Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 90 (2005) 17–21
Food Chemistry
www.elsevier.com/locate/foodchem

Components and antioxidant activity of polysaccharide conjugate from green tea

Haixia Chen [a,b], Min Zhang [b], Bijun Xie [b,*]

[a] Institute of Marine Drug and Food, Ocean University of China, Qingdao 266003, PR China
[b] Department of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China

Received 21 March 2003; received in revised form 3 March 2004; accepted 3 March 2004

Abstract

Tea polysaccharide conjugate (TPC) has been used to cure diabetes in China. In order to characterize the chemical properties and activities of TPC, the TPC was isolated and purified from lower grade green tea. The monosaccharide components and the average molecular weight of TPC were analyzed by gas chromatography (GC) and high performance liquid chromatography (HPLC) methods, respectively. The antioxidant activity of TPC was tested using a deoxyribose assay, a photoreduction of nitro blue tetrazolium (NBT) assay and lipid peroxidation inhibition assay. The antioxidant activity on alloxan-induced oxidative damage was also tested. TPC was a conjugate with the molecular weight (MW) of 120 kDa. TPC exerted significant inhibitory effects on hydroxyl and superoxide radicals and lipid peroxidation with IC_{50} values of 101, 145 and 238 μg ml^{-1}, respectively. TPC could also improve the activity of superoxide dismutase (SOD) ($p < 0.05$). These results suggested that TPC was a potent antioxidant and there appeared to be a direct connection between antioxidant activity and hypoglycemic activity.

Keywords: Tea polysaccharide conjugate; Components; Antioxidant activity

1. Introduction

Tea consumption benefits health in many ways. Earlier studies have shown that green tea and its polyphenol constituents are effective antioxidants and have pharmacological activities, such as anti-cancer, anti-mutation and anti-atherosclerosis (Andrea & Michael, 1997; Chi, 1997; Zhu, Wang, & Guo, 2001). Lower grade green tea has traditionally been used to cure diabetes in east Asia, especially in China and Japan (Chen & Xie, 2001). However, the content of polyphenols in low-grade tea is low. Increasingly, people are becoming interested in lower grade tea and it has been found that tea polysaccharide is one of the main components related to its hypoglycemic activity. The polysaccharide of green tea has also been reported to have immunological, anti-radiation, anti-blood coagulation, anti-cancer, anti-HIV and hypoglycemic activities (Isiguki, Takakuwa, & Takeo, 1992; Wang et al., 2000; Wang & Wang, 1992; Zhou, Ding, Wang, & Xie, 1997). Great advances have been made in chemical and hypoglycemic studies of tea polysaccharide (Tadakazu, Tomoki, Hitoshi, Fumihisa, & Mistugu, 1998; Wang, Wang, Li, & Zhao, 2001).

Free radicals and active oxygen can induce oxidant damage. Lipid peroxidation, that involves a series of free radical-mediated chain reaction processes, is also associated with several types of biological damage. The role of free radicals and active oxygen is becoming increasingly recognized in the pathogenesis of many human diseases, including cancer, aging, and atherosclerosis (Perry et al., 2000). Until now, studies on tea polysaccharide conjugate (TCA) have mainly focussed on the activities, but the activity mechanism has not yet been well understood. The antioxidant activity of tea polysaccharide conjugate also requires further studies. The correlation between antioxidant activity and hypoglycemic activity of tea polysaccharide conjugate remains unknown.

In this study, a TPC was prepared from lower grade green tea and the components were analyzed. The

* Corresponding author.

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.03.001

antioxidant activity of TPC and the correlation between antioxidant activity and hypoglycemic activity were also investigated.

2. Materials and methods

2.1. Materials

Lower grade green tea was purchased from Xuanen County, Hubei Province, China. Nitro blue tetrazolium (NBT), alloxan and 2-deoxyribose (DR) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trifluoroacetic acid (TFA) and the standard monosaccharides (D-glucose, D-xylose, D-galactose, D-ribose, L-arabinose, D-glucuronic acid) were purchased from Merck Co. (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, MO, USA). Polyamide adsorbent resin (80–100 mesh, separate components according to the polarity and group) was purchased from Nankai University Chemical Co. (Tianjin, China) and dextrans of different molecular weights were from Pharmacia Co. (Uppsala, Sweden). SOD and blood glucose reagent kits were purchased from the Biological Engineering Institute (Nanjing, China). All other chemicals used were of analytical purity.

