JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Characterization and Antioxidant Activity of the Complex of Tea Polyphenols and Oat β-Glucan

Zhen Wu,[†] Jian Ming,[†] Ruiping Gao,[†] Yuxue Wang,[†] Qiang Liang,[†] Huaguang Yu,[*,#] and Guohua Zhao[*,†,‡]

[†]College of Food Science, Southwest University, Chongqing 400715, People's Republic of China

[‡]Food Engineering and Technology Research Centre of Chongqing, Chongqing 400715, People's Republic of China

[#]College of Physics Science and Technology, Yangzhou University, Yangzhou 225002, People's Republic of China

ABSTRACT: Few data are available about the effects of complexation of polyphenols with polysaccharide on their bioavailability. The complex of tea polyphenols (TP) with oat β-glucan was characterized by ultraviolet−visible spectrometry, Fourier transform infrared spectrometry, differential scanning calorimetry, atomic force microscopy, and solid-state ^{13}C NMR spectroscopy. The results indicated that the bonds which governed the interaction between TP and oat β-glucan were strong hydrogen bonds. The in vitro antioxidant activity of TP, β-glucan, their complex, and physical mixture was assessed using four systems, namely, DPPH$^{\bullet}$, OH$^{\bullet}$, and $O_2^{\bullet-}$ scavenging activities and reducing power. The complexation and blending of TP and β-glucan exhibited different impacts on the index of in vitro and in vivo antioxidant capacities. In the concentration range of 0.5−2.5 mg mL^{-1}, the complex had highest $O_2^{\bullet-}$ scavenging activity, whereas the highest OH$^{\bullet}$ scavenging activity was found with the physical mixture. For antioxidant testing in vivo, there was no significant difference between the complex and the physical mixture in terms of glutathione peroxidase activity and levels of malondialdehyde and total antioxidant capacity in serums. However, the complex exhibited much higher activities of superoxide dismutase and glutathione peroxidase in livers than the physical mixture. The present study provided a deeper understanding of the influence of molecular interaction between TP and oat β-glucan on their antioxidant activities.

KEYWORDS: oat β-glucan, tea polyphenols, complex, characterization, antioxidant activity, in vivo

INTRODUCTION

Interactions of polyphenols with other constituents of the food matrix are likely to interfere with the metabolism of polyphenols and should be taken into account in bioavailability studies.[1] Indeed, interactions of polyphenols with protein and digestive enzymes are well-known to reduce protein digestibility and can be expected to alter polyphenols' bioavailability.[2,3] However, few data are available in the literatures about the effects of complexation of polyphenols with polysaccharide on their bioavailability.

Polyphenols bind to polysaccharides, leading to the formation of polyphenol−polysaccharide complexes, which can significantly improve organoleptic properties,[4] influence their extractability,[5,6] disrupt polyphenol−protein interactions,[7,8] provide controlled release of polyphenols in the human body for more efficient nutraceutical usage, and influence recovery of polyphenols from plant crude extracts.[9−12] Furthermore, the existence of noncovalent interaction between proanthocyanidins and apple cell wall material in aqueous solutions has been investigated.[13−16] Later work by Le Bourvellec et al.[5] found that apple polyphenols mainly bound to pectins.

Tea polyphenols (TP) are a large and diverse class of compounds extracted from tea.[17] The potential for consumption of TP to prevent or ameliorate chronic disease is currently the subject of considerable scientific investigation.[18−21] Although a number of mechanisms have been proposed for the beneficial effects of TP in different models of chronic disease, the radical scavenging and antioxidant properties of TP are frequently cited as important contributions.[18−23] However, wide application of TP as antioxidant has been limited because many factors affecting antioxidant activity are as yet unknown. Particularly, food matrix interactions may play a part in determining polyphenol antioxidant activities.[24] Much of the evidence supporting that the antioxidant activities of TP were greatly affected by their interactions with other constituents of the food matrix was derived from assays of antioxidant activities of the complexation of polyphenols with proteins in vitro.[25−27] Evidence that antioxidant activities of TP are directly or indirectly affected by their interactions with polysaccharides is more limited.

Oat β-glucan is a nonstarch polysaccharide composed of β-(1 → 4)-linked glucose units separated every two to three units by β-(1 → 3)-linked glucose. This water-solution dietary fiber has received much attention because of its potential functionalities such as increasing immunity, anticancer activity, and lowering of blood cholesterol, lipids, and blood glucose.[28−30] In Oriental countries, tea and β-glucan-rich cereals, such as barley and oat, are extensively consumed together in a specific diet. However, there are, so far, few reports on the complexation of TP with β-glucan.

In our recent work we have reported that oat β-glucan plays a major role in regulating the free concentration of TP in aqueous solution.[31] However, the influence of molecular interactions between TP and oat β-glucan on their biological activities is still unknown. On the basis of the above considerations the aims of the present study were to prepare the complex of TP with oat β-glucan and investigate its characterization by ultraviolet−visible spectrometry (UV), Fourier transform infrared spectrometry (FT-IR), differential scanning calorimetry (DSC), atomic

Received: July 7, 2011
Revised: September 5, 2011
Accepted: September 5, 2011
Published: September 05, 2011

force microscopy (AFM), and solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Moreover, we investigated the in vitro and in vivo antioxidant activities of TP, β-glucan, their complex, and the physical mixture of TP and β-glucan by using different assay systems.

MATERIALS AND METHODS

Tea Polyphenols. TP samples were obtained from Changsha Active Ingredients Inc. (Changsha, China), with TP content up to 98.38%. HPLC analysis indicated that it contained (−)-catechin (C, 0.07%), (−)-epigallocatechin (EGC, 0.89%), (−)-epicatechin (EC, 0.34%), (−)-epigallocatechin gallate (EGCG, 74.51%), (−)-gallocatechin gallate (GCG, 4.93%), and (−)-epicatechin gallate (ECG, 17.64%).

Oat β-Glucan. Oat β-glucan was bought from Zhengzhou Lion Biological Technology Co. Ltd. (Zhengzhou, China) with a β-glucan content of 77%. The value for the weight-average molecular weight was 9.1×10^5. For details of the procedures, see ref 31.

Pretreatment of Oat β-Glucan. Oat β-glucan (10 mg) was heated at 80 °C with magnetic stirring in 15 mL of distilled water for 2 h until complete solubilization. The solution was centrifuged at 2500*g* for 10 min, and then the supernatant was left to decolorize via actived carbon, followed by the removal of protein by Sevag method.[32] The retained solution was successively dialyzed against running tap water for 24 h and distilled water for 24 h in dialysis bag (Union Carbide) with size exclusion of 1.4 kDa for globular molecules. Then it was precipitated with ethanol (1:5, v/v) and the precipitate collected by centrifugation at 2500*g* for 10 min. After being dried with an Alpha 2-4 LD Plus vacuum freeze-dryer (Christ, Germany) at −40 °C for 12 h, the obtained oat β-glucan was used for all subsequent in vitro and in vivo experiments.

Influence of the Concentration of NaCl and Ethanol on the Adsorption Capacity of TP into Oat β-Glucan. Adsorption experiments were conducted under the optimum condition of temperature of 40 °C, pH 5.56, phosphate-buffered saline (PBS) buffer concentration of 0.13 M, 6 mL of 0.5 mg mL^{-1} oat β-glucan, and 2 mL 0.5 mg mL^{-1} TP solution.[31] For details of the procedures, see ref 31.

Measurement of TP Concentration. To determine the TP concentration of the complex of TP with oat β-glucan, the spectrophotometric method described by Li et al.[33] was used. One milliliter of TP was transferred into a 25 mL volumetric flask to react with 5 mL of dyeing solution (1.000 g of ferrous sulfate and 5.000 g of potassium sodium tartrate tetrahydrate dissolved in 1000 mL of distilled water), 4 mL of distilled water, and 15 mL of buffer (0.067 M potassium phosphate, pH 7.5). Several minutes was required for color development. Absorbance readings were made at 540 nm by a Shimadzu UV−visible 2450 spectrophotometer, using a blank solution prepared with distilled water replacing TP.

Preparation of the Complex and Physical Mixture of Oat β-Glucan and TP. Pretreated oat β-glucan (300 mg) and TP (100 mg) were totally dissolved in 8 mL of PBS buffer and applied into a dialysis bag. It was dialyzed against 30 mL of PBS buffer for 16 h in a capped 50 mL plastic tube for the formation of the complex under the optimum adsorption conditions. For details of the procedures, see ref 31. Then, the solution inside the dialysis bag was successively dialyzed against tap water for 24 h and distilled water for 24 h in a dialysis bag for the removal of phosphate ion. After vacuum freeze-drying of the solution inside the dialysis bag, the dried light-yellow powder was collected as the complex of TP with oat β-glucan. To determine the working curve of UV absorbance (A) − TP concentration (C), the absorbance of the standard TP aqueous solution with different known concentrations was measured. A fitted regression equation, $A = 0.00977 + 8.09C$, was obtained with $R^2 = 0.999$. Then the content of TP in the complex of TP with oat β-glucan was 19.8 μg mg^{-1}.

