We isolated and identified the glyceraldehyde-derived advanced glycation product (AGE) formed from glyceraldehyde and N\(^{\text{α}}\)-acetyl-arginine. A major product was identified as N\(^{\text{α}}\)-acetyl-N\(^{\text{β}}\)-(5-methyl-imidazolin-4-one-2-yl)-ornithine. The compound has been reported as methylglyoxal-derived AGE, MG-H1. This study suggests that MG-H1 is formed through both glyceraldehyde-related and methylglyoxal-related pathways. There is a possibility that MG-H1 becomes an index of injury to glyceraldehyde and methylglyoxal-related enzymes.

**Key words:** glyceraldehyde; advanced glycation end product; glycation; Maillard reaction; methylglyoxal

Proteins are modified by reducing sugars such as glucose in the Maillard reaction, which is also called glycation as the post-translational modification. It is known that the early stage Maillard products, Schiff base (imine) and Amadori rearrangement product (ketoamine) form the carbonyl intermediates. The carbonyl compounds attack the lysine and arginine residues of proteins and the following glycation process is accelerated. At the advanced stage of the reaction, advanced glycation end products (AGE) are formed via dehydration, condensation, cyclization, oxidation, and so on.

Most AGEs are formed by the modification of lysine and arginine residues in the protein. It is known that pyrraline, CML, GLAP, GA-pyridine, and OPLLysine are formed as adduct-type AGE from lysine residue in the protein. Furthermore, 3-deoxyglucosone-derived imidazolones, methylglyoxal-derived imidazolones as MG-H1, and argpyrimidine are formed from arginine residue as adduct-type AGE. Pyrropyridine is detected as a lysine–arginine crosslink.

AGEs are considered to play important roles in the progression of diabetic complications. It has been reported that glyceraldehyde (GLA)-modified bovine serum albumin (BSA) showed neurotoxicity for rat primary cultured cortical neurons and induced over-expression of vascular endothelial growth factor in bovine retinal pericytes. These studies indicate that GLA-modified protein might contribute to the development of diabetic neuropathy and diabetic vascular disease.

Previously we reported that glyceraldehyde-derived pyridinium compound (GLAP) is a cytotoxic-AGE formed in the lysine residues of proteins. Most AGEs are formed in the lysine or arginine residues of proteins, but has not been clarified what GLA-derived AGEs are formed in arginine residues. In this study, we isolated and identified a GLA-derived AGE formed from GLA and N\(^{\text{α}}\)-acetylarginine.

GLA (0.2 mM) and N\(^{\text{α}}\)-acetylarginine (0.1 mM) were dissolved in 0.2 M sodium phosphate buffered solution (pH 7.4). The reaction mixture was incubated at 37°C and 50°C for one week. The incubated mixture was filtered with a cellulose acetate membrane filter (0.20 μm). The filtrate was put on a column on reversed phase HPLC. Analytical HPLC was done under the following conditions: Column, Inertsil ODS-3 (250 × 4.6 mm I.D.); Elution, isocratic of 0.1% TFA from 0 to 15 min and a linear gradient of 0–40% acetonitrile containing 0.1% TFA from 15 to 40 min; flow rate, 1 ml/min; detection, absorbance at 215 nm; temperature, ambient. Figure 1 shows the typical HPLC profile of the reaction mixture of GLA and N\(^{\text{α}}\)-acetylarginine at 37°C (A) and 50°C (B). The major compound (the peak with arrow) was especially increased at 50°C, and showed non-fluorescence (data not shown). The compound was as a lysine–arginine crosslink.

Abbreviations: AGE, advanced glycation end products; BSA, bovine serum albumin; CEL, carboxyethyllysine; CML, carboxymethyllysine; 3DG, 3-deoxyglucosone; GLA, glyceraldehyde; GLAP, glyceraldehyde-derived pyridinium compound; MGO, methylglyoxal; MOLD, methylglyoxal-derived lysine dimer; TFA, trifluoroacetic acid

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collected using preparative HPLC, and the conditions were as follows: Column, Pegasil-ODS column (250 × 10 mm I.D.); elution, isocratic of 0.1% TFA from 0 to 30 min and a linear gradient of 0–40% acetonitrile containing 0.1% TFA from 30 to 120 min; flow rate, 4 ml/min; detection, absorbance at 215 nm; temperature, ambient.

The isolated compound was analyzed by NMR and FAB-MS using the ECP-500 system (500 MHz, JEOL) and a tandem mass spectrometer SX102 (JEOL). The NMR spectral data were as follows: NMR δH (D$_2$O) (numbers in parentheses represent the positions of the assigned carbon positions, as shown in Fig. 2): 1.37–1.41 (6, 3H, m), 1.63–1.76 (4', 2H, m), 1.84–1.92 (3', 2H, m), 1.97 (7', 3H, s), 3.30–3.33 (5', 2H, m), 4.28–4.34 (2', 1H, m). NMR δC (D$_2$O): 15.54 (6, CH$_3$), 21.79 (7', CH$_3$), 24.11 (4', CH$_2$), 27.91 (3', CH$_2$), 41.70 (5', CH$_2$), 52.66 (2', CH), 55.75 (5, CH), 155.93 (2, C), 174.45 (4, C), 175.86 (6', C), 177.52 (1', C). Moreover, these assignments were also verified by $^1$H-detected $^1$H–$^1$H COSY, $^1$H–$^1$H COSY, HMBC, and $^{13}$C DEPT spectra. FAB-MS was recorded with glycerol as a matrix and polyethylene glycol 400 as mass standard. The data showed as an [M + H]$^+$ ion at m/z 271.

NMR and MS study revealed the major compound to be $N^\alpha$-acetyl-$N^\beta$-acetylglycine. The chemical structure is shown in Fig. 2. This compound is already known as MG-H1 formed from methylglyoxal (MGO)-related glycation. This study indicates that MG-H1 as imidazole-type AGE is formed from GLA as well as MGO. The proposed pathway of MG-H1 formation is shown in Fig. 3. The arginine
residue added to $\alpha$ carbon of GLA, and cyclized with dehydration. The intermediate was isomerized to MG-H1 in keto-enol tautomerism. In addition, MG-H1 was formed from GLA via MGO formation. Briefly, MGO was formed from GLA with dehydration. The arginine residue added to MGO, and cyclized with dehydration. The intermediate was isomerized to MG-H1 in keto-enol tautomerism.

MG-H1 is hydrophilic compound, and the MG-H1 formation is temperature-dependent. In this study, the MG-H1 level was 5.5 mm (37 °C) and 11.5 mm (50 °C) respectively. Imidazolone might be formed from MG-H1 as hydro-imidazolone by oxidation, but hydro-imidazolone was detected only in the biological specimen. It has been reported that MG-H1 increased in the hemoglobin and plasma in triose isomerase deficiency. The MG-H1 level was higher than CEL or MOLD as MGO-derived AGE. In this case, MG-H1 might be formed by the modification of lysine with both GLA and MGO. These carbonyl compounds increased in the degradation of sugars, the Maillard reaction, glycolysis, and activation of polyol pathway, under hyperglycemia or catabolic conditions. An in vitro experiment also demonstrated that the MG-H1 content was increased by the inhibition of glyceraldehyde-3-phosphate dehydrogenase.

This study suggests that MG-H1 is formed through both glyceraldehyde-related and methylglyoxal-related pathways. There is a possibility that MG-H1 becomes an index of an injury to glyceraldehyde and methylglyoxal-related enzymes such as glyceraldehyde-3-phosphate dehydrogenase, triose-phosphate isomerase, and glyoxalase.

References


