Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 61 (2005) 399–406
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Synthesis and NMR structural analysis of *O*-succinyl derivative of low-molecular-weight κ-carrageenan

Yun-Peng Jiang*, Xi-Kun Guo, Xiu-Fang Tian

Department of Chemistry, College of Science, Shantou University, Shantou 515063, People's Republic of China

Received 19 November 2004; revised 28 April 2005; accepted 3 May 2005
Available online 25 July 2005

Abstract

Low-molecular-weight (LMW) κ-carrageenan was achieved through mild hydrochloric acid hydrolysis of κ-carrageenan. The acylation of LMW κ-carrageenan was performed by use of tetrabutylammonium (TBA) salt of the anionic polysaccharide fragments, succinic anhydride, 4-dimethylaminopyridine and tributylamine under homogeneous conditions in *N*,*N*-dimethylformamide at 80 °C. Investigation of FT-IR spectrum of the succinylated LMW κ-carrageenan showed that a monoester derivative with succinyl group was formed when LMW κ-carrageenan reacted with succinic anhydride. The ^{1}H and ^{13}C NMR spectroscopy has been used to characterize the fine structure of *O*-succinyl derivative of the LMW κ-carrageenan. The ^{13}C and ^{1}H NMR chemical shifts of disaccharide unit of *O*-succinyl LMW κ-carrageenan have been fully assigned using 2D NMR spectroscopic techniques.

Keywords: Low-molecular-weight κ-Carrageenan; Acylation; Succinic anhydride

1. Introduction

Carrageenans are a family of sulfated polysaccharides prepared by alkaline extraction (and modification) from red seaweed. They have structures that are generally based on linear chains of alternating 3-linked β-D-galactopyranosyl and 4-linked α-galactopyranosyl residues (Janaswamy & Chandrasekaran, 2002; Myslabodski, Stancioff, & Heckert, 1996). Three primary forms (κ-, λ-, ι-) of carrageenan are identified based on the modification of the disaccharide repeating unit resulting from the occurrence of ester sulphate, or anhydride formation in the 4-linked residue (Karlsson & Singh, 1999), and the corresponding IUPAC inspired names and letter codes are carrageenose 4′-sulfate (G4S-DA), carrageenose 2,4′-disulfate (G4S-DA2S), and carrageenan 2,6,2′-trisulfate (G2S-D2S,6S). The idealized chemical repeat of κ-carrageenan is 3-linked β-D-galacto-pyranosyl 4-sulfate alternating 4-linked 3,6-anhydro-α-D-galactopyranosyl.

The carrageenans are the first natural sulfated polysaccharides found to have anti-human immunodeficiency virus (HIV) activity (Nakashima et al., 1987). Interest in the biological activity and pharmaceutical value of sulfated polysaccharides has increased in the last decade. The sulfated polysaccharides, including heparin, dextran sulfate, pentosan polysulfate and others, have been shown to inhibit the replication of HIV (Witvrouw, Desmyter, & De Clercq, 1994). In general, the anti-HIV activity of these compounds is correlated to their anionic character. Increasing in density of negative charge of sulfated polysaccharides favours their anti-HIV activity (Herold et al., 1995). On the other hand, the flexible of backbone of sulfated polysaccharides affect the anti-HIV activity of these compounds, too. The less flexible the backbone of sulfated polysaccharides is, the higher anti-HIV activity of these compounds. However, the flexible of carrageenan molecular, for instance irregular helical form, was found to increase with increasing 3,6-anhydro-α-D-galactopyranosyl units, so the anti-HIV activity of λ-carrageenan is higher than that of κ-carrageenan (Carlucci et al., 1997).

We undertook the present work on *O*-succinyl derivative of κ-carrageenan fragments for the following purpose: introduction of free carboxyl into LMW κ-carrageenan was executed by reaction of monocarboxyl of succinic

* Corresponding author. Tel.: +86 754 290 3659; fax: +86 754 290 3276.
E-mail address: jyp@stu.edu.cn (Y.-P. Jiang).

0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.05.016

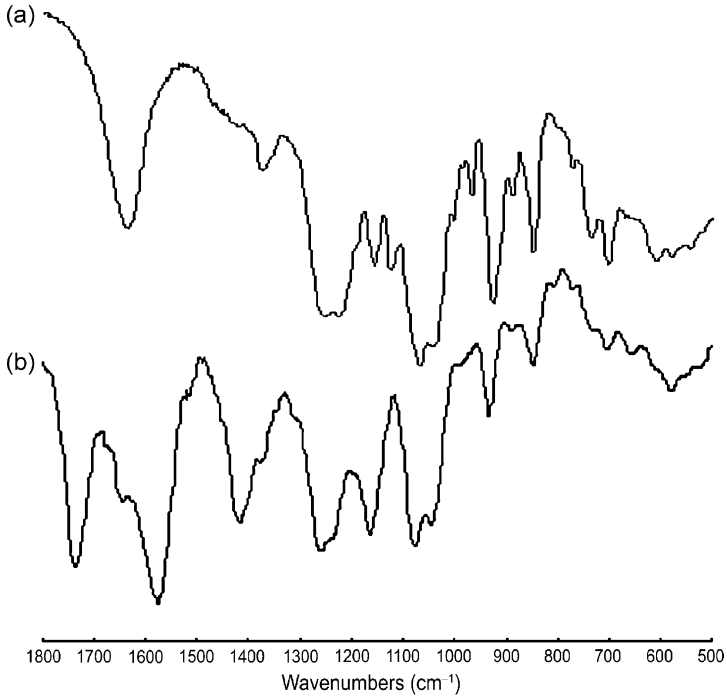

Fig. 1. FT-IR spectra of LMW κ-carrageenan and its *O*-succinyl derivative. (a) Starting LMW κ-carrageenan; (b) *O*-succinyl LMW κ-carrageenan.

