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Short communication

Simultaneous determination of two Amadori compounds in Korean red ginseng (*Panax ginseng*) extracts and rat plasma by high-performance anion-exchange chromatography with pulsed amperometric detection

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Abstract

A new simple, rapid and sensitive high-performance anion-exchange chromatography method with pulsed amperometric detection (HPAEC–PAD) was developed and validated for the simultaneous determination of two Amadori compounds, arginyl-fructose and arginyl-fructosyl-glucose in Korean red ginseng (*Panax ginseng*) extracts, rat plasma. Separation of the two target analytes was efficiently undertaken on CarboPac PA1 anion-exchange column with isocratic elution (400 mM sodium hydroxide and deionized water (90:10, v/v)) at flow rate 0.7 mL/min within 15 min of single chromatographic run. Under optimized conditions, the detection limits (signal-to-noise ratio equal to 3) were 20 and 25 ng/mL for arginyl-fructose and arginyl-fructosyl-glucose, respectively. Calibration curves of peak area for the two analytes were linear over three orders of magnitude with a correlation coefficients greater than 0.999. The accuracy of the method was tested by recovery measurement of the spiked samples which yielded good results of 94.15–102.62%. This method was successfully applied to the quantification of arginyl-fructose and arginyl-fructosyl-glucose in herbal extracts and in the plasma samples from rat.
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Keywords: High-performance anion-exchange chromatography; Pulsed amperometric detection; Amadori compounds; Red ginseng; Rat plasma

1. Introduction

Panax ginseng, one of the most well known herbal medicines in oriental countries has been widely used as alternative medicine, health food and tonic owing to the belief of its effectiveness in maintaining youth and prolongation of the life span. Major ingredients of ginseng are saponins, ginsenoside, which has been reported to be effective on the cardiovascular and central nervous systems [1–3] and also as an anti-oxidant [4–6]. Other than ginsenosides, amino acid derivatives, arginyl-fructose (Arg-Fru) and arginyl-fructosyl-glucose (Arg-Fru-Glc) have been identified in Korean red ginseng, a steamed ginseng [7], along with their various pharmacological activities such as anti-oxidant, anti-diabetes, anti-obesity and the modulation of glucose transport [8].

Due to the high enrichment of arginine in crude ginseng [9], Arg-Fru and Arg-Fru-Glc are the major Amadori compounds formed by the reaction of maltose, arginine and glucose, arginine respectively during the steaming and heat-drying processes of Korean red ginseng preparation. Amadori compounds are generated by a non-enzymatic glycation reaction between amino acids and reducing sugars, which contributes to the aroma, flavor, taste and color of food and also influences their nutritional and toxicological properties [10]. With the increasing recognition of the various therapeutic effects of Korean red ginseng [11–14], application of Arg-Fru and Arg-Fru-Glc as health food or alternative medicine is being actively sought. In this regard, the development of a reliable analytical method is required for their accurate quantification in herbal extracts and plasma.

The analysis of Amadori compounds, however, is not an easy task, especially in the samples of complex systems such

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as food or biological fluids. Various column chromatographic techniques have been tried for the separation and quantification of Amadori compounds. Previously, a classical amino acid analyzer method with post-column reaction using ninhydrin was applied to the analysis of Amadori compounds [15,16]. This method, however, had several disadvantages such as insufficient separation, poor sensitivity and long analysis time. Derivatization of carbohydrate moiety using such as trimethylsilyation in gas chromatography (GC) was also tried but it still required time-consuming derivatization procedures and complex separation steps due to the generation of tautomeric form [17]. Additionally, HPLC method with a refractive index detector has been tried for analyzing Amadori compounds, but it also has problems such as poor sensitivity and insufficient separation [18]. The method of pre- or post-column derivatization and UV or fluorometric detection by HPLC has been widely used for sugar analysis [19–22] and it has been applied to analyze Amadori compounds. For example, Ryu et al. tried to analyze for Arg-Fru in aged garlic extract by HPLC and post-column reaction with triphenyltetrazolium chloride [23]. However, the method still requires time-consuming clean-up procedures to separate Amadori compounds from the complex matrix prior to the chromatographic step. TLC method using ninhydrin and anisaldehyde reagent has been also reported for sugar and amino acid detection to analyze Amadori compounds [24]. But this method is applied to the qualitative detection of Amadori compounds, not to quantitative.

