Components and Activity of Polysaccharides from Coarse Tea

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Coarse tea contained a high content of polysaccharide complex. Composed of polysaccharide and protein, the polysaccharide complex from tea (TPS) belonged to glycoprotein with the molecular weight () of (10.7−11.0) × 10^4. When mice (7 weeks old, C57BL/8) were injected with TPS, the levels of blood glucose (BG) in normal mice and model mice with high BG were decreased significantly by averages of 13.54 and 22.18%, respectively. The antibody concentration (OD_{413nm}) in the mice injected with 2.4 mg/mL TPS was increased evidently by 44.93% ($p < 0.01$). TPS treatment was beneficial not only for the subsequent production of interleukin (IL) 2 in spleen cells of adjuvant arthritis (AA) rats but also because it prohibited the body from producing too much IL-1 in AA rats. Treatment of diabetes with coarse tea in both China and Japan may be related to TPS and the content of TPS in coarse tea.

Keywords: *Tea; polysaccharide; components; activity*

INTRODUCTION

Diabetes, a chronic disease, endangers human health and is proving difficult to cure. Curing diabetes with coarse tea is a popular folk prescription in both China and Japan (*1*). Why does coarse tea have this function? The reason is still not completely understood. Along with the improvement of people's living standard and the increase in the number of diabetics throughout the world, especially in developing countries, more and more people are becoming interested in coarse tea and its effects on blood glucose (BG).

High BG is one of the markers of diabetes. Tea polysaccharides (TPS) and tea polyphenols (TPP) are the main components in tea, and they play the role of reducing BG in animals (*2*−*6*). The BG reducing activities are almost equal between green tea water extracts (GTWE) and black tea water extracts (BTWE), whereas the content of TPP in GTWE is twice that in BTWE (*7*). However, the contents of TPS in GTWE and BTWE were not significantly different (*8*). The results suggest that reducing BG with tea is closely related to TPS. It is probably because the content of TPS in coarse tea is very high that coarse tea is widely used to cure diabetes in Chinese and Japanese folk medicine. However, this function has not yet been well understood.

Until now the study on the activity and composition of TPS has mainly centered on the gross TPS effect on blood vessels and reducing BG, but a study on the function of purified TPS reducing BG and enhancing antibody levels has not yet been reported. As well, reports of the composition of TPS are seldom seen except for reports about the saccharide part (*9*). The composition of saccharides and protein in purified TPS remains unknown.

Coarse tea is made of mature tea plant [*Camellia sinensis* (L) O. Kuntze] shoots, whereas tea, green tea and black tea, consumed around the world is usually made from tender shoots, such as one or two leaves and a bud. People are becoming less and less interested in coarse tea with inferior flavor as improvements in their standard of living allow them to purchase better quality tea. Coarse tea is left in the warehouse or on the tea plants themselves and wasted. It is necessary for us to use this resource efficiently by studying its content and components and the activity of TPS from coarse tea.

MATERIALS AND METHODS

Chemicals and Reagents. Enzymes and chemicals were purchased from the following companies: trypsin (4000 units/mg) and pectinase (20 units/mg) from Bio Life Science and Technology Co.; ConA from Sigma Chemical Co.; Sephadex G-75, G-150, and G-200 from Pharmacies Fine Chemical. All other chemicals used were of guaranteed grade and from China Chemical Co.; the water used was glass doubly distilled water.

Animals for Experiments. One hundred and twenty C57BL/8 mice and 40 Sprague−Dawley (SD) rats were selected for these experiments. The average weight of the healthy, 7-week-old male mice was 20 ± 2 g; the healthy, 12-week-old male rats were 200 ± 15 g. The animals were housed in a room at 25 ± 1 °C, with a 12 h light−dark cycle, and fed with a complete diet.

Tea for Experiment. Green teas of various grades were purchased from Xihu Tea Co.. The contents of TPP, catechins, and caffeine in the tea samples were measured by conventional methods (*10*) and are shown in Table 1.

