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Protocatechuic acid grafted onto chitosan: Characterization and antioxidant activity

Jun Liu*, Chen-guang Meng, Ye-hua Yan, Ya-na Shan, Juan Kan, Chang-hai Jin

College of Food Science and Engineering, Yangzhou University, Yangzhou 225127, Jiangsu, China

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ABSTRACT

In this study, protocatechuic acid (PA) was grafted onto chitosan (CS) by a carbodiimide mediated cross-linking reaction. The structural characterization, physical property and antioxidant activity of PA grafted CS (PA-g-CS) was investigated. As results, three copolymers with different grafting ratios (61.64, 190.11 and 279.69 mg PAE/g) were obtained by varying the molar ratios of reaction substrates. PA-g-CS showed the same UV absorption peaks as PA at 258 and 292 nm. As compared to CS, PA-g-CS exhibited a decreased band at 1596 cm^{-1} and a new band at 1716 cm^{-1}, suggesting the formation of amide and ester linkages between PA and CS. New proton signals at δ6.77–7.33 ppm were observed on ^{1}H NMR spectrum of PA-g-CS, assigning to the methine protons of PA. Signals at δ 150.8–116.6 ppm on ^{13}C NMR spectrum of PA-g-CS was assigned to the aromatic ring carbon of PA moieties. All the structural information confirmed the successful grafting of PA onto CS. SEM observation showed CS had a smooth surface, while PA-g-CS had a rough surface. TGA revealed the thermal stability of PA-g-CS was lower than CS. Antioxidant activity assays further verified the reducing power and DDPH radical scavenging activity of PA-g-CS was much higher than CS.

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1. Introduction

Reactive oxygen species (ROS) and oxygen-derived free radicals may induce many diseases, such as aging, cancer, atherosclerosis, diabetes and rheumatoid arthritis [1]. Therefore, it is essential to develop and utilize effective antioxidant agents to protect human body from free radicals. In the search of new and effective natural antioxidant agents, several polysaccharides and polysaccharide derivatives have been demonstrated to possess potent antioxidant activities and to be explored as novel potential antioxidants [2–4].

Among various renewable polymers, chitosan (CS) is one of the most important biocompatible polymers. CS is a hetero-polymer consists of β-(1 → 4) linked *N*-acetyl-2-amino-2-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose. CS is also a unique cationic polysaccharide with many other useful properties, such as viscosity, mucoadhesivity, polyelectrolyte behavior, film forming and metal chelating ability [5]. These special properties make CS highly important in food, environmental and biomedical industries [6–8]. However, CS also suffers from a few disadvantages and requires further development to achieve the targeted results and desired range of efficiency. To overcome some of the disadvantages of the pristine chitosan, it is most imperative to functionalize it with suitable functional groups [9]. The presence of active functional primary amino and hydroxyl groups in CS provides a specific platform for side groups' attachment. Till now, many chemical modification methods including acylation, hydroxylation, nitration, alkylation, sulphonation, phosphorylation, xanthation and graft copolymerization have been applied change the surface characteristics of CS [10]. Among various techniques used, graft copolymerization is of the utmost importance. Graft copolymerization of synthetic polymers onto CS can not only enhance the desired properties but also widen the field of the potential applications of CS [11].

Phenolic compounds are widely dispersed throughout the plant kingdom representing about 8000 different phenolic structures. Phenolic compounds are synthesized by plants during development and in response to conditions such as infection, wounding, UV radiation, etc. [12]. Phenolic compounds are also an essential part of human diet and are of considerable interest due to their antioxidant [13,14], antidiabetic [15] and anticancer properties [16]. In recently years, many attempts have been made to conjugate phenolic compounds onto CS through graft copolymerization reaction [17–19]. The main advantage of conjugation phenolic compounds onto CS is that it can introduce desired properties, including antioxidant [20], antimicrobial [21], antidiabetic [22] and antitumor

* Corresponding author.
E-mail address: junliu@yzu.edu.cn (J. Liu).

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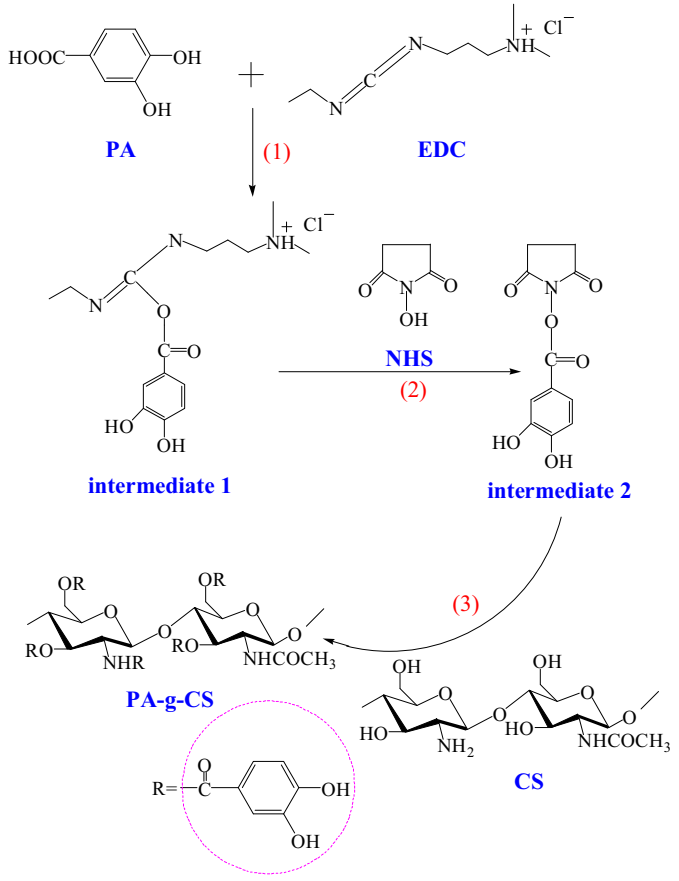

Fig. 1. The schematic reaction mechanism for the synthesis of PA-g-CS by EDC and NHS: (1) Carboxyl groups on PA react with EDC to produce the O-acylisourea intermediate 1; (2) O-acylisourea further reacts with NHS to produce the active ester intermediate 2; (3) Active ester finally reacts with the amino and hydroxyl groups on CS to yield amide and ester bonding product (PA-g-CS).

