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The Formamidopyrimidines: Purine Lesions Formed in Competition With 8-Oxopurines From Oxidative Stress

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Conspectus

DNA is constantly exposed to agents that induce structural damage, from sources both internal and external to an organism. Endogenous species, such as oxidizing chemicals, and exogenous agents, such as ultraviolet rays in sunlight, together produce more than 70 distinct chemical modifications of native nucleotides. Of these, about 15 of the lesions have been detected in cellular DNA. This kind of structural DNA damage can be cytotoxic, carcinogenic, or both, and is being linked to an increasingly lengthy list of diseases.

The formamidopyrimidine (Fapy) lesions are a family of DNA lesions that result after purines undergo oxidative stress. The Fapy lesions are produced in yields comparable to the 8-oxopurines, which, owing in part to a perception of mutagenicity in some quarters, have been subjected to intense research scrutiny. But despite the comparable abundance of the formamidopyrimidines and the 8-oxopurines, until recently very little was known about the effects of Fapy lesions on biochemical processes involving DNA or on the structure and stability of the genomic material.

In this Account, we discuss the detection of Fapy lesions in DNA and the mechanism proposed for their formation. We also describe methods for the chemical synthesis of oligonucleotides containing Fapy•dA or Fapy•dG and the outcomes of chemical and biochemical studies utilizing these compounds. These experiments reveal that the formamidopyrimidines decrease the fidelity of polymerases and are substrates for DNA repair enzymes. The mutation frequency of Fapy•dG in mammals is even greater than that of 8-oxodGuo (8-oxo-7,8-dihydro-2'-deoxyguanosine, one of the 8-oxopurines), suggesting that this lesion could be a useful biomarker and biologically significant.

Despite clear similarities, the formamidopyrimidines have lived in the shadow of the corresponding 8-oxopurine lesions. But the recent development of methods for synthesizing oligonucleotides containing Fapy•dA or Fapy•dG has accelerated research on these lesions, revealing that the formamidopyrimidines are repaired as efficiently and, in some cases, more rapidly than the 8-oxopurines. Fapy•dG appears to be a lesion of biochemical consequence, and further study of its mutagenicity, repair, and interactions with DNA structure will better define the cellular details involving this important product of DNA stress.

Keywords

DNA damage; reaction mechanism; DNA repair; oligonucleotides synthesis

1. Introduction

DNA is constantly damaged by endogenously produced oxidizing species, and is also susceptible to exogenous agents such as ionizing radiation, chemical oxidants, and even sunlight (UVA irradiation). The native nucleotides undergo chemical modification to

produce more than 70 lesions, of which ~15 have been detected in cellular DNA. DNA lesions can be cytotoxic and/or carcinogenic. Furthermore, DNA damage is associated with an increasing number of diseases, as well as aging.¹ With such a large and increasing number of DNA lesions, it has not been possible for scientists to focus on all equally. Chemically modified purines, and in particular 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo, Scheme 1) have garnered a great deal of attention. 8-OxodGuo is often used as a biomarker for oxidative stress, and is thought by some to be highly mutagenic, despite the fact that it is formed in mammalian cells in lower quantities than other lesions (e.g. thymidine glycol, Tg) and is significantly less mutagenic than some lesions (e.g. spiroiminodihydantoin, Sp).^{2,3}

This Account focuses on the formation, biochemistry, and mutagenicity of the formamidopyrimidine (Fapy) lesions (Fapy•dA, Fapy•dG), which are chemically related to 8-oxodGuo and the analogous lesion derived from 2'-deoxyadenosine (8-oxodAdo). Much of the data concerning the formamidopyrimidines is compared to studies on the respective 8-oxopurines, for the reasons stated above. Substituted formamidopyrimidines are formed by alkylation at N7 of the purines, followed by hydrolysis. Such formamidopyrimidines are produced by aflatoxin and other electrophilic species, and their chemistry is described elsewhere.^{4,5}

2. Formation and Detection of Formamidopyrimidine Lesions

The formamidopyrimidines are believed to arise from β -cleavage of the formal C8-hydroxyl radical adduct (illustrated in Scheme 2 for dG oxidation). The nitrogen-centered radical (**1**) is a common precursor for the respective Fapy and 8-oxopurine lesions. The relative amounts of 8-oxodGuo and Fapy•dG (as well as the respective lesions derived from dA) are dependent upon the oxidation conditions in which **1** is produced.⁶ 8-OxodGuo formation requires further one electron oxidation of **1**, whereas Fapy•dG is formally generated by reduction following fragmentation of the radical. Hence, the formamidopyrimidines are of the same oxidation state as the native nucleotides from which they are derived. The Fapy lesions could in principle regenerate the native nucleotides following cyclization and dehydration, but this has not been observed.

Radical **1** was initially proposed to yield Fapy•dG via β -fragmentation. Pulse radiolysis studies indicated that **1** fragmented to the Fapy•dG amino radical precursor with a rate constant of $\sim 2 \times 10^5 \text{ s}^{-1}$.⁷ Recent computational studies suggest that the pathway from **1** to Fapy•dG may be less direct but the hydroxyl radical adduct is a precursor.⁸

The partitioning of **1** as a function of the reaction environment was consistent with studies of oxidatively damaged DNA. Free formamidopyrimidine and 8-oxopurine bases were analyzed by GC/MS following acid hydrolysis of the DNA and persilylation of the material in order to create volatile analytes.⁶ In accordance with the anticipated O₂ dependence (Scheme 2), formamidopyrimidine lesions are favored under anoxic conditions but 8-oxopurines are formed in greater amounts in the presence of O₂ when chromatin is γ -irradiated. Fapy and 8-oxopurine lesions are also formed in comparable quantities when DNA is irradiated at 254 nm, and both are formed in greater amounts than common pyrimidine lesions (e.g. Tg).⁹ However, as expected 8-oxodGuo is formed preferentially over Fapy•dG under oxidative electron transfer conditions.¹⁰ Although the merits of the GC/MS analysis method have been disputed, the effect of O₂ on the ratios of the lesions is consistent with the proposed mechanisms.¹¹ Nonetheless, LC/MS/MS is now the method of choice for quantifying lesions in damaged DNA.¹² Moreover, these data reveal that Fapy•dG is formed in comparable yields under many conditions to 8-oxodGuo, which is thought of as an important biomarker.