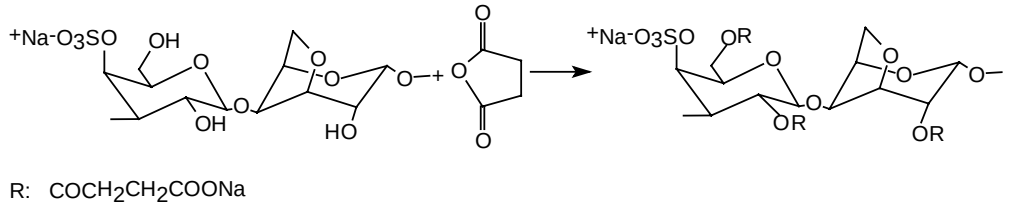

Fig. 2. Synthesis of *O*-succinyl LMW κ-carrageenan.

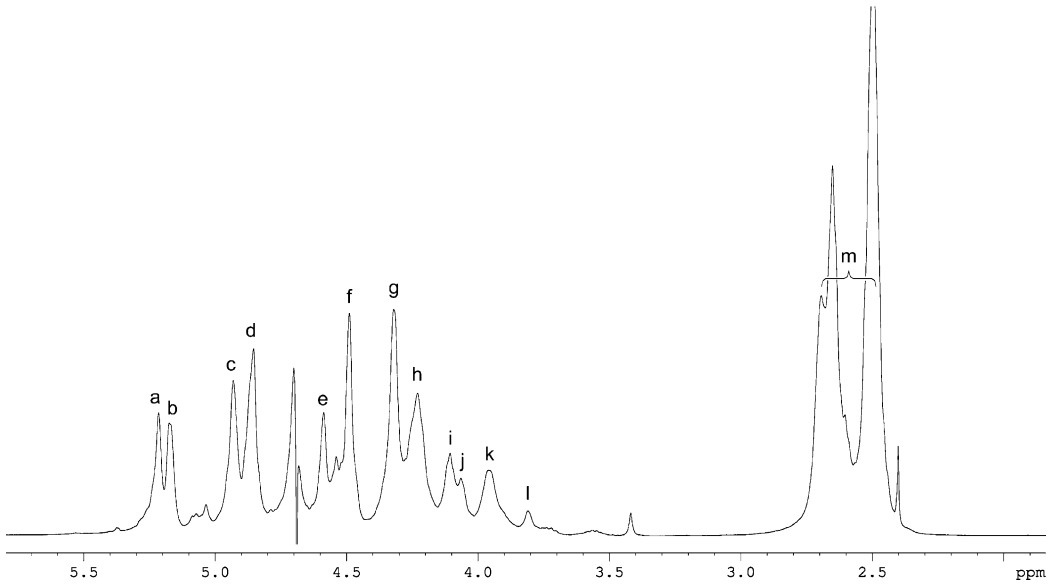

Fig. 3. ^{1}H NMR spectrum of *O*-succinyl LMW κ-carrageenan. (a) DA-1; (b) DA-3 (c) G4S-2; (d) G4S-1 and DA-4; (e) DA-5; (f) G4S-4 and DA-2; (g) G4S-6; (h) G4S-3 and DA-6b; (i) G4S-5; (j) DA-2 unsubstituted; (k) DA-6a; (l) G4S-6 unsubstituted; (m) methylene of acyl chain.

anhydride with hydroxyl groups of κ-carrageenan to increase the density of the negative charge. We describe herein the preparation of succinylated LMW κ-carrageenan. The structure of *O*-succinyl derivative of LMW κ-carrageenan is characterized by using FT-IR and NMR spectroscopy.

2. Experimental

2.1. Depolymerization of κ-carrageenan

κ-Carrageenan was purchased from Yantai Algae Industries (Shandong, China), and purified by the method of Smith et al. (1954). Partial hydrolysis was conducted as follow: a solution of κ-carrageenan (2.5 g) in 0.1 M hydrochloric acid (250 ml) was heated at 60 °C and kept for 1 h. The degradation was terminated by neutralization with 0.1 M sodium hydroxide, and then the solution was dialyzed against distilled water for 6 days. The dialyzed solution was filtered through a Millipore membrane (GS, 0.45 μm), and the filtered solution was concentrated in vacuum to a small volume, and then percolated through a column (100 ml) of 732 type (H^+) cation-exchange resin at 4 °C. The pH of the solution was adjusted to 8 by addition of tetrtabutylammonium and the TBA salt was obtained after lyophilization and drying for 24 h at 40 °C under vacuum.

