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ARTICLE

Effects of Sulfation on the Physicochemical and Functional Properties of a Water-Insoluble Polysaccharide Preparation from *Ganoderma lucidum*

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The sulfation of a water-insoluble *Ganoderma lucidum* polysaccharide (GLP) was successfully carried out with chlorosulfonic acid−pyridine in dimethyl formamide to prepare three sulfated GLP derivatives, named sGLP1, sGLP2, and sGLP3. The chemical structure of the sulfated GLP was confirmed by Fourier transform infrared and ^{13}C NMR analyses. The sGLPs were evaluated for their water solubility, degree of substitution (DS), antioxidant properties, and bile acid-binding capacities. The results showed that sulfation improved the water solubility of GLP and increased its scavenging capacities against hydroxyl and superoxide anion radicals, hydrogen peroxide-scavenging activity, Fe(II) chelating ability, reducing power, and bile acid-binding capacities. It was also observed that the DS may influence the physicochemical and functional properties of sGLPs. For instance, the sulfated GLP with the lowest DS had the greatest bile acid-binding capacity, and the sGLP that had the highest DS showed the lowest bile acid-binding ability under the experimental conditions. The results from this study suggested that sulfation is a possible approach to obtain novel water-soluble derivatives of GLP with improved physicochemical, functional, and biological properties for potential utilization in functional foods or supplemental products.

KEYWORDS: *Ganoderma lucidum*; sulfation; antioxidant activity; water-insoluble polysaccharide; bile acid-binding capacity

INTRODUCTION

Ganoderma lucidum (Leyss.: Fr.) Karst, an edible mushroom, has been used in functional foods and preventive medicines in the Far East for more than 2000 years and has become a popular dietary supplement ingredient in Western countries, with an annual global market value of over $1.5 billion for *G. lucidum* extracts (*1*, *2*). *G. lucidum* is an important economic crop in certain regions of China, from which more than 200 polysaccharides including the water-soluble health *G. lucidum* polysaccharide preparation (GLPP) have been isolated (*3*−*5*). During the preparation of GLPP, water-insoluble *G. lucidum* polysaccharide residues are generated and treated as waste. Developing value-added nutraceutical products from the water-insoluble polysaccharides is in timely demand to improve the profitability of *G. lucidum* production and processing industries and to enhance the local agricultural economy.

Molecular modification may effectively improve physicochemical and biological properties of polysaccharides and has become an emerging research field along with the increasing pursuit of novel nutraceuticals and functional food ingredients. Sulfation is an effective, simple, affordable, and rapid approach to modify the polysaccharide structure for improving water solubility and functional properties of edible polysaccharides (*6*−*9*). In 2001, Bao and others isolated an alkaline-extractable linear α-D-glucan from *G. lucidum* and prepared its sulfated derivatives. The results from this study showed that the sulfated α-D-glucan with a higher degree of substitution (DS) had a greater water solubility but did not necessarily exhibit a stronger immunomodulating ability (*10*). Recently, the effect of sulfation on the antiproliferative activity of a hot water-soluble glycopeptide from *G. lucidum* was investigated (*4*). The sulfated derivative was compared to the original glycopeptide for its antiproliferative activity using L1210 cancer cell lines. The results showed that both the original glycopeptide and its sulfated derivative had significant antiproliferative activity (*4*). In 2009, a (1→3)-β-D-glucan was isolated from *G. lucidum* and was used to evaluate the effect of sulfation on the physicochemical properties of the polysaccharide (*11*). The results suggested that sulfation altered the viscosity of the β-D-glucan and enhanced its water solubility (*11*). These previous studies suggested that there was a potential to improve the functional and biological properties of *G. lucidum* polysaccharides through sulfation. The possibility of sulfation to improve the physicochemical and biological

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properties of the water-insoluble *G. lucidum* polysaccharides for developing novel value-added polysaccharide products has not been evaluated.

In our continuous effort to enhance the profitability of *G. lucidum* production and processing industries, the present study was conducted to investigate the effect of sulfation on water solubility, antioxidant properties, and bile acid-binding capacities of the water-insoluble *G. lucidum* polysaccharide, a byproduct generated during the preparation of the water-soluble health *G. lucidum* polysaccharide (GLPP) (*5*). The antioxidant properties were estimated as the scavenging capacities against hydroxyl and superoxide anion radicals, hydrogen peroxide-scavenging activity, metal chelating ability, and reducing power. The bile acid-binding capacity was evaluated using cholic and chenodeoxycholic acids.