2'-Deoxyguanosine oxidation products are always observed in greater quantities than the respective products derived from dA despite comparable rate constants for hydroxyl radical reaction with the individual nucleosides.¹³ This suggested that 8-oxopurine and formamidopyrimidine lesions result from pathways in addition to hydroxyl radical addition to C8. Ravanat has attributed higher levels of dG oxidation products to mechanisms involving electron transfer and pyrimidine peroxy radicals.¹⁴

MSⁿ is a powerful tool for detecting DNA lesions. However, such instrumentation is not available to all investigators. In addition, in most experiments the DNA is enzymatically digested to nucleosides prior to MS analysis, which prevents determining lesion location.¹⁵ Identifying specific lesions at exact sites within DNA is potentially useful and a challenging problem.^{16,17} A sensitive chemical method for quantifying overall amounts of Fapy•dG and/or 8-oxodGuo was recently developed that takes advantage of these lesions' more facile oxidation than native nucleotides (Scheme 3).¹⁸ 8-OxodGuo (0.75 V) is more readily oxidized than dG (1.29 V), with the oxidation of Fapy•dG (1.05 V) of intermediate magnitude.¹⁹ Demonstration by Burrows that oxidized reactive intermediates are trapped by nucleophiles provided the impetus for developing a selective sensor for 8-oxodGuo and Fapy•dG.²⁰ The oxidized reactive intermediates were covalently trapped or "tagged" using a biotinylated derivative of spermine (2). By varying the oxidant one could tag both lesions (Na₂IrCl₆) or selectively 8-oxodGuo by using a weaker oxidizing agent (K₃Fe(CN)₆).¹⁸ The amount of lesion present was quantified using a streptavidin-horseradish peroxidase fluorescence assay after adsorbing the tagged DNA on the surface of a microtiter plate well.

3. Synthesis of oligonucleotides containing formamidopyrimidine lesions or their analogues at defined sites

Studies on 8-oxopurine lesions were greatly facilitated by their incorporation in oligonucleotides using solid phase synthesis methods.²¹ Fapy lesions exhibit an unusual property for a nucleoside that made synthesizing oligonucleotides containing them challenging. Because the glycosidic bond does not involve a nitrogen atom that is part of the aromatic heterocycle, formamidopyrimidine nucleosides readily epimerize and rearrange to their more stable pyranose forms (Scheme 4).²² Although rearrangement to the pyranose isomer cannot happen in DNA where the 5'-substituent is a phosphate, isomerization presented a challenge when designing a method for synthesizing oligonucleotides containing formamidopyrimidine lesions. It was anticipated that exposure of the lesions' 5'-hydroxyl groups during oligonucleotides synthesis would result in at least partial rearrangement to the unnatural pyranose isomers.

Syntheses were developed that avoid possible rearrangement by including the formamidopyrimidine lesions as components of dinucleotides (Scheme 5).²³⁻²⁵ Rearrangement during phosphoramidite synthesis was avoided by using an electron withdrawing nitro group as a latent formamide (e.g. 4). The nitro group was hydrogenated and formylated (formyl acetic anhydride) after the dinucleotide bond was formed. The original synthesis of Fapy•dG containing oligonucleotides required the use of reverse phosphoramidites, further complicating the synthesis.^{23,24} However, a second generation synthesis of Fapy•dG utilized 5, which was compatible with standard 3' → 5' solid phase oligonucleotides synthesis reagents (Scheme 5).²⁵ Regardless, the syntheses of the dinucleotides are lengthy and limit the oligonucleotides sequences that can be readily prepared.

Rizzo subsequently reported a method for synthesizing N5-alkylated formamidopyrimidine lesions that utilizes monomeric phosphoramidites whose syntheses are shorter than that of 5.²⁶ These researchers incorporated monomeric phosphoramidites into oligonucleotides

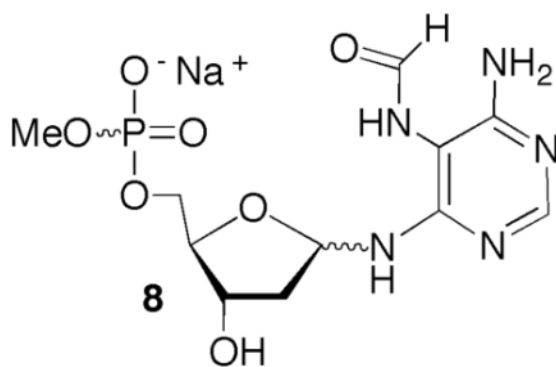
containing as many as 29 nucleotides via solid phase synthesis, and purified the desired furanose isomers by HPLC. This methodology is very attractive, and could greatly improve the synthesis of Fapy containing oligonucleotides if it is applicable to the parent lesions.

Recently, an improved synthesis of oligonucleotides containing Fapy•dG was reported by Francis Johnson (Scheme 6).²⁷ These researchers took advantage of the electronic effect of the 5-nitro group employed in the original research to prevent isomerization.^{23–25} Their innovation was to incorporate the nitropyrimidine within the chemically synthesized oligonucleotides via **6** and postsynthetically introduce Fapy•dG in the oligonucleotides (**7**) by reducing the nitro group and formylating the resulting amine. Each postsynthetic step was carefully monitored by mass spectrometry. In addition to not requiring a lengthy preparation of a dinucleotide phosphoramidite, this method enables the synthesis of any oligonucleotide sequence from a single precursor.

