Production of arginyl-fructosyl-glucose during processing of red ginseng

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Abstract

Arginyl-fructosyl-glucose (Arg-Fru-Glc), a new nitrogenous compound, was produced by the Maillard reaction during heat treatment for preparation of red ginseng from fresh one. Although arginine and maltose were found in Aconiti Tuber, Angelicae Tuhon Radix, Angelicae Radix and Astragali Radix, only a trace amount of Arg-Fru-Glc was produced by heat treatment of these crude drugs. It was found that absence of water and acidic conditions (pH＜3.0) were necessary for completion of the Maillard reaction between arginine and maltose. In ginseng, the amount of uronic acid, an acidic carbohydrate, increases as cultivation time is prolonged. No Arg-Fru-Glc was found in rat plasma after oral administration of this compound, whereas Arg-Fru was detected after oral administration of Arg-Fru.

On the basis of these experimental results, the mechanism of Arg-Fru-Glc production in ginseng and its physiological significance were discussed.

Key words red ginseng, white ginseng, uronic acid, arginyl-fructosyl-glucose.
Abbreviations Arg-Fru-Glc, arginyl-fructosyl-glucose ; Arg-Fru, arginyl-fructose.

Introduction

Panax ginseng C.A. MEYER has been used for thousands of years as an herbal medicine in East Asia including China, Korea and Japan. In the oldest known Chinese Pharmacopea, "Shen Nong Ben Tsao Ching", it was suggested to be a divine herb for maintaining youth and prolonging life. There have been extensive physiological and biochemical studies on the mechanisms responsible for the effects of ginseng in the body. Most of these studies have been done on the saponin fraction of ginseng.[1-6]

Recently, however, we have isolated physiologically active substances from non-saponin fractions of Korean red ginseng. One of them was an acidic polysaccharide that inhibited the lipolytic action of toxohormone-L in cancerous ascites fluid of sarcoma 180-bearing mice.[7] Others were adenosine and pyroglutamic acid, which inhibited epinephrine-induced lipolysis and stimulated insulin-mediated lipogenesis from glucose in fat cells.[8] In the course of these experiments, we discovered a new nitrogenous compound identified as arginyl-fructosyl-glucose (Arg-Fru-Glc) from the non-saponin fraction of Korean red ginseng.[9]

In contrast to Korean red ginseng, Korean white ginseng was found to contain an extremely low level of this nitrogenous compound. We proved that Arg-Fru-Glc was produced by the Maillard reaction between maltose and arginine during the heating process involved in the preparation of red ginseng.

The present investigation was done to clarify the factors required for production of Arg-Fru-Glc in ginseng and the mechanism of intestinal absorption of this compound.

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Materials and Methods

Korean ginseng : Red ginseng powder (*Panax ginseng* C.A. MEYER) was kindly provided by Nikkan Korai Ninjin Co. Ltd. (Kobe, Japan) and Korea Ginseng and Tobacco Research Institute (Taejon, Korea). White ginseng preparations obtained after cultivation of the plants for 1,2,3,4,5 and 6 years were kindly supplied by Dr. Hoon Park of the above institute. Freshly prepared 6-year-old ginseng roots were provided by Prof. Yinan Zheng, Department of Medicinal Plants, Jilin Agricultural University, China.

Other materials : Aconiti Tuber, Angelicae Tuhon Radix, Angelicae Radix and Astragali Radix were kindly supplied by Dogendo Pharmacy, Matsuyama, Japan. These materials were prepared by sun-drying.

Amino acid analysis : One g of each material was mixed with 10 ml of water and the mixture was stirred at 4°C for 12 h, followed by centrifugation at 10,000×g for 20 min. One ml of 5-sulfosalicylic acid solution (2 %) was added to 0.5 ml of the supernatant fraction. After centrifugation, an aliquot of the resulting supernatant was subjected to amino acid analysis (Hitachi type 835 apparatus). Amino acids and related compounds were detected by the ninhydrin reaction.

Estimation of maltose content : One g of each material was mixed with 10 ml of water and the mixture was stirred at 4°C for 12 h. After centrifugation, the supernatant was subjected to HPLC using a YMC-Pack PA-03 (250×4.6 mm I.D.) and 75 % CH_3CN as the eluent, and the eluate was analyzed with a TOSOH RI-8010.

