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# Antioxidant activities of different fractions of polysaccharide conjugates from green tea (*Camellia Sinensis*)

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**Abstract**

Three fractions of water-soluble polysaccharide conjugates, coded as TPC-1, TPC-2, and TPC-3, were isolated and purified from low-grade green tea (*Camellia sinensis*) by absorbent chromatography and ion exchange chromatography. Their chemical and physical characteristics were determined by chemical methods, gas chromatography and size exclusion chromatography with laser light scattering. Deoxyribose assay, photoreduction of Nitro Blue Tetrazolium (NBT) assay and lipid peroxidation inhibition assay were applied to test the antioxidant activities of tea polysaccharide conjugates *in vitro*. The results indicated that the three polysaccharide conjugates were heteropolysaccharides bounded with protein. TPC-1 was composed of L-arabinose (Ara), D-ribose (Rib), D-xylose (Xyl), D-glucose (Glc), D-galactose (Gal) and D-mannose (Man). TPC-2 was only composed of four monosaccharides: Ara, Rib, Glc and Man. There was no Man detected in TPC-3. The protein contents of TPC-1, TPC-2, and TPC-3 were 2.8%, 3.8% and 4.0% and the molecular weights were $26.8 \times 10^4$, $11.8 \times 10^4$, $4.2 \times 10^4$, respectively. TPC-3 showed the highest antioxidant activities among the three fractions of polysaccharide conjugates, with an $IC_{50}$ of 182 μg/ml for the deoxyribose assay, and 93 μg/ml for the photoreduction of NBT assay, values which were lower than those of TPC-1 and TPC-2 ($P < 0.01$). The effects of the molecular weight and protein content of the polysaccharide conjugates on the improvement of the bioactivities appeared to be significant.


*Keywords:* Tea polysaccharide conjugate; Composition; Molecular weight; Antioxidant activities

## 1. Introduction

Polysaccharides and their conjugates, used in the food industry and in medicine for a long time, have attracted much attention, in recent years, due to their biological activities. Tea polysaccharide conjugate is one of the main bioactive components of green tea, especially low-grade green tea. The contents of tea polysaccharide conjugates in low-grade green tea vary from 0.4% to 1.5% and they are one of the main components possessing hypoglycaemic activity (Chen & Xie, 2002; Ding, He, & Jie, 2005; Wang, Xie, Cai, & Yang, 1995). Great advances have been made in chemical and bioactive studies of tea polysaccharide conjugates in the last ten years (Chen, Zhang, & Xie, 2004a, 2004b, 2005; Tadakazu, Tomoki, Hitoshi, Fumihisa, & Mistugu, 1998). Tea polysaccharide conjugates have been reported to have immunological, anti-radiation, anti-blood coagulation, anti-cancer, anti-HIV and hypoglycaemic activities (Chen & Xie, 2001; Isiguki, Takakuwa, & Takeo, 1991; Zhou, Ding, Wang, & Xie, 1997). Structural analysis of tea polysaccharide conjugates revealed that they contain neutral sugars, uronic acid and protein.

The bioactivity of polysaccharides depends on their chemical characteristics. In our previous studies (Chen et al., 2004a), tea polysaccharide conjugates were found to exhibit antioxidant activities, and there was a direct

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relationship between the uronic acid contents and the radical-scavenging effects of tea polysaccharide conjugates. In this study, we attempted to investigate the molecular weights and chemical compositions, as well as the antioxidant activities, of the polysaccharide conjugate fractions isolated from green tea. Different polysaccharide conjugate fractions were obtained by a series isolation procedure. The effects of molecular weights and chemical compositions of tea polysaccharide conjugate fractions on their antioxidant activities *in vitro* were evaluated.