MATERIALS AND METHODS

Chemicals and Reagents. Chlorosulfonic acid (CSA), pyridine, sodium hydroxide, and hydrochloric acid were purchased from Nanjing Chemical Reagent Co. Ltd. (Nanjing, China), while 30% hydrogen peroxide was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Sodium salicylate, β-nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), nitroblue tetrazolium chloride (NBT), ascorbic acid, deuterated dimethyl sulfoxide (DMSO-d_6), cholestyramine, individual bile acids (cholic and chenodeoxycholic acids), diphorase, nicotinamide adenine dinucleotide (NAD), and 3-α hydroxysterol dehydrogenase were purchased from Sigma-Aldrich (St. Louis, MO). GLP, a water-insoluble heteroglycan, composed of glucose, mannose, and galactose at a ratio of 37.1:1.0:6.6, was obtained as a byproduct from the preparation of a bioactive polysaccharide named GLPP (*12*). Details about GLPP were described in our earlier studies (*5*). All other chemicals and solvents were analytical grade and used without further purification.

Preparation of Sulfated Derivatives. Sulfation of GLP was performed according to a previously reported procedure with minor modifications (*13*). Briefly, the sulfation reaction was carried out in a three-neck conical flask with stirring at a selected temperature. GLP (500 mg) was suspended in dry dimethyl formamide (DMF), the mixture was stirred for 30 min, and then, CSA and pyridine complex at different ratios (1:1, 1:2, and 1:4, v/v) were added. After the reaction was finished, each reaction mixture was quickly cooled to room temperature using an ice bath, neutralized with 20% NaOH solution, and dialyzed for 72 h with distilled water. The dialyzate was concentrated under reduced pressure below 45 °C and precipitated with anhydrous alcohol. The precipitates with variable sulfur contents were collected after drying over phosphorus pentoxide under a reduced pressure.

Structural Characterization. Sulfation of GLP was confirmed by sulfur content determination and Fourier transform infrared (FT-IR) and ^{13}C NMR spectroscopies. Sulfur content determination was performed according to a previously described barium chloride−gelatin nephelometery with slight modifications (*14*). A calibration curve was prepared with sodium sulfate as a standard. The DSs, which indicated the average number of sulfonic groups attached to a glucose unit, were calculated according to the following formula (*7*):

$$\mathrm{DS} = \frac{162 \times \frac{S\%}{32}}{100 - \left(\frac{102}{32} \times S\%\right)}$$

FT-IR spectra were recorded on a Nicolet-170X spectrophotometer (Nicolet, United States). ^{13}C NMR spectra were recorded at 400 MHz using a Bruker DRX-400 NMR Spectrometer (Bruker, German). GLP was dissolved in DMSO-d_6, and its sulfated derivative was dissolved in D_2O and examined at 30 °C.

Water Solubility Test. The solubility was measured at ambient temperature according to the Chinese Pharmacopoeia (*15*).

HO$^\bullet$-Scavenging Activity Estimation. The HO$^\bullet$-scavenging activity was analyzed as described previously (*16*). The reaction mixture contained 1 mL of $FeSO_4$ (1.5 mM), 0.7 mL of H_2O_2 (6 mM), 0.3 mL of sodium salicylate (20 mM), and 1 mL of different concentrations of samples (GLP and three derivatives). Ascorbic acid was included as an antioxidant standard for comparison. After incubation for 1 h at 37 °C, the absorbance at 562 nm was detected for calculating the HO$^\bullet$-scavenging capacity. The percentage of scavenging effect was calculated as

$$\%\ \mathrm{HO}^\bullet\ \text{scavenged} = [1 - (A_1 - A_2)/A_0] \times 100\%$$

where A_1 is the absorbance of the sample or ascorbic acid and A_0 is the absorbance of the solvent control, whereas A_2 is the absorbance of the reagent blank without sodium salicylate.