Preparation of TPS. Preparation of TPS referred to the method of Mizumo et al. (*11*) was as follows: The tea infusion of green tea of various grades was prepared respectively in the light of ISO-1574-1980 standards. The tea extract was concentrated in a rotary evaporator under reduced pressure, sedimented by alcohol containing 5% water for 24 h, and then

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Table 1. Contents of TPP, Catechins, Caffeine, and TPS in Various Grades of Green Tea (Grams per 100 g)

	grade[a]					
	first	second	thirst	fourth	fifth	sixth
TPP	22.03 ± 0.31	23.70 ± 0.97	21.10 ± 1.01	18.55 ± 0.31	19.05 ± 0.37	17.23 ± 0.85
catechins	14.07 ± 0.97	14.01 ± 0.78	12.56 ± 0.51	9.87 ± 0.71	9.63 ± 0.91	8.33 ± 0.79
caffeine	4.00 ± 0.21	4.09 ± 0.37	3.85 ± 0.11	3.25 ± 0.71	2.87 ± 0.05	3.01 ± 0.07
TPS	0.23 ± 0.10	0.29 ± 0.12	0.31 ± 0.17	0.46 ± 0.21	0.49 ± 0.15	0.58 ± 0.15

[a] The first grade tea consisted of 90% one leaf and a bud and 10% two leaves and a bud; the second grade, 60% one leaf and a bud and 40% two leaves and a bud: the third grade, 30% one leaf and a bud, 30% two leaves and a bud, and 40% three leaves and a bud; the fourth grade, 10% one leaf and a bud, 20% two leaves and a bud, 50% three leaves and a bud, and 20% tender banjhi; the fifth grade, 10% two leaves and a bud, 30% three leaves and a bud, 50% tender banjhi, and 10% leaves; the sixth grade, 80% tender banjhi and 20% leaves.

centrifuged (20 min, 5000*g*). The sediment was washed with ethanol, acetone, and ether alternately five times to exclude lipid completely. The sediment was dissolved in water before trypsin was added and hydrolysis at 38 °C for 36 h. Boiling water was used to stop the enzymatic reaction. The mixture was dialyzed for 24 h with running water and distilled water, respectively. Then pectinase was added, and the mixture was hydrolyzed at 40 °C and pH 4.0 for 18 h before it was deproteinized according to Sevag's method and dialyzed once more before it was sedimented by alcohol. The gross extract of TPS was obtained by vacuum-drying. The gross extracts of TPS from tea of various grades were respectively dissolved in water and separated through a Sephadex G-75 gel column with water at a flow rate of 0.35 mL/min. The protein eluted was determined automatically at 280 nm. The amount of saccharides per tube was determined by using the sulfuric acid−phenyl hydroxide method at 490 nm. The sugar part of high molecular weight was gathered and sedimented with alcohol and then purified through a Sephadex G-150 gel column with water at a flow rate of 0.12 mL/min. The eluent was collected, sedimented, and dried according to the same method mentioned above. Thus, the purified TPS was obtained and weighed (Table 1).

Assay of Purity and Molecular Weight of TPS from Coarse Tea. Four milligrams of TPS was dissolved in 1.5 mL of water, loaded on a Sephadex G-200 gel column equilibrated with 0.1 mol/L NaCl, and then eluted with 0.1 mol/L NaCl at the rate of 0.12 mL/min. The elutions of standard molecular weight of dextrans were carried out in the same manner.

Two milligrams of TPS was dissolved in 1.0 mL of water. The purity and the moleuclar weight of TPS were determined by HPLC on a Varian LC 5060 HPLC apparatus. The apparatus is equipped with UV-100 ultraviolet absorption and an RI-3 refractive index detector to assay the purity and the molecular weight of TPS, respectively, both of which used a TSK gel-2000SW column (7.5 × 300 mm).

Component of Protein and Polysaccharide in the TPS from Coarse Tea. The TPS was hydrolyzed with 6 mol of HCl in sealed evacuated tubes at 115 °C for 24 h. The components of amino acids of the protein in TPS were assayed with a Hitachi 835 amino acid analyzer. The component of monosaccharide of the polysaccharide in TPS was assayed according to the following steps: 10 mg of TPS was dissolved in 4.0 mL of 2 mol/L CF_3COOH, sealed, and dissociated at 100 °C for 8 h. The dissolved liquid (2.0 mL) was added to 1 drop of NH_3, 1.0 mL of insitol, and 30 mg of $NaBH_4$; after one night Dowex 50w-x8(H^+) was put in and filtered, and then 0.1 mL of trimethylsilyl ethers was added and dissolved adequately. The monosaccharide of polysaccharide in TPS was analyzed according to the method of Bradbury et al. (*12*) with a gas chromatograph (Shimadzu GC-9A, Japan).