## 2. Materials and methods

### 2.1. Materials

Low-grade green tea was purchased from Xuanen County Tea Factory, Hubei Province, China. It was identified as grade six crude green tea, according to the Green Tea Quality Standard (GH016-84) of China. Trifluoroacetic acid (TFA) and the standard monosaccharides (D-glucose, D-xylose, D-galactose, D-ribose, L-arabinose) were purchased from Merck Co. (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, MO). Polyamide adsorbent resin (80–100 mesh) was purchased from Nankai University Chemical Co. (Tianjin, China) and dextrans of different molecular weights were from Pharmacia Co. (Uppsala, Sweden). Galacturonic acid, nitro blue tetrazolium (NBT), and 2-deoxyribose were purchased from Sigma. Diethylaminoethyl cellulose (DEAE cellulose) was purchased from Shanghai Chemical Co. (Shanghai, China). Freshly prepared deionised and redistilled water was prepared in our laboratory.

### 2.2. Isolation of polysaccharide conjugates fractions

Tea polysaccharide conjugates fractions were extracted and isolated from the green tea. Low-grade green tea powders (100 g) were mixed with 500 ml of 80% (v/v) ethanol and shaken at 30 °C for 24 h, to remove most of the polyphenols and monosaccharides. After the mixture was filtered, the residues were dried in air and then extracted three times with hot water (70 °C); (1:20, w/v). The tea extract was concentrated in a rotary evaporator under reduced pressure, precipitated by 95% (v/v) ethanol at 4 °C for 24 h, and then centrifuged (5000*g*, 10 min). The precipitate was vacuum freeze-dried, and 2.9 g of crude tea polysaccharide conjugates were obtained (Chen et al., 2004a, 2004b). Crude tea polysaccharide conjugates were dissolved in water and separated by a polyamide adsorption resin column (60 cm × 3.0 cm i.d.) with water as eluant at a flow rate of 0.4 ml per min. The main polysaccharide fractions were combined and precipitated with 95% (v/v) ethanol and then lyophilised. A DEAE-cellulose column (50 cm × 2.5 cm i.d.) was then used to give three acid polysaccharide conjugate fractions, coded TPC-1, TPC-2, and TPC-3, with a NaCl gradient (from 0.1 to 1.0 M) as eluant.

### 2.3. Components analysis

The carbohydrate contents of the samples were determined by the phenol–sulfuric acid method, using glucose as standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The protein contents in the tea polysaccharide conjugate fractions were measured according to Bradford's method, using bovine serum albumin (BSA) as the standard (Bradford, 1976). The uronic acid contents of different polysaccharide conjugate fractions were quantified by HPLC on a Sugar-Pak I column with a 0.1 mM calcium disodium ethylenediaminetetraacetic acid solution as the mobile phase and refractive index detection, as described by Chen et al. (2004a).

Gas chromatography (GC) was used for identification and quantification of monosaccharides in tea polysaccharide conjugate fractions. GC with flame ionization detection (FID) was performed on a Shimadzu GC-6A instrument (Japan), with a WCOT column containing OV1701 (30 m × 0.32 mm × 0.5 μm). Firstly, the polysaccharide conjugate (10 mg) was hydrolysed with 4 ml of 2 M TFA at 120 °C for 2 h, and then hydrolysed products were dried at reduced pressure. Derivation was then carried out using a trimethylsilylation reagent, according to the method of Chaplin and Kennedy (1994). The GC injection temperature was 250 °C and FID temperature was 250 °C. Column temperature was programmed from 150 °C to 180 °C at 20 °C per min, then increased to 240 °C at 2 °C per min and held for 5 min at 240 °C.

### 2.4. Molecular weight determination

Molecular weights of the tea polysaccharide conjugate fractions were determined by size exclusion chromatography with laser light scattering, as described by Zhang, Zhang, Cheung, and Ooi (2004). Size exclusion chromatography (SEC) combined with a multi-angle laser photometer (DAWN DSP, Wyatt Technology Co., USA) was performed in a system with a p100 pump (Thermo Separation Products, San Jose, CA.), equipped with PSW4000 column (TSK) and interferometric refractometer at 25 °C. The carrier solution was 0.2 M sodium chloride, and the samples were dissolved in 0.2 M sodium chloride with stirring. The carrier and sample solutions were made dust free by passing through a 0.45 μm Millipore filter and degassed before use. The injection volume was 200 ml, and the flow rate was 0.8 ml/min. The calibration of the photometer was done with ultra-pure toluene, and the normalisation of the refractive index (RI) detector was done with bovine albumin monomer (Sigma A-1900). The specific RI increment ($dn/dc$) at 633 nm and 25 °C was determined using an interferometric refractometer (Optilab/903, Wyatt Technology, USA). The $dn/dc$ value was averaged to 0.128 ml/g and was assumed to be constant during the sample elution. Astra software was utilised for data acquisition and analysis.