$O_2^{\bullet-}$-Scavenging Activity Assay. $O_2^{\bullet-}$ was generated in the PMS-NADH system containing 1 mL of 10 μM PMS, 1 mL of 100 μM NADH, 50 μL of 600 μM NBT in 0.1 M PBS at pH 7.8, and 1 mL of sample or ascorbic acid solution or Tris-HCl buffer (for the control) as described previously (*17*). The reaction was initiated by adding PMS. After the mixture was incubated at room temperature for 5 min, the reaction was stopped by adding 50 μL of 1 M HCl, and the absorbance was measured at 560 nm against the blank. The capability of scavenging $O_2^{\bullet-}$ was calculated as:

$$\%O_2^{\bullet-}\ \text{scavenged} = (1 - A_1/A_0) \times 100\%$$

where A_0 is the absorbance of the control reaction (without sample) and A_1 is the absorbance of the sample or ascorbic acid reaction.

Determination of H_2O_2-Scavenging Activity. The H_2O_2-scavenging capacity was measured according to a reported procedure with slight modifications (*18*). H_2O_2 (1.0 mL, 0.1 mM) and 1.0 mL of testing sample (GLP, three derivatives, and ascorbic acid at different concentrations) were mixed, followed by the addition of 100 μL of 3% ammonium molybdate, 10 mL of H_2SO_4 (2 M), and 7.0 mL of KI (1.8 M). The mixture was titrated with $Na_2S_2O_3$ (5 mM) until the yellow color disappeared. The scavenging effect was calculated as:

$$\%H_2O_2\ \text{scavenged} = (V_0 - V_1)/V_0 \times 100\%$$

where V_0 is the volume of $Na_2S_2O_3$ solution used to titrate the control mixture without sample and V_1 is the volume of $Na_2S_2O_3$ solution titrate of the mixture-containing sample.

Chelating Capacity Assay. The ferrous ion chelating potency of samples (GLP, three derivatives, and ascorbic acid at different concentrations) was determined according to the method described by Decker and Welch (*19*). Sample or ethylenediaminetetraacetic acid (EDTA) solution (1 mL) was mixed with 50 μL of ferrous chloride (2 mM) and 0.2 mL of ferrozine (5 mM), shaken well, and allowed to stay still for 10 min at room temperature, and the absorbance of the mixture was determined at 562 nm. EDTA was included as a positive control. The ion chelating activity was calculated as:

$$\text{chelating rate} = [1 - (A_1 - A_2)/A_0] \times 100\%$$

where A_0 is the absorbance of the control (without sample), A_1 is the absorbance in the presence of the sample, and A_2 is the absorbance without ferrozine.

Reducing Power Measurement. The reducing power was quantified following a method described earlier (*20*). Briefly, 2.5 mL of sample (GLP, three derivatives) or ascorbic acid solution 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 adding 2.5 mL of trichloroacetic acid 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. Ascorbic acid was used as the positive control. A higher absorbance of the reaction mixture indicates a stronger reducing power of the sample.

Bile Acid-Binding Capacity Assay. The bile acid-binding capacities of sGLPs were determined following a previously reported procedure (*21*). Briefly, 50 mg of sample was treated with 0.5 mL of 0.01 M HCl, which simulated gastric condition, and was incubated at 37 °C for 60 min with continuous shaking (50 rpm). The solution was adjusted to pH 7.0 by adding 0.05 mL of 0.1 M NaOH and mixed with 2.5 mL of 400 μM bile acid stock solution prepared in 0.01 M phosphate buffer (pH 7.0), which simulated the intestinal condition. The mixture was incubated for another

Table 1. Sulfur Content, DS, Yield, and Water Solubility[a]

product code	sulfur content (%)	DS	yield (w/w, %)	water solubility[b] (mg/mL)
sGLP1	8.89 a ± 0.10	0.63	79.96 a ± 0.03	100 a ± 7.07
sGLP2	12.00 b ± 0.16	0.98	136.34 b ± 0.04	101 a ± 1.41
sGLP3	16.24 c ± 0.13	1.70	135.72 b ± 0.03	100 a ± 2.83

[a] Data are expressed as means ± SDs. Values carrying the same letters are not significantly different ($P > 0.05$). sGLP1, sGLP2, and sGLP3 represent different sulfated GLPs, respectively. [b] The solubility test was conducted at ambient temperature according to the Chinese Pharmacopoeia (2005 edition).

