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IDENTIFICATION OF N^2-(1-CARBOXYMETHYL)GUANINE (CMG) AS A GUANINE ADVANCED GLYCATION END PRODUCT

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Abstract: The reaction of D-glucose with 9-methylguanine produces N^2-fructosyl-9-methylguanine, which undergoes oxidation to produce N^2-(1-carboxymethyl)-9-methylguanine as a major adduct.

Protein-bound Amadori products (AP) form in vivo by the reaction of D-glucose with the free amino group of lysine or the N-terminus of the polypeptide chain. Over time, these adducts undergo further dehydration, oxidation, and degradation to form irreversibly-bound Maillard products known as advanced glycation endproducts or AGEs. One AGE, carboxymethyllysine (CML), has been proposed to form in part by oxidative cleavage of fructosylysine between C-2 and C-3 of the carbohydrate moiety.[1] CML accumulates in human lens and collagen as a function of age and ambient glycemia, and can be readily measured on diverse plasma and urinary proteins.[2]

DNA nucleotide bases also undergo advanced glycation reactions.[3] Although the aromatic character of the purine and pyridinium rings renders the primary amino groups of nucleotides less reactive toward reducing sugars than the corresponding amino groups of amino acids, the long half-life of DNA in post-mitotic cells would favor the accumulation of DNA-linked AGEs in vivo. In vitro studies have shown that DNA reacts with glucose and glucose-derived reactive intermediates to produce covalent addition products that can cause mutations in bacteria.[4-6] Reducing sugars also have been shown to cause mutations in plasmids that have been transfected into mammalian cells.[7] In both prokaryotic and eukaryotic systems, AGE-modification leads to the transposition of host-derived, genomic elements, and it has been suggested that AGE-mediated DNA damage may play an important role in the chromosomal alterations associated with aged cells and oncogenic transformation.[7]

A spectrum of products that range from reversible modifications involving Schiff bases and APs to irreversible adducts can form from the reaction of nucleotides or their bases with glucose, ribose, or glucose 6-phosphate.[8-10] We recently reported a novel nucleotide base modification, N^2-(1-carboxyethyl)-9-methyl-guanine (CEmG), that forms as the predominant product of the reaction of glucose with 9-methylguanine (9-mG) in vitro.[9] Preliminary data indicate that the carboxyethyl modification is mutagenic in a model eukaryotic assay system and produces DNA alterations which are similar to those observed after the transfection of AGE-modified DNA.[9]

We describe herein the formation of the Amadori oxidation product, N^2-(1-carboxymethyl)-9-methylguanine (CMmG), after the reaction of 9-mG with D-glucose under aerobic conditions. 1 mmol of 9-mG was incubated with 10 mmol of D-glucose in phosphate buffer (30 mL; pH 7.4) for four weeks and new products identified by reverse-phase HPLC.[11] Mass spectroscopic analysis studies of these new products showed, in addition to the 9-mG AP (N^2-fructosyl-9-methylguanine), a product with a molecular ion at 224 $[MH]^+$, an increase of 59 Da compared to 9-mG. The mass of this adduct differed from that of CEmG (*m/z* 237). The ^{1}H NMR spectrum (DMSO-d_6) was simple and featured the characteristic lower field singlet at 3.91 ppm due to the methylene protons. Also present were two singlets at 3.5 and 7.7 ppm arising from the 9-methyl and the aromatic proton on C-8 of guanine. The exchangeable

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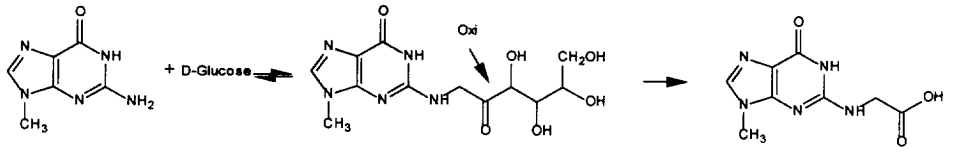
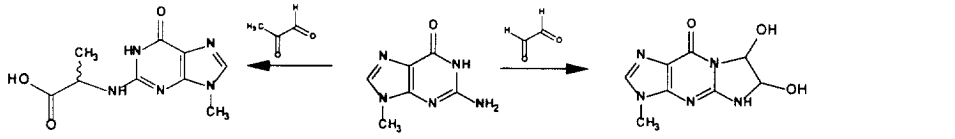
N^1 and N^2 protons gave rise to broad, one proton resonances at 10.5 and 7.2 ppm, respectively. The electrospray MS had a consistent, exact mass for $C_8H_9N_5O_3$. Taken together, these data are consistent with a structure bearing the carboxymethyl adduct (Eq 1). In a long-term incubation (36 weeks) of the above reaction, CMmG was formed in 43% yield. The formation of CMmG was also observed upon heating the reaction mixture at 50 °C for 96 h.



In contrast to the pathway leading to CEmG, CMmG was not detected by the reaction of AP (1-*N*-propylamino-*N*-D-fructose) with 9-mG. While CEmG can form by the reaction of 9-mG with methyglyoxal (α-oxypropanal)[9], the corresponding reaction between 9-mG and glyoxal, a proposed intermediate in the formation of CML,[12] produces a cyclic adduct (Eq 2). The formation of this glyoxal adduct was confirmed by ^{1}H NMR, MS, and by sodium periodate oxidation, and the data were consistent with a prior report.[13] We also prepared CMmG by the reaction of 9-mG with glyoxalic acid under reducing conditions ($NaBH_4$), and the resulting ^{1}H NMR and MS data were consistent with that recorded for CMmG.



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11. HPLC was conducted using a Hewlett-Packard Model 1090 and Waters Instruments (Waters 626 pump and Waters 490 E multiwavelength detector). Electrospray ionization (ESI) samples were run on a Quattro triple quadrupole mass spectrometer. Loop injection sampling used an ABI model 140B syringe pump employing H_2O/CH_3CN (1/1) at a flow rate of 15 μL/ min, a Rheodyne model 7125 valve with a 10 μL loop, and a Micromass Megaflow ESI probe using nitrogen for the nebulizer/drying gas. LC/MS samples were run employing the ABI pump and Rheodyne valve with a 20 μL loop at a flow rate of 50 μL/min.
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