C-nucleoside and carbocyclic analogues of the formamidopyrimidine lesions have also been synthesized (Scheme 7).^{24,28,29} Oligonucleotides containing formamidopyrimidine analogues are readily synthesized using monomeric phosphoramidites via standard solid-phase synthesis methods because the molecules cannot rearrange or epimerize. These configurationally stable Fapy analogues are useful probes, particularly in templates for co-crystallization studies with base excision repair proteins, and are potentially useful as repair enzyme inhibitors.^{29–31}

4. Chemical Properties and Physical Effects on Duplexes of Formamidopyrimidine Lesions

The cleavage of the purines' imidazole ring to produce formamidopyrimidines releases the glycosidic nitrogen lone pair electrons from the aromatic systems. This has significant effects on the lesions' chemical properties, including increased deglycosylation rates compared to native nucleotides. The half-life for hydrolysis of the Fapy•dA nucleotide monophosphate (**8**) was ~4 days at 37 °C.³² Deglycosylation rate constants at 55 °C for **8** and Fapy•dA in an oligonucleotide were within experimental error of one another ($t_{1/2} \sim 21$ h), suggesting that the local environment had no effect on the reaction. Fapy•dG underwent deglycosylation even more slowly ($t_{1/2} > 500$ h at 55 °C) than Fapy•dA, and the glycosidic bond's stability was corroborated in similar studies on a protected form of the nucleoside.^{32,33}



Acyclic imine **3** (Scheme 4) may be an intermediate in the interconversion of Fapy anomers and its hydration would explain the large negative entropy of activation for deglycosylation.³² The availability of the nitrogen lone pair greatly facilitates epimerization at the formamidopyrimidine anomeric center and **8** equilibrates within 7 h at 25 °C in buffer

(pH 7.5).³² A protected form of Fapy•dG behaves similarly.³³ The distributions of anomers in duplexes were determined indirectly by taking advantage of endonuclease IV's (Nfo) selective cleavage of α -nucleotides in DNA.³⁴ Selective incision of DNA containing α -formamidopyrimidines was extrapolated from experiments using α - and β -C-Fapy•dA.³⁵ DNA containing α -C-Fapy•dA was incised ($k_{\text{cat}}/K_m = 4.1 \pm 0.2 \times 10^{-3} \text{ nM}^{-1}\text{min}^{-1}$), whereas β -C-Fapy•dA incision was negligible even in the presence of high concentrations of Nfo. When oligonucleotides containing mixtures of anomers of Fapy•dA or Fapy•dG were hybridized at 55 °C Nfo incision provided 14% as the upper limit of α -Fapy•dA content and <5% α -Fapy•dG in its respective duplex. Iterative hybridization of Fapy•dA containing oligonucleotides at 55 °C, followed by Nfo digestion indicated that this was an equilibrium mixture. Equilibration in the duplex was slower than that observed for **8**, possibly due to base pairing and other constraints.

Recently, Johnson prepared duplexes containing either α - or β -Fapy•dG by separating oligonucleotides containing individual anomers, hybridizing with excess complement at room temperature, and purifying by HPLC.²⁷ Both anomers of Fapy•dG reduced the UV-melting T_m of the duplexes relative to an otherwise identical one containing a dG•dC base pair. However, α -Fapy•dG was considerably more destabilizing ($\Delta\Delta G = -5.3 \text{ kcal/mol}$) than β -Fapy•dG ($\Delta\Delta G = -1.5 \text{ kcal/mol}$). A larger decrease in duplex stability ($\Delta\Delta G = -3.3 \text{ kcal/mol}$) was observed using a duplex containing >95% β -Fapy•dG that was synthesized by the dinucleotide phosphoramidite method.^{35,36} The differences may be ascribable to the distinct sequences and lengths of the duplexes used in the experiments. The earlier studies also indicated that duplexes containing Fapy•dG were more tolerant of mismatches than those containing the native nucleotide. For instance, duplexes containing Fapy•dG opposite dA or dG were more stable by 1.5 kcal/mol than the respective complexes containing dG. Fapy•dA had a similar effect on duplex stability. Compared to dA, Fapy•dA destabilized a 12-nucleotide duplex containing thymidine opposite it by ~2.6 kcal/mol.³⁷ Furthermore, duplexes containing purines opposite Fapy•dA were more stable than the respective complexes containing dA.

5. Repair of DNA Containing Formamidopyrimidine Lesions

Base excision repair (BER) is a primary mechanism for removing DNA lesions produced by oxidative stress. This is a multi-step, multi-enzyme process during which the damaged nucleotide is removed in the first step by a DNA glycosylase.³⁸ Some glycosylases possess a second active site, which is responsible for cleaving the abasic site formed upon removal of the modified nucleobase via a lyase mechanism. To initiate correct repair the DNA glycosylase must discriminate between base pairs containing the lesion opposite the nucleotide normally base paired with the undamaged nucleotide and those that comprise a promutagenic base pair (Scheme 8). Excision of the damaged nucleotide opposite the "incorrect" native nucleotide ("misrepair") removes any memory of the original nucleotide, resulting in the formation of a premutagenic base pair. Other glycosylases are responsible for incising incorrect native nucleotides opposite DNA lesions.³⁸

The DNA glycosylases responsible for removing damaged nucleotides tend to have broad substrate specificities. In *E. coli*, formamidopyrimidine DNA glycosylase (Fpg) acts on modified purines, including its namesake lesions.⁴¹ This glycosylase also acts as a lyase resulting in β,δ -elimination and the release of cleaved DNA containing 5'- and 3'-phosphate termini. Fpg efficiently excises Fapy•dA and does not discriminate between substrates containing different nucleotides opposite the lesion (Table 1).³⁹ The specificity constant for Fapy•dA excision opposite dT is approximately an order of magnitude greater than that for Fpg reaction with DNA containing an 8-oxodGuo:dC base pair.⁴⁰ Fpg also excises Fapy•dG more efficiently than 8-oxodGuo when the lesions are opposite dC (Table 1). In contrast to

Fapy•dA, the enzyme distinguishes between Fapy•dG and 8-oxodGuo that are base paired with dC or dA.^{40,41} Duplexes containing the lesions opposite dC are acted on by Fpg more than 17-times as efficiently than those containing dA opposite 8-oxodGuo or Fapy•dG.