2.2. Preparation of TPC

Lower grade green tea (100 g) was mixed with 500 ml ethanol at a concentration of 80% (v/v) and was shaken at 30 °C for 24 h to exclude most of the polyphenols and monosaccharides. After filtration, the residues were dried in air and then extracted with hot water three times (1:20, w/v). The tea extract was concentrated in a rotary evaporator under reduced pressure, precipitated with 95% (v/v) ethanol at 4 °C for 24 h, and then centrifuged (10 min, 5000*g*). The sediment was vacuum-dried and 2.9 g crude TPC were obtained. Crude TPC was then separated by an adsorption column (600 × 30 mm i.d.) packed with polyamide resin (Nankai University Chemical Co., Tianjin, China). Polyamide was a polymer of hexanolactam. to remove polyphenolic compounds. Briefly, the adsorption column was primed by washing with five column volumes of 0.5 M NaOH, followed by five column volumes of 0.5 M HCl and 95% (v/v) ethanol was applied last. Before changing the wash solutions, 20 column volumes of distilled water were applied to wash the adsorption column. The adsorption column was then equilibrated with destilled water. 2.9 g of crude TPC (dissolved in 0.5 ml distilled water) was applied to the column and was then eluted with distilled water, at a flow rate of 0.4 ml min^{-1}. The amount of glycan per fraction was determined by using the sulfuric acid–phenol method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), and the protein eluted was determined automatically by monitoring absorbance at 280 nm (Chinpal, Frederick, Page, & James, 2002). The glycan fractions were collected and precipitated with 95% (v/v) of ethanol and then lyophilized. Purified TPC (0.98 g) was obtained.

2.3. Determination of the molecular weight

Tea polysaccharide conjugate (2.0 mg) was dissolved in 0.2 M NaAc–AcOH (pH 6.0) solutions, applied to a gel-filtration chromatographic column of TSK G3000SW (Bio-Rad, Richmond, CA, USA), maintained at a temperature of 30 °C, eluted with the same buffer at a flow rate of 1.0 ml min^{-1} and detected by a refractive index detector. Preliminary calibration of the column was conducted using dextrans of different molecular weight. Multichrom with GPC software (Thermo Lab-Systems, Shrewsbury, MA, USA) was used to acquire and analyze the molecular weight data.

2.4. Analysis of monosaccharides

Tea polysaccharide conjugate (10 mg) was dissolved in 4 ml of a 2 M TFA and hydrolyzed at 140 °C for 2 h, then the monosaccharides were reduced and converted to the corresponding aditol acetates according to the method of Chaplin and Kennedy (1994). Analytical GLC was performed with a Shimadzu GC-6A (Japan) gas chromatography (30 m × 0.32 mm i.d. WCOT column containing OV1701). The analytical conditions were 20 min at 150 °C, then raised to 240 °C at 2 °C min^{-1}. Uronic acid was determined by the sulfuric acid-carbazole method, using glucuronic acid as standard (Bitter & Muir, 1962).

2.5. Analysis of amino acids

Tea polysaccharide conjugate (10 mg) was dissolved with 6 M HCl and hydrolyzed at 110 °C for 18 h. The amino acid constituents were analyzed by the HPLC AccQ method (Mariona, Torbjorn, & Jan, 1997). Separations were carried out on a (150 × 3.9 mm i.d. AccQ Tag C_{18}) reversed-phase column (Waters, Mildford, MA, USA), with a flow-rate of 1.0 ml min^{-1} and performed at 40 °C, eluted with mobile phase A (KH_2PO_4 0.04 M) and mobile phase B (acetonitrile/water = 60/40 (v/v)) by gradient elution. Preliminary calibration of the column was conducted using 24 standard amino acids (Waters, Mildford, MA, USA).

