Carbohydrate Polymers 96 (2013) 9–14

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Purification and characterization of D-Gal-6-sulfurylase from *Eucheuma striatum*

Xiaojuan Qin, Chaoyang Ma, Zaixiang Lou, Aimei Wang, Hongxin Wang*

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi 214122, PR China

ARTICLE INFO

Article history:
Received 11 January 2013
Received in revised form 16 March 2013
Accepted 20 March 2013
Available online 28 March 2013

Keywords:
Kappa-carrageenan
Eucheuma striatum
D-Gal-6-sulfurylase
Desulfation
Gel strength

ABSTRACT

D-Gal-6-sulfurylase catalyzing the conversion of μ-carrageenan into κ-carrageenan was extracted from *Eucheuma striatum* and purified by ammonium sulfate precipitation, hydrophobic interaction chromatography and ion exchange chromatography. The purified enzyme was a monomeric protein with a molecular mass of about 65 kDa as shown in SDS–PAGE. The maximum activity of the enzyme was observed at pH 7.0 and temperature 40 °C. K_m value for μ-carrageenan was 4.31 mM, and the corresponding V_{max} was 0.17 mM min^{-1}. The carrageenan treated with 10 U of the purified enzyme exhibited 7.1-fold increase in gel strength with a removal of 30% sulfate groups. ^{1}H NMR spectral analysis of the control and enzyme treated carrageenan confirmed the conversion of μ- into κ-carrageenan and highlighted the specificity of Gal-6-sulfurylase for μ-carrageenan. This Gal-6-sulfurylase provides an eco-friendly and alternative for alkali treatment method to produce high gel strength κ-carrageenan.

1. Introduction

Carrageenans are water-soluble sulfated polysaccharides extracted from numerous red algae (Rhodophyta; Doyle, Giannouli, Rudolph, & Morris, 2010). They share a common galactan backbone of alternating disaccharide-repeating 1,3-linked-β-D-galactopyranose (β-Gal, unit G) and 1,4-linked-α-D-galactopyranose (α-Gal, unit D) forming a linear chain (Jouanneau et al., 2010). The difference in the number and position of sulfated esters (S) and by the occurrence of 3,6-anhydro ring in the α-linked residues (An Gal, unit A) give rise to three major types of carrageenans sorted with assigned Greek letters; κ-carrageenan (kappa, G4S-DA), ι-carrageenan (iota, G4S-DA2S) and λ-carrageenan (lambda, G2S-D2S, 6S; Knutsen, Myslabodski, Larsen, & Usov, 1994; Rees, 1970; Usov, 1998). The type of κ-carrageenan due to its physical, thermal, and chemical properties as well as its ability to interact with other food polymers, such as starch and proteins, is widely used as gelling, stabilizing, and viscosity-binding agents (Usov, 2011) in food, cosmetics and pharmaceutical industries (Bravo, Arimon, Valle-Delgado, Garcia, & Durany, 2008; Huang, Kennedy, Li, Xu, & Xie, 2007).

However, the native or unprocessed κ-carrageenan has very heterogeneous chemical structures, and usually contains the fraction of the biosynthetic precursor named μ-carrageenan (G4S-D6S; Bellion, Brigand, Prome, Welti, & Bociek, 1983; Van de Velde & De Ruiter, 2002). Evidence from X-ray diffraction (Anderson, Campbell, Harding, Rees, & Samuel, 1969) and optical rotation (Lawson & Rees, 1970; Mckinnon, Rees, & Williamson, 1969; Rees, Steele, & Williamson, 1969) indicates that the presence of sulfated esters in μ-carrageenan deteriorates its gel strength by blocking the formation of double helices among the molecules. Alkali treatment could remove the sulfate groups from μ-carrageenan to enhance the gel strength (Rees, 1972; Yaphe & Duckworth, 1972). The widely used industrial step involves soaking the red algae in a strong solution of alkali hydroxide (up to 10% NaOH/KOH) at elevated temperature (70–90 °C) for a few hours (up to 5 h; Al-Alawi, Al-Marhubi, Al-Belushi, & Soussi, 2011; Freile-Pelegrín & Robledo, 2007; Viana, Noseda, Duarte, & Cerezo, 2004). The alkali treated carrageenans generally exhibit higher gel strength than the native extracts, however, this treatment has disadvantages including the decrease of carrageenan yields, and generation of an alkali effluent (Freile-Pelegrín & Robledo, 2007; Freile-Pelegrín, Robledo, & Azamar, 2006). Thus, there is a growing need to develop eco-friendly technologies for recovery of products from bioresources. Recently, an alternative method of subjecting the temperate red seaweed to dark has been used to improve the gel quality of carrageenan, however, this dark treatment reaction taken a long time (more than 10 days; Villanueva, Hilliou, & Sousa-Pinto, 2009).

The enzymatic catalysis for improving the gel strength of carrageenan has been reported. The reported enzymes include D-Gal-2,6-sulfurylase and sulfohydrolase. The former enzyme from *Choudrus crispus* could catalyze the conversion of ν- into ι-carrageenan (Genicot-Joncour et al., 2009), however, this enzyme was specific to ν-carrageenan and was inactive on μ-carrageenan.