TPS Dose for Experiment. The average coarse tea consumption was estimated at 30 g/day for a 60 kg diabetic (*13*), and the TPS content in coarse tea was 0.5%. The TPS doses for the experiments were set at about 6, 12, and 20 times more than the human dose; thus, 15, 30, and 50 mg of the TPS/kg of body weight were determined as the injecting doses for the experimental mice or rats.

Estimation of TPS Bioactivities. A total of 40 mice were divided into a control group and different dose experimental groups. The experimental groups separately received injections of 1.2, 2.4, and 4.0 mg/mL TPS (0.25 mL/mouse·day), while the control group received 0.25 mL of water, which was continued for 5 days. On the first day the mice received an injection of sheep red blood cells (SRBC). On the sixth day the blood of the mice in different treatment groups was taken, and then the blood serum was separated and diluted to five groups of different serum concentrations by saline; 2.5 mL of the blood serum of different concentrations was mixed in per hole separately with 0.5% suspension of 2.5 mL of SRBC and then incubated at 37 °C for 3 h. The antibody concentration in the blood serum was expressed by the optical density (OD_{413nm}) value that was assayed and computed according to a method described previously (*8*).

A total of 80 mice were divided into 8 groups randomly, of which 4 groups were injected with 50 mg/kg of body weight of alloxan and turned into high BG model. Three groups of normal mice received, respectively, various concentrations (1.2, 2.4, and 4.0 mg/mL) of TPS (0.25 mL/mouse·day) while the control group received water. Three groups of model mice received 4.0, 6.0, and 8.0 mg/mL TPS (0.25 mL/mouse·day) while the control group received 0.25 mL of water. After 12 h, the content of BG of all the mice was tested.

A total of 40 rats were divided randomly into 5 groups. Four of them were induced to be adjuvant arthritis (AA) model according to the method of Perper et al. (*14*). The effects of TPS on the ConA-induced proliferation reaction of spleen lymphocytes were determined according to the method of Yamamoto et al. (*15*). The final concentrations of TPS, respectively, were 1.2, 2.4, and 4.0 mg/mL with ^{3}H-TdR activity of 7.4×10^3 Bq per hole. The effects of TPS on interleukin (IL) 1 and IL-2 were tested by conventional methods (*16 17*).

Statistical Analysis. Results are expressed as means ± SD for observation of the indicated number (n). Statistical significance of differences between means was assessed by variance analysis (ANOVA). Values of $p < 0.05$ were considered to be significant and values of $p < 0.01$ very significant.

RESULTS AND ANALYSIS

TPS in Coarse Tea. Teas are graded according to the materials of the teas. The material for the first-grade tea was tenderer than that of the sixth, because the first-grade tea consisted mainly of one leaf and one bud, whereas the sixth consisted mainly of banjhi leaves, that is, coarse tea. The contents of TPP, catechin, and caffeine in the sixth-grade tea were less than those in the first-grade tea by 20, 40, and 25%, respectively, but the content of TPS in the sixth-grade tea was twice as high as that of the first one (Table 1). The results indicate that treating diabetes with coarse tea in folk medicine may be related to its high content of TPS.

TPS, a Glycoprotein. The elution profile of the gross extract of TPS on Sephadex G-75 shows that gross TPS is composed of two parts, a low molecular weight one and a high molecular weight one, which contained large amounts of saccharides and proteins. The elution profiles of the high molecular weight part obtained from

