Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 61 (2005) 441–445
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

O-maleoyl derivative of low-molecular-weight κ-carrageenan: Synthesis and characterization

Yun-Peng Jiang, Xi-Kun Guo*

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

Received 31 October 2004; revised 12 May 2005; accepted 16 June 2005
Available online 8 August 2005

Abstract

Low-molecular-weight (LMW) κ-carrageenan was achieved through mild hydrochloric acid hydrolysis of κ-carrageenan. The acylation of low-molecular-weight κ-carrageenan was performed by use of tetrabutylammonium (TBA) salt of the anionic polysaccharide fragments, maleic anhydride, 4-dimethylaminopyridine and tributylamine under homogeneous conditions in *N*,*N*-dimethylformamide at 60 °C. Analysis of FT-IR spectrum of *O*-maleoyl κ-carrageenan showed that a monoester derivative with maleoyl group was formed when LMW κ-carrageenan reacted with maleic anhydride. Investigation of ^{1}H NMR spectroscopy revealed that the maleoylation took place regioselectively at C-2 of the κ-carrageenan 3-linked unit.

Keywords: Low-molecular-weight κ-carrageenan; Acylation; Maleic 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). 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 (Carlucci et al., 1997). However, the flexible of carrageenan molecular, for instance irregular helical form, was found to increase with increasing 3,6-anhydro-α-D-galactopyranosyl units (Rees, 1977; Yalpani, 1988).

We undertook the present work on *O*-maleoyl derivative of κ-carrageenan fragments for the following purpose: (a) introduction of free carboxyl into low-molecular-weight κ-carrageenan was executed by reaction of monocarboxyl of maleic anhydride with hydroxyl groups of carrageenan to increase the density of the negative charge. (b) Rigidity of the carrageenan molecule was increased through introducing a double bond. We describe herein the reaction

* Corresponding author. Tel.: +86 754 2902223; fax: +86 754 2903276.
E-mail address: xkguo@stu.edu.cn (X.-K. Guo).

0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.06.012

procedure of the degraded κ-carrageenan with maleic anhydride. The structure of *O*-maleoyl derivative of low-molecule-weight κ-carrageenan is characterized by using FT-IR and ^{1}H 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, Cook, and Neal (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 1.5 h. The degradation was terminated by neutralization with 0.1 M sodium hydroxide, and then the solution was dialyzed (Spectra/por1, diameter 25.5 mm, molecular weight cut-off 6000–8000) 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 cation-exchange resin (H^+, 16–50 mesh, SCRC, China) at 4 °C. The pH of the solution was adjusted to eight by addition of tetrabutylammonium and the TBA salt was obtained after lyophilization and drying for 24 h at 40 °C under vacuum.

2.2. O-maleoyl derivative of LMW κ-carrageenan

A solution of κ-carrageenan TBA salt (898 mg, corresponding to 4.31 mmol of OH group) in dry *N*,*N*-dimethylformamide (DMF) (20 ml) under N_2 flow was added to 4-dimethylaminopyridine (131 mg, 1.03 mmol), maleic anhydride (1.27 g, 12.9 mmol), and tributylamine (3. 10 ml, 12.9 mmol). The solution was heated at 60 °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 1.5 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*-maleoyl 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 an optically clear pellet. The spectral resolution was 4 cm^{-1}.

^{1}H NMR spectra of D_2O solutions were recorded at 35 °C on a Bruker AVANCE DRX-500 spectrometer with a 5-mm ^{1}H probe. The parameters were as follows: pulse angle: 90 °C; acquisition time: 2 s; relaxation delay: 2 s; scans: 120; spectral width: 6000 Hz. Chemical shifts (δ) were given in ppm relative to the water peak ($\delta = 4.68$ ppm).

3. Results and discussion

3.1. Depolymerization of κ-carrageenan

Common methods for depolymerization of carrageenan include acid hydrolysis (Hjerde, SmidsrØd, & Christensen, 1996; Karlsson & Singh, 1999; Yu et al., 2002), reductive hydrolysis (Falshaw, Furneaux, & Wong, 2003; Stevenson & Furneaux, 1991), methanolysis (Knutsen & Grasdalen, 1992) and active oxygen species fragmentation (Yamada, Ogamo, Saito, Uchiyama, & Nakagawa, 2000; Yamada et al., 1997). κ-Carrageenan is stable to desulphation during mild acid hydrolysis (Karlsson & Singh, 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. 1a. 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.

