STUDIES ON BIOLOGICALLY ACTIVE SUBSTANCES IN NON-SAPONIN FRACTION OF KOREAN RED GINSENG

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ABSTRACT

Acidic polysaccharide from Korean red ginseng was found to inhibit pancreatic lipase activity and cause reduction of plasma triglyceride level after oral administration of corn oil emulsion to rats. Thus acidic polysaccharide may reduce plasma triglyceride through its inhibitory action on pancreatic lipase and successive inhibition of intestinal absorption of fat due to reduction of lipolysis.

In the course of this experiment, we found an unknown ninhydrin positive substance in Korean red ginseng. The unknown substance was identified to be arginyl-fructosyl glucose(Arg-Fru-Glc). Content of this new compound was 5.37% in Korean red ginseng powder. Sucrase and maltase activities in mucous layer of rat jejunum were found to be inhibited by Arg-Fru-Glc. Physiological significance of the new compound was discussed based on these experimental results.

INTRODUCTION

Panax ginseng is a medicinal plant long used in the treatment of various pathological states including general complaints such as headache, shoulder ache, chilly constitution, anorexia and diabetes.

In 1990, we isolated "selective modulators" from non-saponin fraction of Korean red ginseng. These are adenosine and pyro-glutamic acid which inhibit epinephrine-induced lipolysis and stimulate insulin-mediated lipogenesis from glucose in rat adipocytes[1]. In addition to these substances, we isolated an inhibitory substance toward Toxohormone-L from the non-saponin fraction[2]. Toxohormone-L is secreted from various cancer cells and has two actions: lipolytic and anorexigenic, which may cause reduction of body fat in cancer patients[3]. The inhibitory substance toward the actions of Toxohormone-L was found to be a pectin-like α-1, 4 polygalacturonan backbone with ace-toxyl groups, and so was an acidic polysaccharide.

In the present investigation, we search for other biological activities of this acidic polysaccharide. In the course of this experiment, a new compound was found from the non-saponin fraction. The present investigation also describes preparation and identification of this new compound, and its physiological significance.

MATERIALS AND METHODS

Animals: Young male Wistar king rats, weighing 160 to 200 g, were allowed free access to the standard laboratory diet and water.

Red 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.

Other materials: [^{14}C(U)]-sucrose was from Du Pont-New England Nuclear. [^{14}C(U)]-maltose was from Amersham. Triglyceride E-test kit was from Wako Pure Chemical Industries, Ltd.(Osaka, Japan). Procine pancreatic amylase was from Sigma(St. Louis, MO).

Measurement of lipase activity: Lipase activity was determined with Triolein as the substrate by measuring oleic acid release. Triolein(79.4mg) was sonicated with EPL(9.55 mg) and sodium taurocholate(5mg) in 10mℓ of 0.1M N-Tris-(hydroxymethyl)methyl-2-aminoethanesulfonic acid(TES) buffer (pH 7.0) contained 0.1M NaCl in an ice bath. The assay system contained the following components in a total volume of 0.2mℓ: 0.1mℓ of triolein substrate emulsion, 0.05ml of the above TES buffer or acidic polysaccharide dissolved in the buffer and 0.05mℓ of enzyme solution dissolved in the buffer. Reaction at 37℃ were started by adding the enzyme solution and stopped by 3mℓ of chloroform-heptane-methanol 49: 49: 2(v/v/v). Oleic acid released was estimated with copper reagent and bathocuproine by the method of Zapf *et al.*[4].

Oral administration of corn oil to rats: Twenty male rats were divided into two groups. One (10 rats) was control group and the other (10 rats) treated one. The rats of control group were administered with 0.8mℓ of water and 1mℓ of lipid emulsion which was sonicated mixture of corn oil (6mℓ), sodium cholate(80mg), cholesterololeate(2g) and water(6mℓ). The rats of treated grorp were administered with 0.8mℓ of acidic polysaccharide solution(125mg/mℓ) and 1mℓ of the lipid emulsion.

Estimation of sucrase and maltase activities: Thirty cm of jejunum were removed from male rats weighing 200g. After washing with saline solution, the mucous layer was scraped out from the jejunum, added with 10mℓ of 80mM phosphate buffer(pH 7.0) and homogenized. The homogenate was centrifuged at 3,500×g for 10min and the resultant supernatant was used as enzyme solution of sucrase and/or maltase. Assay

mixture for sucrase or maltase was consisted of 80$\mu\ell$ of 5mM ^{14}C - sucrose(1.6μCi) or 5mM ^{14}C - maltose(1.6μCi), 10$\mu\ell$ of test solution and 10$\mu\ell$ of the enzyme solution. The mixture was incubated for 30min at 37℃ and boiled for 2min to stop the reaction. Five $\mu\ell$ supernatant of the reaction mixture was spotted on a TLC plate and developed with aceton and water(9 : 1, v/v). Radioactivity of the liberated monosaccharide was counted. Enzyme activity was expressed as liberated monosaccharide nmol/min/mg protein for sucrase and as liberated glucose μmol/min/mg protein for maltase.