Intestinal absorption of Arg-Fru-Glc and Arg-Fru : Male Wistar-King rats, weighing 180 to 200 g, were given a standard laboratory diet and water *ad lib.* All were subjected to an overnight fast before the experiments. Two ml of saline solution containing Arg-Fru-Glc or Arg-Fru was orally administered to the rats, and blood was collected from the abdominal vein 1 h and 2 h later. The plasma prepared from the blood was sujected to amino acid analysis after deproteinization.

Results

In the previous study, we found that red ginseng contained a large amount of Arg-Fru-Glc (42.8 mg/g) whereas white ginseng possessed only a trace amount.[9] Furthermore, we clarified that Arg-Fru-Glc was produced from arginine and maltose by the Maillard reaction during the processing from fresh ginseng to the red one. In addition to white ginseng, arginine and maltose were found in various dried plants such as Aconiti Tuber, Angelicae Tuhon Radix, Angelicae Radix and Astragali Radix, as shown in Table I. Then, these plants including white ginseng, were subjected to heat treatment at 100°C for 2 h and estimation of their Arg-Fru-Glc contents was made. Before the heat treatment, white ginseng did not contain any Arg-Fru-Glc, whereas 3.6 mg/g of the compound was found after the treatment. Although other plants contained both arginine and maltose, only a trace amount of Arg-Fru-Glc (0.4-0.0 mg/g) was produced by the heat treatment. These results suggest that some factors other than arginine and maltose may participate in the production of Arg-Fru-Glc.

It is well known that water is liberated in the Maillard reaction of carbohydrates with amino acids. Therefore, it is likely that water inhibits the synthesis

Table I Arginine and maltose contents of various medicinal plants.

Medicinal plants	Arginine (mg/g)	Maltose (mg/g)	Arg-Fru-Glc after heat treatment (mg/g)
White ginseng	10.4	7.8	3.6
Aconiti tuber	9.6	7.2	0.3
Angelicae tuhon radix	14.4	8.8	0.4
Angelicae radix	17.6	3.1	0.0
Astragali radix	2.0	0.9	0.0

Arginine and maltose were estimated as described in "Materials and Methods". Heat treatment was carried out at 100 °C for 2 h. White ginseng was prepared after 6 years of cultivation. Values are means of three separate assays.

of Arg-Fru-Glc from arginine and maltose through this reaction. This was found to be the case, as shown in Fig. 1. Arg-Fru-Glc synthesis by the Maillard reaction was considerably inhibited by addition of water to the mixture of arginine and maltose in glacial acetic acid.

The pH of the reaction mixture was also a factor which affected the synthesis of Arg-Fru-Glc. As shown in Fig. 2, Arg-Fru-Glc was found to be synthesized at a pH below 3.00 in acetate buffer but not at over pH 3.50. These results indicate that absence of water and acidic conditions are essential factors for the synthesis of this nitrogenous compound through the Maillard reaction.

It is well known that the processes of dehydration and heat treatment are repeated in the preparation of red ginseng from fresh one. These processes thus provide suitable conditions for the synthesis of Arg-Fru-Glc. Generally, red ginseng is prepared from fresh ginseng roots that have been cultivated for 6 years.

Previously, we found that Korean red ginseng contained an acidic polysaccharide consisting mainly

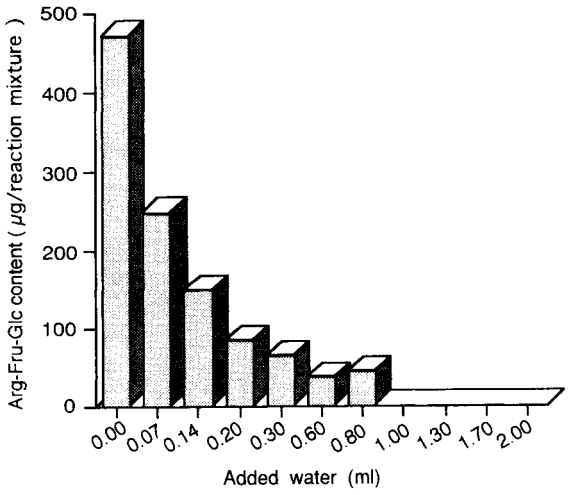

Fig. 1 Inhibitory action of water on synthesis of Arg-Fru-Glc from arginine and maltose by the Maillard reaction.

