Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2017–2022

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

REACTION OF GUANOSINE WITH GLUCOSE UNDER OXIDATIVE CONDITIONS

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Received 14 April 1998; accepted 25 June 1998

Abstract: Incubation of glucose with guanosine under oxidative conditions at 37 °C or 70 °C leads to the formation of a main product, which can be detected by HPLC/DAD. The compound was isolated and identified as N^2-carboxymethyl-guanosine (CMG). To confirm the structure, CMG was also synthesized from glyoxal and guanosine.

INTRODUCTION

Formation of covalent adducts between potentially genotoxic substances and DNA is considered as a crucial step for development of genetic disorders.[1] It was found that there is relatively high correlation between the capability of a compound to form DNA adducts and its cancerogenic potency.[2] Therefore considerable efforts have been made to identify DNA modifications which are formed from potentially mutagenic and cancerogenic substances. Particularly guanosine residues are modified, e.g. by the attack of electrophils to the 2-NH_2-group.[3,4] More recently it was shown that guanosine can react also with carbohydrates such as glucose, ribose, or ascorbic acid resulting in the formation of covalently bound adducts. Several products have been isolated and identified as N^2-(glucosyl)-guanosine,[5] N^2-(1-carboxy-3,4,5-trihydroxypentyl)-guanosine (CTPG),[6,7] N^2-carboxyethyl-guanosine (CEG),[8,9] N^2-(1-carboxy-3,4-dihydroxybutyl)-guanosine,[10] N^2-(1-carboxy-3-hydroxypropyl)-guanosine,[11] or the analogous derivatives of guanine or 2-deoxyguanosine. In the presence of primary amines such as lysine or propylamine the DNA-sugar interaction is accelerated[12] and crosslink products between guanosine and the amine are formed.[10]

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0960-894X/98/$ - see front matter
PII: S0960-894X(98)00343-6

There is evidence that the reaction of sugars with DNA (DNA glycation) causes increased mutation rates in bacteria cells[13] and mammalian cells.[14] Furthermore it was suggested that nonenzymatic glycation of DNA contributes to age-related increase in DNA mutations[15] and diabetes-associated teratogenesis.[16]

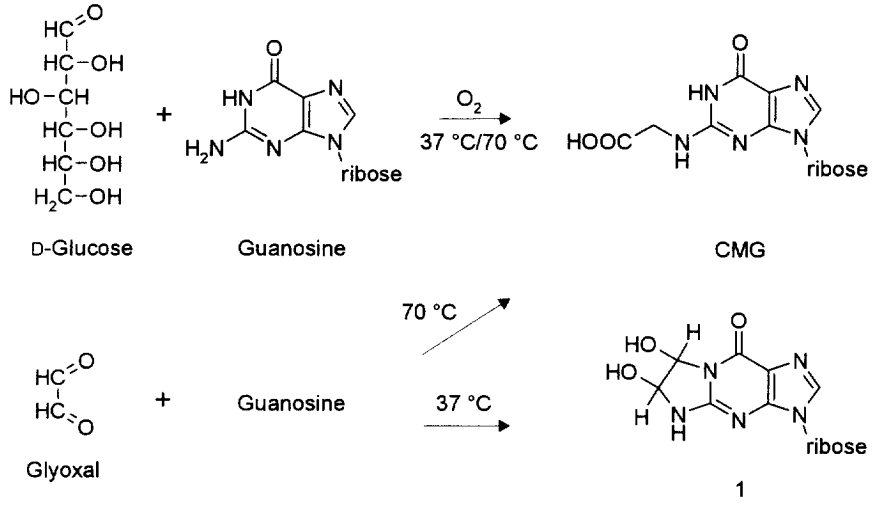
Genomic DNA, on the other hand is subjected to extensive oxidative stress resulting in approximately 10^4-10^5 base modifications per cell and day.[17] Since it is known that the presence of oxygen generally favours glycation,[18,19] we have investigated the reaction of guanosine with glucose under oxidative conditions and isolated and identified a major product which is formed.

RESULTS AND DISCUSSION

Guanosine was incubated with glucose at 37 °C in the presence of oxygen[20] and the reaction mixture was analyzed by HPLC with diode array detection.[21a] As a control the mixture was treated in the same way, but under anaerobic conditions.[22] Glucose turned out to be more active as glycating agent in the presence of oxygen than without. 6.7 % of the guanosine was converted into glycated derivates after 3 weeks under oxidative conditions, whereas under anaerobic conditions only 4.0 % of the educt was modified. Oxidation products of guanosine, particularly 8-hydroxy-guanosine could not be detected under the conditions applied here.