Physical mixtures of TP and oat β-glucan at the same molar rate as the complex were prepared by mixing accurately weighed amounts until a fully homogeneous mixture was obtained. All physical mixtures were stored in a desiccator until further evaluation.

Physicochemical Characterization. *UV Spectrometry.* Samples were dissolved in distilled water with magnetic stirring until complete solubilization. UV spectroscopy was recorded using a UV-2450 spectrophotometer (Shimadzu, Japan) in the range 200−400 nm.

FT-IR Spectrometry. FT-IR spectra of samples were obtained using a Spectrum 100 FT-IR spectrophotometer (Perkin-Elmer, USA) in the range 4000−350 cm^{-1} by the KBr method.

Differential Scanning Calorimetry. DSC measurements were carried out with a thermal analyzer (Netzsch DSC 200 PC, Bavaria, Germany). About 6 mg of the dried, ground samples was placed in an aluminum sample pan. The sample was heated to 150 °C and held for 2 min to eliminate the previous heat history and was subsequently cooled to 50 °C at 10 °C min^{-1}. Then, the samples were heated again from 50 to 275 °C at a heating rate of 10 °C min^{-1} in a nitrogen atmosphere, and the DSC curves were recorded using a sealed empty pan as reference.

Atomic Force Microscopy. The samples were mixed with doubly distilled water to a final concentration of 0.4 mg mL^{-1}. A small volume of these solutions was sprayed onto freshly cleaved mica disks and allowed to dry in air before imaging. Images were obtained using an AFM (Veeco, USA) in contact mode with silicon nitride levers.

Solid-State ^{13}C NMR Experiments. All of the solid-state NMR experiments were carried out at $B_0 = 9.4$ T on a Bruker AVANCE III 400 WB spectrometer. The corresponding ^{13}C resonance frequencies were 100.6 MHz. Samples were packed in a 7 mm ZrO_2 rotor and spun at the magic angle (54.7°), and the spin rate was 7 kHz. ^{13}C CP/MAS NMR spectra were recorded with a contact time of 1.2 ms and a recycle delay of 2 s. The ^{13}C chemical shift was externally referenced to the high-field resonance of hexamethylbenzene at 17.17 ppm as a secondary reference.

Determination of Antioxidant Activities in Vitro. *Reducing Power Assay.* The reducing power of samples was determined according to the method described by Xu et al.[34] with a few modifications. Briefly, 2.5 mL of reaction mixture, containing sample (the complex, 0.5−4.5 mg mL^{-1}; physical mixture, 0.5−4.5 mg mL^{-1}; oat β-glucan, 0.49−4.41 mg mL^{-1}; or TP 0.01−0.09, mg mL^{-1}) in phosphate buffer (0.2 M, pH 6.6), was incubated with 2.5 mL of potassium ferricyanide (1%, w/v) at 50 °C for 20 min. The reaction was terminated by 2.5 mL of TCA solution (10%, w/v). Then 5 mL of distilled water and 1 mL of ferric chloride (0.1%, w/v) were added to the reaction mixture. The absorbance was measured at 700 nm with distilled water as blank.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Assay. The DPPH (Wako Pure Chemical Industries, Tokyo, Japan) radical scavenging activity was measured by using the method reported by Qiao et al.[35] with minor modification. A 2 mL sample solution was mixed with 2 mL of 0.2 mM DPPH$^{\bullet}$ in ethanol. Then, the mixture was shaken and allowed to stand at room temperature in the dark for 30 min. The absorbance of the mixture was measured at 517 nm using a spectrophotometer. DPPH radical scavenging activity was calculated by the equation

$$\text{DPPH radical scavenging activity } (\%) = [A_0 - (A_1 - A_2)]/A_0 \times 100\% \quad (1)$$

where A_0 is the absorbance of the control (water instead of the sample solution), A_1 is the absorbance of the sample, and A_2 is the absorbance of the sample under identical condition as A_1 with ethanol instead of DPPH$^{\bullet}$ solution.

Hydroxyl Radical Scavenging Assay. The scavenging activities of hydroxyl free radicals were assayed according to a modified Fenton reaction protocol.[36] The assay system consisted of 1.4 mL of 0.02 mM crystal purple (Kelong Chemical Reagent Co., Chengdu, China) solution, 1.0 mL of 5 mM Fe^{2+} solution, 1 mL of Tris-HCl (pH 5.5), 1 mL of

2.5 mM H_2O_2, and 2 mL of the aqueous solution of tested samples. The final volume of 10 mL was achieved by adding distilled water. The concentrations of the sample solutions were 0.5−4.5 mg mL^{-1} for the complex and physical mixture, 0.49−4.41 mg mL^{-1} for oat β-glucan, and 0.01−0.09 mg mL^{-1} for TP. The scavenging activities of hydroxyl free radical were evaluated by the formula

$$\text{hydroxyl radical scavenging rate (\%)} = [(A_2 - A_1)/(A_0 - A_1)] \times 100\% \quad (2)$$

where A_0 was the absorbance of the negative control (without Fe^{2+} and H_2O_2) at 580 nm, A_1 was the absorbance of the positive control (without test compound), and A_2 was the absorbance in the presence of the test compound.

Superoxide Radical Scavenging Assay. Superoxide radical scavenging capacities of samples were examined by a pyrogallol autoxidation system with minor modification.[37] Briefly, 9 mL of Tris-HCl buffer solution (50 mM, pH 8.2) was incubated in a water bath at 25 °C for 20 min, and then 40 μL of pyrogallol solution (45 mM, prepared in 10 mM HCl), which was also preincubated at 25 °C, was added. The mixture was incubated at 25 °C for 3 min, and then a volume of 50 μL of ascorbic acid was dripped into the mixture to terminate the reaction. The absorbance at 420 nm marked as A_0 was measured 5 min later, and this A_0 indicated the speed of pyrogallol autoxidation. The A_1 autoxidation speed was obtained using the above method and with the addition of 2 mL of various concentrations of test compounds into the Tris-HCl buffer solution. A blank control of reagent was obtained as A_2. The scavenging activity was calculated according to the following formula:

$$\text{superoxide radical scavenging rate (\%)} = [1 - (A_1 - A_2)/A_0] \times 100\% \quad (3)$$

Determination of Antioxidant Activities in Vivo. The animal study was approved by the Chongqing Experimental Animal Committee (Chongqing, China). The assay of antioxidant activities in vivo was carried out according to the reported method with some modifications.[38] Sixty Kunming mice (half male and half female, 8 weeks old), grade of specific pathogen free with body weight (BW) of 20 ± 2 g, were used in this study. The mice were housed under conditions of 25 ± 1 °C, 50 ± 10% humidity, and 12:12 light/dark cycle (light on at 8:00 a.m.), with free access to water and a standard diet. After adapting to their environment for 1 week, these mice were randomly divided into six groups with 10 mice each (5 males and 5 females separately). Mice in group C (normal control group) were treated with 0.9% sodium chloride (NaCl, 25 mL/kg BW) per day by hypodermic injection and gastric gavage. Mice in group MC (model control group) were treated with 9% D-galactose (Sigma Chemical Co., St. Louis, MO) (D-Gal, 25 mL/kg BW) by hypodermic injection and 0.9% NaCl (25 mL/kg BW) by gastric gavage per day. Mice in groups TP, BG, C-TP-BG, and M-TP-BG were treated with 9% D-Gal (25 mL/kg BW) by hypodermic injection and received fixed doses of TP (5.94 mg/kg BW/day), β-glucan (294.06 mg/kg BW/day), their complex (300 mg/kg BW/day), and physical mixture (300 mg/kg BW/day), respectively, by gastric gavage, once a day for 15 consecutive days.

After overnight fasting following the last drug administration, the mice were weighed and killed by decapitation. Blood samples were harvested immediately in centrifuge tubes. After 1 h, the blood samples were centrifuged at 4000*g* for 10 min to afford the sera required. The liver was excised, weighed, and homogenized immediately in ice-cold 0.9% NaCl solution (0.1 g tissue/mL solution). The suspension was centrifuged as mentioned above, and the supernatant was collected for further analysis. All above treatments were done at 4 °C. Activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), level of malondialdehyde (MDA), and total antioxidant capacity (TAOC) were measured by using commercial reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the instruction manuals.

