists the ratios of $k_{cat}/K_{m(app)}$ (dCTP incorporation) to $k_{cat}/K_{m(app)}$ (dATP incorporation). This ratio is a measure of the fidelity of the polymerase at that position in the template, and the larger the number, the higher the fidelity. For wild-type and both mutant polymerases, there is a large bias for correct (dCTP) incorporation opposite dG. The dC:dA incorporation efficiency ratio was 4.1 $\times$ $10^5$ for wild-type pol $\beta$, and 3.6 $\times$ $10^5$ for N279A, indicating no significant change in fidelity. The dC:dA incorporation efficiency ratio for R283A was 65, 6000-fold lower, indicating a very large decrease in fidelity.

Incorporation of dCTP opposite 8-oxodG by wild-type pol $\beta$ has a ~3-fold higher $K_{m(app)}$, but almost no decrease in $k_{cat}$, resulting in only a 3.4-fold decrease in overall efficiency compared to incorporation opposite dG (Table 1). For the N279A polymerase, there is a 4-fold increase in $K_{m(app)}$ and a slightly lowered $k_{cat}$, resulting in a 5-fold decrease in overall efficiency compared to incorporation opposite dG. For the R283A polymerase, there is a small increase in the already high $K_{m(app)}$ and a 5-fold decrease in $k_{cat}$, resulting in an almost 8-fold decrease in efficiency. Incorporation of dCTP opposite 8-oxodG by wild-type pol $\beta$ is favored ~2-fold over incorporation of dATP opposite 8-oxodG (Table 2). Incorporation of dATP opposite 8-oxodG by wild-type pol $\beta$ is 62 000 times more efficient than incorporation of dATP opposite dG. Neither the R283A mutation nor the N279A mutation had a significant effect on the dCTP:dATP incorporation efficiency ratio opposite 8-oxodG (Table 2).

*Effect of Asn279 Mutation on the Incorporation of 8-OxodGTP*. To determine if the pol $\beta$ active site modulates the preference for incorporation of 8-oxodGTP opposite a template dC or dA, we measured the steady-state incorporation of 8-oxodGTP with wild-type pol $\beta$ and the N279A mutated polymerase (Table 3). Pol $\beta$ structural studies show that Asn279 interacts with the incoming dNTP (*9*). In these experiments we used a slightly different sequence in order to measure incorporation opposite dA and dC. The changes in the oligonucleotide sequence are within the gap: TTCG*A for dCTP and dATP incorporation (Figure 2, PT, 4P, 4NP) compared with AAG(A/C)T for dGTP and 8-oxodGTP incorporation (Figure 2, 4P). These changes were made in the sequence to ensure that only single-nucleotide incorporation occurred, and to lessen the possiblity that a T·G mispair

Table 1: Kinetic Parameters for the Incorporation of dCTP or dATP Opposite dG and 8-OxodG by DNA Polymerase $\beta$ Using a Four-Nucleotide Phosphorylated Gap Substrate

| | wild-type pol $\beta$ | | | N279A pol $\beta$ | | | R283A pol $\beta$ | | |
|---|---|---|---|---|---|---|---|---|---|
| template:dNTP | $K_{m(app)dNTP}$ ($\mu$M) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_{m(app)dNTP}$ ($min^{-1}$ $\mu M^{-1}$) | $K_{m(app)dNTP}$ ($\mu$M) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_{m(app)dNTP}$ ($min^{-1}$ $\mu M^{-1}$) | $K_{m(app)dNTP}$ ($\mu$M) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_{m(app)dNTP}$ ($min^{-1}$ $\mu M^{-1}$) |
| dG:dCTP | 0.7 ± 0.2 | 27 ± 1.3 | 38 ± 9.1 | 59 ± 26 | 4.6 ± 0.96 | 0.077 ± 0038.4 | 1000 ± 170 | 0.15 ± 0.014 | $15 \pm 2.9 \times 10^{-5}$ |
| dG:dATP | 200 ± 65 | 0.019 ± 0.0018 | $9 \pm 3 \times 10^{-5}$ | 1700 ± 1200 | $3.8 \pm 1.4 \times 10^{-3}$ | $2.2 \pm 1.7 \times 10^{-6}$ | 180 ± 64 | 41 ± 4.8 × $10^{-5}$ | $0.23 \pm 0.086 \times 10^{-5}$ |
| 8-oxodG:dCTP | 1.9 ± 0.36 | 20.8 ± 0.97 | 11 ± 2.2 | 126 ± 39 | 2.4 ± 0.45 | 0.019 ± 0.007 | 1397 ± 583 | $0.028 \pm 680 \times 10^{-5}$ | $2.01 \pm 0.97 \times 10^{-5}$ |
| 8-oxodG:dATP | 6 ± 1.5 | 36 ± 3 | 6 ± 1 | 50 ± 17 | 1.5 ± 0.25 | 0.03 ± 0.01 | 263 ± 49 | 220 ± 14 × $10^{-5}$ | $0.84 \pm 0.17 \times 10^{-5}$ |