2.2. O-succinyl derivative of LMW κ-carrageenan

A solution of κ-carrageenan TBA salt (547 mg, corresponding to 2.87 mmol of OH group) in dry *N*,*N*-dimethylformamide (DMF) (20 ml) under N_2 was added to 4-dimethylaminopyridine (81.3 mg, 0.82 mmol), succinic anhydride (847 mg, 8.63 mmol), and tributylamine (2.07 ml, 8.63 mmol). The solution was heated at 80 °C for 6 h. After the reaction, the mixture was cooled to 4 °C in ice water, and then a cold saturated ethanolic solution of sodium acetate (150 ml) was added. The mixture was allowed to stand for 1 h at 4 °C. After centrifugation, the precipitate was dissolved in distilled water, and dialyzed against 5% aq sodium hydrogen carbonate for 2 days, followed by distilled water for 4 days. The solution was lyophilized to give the *O*-succinyl LWM κ-carrageenan.

2.3. Characterization

FT-IR spectra were obtained by using a Nicolet Magna 750 spectrometer. Sample was ground together KBr in an agate mortar before pressing to form a optically clear pellet. The spectral resolution was 4 cm^{-1}.

^1H spectrum of D_2O solution was recorded at 35 °C on a Bruker AVANCE DRX-500 spectrometer with a 5-mm ^1H probe. The parameters were as follows: pulse angle: 90°; acquisition time: 3 s; relaxation delay: 2 s; scans: 32; spectral width, 5251 Hz. Chemical shifts (δ) were given in

Table 1
Assignment of *O*-succinyl LMW κ-carrageenan shifts (ppm) in the ^1H and ^{13}C NMR spectra

	G4S (3-linked unit)						DA (4-linked unit)						Carboxyl of easter linkage	Free carboxyl	Methylene
	1	2	3	4	5	6	1	2	3	4	5	6			
Proton	4.85	4.93	4.23	4.48	4.11	4.32 (3.81)	5.22	4.49 (4.07)	5.18	4.85	4.59	3.96[a]/4.23[b]			2.50–2.70
Carbon	100.8	73.8	76.3	79.5	73.0	64.4 (62.1)	93.9	77.8 (70.6)	71.2	71.5	77.7	70.6	175.4–176.1	176.8–182.1	31.71–33.1

Chemical shits of proton and carbon unsubstituted are shown in parenthesis.
[a] DA H-6a.
[b] DA H-6b.

ppm relative to the water peak ($\delta = 4.68$ ppm). ^{13}C NMR of MeOD solution was run at 35 °C using a Bruker AVANCE DRX-500 spectrometer with a 5-mm ^{1}H probe (125 MHz, 9036 scans, 1 s acquisition time, 1.8 s relaxation delay time, 30,303 Hz spectral width).

$^{1}H/^{1}H$ COSY measurement was performed with a Bruker AVANCE DRX-500 apparatus with a 1024×512 data matrix. A 4194 Hz spectral width was used in both dimensions. The spectrum was processed by using a sine-bell filtering function in both dimensions after zero-filling to a 1024×512 matrix. $^{1}H/^{13}C$ HMQC spectrum was acquired using a 1024×256 data matrix on a Bruker AVANCE DRX-500 spectrometer, and an acquisition time was 0.157 s.

3. Results and discussion

3.1. Depolymerization of κ-carrageenan

Common methods for depolymerization of carrageenan include acid hydrolysis (Hjerde, SmidsrØd, & Christensen, 1996; Karlsson et al., 1999; Yu et al., 2002), reductive hydrolysis (Falshaw, Furneaux, & Wong, 2003; Stevenson & Furneaux, 1991), methanolysis (Knutsen & Grasdalen, 1992), and active oxygen species fragmentation (Yamada et al., 1997; Yamada, Ogamo, Saito, Uchiyama, & Nakagawa, 2000). κ-Carrageenan is stable to desulphation during mild acid hydrolysis (Karlsson et al., 1999; Yu et al., 2002). Therefore, depolymerization of κ-carrageenan was performed in 0.1 M hydrochloric acid at 60 °C. FT-IR spectrum of LMW κ-carrageenan is shown in Fig. 1(a). Absorption peaks appearing at 1250 and 850 cm^{-1} are contributed to S=O of sulfate esters and C–O–S of axial secondary sulfate on C-4 of galactose, respectively. Meanwhile, the band at 930 cm^{-1} is characteristic of C–O of 3, 6-anhydro-D-galactose. These characteristic absorption peaks found in the FT-IR spectra are indicative of the structural integrity of κ-carrageenan in the degradation process.