60 min at 37 °C and then centrifuged for 10 min at 20000 *g*, and the supernatant was collected for unbound bile acid determination. Quantification of the unbound bile acids was conducted using a commercial kit from Sigma-Aldrich. The final assay mixture was added with 100 μL of supernatant or bile acid standards, 125 μL of 1.22 mM NAD, 125 μL of 5 mM NBT, 100 μL of 625 units/13-α hydroxysterol dehydrogenase, and 100 μL of 625 units/1 diphorase. The mixture was incubated for 60 min at ambient temperature. After incubation, 100 μL of 1.33 M phosphoric acid was added to stop the reaction, and the absorbance of each reaction mixture was measured at 530 nm. The phosphate buffer without bile acid was used for a reagent blank, and cholestyramine resin was used as a positive control to verify the enzyme. The levels of unbound bile acids were obtained using a standard curve prepared with each of the two pure bile acids, which were cholic and chenodeoxycholic acids. The bile acid-binding capacity (mg/g sample) was calculated against a reagent blank. Duplicate tests were performed for each sample against each bile acid.

Statistical Analysis. Measurements were conducted in triplicate. Data were reported as means ± standard deviations (SDs) for triplicate determinations. Analysis of variance and Tukey's tests were performed (Minitab for Windows, Version 13, Minitab Inc., PA) to identify differences among means. Statistical significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Characterization of Sulfated GLPs. CSA−pyridine was used to prepare sulfated GLPs in the present study. CSA−pyridine is a widely used sulfation agent for polysaccharides because of its high reaction yield, high DS, and the fact that it is less destructive to polysaccharides, although other sulfation agents such as sulfuric acid, sulfur trioxide−pyridine, and sulfur trioxide−dimethylacetamide could also be used (*9*, *13*). Three sulfated GLPs, sGLP1−3, were prepared with a yield of 80−136% (**Table 1**). The DS of the three sulfated GLP ranged from 0.63 to 1.70 (**Table 1**). All three sulfated derivatives had excellent water solubility of about 100 mg/mL (**Table 1**), confirming the previous observation that sulfation may enhance water solubility of *G. lucidum* polysaccharides (*4*, *10*, *11*).

Sulfation of GLP was confirmed using FT-IR and ^{13}C NMR analyses. **Figure 1** presents the FT-IR spectra of GLP and sGLP in the 400−4000 cm^{-1} region. Both GLP and sGLP had the typical absorption peaks assigned to the saccharide moiety at 3422, 2920, 1641, 1076, 1042, and 897 cm^{-1}. In comparison with GLP, two new characteristic absorption bands appeared in the FT-IR spectrum of sGLP, one at 1240 cm^{-1} describing an asymmetrical S=O stretching vibration and the other at 816 cm^{-1} representing a symmetrical C−O−S vibration associated with a C−O−SO_3 group (*22*). These special IR peaks indicated that sulfonic groups were successfully introduced in the GLP molecule.

Peak assignments from the ^{13}C NMR spectra of GLP and sGLP are shown in **Figure 2**. GLP is a complex polymer mainly composed of β-(1→3)-, β-(1→6)-, or β-(1→3,6)-linked glycoside according to our previous research (*12*). The strong signal around δ 39 ppm was assigned to the solvent of DMSO-d_6 in GLP's spectrum. A more complicated spectrum was observed in the spectrum of sGLP because of the introduction of electronegative

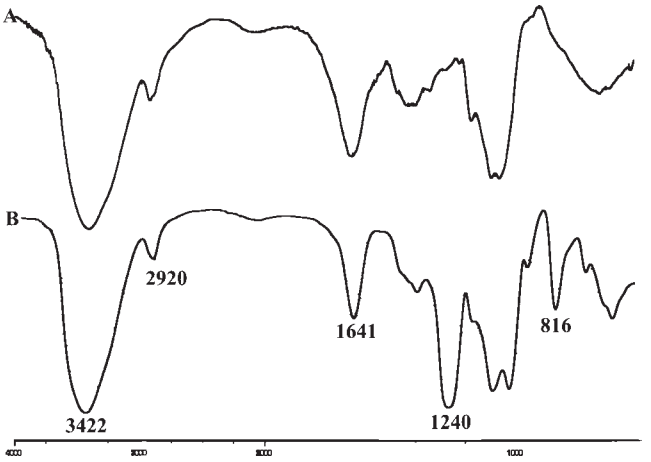

Figure 1. IR spectra of (**A**) GLP and (**B**) sGLP. GLP is the water-insoluble GLPP, and sGLP stands for the sulfated GLP.