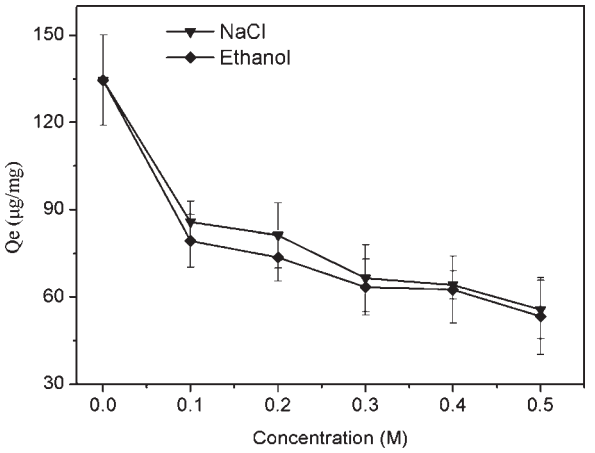

Figure 1. Effects of the concentration of NaCl and ethanol on the adsorption capacity of tea polyphenols (TP) into oat β-glucan under the optimized conditions of temperature, 40 °C; pH 5.56; phosphate-buffered saline (PBS) buffer concentration, 0.13 M; 6 mL 0.5 mg mL^{-1} oat β-glucan; and 2 mL 0.5 mg mL^{-1} TP solution. Each value represents the mean ± SD ($n = 3$). *Qe* represents the adsorption capacity of TP into oat β-glucan (μg mg^{-1}).

Statistical Analysis. Analysis of variance (ANOVA) accompanied with least-significant difference (LSD) test (SPSS, version 15) was conducted to identify the significant difference. Differences with $p < 0.05$ were significant statistically.

RESULTS AND DISCUSSION

Effects of the Concentration of NaCl and Ethanol on the Adsorption Capacity of TP into Oat β-Glucan. The influence of physicochemical parameters such as pH, PBS buffer concentration, and temperature have been investigated.[31] To further confirm involvement of hydrophobic interaction and hydrogen bond during complex formation, *Qe* (adsorption capacity) was determined with varying ionic strength and ethanol concentration.[13,39] Generally, hydrophobic interactions between organic molecules increased with ionic strength.[40] As shown in Figure 1, the complexation of TP and β-glucan decreased with increasing ionic strength, which suggested that hydrophobic interactions had little sense on the interaction between TP and β-glucan. The amount of TP bound also decreased with increased ethanol concentration (Figure 1). Ethanol exhibited not only proton-donor but also proton-acceptor properties, creating the possibility for formation of a hydrogen bond with TP at the OH group of the ring.[39] Meanwhile, the presence of ethanol during complexation exerted dramatic effects on the substrates. First, ethanol caused conformational changes of β-glucan by promoting formation of intramolecular hydrogen bonds,[41] to result in a decrease in the number of free hydroxyl groups of β-glucan available for complexation. Second, TP could form stronger polar and apolar interactions with ethanol than with water, which retarded the transfer of TP from solution onto β-glucan to form the complex. This was supported by the fact that aqueous ethanol was more effective than water in the extraction of TP.[42] Le Bourvellec et al.[13] investigated the influence of ionic strength and the presence of

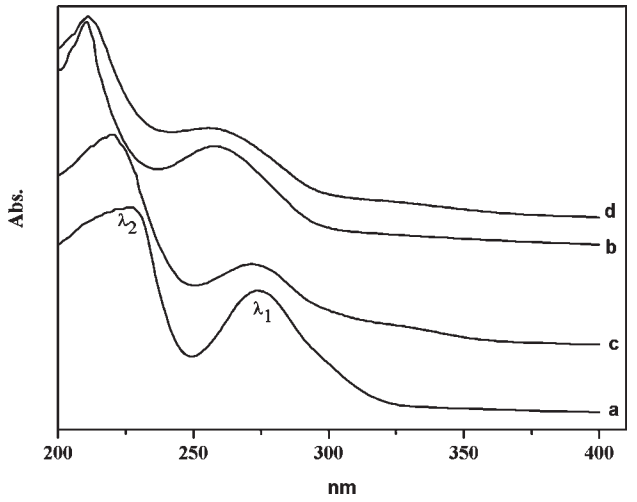

Figure 2. Ultraviolet−visible (UV) spectra of tea polyphenols (TP) (a), oat β-glucan (b), the physical mixture (c) of TP and β-glucan, and the complex (d) of TP with β-glucan. λ_1 = 273 nm; λ_2 = 228 nm.

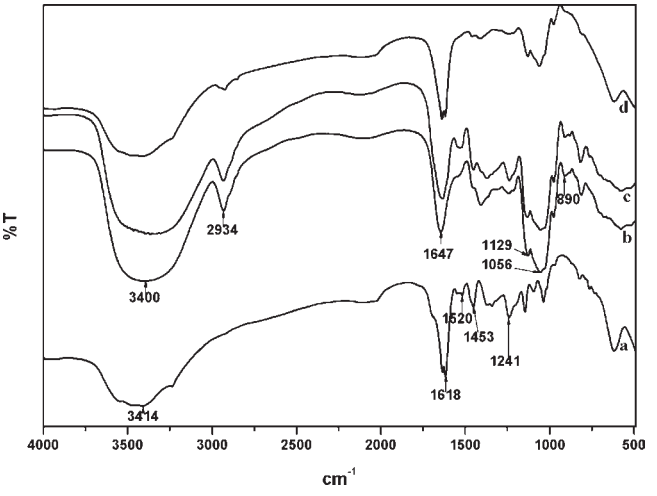

Figure 3. Fourier transform infrared spectrometry of tea polyphenols (TP) (a), oat β-glucan (b), the physical mixture (c) of TP and β-glucan, and the complex (d) of TP with β-glucan.

ethanol on the adsorption capacity of procyanidins in apple cell wall material and found similar results.

Physicochemical Characterization of the Complex TP with β-Glucan. *UV Absorption Spectrum.* In the spectrum of TP in water solution (Figure 2a), two λ_{max} value were found at 273 nm (λ_1, $\pi-\pi^*$ transition of the phenolic group) and 228 nm (λ_2, $\pi-\pi^*$ transition of the phenyl ring). The typical peak reported for TP at 273 nm was identified in the spectra of both the physical mixture (Figure 2c) and TP (Figure 2a). The spectra are related to the $\pi-\pi^*$ transitions within the aromatic ring of TP molecules.[43] In comparison with TP absorption spectra, the band at 273 nm totally disappeared in the spectrum of the complex (Figure 2d), which can be explained by the extension of the conjugated system with the complexation. Li et al.[44] reported an inclusion complex of phenolic compounds with β-cyclodextrin and found similar results by UV−visible absorption spectrometry.

FT-IR Analysis. The FT-IR spectrum of TP (Figure 3a) consisted of the prominent absorption bands of the hydroxyl group

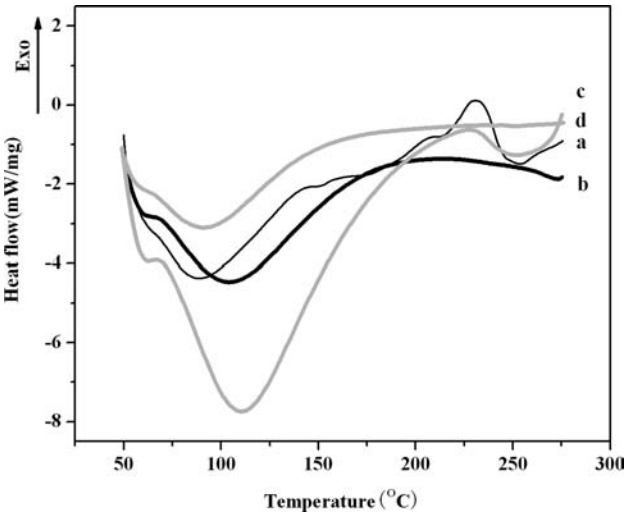

Figure 4. Differential scanning calorimetry curves of tea polyphenols (TP) (a), oat β-glucan (b), the physical mixture (c) of TP and β-glucan, and the complex (d) of TP with β-glucan.

(3414 cm^{-1}) and of an aromatic nucleus (1618, 1520, 1453, 1241 cm^{-1}). However, the bands at 1520 and 1453 cm^{-1} totally disappeared in the spectrum of the complex (Figure 3d). These peaks were found to overlap with the peaks in a broad band ranging from 1600 to 1450 cm^{-1}. This indicated interaction between the hydroxyl groups of polyphenols and the oxygen atoms of the cross-linking ether bonds of β-glucan.[45] Furthermore, some small characteristic absorption peaks of TP between 1000 and 1500 cm^{-1} were almost masked by that of oat β-glucan. The basic characteristics of oat β-glucan (Figure 3b) were at 3400 cm^{-1} (strong and wide O−H stretch), 2934 cm^{-1} (C−H stretch), and 1056 cm^{-1} (C−O stretch). Correspondingly, the FT-IR spectrum of the complex of TP with oat β-glucan showed the typical signals of glucose polysaccharides.[46,47] A decrease in intensity of the main band at 3413 cm^{-1} in the FT-IR spectrum of the complex of TP with β-glucan was due to a decrease in the number of free hydroxyl groups, indicating formation of hydrogen bonding between polyphenol hydroxyl groups and the hydroxyl groups of β-glucan. Additionally, the FT-IR spectrum of the physical mixture (Figure 3c) did not differ significantly from those of the single components.