These reactivity patterns were affirmed under single-turnover conditions in which dissociation of the protein is not a factor (Table 2).⁴² Fapy•dG was excised by Fpg ~5-fold more rapidly when it was opposite dC than when paired with dA. The selectivity for incision of 8-oxodGuo paired with dC versus dA was more than 30. Analysis of Fapy•dA incision by Fpg under single-turnover conditions also showed that the lesion was very efficiently excised but the enzyme paid no heed to the identity of the opposing nucleotide. Fapy•dA, Fapy•dG, and 8-oxodGuo are treated qualitatively differently by hOGG1, the functional human homologue of Fpg. hOGG1 hydrolyzes the glycosidic bond in Fapy•dA much less efficiently than does Fpg but it too does so indiscriminately with respect to the opposing nucleotide. Fapy•dG and 8-oxodGuo are cleaved much more rapidly by hOGG1, and the enzyme recognizes DNA containing Fapy•dG:dC almost 50-times more effectively than it does an otherwise identical duplex containing a dA opposite the lesion. Discrimination against 8-oxodGuo:dA is so great that glycosylase activity is not detected, whereas the lesion is incised with comparable efficiency as Fapy•dG when paired with dC.

The preference for Fapy•dG versus Fapy•dA is reversed by the yeast glycosylase, Ntg1 (Table 2).⁴² Ntg1 incises the latter between ~30 and 150 times more efficiently, but the enzyme shows little selectivity with respect to opposing nucleotide for either lesion. In contrast to hOGG1 and Fpg, Ntg1 does not incise 8-oxodGuo opposite any nucleotide. The selectivity by Ntg1 for formamidopyrimidine lesions over 8-oxodGuo is consistent with the enzyme's similar substrate specificity to endonuclease III (Nth).³⁸ Nth typically recognizes pyrimidine lesions. It also incises Fapy•dA and Fapy•dG, but not 8-oxodGuo. Although it does not strongly discriminate between substrates with different opposing nucleotides, it preferentially acts on duplexes containing purines opposite formamidopyrimidines.⁴³ Endonuclease VIII (Nei) is another glycosylase, which recognizes pyrimidine lesions that also incises Fapy•dA and Fapy•dG. Nei preferentially incises Fapy•dA over Fapy•dG, recognizes both lesions more efficiently than does Nth, and also exhibits a preference for substrates containing opposing purines. Fapy (but not 8-oxopurine) incision by Nth and Nei may be a reflection of their chemical structures, which can be categorized as homo-pyrimidines.

Indiscriminate incision by various glycosylases of duplexes containing different native nucleotides opposite some of the lesions could indicate that the respective molecules threaten genomic stability. Alternatively, repair enzymes may incorrectly read some promutagenic base pairs in a test tube because they are not formed in a cell. For instance, 8-oxodGuo does not induce polymerases to misincorporate dT and dG opposite it in cells, yet the lesion is most efficiently excised by Fpg from duplexes containing it base paired with these nucleotides.^{41,44,45} Hence, one should correlate base excision repair enzyme selectivity with polymerase enzyme activity and mutagenicity studies.

X-ray crystallography provides an explanation for how the enzymes select for the correct base pair. Base excision repair enzymes typically bind the DNA lesion in an extrahelical (flipped out) conformation. Fpg and hOGG1 bind flipped out *syn*-8-oxodGuo and utilize hydrogen bonding to the N7-hydrogen in the lesion to distinguish it from the undamaged native nucleotide.^{46,47} Discrimination against a promutagenic base pair containing dA opposite the lesion is achieved by utilizing an Arg to hydrogen bond to dC but not dA. The estranged dC is also hydrogen bonded to Arg in a complex in which Fpg recognizes a cFapy•dG:dC base pair.³⁰ However, cFapy•dG is bound in the *anti*-conformation resulting in a different set of hydrogen bonding interactions with Fpg than 8-oxodGuo.

The ability of Fpg to distinguish between 8-oxodGuo and Fapy•dG base paired with dA or dC, is complemented by an enzyme that removes the incorrect native nucleotide opposite the lesions. The mismatch repair protein, MutY selectively removes dA when it is opposite dG or 8-oxodGuo.⁴⁸ MutY, Fpg, and a third enzyme, MutT, which acts on nucleotide triphosphates, comprise the “GO” family of base excision repair enzymes.³⁸ In contrast to the glycosylases that incise DNA lesions, MutY extrudes dA from the duplex and hydrolyzes its glycosidic bond.⁴⁹ MutY incises dA opposite Fapy•dG ($k_{\text{obs}} = 4.2 \pm 0.5 \text{ min}^{-1}$) more efficiently than from a dG:dA ($k_{\text{obs}} = 1.6 \pm 0.2 \text{ min}^{-1}$) base pair but less so when 8-oxodGuo ($k_{\text{obs}} = 16.0 \pm 2.0 \text{ min}^{-1}$) is present.⁴⁰

6. Formamidopyrimidine Lesions' Effects on Polymerase Enzymes and Their Mutagenicity in Cells

Prior to the independent synthesis of oligonucleotides containing Fapy•dA and Fapy•dG or their analogues, the effects of formamidopyrimidines on polymerase fidelity were extracted from experiments in which DNA was randomly oxidized or methylated (followed by alkaline hydrolysis to produce N-methylformamidopyrimidines).⁵⁰ The effects of Fapy lesions on polymerases were inferred from correlations between mutation spectra and overall quantities of the respective lesions as determined by mass spectrometry. Such experiments lack chemical precision. In addition, extrapolating results concerning N-methylformamidopyrimidines to Fapy•dA and Fapy•dG is risky because the molecules present different base pairing possibilities.

