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DNA polymerase structure-based insight on the mutagenic properties of 8-oxoguanine

William A. Beard, **Vinod K. Batra**, and **Samuel H. Wilson**

Abstract

An aerobic environment burdens DNA polymerase substrates with oxidized substrates (DNA and nucleotide pools). A major promutagenic lesion resulting from oxidative stress is 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxoG). Guanine oxidation alters the hydrogen bonding properties of the base and glycosidic-preference of the nucleotide. The favored glycosidic *syn*-conformation exposes the Hoogsteen edge of the base for hydrogen bonding with adenine during DNA synthesis. The cell has recognized the threat of this lesion and has evolved an intricate surveillance system to provide DNA polymerases with unmodified substrates. Failure to do so leads to transversion mutations. Since the mutagenic properties of the base are dictated by the *anti-syn* conformation of the nucleotide, the molecular interactions of 8-oxoG in the confines of the DNA polymerase active site are expected to influence its coding potential. Recent structural characterization of DNA polymerases from several families with this lesion in the nascent base pair binding pocket has provided insight to the mutagenic properties of this modified nucleotide. These structures reveal that flexibility around the template binding pocket can permit 8-oxoG to assume an *anti*- or *syn*-conformation and code for cytosine or adenine incorporation, respectively. In contrast, the binding pocket for the incoming nucleotide does not have this flexibility so that 8-oxodGTP insertion opposite cytosine is strongly discouraged.

1. Introduction

Oxidative stress leads to the production of multiple toxic chemicals that can threaten the integrity of genomic DNA, ultimately leading to mutations and chromosome instability. These events are known to be adverse for cells and are considered to be extremely important in the balance between human health and disease, especially regarding chronic conditions and diseases such as cancer and aging [1]. Organisms throughout nature maintain elaborate and diverse mechanisms for protecting themselves against the adverse genotoxic consequences of oxidative stress. A major lesion found in DNA and dNTP pools exposed to reactive oxygen species (ROS) is the promutagenic lesion 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxoG or Go). Elegant biochemical and genetic studies in *E. coli* and yeast defined key elements of a multifaceted cellular defense system to reduce 8-oxoG mutagenesis and is often referred to as the “GO system” (Fig. 1). Importantly, the conserved nature of this system underscores the deleterious nature of this lesion [2–7].

The early genetic and biochemical studies of cellular mutagenesis associated with oxidative stress led to the discovery in 1983 by Nishimura and associates of the oxidized deoxynucleotide called 8-oxoG [7]. This early work led to an understanding of the special

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coding property of 8-oxoG when acting as a substrate (template DNA or incoming nucleotide) for the replicative and repair DNA polymerases. Thus, oxidative stress leads to production of 8-oxodGTP in the dNTP pool and 8-oxoG in DNA. Since 8-oxoG is found to base pair with adenine in addition to cytosine, an explanation for the mutagenic consequences of 8-oxoG becomes apparent. The discovery of three *E. coli* genes, known as MutM, MutY and MutT, that comprised the original GO system, along with the subsequent characterization of their gene products, were instrumental in sparking the development of a research field focused on the repair of simple base lesions, base excision repair (BER). Finally, the BER process and components of the GO system are conserved from microorganisms to higher organisms, including humans [6,7]. Knowledge of the impact of oxidative stress on human health and, in particular, the pathobiology of chronic disease such as cancer [8], is continuing to emerge, but its importance may be even greater than we currently appreciate.

Because the mutagenic effects of 8-oxoG are mediated by the action of DNA polymerases, structural studies of DNA polymerases with 8-oxoG provide insight into DNA polymerase substrate specificity (fidelity and mutagenesis) and mechanism. Similarly, studies of the GO system link our understanding of BER to mutagenesis and human health. These linkages have enhanced enthusiasm for medical research on DNA polymerase mechanism and BER, and both areas have enjoyed considerable growth in recent years.

2. Mutagenesis and the GO system

2.1 Oxidative DNA damage

DNA is under continual threat of attack by ROS generated during aerobic respiration. In the initial step, oxygen radicals generated by oxidative stress produce the 8-oxoG lesion in DNA. Features of the *E. coli* GO system as it relates to the repair of 8-oxoG in DNA are illustrated in Fig. 1A. This scheme depicts a model where the oxidized base is removed by a damage specific DNA glycosylase (*E. coli* MutM, human OGG1) thereby initiating BER. If 8-oxoG escapes repair and a replicative DNA polymerase misinserts adenine opposite 8-oxoG, an alternate BER pathway is initiated by a DNA glycosylase (*E. coli* MutY, human MYH) that will remove the inappropriate adenine. DNA polymerase (po1) β gap-filling DNA synthesis will result in a DNA substrate for OGG1 (8oxoG–C) or MYH (8-oxoG–A). Thus, pol β can maintain the lesion-containing mutagenic base pair by inserting A during BER (as illustrated by the double-headed arrow in Fig. 1A). Therefore, BER in general and DNA synthesis by pol β, are viewed as important modulators in the mammalian GO system. Replication of the unrepaired adenine-containing strand results in a G to T transversion.