DSC Analysis. DSC is a method that confirms the formation of a complex in the solid state.[48] In the present work DSC was applied to the analysis of TP, oat β-glucan, their complex, and the physical mixture of TP and oat β-glucan. All obtained thermograms presented a broad endothermic peak around 100 °C (Figure 4), which was attributed to the elimination of water from the subjects. Clearly, the central temperatures corresponding to the peaks of β-glucan and the physical mixture were 105 and 110 °C, respectively (Figure 4b,c). They were much higher than that (88 °C) of TP and the complex (Figure 4a,d). The difference in these temperature data reflected the difference in water-holding (a_w) properties of the subjects. For TP and the physical mixture, an extra broad endothermic peak was located approximately at the melting points of monomers of TP.[49] However, this peak disappeared in the thermogram of the complex, which suggested the formation of the complex between TP and β-glucan. Furthermore, this observation indicated that TP was not present in the complex as its original aggregating form.

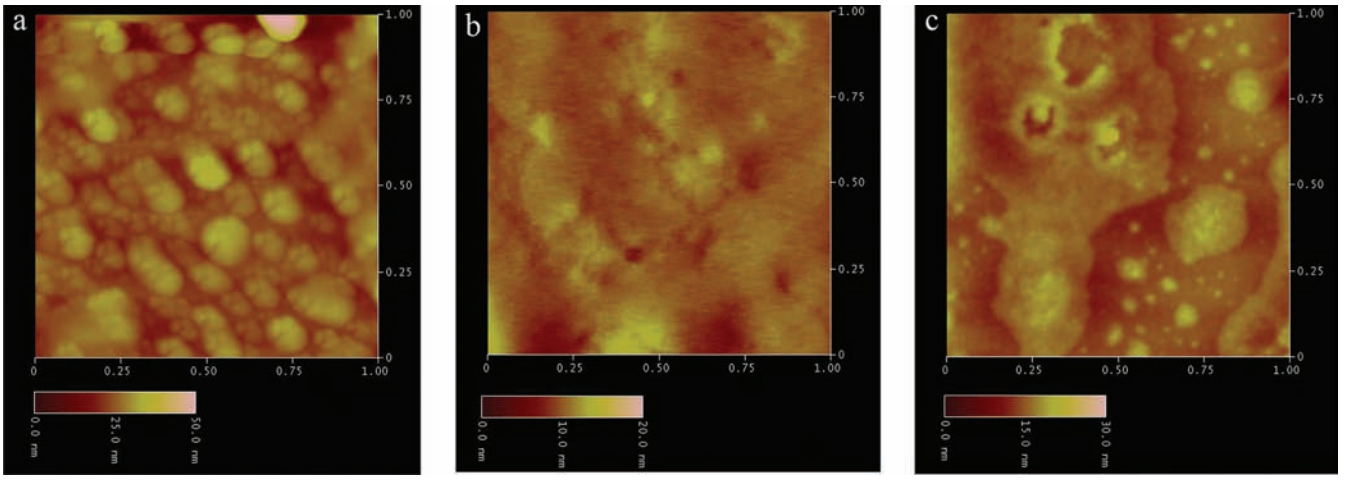

Figure 5. Atomic force microscopy images of oat β-glucan (a), the complex (b) of tea polyphenols with β-glucan, and the physical mixture (c) of tea polyphenols and β-glucan. The size of images is 1 μm × 1 μm.

AFM Analysis. Figure 5a shows the small spherical particles of aggregation or entanglement of oat β-glucan molecules, which ranged in size from about 50 to 200 nm in diameter. It was not possible to determine precisely the size of these particles from the particle diameter due to the inevitable broadening effects of the tip probe,[50] which added on a constant value of 10 nm to the size of each particle. However, the height of the particle is unaffected by probe width.[51] Therefore, the height is a more accurate reflection of the particle diameter. Heights varied from about 2 to 10 nm. To get higher resolution AFM images, β-glucan solution was dropped onto the surface of mica. The conformation of β-glucan may change when it is adsorbed onto the mica surface, so β-glucan conformation observed by AFM does not always reflect the real conformation in solution. Figure 5b,c shows that the AFM image of the complex was markedly different from that of physical mixture, which consisted of a conglomerate of irregular structural features. Previous research confirmed the behavior of self-assembly of β-glucan.[50] The difference in images of β-glucan and the complex indicated that the complexation of TP damaged the spherical aggregates of β-glucan. The complexation of TP onto β-glucan resulted in regular, continuous, and partly homogeneous units for oat β-glucan, indicating the interference of TP on the self-assembly of β-glucan. Combined with the results from DSC, it was concluded that the complexation of TP and β-glucan brought out significant changes in the present state of the substrates.

Solid-State ^{13}C NMR Spectroscopy. Solid-state ^{13}C CP/MAS NMR is an ideal technique for investigating static interactions, because these interactions could cause additional peaks to appear in the solid-state NMR spectrum as a result of the changes to the conformation of carbon atoms in both the complexed molecules and the surrounding host matrix.[52] For the dry oat β-glucan sample, the spectrum (Figure 6a) contained a number of broad peaks of which only those assignable to C1 and C6 carbons were differentiated clearly. The two shoulder peaks at 103.2 and 62.5 ppm were assigned to C1 and C6 carbons, respectively. Similar results have been reported by Morgan et al.[50] The two peaks at 155.5 and 143.7 ppm in the spectrum of the physical mixture (Figure 6c), due to the carbons in the aromatic rings of TP molecules,[53] are relatively more intense compared to the peaks in the spectrum of the complex (Figure 6b). Evidently the complexation

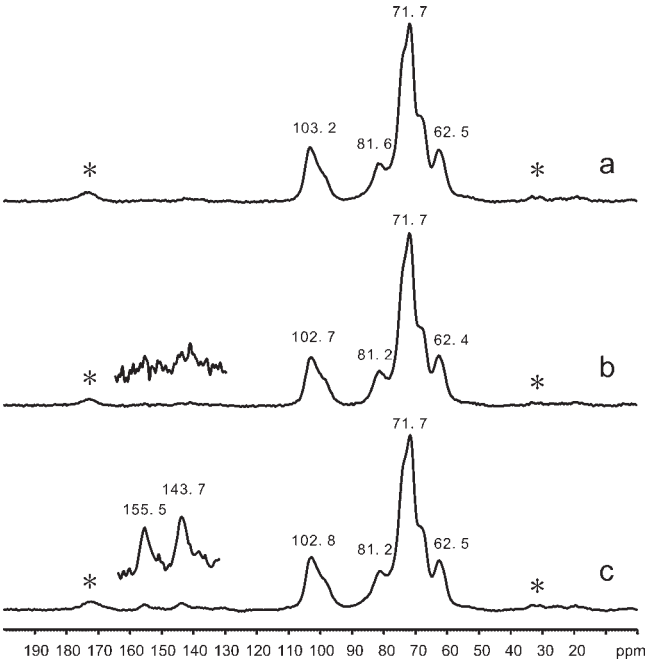

Figure 6. Solid-state ^{13}C NMR spectra of oat β-glucan (a), the complex (b) of tea polyphenols with β-glucan, and the physical mixture (c) of tea polyphenols and β-glucan.

process causes slight shifts for all signals, indicating that TP was complexed onto the oat β-glucan molecules. Additionally, the interaction does not cause a major change in the conformation of oat β-glucan molecules. Similarly, Bowles et al.[52] used ^{13}C CP/MAS NMR to examine bile acid and Congo red absorption to barley β-glucan. Gunness et al.[54] observed chemical shift changes for many bile salt resonances in the presence of barley β-glucan.

The above results confirmed the formation of the complex of TP and oat β-glucan. As known to all, this kind of complexation always resulted in changes of the bioactivities of its substrates. To clarify this action model for the complexation of TP and oat β-glucan, the in vivo and in vitro antioxidant capacities were

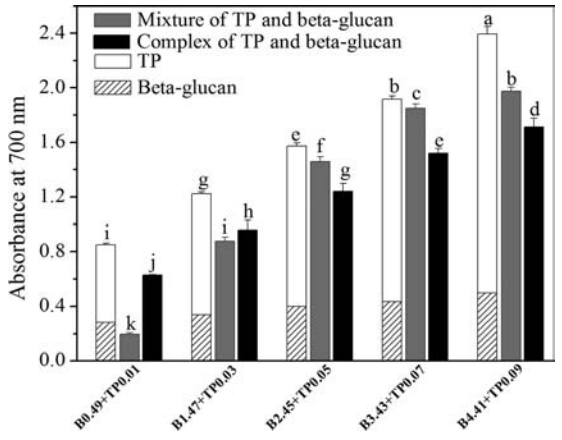

Figure 7. Reducing power of the physical mixture and complex of oat β-glucan and TP compared to the sum of reducing power determined in experiments with single samples. Values are given as the means of three independent experiments, and error bars represent the SD. Different lower case letters indicate significant difference ($p < 0.05$). B, oat β-glucan; TP, tea polyphenols. The number after each sample abbreviation is the concentration of the sample (mg mL^{-1}). High absorbance indicates high reducing power.

