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Substrate Specificity of Fpg (MutM) and hOGG1, Two Repair Glycosylases

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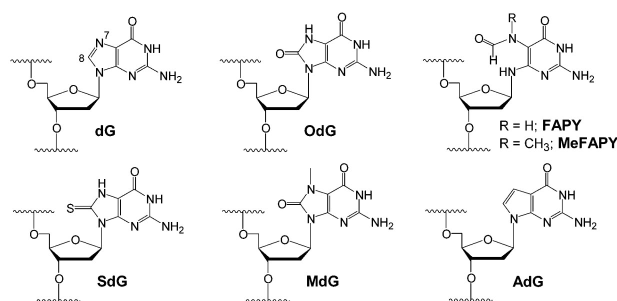
Reactive oxygen species (ROS) arise from chemical carcinogens,[1] radiation,[2] and normal metabolic respiration[3] and react most prominently[4] with 2′-deoxyguanosine (dG) to form 8-oxo-2′-deoxyguanosine (OdG).[5] Owing to its ability to form base pairs with dA,[6,7] OdG is mutagenic and leads to dG → T transversions in vivo.[8] To protect itself from OdG lesions, *E. coli* has evolved enzymes which remove the 8-oxoguanine base from OdG:dC base pairs and the adenine base from OdG:dA base pairs as well as hydrolyze OdGTP. Together, these three enzymes, Fpg (also known as MutM), MutY, and MutT, respectively, make up the cellular GO repair system which limits the abundance and promutagenicity of OdG.[9] In addition, human functional homologues of Fpg, MutY, and MutT have been characterized and designated hOGG1, MYH, and MTH1.[10]

Much research has focused on Fpg[11,12] and hOGG1[13,14] and their structure and substrate specificity. It has been shown that both enzymes are inactive on dG despite differing from OdG at only the N7 and C8 positions (see Supporting Information).[15] Crystal structures of both enzymes have revealed the presence of a hydrogen bond between a backbone carbonyl (Ser220[16] and Gly42[17] in Fpg and hOGG1, respectively) and the N7-hydrogen of OdG. Since there is no N7-hydrogen in dG, it has been argued that this hydrogen bond may be responsible for the observed substrate specificity; only dG derivatives with an N7-hydrogen can make the requisite enzyme interaction and properly fit into the active site. However, published biochemical studies suggest a more complex picture. These studies have shown that both Fpg and hOGG1 are also active with the naturally occurring imidazole ring opened lesion 2,6-diamino-4-hydroxy-5-*N*-methyl-formamidopyrimidine (MeFAPY),[15] even though it does not contain an equivalent N7-hydrogen. MeFAPY does contain an equivalent C8-oxygen however (which is also not present in dG) thus raising the possibility that this atom may be used for substrate recognition instead. To more fully address the mode by which both enzymes identify their substrate, we tested their activity with various dG/OdG analogues which varied at only the N7 and/or C8 positions. These analogues included 8-thio-2′-deoxyguanosine (SdG), 7-methyl-8-oxo-2′-deoxyguanosine (MdG), and 7-deaza-2′-deoxyguanosine (AdG).

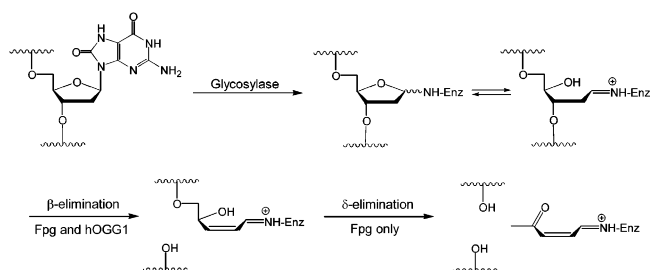
SdG,[18] which contains a N7-hydrogen but lacks a C8-oxygen, and MdG,[19] which contains a C8-oxygen but lacks a N7-hydrogen, have both been previously synthesized and incorporated into oligonucleotides. Similar procedures were used to incorporate both nucleosides into the 25 nucleotide long oligonucleotide 5′-dCATCGATACGATCTXCCTCTCTCTC-3′, where X was SdG or MdG. Oligonucleotides with the same sequence but where X was OdG or AdG, which lacks both a N7-hydrogen and C8-oxygen, were purchased from Eurogenetics Inc. or SigmaGenosys, respectively. All four oligonucleotides were then purified by gel electrophoreses and HPLC before being radiolabeled at their 5′-end and annealed with the complementary sequence 5′-dGAGAGAGAGGCAGATCG-



TATCGATG-3′. The duplexes were then treated with either Fpg or hOGG1 for 2 h before the addition of a 96% formamide stop solution.

Before addressing the results of these studies, it should be noted that Fpg and hOGG1 have both glycosylase and apurinic (AP) lyase activities; thus they not only remove the 8-oxoguanine base by breaking the glycosidic bond, but also cleave the backbone of the resulting AP site (Scheme 1). Interestingly while both enzymes catalyze β-elimination, detaching the 3′-oxygen from the AP sugar, only Fpg also catalyzes δ-elimination, detaching the 5′-oxygen as well.[15]

Scheme 1. Generalized Mechanism of Fpg and hOGG1[a]



[a] In the first step of the reaction, a terminal proline (Fpg)[20] or Lys249 (hOGG1)[21] attacks the 1′C, resulting in removal of the 8-oxoguanine base. A Schiff base forms, and β-elimination only (hOGG1) or β- and δ-elimination (Fpg) lead to backbone cleavage.