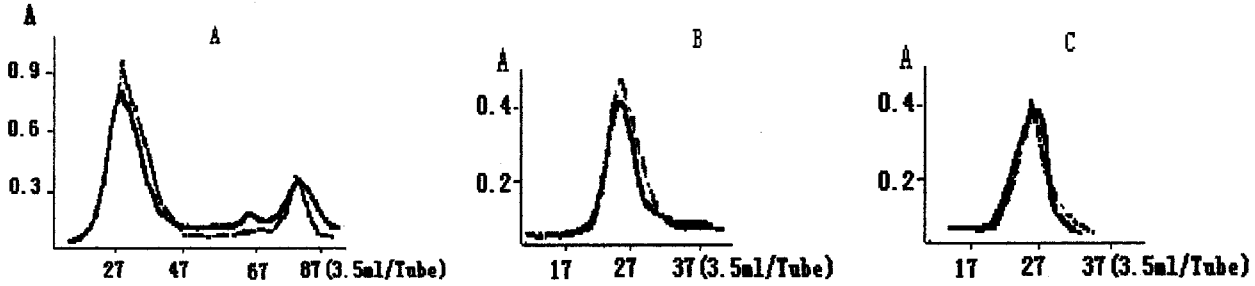

Figure 1. Elution profiles of TPS on Sephadex G-75 (A), Sephadex G-150 (B), and Sephadex G-200 (C) columns: solid line, 280 nm; dotted line, 490 nm.

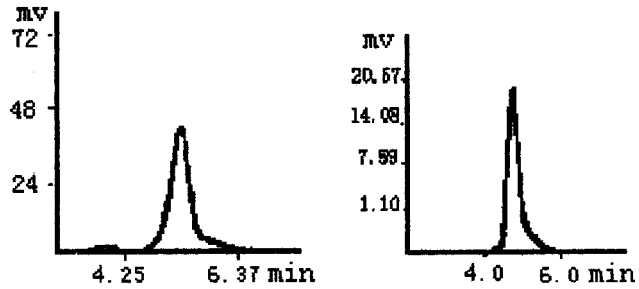

Figure 2. Chromatogram of TPS purified by Sephadex G-150 gel column on HPLC: left, profile measured by a RI-3 detector; right, profile measured by a UV-100 detector at 280 nm.

Table 2. Elution Data of Different Molecular Weights on a Sephadex G-200 Column and HPLC

sample name		Sephadex G-200			HPLC
	MW ($\times 10^4$)	elut vol (mL)	V_e/V_o	Log M	RT (min)
Blue detran	>200	68 (V_o)			
Dextran T500	50	73 (V_e)	1.07	5.70	5.017
Dextran T200	20	85 (V_e)	1.25	5.30	
Dextran T100	10	100 (V_e)	1.47	5.00	5.300
Dextran T70	7				5.378
Dextran T40	4				5.791
Dextran T35	3.5	125 (V_e)	1.84	4.54	
Dextran T15	1.5	140 (V_e)	2.06	4.18	
TPS		99 (V_e)	1.45	5.04	5.289

Table 3. Effect of TPS on Blood Agglutinin in Mice (n = 10, $\bar{x} \pm$ SD)

	TPS concn			
	control	1.2 mg/mL	2.4 mg/mL	4.0 mg/mL
antibody concn (OD_{413nm})	2.3 ± 0.8	3.0 ± 0.5	3.7 ± 0.9^b	3.3 ± 0.8^a

[a] Compared with the control group $p < 0.05$. [b] Compared with the control group $p < 0.01$ (same as Table 4).

both the Sephadex G-150 and Sephadex G-200 columns show that the peak shapes of saccharide and protein on Sephadex G-150 and Sephadex G-200 columns are similar and the peak positions of the saccharide and protein are nearly overlapping (Figure 1). This indicates that TPS is a complex of saccharide and protein, hence, a glycoprotein, and that the proteins and saccharides were tightly bound and difficult to separate from each other.

Molecular Weight of TPS. The elution profiles of the purified TPS on the Sephadex G-200 gel column and HPLC suggest that the TPS was quite pure (Figure 2). The data of the molecular weight of TPS obtained by Sephadex G-200 gel column and HPLC are shown in Table 2. The molecular weight determined by HPLC was $\sim 11 \times 10^4$ and the one obtained by Sephadex G-200 gel column, 10.7×10^4. Clearly these are quite similar.

Components in TPS. The monosaccharides of the saccharide in TPS were composed of Ara, Xyl, Fuc, Glc, and Gal. Their mole percentages were 6.49, 2.60, 6.53, 43.27, and 41.11%, respectively.

The amino acid composition of the protein in TPS was Ala plus Gly, 17.89% (the figure after the amino acid name standing for mole percentage in the total moles of amino acids); Val, 5.63%; Leu, 5.93%; Ser, 6.10%; Thr, 3.39%; Met, 0.55%; Asp, 18.27%; Glu, 24.23%; Lys, 4.97%; Arg, 2.87%; Phe, 1.74%; Tyr, 1.57%; His, 0.55%; Pro, 3.34%; and Hypro, 2.95%. It is evident that TPS contained many natural amino acids and that the content of amino acids with one amino and two carboxyls was higher.

