



Kappa carrageenan from the red alga *Kappaphycus striatus* cultivated at Vanphong Bay, Vietnam: physicochemical properties and structure

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Abstract

The red alga *Kappaphycus striatus* “sacol” brown strain is an economically important species and extensively cultivated at Vanphong Bay, Vietnam. To elucidate the carrageenan quality, the chemical profile of the alkali-modified carrageenan from this alga was investigated by FT-IR, ¹H and ¹³C NMR spectroscopies, and methods of analysis. The structural components of the polysaccharide mainly consisted of 3,6-anhydro- α -D-galactose and β -D-galactose-4-sulfate. FT-IR and ¹H and ¹³C NMR spectra indicated that the polysaccharide was composed predominantly of kappa carrageenan with a small amount of iota carrageenan and 6-O-methylated carrageenan. The red alga *K. striatus* is good source of carrageenan for application in food or medicine.

Keywords FT-IR · Kappa carrageenan · *Kappaphycus striatus* “sacol” brown strain · Rhodophyta · NMR · Properties

Introduction

Carrageenans are mainly extracted from different species of Rhodophyta cultivated or harvested naturally from the genera *Agardhiella*, *Chondracanthus*, *Chondrus*, *Eucheuma*, *Gigartina*, *Hypnea*, *Iridaea*, *Kappaphycus*, *Sarconema*, and *Solieria* (Zia et al. 2017). *Eucheuma* and *Kappaphycus* are most commonly cultivated across Malaysia and Southeast Asia as a source of carrageenan (Hurtado et al. 2014; Hung et al. 2019). Carrageenans are mainly composed of D-galactose residues linked alternately in 3-linked- β -D-galactopyranose and 4-linked- α -D-galactopyranose units and are classified according to the degree of substitution that occurs on their free hydroxyl groups. There are three main types of carrageenan, which vary in their degree of sulfation.

Kappa carrageenan is composed of alternating 3-linked β -D-galactose-4-sulfate and 4-linked 3,6-anhydro- α -D-galactopyranose having one sulfate group per disaccharide repeating unit. Iota carrageenan is composed of alternating 3-linked β -D-galactose-4-sulfate and 4-linked 3,6-anhydro- α -D-galactose-2-sulfate having two sulfate groups per disaccharide repeating unit. Lambda carrageenan has three sulfate groups per disaccharide unit but do not exhibit any 3,6-anhydride bridges contrary to kappa and iota carrageenan. The main differences which effect on the properties of different carrageenans are the number and position of ester sulfate groups and the content of 3,6-anhydro-galactose (Campo et al. 2009). Carrageenans are classically used as agents for the induction of experimental inflammatory pain and have also been shown to have several potential pharmaceutical properties including antitumor, immunomodulatory, antihyperlipidemic, and anticoagulant activities (Campo et al. 2009; Bixler 2017). Recently, carrageenans and their derivatives have been used in controlled drug delivery systems, as well as enhancing dissolution rates of poorly soluble substances (Li et al. 2014; Yegappan et al. 2018).

The red alga, carrageenophyte, *Kappaphycus striatus* is an economically important food species and extensively cultivated in Vietnam as a source of not only carrageenan but also bioactive compounds for biochemical and medicinal application (Hung et al. 2011, 2015, 2019; Viet et al. 2019; Hung and Trinh 2021). However, little is known about the properties and

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structure of carrageenans from this alga in Vietnam. Thus, the objective of the present research was to evaluate primarily the properties and structure of kappa carrageenan from the red alga *K. striatus* “sacol” brown strain cultivated at Vanphong Bay, Vietnam, which will provide more valuable information of the carrageenan group from the red algae, carrageenophytes, for applications in food or medicine.

Materials and methods

Materials

The red alga *Kappaphycus striatus* “sacol” brown strain (F. Schmitz) Doty ex P. Silva was collected over a cultivation period of 60-day intervals at Vanphong Bay (120°29' N, 109°10' E) of Vietnam in 2019, brought to the laboratory, washed with tap water to eliminate sand, debris, and epiphytes, dried by sunlight, and kept at -20°C until used.