### 2.5. Scavenging effects of tea polysaccharide conjugates on hydroxyl radicals

Scavenging effects of tea polysaccharide conjugates on hydroxyl radicals were 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), various concentrations of tea polysaccharide conjugate samples, $H_2O_2$ (1 mM) and ascorbic acid (100 μM). Solutions of $FeCl_3$ and ascorbic 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, the reaction mixtures were then heated in a boiling water bath for 15 min. The absorbance of the resulting solution was measured spectrophotometrically at 532 nm.

### 2.6. Scavenging effects of tea polysaccharide conjugates on superoxide radicals

The scavenging effects of tea polysaccharide conjugates on superoxide radicals were assayed by using the method of photoreduction of NBT with some modifications (Stewar & Beewley, 1980). Reaction mixtures in a final volume of 3.0 ml contained the following reagents at final concentrations: 13 mM methionine, 10 mM riboflavin, 75 μM NBT, 100 mM EDTA, 50 mM phosphate buffer (pH 7.8), and various concentrations of tea polysaccharide conjugates samples. The colour was developed by illumination of the mixtures at 3000 Lux for 30 min and then the absorbance was measured at 560 nm. The capability of scavenging the superoxide radical was calculated using the following equation:

$$\text{Scavenging effect}\ (\%) = (1 - A_{\text{sample560 nm}}/A_{\text{control560 nm}}) \times 100$$

### 2.7. Effects of tea polysaccharide conjugates on lipid peroxidation

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 Center, Hubei, China) were sacrificed, and the livers were excised, rapidly washed and homogenised in 8 volumes (v/w) of 5 mM Tris–HCl buffer (pH 7.4), containing 0.25 M sucrose and 0.1 mM EDTA. Subcellular 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 the tea polysaccharide conjugate samples 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 530 nm, and the percent inhibition of lipid peroxidation of samples was calculated.

### 2.8. Statistics

All the data were expressed as mean ± standard deviation (SD) of three replicates, and 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. Chemical composition of the tea polysaccharide conjugates

The yields of the three tea polysaccharide conjugates fractions were 0.1%, 0.2% and 0.2%, respectively. The protein, neutral sugar, uronic acid contents and monosaccharides composition of the polysaccharide conjugates are summarised in Table 1. No polyphenols existed in the tea polysaccharide conjugate fractions. The polysaccharide conjugate TPC-1 had a higher neutral carbohydrate content (46.9% in TPC-1 and 39.8% in TPC-3) but lower protein content (2.8% in TPC-1 and 4.0% in TPC-3) than TPC-2 and TPC-3. The uronic acid contents evaluated in TPC-1, TPC-2, and TPC-3 increased gradually from 30.0% to 51.8%, which correlated with increasing ion exchanging ability of the eluent. According to GC analysis, TPC-1 was composed of arabinose (Ara), ribose (Rib), xylose (Xyl), glucose (Glc), galactose (Gal) and mannose (Man) with molar ratios of 3.3:2.0:2.7:4.1:1.0:2.5. TPC-2 was only composed of four monosaccharides: Ara, Rib, Glc and Man. There was no Man detected in TPC-3.