3.2. O-succinyl derivative of LMW κ-carrageenan

Yamada et al. (2000) synthesized *O*-acylated derivatives of LMW κ-carrageenans by introducing straight chain carboxylic acids of different length (C4, C6, C12). Three free hydroxyl groups of κ-carrageenan were all acylated by using *N*,*N*-dimethylformamide as the solvent in the presence of 4-dimethylaminopyridine and tributylamine as catalysts at room temperature. We found that *O*-succinyl derivative of LMW κ-carrageenan was not obtained under these conditions. Petitou et al. (1992) acylated the tributylammonium salt of dermatan sulfate using succinic anhydride as acylating agent and DMF as solvent in the presence of a catalytic amount of 4-dimethylaminopyridine at 60 °C. Under similar reaction conditions, succinylation of the tetrabutylammonium salt of LMW κ-carrageenan proceeded successfully at 80 °C. The reaction formula for LMW κ-carrageenan reacted with succinic anhydride is shown in Fig. 2. Fig. 1(b) shows the FT-IR spectrum of product after the reaction for 6 h. Observation of the region around 1740 cm^{-1} indicates the carbonyl stretching vibration of ester linkage. The absorption band at 1590 and 1430 cm^{-1} are attributed to asymmetrical stretching vibration and symmetrical stretching vibration of $-COO^{-}$ of carboxylate, respectively (Ke & Dong, 1998). This revealed that a monoester derivative with succinyl group was formed when LMW κ-carrageenan reacted with succinic anhydride.

3.3. ^{1}H NMR spectroscopy

The ^{1}H NMR spectrum of the starting low-molecular-weight κ-carrageenan (not shown) was very similar to

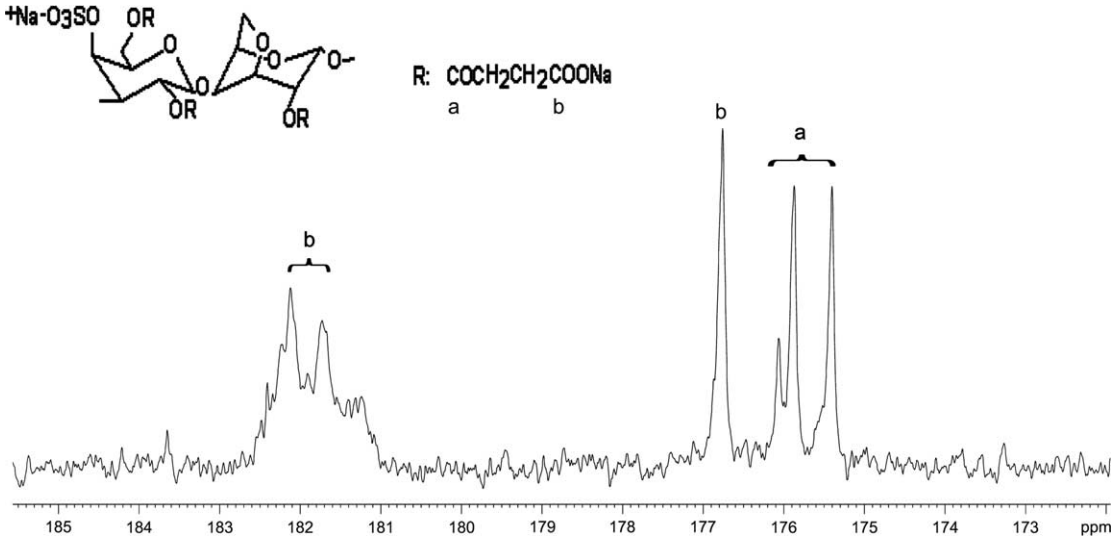

Fig. 4. ^{13}C NMR spectrum of carbonyl region of *O*-succinyl LMW κ-carrageenan.

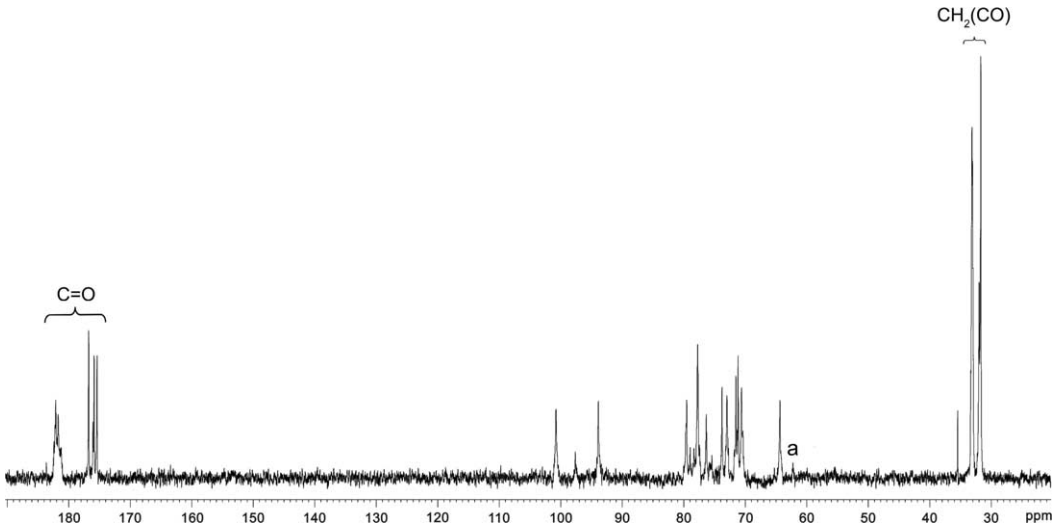

Fig. 5. Full-range ^{13}C NMR spectrum of *O*-succinyl LMW κ-carrageenan. (a) G4S-6 unsubstituted.