2.6. Assay for the scavenging effect on hydroxyl radicals

The deoxyribose method for determining the rate of reaction of hydroxyl radical with antioxidant was performed, as described by Halliwell, Gutteridge, and Aruoma (1987). Reaction mixtures in a final volume of

1.0 ml contained deoxyribose (60 mM), KH_2PO_4–KOH buffer (pH 7.4, 20 mM), $FeCl_3$ (100 μM), EDTA (100 μM), H_2O_2 (1 mM) and ascorbate acid (100 μ M). Solutions of $FeCl_3$ and ascorbate acid were made up immediately before use. After incubation at 37 °C for 1 h, the colour was developed by adding 1 ml of 1% thiobarbituric acid (TBA) (w/v) and 1 ml of 25% (v/v) HCl, which was then heated in a boiling water bath for 15 min. The absorbance of the resulting solution was measured spectrophotometriclly at 532 nm.

2.7. Assay for the scavenging effect on superoxide radicals

The assay was performed by using the method of photoreduction of NBT (Stewar & Beewley, 1980) but with some modifications. Reaction mixtures contained, in a final volume of 3.0 ml, the following reagents at final concentrations, 13 mM methionine, 10 mM riboflavin, 75 μM NBT, 100 mM EDTA, 0.05 M phosphate buffer (pH 7.8), and various concentrations of sample. The colour was developed by illumination of the mixtures at 3000 Lux for 30 min and then measuring the absorbance at 560 nm.

2.8. Lipid peroxidation inhibition assay

The assay was performed by using the method described by Mee, Han, and Ha (2001) but with some modifications. Ten 12-week-old mice (Laboratory animal centre, Hubei, China) were sacrificed, and the livers were excised, rapidly washed and homogenized in eight volumes (v/w) of 5 mM Tris–HCl buffer (pH 7.4) containing 0.25 M sucrose and 0.1 mM EDTA. Subcelluar fractionation was carried out using differential centrifugation, and the pelleted microsomes were diluted with the same buffer. Reaction mixtures contained 10 μl of the mice microsomes, 500 μl of 0.1 mM Tris–HCl buffer (pH 7.4) containing 12.5 μM $FeSO_4 \cdot 7H_2O$, and 40 μl of 2 mM H_2O_2. Various concentrations of sample were added to the reaction mixture and incubated at 37 °C for 1 h, followed by centrifugation (3000*g*, 10 min). After the addition of 1.0 ml of the TBA reagent to the supernatant, the tubes were placed in a boiling water bath for 15 min. Absorbance was then measured at 532 nm, and the percent inhibition of lipid peroxidation of samples was calculated. This assay was performed without $FeSO_4 \cdot 7H_2O$ and H_2O_2 to assess inhibitory effect on lipid spontaneous peroxidation.

2.9. Estimation of antioxidant activity in alloxan-induced hyperglycemic mice

Forty adult male Kunming mice, weighing 18–22 g, were used. The mice were housed in a temperature-controlled room with a 12-h light and 12-h dark cycle. The animals were kept in the experimental animal room for seven days with free access to food and water. They were fasted for 24 h before experimentation but with free access to tap drinking water. The mice were divided into four groups randomly, of which three groups were injected with 200 mg/kg of body weight of alloxan and turned into hyperglycemic mice. Two groups of model high blood glucose mice received 200 and 400 mg/kg TPC while the control groups received the same volume of water alone. After giving TPC six days, the activity of SOD and the content of blood glucose of all the mice were estimated according to the method of Xu, Bian, and Chen (2002).

2.10. Statistical treatment of data

All the data were expressed as means ± standard deviation (SD) of three replications, and the Student's test was used for the statistical analysis. The values were considered to be significantly different when the P value was less than 0.05.

3. Results

3.1. Components of TPC

Tea polysaccharide conjugate was extracted from the lower grade green tea with water and then purified by polyamide adsorbent chromatography. TPC was eluted as a single symmetrical peak, corresponding to a molecular mass of more than 120 kDa, as determined by HPLC method, which indicated that the polysaccharide was homogeneous.

