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The Study on Purification and Antioxidation Effects of Glycosaminoglycan from Patinopecten Yessoensis Waste

Qingman Cui [+], Guangjing Li and Chunying Yuan

Key Laboratory for Marine Chemistry and Resource, College of Marine Science and Engineering, Tianjin University of Science & Technology, Tianjin 300457, China

Abstract. Purification and antioxidation effects of glycosaminoglycan from *Patinopecten yessoensis* waste were studied, the results showed that the purity of glycosaminoglycan was 90.56%, and glycosaminoglycan contained -OH(3424 cm^{-1}),-COO-(1653 cm^{-1}), -NH(1567 cm^{-1}),-SO2(1414 cm^{-1}),C-O-C(sugar ring, 1044 cm^{-1}) and other characteristic groups. Glycosaminoglycan had a good scavenging capacity of DPPH free radical and reducing capacity, a good ability of metal chelating.

Keywords: *Patinopecten yessoensis* waste, Glycosaminoglycan, Antioxidant effects

1. Introduction

Glycosaminoglycan, also known as glycosaminoglycans and acidic polysaccharide, a kind of heteropolysaccharide, has a wide range of biological functions. Some scholars had isolated and purified glycosaminoglycan from the whale shark cartilage, starfish, sea cucumber body wall, scallops, clams, abalone, sea hares, Mactra veneriformis and Bullacta exarata and so on marine animals, and their chemical composition and functional activities were studied partly[1-8].

Patinopecten yessoensis belongs to *Lamellibranchia, Pterioida, Pectinidae, Patinopecten,* is cold water shellfish, originated in Japan and Korea, now artificial breeding and proliferation production of *P. yessoensis* had been proceeded in Shandong province, Liaoning province and other northern coastal areas. *P. Yessoensis* is rich in unsaturated fatty acids of EPA and DHA, have also good effects on body weakness, loss of appetite, malnutrition and other diseases.Yunhai Yu et al [9] had extracted sulfated polysaccharides from *P. Yessoensis*, and studied its monosaccharide composition and anti-clotting effect in vitro, Hongling Yin et al [10] had extracted polysaccharides with scavenging activity of hydroxyl radical from *P. Yessoensis*. In order to further expand the functionality of *P. Yessoensis*, make full use of *P. Yessoensis waste*, purification and antioxidation effects of glycosaminoglycan from *P. yessoensis* waste had been studied.

2. Materials and Methods

2.1. Materials

P. Yessoensis was purchased from Tanggu farmers market.

The experimental reagents: *Bacillus subtilis* neutral protease (enzyme activity 60000 U/g), trypsin(1:250), alcian blue 8GX, ethanol, acetone, sodium acetate, CTAB (hexadecyl trimethyl ammonium bromide) were of analytical grade.

The main instruments: T6 UV spectrophotometer, RE-3000 rotary evaporator, TGL-16M high-speed refrigerated centrifuge, SCIENTZ-ⅡD ultrasonic crusher, FD-1D-50 vacuum freeze dryer, Tu-1810SPC spectrophotometer, etc.

[+] Corresponding author. Tel.: +86 22 6060 1462; fax: +86 22 6060 0358.
E-mail address: cqm80@163.com.

2.2. Experimental Methods

2.2.1. The extraction and purification of the glycosaminoglycan

P. yessoensis waste was homogenized (solid-liquid ratio of 1:2), added 0.4% neutral protease and trypsin respectively, enzymatic hydrolysis for 3.5 h, enzyme inactivation, 8000 r/min centrifugation for 10 min, deproteinization through isoelectric point and trichloroacetic acid, supernatant was added ethanol(final concentration of 60% ethanol), and alcohol precipitation 24 h at 4 ℃, the precipitate was separated by centrifugation, washed with anhydrous ethanol and acetone and dried under vacuum.

The crude glycosaminoglycan was hydrolyzed again with 0.4% trypsin, deproteinization, CTAB combination, dialysis and alcohol precipitation.

2.2.2. Determination of glycosaminoglycan content

Glycosaminoglycan content was determined by alcian blue colorimetric method [11].

2.2.3. The spectral analysis of glycosaminoglycan

1mg/mL Glycosaminoglycan solution was formulated, scanned in the wavelength range of 190-800 nm.