Extraction of carrageenan

Alkali-modified carrageenan extraction was carried out according to the method of Ohno et al. (1994). Dry alga (20 g) was incubated in 400 mL 6% KOH at 80°C for 2 h. The algae were then washed and extracted in 400 mL distilled water at 90°C for 2 h. Thereafter, the solution was filtrated and the extract was gellified with 0.2% KCl, frozen overnight and then thawed, washed with fresh water at least twice, and dried at 60°C to constant weight.

Prior to determining the chemical composition and structures, the carrageenan sample was repurified by dialysis using a Spectrapor membrane with Mw cut-off 12,000–14,000 Da, against Milli-Q water for 24 h with frequent changes of water. After dialysis, the sample was filtrated by filter (0.45 μm) and precipitated by absolute ethanol, dried at 60°C , and milled into powder.

3,6-Anhydrogalactose content determination

3,6-Anhydrogalactose content in polysaccharide was determined by the resorcinol method of Yaphe and Arsenault (1965) using D-fructose as a standard. Briefly, 2 mL of each fructose solution at various concentrations, polysaccharide (100 $\mu\text{g mL}^{-1}$), and distilled water was transferred to a boiling tube and covered with a glass marble. The tubes were placed in an ice bath, and 10 mL of the resorcinol reagent (9 mL of resorcinol 1.36 mM, 1 mL of acetal 2.78 mM, and 100 mL of concentrated HCl) was added to each tube. The contents were mixed and cooled in an ice bath for at least 3 min. The tubes were placed in a 20°C water bath for 4 min and then heated for 10 min at 80°C . It was then cooled for 1.5 min in an ice bath, and the absorbance was measured at 555 nm. 3,6-

Anhydrogalactose content was multiplied to a value of 1.087. The experiments were carried out in triplicate and given as average values for each test solution.

Sulfate content determination

Sulfate content was determined according to the method of Terho and Hartiala (1972) using Na_2SO_4 as a standard. Briefly, 0.5 mL of each Na_2SO_4 standard solution at various concentrations, polysaccharides (100 $\mu\text{g mL}^{-1}$), and distilled water was pipetted into test tubes and 2.0 mL of absolute ethanol was added to each tube. One milliliter of BaCl_2 buffer (10 mL of acetic acid 2 M, 2 mL of BaCl_2 0.005 M, and 8 mL of NaHCO_3 0.02 M made up to 100 mL with absolute ethanol) and 1.5 mL of sodium rhodizonate solution (5 mg of rhodizonate natri dissolved in 20 mL distilled water made up to 100 mL with absolute ethanol) were added to each tube and shaken well. The tubes were allowed to stand 10 min in the dark at room temperature, and the absorbance was measured at 520 nm in 30 min. The experiments were carried out in triplicate and are given as average values for each test solution.

Gelling and melting temperature determination

Gelling and melting temperatures were determined according to the method of Craigie and Leigh (1978) using 1.5% carrageenan solution in 0.2% KCl. The gelling temperature was measured by pouring a hot carrageenan solution (10 mL, w/v) into a test tube (diameter 22 mm, height 142 mm). A thermometer (0.1 $^{\circ}\text{C}$ divisions) was inserted into the test tube, and then, the solution was allowed to cool at a rate of $0.5^{\circ}\text{C min}^{-1}$. Glass beads (3.0 mm) were periodically introduced onto the surface of the solution. The gelling temperature was recorded when the bead failed to sink through the gel. The gelled sample was equilibrated overnight at 20.0°C , and melting temperature was measured by placing a glass bead on the surface of the gel in a test tube clamped in a water bath and warmed at the rate of $0.5^{\circ}\text{C min}^{-1}$. Melting temperature was recorded when the glass bead sank to the bottom. The experiments were carried out in triplicate and are given as average values for each test solution.