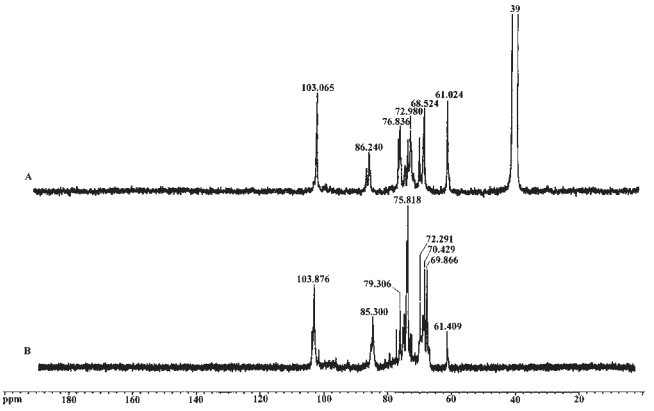

Figure 2. ^{13}C NMR spectra of (**A**) GLP and (**B**) sGLP. GLP is the water-insoluble GLPP, and sGLP stands for the sulfated GLP.

sulfate ester groups. The carbons directly attached with sulfonic groups might shift to lower field position, while the others indirectly attached to sulfonic groups would shift to higher field position (*23*). The decreased intensity of the peak at δ 61.409 ppm indicated that the hydroxyl group on C-6 was partially substituted with a sulfonic group. Peaks at δ 79.306 ppm and δ 75.818 ppm showed that C-2 and C-4 might also be partially substituted. Furthermore, a few peaks around 103.876 ppm could be assigned to C-1, because the substitution at C-2, C-4, or C-6 may influence the chemical shift of the adjacent C-1, leading to the splitting of the C-1 carbon signal (*7*, *23*, *24*). Interestingly, the peak at δ 75.818 for substitution at C-4 position was much stronger than other peaks, which indicated that the C-4 position was more reactive than other positions. This can be explained by the polysaccharide's special structure. The polysaccharide mainly contains β-(1→3)-, β-(1→6)-, or β-(1→3,6)-linked glycoside, which means that C-4 or C-2 positions have more free hydroxyl groups while the hydroxyl groups attached to C-3 or C-6 position might be linked to another glucosyl residue. As compared to the C-2 position, substitution happened more in the C-4 position probably because of the influence of steric hindrance. The ^{13}C NMR spectrum of sGLP also indicated that sulfation of GLP was achieved under the experimental conditions.

HO•-Scavenging Activity. HO• is the most harmful reactive oxygen species (ROS) and is involved in the oxidative injury of biomolecules including carbohydrates, proteins, lipids, and DNA in cells, causing tissue damage or cell death. Removing HO• is

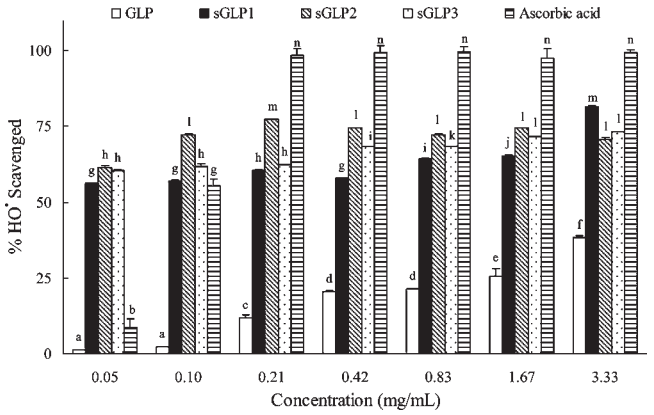

Figure 3. Hydroxyl radical-scavenging activities of GLP and sulfated GLP. GLP is the water-insoluble GLPP, and sGLP stands for the sulfated GLP. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P > 0.05$).