KBr pellets (the ratio of the mass of glycosaminoglycan with potassium bromide is about 1:200), infrared scanning in the wavelength range of 4000-400 cm^{-1}.

2.2.4. Determination of scavenging capacity of DPPH (1,1-Diphenyl-2-picrylhydrazyl radical 2,2- Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl) free radical

Five different mass concentrations of glycosaminoglycan solution were formulated, 2 mL glycosaminoglycan solution was added 2 mL DPPH ethanol solution (2 mmol/L), mixed and reacted for 30 min at room temperature, the absorption value was measured at 517 nm (Ai). The sample was replaced by ethanol, the absorption value (Ao) was measured, meanwhile 2 mL of sample solution was mixed with 2mL anhydrous ethanol, and the absorption value(Aj) of mixture was measured, scavenging rate *I* was calculated as follow:

$$I\% = (Ao\text{-}Ai\text{-}Aj) / Ao$$

Vitamin C (Vc) as positive control, experiment was repeated three times.

2.2.5. Determination of reducing capacity

According to Oyaizu method[12], five different mass concentrations of glycosaminoglycan solution(0.1, 0.2, 0.3, 0.4, 0.5 mg/mL) were formulated, 2.5 mL glycosaminoglycan solution was added 2.5 mL pH6.6 phosphate buffer, 2.5 mL 1% potassium ferricyanide sequentially, water bath for 20 min at 50 ℃, and then added 2.5 mL 10% trichloroacetic acid, 3000 r/min centrifugation for 10min, 2.5 mL of the supernatant was added 2.5 mL distilled water and 0.5 mL 10% ferric chloride, settled the mixed liquid for 10 min, the absorption value was measured at 700 nm. Vitamin C(Vc) as positive control, experiment was repeated three times.

2.2.6. Determination of metal chelating ability

According to Decker method [13] with some modification. 1 mL Different concentrations of glycosaminoglycan solution was added to the sample tube, then add 3.7 mL of distilled water, 0.1 mL 2 mmol/L $FeCl_2$ solution, and finally added 0.2 mL 5 mmol/L Ferrozine to start the reaction, distilled water was insteaded of the glycosaminoglycan solution in blank tube, distilled water was insteaded of the $FeCl_2$ solution in standard tube, three groups were settled for 10 min at room temperature, the absorption value was measured at 562 nm, EDTA(ethylene diamine tetraacetic acid) as positive control, experiment was repeated three times, the metal chelating rate (%) was calculated as follow:

$$\text{Metal chelating rate (\%)} = [A_{blank}\text{-}(A_{sample}\text{-}A_{standard})] / A_{blank} \times 100$$

3. Results and Analysis

3.1. UV Absorption Spectra of the Glycosaminoglycan

The purified glycosaminoglycan from *P. yessoensis* waste was scanned with UV spectrophotometer, UV

absorption spectrum was shown in Fig.1. the purified glycosaminoglycan had no obvious absorption peak at about 280 nm and 260 nm, indicated that prepared glycosaminoglycan had a high purity, and had the strong absorption peak at 203nm.