Viscosity-average molecular weight and gel strength determination

The viscosity-average molecular weight (MW) was obtained from viscometry and extrapolation. The sample was dissolved at an initial concentration of 0.1% in 0.1 M NaCl. Viscosity measurements at different diluted concentrations of carrageenan in 0.1 M NaCl were done at $25 \pm 0.1^{\circ}\text{C}$ in Ubbelohde viscometers. From efflux time of polymer solution (t) and that

of solvent 0.1 M NaCl (t_o), relative viscosity $rel = t/t_o$ was obtained. Specific viscosity was calculated from the relationship $sp = rel - 1$. Reduced viscosity for a set of polymer solutions was calculated at different concentrations (g mL⁻¹). Intrinsic viscosity was then obtained from common ordinate intercept on extrapolation of plots of reduced viscosity versus concentration (Pal et al. 2008). MW was calculated according to the Mark–Houwink equation for kappa carrageenan in 0.1 M NaCl at 25°C:

$$[\eta] = K \cdot M_w^\alpha,$$

where $K = 8.84 \times 10^{-3}$ and $\alpha = 0.86$ (Vreeman et al. 1980).

Gel strength was measured according to the method of Ohno et al. (1994). 1.5% solution (1.5 g carrageenan in 100 mL of 0.2% KCl) was heated at 90°C for 30 min. After that, solutions were poured into petri disks (diameter: 94 mm and disk height: 14 mm) and kept at room temperature for 24 h. To measure gel strength, the gel was preincubated at 20°C for approximately 2 h and measured on a rheometer (CR-500DX; Sun Scientific, Japan) with a diameter plunger: 12 mm, gel thickness: 10 mm, and table speed: 60 mm min⁻¹. The assays were carried out in triplicate and are given as average values.

FT-IR spectroscopy

Films were prepared by drying 5 mL (5 mg mL⁻¹) of sample solutions on a polyethylene surface at 60°C, mixed with KBr, pressed into a homogenous pellet and put into the sample holder of the FT-IR spectrometer. The spectral bands were recorded in the range of 4000 to 500 cm⁻¹ on a Bruker mode ALPHA spectrometer.

¹H and ¹³C nuclear magnetic resonance (NMR) spectroscopies

Carrageenan (10 mg) was dissolved in 1 mL of D₂O + 1% CD₃COOD, and 10 mM of 2,2-dimethyl-2-silapentane-3,3,4,4,5,5-d₆-5-sulfonate (DSS) was used as an internal standard. The sample was loaded into a 5 mm O.D. nuclear magnetic resonance tube. The ¹H NMR spectrum was recorded at 70°C and operated at 500.24 MHz on a Bruker Avance 500 III spectrometer. Sixty-four scans were accumulated at a pulse angle of 90° with an interpulse delay of 6.50 s and an

acquisition time of 2.04 s. The ¹³C NMR spectrum was measured at 70°C and operated at 125.78 MHz, accumulated over 20,000 scans with an interpulse delay of 6.50 s and an acquisition time of 1.04 s. Chemical shifts were corrected relative to the internal standard of DSS ($\delta = 0.000$ ppm for both ¹H and ¹³C NMR) (Van de Velde et al. 2004).

Results and discussion

Chemical composition and properties of polysaccharide

Carrageenan yield, 3,6-anhydrogalactose and sulfate contents, gelling and melting temperatures, gel strength, and viscosity-average molecular weight of carrageenan are shown in Table 1. The data are in range of those reported for the carrageenans from the genera *Kappaphycus* and *Eucheuma* with carrageenan yields from 18.8 to 58.3%, 3,6-anhydrogalactose content from 15 to 40%, ester sulfate from 23.1 to 34.5%, gelling temperature from 32.7 to 34.5°C, melting temperature from 52 to 56°C, molecular weight from 100 to 700 kDa, and gel strength from 505 to 1158 g cm⁻² (Santos 1989; Ohno et al. 1996; Mendoza et al. 2002; Hayashi et al. 2007; Hurtado et al. 2008; Periyasamy et al. 2014; Hung et al. 2019).

The differences in the yields, molecular weights, chemical compositions, and qualities between carrageenan samples can be attributed to the extraction methodology used in each study and the time of algal harvest (Periyasamy et al. 2014; Zuldin et al. 2016; Hung et al. 2009, 2019; Viet et al. 2019).

