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Structural and compositional characteristics of hybrid carrageenans from red algae *Chondracanthus chamissoi*

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ABSTRACT

Two polysaccharides CCC and CCH were extracted from red algae *Chondracanthus chamissoi* by cold water and hot water, respectively. Characterization of the structure by chemical and spectroscopic methods showed that CCC was a hybrid carrageenan composed of κ-carrageenan (35%) and ι-carrageenan (43%) along with its precursor μ-carrageenan (22%) and CCH was an ideal κ-carrageenan. Oligosaccharides from CCH and CCC were prepared and their structural sequences determined by ES-CID-MS/MS gave further insight into the structural characteristics of hybrid carrageenans from *C. chamissoi*. Some hybrid oligosaccharides, e.g., κ–κ, κ–μ and κ–ι, were obtained with mild acid hydrolysis of CCC. Then, a regular even-numbered oligosaccharides generated with reductive acid hydrolysis of CCH confirmed it an ideal κ-carrageenan.

1. Introduction

Carrageenans are highly sulfated galactans occurring in the cell walls of red algae (Rhodophyta) with a linear backbone of alternating 3-linked β-D-galactopyranose (G-unit) and 4-linked α-D-galactopyranose (D-unit). Classification of carrageenans is based on the occurrence of 3,6-anhydro form (A unit) in the 4-linked galactose residues and the pattern of sulfation. The most common types of carrageenans are traditionally called κ-, ι- and λ-carrageenan with different biose units of -[G4S-A]-, -[G4S-A2S]- and -[G2S-D2S6S]-, respectively (Knutsen, Myslabodski, Larsen, & Usov, 1997). However, native carrageenans are rarely in their uniformed or ideal form. The diversity of carrageenan is attributed to a mixed combination of different biose units or copolymeric chains. The most classical copolymers of carrageenan are those found in native κ- and ι-carrageenan chains that usually contain components of their biosynthetic precursors, e.g., μ- and ν-carrabiose with the biose unit of -[G4S-D6S]- and -[G4S-D2S6S]-, respectively (Jouanneau, Boulenguer, et al., 2010; Jouanneau, Guibet, et al., 2010). The structural heterogeneity of carrageenan composition, which depends on algal source, life stage, and extraction procedure (Pereira & van de Velde, 2011; Vandevelde, 2008) offers a wide range of physiochemical properties and biological activities, including gelling, thickening abilities (Vandevelde, 2008), antiviral (Lee, Takeshita, Hayashi, & Hayashi, 2011), antitumor activities (Zhou, Sheng, Yao, & Wang, 2006) and immunomodulatory activities (Stephanie, Eric, Sophie, Christian, & Yu, 2010).

Chondracanthus chamissoi is a benthic marine red algae and distributed from Paita, Peru (5°S) to Ancud, Chile (42°S). As an important source of income in Chile, *C. chamissoi* is widely used as raw material by the carrageenan industry and exported to many countries as edible seaweed (Buschmann, Correa, Westermeier, Hernández-González, & Norambuena, 2001). The harvesting of the red algae from natural populations has been and will be restricted by several authorities and thereby, stimulating the research in seaweed production by aquaculture (Bulboa, Macchiavello, Oliveira, & Fonck, 2005). Although earlier report by (Bixler, 1996) has shown polysaccharides from *C. chamissoi* contain κ and ι-carrageenan, their precise chemical structures are still to be elucidated. The aim of this study is structural characterization of two different carrageenans extracted from *C. chamissoi* in Chile.

Abbreviations: ES-MS, electrospray mass spectrometry; CID, collision-induced dissociation; GC–MS, gas chromatography–mass spectrometry; MMB, 4-methylmorpholine borane; CTMS, chlorotrimethylsilane; DP, degree of polymerization; Gal, galactose; anGal, 3,6-anhydrogalactose; A, 4-linked α-3,6-anhydrogalactose; Aol, 4-linked α-3,6-anhydrogalactitol; A2S, 4-linked 2-*O*-sulfated-α-3,6-anhydrogalactose; D, 4-linked α-D-galactopyranose; D6S, 4-linked 6-*O*-sulfated-α-D-galactose; G, 3-linked β-D-galactopyranose; G4S, 3-linked 4-*O*-sulfated-β-D-galactose.