Recently, a new method using high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC–PAD) [25–30] or high-performance anion-exchange chromatography with mass spectrometer (HPAEC–MS) [31,32] has been developed for the determination of sugars. HPAEC–PAD method employs alkaline condition where affinity between sugar and quaternary amine stationary phase occurs. Under alkaline conditions, weak-acidic sugars are present as anions, which can be separated and detected by anion-exchange column with PAD. This method yielded good resolution, high sensitivity and selectivity for the analysis of sugar [33,34], which can be extended to the analytical method for Amadori compounds. Davidek et al. [35] developed a method for the separation and simultaneous determination of Maillard precursors and Amadori compounds in Maillard reaction model system. This analytical method, however, has a limited application in much simpler model systems. Up to now, there is no report of the simultaneous determination of hexose-derived and di-hexose-derived Amadori compounds in complex matrix such as natural herbal extracts or biological fluids by high-performance anion-exchange chromatography with pulsed amperometric detection.

In the present study, we developed a new analytical method which can determine the Amadori compounds, Arg-Fru and Arg-Fru-Glc simultaneously using HPAEC–PAD. This method can be applied for the simple, rapid, sensitive and simultaneous determination of Arg-Fru and Arg-Fru-Glc in the two complex matrices, herbal extracts and biological samples such as plasma, at the same time. We believe that with the present study, a useful and versatile analytical method has been provided for the study of Arg-Fru and Arg-Fru-Glc, which can be extended to the research for other Amadori compounds.

2. Experimental

2.1. Chemicals and reagents

L-Arginine, maltose monohydrate, D-glucose, *N*-acetyl neuraminic acid, perchloric acid, ninhydrin and trichloroacetic acid were from Sigma (St. Louis, MO). Formic acid was purchased from Fluka (Burchs, Switzerland). Ammonia solution (28%) and butanol were from Junsei (Junsei Chemical Co., Ltd, Japan). Methanol and acetonitrile were HPLC grade from Burdick & Jackson (Honeywell International Inc., Muskegon, MI). 50% (w/w) sodium hydroxide solution was from Fisher Scientific (Fair Lawn, NJ) and the water used was ultra-pure deionized water (18.2 MΩ cm) produced from Millipore Milli-Q Gradient system (Millipore, Bedford, MA). All other reagents used were of highest grade available.

2.2. Synthesis of arginyl-fructose and arginyl-fructosyl-glucose

Arg-Fru (**1**), Arg-Fru-Glc (**2**) (Fig. 1) was prepared according to the previous report [36]. Mixture of maltose, L-arginine and glucose, L-arginine were dissolved in glacial acetic acid, respectively, and stirred for 1 h at 70–80 °C. After cooling to room temperature, the resultant slurry was centrifuged at 1000 × *g* for 10 min and the supernatant was concentrated to dryness. The dried sample was dissolved in 50 mL of water and applied to a cation exchange column (CG-Amberlite IR-120-1, Fluka, Burchs, Switzerland). After washing with 200 mL of water, 300 mL of 0.5% ammonia solution was eluted and the effluent was freeze-dried. Then the dried sample was purified using silica gel column (Kieselgel 160, 70–230 mesh, Merck, Germany) with butanol–acetic acid–water (2:1:1) solution. The fractions were identified by TLC with ninhydrin reagent. Resultant compounds **1** and **2** were obtained as off-white powder. Structures of synthesized **1** and **2** were confirmed using NMR and LC–MS by which purities were confirmed to be over 95%. The NMR spectral data of the synthesized **1** and **2** were identical with the previously reported data and mass of **1** and **2** was analyzed to be *m/z* 337 and *m/z* 449 ([M+H]), respectively, in LC–MS analysis (Fig. 2). The LC–MS analysis was performed using a Finnigan LCQ Deca XP ion trap mass spectrometer with ESI positive mode and surveyor HPLC system (ThermoQuest, San Jose, CA).

2.3. High-performance anion-exchange chromatography coupled with pulsed amperometric detection

The analysis was performed by Dionex ion chromatography system (ICS2500, Dionex, Sunnyvale, CA) composed of autosampler (AS 50 with a 25 μL sample loop), gradient pump (GP-50) with on line degasser and electrochemical detector (ED-50). The chromatographic separation was