Statistical analysis : Student's t test was used to determine the significance of differences.

RESULTS AND DISCUSSION

Effect of acidic polysaccharide on intestinal absorption of fat : Acidic polysaccharide was prepared from Korean red ginseng by the method reported previously[2]. The acidic polysaccharide was found to inhibit dose - dependently pancreatic lipase activity as shown in Fig. 1. When corn oil emulsion was orally administered to rats, plasma triglyceride level was gradually elevated and reached at its maximal level at 4h after the administration. On the other hand, oral administration of corn oil emulsion and acid polysaccharide caused less increase in plasma triglyceride level than that of control group(Fig. 2). It was found that there was no difference in lipoprotein lipase activities of post - heparin plasma between control and acidic polysaccharide groups at 4h after oral administration of corn oil emulsion.

These results suggest that acidic polysaccharide causes reduction of plasma triglyceride level through its inhibitory action on pancreatic lipase and successive inhibition of intestinal absorption of fat due to reduction of lipolysis. It seems likely that such an action of acidic polysaccharide may improve hyperlipaemia in man and thus be good for prevention of obesity and atherosclerosis. Acidic polysaccharide content is extremely higher in red ginseng than in white one

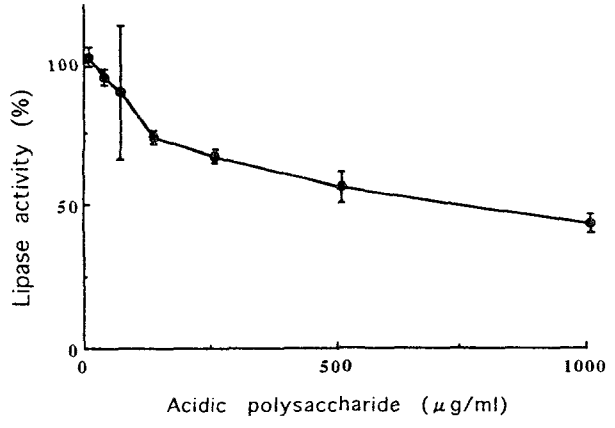

Fig. 1. Effect of acidic polysaccharide on pancreatic lipase activity.
Each point represents as means ± SE of three separate assays.

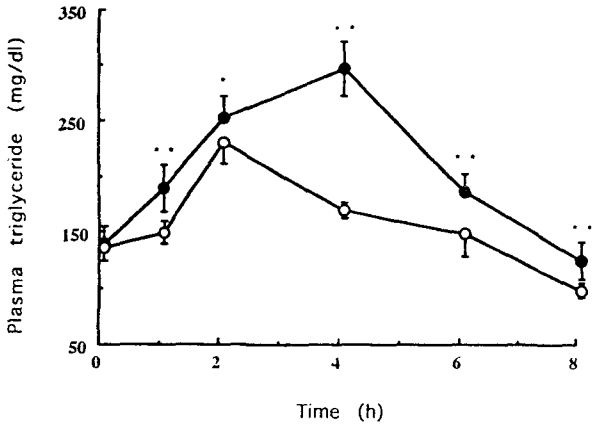

Fig. 2. Effect of acidic polysaccharide on plasma triglyceride of rats after oral administration of corn oil emulsion.
Each point represents as means ± SE of ten separate experiments.
●—● : control group, ○—○ : acidic polysaccharide group.
* : $p<0.05$, * * : $p<0.01$.

(Table 1). Furthermore, big roots of red ginseng contains higher amount of acidic polysaccharide than the small roots, suggesting that big roots of red ginseng might be the most effective ginseng products for improvement of hyperlipaemia.

Table 1. Acidic polysaccharide content of various ginseng products.

Ginseng		Acidic polysaccharide content	
		mg/g	%
White	Big roots	6.27	0.63
	Small roots	8.62	0.86
Red	Big roots	74.65	7.46
	Small roots	47.84	4.78

Discovery of new compound in Korean red ginseng : Korean red ginseng powder was added with 10 volume of water and mixed for 12h at 4℃. The resultant water extract was dialyzed against water. The outer dialysate was concentrated and subjected to analysis with Hitachi Amino Acid Analyzer - 835. Unknown ninhydrin postive peak was found around the peak of β - alanin. The unknown peak was collected and desalted with reverse - phase HPLC (ODS - 120T). Arginine was found to be liberated from the unknown peak fraction after treatment with 3N ammonia for 1h at 100℃, suggesting that arginine was one of the components in the unknown peak fraction.

Analysis with ^{13}C - NMR spectrum suggests existence of disaccharide in addition to arginine in this unknown fraction : {δ24.71(CH_2), 27.26(CH_2), 41.23(CH_2), 53.14(CH_2), 61.30(CH_2), 63.13(CH), 64.76(CH_2), 69.62(CH), 70.02(CH), 70.34(CH), 72.49(CH), 73.19(CH), 73.55(CH), 78.38(CH), 96.19(C), 101.38(CH), 157.57(C), 173.52(C)}.