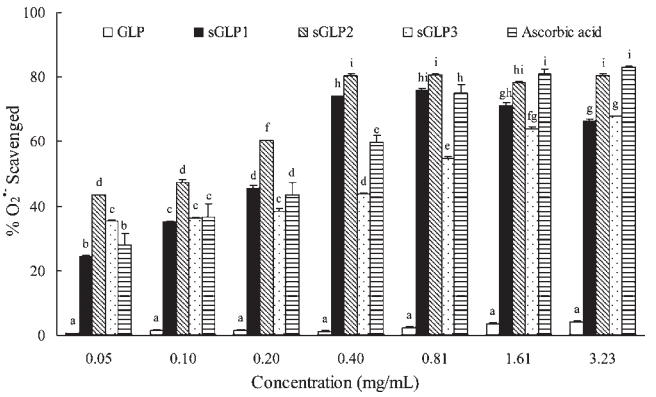

Figure 4. Superoxide radical-scavenging activities of GLP and sulfated GLP. GLP is the water-insoluble GLPP, and sGLP stands for the sulfated GLP. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P > 0.05$).

important for the protection of biological systems (*25*). The sGLPs were compared to GLP and ascorbic acid, a known antioxidant compound, for their HO•-scavenging capacities. As shown in **Figure 3**, all three sulfated derivatives exhibited greater HO•-scavenging capacity than GLP at all seven testing concentrations. sGLP2 with a DS value of 0.98 exhibited the strongest HO•-scavenging capacity at all testing concentrations except 3.33 mg/mL, suggesting that the degree of sulfation may not be directly associated with HO•-scavenging capacity of sGLP, and the molecular structure change induced by sulfation may also contribute to their overall radical-scavenging capacity. Furthermore, at 0.05 and 0.10 mg/mL concentrations, sulfated derivatives showed a higher HO•-scavenging capacity than ascorbic acid, but ascorbic acid showed a stronger scavenging capacity than GLP and the three sGLPs at the concentrations of 0.21−3.33 mg/mL (**Figure 3**). In addition, the HO•-scavenging capacity of GLP was more dose-dependent than that of its sulfated derivatives (**Figure 3**).

$O_2^{\bullet-}$-Scavenging Activity. $O_2^{\bullet-}$ is also a highly reactive chemical species and can be generated under regular physiological conditions. In addition to direct attack of important biological molecules, $O_2^{\bullet-}$ may also be involved in the formation of singlet oxygen and hydroxyl radicals, which may increase local oxidative stress and initiate cellular damage and pathological incidents such as arthritis and Alzheimer's disease (*26*). Three sulfated derivatives were compared to GLP and ascorbic acid for their $O_2^{\bullet-}$-scavenging capacity. As shown in **Figure 4**, all three sulfated GLP derivatives had stronger $O_2^{\bullet-}$-scavenging capacities than GLP at all seven tested concentrations and might exhibit stronger $O_2^{\bullet-}$-scavenging capacity than ascorbic acid at the lower testing concentrations of 0.05−0.10 mg/mL. These data suggested that sulfation might be a possible approach to enhance $O_2^{\bullet-}$-scavenging capacity of GLP. Similar to the observation for HO•-scavenging capacity, sGLP2 with a middle DS value also had the greatest $O_2^{\bullet-}$-scavenging capacity among the three sulfated GLP derivatives. Furthermore, sGLP2 showed a stronger or the same ability as ascorbic acid to react with and quench $O_2^{\bullet-}$ in the system, suggesting that it may be possible to obtain a novel sGLP with strong radical-scavenging capacity through optimization of the degree of sulfation.

H_2O_2-Scavenging Activity. Hydrogen peroxide is an oxidative agent and is involved in the formation of other ROS molecules such as hydroxyl radical in biological systems. Hydrogen peroxide is one of the major inducers for cellular aging and may

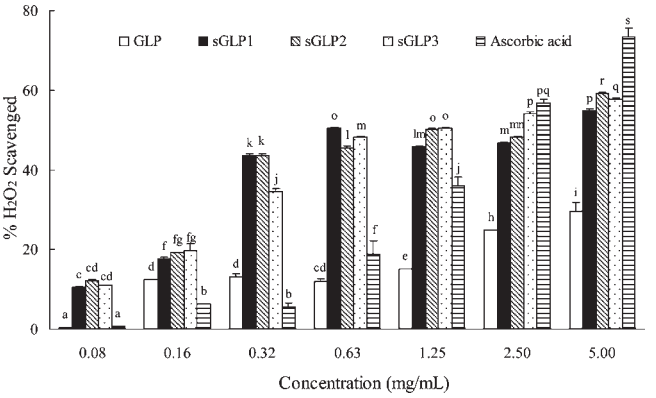

Figure 5. Hydrogen peroxide-scavenging activities of GLP and sulfated GLP. GLP is the water-insoluble GLPP, and sGLP stands for the sulfated GLP. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P > 0.05$).

