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Effects of ultrasound on molecular properties, structure, chain conformation and degradation kinetics of carboxylic curdlan

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ABSTRACT

In this study, high-intensity ultrasound (20 kHz), a simple, effective and without any additive method, was used to the degradation of carboxylic curdlan (Cc) produced by 4-acetamido-TEMPO-mediated oxidation. The effects of ultrasound on molecular properties, structure and chain conformations of Cc were investigated by viscometry, size-exclusion chromatography with multiangle laser-light scattering (SEC–MALLS) analysis, as well as FTIR and NMR spectroscopies. The results indicated that the intrinsic viscosity [η] and the weight-average molecular weight (M_w) of Cc decreased obviously after ultrasound, and a uniform and narrow distribution of degradation product was obtained. The *z*-average radius of gyrations (R_g) firstly increased and then decreased as the sonication time prolonged. Ultrasound destroyed the hydrogen bonds resulting in the transition from compact random coil conformation to more flexible and even shorter extended chains. Ultrasonic treatment could not alter the primary chemical structure of Cc molecules according to the structural analysis by FTIR and NMR spectroscopies. Degradation kinetics based on Schmid model was applied to estimate the degradation rate constant *k*. It was found that the *k* value of Cc decreased with increasing the polymer concentration from 0.05 to 0.2% (w/v).

1. Introduction

Polysaccharides represent a major class of biomacromolecules. In addition to their well-known functions as structure and storage molecules in the cells of living organisms, polysaccharides play important and specialized roles in living organisms such as by acting as immunomodulators or biological response modifiers (BRMs) of the innate immune responses. Natural polysaccharides from plants and microbial sources have been widely explored for their antitumor and immunomodulatory properties and other health benefits (Zong, Cao, & Wang, 2012). Because of strong antitumor and immumodulatory activities, a variety of the purified β-glucans and polysaccharide–protein and –peptide complexes (PSPs) from mushrooms (fungi) have found clinical applications for immunotherapy and cancer treatment (Giavasis, 2014; Stachowiak & Reguła, 2012; Zhang, Cui, Cheung, & Wang, 2007), such as Lentinans from *Lentinus edodes* (Xu, Yan, Tang, Chen, & Zhang, 2014), polysaccharide D-fraction and MD-fraction from *Grifola frondosa* (Boh & Berovic, 2007), and PSP from *Coriolus versicolor* (Cui, 2003).

In general, biological activities of polysaccharides are closely related to their chemical composition, configuration and chain conformation, as well as their physical properties. Thus, differences in bioactivities can be correlated with solubility in water, molecular weight, branching ratio, chemical structure and chain conformation (Yang & Zhang, 2009). Especially, polysaccharides in an aqueous solution exhibit various forms of chain conformation, most commonly random coil and various helical forms, single helix, double helix and triple helix, which are important to their properties and functions. Previous studies have suggested that the solution property and chain conformation of polysaccharides are important factors for their bioactivities, though the specific bioactive conformation forms varied with the polysaccharide structures and also with the bioassay systems. However, a number of high-molecular weight (high-M_w) polysaccharides exhibit poor solubility, high viscosity and unstable physicochemical properties, which are unfavorable for clinical uses. Moreover, some polysaccharides with high-M_w tend to form aggregates in solution that may mask the behavior of individual macromolecules and influence the bioactivities and functions. It is desirable to improve the solubility and chain conformation and reduce the viscosity of polysaccharides in aqueous solutions by modification of the polysaccharide molecules. Furthermore, most studies have demonstrated that biological activities of polysaccharides are obviously improved by molecular modifications or degradations (Xing et al., 2005).

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Several methods have been applied to degrade polysaccharides, including chemical degradation, enzymatic hydrolysis, and physical depolymerization (Liu, Bao, Du, Zhou, & Kennedy, 2006). Amongst of them, physical treatments, such as various radiation means of γ-ray, ultraviolet light, microwave and power ultrasound (Vodeničarová, Dřímalová, Hromádková, Malovíková, & Ebringerová, 2006), are considered as the most convenient and frequently used technologies that are few or no chemicals need to be added to the polysaccharide solutions and the degraded products are easier to recover and purify. It is interesting and important to point out that ultrasonic treatment is one of the most promising, effective and environmentally friendly approaches for the degradation of polymer in aqueous medium (Suslick & Price, 1999). Up to now, there are a number of polysaccharides and their derivatives, such as dextran (Zou et al., 2012), chitosan (Czechowska, Rokita, Lotfy, Ulanski, & Rosiak, 2005), pullulan (Ohta, Kato, & Kawahara, 1984), fucoidan (Guo et al., 2014), xylogucan (Vodeničarová et al., 2006), carrageenans (Lii, Chen, Yeh, & Lai, 1999), pectin (Zhang et al., 2013) and carboxymethycellulose (Grönroos, Pentti, & Hanna, 2008), have been used for ultrasonic degradation to improve their physical and chemical properties such as solubility, viscosity and molecular weight. Moreover, these studies mainly focus on effects of ultrasonic degradation on viscosity, molecular weight, chemical structure and degradation kinetics. However, to our best knowledge, there have been no or only few efforts have been made to elucidate changes in the chain conformations of polysaccharides before and after ultrasound.