* Corresponding author. Tel.: +86 510 85917795; fax: +86 510 85876799.
E-mail address: whx200720082009@yahoo.cn (H. Wang).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.03.061

The latter enzyme was reported to catalyze the conversion of μ-carrageenan into κ-carrageenan by removing the sulfate groups (Wong & Craigie, 1978), but so far the purified enzyme was not obtained yet. In this paper, the D-Gal-6-sulfurylase from *Eucheuma striatum* was purified by sequential chromatography procedure to a natural electrophoretic homogeneity for the first time. The purified Gal-6-sulfurylase exhibited significant advantages in purity and enzymatic activity for μ-carrageenan. In addition, Gal-6-sulfurylase treated with carrageenan could obviously improve the gel strength. Therefore, the Gal-6-sulfurylase was purified in this study to provide an eco-friendly and alternative for alkali treatment method to produce high gel strength κ-carrageenan.

2. Experimental

2.1. Materials

E. striatum was provided by Hainan Provincial Fisheries Research Institute and collected in May 2011 on the shore at Hainan Island, China. The specimens were carried in a cool pack to the laboratory under cool conditions, frozen in liquid nitrogen and stored at −80 °C. Unmodified κ-carrageenan was extracted and purified from *E. striatum* according to the method of Freile-Pelegrín et al. (2006). The polysaccharide was composed of about 11.2% μ-carrageenan moieties according to ^{1}H NMR analysis. The present hybrid carrageenans were used to screen and study Gal-6-sulfurylase activities. Ultra pure water used for experiment was prepared in laboratory from a Milli-Q system (Millipore, USA). All other chemicals were analytical grade and obtained from Sigma (St. Louis, MO, USA).

2.2. Extraction and purification of Gal-6-sulfurylase

Frozen *E. striatum* was ground in liquid nitrogen and allowed to thaw in cold extracting buffer (50 mM Tris–HCl, pH 9.5, 500 mM KCl, and 10 mM 2-β-mercaptoethanol) at a proportion of 1:3 (w/v). The suspension was gently stirred overnight at 4 °C, and all of the following fractionation and purification steps were performed at this temperature. The suspension was centrifuged at 12,000 × g for 15 min and proteins in the supernatant were precipitated with 80% ammonium sulfate saturation. Precipitate was collected after centrifugation at 15,000 × g for 15 min and dissolved in buffer A (50 mM Tris–HCl, pH 7.1, and 10 mM 2-β-mercaptoethanol). The solution, containing some polysaccharides as well as the active protein, was dialyzed against same buffer for 24 h with the change of buffer at an interval of 4 h. The dialyzed enzyme solution was brought to 30% $(NH_4)_2SO_4$ saturation and loaded on Phenyl Sepharose 6 Fast Flow column previously equilibrated with buffer B (50 mM Tris–HCl buffer, pH 7.1, 30% $(NH_4)_2SO_4$, and 10 mM 2-β-mercaptoethanol). The gel was washed with buffer B at a flow rate of 1.5 mL min^{-1} until effluent A_{280} was negligible. Elution of the bound proteins was achieved by applying a linearly decreasing gradient from 30% to 0% $(NH_4)_2SO_4$ at a flow rate of 1.5 mL min^{-1}. All of the active fractions were pooled and dialyzed for 36 h in buffer A.

The desalted enzyme solution was further purified with DEAE Sepharose CL-6B column previously equilibrated with buffer A. The column was washed with buffer A to remove the unbound proteins and eluted at a flow rate of 1 mL min^{-1} with an increasing step gradient of NaCl in buffer A. The active fractions were collected and dialyzed about 6 h against buffer A.

At each step of purification, the active fractions were analyzed by SDS–PAGE (Laemmli, 1970) using 12% polyacrylamide gel and molecular weight markers of 14.4–97.4 kDa (Genei, Bangalore) were also run simultaneously at l20 V. Protein quantification was performed according to Bradford method using bovine serum albumin as a standard (Bradford, 1976).

2.3. Gal-6-sulfurylase activity assay

Gal-6-sulfurylase activity was determined by measuring the concentration of sulfate released after incubating with hybrid carrageenans. The standard reaction mixture contained 100 μL of each protein fraction in 50 mM Tris–HCl, pH 7.1, and 100 μL of hybrid carrageenans (1.4%, w/v) in the same buffer. The reaction was conducted at 45 °C for 6–15 h and terminated by the addition of 0.2 ml of 6 N HCl. The fraction taken at zero time was used as a reference. The release of sulfate was measured by high-performance anion-exchange chromatography (HPAEC; Genicot-Joncour et al., 2009). One unit of the Gal-6-sulfurylase activity is defined as the amount of enzyme causing production of 1 μmol sulfate per minute at optimal conditions of temperature and pH.

