The Formation of Argpyrimidine in Glyceraldehyde-Related Glycation

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Three major glyceraldehyde-related advanced glycation end products (AGEs) were formed from a mixture of \( N^{N/C_{11}} \)-acetyllysine, \( N^{N/C_{11}} \)-acetylarginine, and glyceraldehyde. Two of the compounds were MG-H1 and GLAP, as previously reported, and the other compound was identified as \( N^{N/C_{11}} \)-acetyl-\( N^{N/C_{14}} \)-(5-hydroxy-4,6-dimethyl-pyrimidin-2-yl)-ornithine, argpyrimidine (APN). APN is a modification product of arginine residue, but it did not form from glyceraldehyde with arginine residue. The coexistence of lysine residue was necessary to APN formation.

Key words: glyceraldehyde; argpyrimidine; methylglyoxal; glycation; Maillard reaction

Glycation, which occurs in post-translational modification processes, is based on the amino-carbonyl reaction. This reaction is also called the Maillard reaction, and irreversible products are formed as advanced glycation end products (AGEs) at advanced stages of the reaction. AGEs are thought to have physiological effects in the progression of diabetic complications through binding to the AGE receptor. Various carbonyl-derived AGEs have been reported, but known AGEs may be only a part of all AGEs. We have reported GLAP and MG-H1 as glyceraldehyde-derived AGEs.\(^{2,3}\) GLAP is formed from GLA with lysine residue, and MG-H1 is formed from GLA with arginine residue. In this study, we isolated and identified GLA-related AGEs, which are generated in the coexistence of lysine residue and arginine residue.

Glyceraldehyde (GLA, 50 mM), \( N^{N} \)-acetyllysine (NAcLys, 25 mM), and \( N^{N} \)-acetylarginine (NAcArg, 25 mM) were obtained from Sigma-Aldrich (St. Louis, MO), and were dissolved in 0.2 M sodium phosphate buffered solution (pH 7.4). The mixture was incubated at 37 °C for one week, and then the mixture was filtered with a cellulose acetate membrane filter unit (DISMIC, 0.20 μm, Advantec, Tokyo). This solution was analyzed by reversed phase HPLC. HPLC was done with the 2996 HPLC system (Waters, Milford, MA), which consisted of Pump 1525, Degasser DG2, Auto Sampler 717 plus, Photodiode Array Detector 2996, and the Empower microcomputer program. The HPLC conditions were as follows: A 5-μl sample was put on a Cosmosil 5C18-MS column (150 × 4.6 mm I.D., Waters), and eluted with linear gradient of 0.05% TFA (A)–80% acetoni-trol (B) from 0 min (100% A) to 45 min (50% B in A) at a flow rate of 1 ml/min. The column temperature was ambient.

Typical HPLC profiles are shown in Fig. 1A (absorbance at 215 nm), B (291 nm), and C (330 nm). The four major peaks were named P1, P2, P3, and P4. P1 and P2 have been reported to be GLA-derived AGEs, MG-H1 (P1) and GLAP (P2). P3 and P4 were yellow unidentified compounds. In this paper, identification of P3 was reported (identification of P4 is underway).

For identification, P3 was purified by cellulose column chromatography. A reaction mixture of GLA, NAcLys, and NAcArg was mixed with two volumes of 2-propanol. The solution was filtrated and put on a microcrystalline cellulose (approximately 90 g of Avicel, Merck, Darmstadt, Germany) column (500 × 30 mm I.D.), which has equilibrated with 2-propanol-water (2:1). The sample was eluted with the same solution, and 10-ml fractions were collected. Each fraction was analyzed by cellulose partition TLC (Merck) with 2-propanol-water (3:1) as the solvent. P3 was detected with a TLC-lamp (366 nm), which showed Rf 0.86. The fraction was concentrated under reduced pressure and...
then lyophilized. Finally, 10.7 mg of P3 compound as a yellow powder was obtained from 30 ml of reaction mixture.

UV-visible and fluorescence spectra of P3 were measured with a spectrophotometer U3310 (Hitachi, Tokyo) and a fluorescence spectrometer F3000 (Hitachi). The UV-visible spectra of the P3 showed maximum peaks at 235 and 313 nm. Fluorescence spectra showed maximum peaks at 325 nm (excitation) and 385 nm (emission).