published values (Knutsen & Grasdalen, 1992; Yu et al., 2002), showing the major signals consistent with diads G4S-DA belonging to κ-carrageenan. The ^{1}H NMR spectrum of *O*-succinyl LMW κ-carrageenan was well resolved (Fig. 3). Due to overlap of some protons, only 10 major signals at 3.5–4.4 ppm region are visible for the major disaccharide repeating unit of *O*-succinyl LMW κ-carrageenan. A multiplet was present at 2.50–2.70 ppm region, corresponding to the methylene proton of succinyl group. The methylene proton number was obtained by

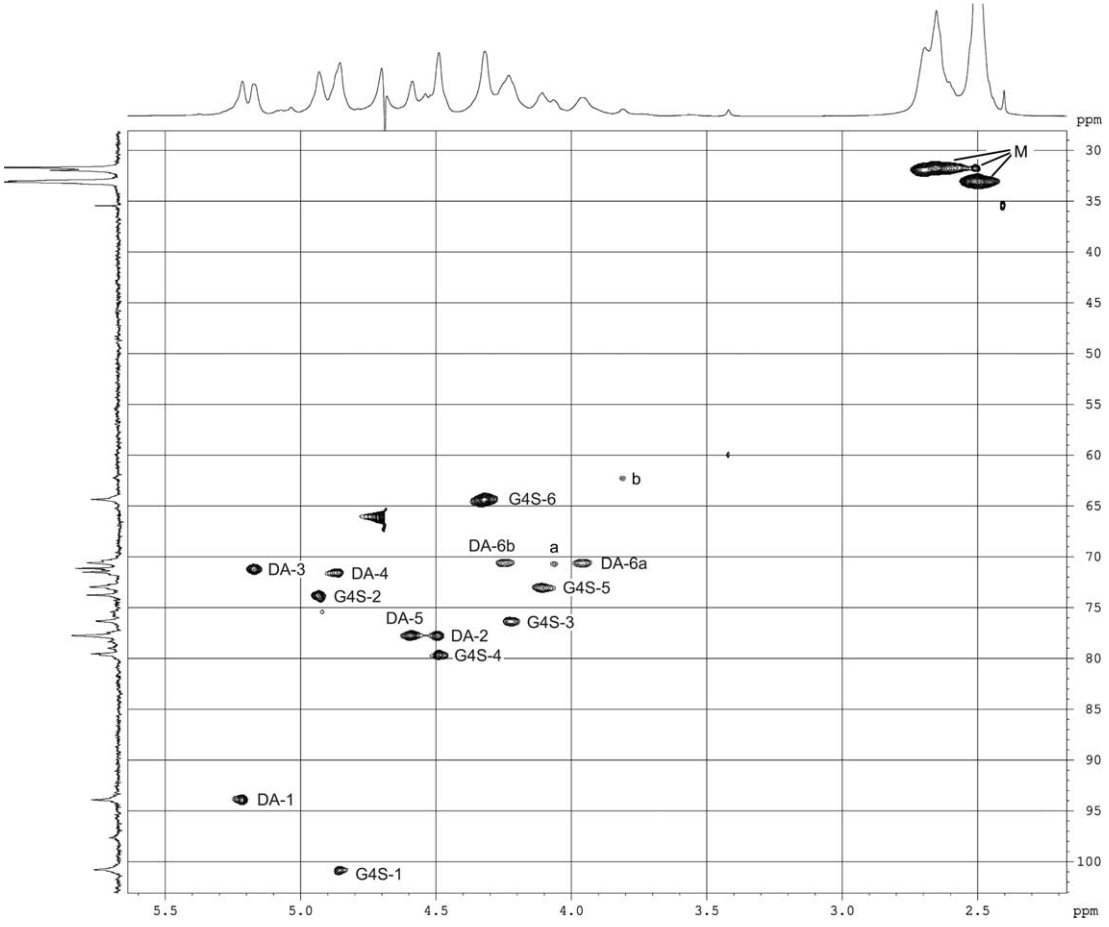

Fig. 6. ^{1}H/^{13}C HMQC spectrum of *O*-succinyl LMW κ-carrageenan. (a) H-2/C-2 of DA-2 unsubstituted; (b) H-6/C-6 of G4S-6 unsubstituted; (M) Methylene of acyl chain.

integrating the proton signals of the acyl chain. The degree of substitution of the product is given by:

DS = proton number of $\delta 2.50 - 2.70/4 = 10.9/4 = 2.7$

Small signals at 3.81 and 4.07 ppm were assigned to G4S-6 and DA-2 protons unsubstituted (Fig. 3, Table 1), and the corresponding proton numbers are 0.25 and 0.22, respectively.