Synthetic templates containing Fapy•dA or Fapy•dG flanked by a 5'-thymidine (dT) and 3'-dA were used to examine their effects on the Klenow exo^- fragment of DNA polymerase I from *E. coli*, a processive enzyme that is often employed in studies on modified nucleotides (Table 3). “Correct” nucleotides (dT, dC respectively) were incorporated opposite Fapy•dA and Fapy•dG moderately more slowly than they were opposite the corresponding native nucleotides.^{36,37} The incorporation efficiency ($V_{\text{max}}/K_{\text{m}}$) of dT opposite Fapy•dA was ~30% that of the native nucleotide.³⁷ 2'-Deoxycytidine was incorporated ~50-times less readily opposite Fapy•dG by Klenow exo^- than it is opposite dG (Table 3), and the resulting complex is extended ~80-fold less rapidly than the native one.³⁶ This blocking effect is small compared to that of an abasic site (AP).⁵¹

Polymerase fidelity also decreased when acting on templates containing the formamidopyrimidine lesions. The misincorporation frequency ($F_{\text{ins}} = (V_{\text{max}}/K_{\text{m}})_{\text{Incorrect}} / (V_{\text{max}}/K_{\text{m}})_{\text{Correct}}$) for dA by Klenow opposite Fapy•dA increased ~5-fold compared to when the undamaged, native nucleotide is in the template.³⁷ However, this still accounts for less than 2% of the incorporation events opposite the lesion. Similar studies on Fapy•dG revealed a greater increase in dA misincorporation opposite the lesion compared to the native nucleotide, but the F_{ins} was dependent upon the 3'-adjacent nucleotide. 2'-Deoxyadenosine was misincorporated opposite Fapy•dG less than 1% of the time when the lesion was present in the 5'-T-Fapy•dG-T sequence.⁴⁴ However, the F_{ins} approached 5% in 5'-T-Fapy•dG-A (Table 3), and subsequent extension was only ~2-fold less efficient than when dC was incorporated opposite the lesion.³⁶

The results of replication studies in *E. coli* using the restriction endonuclease and postlabeling (REAP) method aligned with the lower range of F_{ins} in Klenow experiments.^{44,52} In REAP experiments the lesion is strategically introduced at a defined site within a single-stranded plasmid, and the quantity and sequence of progeny plasmids is analyzed. The bypass efficiency, which is a measure of how readily a lesion containing template is replicated was determined by comparing the quantity of progeny from cells transfected with the plasmid containing the lesion to those from plasmid containing the

native nucleotide. Fapy•dG bypass efficiency was consistently approximately one-third lower than that of 8-oxodGuo in 4 sequence contexts.⁴⁴ Furthermore, the bypass efficiency of 8-oxodGuo responded positively to SOS-induction of bypass polymerases, but had no statistical effect on Fapy•dG, suggesting that these enzymes are not involved in the replication of templates containing the latter. Fapy•dG was also less mutagenic than 8-oxodGuo in all 4 sequences examined. 2'-Deoxyadenosine was the only nucleotide misincorporated opposite either lesion above background levels and never reached 2% in sequences containing Fapy•dG. In contrast, the levels of 8-oxodGuo → dT transversions resulting from bypass of 8-oxodGuo ranged from 3.1 – 9.8%.

In contrast, Fapy•dA and 8-oxodAdo exhibited no mutagenicity in *E. coli* or mammalian cells.⁵³ However, Fapy•dG → T transversions were as high as 29.6% when using the vector containing the 5'-T-Fapy•dG-T in simian kidney COS-7 cells. The mutation frequency was ~4-fold higher in this sequence than in 5'-T-Fapy•dG-A. In addition, dA was incorporated opposite Fapy•dG ~25% more frequently in both sequences than when 8-oxodGuo was present in the template. This was a striking observation considering that 8-oxodGuo is regarded to be a highly mutagenic DNA lesion.

Premutagenic base pairs also result from incorporation of damaged nucleotides into DNA by polymerases.⁵⁴ In the case of 8-OxodGuoTP MutT cleanses the nucleotide pool by hydrolyzing the phosphate.⁵⁵ Fapy•dGTP is accepted as a substrate by Klenow fragment resulting in Fapy•dG ($V_{\max}/K_m = 1.2 \pm 0.5 \times 10^4 \text{ M}^{-1}\text{min}^{-1}$) incorporation opposite dC ~1,600-times less efficiently than is dGTP ($V_{\max}/K_m = 2.0 \pm 0.3 \times 10^7 \text{ M}^{-1}\text{min}^{-1}$).⁵⁶ Klenow misincorporates the lesion opposite dA 1 out of every 250 events ($V_{\max}/K_m = 4.8 \pm 1.6 \times 10^2 \text{ M}^{-1}\text{min}^{-1}$). These data suggest that if Fapy•dGTP is present at 1% of the concentration level of dGTP, Fapy•dG will be incorporated opposite 1 out of every 2.5×10^7 dA's, resulting in ~100 Fapy•dG-dA base pairs per cell. Although there is no evidence Fapy•dG incorporation occurs in cells via this pathway, MutT is unlikely to prevent it. Fapy•dGTP ($k_{\text{cat}}/K_m = 3.8 \pm 1.3 \times 10^2 \text{ M}^{-1}\text{s}^{-1}$) hydrolysis by MutT is more than 100-fold slower than is dGTP ($k_{\text{cat}}/K_m = 4.3 \pm 0.9 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$) and greater than 10,000 less efficient than 8-oxodGuoTP ($k_{\text{cat}}/K_m = 5.4 \pm 0.7 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$).⁵⁶

