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The combination between cations and sulfated polysaccharide from abalone gonad (*Haliotis discus hannai* Ino)

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ABSTRACT

Effects of cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) on the structure of abalone gonad sulfated polysaccharide (AGSP) were studied by means of Congo red test, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM). The results showed that the local ordered helical conformation was observed in AGSP. The cations could combine with anionic groups in AGSP by ionic bonds, which caused the weakening of electrostatic repulsion and crosslinking of molecular chains. Furthermore, the effects of divalent cations on the conformation were more obvious than that of monovalent cations, and divalent cations led to the aggregation of AGSP due to the more interaction sites. In short, AGSP molecular chains were crosslinked through ionic bonds after adding cations, and, divalent cations could induce the aggregation of AGSP by electrostatic interactions. This study will provide valuable insights for the further research on AGSP conformation.

1. Introduction

Pacific abalone (*Haliotis discus hannai* Ino), a single-shelled marine mollusk and an aquatic economic species, is widely cultured in Yellow Sea, China. It is abundant of nutrients (Dong et al., 2012; Li et al., 2011; Wang et al., 2014; Zhou, Tang et al., 2012; Zhu et al., 2009) and is popular in East Asia. However, plenty of abalone viscera are abandoned in the process of commercial production, which contain many bioactive compounds, such as peptides (Zhou, Zhu et al., 2012), polysaccharides (Sun et al., 2010; Wu et al., 2013; Zhu et al., 2008, 2010, 2011), lipid (Zhou, Tong et al., 2012). It is also a vital research field of seafood attracting lots of researchers. Polysaccharides were abundant in abalone viscera, which had excellent bioactivities, including antioxidant (Zhu et al., 2008), free radical scavenging (Zhu et al., 2010), antitumor and immunomodulation (Sun et al., 2010), cholecystokinin-releasing (Zhu et al., 2011) and cell proliferation (Wu et al., 2013) activities. In our previous studies, a novel water-soluble abalone gonad sulfated polysaccharide (AGSP) was isolated and confirmed that it had →4)-β-GlcA(1 → 2)-α-Man(1 → disaccharide repeating units (Wang et al., 2015). Our current research showed that backbone chain of AGSP also had →3)-β-GlcA(1 → 3)-α-Gal(1→ repeating units with many Gal and sulfate groups in branch (Song et al., 2018). Moreover, it was found that an abalone gonad polysaccharide fraction (named AGP-32) could promote mice splenic lymphocyte proliferation (Yang et al., 2015), and all the three fractions (named AGP-31, -32, -33) could stimulate cholecystokinin secretion in STC-1 cells (Zhao et al., 2016).

It is well known that the change of conformation may influence biological activities of polysaccharides (Yi et al., 2012; Zhang, Li, Xu, & Zeng, 2005). Besides, the conformation of polysaccharides was influenced by environmental conditions such as pH, cations, temperature, etc. It was reported that five types of metal ions caused appreciable changes in the anion conformations of heparin oligosaccharides through the displacement of metal ions from their sites (Remko, Vanduijnen, & Broer, 2013), and a calcium-rich crude polysaccharide was easier to aggregate and keep viscosity as well as weak gelling properties because of interactions between Ca^{2+} and polysaccharide chains (Yin et al., 2012, 2015). In addition, the effects of metal cations on polysaccharide conformation were varied especially on acidic polysaccharides. For instance, the gel strength of kappa-carrageenan, a sulfated polysaccharide, was enhanced by K^+, which was caused by the intramolecular cation bridge through K^+ between a sulfate group and an adjacent ring-oxygen atom with an electrostatic force of attraction,

Abbreviation: AGSP, abalone gonad sulfated polysaccharide; XRD, X-ray diffraction; FTIR, Fourier transform infrared spectroscopy; SEM, scanning electron microscope; AGP, abalone gonad polysaccharide; MWCO, molecular weight cut-off; λ_{max}, maximum absorption wavelength

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whereas Ca^{2+} had little effects (Tako & Nakamura, 1986; Tako, Nakamura, & Kohda, 1987).

Although the primary structure and biological activities of abalone gonad polysaccharides have been discussed, the studies on the effects of metal cations on conformation of these polysaccharides are rarely found in literature. As an uronic acid-containing sulfated polysaccharide, abalone gonad polysaccharide probably interacted with metal cations. The current study was to explore the molecular interactions between AGSP and metal cations including Na^+, K^+, Ca^{2+} and Mg^{2+}. The conformation changes of AGSP with different concentrations of cations were investigated by means of Congo red test, XRD, SEM and FTIR.

2. Materials and methods

2.1. Materials and chemicals

AGSP was extracted from freeze-dried powders of abalone gonad supplied by Dalian Zhangzidao Group Co. (Liaoning Province, China). Trypsin 1:250 from porcine pancreas and papain > 2000 U/mg were purchased from Sangon Biotech (Shanghai) Co. (Shanghai, China). D204 anion exchange resin was purchased from Zhejiang Zhengguang Industrial Co. (Zhejiang, China). Congo red was purchased from Shanghai Macklin Biochemical Co. (Shanghai, China). Other reagents used were of analytical grade.