3.4. ^{13}C NMR spectroscopy

^{13}C NMR studies on acetylated derivatives of oligo- and polysaccharides have demonstrated that the chemical shift of carbonyl carbon signals is remarkably sensitive to their position of substitution in the sugar residue, to the type of sugar residue, and to the mode of the linkage between them (Goux & Weber, 1993). The carbonyl region of the ^{13}C NMR spectrum of *O*-succinyl LMW κ-carrageenan is shown in Fig. 4. The carbonyl carbon signal of ester linkage was observed as a triplet (175.4, 175.9 and 176.1 ppm) in D_2O. The corresponding carbonyl carbon signal of free carboxyl could be divided into three regions (176.8, 181.7 and 182.1 ppm).

A full-range ^{13}C NMR spectrum of *O*-succinyl LMW κ-carrageenan is shown in Fig. 5. The carbon signal of disaccharide repeating unit of κ-carrageenan substituted group at 60–110 ppm. In addition, there are signals at 31.7–33.1 ppm corresponding to the succinyl methylene carbons. A small signal is observed at 62.2 ppm corresponding to G4S-6 carbon unsubstituted.

3.5. 2D NMR spectroscopy

For the 3-linked (G4S) unit of *O*-succinyl LMW κ-carrageenan, if the resonances at 100.8 ppm and 64.4 ppm are assigned to C-1 and C-6, then H-1 and H-6 can be located at 4.85 and 4.32 ppm, respectively, from the $^{1}H/^{13}C$ HMQC spectrum (Fig. 6, Table 1). The H-2 signal at 4.93 ppm shows correlations to H-1 and to H-3 at 4.23 ppm in the $^{1}H/^{1}H$ COSY spectrum (Fig. 7). The coupling constant between H-3 and H-4 protons in G4S unit was too small to give a correlation. The H-6 coupling with H-5 leads to the identification of the H-5 at 4.11 ppm. H-4 at

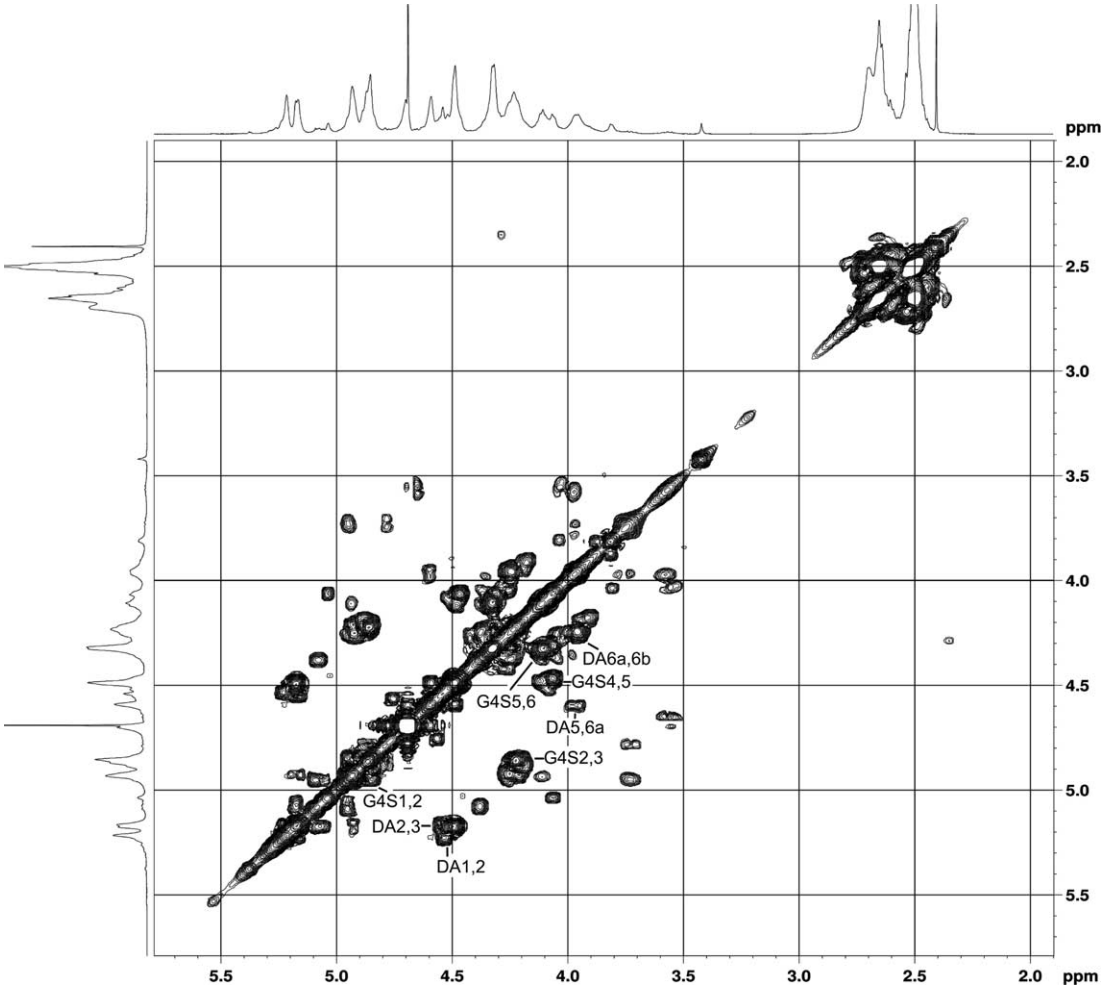

Fig. 7. $^{1}H/^{1}H$ COSY spectrum of *O*-succinyl LMW κ-carrageenan.