2.4. Enzymological experiments with Gal-6-sulfurylase

Buffer solutions for the determination of pH dependence of D-Gal-6-sulfurylase activity were prepared as follows: 0.1 M acetic acid/0.1 M sodium acetate (pH 4.0–6.0), 0.1 M Tris–HCl (pH 6.0–9.0), and 0.1 M glycine–NaOH (pH 9.0–12.0). The optimum temperature for the purified Gal-6-sulfurylase activity was measured at pH 7.0 over a temperature range of 10–60 °C. Different metal ion and organic solvents were studied to examine their effect on the enzymatic activity. Apparent K_m and V_{max} were determined using a Lineweaver–Burk plot. Kinetic data for the Gal-6-sulfurylase reaction with μ-carrageenan as a substrate was obtained with substrate concentrations ranging from 0.1% to 2.5% (w/v), corresponding to 0.27–6.76 mM μ-carrabiose units. The reactions were performed at the optimum conditions (pH 7.0 and 40 °C).

2.5. Determination of sulfate content and gel strength of carrageenan

For the determination of gel strength 1.4% (w/v) solution of carrageenan was prepared in 50 mM Tris–HCl (pH 7.0) buffer. After incubation of hybrid carrageenans with 0–12 U of purified Gal-6-sulfurylase at 40 °C for 15 h, the content of sulfates and gel strength of carrageenan were determined. The amount of sulfate content in sample was determined by the method of Verma, Swaminathan, and Sud (1977). Gel strength (g/cm^2 at 20 °C) was measured on discs of carrageenan (6 cm diameter, 3 cm height) using Texture Analyzer (Model TAXT2J/25, Stable Micro System, Godalming, Surrey, UK) with a 2 cm^2 probe area and operating at a crosshead speed of 0.5 mm s^{-1}. Gel was aged overnight at 4 °C and equilibrated for 20 min at 20 °C before measurement. Gel strength was measured as the required weight to break the gel.

2.6. ^{1}H NMR spectroscopy

The composition of hybrid carrageenans was determined by ^{1}H NMR spectroscopy. One-dimensional ^{1}H NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer equipped with an indirect 5-mm gradient probehead ^{1}H/^{13}C/^{31}P at an ambient temperature. Prior to analysis, samples were dissolved at concentration of about 10 mg mL^{-1} in D_2O. Chemical shifts (δ) are expressed in ppm in reference to an internal standard (DSS). No suppression of the HOD signal was performed.

Table 1
Purification yields of Gal-6-sulfurylase.

Purification step	Protein (mg)	Total activity (U)	Specific activity (μM mg^{-1} min^{-1})	Recovery (%)	Purity
Crude extract	196.70	6332.58	53.66	100.00	1.00
$(NH_4)_2SO_4$ fraction	182.24	5364.72	49.06	84.72	0.91
Phenyl Sepharose	35.60	3940.44	184.46	62.22	3.44
DEAE Sepharose	6.41	1399.92	363.92	22.11	6.78

3. Results

3.1. Purification of Gal-6-sulfurylase

The Gal-6-sulfurylase from *E. striatum* was purified following a combination of ammonium sulfate precipitation, hydrophobic interaction chromatography and ion exchange chromatography. Results from a typical Gal-6-sulfurylase purification experiment are presented in Table 1. About 85% of the enzyme activity was precipitated with 80% saturation of ammonium sulfate. After Phenyl Sepharose 6FF chromatography, the fraction of 22–24% saturation of ammonium sulfate recovered most (about 62%) of Gal-6-sulfurylase activity and removed almost 82% of protein (Fig. 1a). Most red phycoerythrin pigments were removed at this step according to SDS–PAGE (Fig. 2). Further purification was achieved by subjecting the pooled enzyme fraction to a DEAE Sepharose CL-6B column (Fig. 1b). In this step, fractions eluted by 350–600 mM NaCl in 50 mM Tris–HCl buffer resulted in a 6.78-fold purity with a yield of 3.26% and a specific activity of 363.92 μM/mg protein as compared to the crude extract.