FT-IR analysis of polysaccharide

The FT-IR spectral profile of polysaccharide revealed the typical absorption bands related to sulfated polysaccharide of kappa carrageenan (Fig. 1). The absorption bands at 930, 850, and 1260 cm⁻¹ proved the existence of C–O–C of 3,6-anhydro-D-galactose, C₄–O–S stretching, and O=S=O symmetric vibrations of β-D-galactose-4-sulfate, respectively, for kappa carrageenans (Santos 1989; Mendoza et al. 2002; Aguilan et al. 2003; Chiovitti et al. 2008; Arman and Qader 2012; Souza et al. 2018). The band at 1640 cm⁻¹ was related to the structural water deformation (Kacurakova and Wilson 2001). The presence of bands at 1159, 1070, 2934, and 3411

Table 1 Yield and composition of carrageenan extracted from *K. striatus* “sacol” brown strain

Yield (% w/w) ^a	3,6-AGb (% w/w) ^c	Sulfate (% w/w) ^c	Gelling temp. (°C)	Melting temp. (°C)	Gel strength (g cm ⁻²)	MW (kDa) ^d
36.6 ± 3.5	32.4 ± 0.5	24.3 ± 0.8	34.4 ± 0.9	55.6 ± 1.6	715 ± 45	367 ± 21

^a Based on the dry weight of the alga; ^b AG, anhydrogalactose; ^c calculated on the carrageenan fraction; ^d MW, viscosity-average molecular weight; data are presented as the mean ± SEM ($n = 3$)

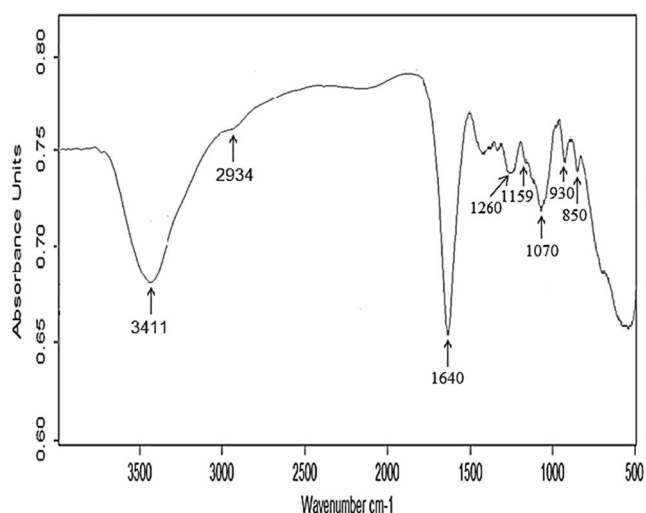


Fig. 1 FT-IR spectrum of carrageenan extracted from *Kappaphycus striatus* “sacol” brown strain

cm^{-1} (Fig. 1) was assigned to the bridge $-\text{O}$ stretch, stretch vibrations of $\text{C}-\text{O}$, $\text{C}-\text{H}$, and $\text{O}-\text{H}$ existing, respectively (Arman and Qader 2012; Souza et al. 2018).

^1H and ^{13}C NMR spectroscopies

The signal assignments of the ^1H NMR spectrum (Fig. 2a) of polysaccharide from *K. striatus* “sacol” brown strain are summarized in Table 2. The signals at 5.10 and 4.64 ppm indicated the α -anomeric protons of 3,6-anhydro- α -D-galactose and β -D-galactose-4-sulfate as reported for kappa carrageenans (Aguilan et al. 2003; Villanueva and Montano 2003; Campo et al. 2009; Cosenza et al. 2014). Additional proton signals at 3.62, 3.98, 4.84, 3.80, and 3.97 ppm of β -D-galactose-4-sulfate and at 4.14, 4.52, 4.53, 4.61, and 4.31 ppm of 3,6-anhydro- α -D-galactose residues matched the ^1H NMR assignments for kappa carrageenans (Aguilan et al. 2003; Campo et al. 2009; Cosenza et al. 2014). In addition to the methyl signal, a signal is observed at 3.43 ppm in the ^1H NMR spectrum, indicated methylation in the C-6 position of D-galactose-4-sulfate of kappa carrageenans (Mendoza et al.