The monosaccharide constituents of TPC were determined by GLC analysis. The H_2SO_4-carbazole action proved that TPC contained uronic acid. As a result, the relative proportions of Ara, Rib, Xyl, Glc, Gal and glucuronic acid were in mole ratios of 1.00:0.77:2.65:0.88:0.42:2.13. Amino acid analysis indicated that the protein portion of TPC consisted of 15 amino acids: Asp 0.59, Ser 0.37, Glu 0.94, Gly 1.16, Arg 0.34, Thr 0.37, Ala 0.28, Pro 0.35, Cys 0.30, Tyr 0.38, Val 0.35, Lys 0.47, Ile 0.30, Leu 0.45, Phe 0.41. The results showed that TPC was a conjugate with portions of polysaccharide and protein.

3.2. Scavenging effect on hydroxyl radical

As shown in Table 1, TPC was found to have the ability to scavenge hydroxyl radicals at concentrations between 33 and 167 μg ml^{-1}. The scavenging effects of TPC increased with increasing concentration. The IC_{50} value of TPC, for hydroxyl radicals, was 101

Table 1
Scavenging effects of TPC on hydroxyl radicals (·OH)

Concentration (μg ml^{-1})	·OH generation (A_{532})	Inhibition (%)
Control	0.922 ± 0.098	–
33	0.632 ± 0.002^{a}	31.5
67	0.499 ± 0.015^{b}	45.9
100	0.447 ± 0.011^{b}	51.5
133	0.393 ± 0.009^{b}	57.4
167	0.354 ± 0.023^{b}	61.6

$n = 3$, $\bar{x} \pm$ SD.
[a] $p < 0.05$. vs control.
[b] $p < 0.01$ vs control.

μg ml^{-1}. Results showed that TPC inhibited the formation of hydroxyl radicals.

3.3. Scavenging effect on superoxide radical

Superoxide radical can be generated by photochemically-reduced flavins and it can reduce nitroblue tetrazolium to blue formazan, measured as a rise in absorbance at 560 nm, which can represent the content of superoxide radicals. After the addition of TPC, the formation of formazan was inhibited. The scavenging effects are shown in Fig. 1. There was a concentration-effect relationship with the increasing concentration. The IC_{50} value of TPC for superoxide radicals was 145 μg ml^{-1}.

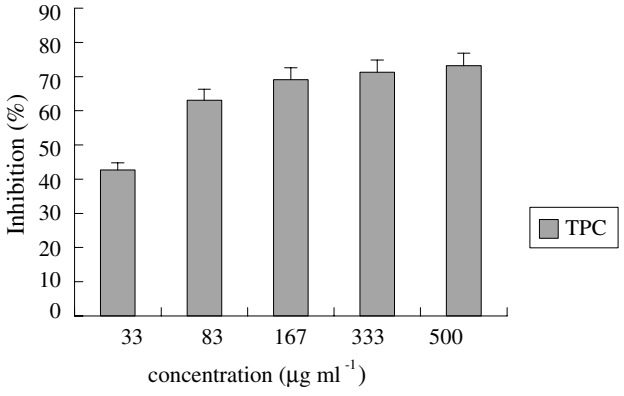

Fig. 1. Scavenging activities of TPC on superoxide radicals (·O_2^-).

3.4. Lipid peroxidation inhibition

As shown in Table 2, in the mouse microsomal lipid peroxidation system as well as in the iron-induced microsomal lipid peroxidation system, TPC showed significant antioxidant activity. Inhibition rose from 28.5% to 53.3% with the concentration increasing from 15 to 294 μg ml^{-1} in the lipid spontaneous peroxidation system ($IC_{50} = 238$ μg ml^{-1}). In the iron-induced mouse microsomal lipid peroxidation system, TPC showed higher antioxidant activity than it did in the spontaneous peroxidation system. In the preliminary assay of iron-induced lipid peroxidations, the presence and involvement of metal ions and oxygen induced generation of free radicals. The results suggested that there was a combination between TPC and metal ions, which could interfere with the free radical reaction chains.