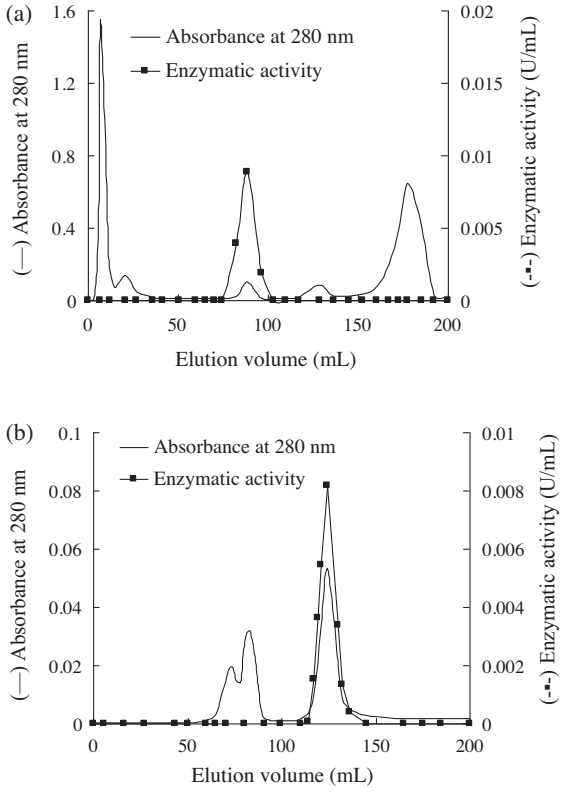

Fig. 1. Purification of Gal-6-sulfurylase by Phenyl Sepharose 6FF (a), and DEAE Sepharose CL-6B (b); *Solid line*, protein absorbance at 280 nm; *Filled square*, Gal-6-sulfurylase activities determined by HPAEC using μ-carrageenan as a substrate.

3.2. Properties of Gal-6-sulfurylase

The samples of each stage of purification were analyzed by SDS–PAGE (Fig. 2). The purified Gal-6-sulfurylase revealed a single band indicating that the enzyme was purified to apparent homogeneity. The molecular weight of the purified Gal-6-sulfurylase was estimated to be 65 kDa, which is similar to the Gal-2,6-sulfurylaseIfrom *Chondrus crispus* (65 kDa; Genicot-Joncour et al., 2009). The results of SDS–PAGE revealed monomeric protein to be the active conformation of the enzyme.

The pH and temperature dependence of the purified enzyme is shown in Fig. 3. Enzyme activity for removal of sulfate esters in carrageenan was determined by analyzing the content of sulfate released in the assay mixture. The optimal pH of Gal-6-sulfurylase was 7.0, though the enzyme activity showed a broad pH range from pH 6.0 to 9.0 (Fig. 3a). Activity at pH 6.0 and 9.0 was 84% and 80%, respectively, of maximum activity. The enzyme activity against carrageenan was measured at different temperatures at pH 7.0 (Fig. 3b). Enzyme activity gradually increased in the temperature range from 20 to 40 °C and optimum at 40 °C. The enzyme activity reduced to almost half of the optimum at 15 °C and 55 °C. The enzyme activity was completely absent at temperatures above 60 °C due to thermal denaturation of the enzyme. K_m and V_{max} of the purified Gal-6-sulfurylase were determined

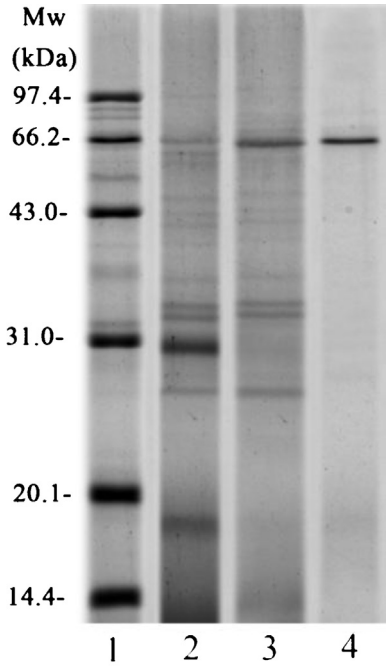

Fig. 2. SDS–PAGE of the intermediate fractions and the purified Gal-6-sulfurylase from *Eucheuma striatum*. *Lane 1*, Protein marker with molecular weight ranging from 97.4 to 14.4 kDa (14.4 kDa, hen egg white lysozyme; 20.1 kDa, trypsin inhibitor; 31 kDa, bovine carbonic anhydrase; 43 kDa, rabbit actin, 66.2 kDa, BSA and 97.4 kDa, rabbit phosphorylase B); *Lane 2*, 80% $(NH_4)_2SO_4$ fraction; *Lane 3*, Phenyl Sepharose 6FF fraction; *Lane 4*, DEAE Sepharose CL-6B fraction.