Arginine (1 mg) and maltose (2 mg) were dissolved in 2 ml of glacial acetic acid containing various amounts of water. The mixture was subjected to heat treatment (100 °C, 1 h) and then concentrated to dryness. The dried material was dissolved in 3 ml of water and the mixture was subjected to estimation of Arg-Fru-Glc content by an amino acid analyzer. Values are means of three separate assays.

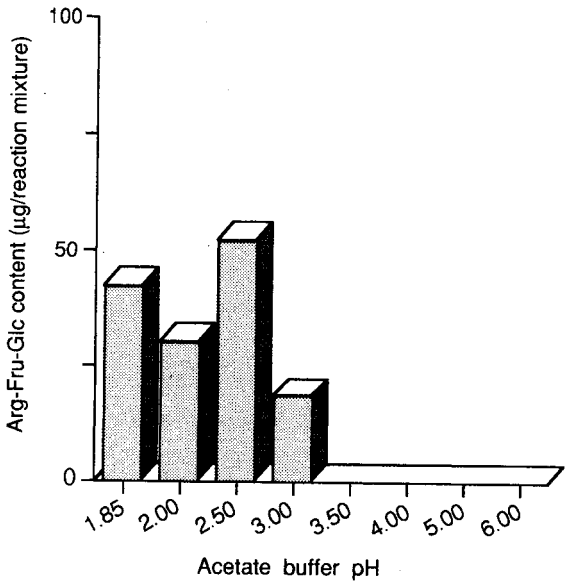

Fig. 2 Effect of acetate buffer pH on synthesis of Arg-Fru-Glc from arginine and maltose by the Maillard reaction.

Acetate buffers (5 M) with various pH values were prepared from 5 M sodium acetate and 5 M acetic acid. Arginine (1 mg) and maltose (2 mg) were dissolved in 2 ml of each acetate buffer. The mixture was subjected to heat treatment (100°C, 1 h) and then concentrated to dryness. The dried material was dissolved in 3 ml of water and mixture was subjected to estimation of Arg-Fru-Glc content by an amino acid analyzer. Values are means of three separate assays.

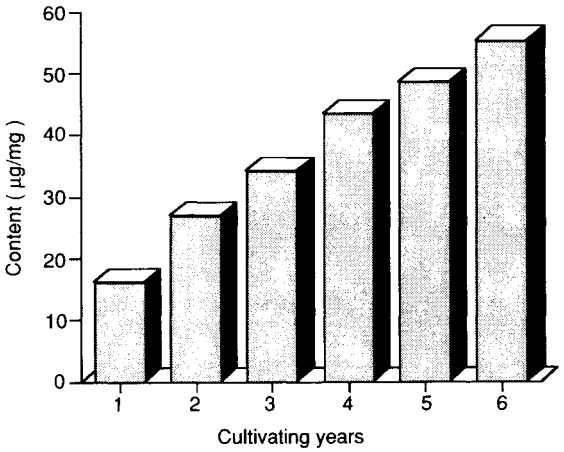

Fig. 3 Uronic acid content as a function of years of cultivation.

Ten mg of white ginseng powder was dissolved in 10 ml of water. One ml of the mixture was subjected to estimation of uronic acid by the method of Dische.[10] Values are means of three separate assays.

of uronic acid or acidic carbohydrate.[7] The uronic acid content of white ginseng was found to increase with prolongation of the cultivation period, as shown in Fig. 3. Six-year-old ginseng contained about 3 times more uronic acid than 1-year-old roots. Therefore, it seems likely that 6-year-old ginseng roots are more acidic than 1-year-old material, thus providing better conditions for production of Arg-Fru-Glc.

Experiments were then designed to clarify the mechanism of the intestinal absorption of Arg-Fru-Glc. For this, an attempt was made to synthesize Arg-Fru-Glc and Arg-Fru by the Maillard reaction. Ten g of arginine and 20 g of carbohydrate (maltose or glucose) were dissolved in 50 ml of glacial acetic acid, stirred for 20 min at 80°C and then centrifuged at 1,000×g for 10 min.