2002; Aguilan et al. 2003; Villanueva and Montano 2003). Moreover, a weak signal at 5.31 ppm (Fig. 2a) was assigned to the anomeric proton of 4-linked α -D-galactose-6-sulfate residues of the biological precursor to kappa carrageenans (Mendoza et al. 2002; Aguilan et al. 2003). The weak signal at 5.37 ppm was assigned to the α -anomeric proton at C-2 of 3,6-anhydro- α -D-galactose-2-sulfate (Mendoza et al. 2002; Aguilan et al. 2003), indicating the presence of iota carrageenan in *K. striatus* carrageenan component.

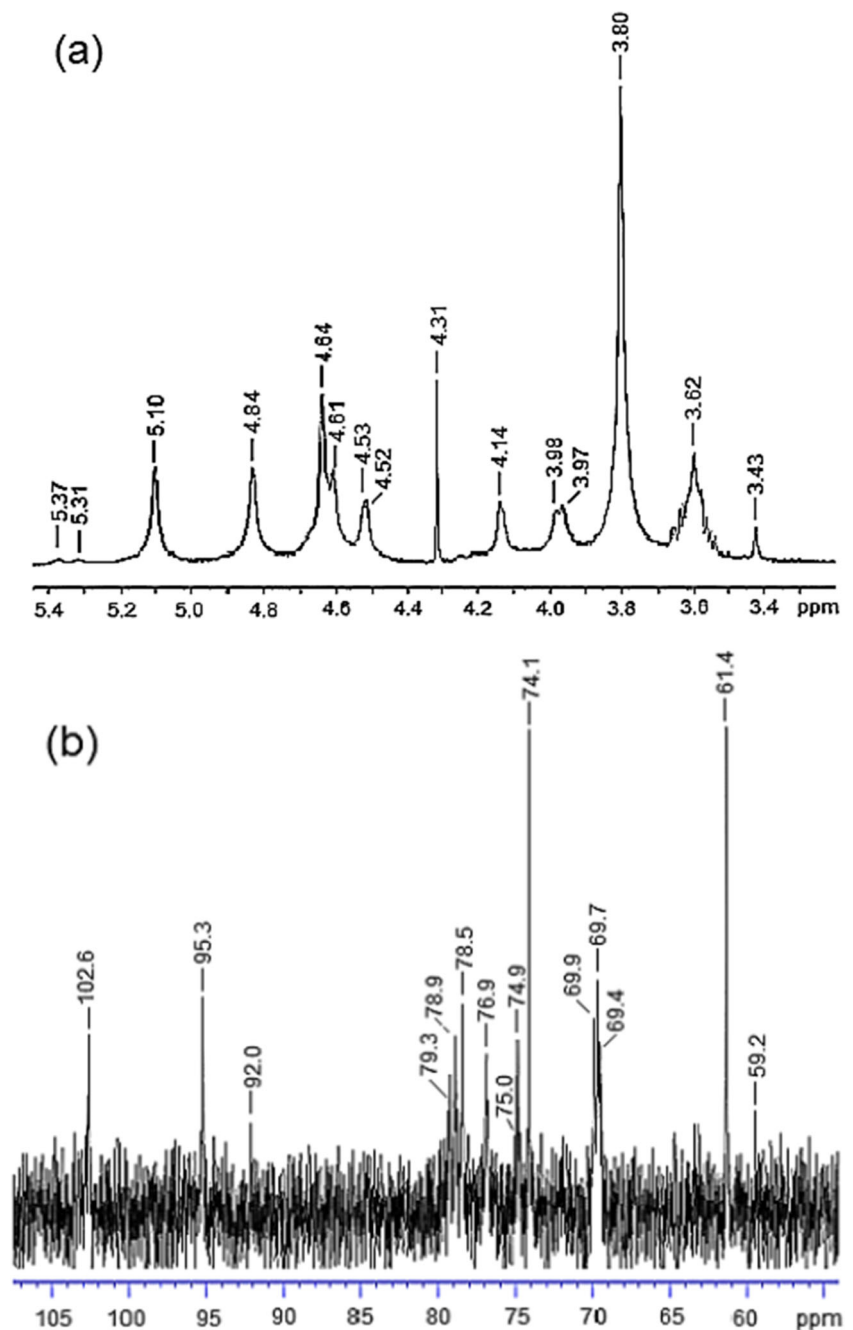
The chemical shifts in the ^{13}C NMR spectrum of polysaccharide (Fig. 2b) corresponded to the signals of the carbon atoms in the disaccharide repeating unit (Table 2) (Aguilan et al. 2003; Chiovitti et al. 2008; Cosenza et al. 2014). The major signals at 102.6 and 95.3 ppm were assigned to the anomeric carbons of β -D-galactose-4-sulfate and 3,6-anhydro- α -D-galactose, respectively (Mendoza et al. 2002; Aguilan et al. 2003; Villanueva and Montano 2003; Chiovitti et al. 2008; Cosenza et al. 2014). On the other hand, the carbon signals at 69.7, 78.9, 74.1, 74.9, and 61.4 ppm of D-galactose-4-sulfate and at 69.9, 79.3, 78.5, 76.9, and 69.4 ppm of 3,6-anhydro-D-galactose corresponded to those of C-2, C-3, C-4, C-5, and C-6 in the ^{13}C NMR spectrum of kappa carrageenans (Aguilan et al. 2003; Chiovitti et al. 2008; Cosenza et al. 2014). A signal at 59.2 ppm is seen in this spectrum, which was assigned to the methoxyl group on C-6 of the β -D-galactose-4-sulfate (Estevez et al. 2000; Mendoza et al. 2002; Aguilan et al. 2003). It has been reported that the carrageenan extracts from *Kappaphycus* were composed predominantly of kappa carrageenan with trace amounts of iota carrageenan. In fact, the signals at 92.0 and 75 ppm in the ^{13}C NMR spectrum (Fig. 2b) were assigned to the anomeric carbons for C-1 and C-2 of 3,6-anhydro- α -D-galactose-2-sulfate (Mendoza et al. 2002; Aguilan et al. 2003), which showed the presence of iota carrageenan in carrageenan component of this alga. The chemical shift values of NMR spectra in this study were identical to those of kappa carrageenan and are in