Bioactivities of TPS. Amounts of 1.2−4.0 mg/mL of TPS could enhance immunization of the mice. The antibody concentration (OD_{413nm} value) of the mice treated by 2.4 mg/mL increased by 60.87% compared with the control group, which was very significant (Table 3). TPS evidently reduced the content of BG in the mice, especially that of model mice with high BG induced by alloxan (Table 4). The model of AA rats is a type of animal model with immunization disorder and is usually used to select anti-inflammation immunization drugs. The data in Table 5 show that TPS could not only increase but also control immunization. The control effect was bidirectional. The first was that the TPS could help produce IL-2 in spleen cells of AA rats, and the other was that the TPS would control the production of too much IL-1 in AA rats.

DISCUSSION

TPS has more bioactivity that can benefit diabetics besides the above, for example, the effect of protecting the body from radiation, delaying thrombosis time, improving the blood vessel system, and decreasing lipid content (*9*). However, it is only under higher concentrations of TPS that TPS have significant effects in decreasing BG, increasing immunization, and so on. Generally speaking, the TPP in high concentration tastes bitter, and caffeine in high concentration can make people overexcited (*1*). The TPS concentration in coarse tea is very high, whereas the TPP and caffeine

Table 4. Effect of TPS on BG in Normal Mice and Model Mice (n = 10, $\bar{x} \pm$ SD)

normal mice				model mice			
control	1.2 mg/mL	2.4 mg/mL	4.0 mg/mL	control	4.0 mg/mL	6.0 mg/mL	8.0 mg/mL
9.6 ± 0.6	8.8 ± 0.7	8.1 ± 0.4^a	8.0 ± 0.6^b	17.9 ± 1.3	16.7 ± 1.4	13.0 ± 2.1^a	12.1 ± 1.5^b

Table 5. Effect of TPS on Immunization of Rats with Adjuvant Arthritis ($n = 8$, $\bar{x} \pm$ SD)

	content ($\times 10^4$ cpm) of ^{3}H-TdR combined		
group	proliferation	IL-2 activity	IL-1 activity
normal rats	2.06 ± 0.41^a	0.70 ± 0.12^a	0.51 ± 0.19^a
AA rats	0.91 ± 0.32^b	0.35 ± 0.10^b	1.21 ± 0.31^b
AA rats + 1.2 mg/mL	1.08 ± 0.41^b	0.39 ± 0.11^b	1.05 ± 0.31^b
AA rats + 2.4 mg/mL	1.35 ± 0.39^{cb}	0.49 ± 0.14^{cb}	0.91 ± 0.21^{cb}
AA rats + 4.0 mg/mL	1.15 ± 0.47^b	0.39 ± 0.12^b	0.96 ± 0.21^b

[a] Compared with the AA rat group $p < 0.01$. [b] Compared with the normal rat group $p < 0.01$. [c] Compared with the AA rat group $p < 0.05$.

contents in it are very low (Table 1). This may be the reason people in China and Japan use coarse tea for the treatment of diabetes.

Early studies (*5*, *6*, *9*, *18*) on TPS were mainly focused on its bioactivity, its composition, and molecular weight of saccharide in the gross product of TPS, but the reported results do not agree with each other. Monosaccharides were mainly Glc, Gal, and Ara, and the molecular weight was $\sim 4 \times 10^4$. Whether the protein is bound in TPS has not been reported. Figures 1 and 2 and Table 2 indicate that the polysaccharide complex belongs to a kind of macromolecular glycoprotein. The molecular weight of TPS in coarse tea was higher than that of earlier reports, and the composition of monosaccharides was also different. These discrepancies may be due to the facts that the materials of the coarse tea were mature and the preparation and purifying methods were different from those in early studies.

The amount of TPS used in this experiment was much larger than that in 30 g of coarse tea. A diabetic has to drink at least 30 g of coarse tea per day, which is not easily accepted by diabetics because the flavor of coarse tea is inferior and preparing the tea infusion is also boring. When the gross TPS was mixed with food, a healthy and antidiabetes food was produced and was well received by patients (*6*). With the abundant resource and low price in tea-producing countries, preparing and using TPS from coarse tea can be of great value.

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