7. Conclusions

The formamidopyrimidines have existed in the shadow of the respective 8-oxopurine lesions, despite being formed in comparable quantities when DNA is exposed to oxidative conditions and the belief that they are at least partly derived from common intermediates. The greater number of studies on 8-oxodGuo is most surely correlated with the availability of oligonucleotides containing the lesion via solid phase synthesis.²¹ The development of methods for synthesizing oligonucleotides containing Fapy•dA or Fapy•dG has accelerated research on these lesions. These investigations have revealed that the formamidopyrimidines are repaired as efficiently and in some cases more rapidly than the respective 8-oxopurines. Limited studies in mammalian cells indicate that Fapy•dG is slightly more mutagenic than 8-oxodGuo. Overall, the Fapy lesions present challenges to polymerases and repair enzymes that are comparable to those that arise from 8-oxopurines in DNA. A great deal still needs to be learned about the mutagenicity, repair, and effects of the formamidopyrimidines on DNA structure. However, Fapy•dG appears to be a lesion of biochemical consequence, and like 8-oxodGuo, should be a useful biomarker of oxidative stress.

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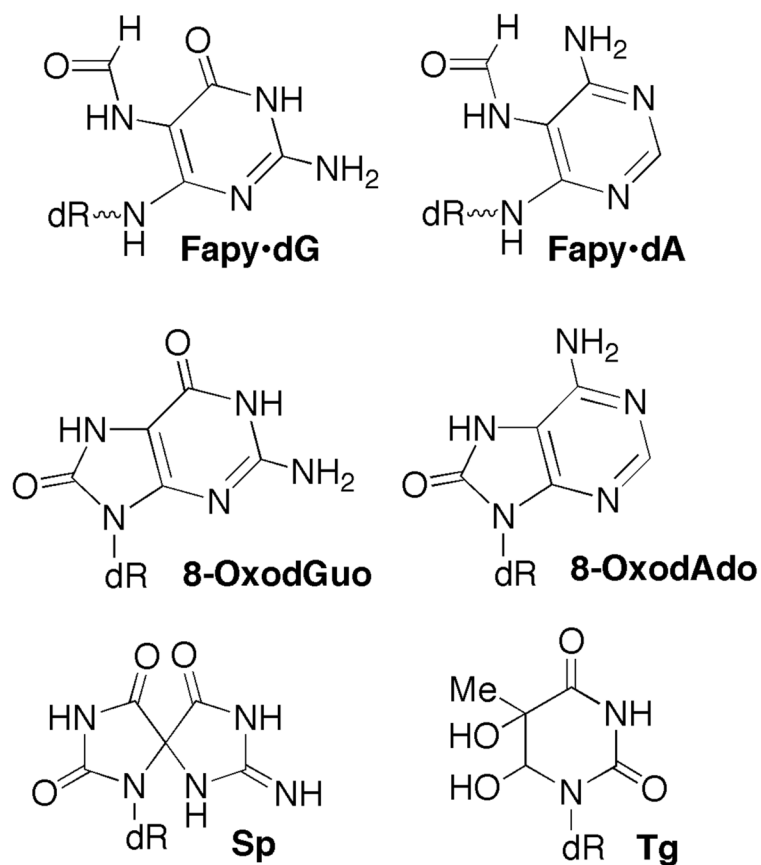
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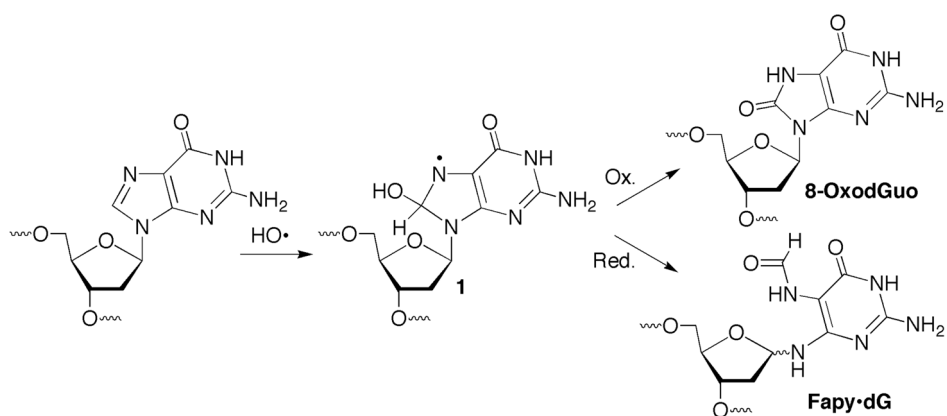
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Biography