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2. Experimental

2.1. Materials

The red seaweed *C. chamissoi* (Chile) was purchased from Kunshan Yihong Seaweed Co. Ltd. (Jiangsu, China). κ-Carrageenan standard, methylmorpholine-borane (MMB), $NaBD_4$ and ion-exchange resin Amberlite IR120 (H^+ form) were purchased from Sigma (Shanghai, China). Superdex Peptide HR column (1.0 cm × 30 cm) was purchased from Pharmacia Bioscience (Uppsala, Sweden). Fused-silica capillary columns DB-225 (30 m × 0.32 mm × 0.25 μm) and DB-225MS were purchased from J&W Scientific (Folsom, USA).

2.2. Extraction of polysaccharides

Extraction of polysaccharides was carried out as previously described by Yang et al. (2011). Briefly, the power of *C. chamissoi* was treated with 85% ethanol for 3 h at 80 °C (3 times) to remove lipids and the residue was dried. The residue was extracted with cold water for 3 h (3 times). The supernatant was combined, evaporated and precipitated with 4 volumes of ethanol. The precipitated polysaccharide (CCC) was dialyzed (7 kDa MWCO) against water and freeze-dried. The residue was further extracted with hot water at 80 °C using the above mentioned procedures and the polysaccharide CCH was obtained.

2.3. General analysis

Total sugar content was determined by the phenol–sulfuric acid method using galactose as standard (Cuesta, Suarez, Bessio, Ferreira, & Massaldi, 2003). The content of crude protein was determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). The anGal content was determined by the resorcinol method using fructose as standard (Yaphe & Arsenault, 1965). The sulfate content was determined by $BaCl_2$-Gelatin method (Dodgson & Price, 1962).

The purity and relative molecular weight (MW) of polysaccharides were determined by gel filtration chromatography on a PL aquagel OH column eluted with 0.2 mol/L Na_2SO_4 at a flow rate of 0.5 mL/min at 35 °C. The column was calibrated with Dextran standards, and the corrected regression equation was $y = -1.8773x + 24.376$ ($R^2 = 0.9997$).

The monosaccharide composition was determined quantitatively as their peracetylated alditols obtained by reductive hydrolysis followed by acetylation as described by Stevenson and Furneaux (1991). The resulting alditol acetates was analyzed by GC (Agilent HP5890 II, USA) using a fused-silica capillary column DB-225.

2.4. Spectroscopic analysis

FTIR spectra of polysaccharides CCC and CCH prepared as KBr pellets were recorded with a Nicolet Nexus 470 Thermo instrument. The ^{13}C NMR spectra were measured with a JNM-ECP 600 spectrometer at 25 °C. The polysaccharides were dissolved in D_2O and acetone-d_6 was used as an internal standard (Yang et al., 2011).

2.5. Desulfation and methylation

Desulfation of polysaccharides CCC and CCH was operated for three times continuously as described by Nagasawa, Inoue, and Kamata (1977). The sulfate content was determined as 5.6% and 6.7% by $BaCl_2$-Gelatin method, respectively. The desulfated polysaccharides were further purified on a Q-Sepharose FF column to remove small amount of sulfated polysaccharides, and the desulfated polysaccharides dsCCC and dsCCH were used for methylation analysis. Methylation was performed according to previous method by Hakomori (1964). The methylated polysaccharide was hydrolyzed and acetylated. Thereafter, partial methylated alditol acetates were analyzed by GC–MS equipped with a DB-225MS fused-silica capillary column.

2.6. Preparation and purification of oligosaccharides

Mild acid hydrolysis of κ-carrageenan standard (10 mg/mL) was carried out with 0.1 M H_2SO_4 at 60 °C for 1.5 h. Hydrolysis of polysaccharide CCC was extended to 3 h. Reductive hydrolysis of polysaccharide CCH was carried out with addition of 0.2 M MMB at 60 °C for 1.5 h. The reaction was terminated by neutralization with 2 M NaOH before analysis.