### 3.2. Molecular weight determination

Usually, the signals in the SEC chromatograms detected by laser light scattering (LLS) detector are correlated to the molecular weight (MW) and molecular size (MN) of the samples. The experimental results of MW and MN from

Table 1
Composition of tea polysaccharide conjugates (TPC)

| | TPC-1 | TPC-2 | TPC-3 |
|---|---|---|---|
| Protein (wt%) | 2.8 | 3.8 | 4.0 |
| Neutral sugar (%) | 46.9 | 45.3 | 39.8 |
| Uronic acid (%) | 30.0 | 47.6 | 51.8 |
| *Sugar components (mol%)* | | | |
| L-arabinose | 3.3 | 1.9 | 4.5 |
| D-ribose | 2.0 | 1.6 | 2.0 |
| D-xylose | 2.7 | nd[a] | 2.8 |
| D-glucose | 4.1 | 2.7 | 1.8 |
| D-galactose | 1 | nd | 1 |
| D-mannose | 2.5 | 1 | nd |

[a] nd: not detected.

Table 2
Molecular weight (MW) and molecular size (MN) of tea polysaccharide conjugates (TPC)

| Sample | MW | MN | MW/MN |
|---|---|---|---|
| TPC-1 | 268,000 | 204,000 | 1.3 |
| TPC-2 | 118,000 | 26,000 | 4.5 |
| TPC-3 | 42,000 | 16,000 | 2.6 |

TPC-1, TPC-2, TPC-3: NaCl elute fractions by DEAE-cellulose chromatography.

SEC-LLS for tea polysaccharide conjugates in 0.2 M sodium chloride at 25 °C are shown in Table 2. The molecular weights of TPC-1, TPC-2, and TPC-3 were 268,000, 118,000 and 42,000, respectively. The MW/MN value of TPC-1 was closer to 1 than that of the other two polysaccharide conjugate fractions, indicating that TPC-1 was more homogeneous.

### 3.3. Scavenging effects of tea polysaccharide conjugates on hydroxyl radicals

The three polysaccharide conjugate fractions were found to have the ability to scavenge hydroxyl radicals at concentrations between 33 and 167 μg/ml (Fig. 1). The scavenging effects of tea polysaccharide conjugates increased with increasing concentration. The $IC_{50}$ values of TPC-1, TPC-2, and TPC-3 for hydroxyl radicals were 184 μg/ml, 158 μg/ml and 93 μg/ml, respectively. There was a significant difference between the scavenging effects of TPC-1, TPC-2 and TPC-3 on hydroxyl radicals ($P < 0.01$: TPC-3 exhibited the highest scavenging effects).

### 3.4. Scavenging effects of tea polysaccharide conjugates on superoxide radicals

The scavenging effects of different fractions of tea polysaccharide conjugates on superoxide radicals are shown in Fig. 2. The tea polysaccharide conjugates again showed a dose-response relationship. TPC-3 exhibited the highest inhibition effects with an $IC_{50}$ value of 182 μg/ml, which was lower than that of TPC-1 and TPC-2 ($P < 0.01$).

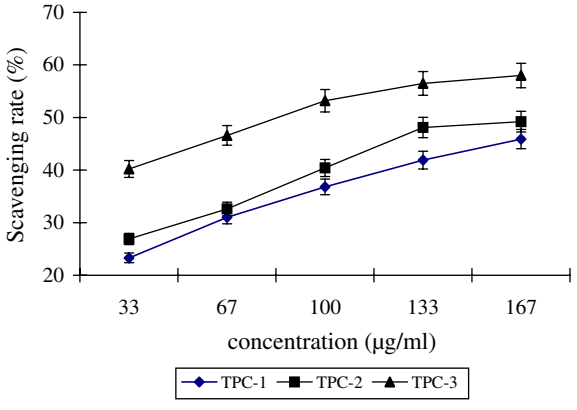


Fig. 1. Scavenging effects of tea polysaccharide conjugates on hydroxyl radicals.

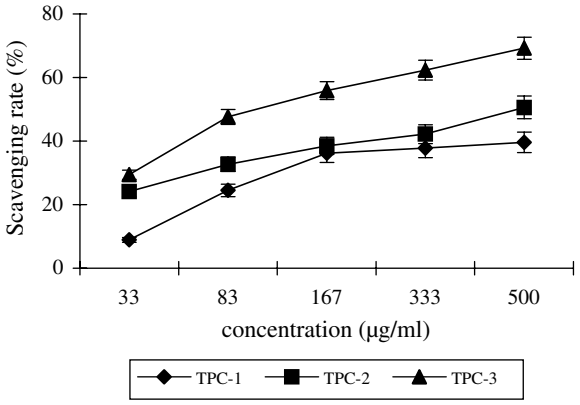


Fig. 2. Scavenging effects of tea polysaccharide conjugates on superoxide radicals.