Table 2 Chemical shifts in the NMR spectra of carrageenan extracted from *K. striatus* “sacol” brown strain

Repeating units	^1H NMR						
	Sugar	H-1	H-2	H-3	H-4	H-5	H-6
D-Galactose-4-sulfate	3-linked	4.64	3.62	3.98	4.84	3.80	3.97
3,6-Anhydro-D-galactose	4-linked	5.10	4.14	4.52	4.53	4.61	4.31
Repeating units	^{13}C NMR						
	Sugar	C-1	C-2	C-3	C-4	C-5	C-6
D-Galactose-4-sulfate	3-linked	102.6	69.7	78.9	74.1	74.9	61.4
3,6-Anhydro-D-galactose	4-linked	95.3	69.9	79.3	78.3	76.9	69.4

H1–6 shows the proton numberings; C1–6 shows the carbon numberings

Fig. 2 **a** ^1H and **b** ^{13}C NMR spectra of carrageenan extracted from *Kappaphycus striatus* “sacol” brown strain



good agreement with the values reported (Aguilan et al. 2003; Campo et al. 2009).

The polysaccharide from the red alga *K. striatus* “sacol” brown strain is composed predominantly of kappa carrageenan with a small amount of iota carrageenan. The physicochemical properties and structure of kappa carrageenan were similar to those of carrageenans from the red algae, carrageenophytes, reported, indicating that the red alga *K. striatus* may promise to be a good source of carrageenans to apply in food or medicine. However, to obtain a better understanding of

physicochemical properties and chemical compositions of the red alga *K. striatus* cultivated, we need further detailed studies.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10811-021-02415-1>.

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