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NOVEL MODIFICATIONS OF N^{α}-BOC-ARGININE AND N^{α}-CBZ-LYSINE BY METHYLGLYOXAL

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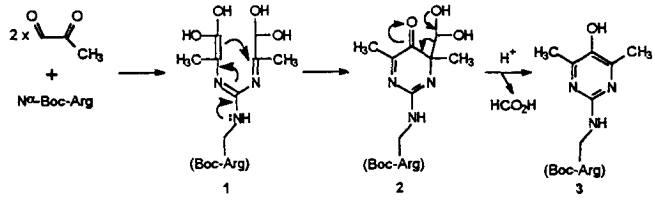
Abstract. Two molecules of methylglyoxal react *in vitro* with one molecule of arginine to form a pyrimidinium adduct or with two molecules of lysine to form an imidazolium-based crosslink.

Methylglyoxal (α-oxopropanal, pyruvaldehyde; MG) forms *in vivo* by elimination of phosphate from dihydroxyacetone phosphate and glyceraldehyde 3-phosphate and also by the Maillard process (advanced glycation).[1,2] Increased levels of MG *in vivo* during hyperglycemia have been reported, suggesting that this reactive 3-carbon dicarbonyl may contribute to tissue damage and certain long-term complications of diabetes.[3]

We recently described a novel nucleotide base modification produced by the incubation of guanine with glucose, Amadori product or MG.[4] MG also crosslinks proteins[5] and reacts with arginine, lysine, and cysteine residues. [6,7] Moreover, MG reacts with aminoguanidine, an advanced glycation inhibitor,[8] to form a stable triazine.[9]

Dicarbonyl sugars such as 3-deoxyglucosone are known to crosslink (polymerize) proteins at a higher rate than glucose.[10] MG is known to modify the guanidino moiety of arginine to form imidazole-type compounds.[7] When we tried to synthesize the imidazolone adduct described in a recent report,[7] we isolated a new adduct that was not previously published. This adduct was fluorescent and displayed an excitation and emission maxima of λ_{max} 320 nm and λ_{max} 384, respectively. The ^{1}H NMR spectrum revealed the presence of a new singlet peak at δ 2.32 integrated for six protons. The ESMS and FABMS showed a molecular ion at *m/z* 355.2. The plausible mechanism for the formation of this compound can be explained via double Schiff base adducts (1) followed by self condensation to arrive at 2. Cleavage of formic acid will result in formation of the pyrimidinium adduct (3).

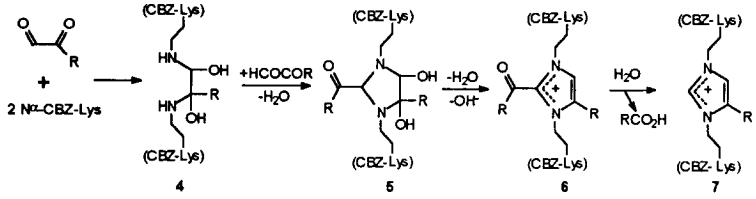


Eq. 1

We also investigated the possible crosslinking potential of MG in a model incubation system involving equimolar amounts of N^{α}-CBZ-lysine and MG dissolved in 0.2 M phosphate buffer (pH 7.3). After 24 h incubation at 37° C, the color of this solution turned to dark brown. A reverse-phase HPLC profile of an aliquot of this incubation showed three major peaks. By LC-MS, one of these products produced a molecular ion of *m/z* 610. The yield of formation of this particular compound was found to increase dramatically upon incubation at pH 5. The ^{1}H NMR spectrum of the new adduct revealed the presence of two lysines, one methyl, and 2CH groups incorporated into one molecule of an imidazolium compound (7, R = CH_3) based on the integration of the methyl group of MG versus the aromatic protons of the CBZ moiety. In addition to the 10 aromatic protons, one non-exchangeable proton appeared downfield in the ^{1}H NMR spectrum at 8.86 ppm and a second was concealed beneath the aromatic protons at 7.32-7.34. Fortunately, 6 N HCl hydrolysis of compound 7 (R = CH_3) left the imidazolium moiety intact and the

desired hydrolysis of the CBZ moiety was achieved. ^{1}H NMR of the hydrolyzed adduct showed the expected downfield protons at 8.64 and 7.19 ppm.

During the course of this work, a report appeared describing the formation under similar conditions of a cognate of **7** differing in α-side chains.[11] Those authors proposed a mechanism involving loss of the acyl group prior to imidazolium formation.[11,12] We would like to propose a more likely mechanism for the formation of **7**, for which there is literature precedent.[13] In this mechanism, **7** would arise *via* initial formation of a bis-hemiaminal (**4**, R = CH_3) of two lysine molecules with one MG, followed by cyclization with another MG molecule to give **5** (R = CH_3) (Eq 2). Dehydration and loss of hydroxide from 2 would produce a 2-acyl-4-imidazolium compound (**6**, R = CH_3); a compound of this type would be expected to undergo a known hydrolytic deacylation reaction to produce the 2-unsubstituted imidazolium derivative **7** (R = CH_3).



Eq. 2

A Lys-Lys crosslink of type **6** (R = 2-furanyl) was previously proposed on the basis of the isolation of 4(5)-furanyl-2-furoylimidazole from acid hydrolysates of glucose-protein incubations by Pongor *et al.*[14] Subsequently, it was found that the N^{α}-BOC analog of **7** (R = 2-furanyl) was formed on reaction of lysine with (2-furanyl)glyoxal.[15] Thus, there is now substantial data that any crosslinks of type **6**, if formed under aqueous conditions, would rapidly be converted to **7**.

The present findings suggest that MG-mediated crosslinking reactions may contribute to the increase in protein modification that affects aged and diabetic tissues. Our results also lend further support to the suggestion[13] that other α-oxoaldehydes found *in vivo* may be able to form crosslinks of this type (e.g., **7**, R = CH_2-$(CHOH)_3$-H, from 3-deoxyglucosone), a possibility which warrants further study.

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