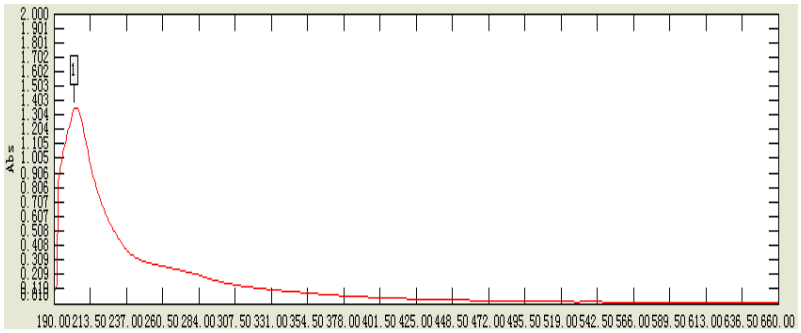

Fig. 1: Ultraviolet absorption spectrum of glycosaminoglycan

3.2. Infrared Spectroscopy Analysis of Glycosaminoglycan

Infrared spectroscopy of glycosaminoglycan was shown in Fig.2. Glycosaminoglycan had strong or weak absorptions at seven places, which were 3424 cm^{-1}, 2936cm^{-1}, 1653 cm^{-1}, 1567 cm^{-1}, 1414 cm^{-1}, 1240 cm^{-1},1041 cm^{-1}, the first one indicated that it had stretching vibration of O-H bond of hydroxyl and N-H bond of amino, the second one indicated that it had stretching vibration of C-H bond of methyl, the third one indicated that it had stretching vibration of C-O of hydroxyl and acetamido and bending vibration of N-H, the fourth one indicated that it had bending vibration of N-H, the fifth one indicated that it had stretching vibration of C-O bond of carboxyl, the sixth one indicated that it had asymmetric stretching vibration of S=O bond of sulfonyl, the seventh one indicated that it had stretching vibration of C-O-S bond of sulfonyl. From the analysis of infrared spectroscopy, it was showed that: the glycosaminoglycan contained the characteristic groups of -OH(3424 cm^{-1}) ,-COO-(1653 cm^{-1}), -NH(1567 cm^{-1}), -SO_2(1414 cm^{-1}), C-O-C (sugar ring, 1044 cm^{-1}) etc.

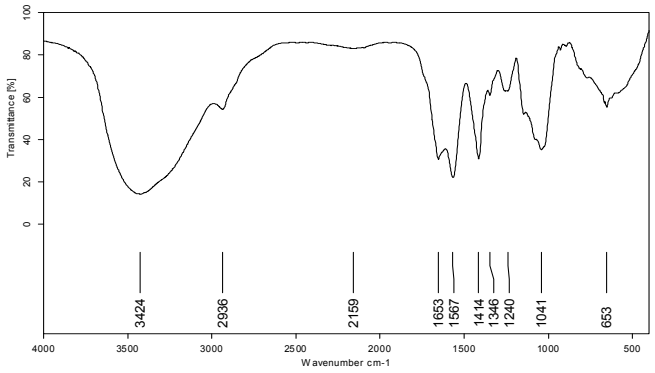

Fig. 2: Infrared spectroscopy of glycosaminoglycan

3.3. Scavenging effect of Glycosaminoglycan on DPPH Free Radical

Scavenging effect of glycosaminoglycan on DPPH free radical increased with the mass concentration within the range of 0.1-0.5 mg/mL, and there was a good positive linear correlation(Fig.3.), the linear equation of Vc: y=108.24 x+48.516 R^2= 0.9255, the linear equation of glycosaminoglycan: y=86.78 x+38.85 R^2=0.9165. Using median scavenging mass concentration as the evaluation index of scavenging free radical ability, median scavenging mass concentrations of Vc and glycosaminoglycan were 0.014 mg/mL and 0.128mg/mL respectively, scavenging effect of Vc on DPPH free radical was obviously stronger than that of glycosaminoglycan.

3.4. Reduction Ability of Glycosaminoglycan

The absorption value and mass concentration of Vitamin C and glucosamineglycan showed closely positive correlation(Fig.4:), linear equations were respectively: y =0.53x+0.085 R^2 =0.9646, y =1.09x +0.145 R^2 = 0.9685. Reduction ability of Vc was obviously bigger than that of glycosaminoglycan.