Curdlan is a bacterial polysaccharide produced by *Alcaligenes faecalis* that has been of significant recent interest due to its interesting and valuable rheological properties and its inherent bioactivity (Lehtovaara & Gu, 2011; Zhang & Edgar, 2014). However, curdlan is insoluble in water, which limits its applications in food industry and biomedicine. More recently, our group has successfully prepared carboxylic curdlan (Cc) derivatives bearing the β-1,3-polyglucuronic acid structure by using 4-acetamido-TEMPO/NaClO/$NaClO_2$ system under mild conditions (Yan et al., 2014). The Cc produced by 4-acetamido-TEMPO-mediated oxidation under pH 4.8 and 40 °C for 4 h had the higher M_w (>500 kDa) and the poor solubility exhibited as a compact random coil structure in an aqueous medium. Therefore, the present study aims to investigate and evaluate the effects of ultrasonic degradation on viscosity, molecular weight, structure and chain conformations of Cc at different concentrations. Meanwhile, the degradation kinetics and mechanism were also discussed.

2. Materials and methods

2.1. Materials and chemicals

Commercial curdlan was obtained from Wako Pure Chemical Co., Japan. Carboxylic curdlan (Cc) bearing the β-1,3-polyglucuronic acid structure was prepared with 4-acetamido-TEMPO-mediated oxidation at pH 4.8 and 40 °C for 4 h based on our previous work (Yan et al., 2014). The carboxylate content of Cc was 1.98 mmol/g by electric conductimetric titration (Saito & Isogai, 2004). The weight-average molecular weight (M_w) and molecular weight distribution (M_w/M_n) of Cc were 9.50×10^5 g/mol and 2.30, respectively, as determined by the SEC–MALLS analysis described in Section 2.4. All other chemicals and solvents were of laboratory grade and used without further purification.

2.2. Ultrasonic degradation

Ultrasonic degradation experiments were performed with a Model VCX 750 processor (Sonics & Materials Inc., Newton, USA) of 20 kHz frequency and 750 W maximum output power. 0.05%, 0.1% and 0.2% (w/v) of Cc solutions were prepared with deionized water to investigate the influence of concentration on Cc ultrasonic degradation. The Cc solution (20 mL) was placed in 50-mL plastic centrifuge tube. The ultrasound probe (13 mm diameter) was submerged into the solution at a fixed depth of 2.0 cm, and the output power of ultrasonic setup was fixed at 600 W in all the experiments. The sample tube was placed in an ice bath during the ultrasonic treatment to maintain an average temperature of 40 ± 5 °C over the treatment period. After ultrasonic treatment for selected periods of time (5–90 min), the Cc solutions were centrifuged at 10,000 rpm for 30 min and then freeze dried and stored at 20 °C before use.

2.3. Intrinsic viscosity determination

Intrinsic viscosity [η] was determined by the serial dilution method (Yan et al., 2009) and the viscosity of diluted Cc was measured in 0.1 M NaCl aqueous solution at 30 ± 0.1 °C with an Ubbelohde viscometer (0.5–0.6 mm capillary diameter). The kinetic energy correlation was always negligible. The [η] value of each sample was estimated by the Huggins and Kraemer equations,

$$\frac{\eta_{sp}}{c} = [\eta] + k' \times [\eta]^2 \times c; \quad \ln \frac{\eta_r}{c} = [\eta] - \beta \times [\eta]^2 \times c \tag{1}$$

where k' and β are constants for a given polymer at a given temperature in a given solvent, c the polymer concentration, η_{sp}/c the reduced specific viscosity, $(\ln \eta_r)/c$ the inherent viscosity.

2.4. Size-exclusion chromatography with multiangle laser-light scattering (SEC–MALLS) analysis

The weight-average molecular weight (M_w), number-average molecular weight (M_n), molecular weight distribution (M_w/M_n), z-average radius of gyration (R_g), and chain conformation of Cc samples before and after ultrasonic treatment were determined by using SEC–MALLS (DAWN HELEOS II, λ = 658 nm: Wyatt Technologies Corporation, USA) on an Agilent 1100 system equipped with two SEC columns (OHpak SB-806 M HQ and SB-805 HQ, 8 mm Φ × 30 cm, Shodex, Japan) in 0.1 M NaCl at 25 °C and a refractive index detector. In the typical analysis, 100 μL aliquot of the sample solution (0.1%, w/v) was injected with 0.1 M NaCl as the mobile phase at a flow rate of 0.5 mL/min, with a refractive index increment (dn/dc) of 0.125 mL/g (Shibata, Yanagisawa, Saito, & Isogai, 2006). Data acquisition and analysis were performed using on-line Astra software (Wyatt Technologies, USA). Details of the SEC–MALLS system used in this study and the operation conditions are described in our previous work (Yan et al., 2014).

2.5. FTIR and NMR spectroscopies

The FTIR spectra of Cc samples before and after ultrasonic treatment were determined using a Nexus 670 FTIR spectrometer (Thermo Nicolet Co., USA) in the wavenumber range from 500 cm^{-1} to 4000 cm^{-1} with KBr pellets and referenced against air. For NMR analysis, Cc and its products (30 mg) were dissolved in 0.5 mL of D_2O. The ^{13}C NMR spectra were recorded by a Bruker AVANCEIII 400 MHz spectrometer (Bruker, Rheinstetten, Germany) at 25 °C. All chemical shifts were expressed in reference to Me_4Si.

3. Results and discussion

3.1. Effect of sonication on intrinsic viscosity

Fig. 1 shows the intrinsic viscosity of the Cc solutions at different concentrations by ultrasonic degradation as a function of sonication time from 5 to 90 min. Irrespective of the concentration