After alkaline hydrolysis (3N ammonia, 1h, 100℃), the unknown fraction liberated glucose and fructose which were identified by GC - MS. Furthermore, only glucose was found to be liberated after treatment of the unknown fraction with α - glucosidase. These results suggest that the unknown fraction is a derivative of fructose with which arginine and glucose were associated. Analysis of the unknown fraction with laser - ionized TOF - MS showed M/Z 498.4 of molecular ion peak which was coincident with its estimated chemical structure ($C_{18}H_{34}N_4O_{12}$). Analysis with ^1H - NMR clarified that proton of C_1 position in glucose split into 3 peaks, δ5.00(d, J=3.66), 5.05(d, J=3.66) and 5.09(d, J=3.66) and fructose existed in the state of α and β - anomers in which pyranose and fura nose forms were equilibrated. These results indicate that glucose does not associate with C_2, C_5 and C_6 positions of fructose.

Arginine was liberated after treatment of the unknown fraction with pancreatic cholesterolester - lipase, indicating that arginine is esterified by fructose at its C_1, C_3 or C_4 position. Analysis with ^{13}C - NMR showed downfield shift at C_1 position of glucose and with ^1H - NMR indicated small J va lue(3.66Hz) at C_1 position of glucose. All these facts suggest that glucose associated with fructose in the way of α 1-1, α 1-3 or α 1-4 bonding. Based on these informations, possible structure of the unknown fraction was presented as shown in Fig. 3.

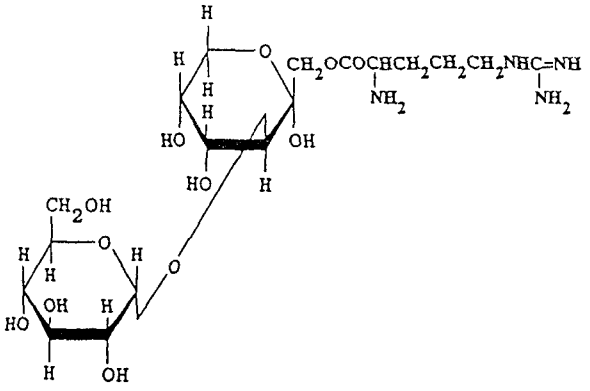

Fig. 3. Estimated structure of unknown substance in Korean red ginseng.

Content of the unknown fraction, or arginyl - fructosyl - glucose(Arg - Fru - Glc) was found to be 5.37% in Korean red ginseng powder. Then, experiments were carried out to clarify physiological significance of this new compound. It was found that sucrase and maltase activities in mucous layer of rat jejunum were inhibited by Arg - Fru - Glc as shown in Fig. 4 and 5. Inhibitory actions of Arg - Fru - Glc on sucrase and maltase may cause prolongation of intestinal absorption of sucrose and maltose and prevent rapid increase in blood glucose, fructose and possibly insulin.

It is well known that rapid increase in blood glucose and insulin accelerates lipogenesis in various tissues such as

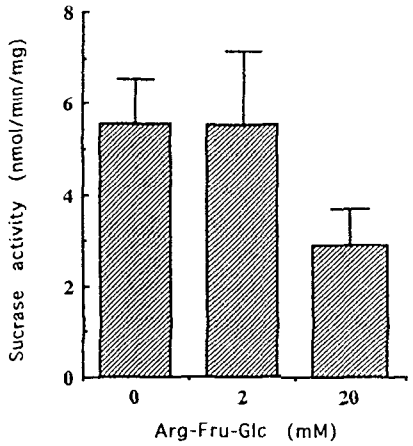

Fig. 4. Effect of Arg - Fru - Glc on sucrase activity. Values are means ± SE of three separate assays.

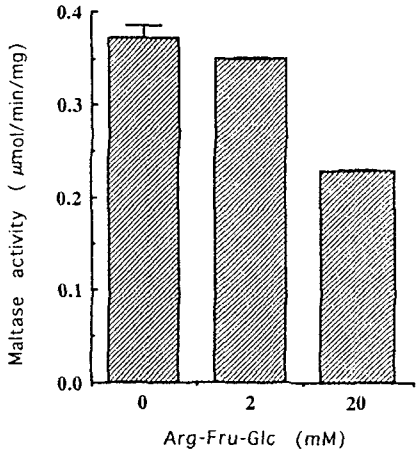

Fig. 5. Effect of Arg - Fru - Glc on maltase activity. Values are means ± SE of three separate assays.

liver and adipocytes and causes fatty liver, hyperlipaemia and obesity. Therefore, it seems possible that Arg - Fru - Glc in Korean red ginseng may protect the appearance of these disorders and improve those pathological states.

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