Mass spectra were recorded with a tandem mass spectrometer SX102 (JEOL, Tokyo). The ionization mode was set to FAB(+) with glycerol (TCI, Tokyo) as a matrix and polyethyleneglycol 200 (Wako) as a mass standard. The FAB-MS measured for the P3 compound showed an \( \frac{M+H}{C_{138}H_{21}N_4O_4} \) ion at \( m/z = 297 \). High-resolution FAB-MS data showed an \( \frac{M+H}{C_{138}H_{21}N_4O_4} \) ion: Calcd. for \( C_{138}H_{21}N_4O_4 \): 297.1563. Found: 297.1553.

For NMR, purified P3 (12 mg) was dissolved in \( \text{D}_2\text{O} \) (0.2 ml) and put in a NMR tube (3 mm I.D., Sigemi, Tokyo). Data were acquired by the ECP-500 system (500 MHz, JEOL) using the NALORAC Probe. As for the result, P3 was identified as \( N^\circ\)-acetyl-\( N^\circ\)-(5-hydroxy-4,6-dimethyl-pyrimidin-2-yl)-ornithine. The chemical structure is shown in Fig. 2. The spectral data were as follows: NMR \( \delta \text{H} (\text{D}_2\text{O}) \) (numbers represent the positions shown in Fig. 2): 1.61–1.73 (4', 2H, m), 1.80–1.87 (3', 2H, m), 1.97 (7', 3H, s), 2.38 (7, 3H, s), 2.38 (8, 3H, s), 3.37–3.40 (5', 2H, t), 4.15–4.17 (2', 1H, q). NMR \( \delta \text{C} (\text{D}_2\text{O}) \): 17.0 (7, \text{CH}_3), 17.0 (8, \text{CH}_3), 21.9 (7', \text{CH}_3), 24.8 (4', \text{CH}_2), 28.7 (3', \text{CH}_2), 40.9 (5', \text{CH}_2), 54.6 (2', \text{CH}), 137.9 (5, \text{C}), 151.0 (6, \text{C}), 151.0 (4, \text{C}), 158.1 (2, \text{C}), 173.8 (6' , \text{C}), 178.6 (1', \text{C}). Additionally, these assignments were also verified by \( ^{13}\text{C} \) DEPT spectra and the \( ^1\text{H}^1\text{H} \) COSY, HMQC, and HMBC techniques. HMBC correlations are shown as arrows in Fig. 2.
Moreover, the structure was verified by the linked-scan method on mass spectrometry with glycerol as a matrix and ultra mark as the mass standard. The product ion was observed at m/z 279 (dehydration), 255 (deacetylation), 237 (dehydration of deacetyl product), 192 (dehydration of deacetyl product), 167, 140, and 100.

The chemical structure of P3 is shown in Fig. 2, and the compound has been reported to be argpyrimidine, a methylglyoxal-derived AGE. MGO-derived AGE was generated in GLA-related glycation.

APN was generated from GLA with AcLys and AcArg. On the other hand, APN was not generated from GLA and AcArg (data not shown). The coexistence of lysine residue was necessary for the generation of APN. These results suggest that MGO was not formed from GLA by dehydration.

It is known that MGO is generated from the Amadori rearrangement product (ketoamine). APN might be generated from MGO, which is formed from the Amadori product. The proposed pathway of APN formation is shown in Fig. 3.

Epsilon amino group of lysine residue adduct to the carbonyl group of GLA, and ketoamine was formed by the Amadori rearrangement reaction via imine formation with dehydration. The Amadori rearrangement product (ketoamine) was isomerized to the intermediate in keto-enol tautomerism. The carbonyl double bond of ketoamine was shifted to the reverse side of the lysine-binding site, and then MGO was formed by lysine elimination. On the other hand, the carbonyl group double bond of ketoamine was shifted to the binding side of the lysine-binding site. Then MGO was formed by lysine elimination. Moreover, two molecules of MGO were adducted by the guanidino group of the arginine residue. APN was formed from this intermediate by cyclization.

Another pathway is proposed in Fig. 3, which is based on experimental evidence. MGO increased rapidly, and then decreased rapidly. MG-H1 also increased rapidly, but APN formation gradually increased. APN formation may be independent of MGO formation. Hence another pathway is proposed, as follows: The guanisino group of the arginine residue adducts to the carbonyl group of two ketoamine, and two lysine residues are eliminated. APN is formed from this intermediate by cyclization.

This study suggests that APN is generated in MGO-related glycation and GLA-related glycation. It has been reported that APN increases in biological specimens, especially the lens protein of cataracts. It is thought that APN is derived from MGO, but some pathways exist in APN formation. APN might in fact be generated by both MGO and GLA. APN might be a marker of both MGO-related glycation and GLA-related glycation.

References

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