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Preparation, characterization, and functional evaluation of proanthocyanidin-chitosan conjugate

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ABSTRACT

In this study, chitosan (CS) was conjugated with proanthocyanidin (PA) by a free radical grafting reaction. The successful synthesis of PA-CS conjugate was confirmed by Fourier transform infrared spectroscopy and proton nuclear magnetic resonance. The optimal molar ratio of PA to CS repeat unit for the preparation of PA-CS was 0.13:1, which led to a high PA content of 381.76 mg PAE/g in PA-CS. The antioxidant assays demonstrated that PA-CS had much stronger radical scavenging activity and reducing power than the native CS. Especially, the half-inhibition concentrations of PA-CS against DPPH and ABTS radicals were only 6.2 μg/mL and 5.9 μg/mL, respectively. In addition, PA-CS showed an alteration in antibacterial activity compared with CS, and the alteration varied with bacterial strain.

1. Introduction

Chitosan (CS), a unique cationic polysaccharide obtained commercially by deacetylation of chitin, has received considerable attention during the last decades due to its favorable properties including biocompatibility, biodegradation, and non-toxicity (Husain et al., 2017; Kumar, 2000). Moreover, compared with other natural biomaterials, CS possesses much more beneficial effects on human health, such as antioxidant, antimicrobial, and antitumor activity (Kalaycioglu, Torlak, Akin-Evingur, Ozen, & Erim, 2017; Subhapradha & Shanmugam, 2017), which endows it great potential in the fields of food, nutraceutical, and biomedicine (Bano, Arshad, Yasin, Ghauri, & Younus, 2017). In recent years, to strengthen the functionality of CS, developing its novel derivatives has become a new pursuit (Lee, Woo, Ahn, & Je, 2014). CS has abundant amino and hydroxyl groups that can be modified easily; to date, a number of CS derivatives have been successfully synthesized and exhibit improved biological properties (Je & Kim, 2006; Sun, Yao, Zhou, & Mao, 2008; Wu et al., 2014). Nevertheless, some of chemical modifications involve multistep organic syntheses, resulting in the formation of undesired byproducts, even detrimental compounds (Curcio et al., 2009). Therefore, developing eco-friendly procedures is crucial for the modification of CS.

Recently, a free radical grafting procedure has been developed for inserting natural antioxidants onto CS (Curcio et al., 2009). Although the mechanism of this approach still needs more evidence to illustrate, the enhancement in the antioxidant properties of the resulting conjugates has been confirmed. Furthermore, because no detrimental products are synthesized, the conjugates prepared by the free radical grafting procedure can be used in the fields of food, nutraceutical, and biomedicine (Hu & Luo, 2016). Accordingly, the free radical grafting procedure becomes an effective and eco-friendly approach for the modification of CS. In addition, the moieties grafted onto CS are natural antioxidants, leading to significant enhancement in the biological properties of the resultants. Among natural antioxidants, polyphenols are the most suitable moieties grafted onto CS due to their versatile beneficial effects on human health as well as their well-documented antioxidant and antibacterial properties (El Gharras, 2009). Up to now, various polyphenols, such as gallic acid (Cho, Kim, Ahn, & Je, 2011), cafferic acid, ferulic acid (Liu, Wen, Lu, Kan, & Jin, 2014), catechin (Zhu & Zhang, 2014), phloroglucinol (Woo & Je, 2013), and epigallocatechin gallate (EGCG) (Moreno-Vasquez et al., 2017), have been successfully grafted onto CS by the free radical grafting reaction. The resulting polyphenol-CS conjugates exhibit better biological properties, including antioxidant and antimicrobial activity, than the unmodified CS. However, the improvement in the biological properties of the conjugates varies with polyphenol. For example, the antioxidant activity of caffeic acid-CS is higher than that of ferulic acid-CS (Liu et al., 2014), whereas ferulic acid-CS shows better antibacterial activity than caffeic acid-CS (Lee et al., 2014).