Table 2: Ratio of dATP/dCTP Incorporation Efficiencies [$k_{cat}/K_{m(app)dNTP}$] Opposite 8-OxodG and dG in the Four-Nucleotide Gap Substrate

| pol $\beta$ | dCTP:dATP ratio ($\times 10^{-5}$) for dG template | dCTP:dATP ratio for 8-oxodG template |
|---|---|---|
| wild type | $4.1 \pm 1.7$ | $1.9 \pm 0.6$ |
| N279A | $3.6 \pm 3.2$ | $0.6 \pm 0.3$ |
| R283A | $(65 \pm 28) \times 10^{-5}$ | $2.4 \pm 1.2$ |

Table 3: Kinetic Parameters for the Incorporation of 8-OxodGTP Opposite dC and dA by DNA Polymerase $\beta$ on a Four-Nucleotide Gap Substrate

| | $k_{cat}/K_{m(app)dNTP}$ ($min^{-1}$ $\mu M^{-1}$) | |
|---|---|---|
| template:dNTP | wild-type pol $\beta$ | N279A pol $\beta$ |
| dC:dGTP | $0.18 \pm 0.04$ | $0.07 \pm 0.01$ |
| dC:8-oxodGTP | $(130 \pm 160) \times 10^{-5}$ | $(40 \pm 6) \times 10^{-5}$ |
| dA:dGTP | $(0.58 \pm 0.08) \times 10^{-5}$ | $(0.14 \pm 0.016) \times 10^{-5}$ |
| dA:8-oxodGTP | $(3200 \pm 370) \times 10^{-5}$ | $(2.8 \pm 0.3) \times 10^{-5}$ |

Table 4: Incorporation Efficiency [$k_{cat}/K_{m(app)dNTP}$] of 8-OxodGTP Opposite dA and dC by Pol $\beta$ on a Four-Nucleotide Gap Substrate

| pol $\beta$ | dA:dC dGTP incorporation ($\times 10^5$) | dA:dC 8-oxodGTP incorporation |
|---|---|---|
| wild type | $3.2 \pm 0.9$ | $24 \pm 4.2$ |
| N279A | $2.1 \pm 0.4$ | $0.07 \pm 0.01$ |

would form after incorporation opposite the target site (A/C).

The incorporation efficiency [$k_{cat}/K_{m(app)dNTP}$] of 8-oxo-dGTP or dGTP opposite dA or dC was measured using a 4-base gap oligonucleotide substrate with a 5′-phosphate group within the gap (Figure 2; 4P**)**. Using this substrate with a template dC, we established an incorporation efficiency of 0.18 $min^{-1}$ $\mu M^{-1}$ for wild-type pol $\beta$ (Table 3). The N279A mutation results in almost a 3-fold decrease in catalytic efficiency. The N279A mutation had a minimal effect on the efficiency of misincorporation of dATP opposite dG, resulting in no significant decrease in fidelity compared to wild-type pol $\beta$.

Incorporation of 8-oxodGTP opposite dC by wild-type pol $\beta$ was 140-fold less efficient than dGTP incorporation (Table 3). Incorporation of 8-oxodGTP opposite dC by N279A is 175-fold less efficient than incorporation of dGTP. Incorporation of 8-oxodGTP opposite dA by wild-type pol $\beta$ is favored 24-fold over incorporation opposite dC and is almost 6000-fold more efficient than incorporation of dGTP opposite dA (Tables 3 and 4). The N279A mutation had almost no effect on the discrimination between dGTP and 8-oxodGTP or on the relative fidelity but dramatically (>1000-fold) decreased the efficiency of incorporation of 8-oxodGTP opposite dA (Table 4). This results in a complete reversal of the dA:dC ratio from a 24:1 preference for incorporation of 8-oxodGTP opposite dA by WT pol $\beta$ to a 14:1 preference for incorporation opposite dC by N279A mutant pol $\beta$ (Table 4).

## DISCUSSION

The central question prompting this investigation was whether a polymerase active site could interact with template 8-oxodG or incoming 8-oxodGTP in a way that favors an anti or syn conformation, thereby determining whether dC or dA will be able to form the most suitable base pair for incorporation. Since polymerases differ in their incorporation efficiencies for dCTP and dATP opposite 8-oxodG (*4*), it seemed reasonable to expect that the polymerase active site can modulate coding, perhaps by altering the conformation of 8-oxodG. Ab inito calculations (*22*) and NMR studies (*23*) identified the 6,8-diketo form of 8-oxodG as the predominant species under physiological conditions. The syn and anti conformers of 8-oxodG are in rapid equilibrium, with the syn conformation being energetically favored. However, the conformation assumed in the active site of the polymerase is not known. Incorporation of 8-oxodGTP has not been studied as thoroughly, but it is likely that its conformation has an effect on whether 8-oxodGTP is incorporated opposite dC or dA. Data presented open the possibility that the polymerase active site alters the equilibrium of conformers of 8-oxodGTP through interactions with Asn279.

