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Copper generated reactive oxygen leads to formation of lysine–DNA adducts

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ABSTRACT

This work describes the addition of a lysine derivative to guanine base in a nucleoside, an oligonucleotide, and to a large DNA that occurs via oxidation by copper generated reactive oxygen species. Nucleophiles present during oxidation leads to the formation of adducts. In this work, 2′-deoxyguanosine is oxidized by copper generated reactive oxygen species in the presence of a lysine derivative, Nα-acetyl-lysine methyl ester. Under these conditions the guanidinohydantoin–lysine adduct is observed in a relative yield of 27% when compared to other guanine oxidation products. MS^2 strongly supports that lysine is added to the 5-position during the formation of guanidinohydantoin–lysine. A fourteen-nucleotide DNA duplex was oxidized under similar conditions. Digestion showed formation of the same guanidinohydantoin–lysine nucleoside. The reaction was then examined on a 392-nucleotide DNA substrate. Oxidation in the presence of the lysine ester showed adduct formation as stops in a primer extension assay. Adducts predominately formed at a 5′-GGG at position 415. Six of the seven sites that showed reaction greater than 3-fold above background were guanine sites. We conclude from this study that copper can catalyze the formation of DNA–protein adducts and may form in cells with elevated copper and oxidative stress.

1. Introduction

DNA is a reactive molecule that when modified gives rise to genetic mutations [1]. It is particularly susceptible to oxidation at guanine base to give a large abundance of lesions [2]. Many guanine modifications are repaired via an intricate repair system [3,4]. Oxidation of guanine by the cellular transition metals copper and iron is a common route for modification. Guanine modification is associated with increased cancer rates [5], chemotherapy drug resistance [6], and error prone polymerization [7]. Lack of repair or oxidative stress greater than the repair capacity will leave the underlying guanine modifications intact within a cell and lead to mutations. It has been shown that guanine modifications can be used as a determinant of oxidative stress in a cell [8].

Recent work showed that excessive oxidative stress in mitochondria leads to the formation of proteolysis-dependent DNA adducts [9]. These adducts are likely DNA–protein crosslinks though it is currently experimentally challenging to identify these types of modifications in cells. Burrows et al. showed that a crosslink between guanine base and lysine can occur when oxidized by photo-oxidants like rose bengal or riboflavin and by stoichiometric one-electron oxidants like Ir(IV) [10]. If these lesions occur in cells then their formation should be catalyzed by reactive oxygen species generated from copper or iron. It should be noted that copper-based reactive oxygen species does not generate the only other known form of DNA–protein crosslinks, a thymidine–tyrosine crosslink [11]. We therefore utilized a copper–hydrogen peroxide system to induce reactive oxygen species and determined if lysine–DNA adducts form.

Cellular oxidative stress is a complex phenomenon that occurs by many pathways. Oxidative stress occurs via one-electron, photo-initiation, singlet oxygen, hydroxyl radical, or two-electron oxidation pathways [12–14]. Copper is a ubiquitous cellular oxidant that reacts with the DNA backbone and the nucleobase [15]. Guanine base is particularly reactive to copper due to its favorable oxidation potential [16]. Copper mediates DNA oxidative lesions via hydroxyl radical (both metal bound or free) and through copper (III) complexes when peroxide is added [17]. The lesions induced by copper can lead to DNA strand breaks or mutagenesis [18]. Identification of guanine–lysine adducts from copper generated reactive oxygen would mean that DNA–protein crosslink formation is competitive with the many other oxidative DNA modifications that occur. Observation of guanine–lysine adducts by common transition metals would infer that these adducts may be formed in cells under high oxidative stress.

Two possible lysine–guanine adducts can be formed (Fig. 1 for structures) [19]. Loss of two electrons by 2′-deoxyguanosine leads to the formation 8-oxo-7,8-dihydro-2′-deoxyguanosine (8OG). 8OG has a more favorable oxidation potential than 2′-deoxyguanosine and once formed is preferentially oxidized. The net loss of four electrons has been shown to form many different lesions including addition by proteins and other nucleophiles [20]. The reaction can form either a spiroiminodihydantoin or a guanidinohydantoin (Gh) derivative (Fig. 1 for structure) [21–24]. Reaction with water has been shown to proceed via a 5-hydroxy intermediate that rearranges to the spiroiminodihydantoin

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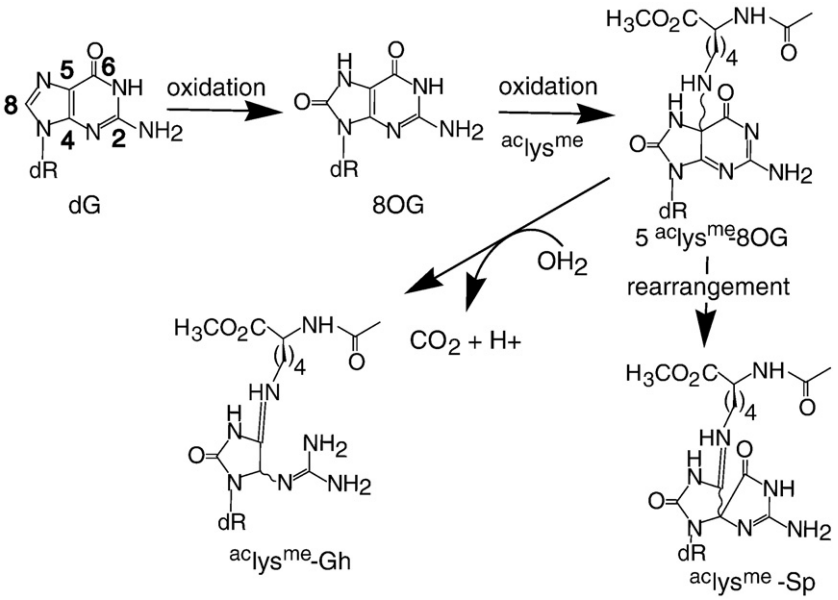