For oligosaccharide preparation, the hydrolysates were separated on a Superdex Peptide column eluted with 0.1 mol/L NH_4HCO_3 at a flow rate of 0.1 mL/min, using a refractive index detector (Yang et al., 2009).

2.7. ES-TOF-MS analysis

Negative-ion electrospray mass spectrometry (ES-MS) analysis on Micromass Q-Tof Ultima instruments (Waters, Manchester, UK) was performed for all oligosaccharides sequence analysis (Yu et al., 2006). Nitrogen was used as the desolvation and nebulizer gas at a flow rate of 500 L/h and 50 L/h, respectively. Source temperature was 80 °C and the desolvation temperature was 150 °C. Samples were dissolved in CH_3CN/2 mmol/L NH_4HCO_3 (1:1, v/v), typically at a concentration of 5–10 pmol/μL, of which 5 μL was loop-injected. Mobile phase (CH_3CN/2 mmol/L NH_4HCO_3, 1:1, v/v) was delivered by a syringe pump at a flow rate of 5 μL/min. Capillary voltage was maintained at 3 kV while cone voltage was 150 eV. For CID-MS/MS product-ion scanning, the collision energy was adjusted between 10 and 80 eV.

In order to get the oligosaccharide sequence, the deuterium reduction was carried out with $NaBD_4$ reagent as described by Yu et al. (2006).

3. Result and discussion

3.1. Extraction and composition analysis of polysaccharides

The cold water soluble polysaccharide CCC from *C. chamissoi* was the major component (25.8%). The yield of polysaccharide CCH extracted with 80 °C hot water was lower (5.2%) than that of CCC. Compared with the κ and ι-carrageenan standard, the chemical compositions of the two polysaccharides are listed in Table 1. Polysaccharides CCC and CCH showed a symmetric peak on a PL aquagel OH column, with average molecular weight of 465 kDa and 299 kDa, respectively. GC analysis showed that CCC and CCH mainly contained Gal and anGal. The ratio of Gal to anGal in CCC was 2.7, which was remarkably higher than κ and ι-carrageenan standard. The sulfate content of CCC was higher than κ- but lower than that of ι-carrageenan standard. Based on the GC analysis result and sulfate content, the structure character of CCC was different to ideal κ- or ι-carrageenans, it was a hybrid-sulfated galactan. CCH had a highly similar sulfate content and monosaccharide composition to κ-carrageenan standard.

3.2. FTIR and ^{13}C NMR analysis of polysaccharides

The FTIR spectra (Fig. 1) of CCC and CCH showed characteristic bands at 1260 cm^{-1} (S=O), 1072 cm^{-1} (Gal) and 930 cm^{-1} (anGal), but the absorption strength was different. The region around 800–850 cm^{-1} is used to identify the position of the sulfate

Table 1
Comparison of physicochemical properties of polysaccharides CCC and CCH from *C. chamissoi*.

Sample	SO_4^{2-}(%)	anGal (%)	Gal (%)	Protein (%)	MW (kDa)	Monosaccharide composition (%) Gal:anGal
CCC	27.8	16.2	50.3	1.1	464.7	2.7:1
CCH	23.1	31.8	36.6	0.8	298.8	1.5:1
κ-Carrageenan	23.5	33.6	38.3	0.6	249.1	1.4:1
ι-Carrageenan	30.5	30.7	35.0	0.7	498.3	1.6:1

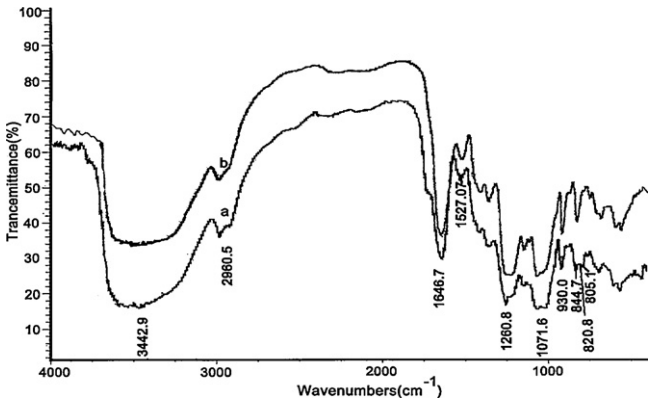

Fig. 1. The FTIR spectra of polysaccharide CCC (a) and CCH (b) from *C. chamissoi*.