2.2 8-oxodGTPase (MutT)

Features of another aspect of the *E. coli* GO system are summarized in Fig. 1B. This scheme depicts a model for the role of 8-oxodGTP in generating an A to C transversion mutation as a function of DNA replication and BER. *E. coli* MutT (human homolog, MTH1) is a 8-oxodGTPase that is responsible for cleansing the dNTP pool of this mutagenic nucleotide. Cellular events that modulate the activity of the MutT enzyme, leading to increased levels of 8-oxodGTP, are known to have a large effect in boosting mutagenesis in *E. coli* [9]. Genetic studies in *E. coli* suggest that 8-oxodGTP misincorporation is significant even in the presence of MutT [10]. If 8-oxodGTP is utilized during DNA replication, it has a high probability of being inserted opposite adenine [11]. Once 8-oxodGTP is incorporated opposite adenine, BER cannot correct this aberrant base pair, but could be involved in the stable fixation of the A to C tranversion mutation (Fig. 1B).

3. Base pairing properties of 8-oxoG

At physiological pH, the major tautomeric form of 8-oxoG has a carbonyl group at C8 and is protonated on N7 [12,13]. Thus, guanine oxidation results in altering the hydrogen bonding capacity of its Hoogsteen edge. Whereas the unmodified deoxyguanine glycosidic torsion angle preference is *anti*, 8-oxoG favors a *syn*-conformation that can form a Hoogsteen base pair with adenine (Fig. 2). The altered glycosidic torsion angle preference is due to steric repulsion between O8 and deoxyribose [14]. Structural characterization of duplex DNA containing 8-oxoG indicates that the glycosidic torsion angle preference is determined by its base pairing partner; being *anti* with a complementary cytosine [15,16] and *syn* when base-paired with adenine [17,18].

Although the 8-oxoG(*syn*)—A(*anti*) base pair does not exhibit Watson-Crick hydrogen bonding, this mispair is well-accommodated within duplex DNA [18]. Likewise, the hydrogen bond acceptors positioned in the DNA minor groove resemble those for a T–A base pair where the carbonyl at C8 of 8-oxoG is predicted to be positioned where the carbonyl of C2 of thymidine would be located [19]. Thus, polymerase-dependent minor groove hydrogen bond scanning would not identify this mutagenic base pair as a substrate for proofreading exonucleases.

4. Influence of 8-oxoG on DNA polymerase β structure/function

4.1 Influence of 8-oxoG on the kinetics of nucleotide insertion and discrimination

As illustrated in Fig. 1, the replicative polymerases (in mammalian cells, pols α, δ and ε) and the BER polymerases (in mammalian cells, primarily po1 β and po1 λ) are expected to influence the mutagenic response to 8-oxoG in DNA and the dNTP pool. To understand the coding potential of 8-oxoG, a number of laboratories have conducted kinetic and structural studies with purified enzymes with 8-oxoG-containing substrates (oligonucleotides or 8-oxodGTP). The results indicate that the propensity to incorporate dCMP versus dAMP opposite template 8-oxoG or insert 8-oxodGTP opposite cytosine or adenine varies significantly depending on the polymerase and DNA sequence context. Published results on these interesting differences from *in vitro* assays are summarized in other articles in this volume.

Due to the ambivalent coding properties of 8-oxoG, the hydrogen bonding potential will depend on the glycosidic *anti-syn* equilibrium and the influence that a DNA polymerase imposes on this equilibrium. Although the coding potential of 8-oxoG is polymerase dependent, 8-oxoG induces effects on nucleotide insertion efficiency that are observed with most polymerases. DNA polymerase nucleotide insertion discrimination or fidelity is determined by how well competing nucleotides are inserted (i.e., ratio of their catalytic efficiencies). In the case of templating 8-oxoG, pol β fidelity would be defined from the ratio of catalytic efficiencies $(k_{cat}/K_m)_{dCTP}/(k_{cat}/K_m)_{dATP}$ (Table 1; Fig. 3, left panel). In this discrimination plot, the vertical line between the plotted catalytic efficiencies (short horizontal lines) represents discrimination or fidelity; the longer the line, the greater the discrimination. It can easily be seen that discrimination is dramatically reduced when the templating base is 8-oxoG rather than an unmodified guanine (G). Most importantly, this type of plot readily exposes the reason for the loss in discrimination; i.e., why the vertical line gets shorter. For pol β, although dCTP insertion efficiency is reduced opposite 8-oxoG relative to guanine, the loss in discrimination is primarily due to the dramatic increase in catalytic efficiency of insertion of dATP opposite 8-oxoG (~4000-fold) [20]. This is probably due to the ambivalent coding properties of 8-oxoG (Fig. 2).