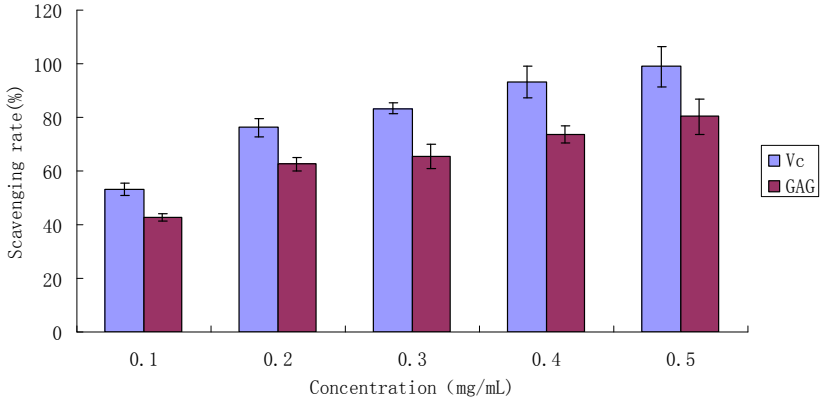

Fig. 3: Scavenging rate of glycosaminoglycan on DPPH free radical

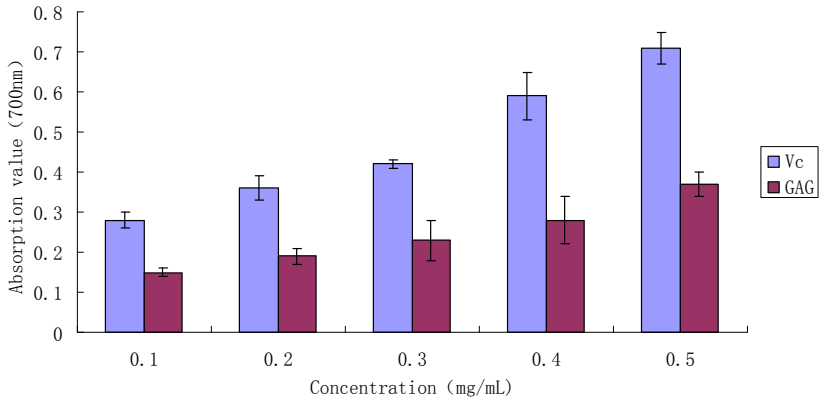

Fig. 4: Reducing ability of glycosaminoglycan

3.5. Effect of Glycosaminoglycan (GAG) on the Metal Chelating Ability

The result was shown in Fig.5. EDTA showed a strong chelating activity, the maximum chelating rate was 99.61% when the concentration was 0.4 mg/mL, followed by a slight decline, the metal chelating ability of glycosaminoglycan showed an increasing trend with the increase of the concentration in the concentrations range 0.2-1 mg/mL, but its chelating rate was lower than that of EDTA..

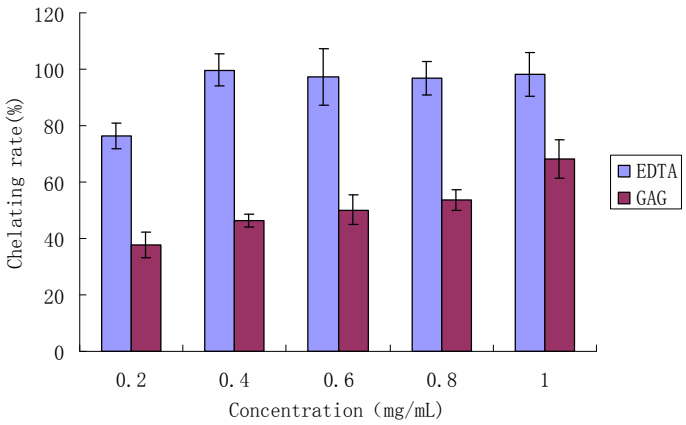

Fig. 5: Metal chelating ability of glycosaminoglycan

4. Discussion

In recent years, antioxidation of the polysaccharide was paid wide attention. Studies had shown that many polysaccharides could increase the activity of antioxidant enzymes, scavenge free radical, inhibit lipid peroxidation, and thus play a protective role of biological membrane[14]. Quanbin Zhang, et al[15] indicated that fucoidan from *L. japonica* showed significant antioxidant activity in vitro. Glycosaminoglycan as a class of polysaccharides, antioxidant effect was very obvious, the research showed that glycosaminoglycan from *P. yessoensis* waste had good scavenging effect of DPPH free radicals and reduction ability.

Some metal ions such as iron ions, copper ions play catalysis in the process of lipid oxidation, therefore substances with metal chelating effect may be play a role of antioxidant indirectly, Ferrozine could combine with Fe^{2+}, and form a purple complex, the purple complex is beginning to fade when there is other competitive complexing agent, therefore combination ability of substances with Fe^{2+} can be evaluated through the complex changes in color. EDTA is an important complexing agent, can chelate a variety of metal ions, this study confirm that EDTA had strong ability to chelate metal, in contrast, metal chelating capacity of glycosaminoglycan from *P. yessoensis* waste was weak.

5. Acknowledgements

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