3.5. Antioxidant activity in alloxan-induced hyperglycemic mice

Injection of alloxan induced severe effects in hyperglycemic mice and the activity of serum SOD was reduced significantly, which means that alloxan induced the high blood glucose and oxidative damage at the same time (Table 3). TPC, at a dose of 200, 400 mg kg^{-1} for six days, continuously reduced the blood glucose level of hyperglycemic mice significantly ($p < 0.05$). For the hypoglycemic activity, the dose of 400 mg kg^{-1}was better than the dose of 200 mg kg^{-1}. The same phenomenon was seen for the increase of serum SOD activity.

4. Discussion

The present study showed that TPC consisted of a polysaccharide part and a protein part. TPC was found to have antioxidant potential according to the evaluation of its free radical-scavenging and lipid peroxidation inhibitory activities. At the same time, TPC showed antioxidant activity and hypoglycemic effects in alloxan-

Table 2
Effects of TPC on liver lipid peroxidation and lipid peroxidation induced by Fe^{2+}–H_2O_2

Concentration (μg ml^{-1})	Lipid spontaneous peroxidation system		Lipid peroxidation induced by Fe^{2+}–H_2O_2	
	TBA-reactive substances (A_{532})	Inhibition (%)	TBA-reactive substances (A_{532})	Inhibition (%)
Control	0.921 ± 0.021	–	1.024 ± 0.064	–
15	0.659 ± 0.024^{a}	28.5	0.328 ± 0.012^{a}	68.0
29	0.619 ± 0.019^{b}	32.8	$0.321 \pm 0.014^{a,b}$	68.7
74	0.552 ± 0.022^{b}	40.1	$0.304 \pm 0.016^{a,b}$	70.3
147	0.545 ± 0.008^{b}	40.8	$0.294 \pm 0.031^{a,b}$	71.3
294	0.491 ± 0.010^{b}	53.3	$0.267 \pm 0.006^{a,b}$	73.9

$n = 3$, $\bar{x} \pm$ SD.
[a] $p < 0.05$ vs control.
[b] $p < 0.01$ vs control.

Table 3
Effects of TPC on blood glucose and SOD of alloxan-induced hyperglycemic mice

Group	Dose (mg kg^{-1} bw)	Blood glucose (mmol l^{-1})	Serum SOD (U ml^{-1})
Control	0	6.89 ± 0.50	106 ± 8.54
Alloxan	200	26.5 ± 1.19^{b}	83.7 ± 8.47^{a}
Alloxan + TPC	200 + 200	$19.1 \pm 1.62^{a,c}$	91.1 ± 9.76
	200 + 400	$17.6 \pm 1.77^{a,c}$	104 ± 6.57^{c}

$n = 10$, $\bar{x} \pm$ SD.
[a] $p < 0.05$.
[b] $p < 0.01$ compared to control group.
[c] $p < 0.05$ compared to alloxan group.

induced diabetic mice. The antioxidant mechanism may be due to the supply of hydrogen by TPC, which combines with radicals and itself forms a stable radical to terminate the radical chain reaction. The other possibility is that TPC can combine with the radical ions that are necessary for radical chain reaction; then the reaction is terminated.

Tea polysaccharide conjugates from lower grade green tea are used to cure diabetes in China and Japan. According to previous reports (Liu, Liu, & Wang, 1999), there are several main mechanisms for polysaccharides to act on the serum glucose level, to decrease the content of liver glycogen, to stimulate the release of insulin, or to influence the activities of metabolizing enzymes. Alloxan is a chemical that can destroy the pancreas by oxidative damage. Therefore, the activity of serum SOD decreases after the injection of alloxan. TPC was found to decrease blood glucose and increase the activity of serum SOD in a similar manner, which suggests that antioxidant activity of TPC is one of the mechanisms of hypoglycemic activity.

References

Andrea, R. P., & Michael, H. G. (1997). Antioxidant properties of catechins and green tea extracts in model food emulsions. *Journal of Agricultural and Food Chemistry, 45*, 4267–4270.