Table 2
The effect of succinyl substitution on the chemical shifts (ppm) of various proton atoms in disaccharide repeating unit of κ-carrageenan

	G4S (3-linked unit)			DA (4-linked unit)		
	O-succinyl LMW κ-carrageenan	Starting LMW κ-carrageenan	Δδ	*O*-succinyl LMW κ-carrageenan	Starting LMW κ-carrageenan	Δδ
H-1	4.85	4.60	0.25	5.22	5.06	0.16
H-2	4.93	3.55	1.38	4.49	4.10	0.39
H-3	4.23	3.97	0.26	5.18	4.48	0.70
H-4	4.48	4.81	−0.33	4.85	4.60	0.25
H-5	4.11	3.76	0.35	4.59	4.56	0.03
H-6	4.32	3.76	0.56	3.96[a]	4.01[a]	−0.05[a]
				4.23[b]	4.18[b]	0.05[b]

[a] DA H-6a.
[b] DA H-6b.

4.48 ppm was then assigned from the H-4, 5 cross-peak. The $^{1}H/^{13}C$ HMQC spectrum allowed the transfer of the H-2, H-3, H-4 and H-5 proton assignments to the corresponding carbon atoms (Fig. 6, Table 1).

For the 4-linked (DA) unit of *O*-succinyl LMW κ-carrageenan, if the resonance at 93.5 ppm is assigned to the anomeric carbon, then the signal at 5.22 ppm can be assigned to H-1 from the $^{13}C/^{1}H$ HMQC spectrum (Fig. 6). The correlation between H-1 and H-2 allows the identification of the H-2 at 4.49 ppm which correlates to the H-3 at 5.18 ppm in the $^{1}H/^{1}H$ COSY spectrum (Fig. 7). The chemical shifts for G4S H-2 and DA H-4 are very similar, but the $^{1}H/^{1}H$ COSY shows G4S H-2 being 0.01 ppm downfield of DA H-4. H-3-H-4 and H-4-H-5 coupling is very small, so that no intense cross-peak was observed. However, the two protons, H-6a at 3.96 ppm and H-6b at 4.23 ppm whose resonance overlaps with that of G4S H-3 located in the same region, were easily identified due to their correlation to C-6 at 70.6 ppm from the $^{1}H/^{13}C$ HMQC spectrum. Furthermore, an H-6a, 6b cross-peak was also observed in the $^{1}H/^{1}H$ COSY spectrum. Although the connectivity between H-6b and H-5 is missing due to small spin–spin coupling, a weak correlation peak between H-6a and H-5 at 4.59 ppm was observed. Having located H-2, H-3, H-5, the corresponding carbon atoms were assigned unambiguously (Fig. 6, Table 1). This then leaves C-4 at 71.5 ppm, and from this, H-4, whose signal overlaps with G4S H-1, was located at 4.85 ppm. Obviously, all connectivities of DA unit, except those between H-3 and H-4, H-4 and H-5 and H-5 and H-6b, are clearly displayed in the $^{1}H/^{1}H$ COSY spectrum.

The effect of succinyl substitution at the 2-position of DA unit and the 2- and 6-positions of G4S unit of LMW κ-carrageenan on the chemical shifts of these protons of its disaccharide repeating unit is shown in Table 2. In general, this substitution has most impact on the chemical shifts of the substituted protons (i.e. DA H-2, G4S H-2 and G4S H-6, the α-effect) and those adjacent to them (i.e. DA H-1, DA H-3, G4S H-1, G4S H-3 and G4S H-5, the β-effect). The signals for H-1, H-2, H-3, H-5 and H-6 of the G4S units were all shifted downfield, while that of H-4 was shifted upfield due to shielding effect of carbonyl of the succinyl groups at the 2- and 6-positions of G4S unit. The H-6a of DA unit was shielded about 0.05 ppm and the H-6b was deshielded about 0.05 ppm. This was attributed to anisotropic effects of carbonyl of the succinyl group at the 2-position of DA unit.

4. Conclusions

O-succinyl LMW κ-carrageenan has been prepared, and DS of 2.7 could be attained under appropriate conditions. Succinyl substitution at different position of G4S-DA is dependent on the position of hydroxyl of sugar residue and the type of sugar residue. The substitution at the 2-position of G4S unit is the highest, followed by substitution at 6-position of G4S unit. The substitution at the 2-position of DA unit is smallest. The ^{1}H and ^{13}C NMR chemical shifts for disaccharide unit of *O*-succinyl LMW κ-carrageenan have been fully assigned using 2D NMR spectroscopic techniques.

Acknowledgements

The authors are grateful for the financial support from Shantou University.