The efficiencies of 8-oxodGTP insertion opposite cytosine and adenine are tabulated in Table 1 and illustrated in Fig. 3 (right panel). Like dATP insertion opposite 8-oxoG, 8-oxodGTP insertion opposite adenine is increased dramatically relative to dGTP insertion. In contrast however, 8-oxodGTP insertion opposite cytosine is decreased dramatically (~1300-fold) so that insertion opposite adenine is preferred by about 10-fold (the dotted vertical line in the plot indicates that discrimination has been reversed; i.e., a non-canonical base pair is preferred). This plot also indicates that although 8-oxodGTP insertion exhibits poor discrimination, the insertion efficiency of 8-oxodGTP opposite adenine is lower than that for dATP insertion opposite a templating 8-oxoG. With this perspective, we now examine DNA polymerase structural attributes that may modulate the *anti-syn* equilibrium of 8-oxoG as a templating (coding) base or an incoming nucleotide.

4.2 Structural aspects of 8-oxoG in the template (coding) binding pocket of a polymerase

DNA polymerases have a modular organization that is often comprised of a polymerase domain and an accessory domain. For pol β, the accessory domain is an amino-terminal lyase domain that has deoxyribose phosphate lyase activity. This enzymatic activity removes a 5′-deoxyribose-phosphate moiety from a BER intermediate and is required to generate a 5′-phosphate required for DNA ligation. The polymerase domain of pol β and replicative DNA polymerases is structurally organized into functionally distinct subdomains referred to as DNA binding (D), catalytic (C), and nascent base pair binding (N) subdomains [21]. These are referred to as thumb, palm, and fingers subdomains, respectively, for right-handed polymerases that possess a nonhomologous catalytic (palm) subdomain. However, the catalytic participants (DNA, dNTP, and metals) can be superimposed indicating that all DNA polymerases utilize a common two-metal ion mechanism for nucleotidyl transfer [22]. DNA polymerase β is very well characterized with over 150 structures in the Protein Data Bank (see [23] for review). The following description will focus primarily on pol β, but unless noted otherwise is also relevant to other DNA polymerases as well.

The nascent base pair binding pocket of DNA polymerases is generally described as `open' in the absence of an incoming nucleotide. Upon binding an incoming nucleotide, the N–subdomain repositions itself to `close' the binding pocket. The result is that the nascent base pair is sandwiched between the polymerase and the primer terminus base pair. The downstream template strand bends abruptly at the templating (coding) base to permit the polymerase access to the nascent base pair. For Y-family DNA polymerases, the conformational adjustments are subtle and the `closed' state results in a spacious binding pocket. The structures of pre-insertion complexes of 8-oxoG paired with an incoming dCTP or ddCTP in the confines of a polymerase nascent base pair binding pocket have been determined for members of four DNA polymerase families: T7 DNA polymerase (A-family) [24], RB69 DNA polymerase (B-family) [25], DNA polymerase β (X-family) [19], and Dpo4 DNA polymerase (Y-family) [26–28]. The fingers or N–subdomain of each of these polymerases is in the closed `active' conformation. These structures indicate that both the modified template base and incoming cytosine are in *anti*-conformations and reveal that only minor local structural changes are needed to accommodate 8-oxoG. For example, the DNA phosphate backbone of the templating 8-oxoG in the pol β structure is repositioned to relieve a steric clash with O8 (Fig. 4A) [19]. In contrast, RB69 DNA polymerase does not require any adjustment in the phosphate backbone of the templating base to accommodate 8-oxoG [25]. The small structural perturbations required to bind dCTP opposite 8-oxoG is consistent with the small decrease in insertion efficiency (Fig. 3, left panel). Structures of T7 [29] and Dpo4 [28] DNA polymerases indicate that a basic side chain is within hydrogen bonding distance to O8 of 8-oxoG(*anti*). Replacing these side chains with alanine results in mutant enzymes that are error-prone; consistent with the idea that the basic side chain stabilizes the *anti*-conformation of 8-oxoG through a hydrogen bond with O8.

Adenine can base pair with 8-oxoG in the templating base position when the *syn*-conformation presents the Hoogsteen edge for replicative base pairing (Fig. 2). Structures of DNA polymerases with an incoming dATP Hoogsteen base paired with a templating 8-oxoG(*syn*) have been determined for the Y-family polymerases Dpo4 [27] and pol κ [30], as well as the K536A mutant of T7 DNA polymerase [29] and the Y567A mutant of RB69 DNA polymerase [31]. With T7 DNA polymerase, Lys536 is within hydrogen bonding distance to O8 of 8-oxoG in an *anti*-conformation, thereby stabilizing the non-mutagenic conformation. Alanine substitution results in an enzyme that preferred the mutagenic insertion of dATP [29] (Fig. 4B). Similarly, Arg332 of Dpo4 DNA polymerase stabilizes the *anti*-conformation of 8-oxoG [28].

