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Preparation of Nucleotide Advanced Glycation Endproducts—Imidazopurinone Adducts Formed by Glycation of Deoxyguanosine with Glyoxal and Methylglyoxal

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Abstract

An analytical procedure was developed for nucleotide advanced glycation endproducts formed by the reaction of glyoxal and methylglyoxal with deoxyguanosine under physiological conditions. For this, the imidazopurinone derivatives, 3-(2'-deoxyribose)-6,7-dihydro-6,7-dihydroxyimidazo[2,3-b]purin-9(8)one (dG-G) and 3-(2'-deoxyribose)-6,7-dihydro-6,7-dihydroxy-6-methylimidazo-[2,3-b]purine-9(8)one (dG-MG), were prepared. Authentic standard and stable isotope-substituted standard adducts were prepared and an isotopic dilution analysis assay methodology was developed using liquid chromatography with tandem mass spectrometry and optimized DNA extraction and nuclease digestion procedures. Analysis of dG-G, dG-MG, and the oxidative marker 8-hydroxydeoxyguanosine in the DNA of cultured human cells and mononuclear leukocytes showed that nucleotide advanced glycation endproducts are major markers of DNA damage in human cells.

Keywords

glycation; DNA; methylglyoxal; glyoxal; deoxyguanosine; 8-hydroxydeoxyguanosine; imidazopurinone

Introduction

Cellular DNA suffers continuous damage from oxidation, deamination, and other processes. 1 One such further process leading to DNA damage is glycation, particularly by the physiological reactive dicarbonyl glyating agents, glyoxal and methylglyoxal, forming nucleotide advanced glycation endproducts (AGEs). Although glycation damage to DNA is associated with mutagenesis and carcinogenesis, the mutagenic potential is low while the cellular protection against glycation is functioning.²

Dicarbonyl glycation of DNA may give rise to significant steady-state levels of nucleotide AGEs relative to the DNA oxidation marker 8-hydroxydeoxyguanosine (8-HOdG). The methylglyoxal-derived imidazopurinone nucleotide AGE, 3-(2'-deoxyribose)-6,7-

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Conflict of Interest

The authors declare no conflicts of interest.

dihydro-6,7-dihydroxy-6-methylimidazo-[2,3-b]purine-9(8)one (dG-MG), has been detected by a ^{32}P -post-labelling technique³ in cellular DNA, and N_2 -(1-carboxyethyl)-2-deoxyguanosine has been detected by liquid chromatography with tandem mass spectrometry (LC-MS/MS) in an alkaline digest of DNA glycated by methylglyoxal.⁴ In this study, we prepared authentic standard imidazopurine adducts, 3-(2'-deoxyribo-6,7-dihydro-6,7-dihydroxyimidazo[2,3-b]purin-9(8)one (dG-G) and dG-MG; Fig. 1), for use in an isotopic dilution analysis LC-MS/MS assay methodology for quantitation of the major nucleotide AGEs derived from glyoxal and methylglyoxal with deoxyguanosine.

Methods

Materials

2'-Deoxyguanosine monohydrate and glyoxal and methylglyoxal solutions (40%) were purchased from Sigma (Poole, Dorset, UK). U- ^{13}C , ^{15}N -2'-Deoxyguanosine (all >98% isotopic purity) was purchased from Cambridge Isotope Laboratories (Andover, MA).

Preparation of dG-G

A mixture of 2'-deoxyguanosine monohydrate (300 mg, 1.05 mmol) and glyoxal (40% solution in water, 185 μL , 92 mg, 1.59 mmol) in water (30 mL) was stirred for 4 days at room temperature. The solution was lyophilized to dryness. The nucleotide AGE product was then purified by reversed-phase high-performance liquid chromatography (HPLC) using a Waters 25 $\times$ 100 mm NOVAPAK C18 (Waters, Elstree, UK), 6- μm pore size cartridge and monitoring the eluate by absorbance at 254 nm. The mobile phase was 50 mM ammonium formate (pH 4.6) with a linear gradient of 0–5% acetonitrile over 30 min. Eluate fractions containing the product were collected, pooled, and lyophilized to dryness.

Preparation of dG-MG

A mixture of 2'-deoxyguanosine monohydrate (300 mg, 1.05 mmol) and methylglyoxal (40% solution in water, 253 μL , 120 mg, 1.67 mmol) in water (30 mL) was stirred for 4 days at room temperature. The solution was lyophilized to dryness and purified by reversed-phase HPLC as described above.