group in carrageenan biopolymer, according to previous literatures (Chopin & Whalen, 1993; Pereira, Sousa, Coelho, Amado, & Ribeiro-Claro, 2003; Tojo & Prado, 2003). The broad peak at 845 cm^{-1}, 820 cm^{-1} and 805 cm^{-1} in Fig. 1a suggested the presence of sulfate ester at the C4 of 3-linked β-D-galactopyranose (G4S), C6 of 4-linked α-D-galactopyranose (D6S) and C2 of 4-linked α-D-3,6-anhydrogalactose (A2S), respectively. So, the structure character of CCC was a mixture of κ-and ι-carrageenan along with some potential precursors. Then, the strong bond at 845 cm^{-1} (G4S) in Fig. 1b indicated that the structure of CCH was similar to κ-carrageenan standard.

The ^{13}C NMR resonances of CCC and CCH were assigned and are shown in Fig. 2, by compared with previous literature (van de Velde, Knutsen, Usov, Rollema, & Cerezo, 2002; Vandevelde, Pereira, & Rollema, 2004). The presence of two major anomeric signals of κ-carrageenan at δ 102.6 (C1 of G4S) and δ 94.7 (C1 of A) in Fig. 2b, indicated that CCH was an ideal κ-carrageenan. Apart from the presence of anomeric signals of κ-carrageenan, the spectrum of CCC (Fig. 2a) also showed anomeric signals corresponding

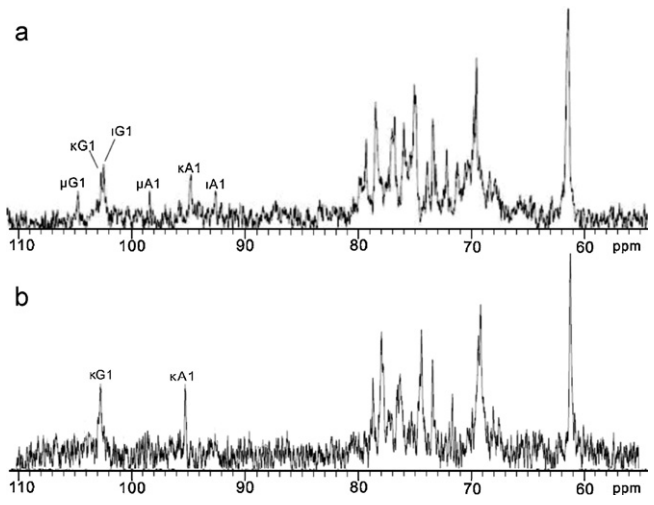

Fig. 2. The ^{13}C NMR spectra of polysaccharide CCC (a) and CCH (b) from *C. chamissoi*.

to ι-carrageenan (δ 102.3 and δ 92.4) and μ-carrageenan (δ 104.6 and 98.5) in relatively abundant amount. The ratio of the κ/ι/μ-carrageenan was determined as 35:43:22, by compared with the peak height of the anomeric signal of 1,3 linked Gal in the spectrum of hybrid polysaccharide CCC.

3.3. Linkage analysis of polysaccharides

The identification and proportions of partially methylated alditol acetates of native and desulfated CCC are listed in Table 2. The native CCC consisted of 1,3- and 1,4-linked units in the approximate molar proportion of 1:1, suggesting the presence of a linear repeating unit of alternating 1,3-linked Gal and 1,4-linked anGal along with low deep of substitution of 1,4-linked Gal. The positions of sulfate group were determined by the linkages analysis of desulfated CCC. The presence of higher amount of 3-linked Gal and 4-linked Gal contents in desulfated CCC suggested that all the 3-linked Gal units were sulfated at C4 and 4-linked Gal were sulfated at C6. Furthermore, the increased proportion of 1,4,5-Ac3-2-Me-3,6-anGal after desulfation indicated that 1,4-linked anGal was partial sulfated at C2, as found in A2S units of ι-carrageenan. Linkage analysis of native CCH showed the presence of 1,3,4,5-Ac_4-2,6-Me_2-Gal and 1,4,5-Ac_3-2-Me-3,6-anGal in equal molar proportions. The absence of 1,3,4,5-Ac_4-2,6-Me_2-Gal in desulfated CCH was attributed to the desulfated derivative of G4S units. So, the structure of CCH was similar to κ-carrageenan with a linear sequence of alternating G4S and A. The methylation results were in good agreement with FTIR and ^{13}C NMR analysis.