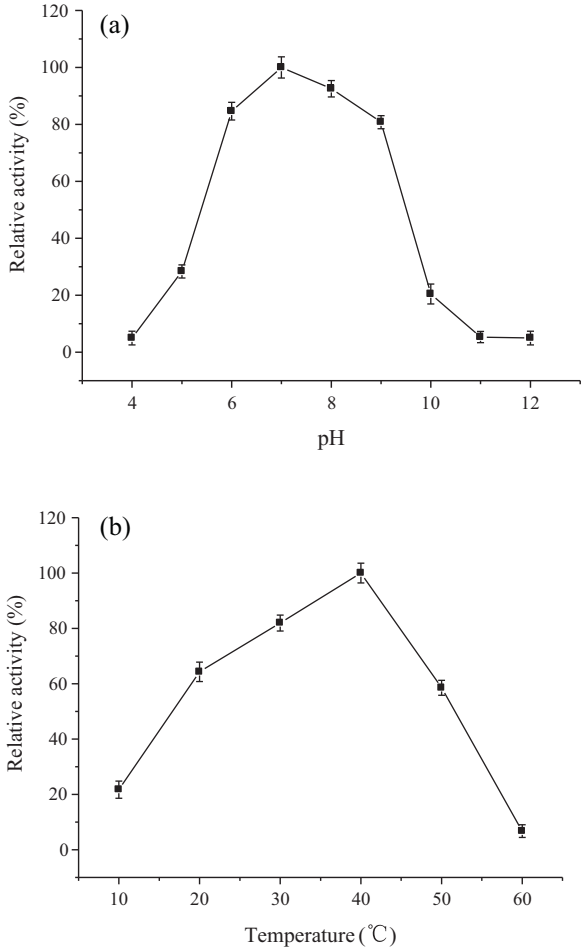

Fig. 3. Effects of pH (a) and temperature (b) on Gal-6-sulfurylase activity using μ-carrageenan as a substrate.

by linear regression, plotting the inverse enzyme activity against the inverse of substrate concentration (Fig. 4). The apparent K_m and V_{max} of Gal-6-sulfurylase were estimated to be 4.31 mM and 0.17 mM min^{-1} respectively at pH 7.0 and 40 °C.

The activity of Gal-6-sulfurylase was investigated in the presence of various metal ions and two inhibitors, which were added at the final concentration of 5 mM. As shown in Table 2, among the metal ions Mg^{2+}, Na^+, K^+, Ba^{2+}, and Mn^{2+} inhibited slightly the enzyme activity, while Hg^{2+} and Pb^{2+} reduced significantly the

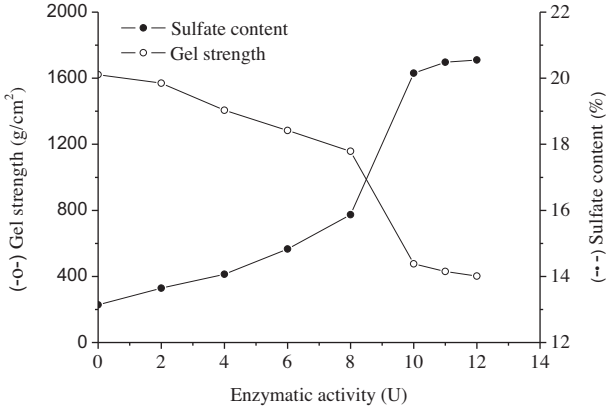

Fig. 4. Effect of Gal-6-sulfurylase activity on sulfate content and gel strength of the carrageenan.

Table 2
Effect of additives on Gal-6-sulfurylase from *Eucheuma striatum*.

Additive	Residual activity (%)
Control	100
Ca^{2+}	103.76 ± 3.24
Mg^{2+}	90.62 ± 2.69
Na^+	65.30 ± 1.94
K^+	79.13 ± 1.75
Ba^{2+}	75.29 ± 1.32
Co^{2+}	102.58 ± 4.26
Mn^{2+}	67.49 ± 3.53
Hg^{2+}	20.41 ± 0.31
Pb^{2+}	33.54 ± 0.47
Zn^{2+}	ND
Cu^{2+}	ND
SDS	98.62 ± 1.2
EDTA	97.08 ± 2.4

activity by 80% and 67%, respectively. The metal ions Zn^{2+}, and Cu^{2+} completely inhibited the activity, while no appreciable change in the activity was observed in the presence of Ca^{2+} and Co^{2+}. The purified Gal-6-sulfurylase was neither inhibited by EDTA nor activated by any metal ion, suggesting that it is not a metalloenzyme. The presence of 5 mM SDS had little effect on enzyme activity, suggesting that it is a stable monomer.

3.3. Effect of Gal-6-sulfurylase activity on sulfate content and gel strength of the carrageenan

Desulfated carrageenan was prepared by reaction with 0–10 U of purified Gal-6-sulfurylase under optimum experimental conditions. As shown in Fig. 4, enzymatic treatment gradually decreased the sulfate content, as well as, increased the gel strength of carrageenan when subjected to 2–10 U of Gal-6-sulfurylase, while no appreciable change in these parameters observed when enzyme concentration was >10 U. The sulfate content of carrageenan decreased remarkably from 20.1% to 14.4% by reaction with 10 U of Gal-6-sulfurylase that corresponded to reduction of about 28.5%. The enzymatic desulfation improved the gel strength as it enhanced its value from 228.4 g/cm^2 in control carrageenan to 1630.2 g/cm^2 in 10 U enzyme treated carrageenan, contributing to almost 7.1-fold increase. Carrageenan samples under different enzyme activities showed a good negative correlation ($r = -0.9562$) between the sulfate content and gel strength: lower sulfate content raises the gel strength.