The main product which is formed from glucose and guanosine under oxidative conditions has the typical UV-absorbance curve of N^2-alkylated guanosine,[8] but it has not been described in literature so far. Under more stringent conditions, such as heating at 70 °C or boiling under reflux the same compound is formed, but in higher yields after shorter reaction time.

Scheme 1



Therefore a mixture of glucose and guanosine was heated for 3 weeks at 70 °C.[23] The pure product was obtained after separation of the reaction mixture by preparative HPLC.[21b] Analysis of ^{1}H NMR, ^{13}C NMR and MS revealed the structure of the new guanosine modification as N^2-carboxymethyl-guanosine (CMG)[24] (Scheme 1).

To confirm the structure of the compound, CMG was also synthesized by the reaction of 200 mg guanosine with 0.5 ml 40% glyoxal for 24 h at 100 °C in 1 ml 1M phosphate buffer[25]. CMG was isolated and purified by preparative HPLC.[20b] Spectral data and retention time at HPLC were identical with those of the compound which was isolated from the glucose/guansoine reaction mixture. Under stringent conditions, such as heating at 100 °C, CMG is a major product which is formed from glyoxal and guanosine (Figure 1A). However, if the same mixture is incubated for several weeks at 37 °C, CMG can not be detected, but another product was obtained (Figure 1B). The structure of this compound was assigned to the cyclic glyoxal-guanosine adduct **1** which has been described in detail[26] (Scheme 1).

Figure 1A

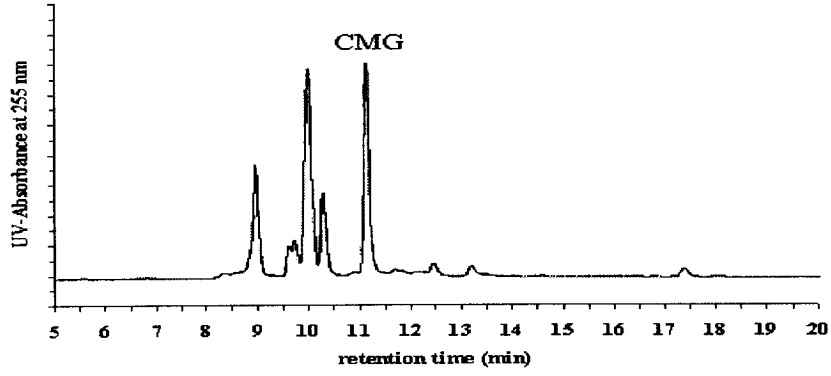
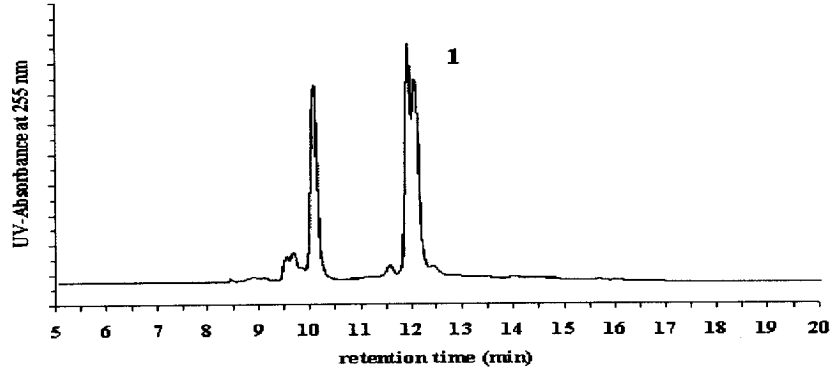
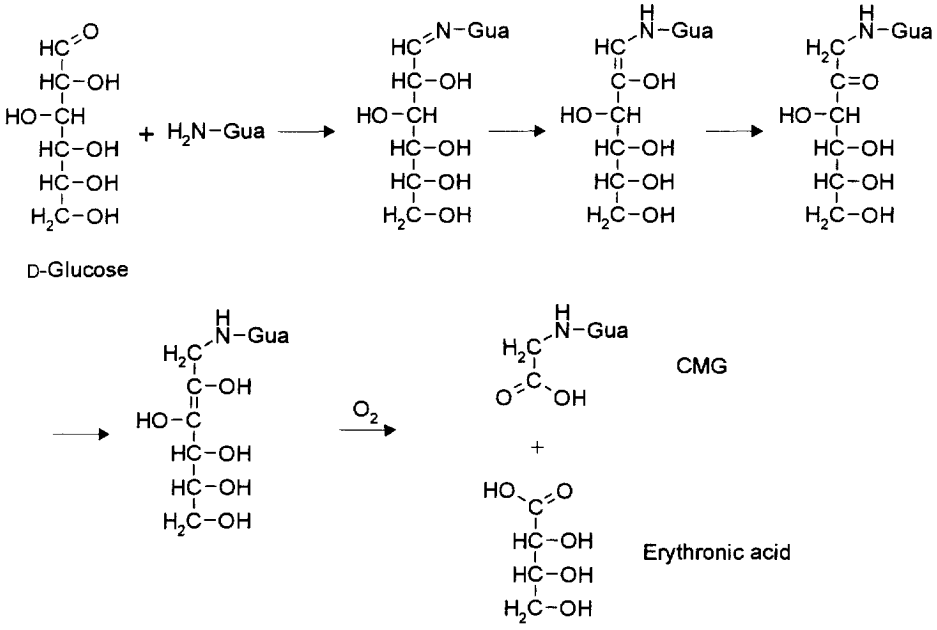