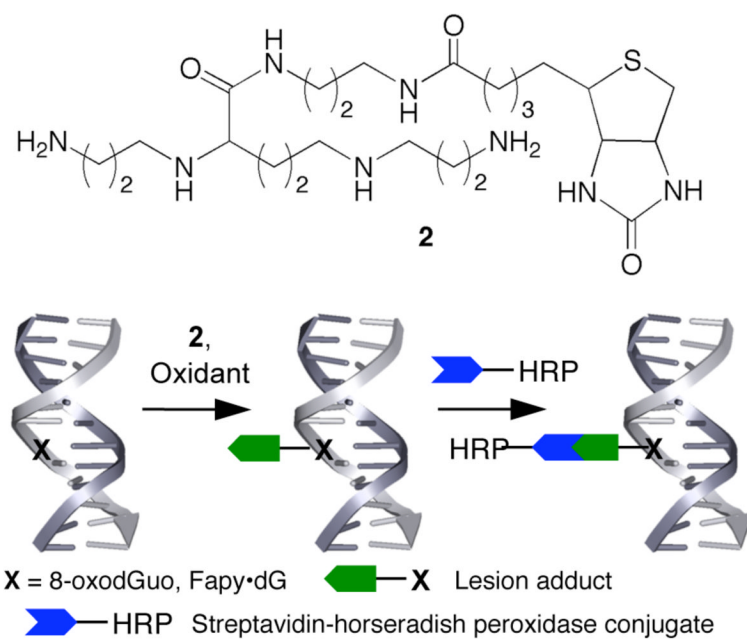
Marc Greenberg received his B.S. in Chemistry from New York University and B.E. in Chemical Engineering from The Cooper Union School of Engineering in 1982. He carried out undergraduate research with Professor David Schuster. After receiving his Ph.D. from Yale University in 1988 under the guidance of Professor Jerome A. Berson, he carried out postdoctoral research as an American Cancer Society fellow with Professor Peter B. Dervan at the California Institute of Technology. He began his independent career in 1990 at Colorado State University and moved to Johns Hopkins University in 2002 where he is a Professor of Chemistry. His research interests encompass fundamental and applied studies on nucleic acid chemistry and biochemistry.

**Scheme 1.**

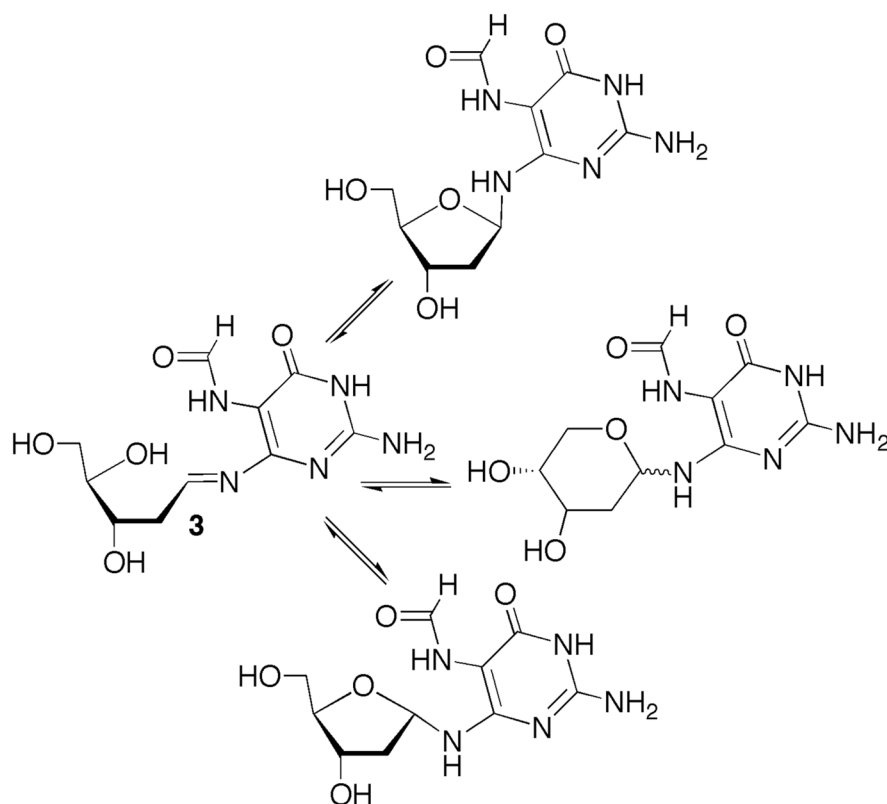
Examples of DNA oxidation products (dR = 2'-deoxyribose).

**Scheme 2.**

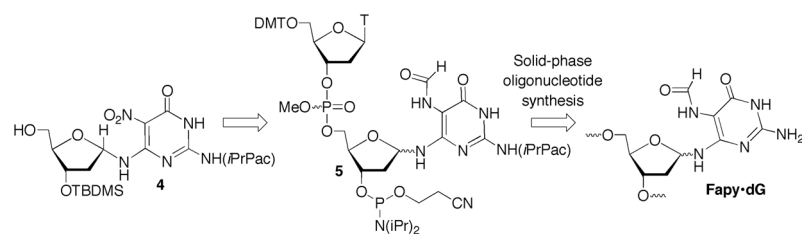
Formation of Fapy•dG and 8-oxodGuo from a common intermediate.

**Scheme 3.**

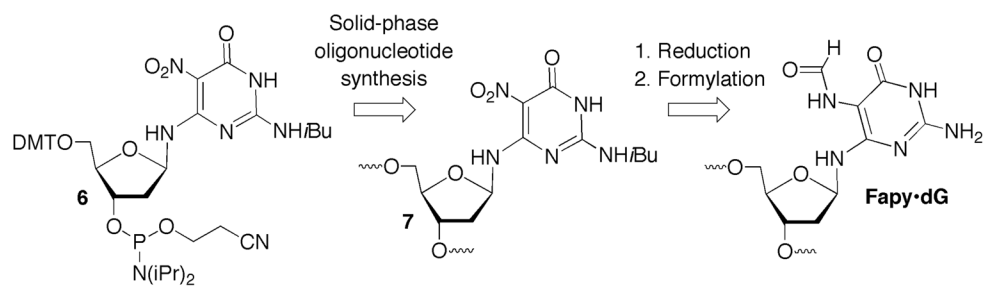
Fluorescence detection of Fapy•dG and/or 8-oxodGuo using **2**.