The results of these studies indicate a larger effect of mutation of either Asn279 or Arg283 to alanine on catalytic efficiency than published steady-state and pre-steady-state kinetic analyses (*11*, *12*, *24*). Steady-state data presented in this paper show that mutation of Asn279 to Ala resulted in a 500-fold decrease in catalytic efficiency. Pre-steady-state analysis shows that the catalytic rate at saturating dNTP concentration ($k_{pol}$) was not significantly affected by the mutation of Asn279 to alanine (*12*). Steady-state kinetics using a poly(dA)/p$(dT)_{10}$ primer-template also indicated that the primary effect of the N279A mutation is a small (<10-fold) increase in the $K_{m(app)}$ and the R283A mutation creates a 5000-fold decrease in efficiency due primarily to a decrease in $k_{cat}$ (*11*). Mutation of Arg283 to alanine, however, results in a 200-fold decrease in catalytic efficiency under pre-steady-state conditions (*24*). Steady-state kinetics of R283A using a poly(dA)/p$(dT)_{10}$ primer-template revealed a 5000-fold decrease in efficiency due primarily to a decrease in $k_{cat}$ (*11*). The data presented here show that mutation of Arg283 to alanine results in a $10^5$-fold decrease in catalytic efficiency. These more dramatic effects of the Asn279 and Arg283 mutations may be detected due to the gapped structure of the substrate.

Mutation of Arg283 to alanine results in a polymerase with a much lowered fidelity. For misincorporation of dATP opposite dG, there is an overall decrease in fidelity of >6000-fold. This is a dramatic decrease in fidelity. The 5-base gap reversion assay detected a 160-fold decrease in fidelity (*11*) and pre-steady-state analysis only a 2-fold decrease (*24*). Since we used only a single sequence context and measured the kinetics of formation for only one mispair, it is unclear whether this large effect pertains to the mutant polymerase in general. Surprisingly, although the Arg283 to alanine mutation has a dramatic effect on fidelity, there is almost no effect on the coding of 8-oxodG.

Wild-type pol $\beta$ has been reported to have an 8-oxodGTP discrimination frequency (DF: the ratio of specificity constants for incorporation of dGTP versus 8-oxodGTP) of $3.9 \times 10^2$ (*25*). DF is equal to $2.3 \times 10^2$ in this study (Table 3), almost 2-fold lower. It should be noted that the substrate used by Kamath-Loeb et al. was a 14/46 primer-template and $Mn^{2+}$ replaced $Mg^{2+}$ in the reaction mixture; this may explain the minor differences between our data (*25*).

There appear to be at least two mechanisms by which pol $\beta$ ensures accurate DNA synthesis. (1) Crystallographic

analysis indicates that gap DNA binds with a 90° kink in the DNA template, exactly at the 5′-phosphodiester linkage of the template base in the polymerase active site (*10*). This sharp bend exposes the base pair on the downstream side of the active site, allowing the base on the template strand to stack with a histidine residue in the 8 kDa domain rather than with its neighboring base in the active site (*10*). This alteration in base-stacking interactions may be relevant to the fidelity of the single-nucleotide gap-filling reaction. Upon binding a dNTP, pol $\beta$ undergoes a conformational change between an open structure and a closed structure through movement of the thumb subdomain (*10*). In this change, the positions of both Arg283 and Asn279 are altered dramatically. Sawaya et al. suggest that thumb movement may be important for fidelity by checking the base pair geometry (*10*). A similar mechanism may be involved during translesion synthesis. However, both the R283A and N279A mutations had no significant effect on 8-oxodG coding. It is possible that some other residue in the active site is important for modulating incorporation opposite 8-oxodG.

The dA:dC incorporation efficiency ratio of 8-oxodGTP was decreased >1000-fold after Asn279 was mutated to alanine, indicating that Asn279 may be involved in orienting the incoming dNTP. If the Asn279 side chain interacts favorably with 8-oxodGTP in the syn conformation, it may result in preferential incorporation opposite dA. We performed a simple modeling experiment where we replaced the ddCTP in the crystal structure (PDB ID code: 1bpy) with 8-oxodGTP (*10*). Rotation around the glycosidic bond from anti to syn revealed that in the syn conformation there is the possibility of hydrogen bond formation between O8 of 8-oxodGTP and ND2 of Asn279. If this H-bond forms during incorporation of 8-oxodGTP it may favor incorporation opposite dA, whereas mutation of asparagine to alanine would eliminate ND2 and the H-bond. An alternative interpretation is that the syn conformation is favored in the absence of any specific interactions and mutation of Asn279 to alanine favors the anti conformation. Modeling studies revealed no obvious interactions between alanine at position 279 and 8-oxodGTP in the anti conformation. However, structural studies will be required to differentiate these possibilities and to confirm or discount the predictions of our modeling studies.

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