3.4. ^{1}H NMR spectra

Neither the release of sulfate nor the increase in gel strength definitely proves the formation of the 3,6-anhydro ring. Nonetheless, Gal-6-sulfurylase activities were unambiguously characterized by ^{1}H NMR experiments performed with the purified protein. The κ-carrabiose moieties (DA-H1) were characterized by the signal of the anomeric proton resonating at 5.11 ppm (Van de Velde, Knutsen, Usov, Rollema, & Cerezo, 2002). Signals at 5.52, 5.32 and 5.26 ppm corresponded to the anomeric proton of ν-carrabiose moieties (D2S, 6S-H1), ι-carrabiose moieties (DA2S-H1) and μ-carrabiose moieties (D6S-H1), respectively. By integrating the anomeric signal, the composition of control carrageenan in κ-, μ-, ι-, and ν-carrabiose moieties was about 73.8%, 11.2%, 14.8%, and 0.1%, respectively (Fig. 5). The enzyme treated carrageenan (10U) was devoid of μ-carrabiose and contained κ-, ι-, ν-carrabiose ratio of about 85.1%, 14.8%, and 0.1%, respectively (Fig. 5). Using the ι-carrabiose moieties as an internal reference, the decrease in intensity of the D6S-H1 signal was equal to the increase in the DA-H1 signal, thus demonstrating that μ-carrabiose moieties were converted into κ-carrabiose moieties. It should be noted that the

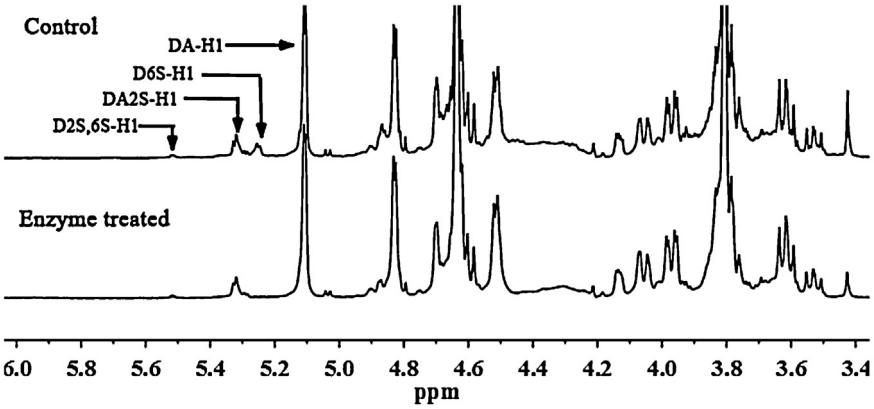

Fig. 5. ^{1}H NMR spectra of control and enzyme treated carrageenans.

intensity of anomeric signal of ν-carrabiose remained constant throughout the incubation experiments, highlighting the specificity of Gal-6-sulfurylase for μ-carrabiose moieties.

4. Discussion

The carrageenan biosynthesis pathway is not fully understood. It is usually accepted that the last step consists the formation of 3,6-anhydro ring found in κ- and ι-carrageenans through the enzymatic conversion of D-galactose-6-sulfate or D-galactose-2,6-disulfate occurring in μ- and ν-carrageenan, respectively. The corresponding enzyme was found firstly in *Porphyra umbilicalis* (Rees, 1961a, 1961b) containing porphyran, and then a few similar studies in several algae containing carrageenans, namely, in *Gigartina stellata* (Lawson & Rees, 1970), *C. crispus* (Genicot-Joncour et al., 2009; Wong & Craigie, 1978), *Calliblepharis jubata* (Zinoun, Diouris, Potin, Floc'h, & Deslandes, 1997) and in *Gracilaria dura* (Shukla, Kumar, Prasad, Reddy, & Jha, 2011) containing agar were reported. The present study for the first time reports the purification of the enzyme from *E. striatum*, which is able to catalyze the desulfation and the cyclization of α-D-Gal-6-sulfate (μ-carrabiose unit) to form α-D-(3,6)-anhydro-Gal (κ-carrabiose unit). Our investigations unambiguously demonstrated that this desulfation event is reliant on the enzymatic reaction that is carried out by single protein. In addition, the purified enzyme was specific to μ-carrageenan and was inactive on ν-carrageenan. This observation is compatible with the nucleophilic substitution reactions proposed for the formation of anhydro ring in alkaline solutions (Ciancia, Matulewicz, & Cerezo, 1997; Viana et al., 2004). Consequently, the term "sulfohydrolase" used for the corresponding enzyme in most of the cited publications seems to be inappropriate, since the reaction is not hydrolysis, but elimination of sulfate. Genicot-Joncour et al. (2009) believes the enzyme belongs to transferase-type enzyme and then named D-Gal-6-sulfurylase (systematic name: L-Gal-6-sulfate: alkyltransferase [cyclizing], EC 2.5.1.5). This terminology is used in the present study.