Proanthocyanidin (PA), a class of natural polyphenols distributed in various vegetables and fruits (Madrigal-Carballo et al., 2009), has potent antioxidant, antimicrobial, and anti-proliferative activity (Zhang et al., 2016). Recently, reactions of PA with CS were investigated. For example, PA was used as a crosslinker to prepare CS-based materials

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(Kim, Nimni, Yang, & Han, 2005; Zhang et al., 2015); PA-CS composites were obtained by electrostatic interactions (Alfaro-Viquez, Esquivel-Alvarado, Madrigal-Carballo, Krueger, & Reed, 2018; Reed, Krueger, & Madrigal-Carballo, 2017). Moreover, PA-CS composites have the ability to prevent the degradation of PA for in vivo applications (Muñoz, Kappes, Roeckel, Vera, & Fernández, 2016) and to form membranes or scaffolds for regenerative medicine, indicating great necessity of investigating the conjugation of CS with PA. However, to the best of our knowledge, little information regarding PA-CS conjugate is found in the literature.

Therefore, the aim of this study was to prepare PA-CS conjugate by the free radical grafting procedure, characterize its physicochemical properties, and analyze its antioxidant and antibacterial activity.

2. Materials and methods

2.1. Materials

CS (degree of deacetylation of 89% determined as described by Hirai, Odani, and Nakajima (1991); molecular weight of 60 015 determined by intrinsic viscosity (Maghami & Roberts, 1988)) was purchased from Zhejiang Aoxing Biotechnology Company, China. PA was obtained from Tianjin Jianfeng Natural Products Company, China. 2,2′-Azinobis(3-ethylbenothiazoline-6-sulphonic acid) diammonium salt (ABTS) was purchased from Solarbio, China. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was provided by TCI Shanghai, China. Other analytical-grade chemicals were provided by Tianjin Bodi Chemicals, China.

Six bacteria used for antibacterial assays were as follows: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 49132, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* CMCC(B) 28001.

2.2. Preparation of PA-CS

PA-CS was synthesized by the free radical grafting procedure as described by Curcio et al. (2009) with modifications. CS was fully dissolved in 100 mL acetic acid solution (2%, v/v) to a final centration of 1% (w/v). Then, 2 mL of H_2O_2 solution (1.0 mol/L) containing 0.108 g ascorbic acid (AsA) was added in the CS solution. After 30-min reaction, PA was added in the CS solution to the following molar ratio of PA to CS repeat unit: 0.03:1, 0.07:1, 0.10:1, 0.13:1, or 0.16:1. Then, the solution was maintained at room temperature for 24 h followed by adjusting to pH 7.0 using NaOH solution (1 mol/L) to precipitate the resulting PA-CS conjugate. Finally, PA-CS was collected by centrifugation at 8000 r/min for 10 min, fully washed with ethanol and distilled water, and freeze-dried for subsequent characterization and functional evaluation.

2.3. Determination of PA content

The PA content in PA-CS was measured using Folin–Ciocalteu procedure (Cho et al., 2011). In brief, 0.5 mL of 1 mg/mL PA-CS solution in 0.1 mol/L acetic acid solution was mixed with 0.5 mL of Folin-Ciocalteu reagent and 2 mL of distilled water. The mixture was maintained in the dark for 5 min followed by adding 2 mL of 7.5% (w/v) Na_2CO_3 solution and shaking for 30 s. Then, the mixture was maintained in the dark at room temperature for 2 h, and the absorbance of the mixture was measured at 760 nm using a UV-1100 spectrophotometer (Mapada, China). The PA content in PA-CS was calculated using a standard curve of PA and expressed as mg of PA equivalences per g (mg PAE/g) of PA-CS.