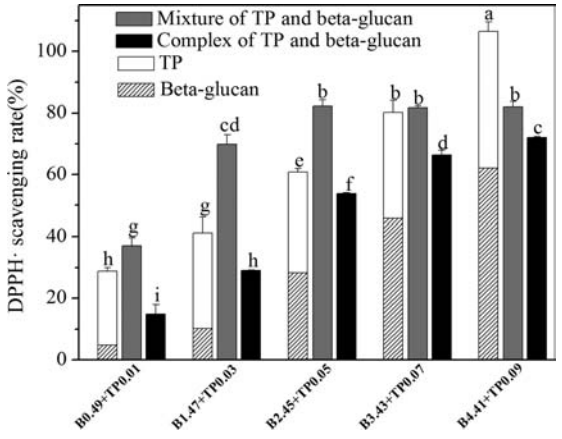

Figure 8. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the physical mixture and complex of oat β-glucan and TP compared to the sum of DPPH radical scavenging activities determined in experiments with single samples. Values are given as the mean of three independent experiments, and error bars represent the SD. Different lower case letters indicate significant difference ($p < 0.05$). B, oat β-gluca; TP, tea polyphenols. The number after each sample abbreviation is the concentration of the sample (mg mL^{-1}).

comparatively investigated with TP and oat β-glucan, their complex, and physical mixture.

Antioxidant Activities in Vitro. *Reducing Power.* As evidenced in Figure 7, the reducing power of the complex and physical mixture was significantly lower than the sum of its two components ($p < 0.05$). This fact reflected that there was no synergistic effect between TP and β-glucan in terms of reducing power. When the complex and physical mixture were compared, the reducing power of the complex was significantly higher than that of the physical mixture when their concentration was lower than 1.5 mg mL^{-1}. However, the reducing power of the physical mixture was higher than that of the complex when the concentration was beyond 1.5 mg mL^{-1}.

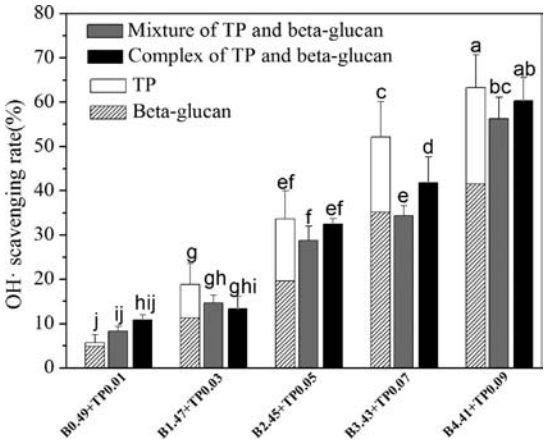

Figure 9. Hydroxyl free radical (OH$^{\bullet}$) scavenging activity of the complex of oat β-glucan and TP compared to the sum of hydroxyl free radical scavenging activities determined in experiments with single samples. Values are given as the mean of three independent experiments, and error bars represent the SD. Different lower case letters indicate significant difference ($p < 0.05$). B, oat β-glucan; TP, tea polyphenols. The number after each sample abbreviation is the concentration of the sample (mg mL^{-1}).

DPPH Radical Scavenging Activities. The synergistic effect on scavenging DPPH radical was obtained when TP at low concentration (<0.05 mg mL^{-1}) was used in combination with β-glucan ($p < 0.05$) (Figure 8). Combination of TP at the concentration of 0.07 mg mL^{-1} with β-glucan exerted slightly synergistic or additive effects ($p \geq 0.05$). However, the use of TP at high level (0.09 mg mL^{-1}) in combination with β-glucan exhibited the negative synergistic (antagonistic) effect on scavenging DPPH radical. Additionally, the physical mixture of TP and β-glucan showed stronger scavenging activity than the complex of TP with β-glucan ($p < 0.05$).

Hydroxyl Radical Scavenging Activities. Among the oxygen radicals, the hydroxyl radical (OH$^{\bullet}$) is the most reactive and severely damages adjacent biomolecules. The combination of TP and β-glucan showed no synergistic effects (Figure 9). However, the hydroxyl radical scavenging activity of the combination of TP with β-glucan approached the sum of hydroxyl free radical scavenging activity of TP and β-glucan when both were at low level, and the difference was not significant ($p \geq 0.05$), thus indicating that the combination of TP and β-glucan had at least an additive effect on scavenging hydroxyl free radical. Again, the data revealed no significant differences between the complex and physical mixture ($p \geq 0.05$). The complex of TP with β-glucan exhibited strong ability to quench hydroxyl radical, which was similar to the physical mixture of TP and β-glucan.

Superoxide Radical Scavenging Activities. Although superoxide ($O_2^{\bullet-}$) is a relatively weak oxidant, it could magnify the cellular damage because it produces other kinds of free radicals and oxidizing agents.[34,55] To compare the $O_2^{\bullet-}$ scavenging capacity of the substrates and complex, it was clearly to find a synergistic effect with concentration lower than 2.5 mg mL^{-1}, but an antagonistic effect with concentration beyond 3.5 mg mL^{-1} (Figure 10). This was a direct result from the different patterns of dose—activity of the substrates and complex. As implied in Figure 10, when concentration was lower than 2.5 mg mL^{-1}, the total $O_2^{\bullet-}$ scavenging capacity of the substrates was dominated by β-glucan; however, it changed into TP when the concentration was beyond

2.5 mg mL^{-1}. The synergistic effect in the lower concentration claimed that associated TP had a higher capacity in $O_2^{\bullet-}$ scavenging than its free form. Similar results were observed for so-called antioxidant dietary fiber.[56] On the contrary, the complexation of TP by β-glucan damaged reasonably the $O_2^{\bullet-}$ scavenging capacity of TP, which resulted in the above-mentioned antagonistic effect. Similarly, Saluk-Juszczak et al.[57] reported that the polyphenolic−polysaccharide conjugates from selected tested medicinal plants were not found to be more effective antioxidants than the solution of pure polyphenol. Additionally, decreased antioxidant capacity was observed for flavonoids when they were bound by food proteins.[26]

Antioxidant Activities in Vivo. The injection of D-Gal into mice induced a senescent phenotype.[58] The aging model shows neurological impairment, decreased activity of antioxidant enzymes, and compromised immune responses.

Effects of test compounds on activities of SOD and GSH-Px and levels of MDA and TAOC in sera in aging mice are shown in Table 1. Apparently, a significant increase in MDA and significant decreases ($p < 0.05$) of antioxidant enzyme activities (SOD and GSH-Px) and TAOC were observed in sera between the treatments of group C (normal control group) and group MC (model control group). This implied the aging activity of D-Gal. The physical mixture of TP and β-glucan treatment inhibited significantly ($p < 0.05$) the formation of MDA and raised the activities of antioxidant enzymes and the level of TAOC in mice sera. As shown in Table 1, the physical mixture of TP and β-glucan treatment, compared to the complex of TP and β-glucan treatment, significantly increased SOD activity in the serum, but showed no significant difference for activities of GSH-Px and levels of MDA and TAOC in sera. However, only the complex of TP with β-glucan treatment completely returned the MDA level in mice serum to normal ($p \geq 0.05$ versu group C).

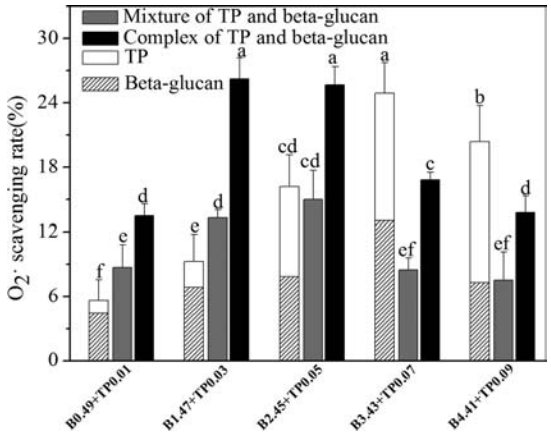

Figure 10. Superoxide anion radical ($O_2^{\bullet-}$) scavenging activity of complex of oat β-glucan and TP compared to the sum of superoxide anion radical scavenging activities determined in experiments with single samples. Values are given as the mean of three independent experiments, and error bars represent the SD. Different lower case letters indicate significant difference ($p < 0.05$). B, oat β-glucan; TP, tea polyphenols. The number after each sample abbreviation is the concentration of the sample (mg mL^{-1}).