Figure 1B



Since it is known that the presence of amines accelerates glycation of guanosine and DNA,[10,12] incubation of glyoxal and guanosine at 37 °C was also carried out in the presence of propylamine[27]. Under these conditions the cyclic adduct **1** is still the main product, but CMG can be detected as a minor component. Thus it can be concluded that at 37 °C glyoxal is not the precursor for CMG formation from glucose as it has been proposed for similar reaction of glucose with proteins.[28] It can rather be suggested that glucose reacts with the 2-NH_2 group of guanosine to form the corresponding glycosylamine, which has been identified as a glycosylation product of guanosine.[6] Several tautomerization steps lead to an endiol which is prone to oxidation resulting in the formation of CMG and erythronic acid (Scheme 2).

Scheme 2



Finally the reaction conditions which lead to the formation of CMG have been investigated more in detail. Under aerobic conditions CMG is the main product at physiological and raised temperatures (Figure 2A). The diastereomers CEG A and B are formed as minor products. CMG can be detected in glucose/guanosine mixture after less than 4 h of heating. If incubation is carried out at 37 °C, product formation can be observed after several days of reaction. Under anaerobic conditions in contrast, CMG is not formed in detectable amounts, but the diastereomeric pair of CEG and CTPG have been identified as reaction products (Figure 2B).

Therefore it can be stated that CMG is the main product when glucose reacts with guanosine in the presence of oxygen. Since oxygen favours DNA glycosylation and DNA is subjected to oxidative stress, it can be assumed that CMG-DNA adducts can contribute to the harmful structural and biological alteration of DNA which can be

induced by glycation. Investigations about the occurrence of CMG in vivo and the role of enzymatic repair systems herein are currently in progress.

Figure 2A

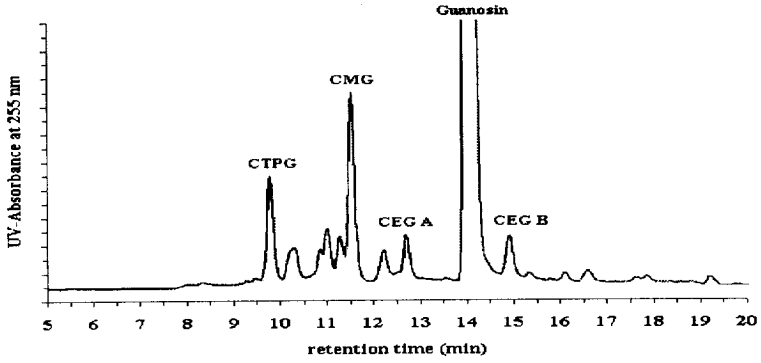
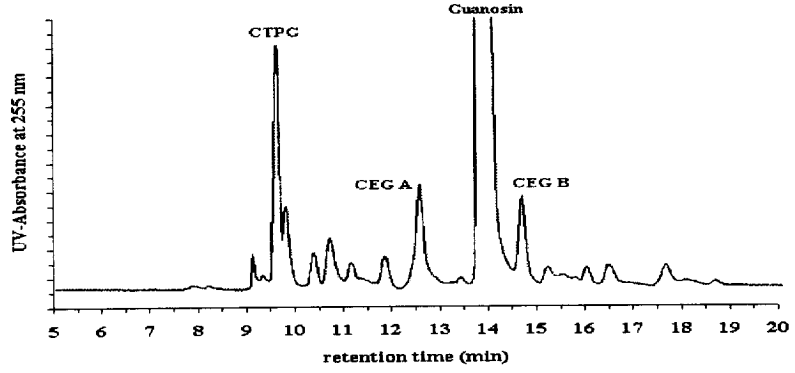