attack many cellular energy-producing systems because of its high penetrating capacity through cell membranes (*18*). All sGLP samples were able to react with and eliminate H_2O_2 under the experimental conditions (**Figure 5**). The sulfated GLPs showed greater H_2O_2-scavenging capacity than ascorbic acid at a concentration range of 0.08−1.25 mg/mL, but ascorbic acid had the greatest H_2O_2-scavenging capacity at 5 mg/mL concentration (**Figure 5**). At concentrations of 0.63 and 1.25 mg/mL, the three sulfated derivatives could eliminate approximately 50% of the H_2O_2 in the testing system, whereas the GLP could only quench about 12% H_2O_2 under the experimental conditions, indicating that sulfation enhanced the H_2O_2-scavenging capacity of GLP.

Chelating Effect against Ferrous Ions. Several mechanisms have been proposed for the antioxidative action of a chemical in biological systems. These may include but are not limited to radical-scavenging, binding of transition metal ion catalyst(s), decomposition or reduction of peroxides, prevention of continued hydrogen abstraction, and induction of antioxidative enzyme activities (*27*). The metal chelating capacity is important since it reduces the concentration of transition metals that may act as catalysts to generate the first few radicals and initiate the radical-mediated oxidative chain reactions in biological or food systems. Ion chelating agents also may inhibit the Fenton reaction and

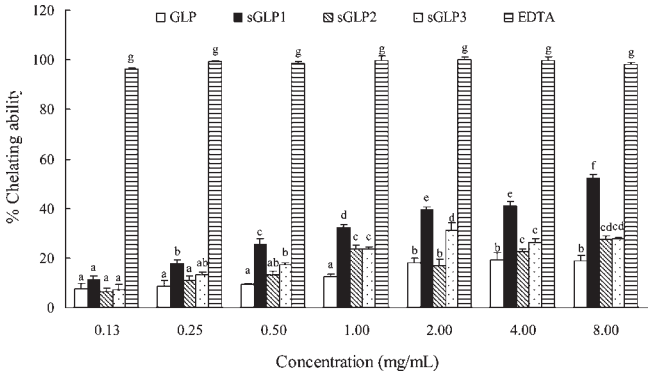

Figure 6. Chelating activity of GLP and sulfated GLP. GLP is the water-insoluble GLPP, and sGLP stands for the sulfated GLP. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P > 0.05$).

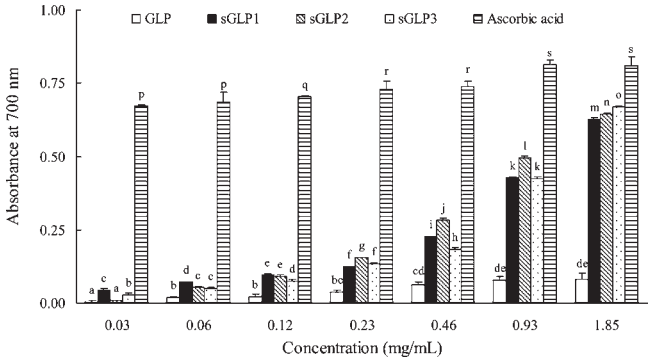

Figure 7. Reducing power of GLP and sulfated GLP. GLP is the water-insoluble GLPP, and sGLP stands for the sulfated GLP. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P > 0.05$).

hydroperoxide decomposition. In the present study, the three sGLPs were compared to GLP and EDTA for their Fe^{2+} chelating capacity. GLP and all three sGLP samples showed a Fe^{2+} chelating activity under the experimental conditions but at a level much lower than that of EDTA (**Figure 6**). The sGLP1 with the lowest sulfation degree had a stronger Fe^{2+} chelating ability than GLP and sGLP2 and sGLP3 at a testing concentration of 0.5 mg/mL and above (**Figure 6**), suggesting that sulfation might enhance the chelating activity of GLP.