The activities of antioxidant enzymes and lipid peroxidation levels in livers are given in Table 2. The activities of SOD and GSH-Px were significantly ($p < 0.05$) greater in group C-TP-BG when compared to group M-TP-BG. There was no significant difference between group C-TP-BG and group M-TP-BG ($p > 0.05$) in terms of the levels of TAOC. However, liver MDA levels in group M-TP-BG were decreased and returned to levels greater than those of group C-TP-BG.

Correlation of Structure and Dose to Antioxidant Activities. To clarify the influence of molecular interactions on the antioxidant activities of TP, it is critical that we better understand how they interact, whether antagonistic, additive, or synergistic.[59,60] In previous studies, Sun-Waterhouse et al.[61,62] found that dietary fibers were shown to affect the antioxidant activities of quercetin. Similarly, it was reported[26] that interactions between TP and proteins found in milk diminished total antioxidant capacity in vitro. In our present study, the complex of TP with oat β-glucan exhibited high antioxidant activities in vitro and in vivo tests. However, these antioxidant effects are not consistent and may depend on the structure and dose. Thus, the relatively low DPPH radical scavenging effect of the complex of TP with β-glucan compared with that of physical mixture may relate to the structure of the complex as well as to the dose provided. Several phenolic compounds, such as luteolin,[63] quercetin,[64] morin,[65] and coumaric acids,[66] which could form intermolecular hydrogen bonds rather than intramolecular hydrogen bonds after complexation, have been reported to improve antioxidant activities upon the formation of complexes.

The major antioxidant enzymes, including SOD and GSH-Px, are the first line of defense against oxidative injury and act cooperatively at different sites in the metabolic pathway of free radicals.[67] MDA, the main product of lipid peroxidation, is an indicator of lipid peroxidation. A lower MDA level suggests that there is less lipid peroxidation and weaker oxidant stress.[68] In the present study, therefore, we measured antioxidant status and

Table 1. Effects of Tea Polyphenols (TP), Oat β-Glucan, Their Complex, and Physical Mixture of TP and Oat β-Glucan on the Activities of SOD and GSH-Px and Levels of MDA and TAOC in Sera in Aging Mice[a]

group[b]	SOD (U/mL)	GSH-Px (U/mL)	MDA (nmol/mL)	TAOC (U/mL)
C	385.73 ± 27.19 b	536.25 ± 29.20 a	11.28 ± 0.57 d	26.72 ± 1.01 b
MC	223.32 ± 14.33 d	294.38 ± 24.45 d	14.62 ± 0.70 a	16.24 ± 0.50 d
BG	320.07 ± 23.89 c	369.38 ± 48.08 c	13.08 ± 1.07 bc	18.50 ± 1.10 c
TP	343.94 ± 13.30 c	341.25 ± 14.22 c	13.59 ± 0.70 ab	19.12 ± 0.68 c
M-TP-BG	481.35 ± 41.21 a	474.38 ± 47.16 b	12.82 ± 1.57 bc	28.78 ± 1.49 a
C-TP-BG	416.98 ± 29.55 b	465.00 ± 27.81 b	12.05 ± 0.70 cd	27.75 ± 1.29 ab

[a] The data are presented as the mean ± SD ($n = 10$) and evaluated by one-way ANOVA followed by LSD test. Different letters (a−d) in same column donate significant difference ($p < 0.05$). [b] Group C, normal control group; group MC, model control group. Groups TP, BG, C-TP-BG, and M-TP-BG received a fixed dose of TP (5.94 mg/kg BW/day), β-glucan (294.06 mg/kg BW/day), their complex (300 mg/kg BW/day), and physical mixture (300 mg/kg BW/day), respectively, once a day for 15 consecutive days.

Table 2. Effects of Tea polyphenols (TP), Oat β-Glucan, Their Complex, and Physical Mixture of TP and Oat β-Glucan on the Activities of SOD and GSH-Px and Levels of MDA and TAOC in Livers in Aging Mice[a]

group[b]	SOD (U/mg protein)	GSH-Px (U/mg protein)	MDA (nmol/mg protein)	TAOC (U/mg protein)
C	44.12 ± 3.07 c	15.37 ± 1.29 bc	0.65 ± 0.09 d	1.15 ± 0.15 a
MC	38.57 ± 2.51 d	12.09 ± 1.01 d	0.99 ± 0.10 a	0.76 ± 0.07 c
BG	51.91 ± 3.18 b	16.77 ± 1.57 b	0.82 ± 0.10 b	0.80 ± 0.13 bc
TP	48.59 ± 3.11 b	14.49 ± 0.68 c	0.67 ± 0.03 cd	0.81 ± 0.12 bc
M-TP-BG	50.52 ± 4.74 b	15.73 ± 0.98 bc	0.65 ± 0.09 d	0.94 ± 0.17 b
C-TP-BG	57.19 ± 4.06 a	18.95 ± 1.42 a	0.76 ± 0.09 bc	0.92 ± 0.05 b

[a] The data are presented as the mean ± SD ($n = 10$) and evaluated by one-way ANOVA followed by LSD test. Different letters (a−d) in the same column donate significant difference ($p < 0.05$). [b] Group C, normal control group; group MC, model control group. Groups TP, BG, C-TP-BG, and M-TP-BG received a fixed dose of TP (5.94 mg/kg BW/day), β-glucan (294.06 mg/kg BW/day), their complex (300 mg/kg BW/day), and physical mixture (300 mg/kg BW/day), respectively, once a day for 15 consecutive days.

lipid peroxidation in D-Gal-induced aging mice. Except for SOD activity, there was no significant difference between the groups of the complex and the physical mixture in terms of GSH-Px activity and levels of MDA and TAOC in serums. However, the complex exhibited much higher activities of SOD and GSH-Px in livers than the physical mixture. The results obtained in vivo indicated that administration of the complex of TP with β-glucan could overcome oxidant injury induced by D-Gal. The mechanisms involved have not yet been established, but the mechanism of action could be related to the molecular interaction between TP and β-glucan.

Furthermore, different mechanisms of association between polyphenols and various polysaccharides were suggested. In the case of cellulosic supports, polyphenol retention would be primarily a surface phenomenon due to hydrophobic interaction.[8,69] On the other hand, dextran gels were able to encapsulate polyphenols inside their pores via hydrogen bonds between the hydroxyl groups of polyphenols and the oxygen atoms of the cross-linking ether bonds, thus encapsulating polyphenols,[31,70,71] as in the apolar cavity of cyclodextrin.[12] Therefore, the bonds that governed the interaction between TP and oat β-glucan were energy bonds of the type strong hydrogen bond.

Additionally, it has been suggested that the radical scavenging ability of polysaccharides was related to the number of active hydroxyl groups in the molecules.[72] Tsiapali et al.[73] found that polyelectrolytes, such as glucan phosphate or sulfate, may have increased scavenging activity. In the present study, the addition of phenolic hydroxyl groups probably increased radical scavenging activities as a result of increasing electron density of electron-donating substituents on the surface of β-glucan. However, compared with DPPH radical scavenging ability of the physical mixture, the low activity of the complex of TP with β-glucan may be associated with the formation of strong intramolecular hydrogen bonds, leading to inhibition of the reactivity of hydroxyl in the polymer chains.

In conclusion, the molecular interaction between TP and β-glucan is of importance from a nutritional viewpoint. This study confirmed that a new complex formed in solution with TP and β-glucan mainly by hydrogen bonds. Importantly, the complexation of TP and β-glucan exerted significant impact on in vivo and in vitro antioxidant capacities of its substrates. This study provided experimental evidence for the formation of polyphenol−polysaccharide complexes in a model solution. These findings are crucial in interpreting the effects of food matrix on the bioavailability of polyphenols and provide a new strategy to improve bioactivities and sensory properties of foods rich in polyphenols and polysaccharides.

AUTHOR INFORMATION

Corresponding Author

*(G.Z.) Phone: +86 23 68 25 03 74. Fax: +86 68 25 19 47. E-mail: zhaogh@swu.edu.cn. (H.Y.) Phone: +86 0514 87 97 54 67. Fax: +86 0514 87 97 54 67. E-mail: hgyu@yzu.edu.cn.

Funding Sources

We acknowledged support from the National Natural Science Foundation of China (Grants 31171654 and 21005069) and the National High-tech R&D Program (863 Program) of China (2011AA100805).

REFERENCES

(1) Cheynier, V. Polyphenols in foods are more complex than often thought. *Am. J. Clin. Nutr.* **2005**, *81*, 223–229.

(2) Baxter, N. J.; Lilley, T. H.; Haslam, E.; Williamson, M. P. Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. *Biochemistry* **1997**, *36*, 5566–5577.