The formation of 3,6-anhydro galactose residues from galactose 6-sulfate during the biosynthesis of galactans is accompanied by the release of sulfate. Consequently, the purification protocol of the Gal-6-sulfurylase was mainly based on their ability to catalyze the release of sulfate, which was assayed by HPAEC. The specific activity of crude extracts was low and this may be due to amounts of anionic polysaccharides with affinity for protein binding liberated into the buffer during enzyme extraction. This problem was partially overcome by using KCl in the isolation medium to suppress the extraction of κ-carrageenan, and to reduce the viscosity of the solution. A relatively high pH was employed in an attempt to maximize the solubility of the protein and minimize its binding to the polysaccharides. The conversion of μ-carrabiose moieties into κ-carrabiose moieties by purified Gal-6-sulfurylase was slow. Under the experimental conditions described, conversion kinetics exhibited a linear rate of sulfate release for incubations of up to 6 h. Our results are in agreement with the previous report from the *C. crispus* where the activity is linear for at least 4 h (Wong & Craigie, 1978). In an attempt to reduce these conversion times, we assayed other incubation conditions by varying enzyme and substrate concentrations. Increasing the enzyme concentration resulted in an increase of Gal-6-sulfurylase activity. This limit, however, could not be passed by the addition of more enzyme (>20 μg). Moreover, higher amounts of carrageenan (>0.7%, w/v) dramatically increased the viscosity of the medium and, as a consequence, affected enzyme efficiency.

The Gal-6-sulfurylase purified in the present study has an optimum pH of 7.0, which similar to the earlier report from the *C. jubata* where the optimum activity of enzyme being at pH 7.1 (Zinoun et al., 1997). On the contrary, the enzyme from *C. crispus* has exhibited the maximum activity at 6.5 (Wong & Craigie, 1978). The optimum temperature of the purified enzyme was 40 °C, which is a little lower than that reported for Gal-2,6-sulfurylase from *C. crispus* (48 °C) (Genicot-Joncour et al., 2009), but higher than that reported for sulfohydrolase from *Gracilaria dura* (35 °C) (Shukla et al., 2011). After incubation with Gal-6-sulfurylase, the proportion of sulfated esters released varied among 1%–30% of the total. Similar observation was made by Zinoun et al. (1997) that less than 20% of the labeled ^{35}SO2- 4 was liberated from the labeled carrageenan precursors. The failure to achieve removal of more than 50% of the sulfated groups might be due to there maining ester's being combined in some other linkage.

5. Conclusions

D-Gal-6-sulfurylase extracted from *E. striatum* was purified and characterized. As shown in the result of SDS–PAGE, the purification of Gal-6-sulfurylase was first achieved to homogeneity. In addition, enzyme treatment could obviously decrease the sulfate content and improve the gel strength of κ-carrageenan. Therefore, the Gal-6-sulfurylase purified in this study could be applied as an alternative for the commonly available alkaline treatment to improve the gel strength of κ-carrageenan. With this in mind, we have initiated efforts to clone the gene encoding Gal-6-sulfurylase to allow its commercial production, and to facilitate its application in the production of κ-carrageenan.

Acknowledgements

This work was partly supported by Research Fund for the Doctoral Program of Higher Education (CXZZ12_0761), and Doctor Candidate Foundation of Jiangnan University.

References

Al-Alawi, A. A., Al-Marhubi, I. M., Al-Belushi, M. S. M., & Soussi, B. (2011). Characterization of carrageenan extracted from *Hypnea bryoides* in Oman. *Marine Biotechnology*, *13*, 893–899.

Anderson, N. S., Campbell, J. W., Harding, M. M., Rees, D. A., & Samuel, J. W. B. (1969). X-ray diffraction studies of polysaccharide sulphates: Double helix models for κ- and ι-carrageenans. *Journal of Molecular Biology*, *45*(1), 85–97.

Bellion, C., Brigand, G., Prome, J. C., Welti, D., & Bociek, S. (1983). Identification and characterization of biological precursors of carrageenans by C-13 NMR spectroscopy. *Carbohydrate Research*, *119*, 31–48.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254.

Bravo, R., Arimon, M., Valle-Delgado, J. J., Garcia, R., & Durany, N. (2008). Sulfated polysaccharides promote the assembly of amyloid $\beta_{1\text{-}42}$ peptide into stable fibrils of reduced cytotoxicity. *Journal of Biological Chemistry*, *283*(47), 32471–32483.

Ciancia, M., Matulewicz, M. A. C., & Cerezo, A. S. (1997). Alkaline modification of carrageenans. Part III. Use of mild alkaline media and high ionic strengths. *Carbohydrate Polymers*, *32*(3–4), 293–295.

Doyle, J. P., Giannouli, P., Rudolph, B., & Morris, E. R. (2010). Preparation, authentication, rheology and conformation of theta carrageenan. *Carbohydrate Polymers*, *80*(3), 648–654.

Freile-Pelegrín, Y., & Robledo, D. (2007). Carrageenan of *Eucheuma isiforme* (Solieriaceae, Rhodophyta) from Nicaragua. *Journal of Applied Phycology*, *20*(5), 87–91.

Freile-Pelegrín, Y., Robledo, D., & Azamar, J. A. (2006). Carrageenan of *Eucheuma isiforme* (Solieriaceae, Rhodophyta) from Yucatán, Mexico. I. Effect of extraction conditions. *Botanica Marina*, *49*(1), 65–71.