A ternary complex structure of pol β has also been determined with 8-oxoG as the templating base and an incoming non-hydrolysable nucleotide analog, dAMP(CH_2)PP (Batra et al., unpublished data). The single-nucleotide gapped templating base 8-oxoG is in a *syn*-conformation and forms a Hoogsteen base pair with the incoming A. The *syn*-conformation of 8-oxoG is stabilized by stacking with an adjacent lysine residue (Lys280). The failure to obtain the ternary complex of pol β in an earlier study may be attributed to a water molecule that occupied the position of the 3′-OH of the dideoxy-terminated primer hastening hydrolysis of the incoming dATP [19].

The efficiency of dATP insertion opposite 8-oxoG by pol β is increased 4000-fold relative to insertion opposite guanine (Table 1; Fig. 3, left panel) [20]. These ternary complex structures indicate that the templating 8-oxoG has assumed the *syn*-conformation and forms a Hoogsteen base pair with adenine. In contrast to a staggered arrangement of bases with a previously reported structure of pol β with an unmodified templating guanine and incoming dATP analog (i.e., dAMP(CH_2)PP) [32], the 8-oxoG(*syn*)–dATP(*anti*) mispair is planar so that the geometry is similar to that expected of a nascent base pair with Watson-Crick hydrogen bonding.

4.3 Structural constraints imposed by the DNA polymerase dNTP binding pocket

As noted above, in addition to DNA, oxygen radicals modify the nucleotide pool producing 8-oxodGTP that can be utilized during DNA replication and repair (Fig. 1B). DNA polymerases generally prefer to insert 8-oxodGTP opposite adenine [11]. This is partly due to a strong discrimination against insertion opposite cytosine and an enhanced insertion opposite adenine (relative to dGTP) (Table 1;Fig. 3, right panel). Left unrepaired, 8-oxodGTP misinserted opposite adenine can result in an A to C transversion (Fig. 1B). To examine the structural basis for 8-oxodGTP-induced mutagenesis during BER, we determined the crystal structure of the ternary complex of pol β with a templating adenine in a single-nucleotide gapped DNA and an incoming 8-oxodGTP [11]. The structure reveals that 8-oxodGTP assumes the *syn*-conformation and forms a Hoogsteen base pair with the templating adenine. Again, in contrast to previously published structures of pol β with active site mismatches, this mispair is planar and exhibits Watson-Crick like geometry, albeit with Hoogsteen hydrogen bonds (Fig. 5).

The *syn*-conformation of 8-oxodGTP is stabilized through Hoogsteen hydrogen bonding with the templating adenine, a hydrogen bond with Asn279, and an intra-molecular hydrogen bond between N2 and a non-bridging oxygen on the α-phosphate. Alanine substitution for Asn279 has been shown to interfere with 8-oxodGTP insertion opposite adenine consistent with destabilizing the *syn*-conformation [33]. With a Watson-Crick base pair in the active site, Asn279 is a hydrogen bond donor to O2 of pyrimidines or N3 of purines. As has been noted [18], the *syn*-conformation of 8-oxoG positions O8 in the DNA minor groove in a similar position as O2 for a Watson-Crick base pair (Fig. 5). Although the nascent base pair exhibits good geometry and minor groove hydrogen bonding, kinetic

analysis indicates that insertion efficiency is reduced considerably relative to that observed for formation of a Watson-Crick base pair (Table 1; Fig. 3, right panel). The structure indicates that the primer terminus is perturbed as a result of its displacement into the major groove when the incoming 8-oxodGTP assumes a *syn*-conformation. Accordingly, the diminished catalytic efficiency is probably due to the altered position (distance and geometry) of the 3′-terminus relative to the incoming nucleotide.

For pol β, and many other DNA polymerases, 8-oxodGTP is preferentially misinserted opposite adenine rather than cytosine (Table 1; Fig. 3, right panel). Even for DNA polymerases that preferentially insert 8-oxodGTP opposite cytosine, insertion efficiency opposite cytosine is dramatically reduced [11]. This is consistent with our inability to crystallize a complex with 8-oxodGTP paired with a templating cytosine. However, modeling an incoming 8-oxodGTP in an *anti*-conformation paired with cytosine indicates that steric repulsion between O8 and its deoxyribose-phosphate would distort the active site. It has long been recognized that 8-oxodG prefers the *syn*-conformation due to steric repulsion with the deoxyribose backbone that the *anti*-conformation could impose [13,14]. Whereas DNA polymerases are able to modulate the backbone position of the templating nucleotide, polymerases do not have the capability of repositioning catalytic participants (i.e., α-phosphate to accommodate incoming 8-oxodGTP in an *anti*-conformation without serious kinetic consequences.