Table 3
Inhibitory activities of tea polysaccharide conjugates (TPC) on liver lipid peroxidation

| Sample | Concentration (μg/ml) | $A_{530}$[a] | Inhibition (%) |
|---|---|---|---|
| | Control | $1.024 \pm 0.064$ | – |
| TPC-1 | 74 | $0.928 \pm 0.036$b | 9.38 |
| | 294 | $0.813 \pm 0.012$b | 20.6 |
| TPC-2 | 74 | $0.917 \pm 0.013$a | 10.5 |
| | 294 | $0.792 \pm 0.019$b | 22.7 |
| TPC-3 | 74 | $0.875 \pm 0.027$b | 14.6 |
| | 294 | $0.789 \pm 0.015$b | 23.0 |

[a] $n = 3$, mean ± SD. a: $p < 0.05$, b: $p < 0.01$ vs control.

### 3.5. Effects of tea polysaccharide conjugates on lipid peroxidation

As shown in Table 3, in the mouse microsomal lipid peroxidation system, tea polysaccharide conjugates showed antioxidant activities at concentrations of 74 μg/ml and 294 μg/ml. Compared with TPC-1 and TPC-2, TPC-3 showed higher inhibitory activities on microsomal lipid peroxidation, but there was no significant difference ($P > 0.05$).

## 4. Discussion

In the past decades, it has been found that the polysaccharides in plants are not only energy resources but play key biological roles in many life processes as well. The structure and mechanisms of pharmaceutical effects of bioactive polysaccharides on diseases have been extensively studied, and more natural polysaccharides with different curative effects have been tested and even applied in therapies (Wang & Fang, 2004).The bioactivities of polysaccharides and their conjugates can be affected by many factors including chemical components, molecular mass,

structure, conformation, even the extraction and isolation methods.

In the present work, low-grade green tea was firstly extracted with 80% (v/v) ethanol before extraction with hot water. The three tea polysaccharide conjugates, although isolated from the same green tea, were different from each other in chemical components and molecular weights. Compared with TPC-1 (MW268000) and TPC-2 (MW118000), TPC-3 (MW42000) exhibited the highest antioxidant activities, according to the deoxyribose assay, the photoreduction of NBT assay and the lipid peroxidation inhibition assay. The results suggested that the molecular weights of polysaccharides played an important role on their bioactivity. The results were similar to Zhang's reports that molecular weight was very important to the anti-tumour bioactivity of mushroom polysaccharides (Zhang, Cheung, & Zhang, 2001).

Natural polysaccharides do not always exist singly in plants, animals and microorganisms, but conjugate with other components, including protein, lipids and nucleic acids, etc. During the isolation of polysaccharides, the non-saccharide components are always removed by chemical or enzymatic methods. Sometimes the polysaccharide conjugates act as a whole in isolation. In the study of tea polysaccharides, we obtained three polysaccharide-protein conjugates, and found they exhibited different antioxidant abilities depending on the protein content. With increasing of the protein content, antioxidant activities of the three polysaccharide-protein conjugates increased. The existence of protein might affect the physico-chemical properties of the polysaccharides and hence their bioactivities.

In this study, several *in vitro* assays were applied to evaluate the antioxidant potential of three tea polysaccharide conjugates. It was found that both protein content and molecular weight of tea polysaccharide conjugates could play an important role in the antioxidant activity. Among the three tea polysaccharide conjugates, a relatively low molecular weight and a high protein content appeared to increase the antioxidant activity. Perhaps tea polysaccharide conjugates with low molecular weight and high protein contents might bind radicals more easily. Further structural analysis and evaluation of the bioactivities of the polysaccharide conjugates from the green tea will be important for their application in food and medicinal fields.

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