2.2. Preparation of AGSP

AGSP was extracted from abalone gonad freeze-dried powders. Abalone gonad freeze-dried powders were dissolved in deionized water (w/v = 1:25) and adjusted to pH 8 by NaOH (6 M), and then 2 wt% trypsin was added. After incubation at 37 °C for 4 h, the mixture was adjusted to 50 °C, and 2 wt% papain was added to incubate for 4 h. Then, the mixture was boiled for 10 min, adjusted to neutral by HCl (6 M), centrifuged at 4000 rpm for 10 min. The supernatant was mixed with D204 anion exchange resins for 6 h with stirring constantly, then the resins were loaded into the column, and elution was performed with 3 M NaCl aqueous solution at a flow rate of 1 mL/min. Fraction was precipitated with 3 times (v/v) of 95% ethanol at 4 °C for 24 h and centrifuged at 4000 rpm for 10 min. The precipitate was dissolved in deionized water, added 1% (w/v) sodium hydrogen sulfite, adjusted to pH 2 by HCl (6 M) and centrifuged at 4000 rpm for 10 min to remove protein. The supernatant was collected, adjusted to pH 8.5 and added 2% (v/v) hydrogen peroxide (30%), stirring constantly for 15 h at room temperature. Then, the mixture was adjusted to pH 6.5, precipitated with 3 times (v/v) of 95% ethanol at 4 °C for 24 h. The precipitate was dissolved in deionized water and dialyzed (MWCO 3500 Da) against flowing tap water (24 h) and deionized water (with more changes of water in 48 h). The retentate was lyophilized to obtain AGSP.

2.3. Preparation of sample solutions

Four salts (NaCl, KCl, $CaCl_2$, $MgCl_2$) were dissolved in pure water to prepare different concentrations of solvents (0.005 M, 0.01 M, 0.05 M, 0.1 M), respectively. Sample solutions (10 mg/mL) were obtained by dissolving accurate weight of AGSP in the various solvents, and AGSP dissolved in pure water was as a control group. Then, all sample solutions were heated for 30 min at 70 °C with oscillated sufficiently in every 10 min. The treated samples were lyophilized for further study.

2.4. Congo red test

Congo red test was performed to investigate conformational transformation of AGSP induced by metal ions. The accurate weight of AGSP was dissolved in 1 mL pure water and different concentrations (0.005 M, 0.01 M, 0.05 M, 0.1 M) of salts solutions (NaCl, KCl, $CaCl_2$, $MgCl_2$) to obtain 2 mg/mL AGSP solutions, respectively. The solutions were mixed with 1 mL Congo red (80 μM) and then added 2 mL NaOH solution to make the final concentration of NaOH to 0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5 M. Pure water without added AGSP was served as the control. The mixtures were equilibrated for 10 min at room temperature. The maximum absorption wavelength λ_{max} was measured by Lambda 35 ultraviolet-visible spectra (Perkin Elmer, Waltham, USA) in the wavelength range of 400–600 nm.

2.5. XRD analysis

XRD is a powerful technique used to unravel polysaccharides structures (Jeddou et al., 2016). The lyophilized sample powders were used to XRD analysis. XRD patterns were obtained using X-ray diffractometer 7000S (Shimadzu, Tokyo, Japan). The patterns with Cu Kα radiation at 40 kV and 30 mA were recorded in the 2θ range of 10–70° at a scanning rate of 5°/min.

2.6. SEM observation

SEM was utilized to observe the surface morphology of the samples. The lyophilized sample powders were fixed onto a copper stub and coated with a thin gold layer, respectively. Then, micrographs were obtained using a JSM-7800F scanning electron microscope (Japan Electron Optics Laboratory, Tokyo, Japan). The samples were observed at the voltage of 3.0 kV.

2.7. FTIR spectra analysis

FTIR spectra were used to explore molecular interaction sites between metal ions and AGSP. The lyophilized sample powders (1 mg) was mixed with dried KBr (100 mg) and pressed into disk for FTIR spectra analysis, respectively. The FTIR spectra were recorded in the frequency range of 400–4000 cm^{-1} on a Frontier FTIR Spectrometer (Perkin Elmer, Waltham, USA), and the resolution was set as 4 cm^{-1}.

3. Results and discussion

3.1. Congo red

Congo red can form complexes with a helical conformation of polysaccharides (Ogawa, Tsurugi, & Watanabe, 1972). As compared with pure Congo red, maximum absorption wavelength λ_{max} of the complex generates bathochromic shift in the wavelength range of 400–600 nm. The interactions of AGSP with Congo red in different solvents were shown in Fig. 1. It appeared an obvious bathochromic shift for AGSP with Congo red in all solvents in comparison to pure Congo red, which showed that AGSP might be a helical conformation. With the increase of concentration of NaOH, λ_{max} of the complex remained stable in pure water. It had been reported that the λ_{max} of complex of polysaccharides with helical conformation could shift to a longer wavelength at low concentrations and drop with the increase of the concentration of NaOH (Liu, Zhu et al., 2016; Yang et al., 2012). Meanwhile, a water-soluble polysaccharide (Zhao et al., 2014) and three exopolysaccharides (Vasconcelos et al., 2008) all showed a stable bathochromic shift even under strong alkaline conditions, which indicated that a highly ordered helical conformation was adopted. It was also reported that hydrogen bonds are main molecular force which maintains the helical conformation of polysaccharides (Leung, Liu, Koon, & Fung, 2006). In comparison, the complex of AGSP remained stable from low concentration to a higher of NaOH. It was inferred that AGSP probably had an ordered helical conformation by hydrogen bonds interaction in pure water.

As shown in Fig. 1a and b, the Congo red test curves of AGSP in NaCl and KCl solutions were nearly consistent with the pure water, and there was no change in different concentrations of salts solutions. It indicated