5. Future directions

Recent progress in understanding the structural basis of 8-oxoG mutagenesis by DNA polymerases has provided insight on how the architecture of the DNA polymerase active site is able to adapt to the Hoogstein base pair. All DNA polymerases can form a Hoogsteen base pair with 8-oxoG(*syn*)—A(*anti*) much more efficiently than a G–A mispair. Ultimately, discrimination will rely on the effect of the *anti*-*syn* equilibrium imposed on 8-oxoG by the DNA polymerase active site. Construction of strategically engineered polymerase active sites that deter the *syn*-conformation of 8-oxoG may be possible, and it will be interesting to investigate the impact of such an altered polymerase on mutagenesis *in vivo*. As noted above, pol β preferentially inserts 8-oxodGTP opposite adenine. Alanine substitution for Asn279 alters discrimination by specifically reducing the efficiency of insertion of the oxidized nucleotide opposite adenine (i.e., destabilizing the *syn*-conformation of 8-oxodGTP) [33]. This results in a change in discrimination where 8-oxodGTP insertion is preferred opposite cytosine (non-mutagenic), albeit at a low efficiency, with the mutant (N279A) enzyme. It remains to be seen whether cellular expression of this mutant would alter the mutagenic response to oxidative stress.

Error free (8-oxoG—C) or error prone (8-oxoG—A) DNA synthesis requires that these mispairs be extended. As noted above, the geometry of both base pairs resemble that of a Watson-Crick base pair. Structures of high-fidelity A-family DNA polymerases with these base pairs situated at the primer terminus (i.e., post-insertion site) confirm this observation and suggest that the 8-oxoG—A mispair could escape proofreading [24,34]. In contrast, structures of the low fidelity Y-family Dpo4 DNA polymerase with 8-oxoG paired with cytosine or adenine at the primer terminus indicate that the 8-oxoG—A mispair displays conformational heterogeneity that could deter error-prone DNA synthesis [35]. A conformationally dynamic primer terminus has also been postulated to inhibit mutagenic insertion opposite an abasic site by pol β [36].

Finally, the discovery of DNA backbone adjustments that accommodate 8-oxoG in the *anti*-conformation raise the notion that DNA-binding auxiliary proteins of DNA replication and BER might have a role in regulating the coding potential of 8-oxoG. The DNA backbone

distortion by 8-oxoG occurs precisely at the boundary where the template strand is dramatically bent to permit the polymerase access to the nascent base pair [23]. Thus, accessory factors that bind to the DNA and restrict backbone distortion could modulate the *anti*-*syn*-conformational equilibrium of 8-oxoG and the attendant mutageneis. An example of such an effect may be suggested by recent observations indicating that pol λ misincorporation of dATP opposite 8-oxoG is influenced by the auxiliary factors PCNA and RP-A [37].

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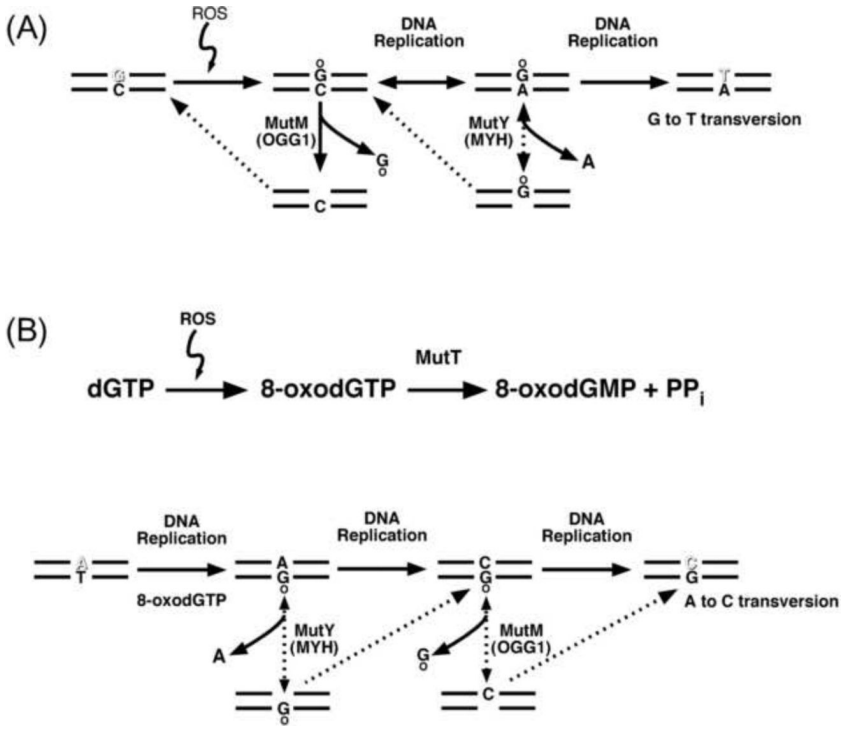

Fig. 1.