Bitter, T., & Muir, H. M. (1962). A modified uronic acid carbazole reaction. *Analytical Biochemistry, 4*, 330–334.

Chaplin, M. F., & Kennedy, J. F. (1994). *Carbohydrate analysis: A practical approach*. London: Oxford university press, pp. 32–33.

Chen, H. X., & Xie, B. J. (2001). Progress on the pharmacological activities of tea polysaccharides. *Journal of Chinese Medicinal Materials, 24*, 65–67.

Chi, H. (1997). Screening of anticarcinogenic ingredients in tea polyphenols. *Cancer Letters, 114*, 153–158.

Chinpal, K., Frederick, L. C., Page, F., & James, M. (2002). Purification and characterization of a doxorubicin-inhibited NADH-quinone (NADH-ferricyanide) reductase from rat liver plasma membranes. *Journal of Biological Chemistry, 277*, 16441–16447.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350–356.

Halliwell, B., Gutteridge, J. M. C., & Aruoma, O. I. (1987). The deoxyribose method: A simple Test-Tube assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Chemistry, 165*, 215–219.

Isiguki, K., Takakuwa, T., & Takeo, T. (1992). Anti-diabetes mellitus effect of water-soluble tea polysaccharide. In *Proceedings of international symposium on tea science* 1991, Shizuka, Japan: The Organizing Committee of ISTS. pp. 240–241.

Liu, C., Liu, Z. Y., & Wang, Q. M. (1999). Studying progress in herbal polysaccharides on hypoglycemic activities. *Journal of Anhui TCM College, 18*, 83–85.

Mariona, R., Torbjorn, L., & Jan, E. L. (1997). Determination of free amino acids in pig plasma by precolumn derivatization with 6-N-aminoquinolyl-N-hydroxysuccinimidyl carbamate and high performance liquid chromatography. *Journal of Chromatography B, 696*, 1–8.

Mee, H. K., Han, J. H., & Ha, C. S. (2001). Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (phyllostachys edulis). *Journal of Agricultural and Food Chemistry, 49*, 4646–4655.

Perry, G., Raina, A. K., Nonomura, A., Wataya, T., Sayre, L. M., & Smith, M. A. (2000). How important is oxidative damage? Lessons from Alzheimer's disease. *Free Radical Biology and Medicine, 28*, 831–834.

Stewar, R. C., & Beewley, J. D. (1980). Lipid peroxidation associated with accelerated aging of soybean areas. *Plant Physiology, 65*, 245–248.

Tadakazu, T., Tomoki, U., Hitoshi, K., Fumihisa, Y., & Mistugu, M. (1998). The chemical properties and functional effects of polysaccharides dissolved in green tea infusion. *Nippon Shokuhin Kagaku Kogaku Kaishi, 45*, 270–272.

Wang, D. F., Li, J., Wang, C. H., Zhao, G. W., Jin, Y., Chen, D. D., & Ye, S. (2000). Study on the component and immune activity of polysaccharides from tea. *Journal of Tea Science, 20*, 45–50.

Wang, D. F., Wang, C. H., Li, J., & Zhao, G. W. (2001). Components and activity of polysaccharides from coarse tea. *Journal of Agricultural and Food Chemistry, 49*, 507–510.

Wang, S. R., & Wang, D. G. (1992). Effects of anti-coagulation and anti-thrombus on the polysaccharides from green tea. *Chinese Traditional and Herbal Drugs, 23*, 254–256.

Xu, S. Y., Bian, R. L., & Chen, X. (2002). *Experimental methods in pharmacology*. Beijing: People's Medical Publishing House, pp. 1523–1526.

Zhou, J., Ding, J. P., Wang, Z. N., & Xie, X. F. (1997). Effect of tea polysaccharides on blood-glucose, blood lipid and immunological function of mice. *Journal of Tea Science, 17*, 75–79.

Zhu, A., Wang, X., & Guo, Z. (2001). Study of tea polyphenol as a reversal agent for carcinoma cell lines' multidrug resistance (study of TP as a MDR reversal agent). *Nuclear Medicine and Biology, 28*, 735–740.