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Spermine Participates in Oxidative Damage of Guanosine and 8-Oxoguanosine Leading to Deoxyribosylurea Formation

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7,8-Dihydro-8-oxo-2′-deoxyguanosine (8-oxoG, Scheme 1) is the primary oxidation product of guanosine and is a biomarker of cellular oxidative damage.[1] 8-OxoG is genotoxic if left unrepaired as it induces G→T transversion mutations.[2,3] Exposure of DNA to reactive oxygen species generates 8-oxoG from guanine residues,[4,5] and further oxidation of 8-oxoG can also occur due to the lower ionization potential of 8-oxoG relative to G.[6] Two of the resulting products, spiroiminodihydantoin (Sp) and guanidinodihydantoin (Gh), are highly mutagenic in vivo.[7] The structural characterization as well as the conditions for formation of Sp and Gh have been studied extensively within a broad range of oxidation systems. With guanosine, $^1O_2$ as well as photoinduced electron-transfer both yield Sp (at pH >7) and Gh (at pH <7).[8−10] One-electron oxidants, including Ir^{IV},[11,12] peroxynitrite ($ONOO^-$),[13] $CO_3^{\bullet-}$,[14] HOCl,[15] and peroxyl radical,[16] also produce Sp and Gh from G or 8-oxoG. 5-OH-OG has previously been implicated as the common intermediate, leading to either Gh or Sp depending on pH and base stacking.[8,12,17] An oxidized quinonoid species, OG^{ox}, is proposed as a precursor subject to nucleophilic addition to form 5-NuH-OG (Scheme 1)[18] by analogy to the urate oxidation pathway.[19,20]

The susceptibility of 8-oxoG to nucleophiles under oxidative conditions is showcased by the covalent cross-linking of a DNA repair protein to dsDNA containing a single 8-oxoG.[21] The observation that oxidized 8-oxoG could be trapped by a lysine in a protein context encouraged us to investigate other biological amines as nucleophiles for their ability to covalently modify oxidatively damaged DNA. In the nucleus, DNA is electrostatically courted by spermine, a ubiquitous polyamine with cellular and nuclear concentrations in the millimolar range.[22] Spermine participates in myriad cellular processes and has been referred to as radioprotective given its ability to mitigate radiation-induced DNA damage.[23−27] We hypothesized that spermine, based on its close association with cellular DNA, would trap oxidized 8-oxoG in the same manner as H_2O leading to Sp and Gh analogues. Under conditions similar to our DNA−protein cross-linking studies, DNA−spermine adducts were readily achieved in 75% yield from B-form self-complementary dsDNA, a Dickerson−Drew dodecamer,[28] containing a single 8-oxoG residue (5′-CGCOAATTCGCG-3′, where O is 8-oxoG). When the spermine concentration was decreased from 100 to 10 μM (equimolar with OG-oligomer), spermine still competed very effectively with solvent as a nucleophile, yielding 50% spermine adducts.

Spermine's proposed method of nucleophilic attack, as shown in Scheme 1, requires the amine to be able to access C-5 of the 8-oxoG base. Previous crystallographic analysis places spermine in the major groove of the Dickerson−Drew dodecamer,[29] although other analytical tools, including photoaffinity cleavage[30] and NMR,[31] show spermine to be more promiscuous with respect to its localization on dsDNA. While a dsDNA template gave high-yielding and reproducible spermine adducts[32] in the presence of a model oxidant, Na_2IrCl_6, a related single-stranded sequence was used in all further studies to simplify DNA adduct characterization by mass spectrometry and HPLC.

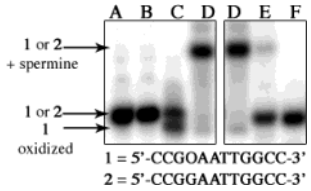
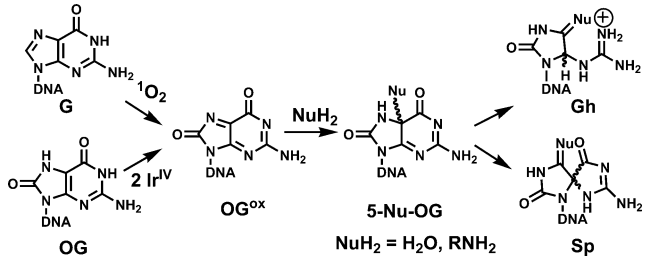


Figure 1. 20% Denaturing polyacrylamide gel electrophoretic study of adducts. (A) **1**, (B) **1** with spermine, (C) **1** with Ir^{IV}, (D) **1** with spermine and Ir^{IV}, (E) **2** with spermine, Rose Bengal, and $h\nu$, and (F) **2** only. Concentrations: [**1** or **2**] = 10 μM; [spermine] = 100 μM; [Ir^{IV} or Rose Bengal] = 100 μM.