DNA repair and replication of 8-oxoG: the GO system. (A) The repair of the oxidized base, 8-oxoG, in DNA is initiated by a DNA damage-specific glycosylase. *E. coli* MutM (human homolog, OGG1) removes 8-oxoG paired with cytosine to purify the genome of this oxidized base. During DNA replication, unrepaired 8-oxoG can code for dCMP or dAMP. To remove misinserted adenines residues, *E. coli* MutY (human homolog, MYH) initiates BER by removing the inappropriate adenine. DNA polymerase β gap-filling DNA synthesis (dashed lines) will result in a DNA substrate for MutM (8-oxoG–C) or MutY (8-oxoG–A). Replication of the unrepaired adenine-containing strand results in a G to T transversion. (B) *E. coli* MutT (human homolog, MTH1) is an 8-oxodGTPase that cleanses the dNTP pools of this oxidized nucleotide. Failure to remove 8-oxoG that has been misinserted opposite A results in an A to C tranversion. As above, pol β gap-filling DNA synthesis (dashed lines) will result in a DNA substrate for MutM (8-oxoG–C) or MutY (8-oxoG–A).

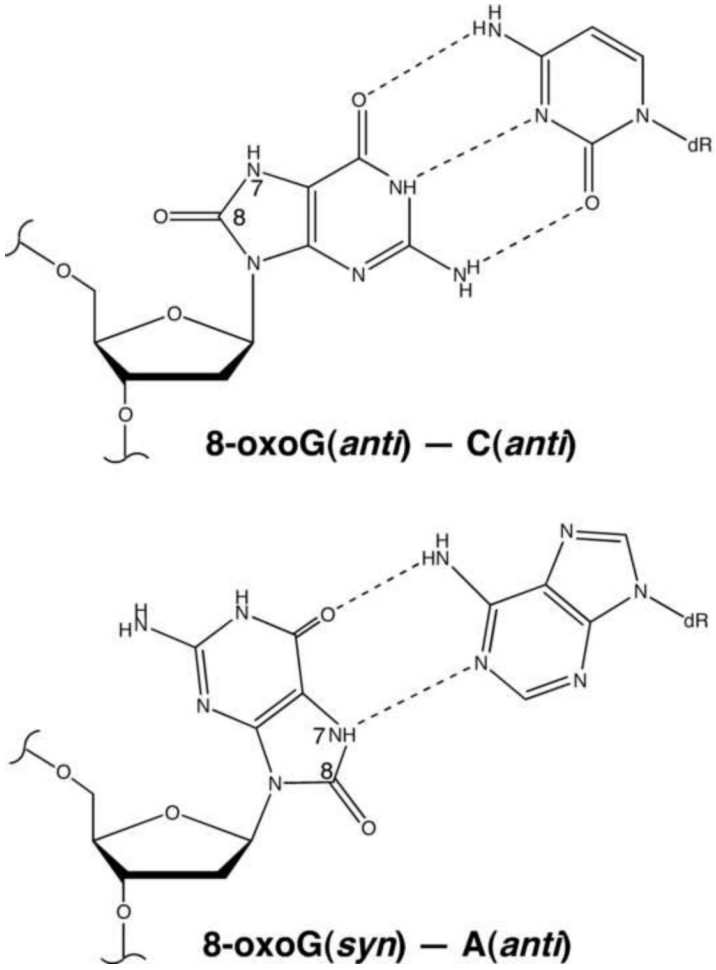

Fig. 2.

Ambivalent coding potential of 8-oxoG. In the *anti*-conformation, 8-oxoG forms a Watson-Crick base pair with cytosine. Oxidation at C8 of guanine results in a carbonyl at C8 and protonation of N7. This alters the hydrogen bonding capacity of the Hoogsteen edge of guanine converting N7 to a hydrogen bond donor that can base pair with adenine. Whereas the unmodified deoxyguanine glycosidic torsion angle preference is *anti*, isolated 8-substituted purine nucleosides favor a *syn*-conformation due to steric repulsion between the deoxyribose and O8 of the modified purine base [13,14].

