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Fapy·dG Instructs Klenow Exo⁻ to Misincorporate Deoxyadenosine

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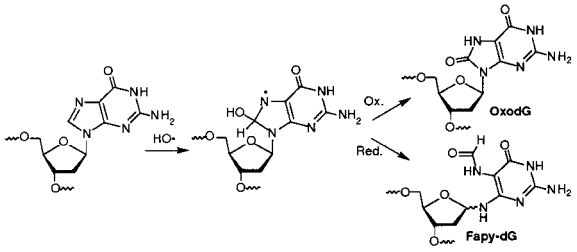
A variety of DNA lesions are formed when the biopolymer is exposed to oxidative stress, such as hydroxyl radical.[1] Inaccurate information transfer resulting from DNA lesions during replication can give rise to mutations. Examining the mutagenicity and repair of DNA lesions and providing a structural basis for these effects are of fundamental importance for understanding their carcinogenicity.[2] We wish to report the first examination of the effects of the formamidopyrimidine derived from deoxyguanosine (Fapy·dG) on a DNA polymerase.

Fapy·dG (*N*-(2-deoxy-α,*β*-D-erythropentofuranosyl)-*N*-(2,6-diamino-4-hydroxy-5-formamidopyrimidine)) and 7,8-dihydro-8-oxodeoxyguanosine (OxodG) arise from a common hydroxyl radical adduct (Scheme 1).[3] The formamidopyrimidines are favored under reductive conditions and during UV irradiation.[4] Fapy·dG is detected in almost 3 times greater amounts than OxodG in a human cancer cell line.[4b] Experiments with oligonucleotides containing OxodG incorporated at defined sites reveal that this lesion induces G→T transversions in vitro and in vivo.[5,6] Subsequent investigations have uncovered an elaborate repair process to protect cells against OxodG.[7] Studies of the effects of Fapy·dG on polymerase activity have utilized the *N5*-methylated analogue, which may present a significantly different hydrogen bonding pattern to an enzyme, and is not produced under biological conditions.[8] Investigations of the effects of Fapy·dG on polymerase and repair enzyme activity have lagged due to the absence of a method for chemically synthesizing oligonucleotides containing this lesion. We recently reported a method for synthesizing oligonucleotides containing an anomeric mixture of Fapy·dG at defined sites and now report the first characterization of its effects on a DNA polymerase, Klenow exo⁻.[9]

Scheme 1



Qualitative analysis of extension of a radiolabeled primer hybridized to a 36mer containing Fapy·dG shows that Klenow exo⁻ pauses when incorporating a nucleotide opposite Fapy·dG and extending the primer one nucleotide past the lesion (Figure 1). However, once polymerization proceeds past the lesion, no other pause sites are detected and full length material is observed. Other lesions affect DNA polymerase activity in a similar manner.[5b,8a,10]

Quantitative analysis of the effect of Fapy·dG on Klenow exo⁻ was determined by using Goodman's standing-start steady-state method.[11] Incorporation of dC opposite Fapy·dG is ~50-fold slower than that opposite dG in an otherwise identical template (Table 1). However, the efficiency of misincorporation of dA opposite the lesion (V_{max}/K_m) is increased ~20-fold compared to the template containing the native nucleotide. When compared to the rate at which Klenow exo⁻ incorporates dC opposite Fapy·dG, the standing start experiment indicates that dA will be incorporated opposite the lesion ~5% of the time (F_{ins}). Klenow exo⁻ misincorporates dA opposite Fapy·dG almost 900 times more frequently than when the template contains dG.