(3) de Freitas, V.; Mateus, N. Nephelometric study of salivary protein-tannin aggregates. *J. Sci. Food Agric.* **2002**, *82*, 113–119.

(4) Vidal, S.; Francis, L.; Williams, P.; Kwiatkowski, M.; Gawel, R.; Cheynier, W.; Waters, E. The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chem.* **2004**, *85*, 519–525.

(5) Le Bourvellec, C.; Guyot, S.; Renard, C. M. G. C. Interactions between apple (*Malus* × *domestica* Borkh.) polyphenols and cell walls modulate the extractability of polysaccharides. *Carbohydr. Polym.* **2009**, *75*, 251–261.

(6) Popa, M. I.; Aelenei, N.; Popa, V. I.; Andrei, D. Study of the interactions between polyphenolic compounds and chitosan. *React. Funct. Polym.* **2000**, *45*, 35–43.

(7) de Freitas, V.; Carvalho, E.; Mateus, N. Study of carbohydrate influence on protein-tannin aggregation by nephelometry. *Food Chem.* **2003**, *81*, 503–509.

(8) Luck, G.; Liao, H.; Murray, N. J.; Grimmer, H. R.; Warminski, E. E.; Williamson, M. P.; Lilley, T. H.; Haslam, E. Polyphenols, astringency and proline-rich proteins. *Phytochemistry* **1994**, *37*, 357–371.

(9) Kalogeropoulos, N.; Yannakopoulou, K.; Gioxari, A.; Chiou, A.; Makris, D. P. Polyphenol characterization and encapsulation in β-cyclodextrin of a flavonoid-rich *Hypericum perforatum* (St John's wort) extract. *LWT−Food Sci. Technol.* **2010**, *43*, 882–889.

(10) Kammerer, J.; Kammerer, D. R.; Jensen, U.; Carle, R. Interaction of apple polyphenols in a multi-compound system upon adsorption onto a food-grade resin. *J. Food Eng.* **2010**, *96*, 544–554.

(11) Huang, J.; Huang, K.; Liu, S.; Luo, Q.; Xu, M. Adsorption properties of tea polyphenols onto three polymeric adsorbents with amide group. *J. Colloid Interface Sci.* **2007**, *315*, 407–414.

(12) Ozawa, T.; Lilley, T. H.; Haslam, E. Polyphenol interactions: astringency and the loss of astringency in ripening fruit. *Phytochemistry* **1987**, *26*, 2937–2942.

(13) Le Bourvellec, C.; Guyot, S.; Renard, C. M. G. C. Non-covalent interaction between procyanidins and apple cell wall material. Part I. Effect of some environmental parameters. *Biochim. Biophys. Acta, Gen. Subj.* **2004**, *1672*, 192–202.

(14) Le Bourvellec, C.; Renard, C. M. G. C. Non-covalent interaction between procyanidins and apple cell wall material. Part II. Quantification and impact of cell wall drying. *Biochim. Biophys. Acta, Gen. Subj.* **2005**, *1725*, 1–9.

(15) Le Bourvellec, C.; Bouchet, B.; Renard, C. M. G. C. Non-covalent interaction between procyanidins and apple cell wall material. Part III. Study on model polysaccharides. *Biochim. Biophys. Acta, Gen. Subj.* **2005**, *1725*, 10–18.

(16) Le Bourvellec, C.; Le Quere, J. M.; Renard, C. M. G. C. Impact of noncovalent interactions between apple condensed tannins and cell walls on their transfer from fruit to juice: Studies in model suspensions and application. *J. Agric. Food Chem.* **2007**, *55*, 7896–7904.

(17) Higdon, J. V.; Frei, B. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 89–143.

(18) Mukhtar, H.; Ahmad, N. Tea polyphenols: prevention of cancer and optimizing health. *Am. J. Clin. Nutr.* **2000**, *71*, 1698–1702.

(19) Yang, C. S.; Chung, J. Y.; Yang, G. Y.; Chhabra, S. K.; Lee, M. J. Tea and tea polyphenols in cancer prevention. *J. Nutr.* **2000**, *130*, 472–478.

(20) Frei, B.; Higdon, J. V. Antioxidant activity of tea polyphenols *in vivo*: evidence from animal studies. *J. Nutr.* **2003**, *133*, 3275–3284.

(21) Chen, D.; Daniel, K. G.; Kuhn, D. J.; Kazi, A.; Bhuiyan, M.; Li, L. H.; Wang, Z. G.; Wan, S. B.; Lam, W. H.; Chan, T. H.; Dou, Q. P. Green tea and tea polyphenols in cancer prevention. *Front. Biosci.* **2004**, *9*, 2618–2631.

(22) Negishi, H.; Xu, J. W.; Ikeda, K.; Njelekela, M.; Nara, Y.; Yamori, Y. Black and green tea polyphenols attenuate blood pressure increases in stroke-prone spontaneously hypertensive rats. *J. Nutr.* **2004**, *134*, 38–42.

(23) Yokozawa, T.; Dong, E. Radical-scavenging activity of green tea polyphenols. In *.Free Radicals in Food*; American Chemical Society: Washington, DC, 2002; Vol. 807, pp 224−240.

(24) Papadopoulou, A.; Frazier, R. A. Characterization of protein-polyphenol interactions. *Trends Food Sci. Technol.* **2004**, *15*, 186–190.

(25) Arts, M. J. T. J.; Haenen, G. R. M. M.; Voss, H. P.; Bast, A. Masking of antioxidant capacity by the interaction of flavonoids with protein. *Food Chem. Toxicol.* **2001**, *39*, 787–791.

(26) Arts, M. J. T. J.; Haenen, G. R. M. M.; Wilms, L. C.; Beetstra, S. A. J. N.; Heijnen, C. G. M.; Voss, H. P.; Bast, A. Interactions between flavonoids and proteins: effect on the total antioxidant capacity. *J. Agric. Food Chem.* **2002**, *50*, 1184–1187.

(27) Riedl, K. M.; Hagerman, A. E. Tannin−protein complexes as radical scavengers and radical sinks. *J. Agric. Food Chem.* **2001**, *49*, 4917–4923.

(28) Bourdon, I.; Yokoyama, W.; Davis, P.; Hudson, C.; Backus, R.; Richter, D.; Knuckles, B.; Schneeman, B. O. Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with β-glucan. *Am. J. Clin. Nutr.* **1999**, *69*, 55–63.

(29) Jenkins, A. L.; Jenkins, D. J. A.; Zdravkovic, U.; Würsch, P.; Vuksan, V. Depression of the glycemic index by high levels of β-glucan fiber in two functional foods tested in type 2 diabetes. *Eur. J. Clin. Nutr.* **2002**, *56*, 622–628.

(30) Wood, P. J. Cereal β-glucans in diet and health. *J. Cereal Sci.* **2007**, *46*, 230–238.

(31) Wu, Z.; Li, H.; Ming, J.; Zhao, G. H. Optimization of adsorption of tea polyphenols into oat β-glucan using response surface methodology. *J. Agric. Food Chem.* **2011**, *59*, 378–385.

(32) Ma, Z.; Wang, J.; Zhang, L. Structure and chain conformation of β-glucan isolated from *Auricularia auricula-judae*. *Biopolymers* **2008**, *89*, 614–622.

(33) Li, P.; Wang, Y.; Ma, R.; Zhang, X. Separation of tea polyphenol from green tea leaves by a combined CATUFM-adsorption resin process. *J. Food Eng.* **2005**, *67*, 253–260.

(34) Xu, J.; Liu, W.; Yao, W.; Pang, X.; Yin, D.; Gao, X. Carboxymethylation of a polysaccharide extracted from *Ganoderma lucidum* enhances its antioxidant activities *in vitro*. *Carbohydr. Polym.* **2009**, *78*, 227–234.

(35) Qiao, D.; Ke, C.; Hu, B.; Luo, J.; Ye, H.; Sun, Y.; Yan, X.; Zeng, X. Antioxidant activities of polysaccharides from *Hyriopsis cumingii*. *Carbohydr. Polym.* **2009**, *78*, 199–204.

(36) Li, J.; Zhang, M.; Zheng, T. The *in vitro* antioxidant activity of lotus germ oil from supercritical fluid carbon dioxide extraction. *Food Chem.* **2009**, *115*, 939–944.

(37) Sfahlan, A. J.; Mahmoodzadeh, A.; Hasanzadeh, A.; Heidari, R.; Jamei, R. Antioxidants and antiradicals in almond hull and shell (*Amygdalus communis* L.) as a function of genotype. *Food Chem.* **2009**, *115*, 529–533.

(38) Ke, C.; Qiao, D.; Gan, D.; Sun, Y.; Ye, H.; Zeng, X. Antioxidant acitivity *in vitro* and *in vivo* of the capsule polysaccharides from *Streptococcus equi* subsp. *zooepidemicus*. *Carbohydr. Polym.* **2009**, *75*, 677–682.