2.4. Characterization of PA-CS

PA-CS was characterized by Fourier transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}H NMR), X-ray diffraction (XRD), thermogravimetric analysis (TGA), and differential scanning calorimeter (DSC). The FTIR spectrum was measured by a VECTOR22 spectrophotometer (BRUKER, Germany) in a wavenumber range of 4000–500 cm^{-1}. The ^{1}H NMR spectrum was recorded by an AVANCE400 spectrometer (BRUKER, Germany), using PA-CS solution in 2% (v/v) CD_3COOD/D_2O. XRD was performed in a 2θ range of 5–60° on a D8 Advance X-ray diffractometer (BRUKER, Germany) with Cu Kα radiation. TGA and DSC were determined by a SDT/Q600 DSC-TGA (TA, USA), heating from 40 °C to 600 °C at a rate of 10 °C/min under nitrogen atmosphere. In addition, the molecular weight of PA-CS was determined by intrinsic viscosity using a RYS-1836 Ubbelohde viscometer (Ruiyisi, China).

2.5. Antioxidant capacity assay

2.5.1. DPPH radical scavenging assay

DPPH radical (DPPH·) scavenging activity was evaluated as described by Moreno-Vasquez et al. (2017). In brief, 2 mL of PA-CS solution was mixed vigorously with an equal volume of DPPH solution (0.1 mmol/L) in ethanol. The solution was stand in the dark at room temperature for 30 min; then, the absorbance of the solution was measured at 517 nm. Sample blank was prepared by replacing the DPPH solution with ethanol to avoid the interference of the sample; replacing the PA-CS solution with ethanol was served as control. The DPPH· scavenging activity (%) was calculated by Eq. (1).

$$\text{DPPH}^{\circ}\text{ scavenging activity} = [1 - \frac{A_1 - A_2}{A_0}] \times 100 \quad (1)$$

where A_1, A_2, and A_0 are the absorbance of the sample, sample blank, and control, respectively.

2.5.2. ABTS radical scavenging assay

ABTS radical ($ABTS^{\cdot+}$) scavenging activity was assayed as described in the previous report (Hu, Wang, Zhou, Xue, & Luo, 2016). ABTS stock (7 mmol/L) was mixed with an equal volume of $K_2S_2O_8$ solution (4.9 mmol/L) followed by standing in the dark at room temperature for 12–15 h. The absorbance of the resulting $ABTS^{\cdot+}$ solution was adjusted to 0.70 ± 0.02 at 734 nm with 10 mmol/L phosphate buffered saline (PBS, pH 7.4). Then, 3 mL of $ABTS^{\cdot+}$ solution was withdrawn and mixed with 50 μL of PA-CS solution. After 6-min reaction, the absorbance of the mixture was measured at 734 nm. The $ABTS^{\cdot+}$ scavenging activity (%) was calculated using the following equation.

$$\text{ABST}^{\cdot+}\text{scavenging activity} = [1 - \frac{A_1 - A_2}{A_0}] \times 100 \quad (2)$$

where A_1, A_2, and A_0 are the absorbance of the sample, sample blank (without the $ABTS^{\cdot+}$ solution), and control (without the PA-CS solution), respectively.

2.5.3. Hydroxyl radical scavenging assay

Hydroxyl radical (·OH) scavenging activity was determined according to the previous report (Su et al., 2013). In brief, 3 mL of PA-CS solution was mixed with 1 mL of phenanthroline (1.5 mmol/L) and 2 mL of PBS (20 mmol/L, pH 7.4), followed by adding 1 mL of $FeSO_4$ (0.5 mmol/L) and 1 mL of H_2O_2 solution (0.1%, v/v). The mixture was incubated at 37 °C for 50 min; then, the absorbance of the mixture was measured at 536 nm. The ·OH scavenging activity (%) was calculated using the following equation.

$$^{\circ}\text{OH scavenging activity} = \frac{A_1 - A_0}{A_2 - A_0} \times 100 \quad (3)$$

where A_1, A_2, and A_0 are the absorbance of the sample, sample blank (without the H_2O_2 solution), and control (without the PA-CS solution), respectively.