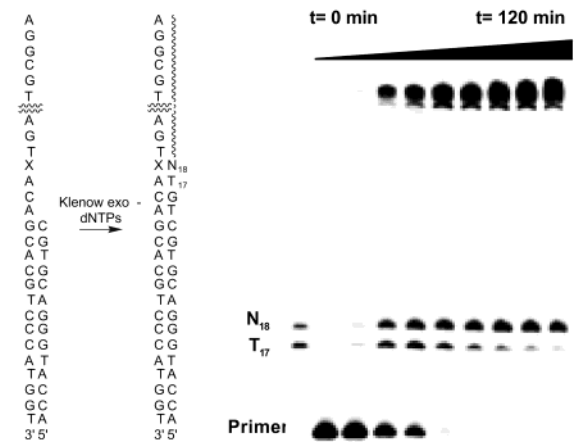
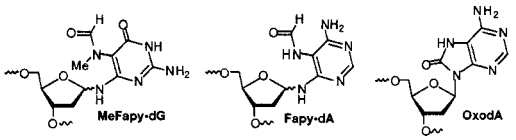


Figure 1. Qualitative analysis of Klenow exo⁻ primer extension opposite a template containing Fapy·dG. Conditions: [DNA] = 50 nM, [Klenow exo⁻] = 11 nM, [dNTPs] = 0.1 mM.



The structure of duplex DNA is very different from that of the complex involving polymerase, DNA, and substrate dNTP. However, duplex stability and polymerase fidelity do correlate in some systems.[12] Indeed, the proclivity for misincorporation of dA opposite Fapy·dG is reflected in the duplex melting thermodynamics of a dodecamer containing the same sequence (Table 2). The duplex containing a Fapy·dG:dA base pair is significantly more stable than the comparable material containing a dG:dA base pair. Furthermore, the duplex containing Fapy·dG:A is destabilized relative to that containing Fapy·dG:C to a much lesser degree than the respective duplexes containing dG opposite dA and dC.

Analysis (Table 3) of the extension of a complement past Fapy·dG also confirms the qualitative effect (Figure 1). Extension of a primer containing dC opposite Fapy·dG is ~70-fold slower than when dG is present in the template. Moreover, we also measured the rate of incorporation of the proper base (dA) when dA, which is the nucleotide most likely to be misinserted opposite Fapy·dG

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Table 1. Comparison of Nucleotide Incorporation Opposite dG and Fapy·dG by Klenow Exo⁻

3′-d(TGG TAC CCT GCA CGA CAX TGA CGT GCA ACT TGC GGA)
5′-d(ACC ATG GGA CGT GCT GT

↓ Klenow exo - dNTPs

3′-d(TGG TAC CCT GCA CGA CAX TGA CGT GCA ACT TGC GGA)
5′-d(ACC ATG GGA CGT GCT GTN

X	dNTP	V_{max} (%·min^{-1})	K_m (μM)	V_{max}/K_m (%·min^{-1}·M^{-1})	F_{ins}[a]
dG	C	5.1	5.2×10^{-3}	9.8×10^{8}	1.0
dG	A	14.0	257	5.4×10^{4}	5.5×10^{-5}
dG	G	6.1	74.2	8.2×10^{4}	8.4×10^{-5}
dG	T	4.7	248	1.9×10^{4}	1.9×10^{-5}
Fapy·dG	C	7.9	0.4	2.0×10^{7}	1.0
Fapy·dG	A	9.9	10.4	9.5×10^{5}	4.8×10^{-2}
Fapy·dG	G	1.0	26.2	3.8×10^{4}	1.9×10^{-3}
Fapy·dG	T	0.3	103	2.9×10^{3}	1.5×10^{-4}

[a] $F_{ins} = (V_{max}/K_m$, X =dG or Fapy·dG, dNTP = T, A, G, or C)/(V_{max}/K_m, X =dG or Fapy·dG, dNTP = C).

Table 2. Comparison of UV-Melting Thermodynamics of Duplexes Containing dG or Fapy·dG[a]

5′-d(TGC AGT XAC AGC)
3′-d(ACG TCA YTG TCG)
X = Fapy·dG or G
Y = A, C, G, or T

X:Y	T_M (°C)[b]	$\Delta G°_{298}$[c]	X:Y	T_M (°C)[b]	$\Delta G°_{298}$[c]
G:C	57.1	18.4	Fapy·dG:C	54.1	15.1
G:A	45.5	13.3	Fapy·dG:A	51.7	14.8
G:G	44.6	11.7	Fapy·dG:G	45.1	13.2
G:T	46.7	13.8	Fapy·dG:T	44.5	12.3

[a] Conditions: PIPES (pH 7.0), 10 mM; $MgCl_2$, 10 mM; NaCl, 100 mM. [b] [Duplex] = 2.2 μM. [c] Units: kcal/mol.