Genicot-Joncour, S., Poinas, A., Richard, O., Potin, P., Rudolph, B., Kloareg, B., et al. (2009). The cyclization of the 3,6-anhydro-galactose ring of ι-carrageenan is catalyzed by two D-galactose-2,6-sulfurylases in the red alga *Chondrus crispus*. *Plant Physiology*, *151*, 1609–1616.

Huang, M., Kennedy, J. F., Li, B., Xu, X., & Xie, B. J. (2007). Characters of rice starch gel modified by gellan, carrageenan, and glucomannan: A texture profile analysis study. *Carbohydrate Polymers*, *69*(3), 411–418.

Jouanneau, D., Guibet, M., Boulenguer, P., Mazoyer, J., Smietana, M., & Helbert, W. (2010). New insights into the structure of hybrid κ-/μ-carrageenan and its alkaline conversion. *Food Hydrocolloids*, *24*(4), 452–461.

Knutsen, S. H., Myslabodski, D. E., Larsen, B., & Usov, A. I. (1994). A modified system of nomenclature for red algal galactans. *Botanica Marina*, *37*, 163–169.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680–685.

Lawson, C. J., & Rees, D. A. (1970). An enzyme for the metabolic control of polysaccharide conformation and function. *Nature*, *227*, 392–393.

Mckinnon, A. A., Rees, D. A., & Williamson, F. B. (1969). Coil to double helix transition for a polysaccharide. *Journal of Chemical Society D*, *674*, 701–702.

Rees, D. A. (1961a). Enzymic desulphation of porphyran. *Biochemical Journal*, *80*, 449–453.

Rees, D. A. (1961b). Enzymic synthesis of 3:6-anhydro-L-galactose within porphyran from L-galactose-6-sulphate units. *Biochemical Journal*, *81*, 347–352.

Rees, D. A. (1970). Structure, conformation, and mechanism in the formation of polysaccharide gels and networks. *Advances in Carbohydrate Chemistry and Biochemistry*, *24*, 267–332.

Rees, D. A. (1972). Shapely polysaccharides. *Biochemical Journal*, *126*(2), 257–273.

Rees, D. A., Steele, I. W., & Williamson, F. B. (1969). Conformational analysis of polysaccharides. III. The relation between stereochemistry and properties of some natural polysaccharide sulfates. *Journal of Polymer Science Part C: Polymer Symposia*, *28*(1), 261–276.

Shukla, M. K., Kumar, M., Prasad, K., Reddy, C. R. K., & Jha, B. (2011). Partial characterization of sulfohydrolase from *Gracilaria dura* and evaluation of its potential application in improvement of the agar quality. *Carbohydrate Polymers*, *85*(1), 157–163.

Usov, A. I. (1998). Structural analysis of red seaweed galactans of agar and carrageenan groups. *Food Hydrocolloids*, *12*(3), 301–308.

Usov, A. I. (2011). Polysaccharides of the red algae. *Advances in Carbohydrate Chemistry and Biochemistry*, *65*, 115–217.

Van de Velde, I. F., & De Ruiter, G. A. (2002). In E. J. Vandamme, S. De Baets, & A. Steinbuchel (Eds.), *Carrageenan* (pp. 254–274). Weinheim, Germany: Wiley-VCH.

Van de Velde, F., Knutsen, S. H., Usov, A. I., Rollema, H. S., & Cerezo, A. S. (2002). ^{1}H and ^{13}C high resolution NMR spectros copy of carrageenans: application in research and industry. *Trends in Food Science & Technology*, *13*, 73–92.

Verma, B. C., Swaminathan, K., & Sud, K. C. (1977). An improved turbidimetric procedure for the determination of sulphate in plants and soils. *Talanta*, *24*(1), 49–50.

Viana, A. G., Noseda, M. D., Duarte, M. E. R., & Cerezo, A. S. (2004). Alkali modification of carrageenans. Part V. The iota–nu hybrid carrageenan from *Eucheuma denticulatum* and its cyclization to iota-carrageenan. *Carbohydrate Polymers*, *58*(4), 455–460.

Villanueva, R. D., Hilliou, L., & Sousa-Pinto, I. (2009). Postharvest culture in the dark: An eco-friendly alternative to alkali treatment for enhancing the gel quality of κ/ι-hybrid carrageenan from *Chondrus crispus* (Gigartinales, Rhodophyta). *Bioresource Technology*, *100*(9), 2633–2638.

Wong, K. F., & Craigie, J. S. (1978). Sulfohydrolase activity and carrageenan biosynthesis in *Chondrus crispus* (Rhodophyceae). *Plant Physiology*, *61*, 663–666.

Yaphe, W., & Duckworth, M. (1972). The relationship between structures and biological properties of agars. In *Proceedings of the International Seaweed Symposium vol. 7* (pp. 15–22).

Zinoun, M., Diouris, M., Potin, P., Floc'h, J. Y., & Deslandes, E. (1997). Evidence of sulfohydrolase activity in the red alga *Calliblepharis jubata*. *Botanica Marina*, *40*, 49–53.