Reducing Power. The reducing power assay measures the electron-donating ability of antioxidants using the potassium ferricyanide reduction method. The reducing capacity of a compound would possibly serve as a significant indicator of its potential antioxidant activity. **Figure 7** compares the reducing power of sulfated GLPs, GLP, and ascorbic acid, with a higher absorbance associated with a greater reducing power. The reducing power of ascorbic acid was 0.813 under the experimental condition, and this value was comparable to that reported in a previous study (*28*). All of the tested sGLPs had greater absorbance values than GLP on the per same weight concentration basis (**Figure 7**). The reducing power of the three sGLPs was in the range of 0.626−0.670 at 1.85 mg/mL, which was about eight times of that for GLP under the same experimental conditions. These data suggested the possibility to enhance the reducing power of GLP through sulfation. Introduction of sulfate group in GLP might enhance the electron cloud density of active hydroxyl

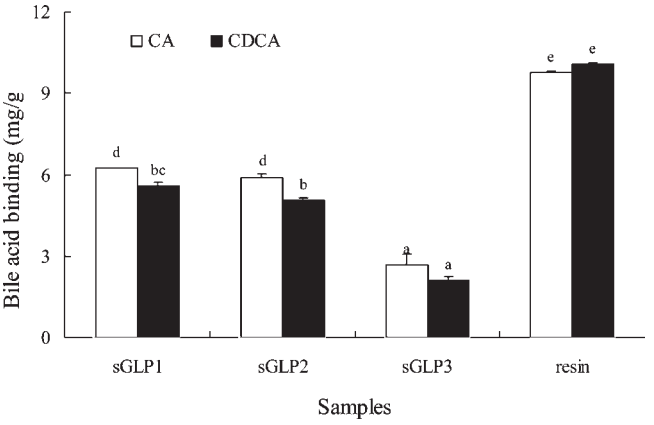

Figure 8. Bile acid-binding capacities of GLP and sulfated GLP. Resin stands for cholestyramine resin, which is included as the positive control. sGLP1, sGLP2, and sGLP3 represent sulfated derivatives of GLP (the water-insoluble GLPP) with different sulfur contents obtained from sulfation. Data are expressed as means ± SDs. Vertical bars represent the SD. Values carrying the same letters are not significantly different ($P > 0.05$).

groups and enhance the molecular electron-withdrawing activity, which can eliminate free radicals and terminate radical-mediated oxidative chain reactions.

Effects of Sulfation on Bile Acid-Binding Properties. Binding of bile acids to polymers may enhance their elimination and promote the conversion of cholesterol to bile acids, which may result in a reduction in the plasma total and LDL cholesterol levels and consequently the risk of cardiovascular diseases. sGLP1, sGLP2, and sGLP3 were evaluated and compared for their in vitro binding capacities against cholic acid (CA) and chenodeoxycholic acid (CDCA), which are the primary bile acids synthesized in liver. All three sulfated GLP derivatives exhibited bile acid-binding capacities (**Figure 8**). The binding capacities of sulfated derivatives seem to be related with their DSs. sGLP1 with the lowest DS showed the greatest binding capacity against CA and CDCA, while sGLP3 with the highest DS had the lowest binding capacity. Additionally, sGLP1 and sGLP2 had binding capacities against both cholic and chenodeoxycholic acids approximately 60 and 55% of that for cholestyramine resin, respectively (**Figure 8**), and stronger than that of acid-treated psyllium under the same experimental conditions (*21*). Cholestyramine resin is a commercial bile acid-binding agent used in supplemental products, whereas psyllium is a cholesterol-lowering agent. The bile acid-binding capacity of sGLPs indicated that sulfation of GLP may lead to water-soluble sGLPs with cholesterol-lowering potential.

In summary, the present study demonstrated that sulfation may be a possible approach to convert the water-insoluble GLPs to their value-added water-soluble derivatives. Sulfation may also improve antioxidant activities and bile acid-binding capacities of the GLPs and may lead to novel, health beneficial, sulfated GLPs for potential utilization in dietary supplements or functional food products. Further in vivo evaluations are recommended to confirm the beneficial health properties of the sGLPs and to examine their possible toxicity to promote their food or supplemental applications.

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