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Novel aldose reductase inhibitory and antioxidant chlorogenic acid derivatives obtained by heat treatment of chlorogenic acid and amino acids

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ABSTRACT

A heating model system (HMS) of chlorogenic acid (CGA) and 20 amino acids was produced by heating at 120 °C for 4 h and evaluated for in vitro antioxidant and aldose reductase (AR). The CGA-glutamic acid (GT) HMS showed high in vitro antiradical activity indicated by $ABTS^+$ (82.37%) and DPPH radical scavenging (83.21%) as well as AR (83.21%) inhibition. The structure of the new compound was established by NMR spectroscopy, as methyl-3-(((*E*)-3-(3,4-dihydroxyphenyl)acryloyl)oxy)-4,5-dihydroxycyclohexanecarboxylate (1) and 4-*O*-caffeoylquinic acid (2) from the CGA-GT HMS. The IC_{50} values of compound 1 for $ABTS^+$, DPPH and AR were 8.21, 56.97 and 3.68 μM, respectively. These activities were similar to or higher than those of known positive controls (5.49, 63.58 and 13.60 μM). We suggest that heat treatment generates novel CGA-GT HMS with increased antioxidant and AR inhibitory effects and contributes to the development of novel functional materials from CGA food products.

1. Introduction

Diabetic complications such as neuropathy, nephropathy, cataracts, and retinopathy are the result of sorbitol accumulation that is produced from glucose by aldose reductase in the polyol pathway (Hwang, Kim, & Lim, 2017). Aldose reductase (AR, EC 1.1.1.21) catalyzes the reduction of glucose to the corresponding sugar alcohol, sorbitol, which is subsequently metabolized to fructose by sorbitol dehydrogenase. AR is an important NADPH-dependent oxidoreductase in the polyol pathway (Choung et al., 2017), which is found in nearly all mammalian cells but at high levels in organs that are affected by diabetic complications such as the lens, retina, and sciatic nerves (Kim, Hwang, Wang, Yu, & Lim, 2017a). AR inhibition is crucial to prevent and address long-term diabetic complications because the increased flux in the polyol pathway leads to sorbitol accumulation in the lens fiber, water influx, generation of osmotic stress, and cataract formation (Kim, Hwang, Suh, & Lim, 2017b). Excessive levels of glucose can induce oxidative stress through AR-dependent (polyol pathway) and AR independent sources, such as stimulated oxidative stress via the auto-oxidation of glucose, of oxidative stress. The increased oxidative stress plays an important role in the pathogenesis of diabetic complications (Tang, Martin, & Hwa, 2012). Therefore, bioactive compounds with both antioxidant and aldose reductase inhibitory activities would be beneficial for prevention and treatment of diabetic complications.

Among polyphenols, chlorogenic acid (CGA, 5-*O*-caffeicquinic acid) is the ester of 3,4-dihydroxycinnamic acid and quinic acid that functions as an intermediate in lignin biosynthesis (Fu et al., 2017). Notably, green coffee bean (GCB) is a primary source of dietary CGA with the highest relative content of this compound (Ky, Noirot, & Hamon, 1997). CGA has received much attention for numerous biological activities that include excellent anti-inflammatory activity in lipopolysaccharide-stimulated RAW 264.7 cells (Hwang, Kim, Park, Lee, & Kim, 2014), inhibition of α-amylase and α-glucosidase linked to type 2 diabetes (Oboh, Agunloye, Adefegha, Akinyemi, & Ademiluyi, 2015), anti-obesity properties, improvement of lipid metabolism in high-fat diet-induced obese mice (Cho et al., 2010), and inhibition of advanced glycation end product formation and associated protein cross-linking (Kim et al., 2011). Hwang et al. (2017) reported CGA possessed AR inhibition and antioxidant activity. In addition, clinical trials have indicated the therapeutic effects of green coffee bean extracts containing CGA against obesity (Flanagan, Bily, Rolland, & Roller, 2014) and diabetes, as well as reduction of risk of type 2 diabetes and cardiovascular disease (Ranheim & Halvorsen, 2005).

Isomerization and transformation of CGA were previously reported

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to generate fourteen derivatives in the temperature range 100–200 °C or in alkaline water solution at room temperature (Dawidowicz and Typek, 2010). The amounts of each component formed were dependent on the heating time and temperature conditions (Dawidowicz and Typek, 2011). Dawidowicz and Typek (2011) reported that the thermal stability of CGA decreased during heating in buffered water solution and that CGA derivatives were formed at various pH levels. The CGA content decreased during heat treatment in the GCB. CGA was easily oxidized and unstable during roasting of GCB (Charlotte, Oruna-Concha, Mottram, Gibson, & Spencer, 2013). The change in CGA stability was due to the transesterification during storage or by heat treatment of food (Goncalves, Moeenfard, Rocha, Estervinho, & Santos, 2017).

It was reported that CGA would form benzacridine derivatives when mixed with respective amino acids in sodium hydrate-buffered solution at pH 9 (Bongartz et al., 2016). However, the influence of various amino acids on antioxidant and aldose reductase inhibitory activities of CGA during heating remains unknown. We determined the $ABTS^+$ and DPPH radical scavenging activities and chemical changes of CGA with amino acids addition during heat treatment in water, and identified the biological activities and structures of the chemical products from CGA-glutamic acid (GT) heating model system (HMS). Additionally, we observed same chemical changes in CGA from the GCB extract by selected GT addition. Our results may be valuable for researchers examining coffee in which CGA derivatives are formed during heat treatment in the presence of amino acids.