The supernatant fraction was dried, dissolved in 50 ml of water and applied to an ion-exchange column (CG-Amberlite IR-120-1, 50 ml). After washing with 200 ml of water, synthesized material was eluted with 300 ml of 0.5 % ammonia solution, and the eluate was freeze-dried. One g of the dried material was dissolved in 5 ml of a mixture of butanol, acetic acid and water (2 : 1 : 1, v/v/v). The resulting solution was then applied to a silica gel column (27 mm×86 cm, kieselgel 160, 70-230 mesh, ASTM) and eluted with the same mixture.

The elution patterns produced after ninhydrin staining are shown in Fig. 4. An aliquot of the eluate in each tube was subjected to analysis with an amino acid analyzer. Arg-Fru-Glc was found to be eluted from tubes Nos. 120 to 145 in (A) of Fig. 4 and Arg-Fru from tubes Nos. 85 to 120 in (B). Various amounts of Arg-Fru-Glc (110 mg, 330 mg and/or 800 mg) thus obtained were dissolved in 2 ml of saline solution, which was then orally administered to rats. Plasma was collected 1 h later, and subjected to amino acid analysis after deproteinization.

Even after administration of a high dose of Arg-Fru-Glc (800 mg/rat), Arg-Fru-Glc was not detectable in the plasma. On the other hand, Arg-Fru was found in plasma after oral administration of 200 mg of Arg-Fru ; 0.83 % of the administered dose 1 h after administration and 1.47 % 2 h after. These results suggest that Arg-Fru-Glc may be digested to Arg-Fru in the small intestine by the action of maltase, and that the resulting Arg-Fru may be absorbed from the intestine.[9]

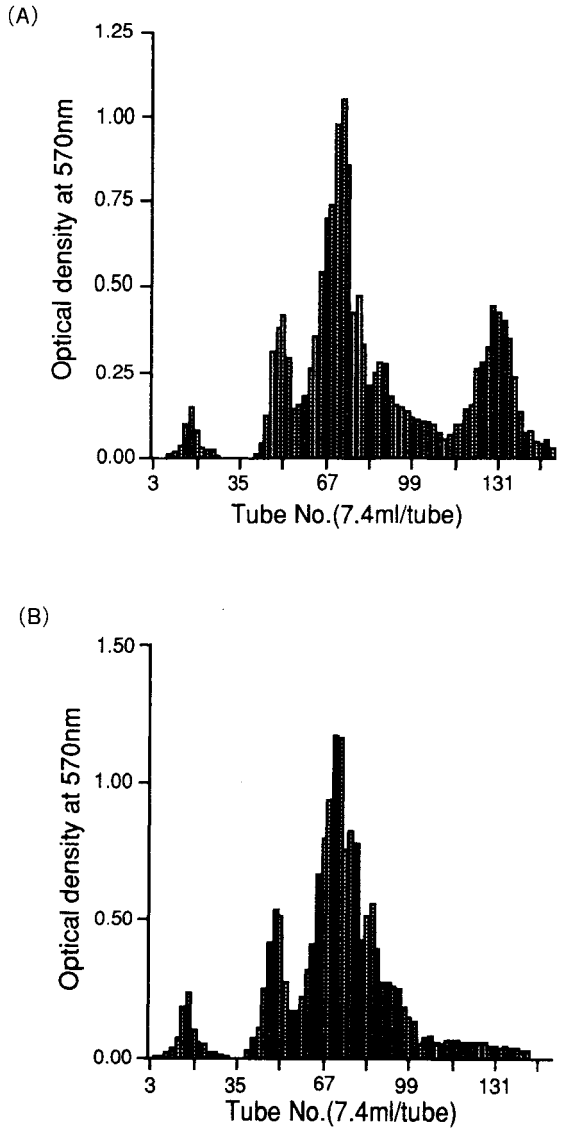

Fig. 4 Separation of Arg-Fru-Glc and Arg-Fru from the products of the Maillard reaction using a silica gel column.

Maillard reactions were carried out as described in the text. The reaction product from arginine and maltose (A) or arginine and glucose (B) was applied to a silica gel column (27 mm × 86 cm, Kieselgel 160, 70 - 230 mesh, ASTM) and eluted with a mixture of butanol, acetic acid and water (2 : 1 : 1, v/v/v). The eluate was analyzed using the ninhydrin reaction at 570 nm and with an amino acid analyzer. Arg-Fru-Glc was found to be eluted from tubes Nos. 120 to 145 in (A) and Arg-Fru from tubes Nos. 85 to 120 in (B).