Based on the above data, the major polysaccharide CCC from *C. chamissoi* was a hybrid carrageenan composed of κ-carrageenan, ι-carrageenan, and μ-carrageenan (precursor of κ-carrageenan), while CCH was an ideal κ-carrageenan.

3.4. Preparation of oligosaccharides and MS analysis

Detailed investigation of oligosaccharide sequences is important for addressing the carrabiose composition and distribution along the hybrid carrageenan. Previous study by Yang et al. (2009) in our laboratory had described the mechanism of mild acid hydrolysis of κ-carrageenan that only odd-numbered oligosaccharides were obtained with mild acid hydrolysis of κ-carrageenan, while even-numbered oligosaccharide alditols were obtained using reductive mild acid hydrolysis. Here, the similar reductive mild hydrolysis technique was used to treat CCH, and series uniform fractions were acquired by gel filtration chromatography on a

Table 2
Methylation analysis of native and desulfated polysaccharide CCC from *C. chamissoi*.

Methylated product	Linkages	Molar ratio	
		Native	Desulfated
1,4,5-Ac_3-2-Me-3,6-anGal	→4)anGal(1→	16.5	33.9
1,2,4,5-Ac_4-3,6-anGal	→4)anGal2S(1→	19.1	1.0
1,3,5-Ac_3-2,4,6-Me_3-Gal	→3)Gal(1→	–	51.1
1,4,5-Ac_3-2,3,6-Me_3-Gal	→4)Gal(1→	–	11.9
1,3,4,5-Ac_4-2,6-Me_2-Gal	→3)Gal4S(1→	53.5	1.2
1,4,5,6-Ac_4-2,3-Me_2-Gal	→4)Gal6S(1→	10.9	0.9

Table 3
Negative-ion ES-MS of oligosaccharide fragments of polysaccharide CCH obtained by reductive mild acid hydrolysis.

Fractions	Found ions (charge)	Theoretical molecular mass (H form)	Assignment	
			DP	Sequences
a1	395.1 (−2)	792.2	4	G4S-A-G4S-Aol
a2	391.7 (−3)	1178.2	6	G4S-A-G4S-A-G4S-Aol
a3	390.0 (−4)	1564.2	8	G4S-A-G4S-A-G4S-A-G4S-Aol
a4	389.0 (−5)	1950.3	10	G4S-A-G4S-A-G4S-A-G4S-A-G4S-Aol
a5	388.4 (−6)	2336.4	12	G4S-A-G4S-A-G4S-A-G4S-A-G4S-A-G4S-Aol
a6	387.9 (−7)	2722.4	14	G4S-A-G4S-A-G4S-A-G4S-A-G4S-A-G4S-A-G4S-Aol
a7	387.6 (−8)	3108.8	16	G4S-A-G4S-A-G4S-A-G4S-A-G4S-A-G4S-A-G4S-A-G4S-Aol