Figure 2B



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20 Guanosine (220 mg) and D-glucose (220 mg) in 2 ml phosphate buffer (1M, pH 7.4) were incubated for 24 d at 37 °C in an open vessel in a shaking water bath. After the reaction the mixture was analyzed by HPLC.
21 a) Analytical HPLC was performed with a Merck L-7100 gradient pump fitted with a 20 µl sample loop and a L-7450 diode array detector including Merck-Hitachi Model D-7000 Chromatography Data Station software. A column packed with LiChrosphere™ (RP-18, 280 x 4 mm i.d., 5 µm particle size) was used. The column was protected with a guard cartridge (25x4mm), packed with the same material as the column. Eluent: gradient elution starting with ammonium formate buffer (5 mM, pH 4.5), ending within 25 min with 50:50 buffer:methanol at a flow rate of 0.8 ml/min. The substances were detected with a diode array detector from 220nm-400nm.
b) Preparative HPLC was performed with a Merck L-6250 preparative pump fitted with a 2 ml sample loop and a Merck L-4000 UV-detector. A column packed with Supelcosil™ (LC-18-DB, 250 x 21.2 mm i.d., 5 µm particle size) was used. A mixture of 96:4 ammonium formate buffer (5mM, pH 7) : methanol was used as solvent at a flow rate of 12 ml/min. Substances were detected at 275 nm.
22 Guanosine (220 mg) and D-glucose (220 mg) were dissolved in 2 ml phosphate buffer (1M, pH 7.4) containing 10 mg DTPA to chelate metal ions, which can catalyse oxidation. The solution was flushed with nitrogen, incubated for 24 d in a clossed vessel in a shaking water bath and analyzed by HPLC.
23 Guanosine (210 mg) and D-glucose (220 mg) were dissolved in phosphate buffer (1M, pH 7.4) and incubated for 24 d in an open vessel at 70 °C in a shaking water bath. The reaction mixture was directly injected into preparative HPLC.
24. Spectral data for CMG: ^{1}H NMR (D_2O, COSY, 400 MHz): δ 3.80 (m, 2H, H-5 rib), 3.90 (s, 2H, CH_2COO^-), 4.11 (m, 1H, H-4 rib), 4.38 (t, J = 5.1 Hz, 1H, H-3 rib), 4.88 (t, J = 5.1 Hz, 1H, H-2 rib), 5.85 (d, J = 5.0 Hz, 1H, H-1 rib), 7.90 (s, 1H, H-8 gua). 13C NMR (D_2O, COSY, DEPT,100 MHz): δ 44.86 (CH_2-N^2), 61.56 (rib-5), 70.16 (rib-3), 72.66 (rib-2), 84.48 (rib-4), 88.74 (rib-1), 116.59 (gua-5), 138.80 (gua-8), 151.37 (gua-4), 152.48 (gua-2), 158.97 (gua-6), 177.41 (COO^-). FAB-MS: m/z (MH^+): 342; UV: λ_{max} = 256 nm.
25 Guanosine (200 mg) and glyoxal (0.5 ml of a 40 % solution in water) were heated in 2 ml phosphate buffer (1M, pH 7.4) in a clossed vessel for 16 h at 100 °C. The reaction mixture was directly used for preparative HPLC.
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27 Guanosine (100 mg), glyoxal (0.25 ml of a 40 % solution in water) and propylamine (250 mg) were dissolved in 1 ml water and the pH was adjusted with phosphoric acid to 7.4. The mixture was incubated for 7 d at 37 °C and analyzed by HPLC.
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