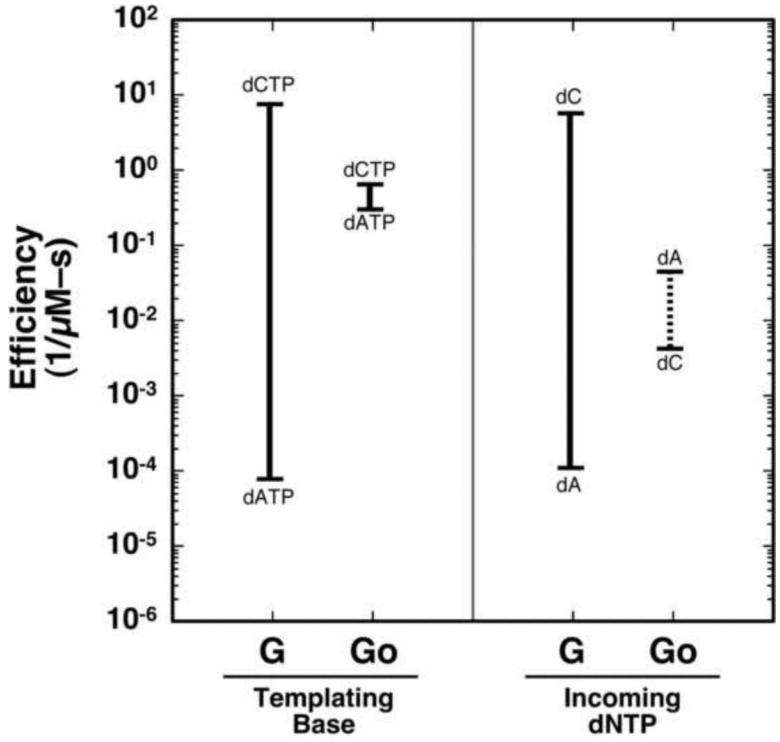

Fig. 3.

Discrimination plot for templating or insertion of 8-oxoG. Discrimination or fidelity is determined from the ratio of catalytic efficiencies for competing substrates (e.g., correct versus incorrect nucleotide insertion). Accordingly, plots of the log of these catalytic efficiencies illustrate the difference (magnitude of discrimination or fidelity; solid or dashed lines). The short horizontal lines mark the catalytic efficiencies for the indicated base pair. The vertical line connecting the alternate substrates is a measure of discrimination or fidelity; the longer the line the greater the discrimination or fidelity. For pol β, insertion of dATP opposite guanine (G) is ~10^5-fold lower than insertion of dCTP [20]. In contrast, when 8-oxoG (Go) is the templating base, discrimination is reduced to 2. This plot illustrates that this loss in discrimination is primarily due to the large increase in catalytic efficiency for insertion of dATP opposite 8-oxoG (i.e., the vertical line denoting the efficiency of dATP insertion is higher when the templating base is Go as compared to G; thus, the horizontal line denoting discrimination or fidelity is much shorter in the case of Go as compared to G). Likewise when the insertion of 8-oxodGTP (dGoTP) is considered, insertion opposite adenine is increased while insertion opposite cytosine is decreased to a large extent (~1300-fold). In this case, insertion of 8-oxodGTP is preferred opposite adenine relative to opposite cytosine ~10-fold (the dashed vertical line indicates that mispair formation is preferred over that of a Watson-Crick base pair).

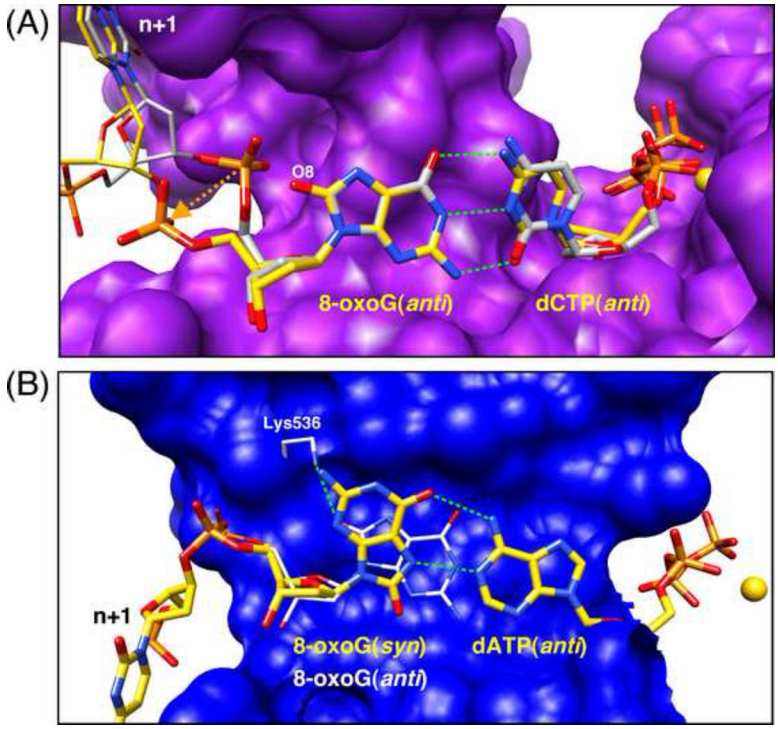

Fig. 4.