Scheme 1. Fate of Guanosine and 8-Oxo-guanosine Oxidation



In a typical experiment, two ssDNA oligomers, 5′-CCGOAATTGGCC-3′ (**1**) and 5′-CCGGAATTGGCC-3′ (**2**), were analyzed independently for their ability to form adducts with spermine upon oxidation. Oxidation of radiolabeled **1** (10 μM) with Na_2IrCl_6 (100 μM) in the presence of excess spermine (100 μM) yielded an adduct at pH 7, visualized as a higher band in the PAGE analysis (Figure 1, lane D). A DNA−spermine adduct was also observed, although less efficiently, when radiolabeled **2** was oxidized by $^1O_2$ from photoactivated Rose Bengal (100 μM) at pH 7 (Figure 1, lane E). This experiment suggests that adducts can be obtained by oxidation of undamaged DNA, although to a lesser extent than from 8-oxoG oxidation.[32]

To establish that the adduct had formed specifically at the 8-oxoG base in **1**, the adduct was isolated from the gel and treated with 0.2 M piperidine; the resulting 5′-[^{32}-P]-labeled 3-nucleotide fragment was observed by PAGE (Figure 2, lane D[3]). The adduct was also heat labile since incubation at 90 °C for 30 min produced nearly complete reversion to a band resembling starting material. In a parallel experiment, the adduct was evaluated by HPLC and ESI-MS[32] where it was confirmed that the major product of the reaction was indeed one spermine molecule appended to the oxidized DNA 12-mer (**1** + spermine − 2H). Interestingly, the only other mass observed for the adduct (lane D) corresponded to a loss of 107 amu from the starting oligomer **1**.

HPLC analysis indicated that the adduct formation, as well as the nature of the adduct itself, was more complicated than

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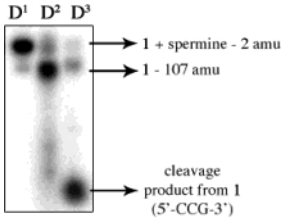
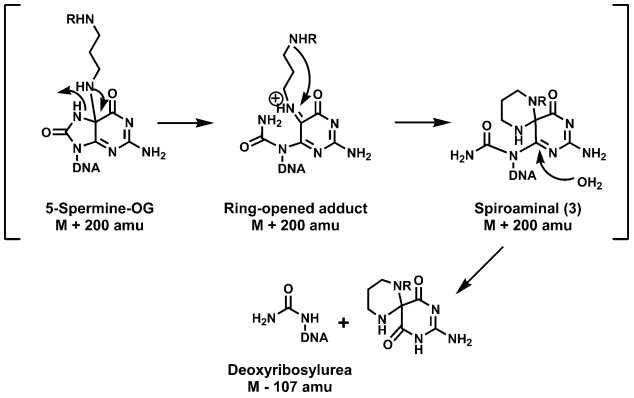


Figure 2. 20% Denaturing PAGE of gel-isolated adducts. (D^1) Isolated adduct from lane **D** in Figure 1, (D^2) heat-treated adduct (90 °C, 30 min), (D^3) alkali-treated adduct (0.2 M piperidine, 90 °C, 30 min).

Scheme 2. Proposed Mechanism for the Formation of Deoxyribosylurea from the Spiroaminal Derived from the DNA−Spermine Adduct[a]



[a] M = mass of parent oligomer **1**.

hypothesized in Scheme 1. The dialyzed reaction mixtures revealed two HPLC peaks for the adduct, suggesting diastereomers or constitutional isomers.[32] Consistent with the PAGE and MS observations, the spermine adducts slowly degraded to an HPLC peak slightly different from the starting oligomer. A kinetic study at 37 °C indicated a $t_{1/2}$ of 12 h.[32] The decomposition of the DNA−spermine adduct proceeds not by simple reversion but by a more destructive pathway involving base fragmentation; we assign the decomposition product as an oligomer containing a deoxyribosylurea lesion (parent oligomer − 107 amu). Taken together, the HPLC, MS, and PAGE data point to an unprecedented mechanism in which the DNA−spermine adduct is not a decorated 8-oxoG but a new heterocycle vulnerable to hydrolysis. We propose the mechanism depicted in Scheme 2 in which the 5-spermine-OG adduct leads to a spiroaminal (**3**) that undergoes slow hydrolysis to deoxyribosylurea.

To gain further support for this mechanism, model studies were conducted by oxidation of an 8-oxoG nucleoside with Na_2IrCl_6 in the presence of 1,3-diaminopropane or 1,4-diaminobutane.[32] While both diamines formed adducts in high yield, 1,3-diaminopropane led to an adduct with a chromophore indicative of 5-NuH-OG (where NuH = 1,3-diaminopropane), or its ring-opened isomer, followed by conversion to a spiroaminal and ultimate hydrolysis to a mass of OG − 107 amu. On the other hand, the adduct formed from 1,4-diaminobutane gave UV−vis and MS fragmentation data consistent with Sp (Nu = $N(CH_2)_4NH_2$).[32] These data support the facile six-membered ring formation of the spiroaminal **3** as the key adduct from spermine that slowly undergoes hydrolysis at C-4. Presumably, the 1,4-diamine adduct does not form the analogous seven-membered ring and therefore undergoes an acyl shift to an Sp-like product instead.

To our knowledge, this is the first characterization of deoxyribosylurea from the oxidation of a guanosine derivative. Deoxyribosylurea is a known degradation species of thymine oxidation via hydrolysis of thymine glycols.[33] In general, a urea lesion possesses mutagenicity and toxicity similar to an abasic site.[34] Ultimately, the appearance of an unlikely DNA damage product in our assessment of spermine's role in DNA oxidation is both unexpected and unprecedented. Spermine, widely held as radioprotective, has been shown in this system to facilitate injurious DNA damage by global disintegration of an 8-oxoguanosine nucleobase to an information-deficient urea lesion.

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Supporting Information Available: Experimental procedures, HPLC, ESI-MS, and kinetic data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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