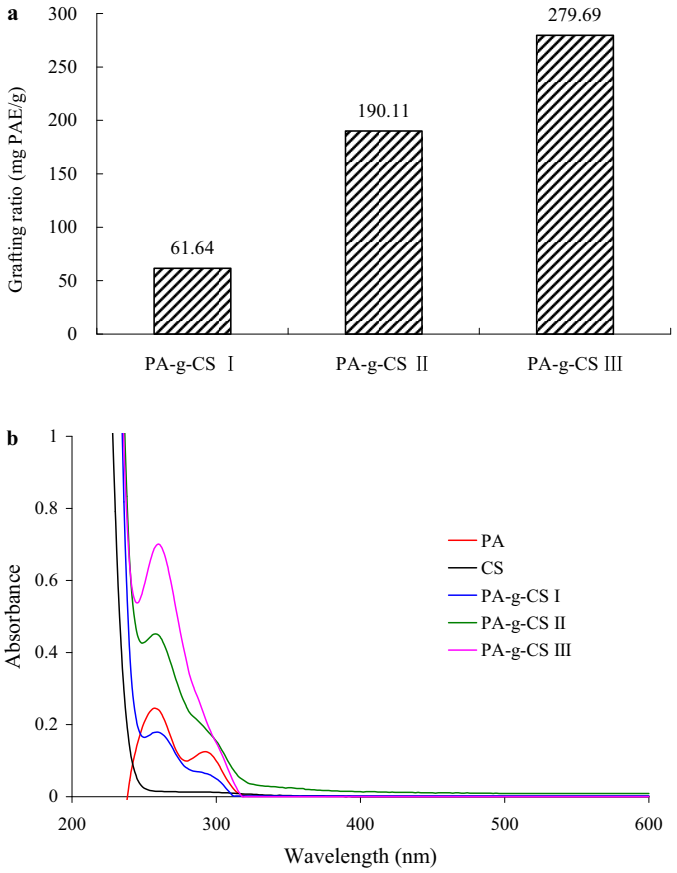

Fig. 2. Grafting ratios (a) and UV–vis spectra (b) of PA-g-CS I, PA-g-CS II and PA-g-CS III.

activities [23] into CS. Till now, several methods including carbodiimide mediated chemical cross-linking, free radical mediated grafting and enzyme catalyzed reaction had been adopted to conjugation phenolic compounds onto CS [20–24]. However, these grafted phenolic compounds are mainly limited to gallic acid [20,24–26], ferulic acid [27–29], caffeic acid [23,26,27,30] and catechin [20–22]. So, little information is available for other phenolic grafted CS [31].

Protocatechuic acid (PA) is a major benzoic acid derivative commonly found in fruits, edible plants and vegetables [32]. PA has been demonstrated to possess many valuable bioactivities, such as antioxidant, antibacterial, anticancer, antiulcer, antidiabetic, antiageing, antifibrotic, antiviral, anti-inflammatory, analgesic, antiatherosclerotic, cardiac, hepatoprotective, neurological and nephro protective activities [33]. In this study, PA was grafted onto CS by a carbodiimide mediated chemical cross-linking reaction. The structural and physical properties of PA grafted CS (PA-g-CS) was characterized by several instrumental methods. The antioxidant activity of PA-g-CS was also evaluated and compared with that of CS.

2. Materials and methods

2.1. Reagents and chemicals

CS (deacetylation degree of 71% and molecular weight of 2.5×10^5 Da) was purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). PA, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-Hydroxysuccinimide (NHS), 2-(*N*-morpholino)ethanesulfonic acid (MES), deuterated acetic acid (CD_3CO_2D), deuterium oxide (D_2O) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were all purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Preparation of PA-g-CS samples

The grafting of PA onto CS was achieved by an EDC/NHS coupling method reported by Pasanphan and Chirachanchai with some modifications [24]. CS (1.22 g, 7.5 mmol) was dissolved in 25 mL of acetic acid solution (0.5%, v/v) and stirred at room temperature overnight to ensure complete dissolution. PA (1.16 g, 7.5 mmol) was dissolved in 5 mL of ethanol and then reacted with EDC (1.44 g, 7.5 mmol) in 20 mL of MES buffer (pH 5.5) to obtain intermediate compound 1. Afterwards, NHS (0.86 g, 7.5 mmol) was added into intermediate compound 1 and the reaction was continued in an ice bath for 1 h to obtain intermediate compound 2. The intermediate compound 2 solution was then gradually added into previously prepared CS solution and reacted for 12 h at room temperature. The final products were dialyzed against distilled water for 72 h to eliminate free acetic acid and isourea by-products, followed by centrifuging at 10,000 rpm for 30 min to remove residual PA. The supernatant was lyophilized to afford PA-g-CS I and stored at 4 °C for further analysis. Similarly, PA-g-CS II and PA-g-CS III were prepared by varying the molar ratios of CS: PA: EDC: NHS (1: 3: 3: 3 for PA-g-CS II; 1: 5: 5: 5 for PA-g-CS III, respectively).

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