Discussion

It is well known since ancient times that fresh ginseng roots can be processed in two ways : sun-drying and steaming. Sun-drying of fresh ginseng produces white ginseng, whereas the process that includes steaming produces red ginseng.

Private preparation of red ginseng has been prohibited by the government of Korea since ancient times, and the government has held a monopoly for red ginseng. However, the pharmaceutical differences between white and red ginseng are unknown. The present communication may provide a clue for clarification of these differences.

In our previous study, Arg-Fru-Glc was found to be synthesized from arginine and maltose by the Maillard reaction during the preparation of red ginseng. Although Aconiti Tuber, Angelicae Tuhon Radix, Angelicae Radix and Astragali Radix contain arginine and maltose, only a trace amount of Arg-Fru-Glc was formed in these dried plants after heat treatment (Table I).

These results suggest that factors other than the substrates for the Maillard reaction may be required for the synthesis of Arg-Fru-Glc during heat treatment. One of these factors was found to be the water content of medicinal plants during heat treatment (Fig. 1). Another was acidic conditions during the treatment (Fig. 2).

In the preparation of red ginseng from white ginseng, steaming and drying are repeated, during which Arg-Fru-Glc synthesis by the Maillard reaction might occur in the presence of a low water content during heat treatment. Usually, red ginseng is prepared from 6-year-old fresh ginseng, in which the uronic acid content is the highest (Fig. 3). Uronic acid will increase the acidity of ginseng roots. Thus it is suggested that 6-year-old ginseng roots may be the best material for preparation of red ginseng.

As reported previously, a trace amount of Arg-Fru-Glc was found in white ginseng ; 1-year-old 0 %, 2-year-old 0 %, 3-year-old 0 %, 4-year-old 0.08 %, 5-year-old 0.09 % and 6-year-old 0.2 %.[9] Thus, the highest amount of Arg-Fru-Glc was found in 6-year-old white ginseng.

We did not examine the water content and acidity of Aconiti Tuber, Angelicae Tuhon Radix, Angelicae Radix and Astragali Radix, and therefore it remains to be clarified why these dried plants fail to produce as much Arg-Fru-Glc as 6-year-old white ginseng (Table I).

In our previous study, Arg-Fru-Glc was found to inhibit maltase activity in the mucous layer of the rat jejunum.[9] In this inhibitory action, breakdown of labelled maltose to glucose by maltase was competitively inhibited by Arg-Fru-Glc, which was also hydrolyzed by maltase to Arg-Fru and glucose. No Arg-Fru-Glc was found in plasma after oral administration of this compound to rats, whereas oral administration of Arg-Fru caused elevation of the Arg-Fru level in plasma. These results suggest that Arg-Fru-Glc may be digested to Arg-Fru and glucose by maltase in the small intestine, and that the resulting Arg-Fru may be absorbed from the intestine.

Recently, Kon *et al.* found that intravenous injection of Arg-Fru into rabbits caused an increase in peripheral blood flow.[11] Furthermore, it has shown that blood vessel nitric oxide synthase acts on Arg-Fru and produces nitric oxide (Ichikawa, Y : personal communication).

Summarizing these experimental results, Arg-Fru-Glc produced during the preparation of red ginseng from fresh one may be digested to Arg-Fru and glucose by maltase in the small intestine and absorbed as Arg-Fru, which may produce nitric oxide through the action of blood vessel nitric oxide synthase and cause an increase in peripheral blood flow.

和文抄録

新鮮な薬用人参から紅参を作成する熱処理の過程で，新規物質であるArg-Fru-Glcがアルギニンとマルトースから生成される。しかし，附子，ウド，当帰，黄耆には，アルギニンやマルトースが存在するにも拘わらず，熱処理によってArg-Fru-Glcはほとんど生成されない。そこで，アルギニンとマルトースからメイラード反応でArg-Fru-Glcが生成する条件について検討したところ，水が存在しないこととpHが3.0以下であることが必要なことが明らかになった。一方，薬用人参中の酸性糖であるウロン酸は栽培年数が増えるほど増加することが判った。Arg-Fru-Glcをラットに経口投与しても血

漿中に Arg-Fru-Glc は出現しないが，Arg-Fru を経口投与すると，血漿中に Arg-Fru が認められた。この様な事実を基にして，薬用人参中の Arg-Fru-Glc の生成やその生理的意義について論じた。

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