DNA polymerase modulation of the conformation of a templating 8-oxoG. (A) The structure of a ternary substrate complex of pol β with a templating guanine (PDB ID 2FMP, gray carbons) [22] was superimposed with the ternary complex structure with a templating 8-oxoG(*anti*) templating nucleotide (PDB ID 1MQ3, yellow carbons) [19]. The complementary incoming correct nucleotides are also shown. The protein surface of the structure of pol β with the oxidized templating nucleotide is purple. The downstream templating nucleotides (n+1) are also shown. The orange sphere represents the nucleotide binding metal (Mg^{2+}). The carbonyl at C8 (O8) is accommodated by an ~180° flip in the phosphate backbone of the templating nucleotide. (B) The structure of a ternary substrate complex of T7 DNA polymerase (exo^{-}) with a templating 8-oxoG(*anti*) (PDB ID 1TK0, gray carbons) [24] was superimposed with the ternary complex structure of the K536A mutant (PDB ID 1ZYQ, yellow DNA carbons) [29]. The *syn*-conformation of 8-oxoG in the mutant structure exposes its Hoogsteen edge for base pairing with the incoming ddATP (yellow carbons). Lys536 is within hydrogen bonding distance to O8 of 8-oxoG and stabilizes the *anti*-conformation. The blue protein surface within 10 Å of 8-oxoG and the downstream templating nucleotide (n+1) of the structure of the mutant complex are shown. The orange sphere represents the nucleotide binding metal (Mg^{2+}).

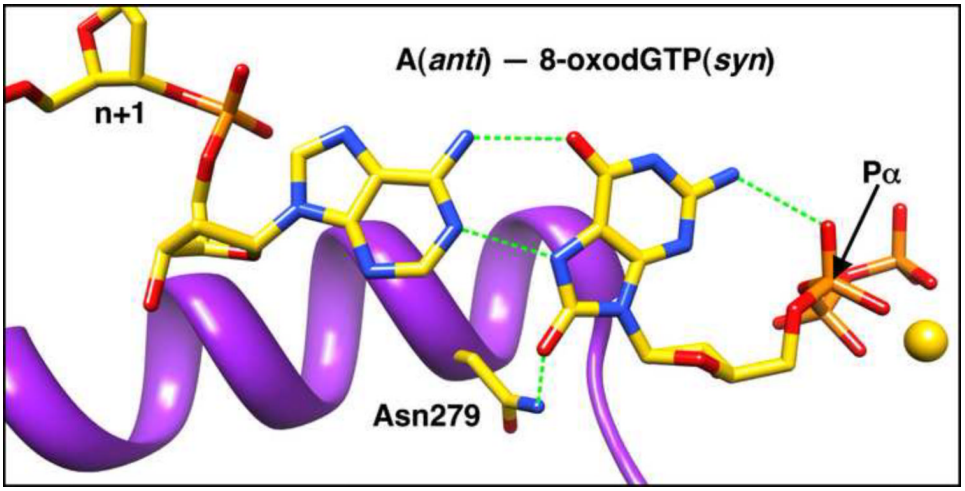

Fig. 5.

Structural features of the *syn*-conformation of 8-oxodGTP in the pol β active site. The nascent base pair is viewed (looking downstream) from the upstream DNA duplex. The sugar of the next templating base is also shown (n+1). The *syn*-conformation of 8-oxodGTP is stabilized through Hoogsteen hydrogen bonding with the templating adenine and a hydrogen bond with Asn279 of α-helix N (purple). Additionally, an intra-molecular hydrogen bond between N2 of 8-oxodGTP and a non-bridging oxygen on the α-phosphate could stabilize the *syn*-confomer.

Table 1

Summary of catalytic efficiencies for pol β-dependent utilization of 8-oxoG substrates [20].

Incoming Nucleotide	Templating Nucleotide	Catalytic Efficiency ($\mu M^{1}\ S^{-1}$)	Fidelity[a]
dCTP	G	6.6	—
dATP	G	7.0×10^{-5}	94,000
dCTP	Go	0.58	—
dATP	Go	0.28	2
dGTP	dC	5.1	—
dGTP	dA	1.0×10^{-4}	51,000
8-oxodGTP	dC	3.9×10^{-3}	—
8-oxodGTP	dA	4.1×10^{-2}	0.1

[a]Fidelity is calculated from the ratio of catalytic efficiencies for correct versus incorrect base pair formation (correct/incorrect). Discrimination is the reciprocal of fidelity (incorrect/correct).