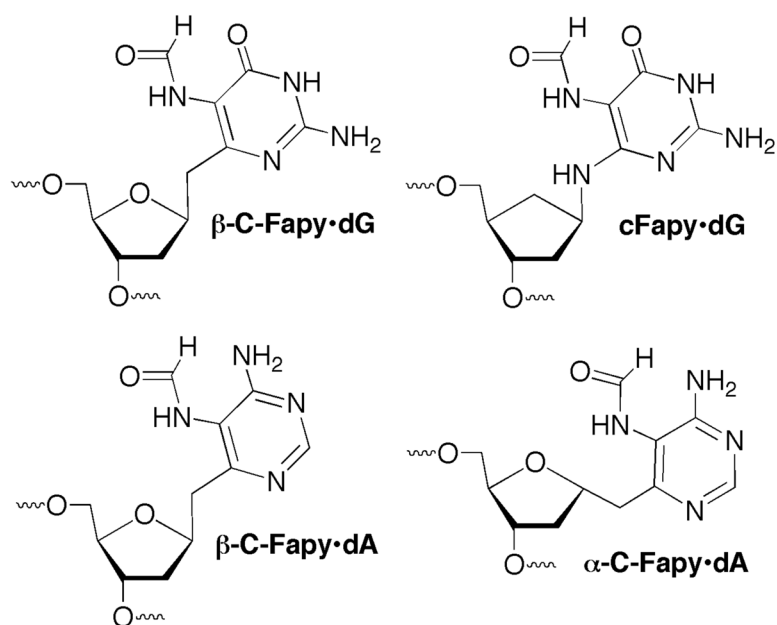
Scheme 4.
Isomerization of Fapy•dG nucleoside.



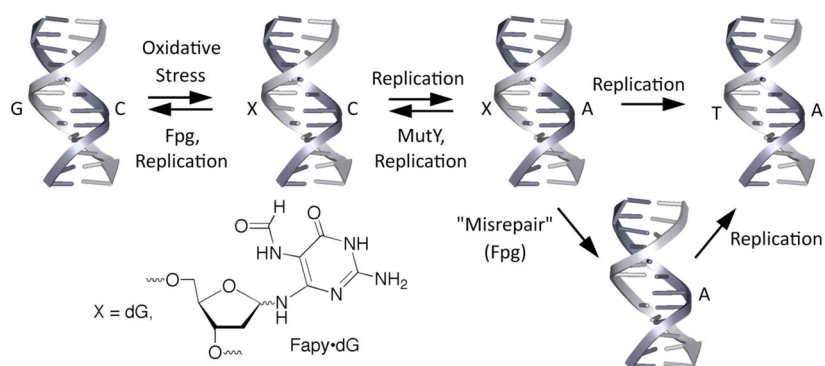
Scheme 5.
Synthesis of oligonucleotides containing Fapy•dG via a dinucleotide phosphoramidite.

**Scheme 6.**

Synthesis of oligonucleotides containing Fapy•dG via postsynthetic modification of a nitropyrimidine.



Scheme 7.
Configurationally stable analogues of Fapy lesions.



Scheme 8.
Formation of a promutagenic base pair by misrepair of a DNA lesion.

Table 1Fapy lesion excision by Fpg.^{39,40}

Base Pair	K_m (nM)	k_{cat} (min ⁻¹)	k_{cat}/K_m (nM ⁻¹ min ⁻¹)
Fapy•dA:T	1.16 ± 0.20	0.12 ± 0.05	0.10 ± 0.05
Fapy•dA:dA	3.34 ± 0.25	0.19 ± 0.13	0.06 ± 0.04
Fapy•dA:dG	4.63 ± 0.41	0.55 ± 0.02	0.12 ± 0.01
Fapy•dG:dC	2.0 ± 0.4	0.14 ± 0.004	0.07 ± 0.01
Fapy•dG:dA	4.7 ± 1.9	0.02 ± 0.01	0.004 ± 0.002

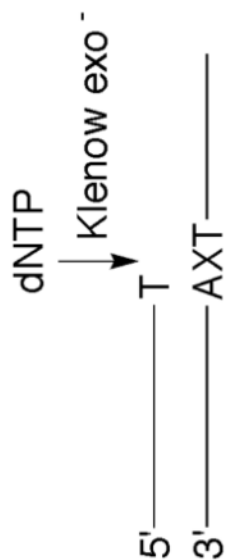
Table 2Incision of purine lesions under single turnover conditions.⁴²

Base Pair	Fpg k_g (min^{-1})	hOGG1 k_g (min^{-1})	Ntg1 k_g (min^{-1})
Fapy•dA:T	103 ± 7	0.21 ± 0.03	141 ± 8
Fapy•dA:dA	99 ± 7	0.34 ± 0.04	99 ± 2
Fapy•dA:dG	64 ± 6	< 0.02	69 ± 6
Fapy•dA:dC	84 ± 2	1.9 ± 0.3	77 ± 5
Fapy•dG:dC	86 ± 10	57 ± 6	0.9 ± 0.4
Fapy•dG:dA	17 ± 1	1.2 ± 0.1	2.4 ± 0.3
8-OxodGuo:dC	14 ± 1	50 ± 7	n.d.
8-OxodGuo:dA	0.6 ± 0.01	< 0.02	n.d.

n.d. = too slow to be determined.

Table 3

Nucleotide incorporation opposite Fapy lesions and native nucleotides by Klenow exo^- .^{36,37}



X	dNTP	V_{max} (%•min ⁻¹)	K_m (μM)	V_{max}/K_m (%•min ⁻¹ •M ⁻¹)	F_{ins}^a
A	T	4.9	0.13	3.8×10^7	1.0
A	A	18.7	147.7	1.3×10^5	3.4×10^{-3}
Fapy•dA	T	3.8	0.34	1.1×10^7	1.0
Fapy•dA	A	14.7	86.7	1.7×10^5	1.5×10^{-2}
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}
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}

^a $F_{ins} = (V_{max}/K_m, X = dG \text{ or Fapy}•dG, dNTP = T, A, G, \text{ or C}) / (V_{max}/K_m, X = dG \text{ or Fapy}•dG, dNTP = C, dA \text{ or Fapy}•dA, dNTP = T)$.