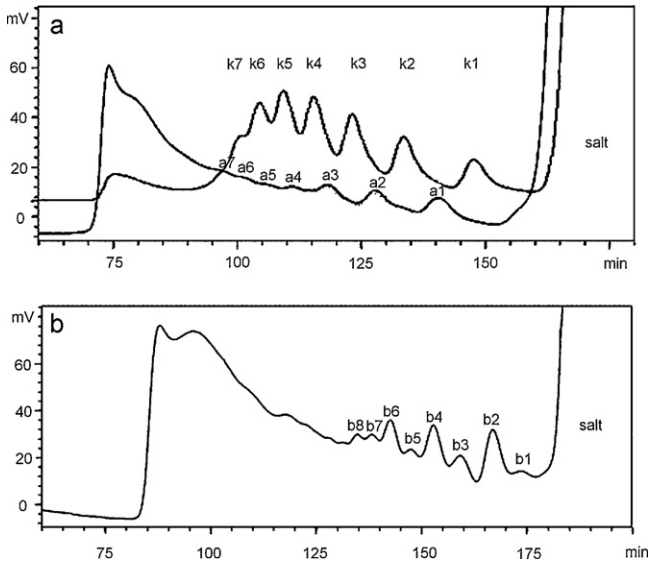

Fig. 3. Gel filtration chromatography of oligosaccharides prepared from polysaccharide CCH (a) and CCC (b).

Superdex Peptide column (Fig. 3a). The elution time of each oligosaccharide alditol of CCH was delayed successively, by compared with κ-carrageenan oligosaccharide (K1–K7) by mild acid hydrolysis. The molecular mass and structures of the oligosaccharides (a1–a7) were determined by negative-ion ES-MS (Table 3). ES-MS analysis of fraction a2 showed a doubly charged ion at *m*/*z* 395.1 with a molecular ion $[M-H]^-$ at *m*/*z* 792.2, suggesting the presence of tetrasaccharide composed of two G4S, one A and one Aol residue, with the sequence of G4S-A-G4S-Aol. A similar regular increment of 386 mass unit (G4S-A) was determined for the next six fractions from DP6 to DP16, successively. All the data showed that oligosaccharides from CCH were a series of even-numbered of κ-carrageenan-derived oligosaccharide alditols. The result was in agreement with previous conclusion (Yang et al., 2009).

For CCC oligosaccharides analysis, eight irregular fractions (Fig. 3b) were obtained by gel filtration chromatography. Based on ES-MS, methylation and NMR analysis, the most possible structural sequences of oligosaccharides are shown in Table 4. Since the hybrid structure of CCC, different DP of oligosaccharides with similar molecular mass and equal charge coexisted in one peak. Take fraction b1 for example, two main ions at *m*/*z* 484 corresponding to a ι-carrabiose (G4S-A2S) and *m*/*z* 502 corresponding to a μ-carrabiose (G4S-D6S) were present in the mass spectrum. Desulfated form of D6S unit was generally present in the sequence of oligosaccharides, suggesting that the sulfation at C6 of D was very labile. The reason for desulfation was discussed in previous study by Ekeberg, Knutsen, and Sletmoen (2001), due to high cone voltage, chemical reactions catalyzed or caused by the mobile phase.

Detailed sequences of hybrid trisaccharides were determined by ES-CID-MS/MS. Since the sulfated oligosaccharides were unstable in the free acid forms, singly charged molecular ion $[M-Na]^-$ was selected as the precursors (Yu et al., 2006). In addition, due to the symmetrical nature of the sequence of trisaccharides, a reducing terminal fragment ion was assigned based on the product-ion spectrum of its alditol after reduction, in which the reducing terminal ions would have a 3 mass increment (Yang et al., 2009). For instance, the precursor-ion spectrum of ι-carrageenan trisaccharide $[M-Na]^-$ at *m*/*z* 772 is shown in Fig. 4a. The present of B/C-ion doublets from nonreducing terminal, e.g., B_1 and C_1 (*m*/*z* 241 and 259), B_2 and C_2 (*m*/*z* 487and 505) and Y/Z-ion doublets with 3 mass increment from reducing terminal clearly identified the sequence

Table 4
Negative-ion ES-MS of oligosaccharide fragments of polysaccharide CCC obtained by mild acid hydrolysis.