As can be seen in Figure 1, SdG, MdG, and AdG were all active with both Fpg and hOGG1. This is interesting since only SdG retains the N7-hydrogen that was deemed important in the crystal structures, while only MdG retains the C8-oxygen that seemed important in the biochemical studies with MeFAPY. These results lead us to believe that Fpg and hOGG1 may discriminate between dG and OdG, at least in part, not by the *presence* of a N7-hydrogen or C8-oxygen, but rather by the *absence* of a fully sp^2-hybridized N7. Not only is the absence of a fully sp^2-hybridized N7 the only steric and electronic similarity at the N7 and C8 positions of OdG, SdG, MdG, and AdG that differs from dG, but it has also been suggested previously that the lone pair of an sp^2-hybridized N7 would be in steric clash with the backbone carbonyl of Ser220 or Gly42 in Fpg[16] or hOGG1,[22] respectively.

To more fully understand the activity of Fpg and hOGG1 with the different analogues, the kinetic and thermodynamic parameters

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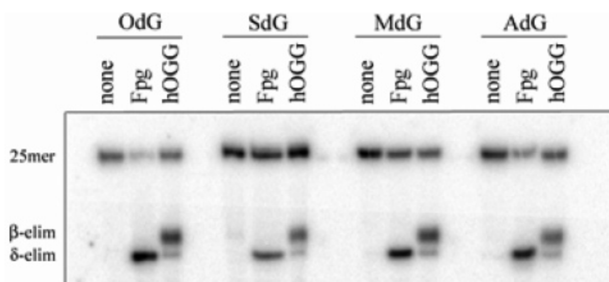

Figure 1. Activity of Fpg and hOGG1 with OdG, SdG, MdG, and AdG. All reactions contained 5 nM duplex and were incubated for 2 h at 37 °C.

Table 1. Kinetic and Thermodynamic Parameters for Reactions with Fpg and hOGG1[a]

	k_{cat} (min^{-1})	K_m (nM)	k_{cat}/K_m (10^{-3})
Fpg-OdG	4.0 ± 0.3	12 ± 3	330
Fpg-SdG	0.027 ± 0.002	225 ± 85	0.12
Fpg-MdG	0.031 ± 0.004	53 ± 8	0.58
Fpg-AdG	3.2 ± 0.4	83 ± 12	39
hOGG-OdG	2.6 ± 0.2	12 ± 4	220
hOGG-SdG	0.027 ± 0.001	151 ± 72	0.18
hOGG-MdG	0.015 ± 0.001	137 ± 37	0.11
hOGG-AdG	0.047 ± 0.001	126 ± 45	0.37

[a] k_{cat} and K_m were determined using single and multiple turnover conditions, respectively. Average k_{cat} and K_m ± standard deviation were determined using three or more individual experiments.

for each reaction were quantified; using single and multiple turnover conditions, both k_{cat} and K_m, respectively, were determined for each reaction. It is important that single turnover conditions are used to determine k_{cat} since this generally gives more accurate data.[23] As can be seen in Table 1, SdG and MdG were poorer substrates for both enzymes as compared to OdG, with roughly 100 times or lower k_{cat}. These results may be explained by the additional steric bulk of the C8-sulfur in SdG or N7-methyl in MdG; either of these groups could prevent proper orientation of the base in the active site. Though there are inherent electronic differences between OdG, SdG, and MdG, these differences do not fully account for the observed rates with Fpg and hOGG since these nucleotides show less than a 2-fold difference in acid-induced depurination after 45 min at 37 °C (see Supporting Information). AdG was a poorer substrate than OdG for hOGG1, but interestingly was almost as active as OdG with Fpg. This may be explained by the active site structure of each enzyme. It has been shown previously that hOGG1 has a much more rigid, preformed active site in comparison to Fpg.[16]

MdG, AdG, and SdG all had lower binding affinities with both enzymes as compared to OdG. Interestingly, MdG and AdG had lower K_m values with Fpg than with hOGG1, again possibly due to the more open active site of Fpg. It is possible the weaker binding of SdG with Fpg may be due, at least in part, to the presence of an alternate tautomer at the N7 and C8 positions. An enol-like tautomer at these sites would create a fully sp^2-hybridized N7 which could then generate steric repulsion with the previously mentioned backbone carbonyl. However solid evidence for such a tautomer is lacking and its presence is thought to be only transitory at best.[24,25]

It should be noted that recently published modeling and crystallographic studies with another naturally occurring dG lesion, 2,6-diamino-4-hydroxy-formamidopyrimidine (FAPY),[26] have indicated that imidazole ring opened bases may align in the active site of Fpg in a different orientation than an 8-oxoguanine base.[27,28] However since the OdG analogues tested in this study all retain an intact imidazole ring derivative, it is likely they align in the Fpg active site in a manner similar to OdG. At this time, no corresponding studies with hOGG1 have been published.

In conclusion, we have tested three OdG analogues with hOGG1 and Fpg and found all three to be active with each enzyme. This suggests broad substrate specificity at the imidazole ring of OdG and lends further insight into the mechanism of substrate recognition by two important repair glycosylases.

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Supporting Information Available: Oligonucleotide synthesis and purification; experimental details for Figure 1 and single and multiple turnover experiments; data and experimental details for acid degradations and control reactions with dG; raw data for Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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