2. Materials and methods

2.1. Chemicals and reagents

Reagents: 2,2′-azino-bis(3-ethylbenzothiazline-6-sulfonic acid) diammonium salt ($ABTS^+$), potassium persulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), nicotinamide adenine dinucleotide phosphate (NADPH), DL-glyceraldehyde dimer, sodium phosphate dibasic anhydrous, sodium dihydrogen phosphate, ammonium sulfate, potassium dihydrogen phosphate, sodium hydroxide, quercetin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), L-ascorbic acid, CGA, and 20 selected amino acids were purchased from Sigma-Aldrich (St. Louis, MO, USA). Human recombinant human AR (HRAR) was purchased from Wako Pure Chemical Industries (Osaka, Japan). All chemicals and reagents used were of analytical grade.

2.2. Preparation of heating model system derived from CGA and 20 amino acids

We prepared 5 mL of CGA (10 mM) and 20 amino acids (50 mM each) at a mole ratio of 1/5 with modification of previously published method, which prepared CGA (14 mM)-amino acid (56 mM) mixture at a mole ratio of 1/5 (Bongartz et al., 2016). The 20 amino acids were individually mixed with CGA solution in 15 mL cap-glass tubes, tightly capped, and heated in a temperature-controlled autoclave apparatus (Jisico, Seoul, South Korea) at 120 °C for 4 h. The optimal conditions for heat treatment were previously determined (Hwang et al., 2011a). We prepared components from the GCB extract (20 mg)-GT (100 mM) using the same CGA and amino acid preparation method for simple analysis. After heating, the reaction mixture was filtered through a 0.45-μm membrane and freeze-dried under vacuum using a Modulyod-115 apparatus (Thermo Electron Co., Waltham, MA, USA).

2.3. HPLC analysis

High-performance liquid chromatography (HPLC) was performed on Agilent 1100 series (Agilent, Sunnyvale, USA) and Thermo LCQ Advantage (Thermo, Waltham, USA) systems equipped with a diode-array detector (DAD). The CGA and 20 amino acids HMS and AR ultrafiltration HPLC (Thermo LCQ Advantage), and GCB-GT HMS (Agilent 1100 series) analysis were conducted on an Eclipse XDB-phenyl column (150 × 4.6 mm, 3.5 μm) coupled to a guard column at 30 °C. Samples (10 μL) were injected into the system. The samples were eluted with acidified water (0.1% formic acid, A-line) and MeOH (B-line), at a flow rate of 0.7 mL/min. The optimized gradient conditions were as follows: 5–100% B at 0–50 min; 100–5% B at 50–55 min; isocratic 5% B at 55–60 min. The detector monitored the eluent at 280 nm.

2.4. AR ultrafiltration HPLC

Ultrafiltration HPLC assay was used to screen for the AR inhibitory constituents in the CGA-GT HMS. Specifically, the CGA-GT HMS (final concentration: 1.0 mg/mL) were incubated with 0.6 M ammonium sulfate and 3.9 μM HRAR in a total volume of 300 μL at 37 °C for 30 min. Then, the incubated mixture was filtered by centrifugation at room temperature and 5167 × *g* for 30 min using the MicroCon YM-10 centrifugal filter unit. The filtrate was subsequently analyzed by HPLC using the methods described in Section 2.3. The sample incubated without HRAR was used as a control. The relative binding affinity of the inhibitors from CGA-GT HMS towards HRAR was defined as the binding degree and was calculated as follows: BD (%) = $(A_a - A_b)/A_a \times 100\%$, where A_a and A_b are the peak areas of a compound interacting without and with HRAR in the HPLC chromatograms, respectively (Wang, Hwang, & Lim, 2017).

2.5. Purification of compounds from CGA-GT HMS

Bioactive constituents of the CGA-GT HMS (200 mg) were purified by using a recycle HPLC method with a gradient system (0–25% MeOH) as the eluent to obtain compounds 1 (5.14 mg) and 2 (4.83 mg).

2.6. NMR analysis

Approximately 4.0–5.0 mg of each compound was dissolved in 600 μL of dimethyl sulfoxide (DMSO)-*d6* and transferred to 3 mm nuclear magnetic resonance (NMR) tubes. ^1H, ^{13}C, and correlation NMR spectra including correlation spectroscopy (COSY), heteronuclear multiple bond correlation (HMBC), heteronuclear single quantum coherence (HSQC), and nuclear overhauser effect spectroscopy (NOESY) were obtained using an Avance DPX 600 spectrometer (Bruker, Billerica, MA, USA). Spectra were obtained at operating frequencies of 600 (^1H) and 150 MHz (^{13}C) with DMSO-d_6, and tetramethylsilane was used as an internal standard. Chemical shifts are reported as δ values.

2.7. $ABTS^+$ radical scavenging assay

Radical scavenging activity was measured by an $ABTS^+$ based assay that was modified slightly from Choung et al. (2017). Briefly, $ABTS^+$ (2.0 mM) and potassium persulfate (3.5 mM) were mixed and diluted 10 times with distilled water. The solution was stored at room temperature in the dark for 24 h before use. 90 μL $ABTS^+$ solution was reacted with 10 μL of each sample at concentrations of 0.05–1.0 mg/mL in DMSO at 750 nm on a microplate reader (EL800 Universal Microplate reader, Bio-Tek Instruments, Winooski, VT, USA) and the activity was recorded after 10 min. Trolox was used as the positive control. The $ABTS^+$ scavenging activity was calculated as follows:

$$\text{Inhibition ratio}\% = [1-(A-B)/(C-D)] \times 100$$

where A is the absorbance of the test sample with $ABTS^+$, B is the test sample without $ABTS^+$, C is the absorbance of the $ABTS^+$ without sample, D is the absorbance of the mixture of buffer and DMSO, without $ABTS^+$ or sample.