Fractions	Found ions (charge)	Theoretical mol mass (H form)	Assignment	
			DP	Sequences
b1	483.0 (−1)	484.0	2	G4S-A2S
	501.0 (−1)	502.0	2	G4S-D6S
b2	322.0 (−2)	646.0	3	G4S-A-G4S
	331.0 (−2)	664.0	3	G4S-D-G4S
b3	241.0 (−3)	726.0	3	G4S-A2S-G4S
	247.0 (−3)	744.0	3	G4S-D6S-G4S
	403.1 (−2)	808.2	4	G4S-A-G4S-D
	289.0 (−3)	870.0	4	G4S-A-G4S-A2S
	295.0 (−3)	888.1	4	G4S-A-G4S-D6S
b4	343.0 (−3)	1032.0	5	G4S-A-G4S-A-G4S
	349.0 (−3)	1050.0	5	G4S-D-G4S-A-G4S/G4S-A-G4S-D-G4S
b5	397.0 (−3)	1194.0	6	G4S-A-G4S-A-G4S-D
b6	352.5 (−4)	1418.2	7	G4S-A-G4S-A-G4S-A-G4S
b7	450.0 (−4)	1804.2	9	G4S-A-G4S-A-G4S-A-G4S-A-G4S
b8	490.5 (−4)	1966.2	10	G4S-A-G4S-A-G4S-A-G4S-A-G4S-D