Results

dG-G

The major product isolated and purified from the reaction of glyoxal with deoxyguanosine was characterized by ^1H and ^{13}C NMR, high-resolution mass spectrometry, and UV and infrared absorbance spectroscopy. ^1H NMR (270 MHz, dimethylsulfoxide [DMSO]- d_6) yielded the following chemical shift δ_{H} (ppm) and coupling constant J (Hz) values: 8.93 (bs, 1H, $N^2\text{-H}$); 8.07 (s, 1H, 8-H); 7.36 (d, 1H, c-H, $J=6.10$); 6.59 (d, 1H, b-H, $J=7.34$); 6.23 (d, $\text{H1}'$, 1H, $J=7.03$); 5.58 (d, d-H, 1H, $J=6.72$); 5.42 (d, 3'-OH, 1H, $J=3.67$); 5.07 (t, 1H, 5'-OH, $J=5.51$); 4.97 (d, 1H, a-H, $J=7.32$); 4.44 (m, 1H, $\text{H3}'$); 3.92 (m, 1H, $\text{H4}'$); 3.63 (m, 2H, $\text{H5}'/\text{H5}''$); 2.35 (m, 1H, $\text{H2}''$). ^{13}C NMR (68 MHz, $\text{DMSO}-\text{d}_6$) gave the following chemical shift δ_{C} (ppm) values: 154.8 (C6); 154.6 (C2); 150.5 (C4); 135.6 (C8); 117.5 (C5); 87.6 (C4'); 84.0 (C-a); 83.6 (C-b); 82.8 (C1'); 70.6 (C3'); 61.5 (C5'). High-resolution mass spectrometry gave a molecular ion with m/z 348.0909 (calculated for $\text{C}_{12}\text{H}_{15}\text{N}_5\text{O}_6\text{Na}$, 348.0915). UV spectrophotometry yielded the following absorbance maxima at wavelength λ_{max} (nm) with extinction coefficient ϵ ($\text{M}^{-1}\text{cm}^{-1}$) values: $\lambda_{\text{max}}=249$ and $\epsilon=14,319\pm214$; and $\lambda_{\text{max}}=275$ and $\epsilon=6,787\pm84$. Infrared spectroscopy (Nujol) gave the following λ_{max} (cm^{-1}): 1713 $\text{V}_{\text{C=O}}$ (s); 1600–1553 $\text{V}_{\text{C=C}}$ (m); 1600 $\delta_{\text{N-H}}$ (m); 1331–1298 $\delta_{\text{O-H}}$ (s); 1229–

1050 V_{C-O} (s); 1331–1050 V_{C-N} (m); 1102–1078 V_{C-O} (s; C-O-C). The percentage yield was 87% (299 mg), based on deoxyguanosine.

dG-MG

The major product isolated and purified from the reaction of methylglyoxal with deoxyguanosine was characterized by 1H and ^{13}C NMR, high-resolution mass spectrometry, and UV and infrared absorbance spectroscopy. 1H NMR (270 MHz, DMSO- d_6) yielded the following chemical shift δ_H (ppm) and coupling constant J (Hz) values: 8.70 (bs, 1H, N^2 -H); 7.95 (s, 1H, 8-H); 7.18 (d, 1H, c-OH, $J=6.72$); 6.21 (d, 1H, b-OH, $J=3.67$); 6.12 (t, 1H, H1', $J=7.02$); 5.36 (d, 1H, d-H, $J=7.32$); 5.31 (d, 1H, 3'-OH, $J=4.27$); 4.97 (t, 1H, 5'-OH, $J=3.67$); 4.34 (m, 1H, H3'); 3.82 (m, 1H, H4'); 3.17 (m, 2H, H5'/H5''); 2.22 (m, 1H, H2''); 1.39 (s, 3H, a-CH₃). ^{13}C NMR (68 MHz, DMSO- d_6) gave the following chemical shift δ_C (ppm) values: 155.0 (C6); 154.1 (C2); 150.4 (C4); 135.4 (C8); 117.6 (C5); 87.6 (C4'); 87.2 (C-c); 84.6 (C-b); 82.8 (C1'); 70.6 (C3'); 61.6 (C5'); 21.0 (C-a). High-resolution mass spectrometry gave a molecular ion with m/z 362.1065 (calculated for $C_{13}H_{17}N_5O_6Na$, 362.1071). UV spectrophotometry yielded the following absorbance maxima at wavelength λ_{max} (nm) with extinction coefficient ϵ ($M^{-1}cm^{-1}$) values: $\lambda_{max}=250$ and $\epsilon=11,202$ 327; and $\lambda_{max}=275$ nm and $\epsilon=7,099\pm78$. Infrared spectroscopy (Nujol) gave the following λ_{max} (cm^{-1}): 1694 $V_{C=O}$ (s); 1602–1553 $V_{C=C}$ (m); 1602 δ_{N-H} (m); 1297 δ_{O-H} (s); 1230–1053 V_{C-O} (s); 1297–1053 V_{C-N} (m); 1096 V_{C-O} (s; C-O-C). The percentage yield was 62 (222 mg), based on deoxyguanosine.

Stable isotopic standards of dG-G and dG-MG: U- $[^{13}C, ^{15}N]$ -2'-deoxyguanosine (98% isotopic purity in each isotope) were used to prepare the isotopically labelled standards of dG-G and dG-MG.

Discussion

In the preparation of glycation adducts of deoxyguanosine with glyoxal and methylglyoxal, we found that imidazopurinone adducts were the major nucleotide AGEs formed. Similar reaction of deoxyguanosine with glyoxal and methylglyoxal under physiological conditions of 37 °C and pH 7.4 gave similar results.

The authentic standard adducts of dG-G and dG-MG, together with stable isotopomers, were used in an isotopic dilution LC-MS/MS assay for quantitation of these adducts in calf thymus DNA, glycated by glyoxal and methylglyoxal, and in DNA extracts of human cells—peripheral mononuclear leukocytes, human leukemia 60 cells, and hepatoma G2 cells. Analysis of dG-G, dG-MG, and the oxidative marker 8-hydroxydeoxyguanosine in DNA of cultured human cells and mononuclear leukocytes showed that nucleotide AGEs are major markers of DNA damage in human cells.

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FIGURE 1. Imidazopurinone nucleotide AGEs, (A) 3-(2'-deoxyriboseyl)-6,7-dihydro-6,7-dihydroxyimidazo[2,3-b]purin-9(8)one (dG-G) and (B) 3-(2'-deoxyriboseyl)-6,7-dihydro-6,7-dihydroxy-6-methylimidazo-[2,3-b]purine-9(8)one (dG-MG).