(39) Umetskaya, V. N.; Trubina, E. L.; Artamonova, O. M. Effect of intermolecular hydrogen bonding on the fluorescence spectra of three-component indole solutions. *J. Appl. Spectrosc.* **1984**, *40*, 159–161.

(40) Wu, J.; Deng, X.; Tian, B. Q.; Wang, L.; Xie, B. J. Interactions between oat β-glucan and Calcofluor characterized by spectroscopic method. *J. Agric. Food Chem.* **2008**, *56*, 1131–1137.

(41) Kurita, O.; Murakami, K.; Fujiwara, T. Chemical modification of polysaccharides by the use of intramolecular associations in polar organic solvents. *Polym. Bull.* **2010**, *65*, 443–454.

(42) Turkmen, N.; Sari, F.; Velioglu, Y. S. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. *Food Chem.* **2006**, *99*, 835–841.

(43) Wang, J.; Cao, Y.; Sun, B.; Wang, C. Characterisation of inclusion complex of *trans*-ferulic acid and hydroxypropyl-β-cyclodextrin. *Food Chem.* **2011**, *124*, 1069–1075.

(44) Li, Z. X.; Ren, R. The preparation for the inclusion complex of paeonol-β-cyclodextrin. *Chin. Pharm. J.* **2004**, *39*, 305–306.

(45) Liang, J.; Li, F.; Fang, Y.; Yang, W.; An, X.; Zhao, L.; Xin, Z.; Cao, L.; Hu, Q. Synthesis, characterization and cytotoxicity studies of chitosan-coated tea polyphenols nanoparticles. *Colloids Surf. B* **2011**, *82*, 297–301.

(46) Wang, S. C.; Bligh, S. W. A.; Zhu, C. L.; Shi, S. S.; Wang, Z. T.; Hu, Z. B.; Crowder, J.; Branford-White, C.; Vella, C. Sulfated β-glucan derived from oat bran with potent anti-HIV activity. *J. Agric. Food Chem.* **2008**, *56*, 2624–2629.

(47) Johansson, L.; Tuomainen, P.; Ylinen, M.; Ekholm, P.; Virkki, L. Structural analysis of water-soluble and -insoluble β-glucans of whole-grain oats and barley. *Carbohydr. Polym.* **2004**, *58*, 267–274.

(48) Karathanos, V. T.; Mourtzinos, I.; Yannakopoulou, K.; Andrikopoulos, N. K. Study of the solubility, antioxidant activity and structure of inclusion complex of vanillin with β-cyclodextrin. *Food Chem.* **2007**, *101*, 652–658.

(49) Shi, J.; Yu, J.; Pohorly, J. E.; Kakuda, Y. Polyphenolics in grape seeds − biochemistry and functionality. *J. Med. Food* **2004**, *6*, 291–299.

(50) Morgan, K. R.; Roberts, C. J.; Tendler, S. J. B.; Davies, M. C.; Williams, P. M. A ^{13}C CP/MAS NMR spectroscopy and AFM study of the structure of Glucagel^{TM}, a gelling β-glucan from barley. *Carbohydr. Res.* **1999**, *315*, 169–179.

(51) Gunning, A. P.; Kirby, A. R.; Morris, V. J. Imaging xanthan gum in air by ac "tapping" mode atomic force microscopy. *Ultramicroscopy* **1996**, *6*, 1–3.

(52) Bowles, R. K.; Morgan, K. R.; Furneaux, R. H.; Coles, G. D. ^{13}C CP/MAS NMR study of the interaction of bile acids with barley β-D-glucan. *Carbohydr. Polym.* **1996**, *29*, 7–10.

(53) Martínez-Richa, A.; Joseph-Nathan, P. Carbon-13 CP-MAS nuclear magnetic resonance studies of teas. *Solid State Nucl. Magn. Reson.* **2003**, *23*, 119–135.

(54) Gunness, P.; Flanagan, B. M.; Gidley, M. J. Molecular interactions between cereal soluble dietary fibre polymers and a model bile salt deduced from ^{13}C NMR titration. *J. Cereal Sci.* **2010**, *52*, 444–449.

(55) Athukorala, Y.; Kim, K.-N.; Jeon, Y. J. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. *Food Chem. Toxicol.* **2006**, *44*, 1065–1074.

(56) Saura-Calixto, F. Antioxidant dietary fiber product: a new concept and a potential food ingredient. *J. Agric. Food Chem.* **1998**, *46*, 4303–4306.

(57) Saluk-Juszczak, J.; Pawlaczyk, I.; Olas, B.; Kolodziejczyk, J.; Ponczek, M.; Nowak, P.; Tsirigotis-Woloszczak, M.; Wachowicz, B.; Gancarz, R. The effect of polyphenolic-polysaccharide conjugates from selected medicinal plants of *Asteraceae* family on the peroxynitrite-induced changes in blood platelet proteins. *Int. J. Biol. Macromol.* **2010**, *47*, 700–705.

(58) Song, X.; Bao, M.; Li, D.; Li, Y. M. Advanced glycation in D-galactose induced mouse aging model. *Mech. Ageing Dev.* **1999**, *108*, 239–251.

(59) Pinelo, M.; Manzocco, L.; Nuñez, M. J.; Nicoli, M. C. Interaction among phenols in food fortification: negative synergism on antioxidant capacity. *J. Agric. Food Chem.* **2004**, *52*, 1177–1180.

(60) Yeh, C. T.; Shih, P. H.; Yen, G. C. Synergistic effect of antioxidant phenolic acids on human phenolsulfotransferase activity. *J. Agric. Food Chem.* **2004**, *52*, 4139–4143.

(61) Sun-Waterhouse, D.; Smith, B. G.; O'Connor, C. J.; Melton, L. D. Effect of raw and cooked onion dietary fibre on the antioxidant activity of ascorbic acid and quercetin. *Food Chem.* **2008**, *111*, 580–585.

(62) Sun-Waterhouse, D.; Melton, L. D.; O'Connor, C. J.; Kilmartin, P. A.; Smith, B. G. Effect of apple cell walls and their extracts on the activity of dietary antioxidants. *J. Agric. Food Chem.* **2007**, *56*, 289–295.

(63) Jullian, C.; Cifuentes, C.; Alfaro, M.; Miranda, S.; Barriga, G.; Olea-Azar, C. Spectroscopic characterization of the inclusion complexes of luteolin with native and derivatized β-cyclodextrin. *Bioorg. Med. Chem.* **2010**, *18*, 5025–5031.

(64) Jullian, C.; Moyano, L.; Yañez, C.; Olea-Azar, C. Complexation of quercetin with three kinds of cyclodextrins: an antioxidant study. *Spectrochim. Acta, Part A* **2007**, *67*, 230–234.

(65) Jullian, C.; Orosteguis, T.; Pérez-Cruz, F.; Sánchez, P.; Mendizabal, F.; Olea-Azar, C. Complexation of morin with three kinds of cyclodextrin: a thermodynamic and reactivity study. *Spectrochim. Acta, Part A* **2008**, *71*, 269–275.

(66) Strazisar, M.; Andrensek, S.; Smidovnik, A. Effect of β-cyclodextrin on antioxidant activity of coumaric acids. *Food Chem.* **2008**, *110*, 636–642.

(67) Yao, D.; Shi, W.; Gou, Y.; Zhou, X.; Yee Aw, T.; Zhou, Y.; Liu, Z. Fatty acid-mediated intracellular iron translocation: a synergistic mechanism of oxidative injury. *Free Radical Biol. Med.* **2005**, *39*, 1385–1398.

(68) Bagchi, D.; Bagchi, M.; Hassoun, E. A.; Stohs, S. J. *In vitro* and *in vivo* generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology* **1995**, *104*, 129–140.

(69) Tang, H. R.; Covington, A. D.; Hancock, R. A. Structure-activity relationships in the hydrophobic interactions of polyphenols with cellulose and collagen. *Biopolymers* **2003**, *70*, 403–413.

(70) Haglund, A. C. Adsorption of monosubstituted phenols on Sephadex G-15. *J. Chromatogr., A* **1978**, *156*, 317–322.

(71) Brook, A. J. W.; Munday, K. C. The interaction of phenols, anilines, and benzoic acids with Sephadex gels. *J. Chromatogr., A* **1970**, *47*, 1–8.

(72) Guo, Z.; Xing, R.; Liu, S.; Yu, H.; Wang, P.; Li, C.; Li, P. The synthesis and antioxidant activity of the Schiff bases of chitosan and carboxymethyl chitosan. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4600–4603.

(73) Tsiapali, E.; Whaley, S.; Kalbfleisch, J.; Ensley, H. E.; Browder, I. W.; Williams, D. L. Glucans exhibit weak antioxidant activity, but stimulate macrophage free radical activity. *Free Radical Biol. Med.* **2001**, *30*, 393–402.