Table 3. Comparison of Primer Extension Past dG and Fapy·dG by Klenow Exo⁻

3′-d(TGG TAC CCT GCA CGA CAX TGA CGT GCA ACT TGC GGA)
5′-d(ACC ATG GGA CGT GCT GTY

↓ Klenow exo - dATP

3′-d(TGG TAC CCT GCA CGA CAX TGA CGT GCA ACT TGC GGA)
5′-d(ACC ATG GGA CGT GCT GTY A

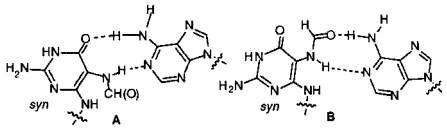
X	Y	V_{max} (%·min^{-1})	K_m (μM)	V_{max}/K_m (%·min^{-1}·M^{-1})	F_{ext}[a]
dG	C	1.1	2.0×10^{-3}	5.5×10^{8}	1.0
dG	A	0.9	374	2.4×10^{3}	4.4×10^{-6}
Fapy·dG	C	6.3	0.8	7.9×10^{6}	1.0
Fapy·dG	A	11.0	3.4	3.2×10^{6}	0.4

[a] $F_{ext} = (V_{max}/K_m$, X =dG or Fapy·dG, Y = A or C)/(V_{max}/K_m, X =dG or Fapy·dG, Y = C).

(Table 1), is at the 3′-terminus of the primer. We found that primer extension is barely affected ($F_{ext} = 0.4$), indicating that replication will not be compromised due to a decrease in polymerase fidelity. In contrast, extension past a dG:dA base pair is more than 200 000 times slower than when the native base pair (dG:dC) is present.

These experiments indicate that Klenow exo⁻ is ~80 million times more likely ($F_{ins} \times F_{ext}$) to produce a duplex containing dA opposite Fapy·dG than dG when replicating comparable templates. Furthermore, copying of the Fapy·dG containing template by Klenow exo⁻ is significantly more efficient than when MeFapy·dG is present, indicating that the hydrogen bonding pattern and/or shape presented by Fapy·dG, which is produced under biological conditions, is significantly different. Klenow exo⁻ fidelity is compromised to a much greater extent by Fapy·dG than it is by MeFapy·dG, Fapy·dA, or OxodA.[8a,13] These experiments indicate

Scheme 2



that Fapy·dG decreases the fidelity of Klenow exo⁻ to a comparable degree as does OxodG, which has received a great deal more attention.[5b]

In contrast to OxodG, the three-dimensional structures of duplexes containing formamidopyrimidines have not been determined.[14] The structural possibilities offered by the formamidopyrimidines are increased compared to those of the respective 8-oxopurines due to scission of the purines' imidazole rings and possible equilibration of anomers.[15] Although the configuration of Fapy·dG in DNA is unknown, experiments with Fapy·dA and configurationally stable C-nucleoside analogues of this latter lesion suggest that the β-anomer is likely responsible for the observations described above.[13a] Fapy·dG instruction of Klenow exo⁻ to incorporate dA can be rationalized by using two syn conformational isomers (Scheme 2). Isomer **A** is analogous to that observed for OxodG:dA base pairs.[14] Fapy·dG can also present a thymine-like hydrogen bonding pattern in the syn conformation by rotating the formamide group (**B**). Whether these base pairing schemes represent the interactions between Fapy·dG and dA remains to be determined. Regardless of the structural basis for the effect of Fapy·dG on Klenow exo⁻, these data indicate that the lesion is premutagenic and its formation could have significant consequences in vivo.

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