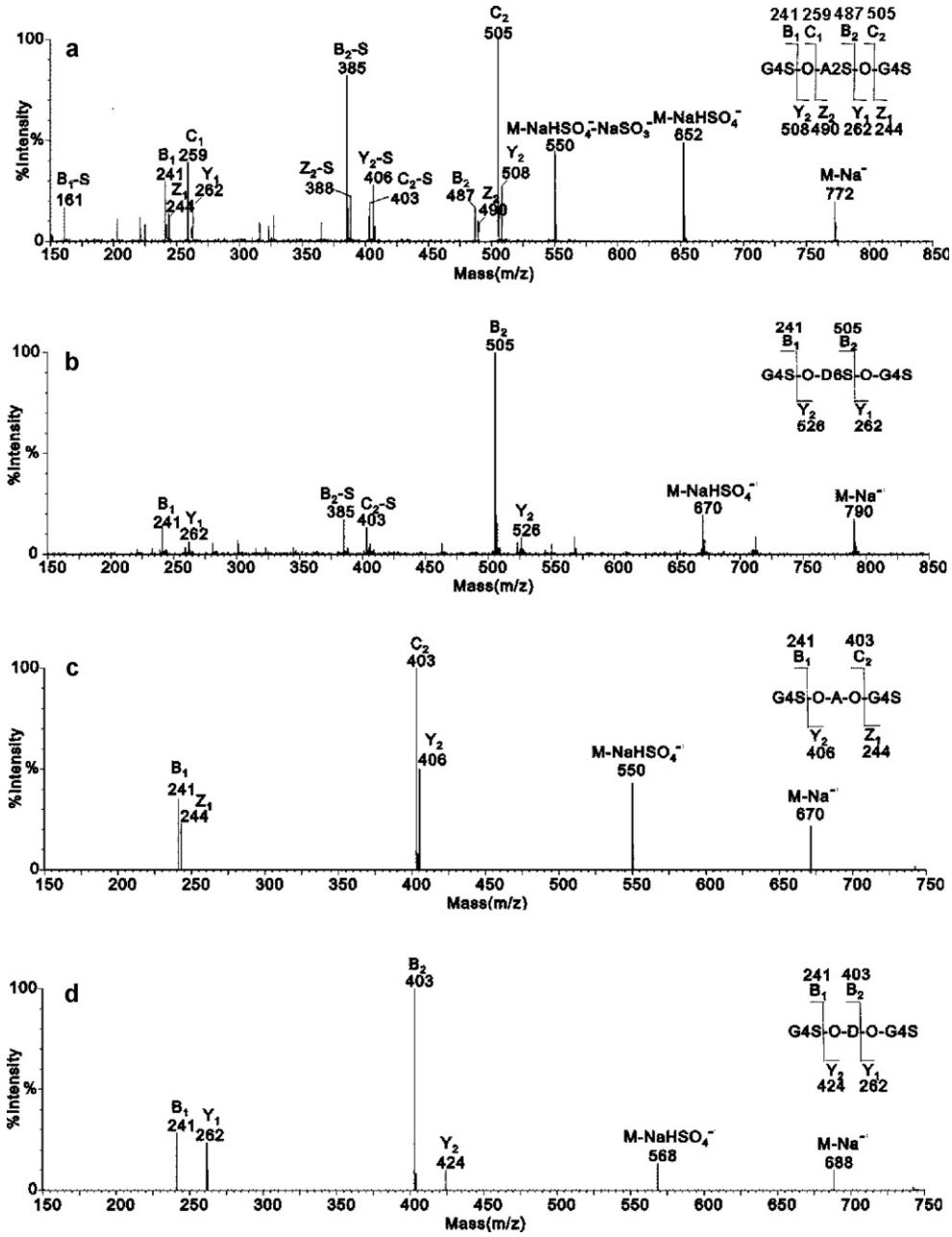

Fig. 4. Negative-ion ES CID MS/MS product-ion spectra of trisaccharides from polysaccharide CCC. (a) Sequence analysis of trisaccharide at *m*/*z* 772; (b) sequence analysis of trisaccharide at *m*/*z* 790; (c) sequence analysis of trisaccharide at *m*/*z* 670; and (d) sequence analysis of trisaccharide at *m*/*z* 688.

as G4S-A2S-G4S. The ion at *m*/*z* 652 was apparent by loss 120 Da from $[M-Na]^-$. The loss of 120 Da was frequently observed for a reducing terminal hexose as a loss of $C_4H_8O_4$ to give a $^{2,4}A$ ion (Chai et al., 2006). However, this A-type cross-ring cleavage ion could not form after reduction due to the monosaccharide ring opening, and therefore this was in further support of the above assignment of the sulfate loss in the form of $NaHSO_4$. Again another sulfate loss in the form of −102 Da (−$NaSO_3$ + H) was observed at *m*/*z* 550. The detailed sequence of other trisaccharides at *m*/*z* 790, 670 and 688 were also confirmed by ES-CID-MS/MS as G4S-D6S-G4S, G4S-A-G4S and G4S-D-G4S, respectively (Fig. 4b–d). By comparing the daughter ions of the four trisaccharide samples, we could conclude that different internal unit (G or A) of trisaccharides, with the presence and position of sulfate groups, largely affected the product-ions of glycosidic cleavage and gave unique fragmentation ions for oligosaccharides in different structure of trisaccharides.

Based on analysis of all oligosaccharide informations of CCC, a series of odd-numbered κ-carrageenan oligosaccharide (DP3-9) were present as major composition. Meanwhile, some hybrid tetrasaccharides composed of κ/ι and κ/μ-carrabiose in small amount occurred in the oligosaccharide mixture. Since the ^{13}C NMR data confirmed that CCC also had a great amount of μ and ι-carrageenan. The result indicated that the hydrolysis condition was just adequate to κ-carrageenan and major ι-carrageenan and μ-carrageenan still existed in the resistant fraction. Previous report by Guibet et al. (2008) had addressed the carrabiose composition and distribution along the hybrid κ/ι carrageenan chains from *C. chamissoi*. Series of homopolymers κ and ι-carrageenan oligosaccharides and hybrid κ/ι-carrageenan oligosaccharides were obtained though in-depth analysis of the hybrid oligosaccharides products with enzymatic degradation by chromatography and spectrometry methods. This gave evidence

that the hybrid κ/ι-carrageenan was glycosidically linked in a single hybrid macromolecule rather than a mixture of homopolymers. Here, structural analysis of CCC oligosaccharides by mild acid hydrolysis and ES-CID-MS/MS showed similar result that the hybrid carrageenan CCC might have three major blocks: a copolymer of κ-carrabiose, hybrid blocks composed of κ/μ/ι-carrabiose in random distribution and μ/ι-carrabioses rich blocks in the resistant fraction. In order to get more precise structural informations, the resistant fraction would be further hydrolyzed with mild acid, in order to get series of μ- and ι-carrageenan oligosaccharides, and then the sequence analysis of oligosaccharides would be confirmed by ES-CID-MS/MS.

4. Conclusions

The results obtained in the present study clearly proved that the structure of polysaccharides from *C. chamissoi* were significant different using different extraction methods. The hot water extracted fraction CCH was an ideal κ-carrageenan. While, the cold water extracted fraction CCC was a hybrid κ/μ/ι-hybrid carrageenan. Since this hybrid carrageenan confers a unique and complicated structure, detailed biological and physiochemical properties will be studied in the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.04.034.

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