Fig. 1. Reaction pathway under investigation. 2′-deoxyguanosine (dG) is oxidized by copper generated reactive oxygen species. The reactive oxygen species react with dG to form 8-oxo-7,8-dihydro-2′-deoxyguanosine (8OG). Further oxidation can lead to the formation of a spiroiminodihydantoin–lysine derivative or a guanidinohydantoin–lysine derivative, $^{acy}Lys^{me}$–Gh. The reaction proceeds through an amine addition intermediate. It should be noted that both $^{acy}Lys^{me}$–Sp and $^{acy}Lys^{me}$–Gh can form lysine addition products at either position 5 or 8. The curved bond is placed at chiral centers formed in the reaction. Position numbers of guanine are listed in bold.

via C6-acyl migration to form a new carbon bond with C4 via low temperature NMR (Fig. 1) [25]. The Gh lesion is thought to form directly from the intermediate by loss of CO_2 at position six [26]. Protein-based nucleophiles have been observed to be covalently bound to DNA under oxidative conditions [10]. As a model of this complex reaction we will characterize guanine–lysine adduct formation in a nucleoside, an oligonucleotide, and a large DNA.

In this work we show that the oxidation of 2′-deoxyguanosine in the presence of a lysine derivative, Nα-acetyl-lysine methyl ester, forms adducts. Evaluation of the reaction by HPLC and tandem MS illustrates that the reaction produces the 5-Nα-acetyl-lysine methyl ester-Gh. A small fourteen-nucleotide duplex was oxidized in the presence and absence of Nα-acetyl-lysine methyl ester. Upon digestion, we observe a nucleoside product with the same mobility as 5-Nα-acetyl-lysine methyl ester-Gh. We then examined the reaction on a large 392-nucleotide DNA substrate. We show that adduct formation occurs at guanine base rich sites and stops replication using a primer extension assay.

2. Materials and methods

2.1. Copper-mediated lysine addition to dG

All experiments were carried out in at least duplicate. All chemical reagents were purchased from Sigma Aldrich unless otherwise noted. Buffers were purchased from Acros Organics. Data was quantified using 32 Karat HPLC software.

Copper-based experiments were mixed in a 2 mL glass HPLC tube. 2′-deoxyguanosine–lysine addition experiments were carried out by mixing the nucleoside, Nα-acetyl-lysine methyl ester, sodium ascorbate, hydrogen peroxide with $CuCl_2$ [800 μL; 250 mM sodium phosphate, 7.5 mM 2′-deoxyguanosine, 30 mM $^{acy}Lys^{me}$, 30 mM hydrogen peroxide, 3 mM sodium ascorbate, and 0.5 mM copper, pH8]. Reagent concentrations were changed as indicated. The samples were directly separated by injection of 5 μL into a Beckman Coulter System Gold with an autosampler and a diode array. An Agilent Zorbax SB-C18 [5 μm, 4.6, 150 mm] column was used for separation of the reaction products by a linear gradient of water, A, and acetonitrile, B, over several m [0% B for 1 m, 14% B over 10 m, 90% B over 6 m]. Absorbance was monitored at 260 nm. Controls excluded the listed reagent.

The MS-identification was performed directly on an HPLC fraction that contained 0.25% acetic acid and 15% acetonitrile. Analyses involved infusion directly into the instrument at 5 μL/m. The mass spectrometry was performed on a Thermo Fisher Scientific LTQ-FT, a hybrid instrument consisting of a linear ion trap and a Fourier transform ion cyclotron resonance mass spectrometer. The entire elutant was introduced into the LTQ-FT using the standard electrospray ionization source for the instrument with a spray voltage of 5 kV and a capillary temperature of 275 °C. Autogain control, AGC, was used and set at 500,000 with a maximum injection time of 1250 ms for FT-ICR full scans. Collision induced dissociation, MS/MS, was executed in the linear trap with an AGC setting of 10,000 and a maximum injection time of 500 ms. FT-ICR full scans were acquired in the positive ion mode at 100,000 resolving power at m/z 400. Mass accuracy errors were below 200 ppb for full scan and below 800 ppb for MS^n. The positive ion MS/MS experiments were performed simultaneously in the linear trap portion of the instrument using helium as a collision gas, isolation widths of 2 amu, a normalized collision energy of 35, a q value of 0.250 and an excitation time of 30 ms.

2.2. Reaction on DNA molecules

Oligonucleotides were purchased from Eurofins MWG Operon and used after a second desalting. The sequence of the 14-nucleotide DNA was 5′-TTCACTGGCCGTCG. The 14-nucleotide DNA and its complement were mixed in equimolar concentrations (250 μL, 100 μM, and 25 mM sodium phosphate pH8) and heated to 95 °C for 3 m. The sample was cooled to room temperature over 30 m. Adduct formation was performed in 75 μL volume as before except the final DNA concentration was 50 μM and the reaction time was 30 m. After the reaction, the duplex was purified by centrispin 10 columns (Princeton Separations) according to the manufacturer's protocol. Hydrolysis was accomplished by incubation of phosphodiesterase 1 and alkaline phosphatase (225 μL, 50 mM Tris, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 1 mM spermine, 0.1 U phosphodiesterase, 5 U AP, pH 9.3) for 30 m. The mixture was injected directly on the HPLC.

Primer extension experiments were carried out using a 392-nucleotide DNA substrate, prepared from pUC 19 plasmid vector (Takara Bio Inc.) by a 30 cycle PCR amplification (55 °C for 30 s, 75 °C