References

Carlucci, M. T., Pujol, C. A., Ciancia, M., Noseda, M. D., Matulewicz, M. C., Damonte, E. B., et al. (1997). Antiherpetic and anticoagulant properties of carrageenans from the red seaweed *Gigartina skottsbergii* and their cyclized derivatives: correlation between structure and biological activity. *Journal Biological Macromolecules*, *20*, 97–105.

Falshaw, R., Furneaux, R. H., & Wong, H. (2003). Analysis of pyruvylated β-carrageenan by 2D NMR spectroscopy and reductive partial hydrolysis. *Carbohydrate Research*, *338*, 1403–1414.

Goux, W. J., & Weber, D. S. (1993). The application of NMR-pattern-recognition methods to the classification of reduced, peracetylated oligosaccharide residues. *Carbohydrate Research*, *240*, 57–69.

Herold, B. C., Gerber, S. I., Polonsky, T., Belyal, J. B., Shaklee, P. N., & Holml, K. (1995). Identification of structural features of heparin required for inhibition of herpes simplex vireus type 1 binding. *Virology*, *206*, 1108–1116.

Hjerde, T., SmidsrØd, O., & Christensen, B. E. (1996). The influence of the conformational state of κ- and ι-carrageenan on the rate of acid hydrolysis. *Carbohydrate Research*, *288*, 175–187.

Janaswamy, S., & Chandrasekaran, R. (2002). Effect of calcium ions on the organization of iota-carrageenan helices: an X-ray investigation. *Carbohydrate Research*, *337*, 523–535.

Karlsson, A., & Singh, S. K. (1999). Acid hydrolysis of sulphated polysaccharides. Desulphation and the effect on molecular mass. *Carbohydrate Polymers*, *38*, 7–15.

Ke, Y. K., & Dong, H. R. (1998). *Handbook of Analytical Chemistry*, vol. 3. Beijing, China: Chemical Industry Press p. 957.

Knutsen, S. H., & Grasdalen, H. (1992). The use of neocarrabiose oligosaccharides with different length and sulphate substitution as model compounds for ^{1}H NMR spectroscopy. *Carbohydrate Research*, *229*, 233–244.

Myslabodski, D. E., Stancioff, D., & Heckert, R. A. (1996). Effect of acid hydrolysis on the molecular weight of kappa carrageenan by GPC-LS. *Carbohydrate Polymers*, *31*, 83–92.

Nakashima, H., Kido, Y., Kobayashi, N., Motoki, Y., Neushul, M., & Yamamoto, N. (1987). Purification and characterization of an avian myeloblastosis and human immunodeficiency virus reverse transcriptase inhibitor, sulfated polysaccharides extracted from sea algae. *Antimicrobial Agents Chemotherapy*, *31*, 1524–1528.

Petitou, M., Coudert, C., Level, M., Lormean, J. C., Zuber, M., Simenel, C., et al. (1992). Selectively *O*-acylated glycosaminoglycan derivatives. *Carbohydrate Research*, *236*, 107–119.

Smith, D. W., Cook, W. H., & Neal, J. L. (1954). Physical studies on carrageenin and carrageenin fractions. *Archives of Biophysics Biochemistry*, *53*, 192–204.

Stevenson, T. T., & Furneaux, R. H. (1991). Chemical methods for the analysis of sulphated galactans from red algae. *Carbohydrate Research*, *210*, 277–298.

Witvrouw, M., Desmyter, J., & De Clercq, E. (1994). Antiviral portrait series: 4. Polysulfates as inhibitors of HIVand other enveloped viruses. *Antiviral Chemistry and Chemotherapy*, *5*, 345–359.

Yamada, T., Ogamo, A., Saito, T., Watanabe, J., Uchiyama, H., & Nakagawa, Y. (1997). Preparation and anti-HIV activity of low-molecular-weight carrageenans and their sulfated derivatives. *Carbohydrate Polmers*, *32*, 51–55.

Yamada, T., Ogamo, A., Saito, T., Uchiyama, H., & Nakagawa, Y. (2000). Preparation of *O*-acylated low-molecular-weight carrageenans with potent anti-HIV activity and low anticoagulant effect. *Carbohydrate Polmers*, *41*, 115–120.

Yu, G., Guan, H., Ioanoviciu, A. S., Sikkander, S. A., Thanawiroon, C., Tobacman, J. K., et al. (2002). Structural studies on κ-carrageenan derived oligosaccharides. *Carbohydrate Research*, *337*, 433–440.

Yun-Peng Jiang Nov 2003–present—Postdoctoral: Biochemical Engineering, Shantou University, Shantou, China. Research on Molecular Modification of Polysaccharides and their bioactivity. *Sep 1999–Aug 2002*—PhD: Chemical Engineering. Tianjin University. Dissertation 'Preparation of Nano-sized Silica/PVA composite ultrafiltration membranes and its application'. *Sep 1992–Jul 1995*—Master: Analytical Chemistry, Hebei University Thesis 'High Performance Liquid Chromatographic Detection of Sulphonamides and Primary Aromatic Amines by Precolumn Derivatisation with Fluorescamine'. *Sep 1986–Jul 1990*—Bachelaor: Department of Chemistry, Neimengguo Normal University.