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N^{ε}-CARBOXYMETHYLLYSINE FORMATION BY DIRECT ADDITION OF GLYOXAL TO LYSINE DURING THE MAILLARD REACTION

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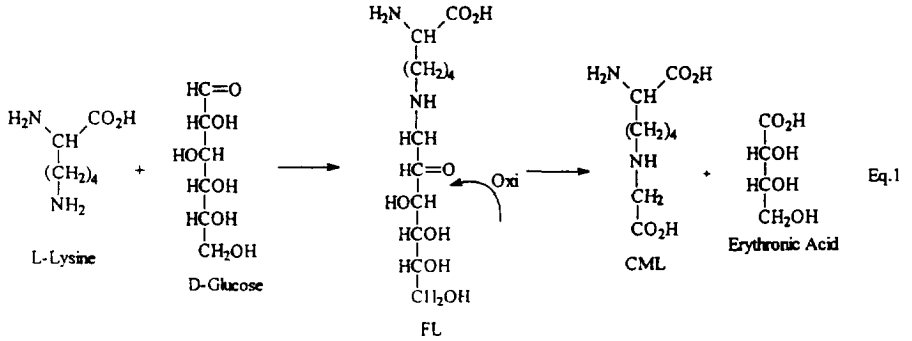
Abstract: Glyoxal, a retro-aldol cleavage product of the Maillard reaction, is a likely intermediate for the formation of N^{ε}-carboxymethyllysine upon incubation with lysine under physiological conditions.

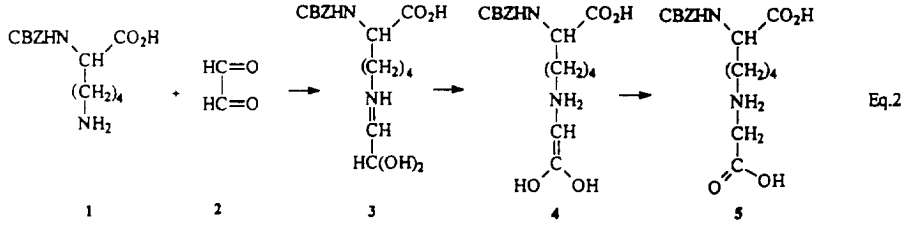
The covalent modification of proteins by reducing sugars *in vivo*, known as advanced glycosylation, follows many of the principles of the Maillard reaction and has been implicated in the pathogenesis of many of the sequelae of chronic diabetes and normal aging.[1,2] This process begins with the nonenzymatic addition of glucose to primary amino groups, first forming freely-reversible Schiff base (SB) adducts, followed by Amadori rearrangement to more stable, N-fructoselysine (FL) products. FL subsequently undergoes rearrangement, dehydration, and fragmentation reactions to produce a host of secondary reactants, including reactive dicarbonyl species such as 3-deoxyglucosone and other reactive diones.[2] Many of these species have been implicated in the formation of the irreversibly-bound chromophores and crosslinks that accumulate on macromolecules which have been aged *in vivo*.[2]

N^{ε}-(Carboxymethyl)lysine (CML) has been detected in human lens, collagen, and urinary proteins and has been proposed to form *in vivo* by oxidative cleavage of FL adducts between C-2 and C-3 of the glucose residue (eq. 1).[3] CML formation from FL *in vitro* was shown to be dependant on phosphate buffer concentration and was inhibited by metal chelators or free radical scavenging systems.



Maillard processes have been recognized for some time to lead to the generation of short-chain, carbonyl-containing fragments such as methylglyoxal, glyceraldehyde, glycolaldehyde or glyoxal.[2] These products arise by retro-aldol-type cleavage reactions of FL and its derivatives. Our recent studies of nucleotide advanced glycosylation, for example, have identified that a major product which forms in the presence of glucose or glucose-6-phosphate is a 3-carbon N^2-carboxyethyl derivative that arises by the direct addition of methylglyoxal to the primary amino group of guanine.[4]

In this communication, we describe an alternative pathway for CML formation in which the source of the carboxymethyl fragment attached to the N^{ε} of lysine is the two-carbon, Maillard reactant glyoxal. Thus, N^{α}-benzoyloxycarbonyl-lysine (**1**) and glyoxal (**2**) in equivalent quantities were incubated in 0.2 M phosphate buffer (pH 7.45) at 37 °C for 28 days. After water evaporation, the residue was subjected to reverse-phase HPLC. Compound **5** was purified as a major product in 23-28% yield and was found to be identical with one synthesized by the reaction of N^{α}-benzoyloxycarbonyl-lysine and iodoacetic acid (20% yield), as assessed by HPLC chromato- graphy, ^{1}H-NMR and MS spectroscopy. A reaction mechanism which would explain the formation of compound **5** is shown in equation 2. A Schiff base (**3**) obtained from glyoxal and N^{α}-benzoyloxy-carbonyl-lysine rearranges via the enamine intermediate (**4**) to give the final compound (**5**).



Eq.2

Incubation of N^{α}-CBZ-lysine with methylglyoxal or glyceraldehyde under similar condition turned brown within 2-5 hours. CML analog was not detected in the early stages and was found in a yield < 1% after 4 weeks. The formation of this brown colour could be a result of self condensation of methylglyoxal or incorporation of lysine in acomplex structure. In contrast to glyoxal, MG is a very reactive compound due to the partial hydration, while in the case of glyoxal complete hydration is considered.[5]

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