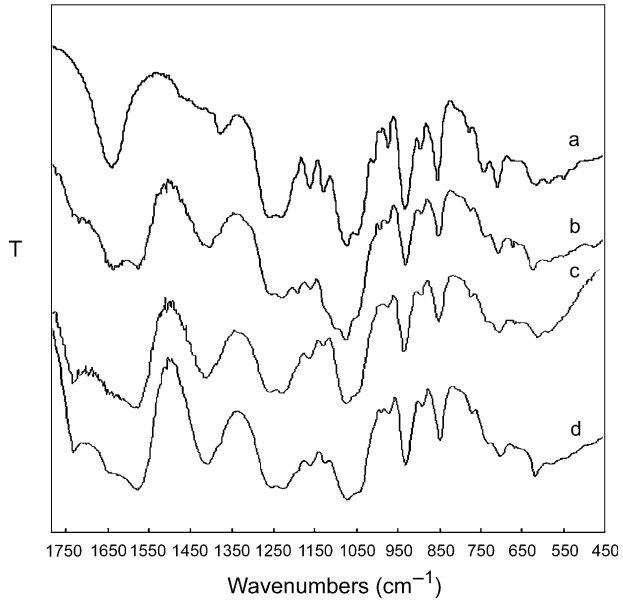

Fig. 1. FT-IR spectra of LMW κ-carrageenan and its *O*-maleoyl derivative obtained under different reaction conditions. (a) LMW κ-carrageenan; (b) product obtained from tributylammonium salt after reaction for 6 h; (c) product obtained from tributylammonium salt after reaction for 9 h; (d) product obtained from tetrabutylammonium salt after reaction for 6 h.

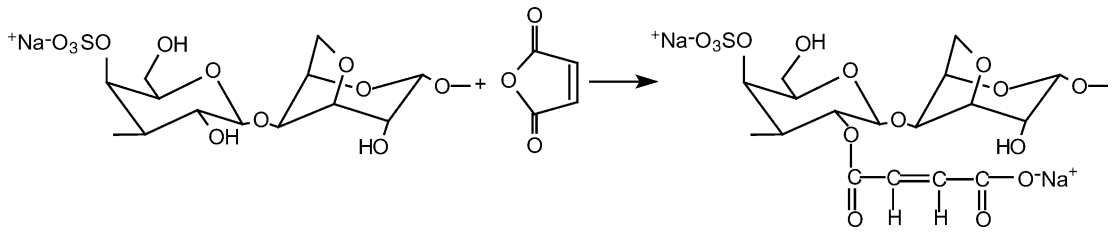

Fig. 2. Synthesis of *O*-maleoyl LMW κ-carrageenan.

3.2. O-maleoyl derivative of LMW κ-carrageenan

Yamada et al. (2000) synthesized *O*-acylated derivatives of low-molecular-weight 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*-maleoyl derivative of LMW κ-carrageenan was not obtained under similar 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 these reaction conditions, maleoylation of LMW κ-carrageenan proceeded successfully. The reaction formula for LMW κ-carrageenan reacted with maleic anhydride is shown Fig. 2. Fig. 1b shows the FT-IR spectrum of product after the reaction for 6h. Observation of the region around 1738 cm^{-1} indicates a smaller proportion of *O*-maleoyl LMW κ-carrageenan (shoulder). The absorption band at 1590 and 1420 cm^{-1} are attributed to asymmetrical stretching vibration and symmetrical stretching vibration of $–COO^-$ of carboxylate (Ke & Dong, 1998). This revealed that a monoester derivative with maleoyl group was formed when LMW κ-carrageenan reacted with maleic anhydride. The absorption peak appearing at 1650 cm^{-1} is assigned to C=C. The FT-IR spectrum of product after the reaction for 9 h is shown in Fig. 1c. With prolonging of reaction time, the intensity of the absorption band at 1738 cm^{-1} was improved, indicating that the DS of maleoyl group increased. This observation was confirmed by an increase in the intensities of absorption bands at 1650 cm^{-1} (C=C), 1590 cm^{-1} and 1420 cm^{-1} ($–COO^-$ of carboxylate).

The FT-IR spectrum of *O*-maleoyl derivative of the tetrabutylammonium salt of LMW κ-carrageenan is shown in Fig. 1d. The intensities of characteristic peaks at 1738 cm^{-1} for ester linkage, at 1650 cm^{-1} for C=C bond, at 1590 and 1420 cm^{-1} for $–COO^-$ of carboxylate are higher than that in Fig. 1b. This revealed that the rate of maleoylation of LMW κ-carrageenan from the tetrabutylammonium salt was higher than that from the tributylammonium salt.

3.3. Characterization of O-maleoyl LMW κ-carrageenan by ^1H NMR spectroscopy

The disaccharide unit regions of the ^1H NMR spectra of κ-carrageenan before and after forming its *O*-maleoyl derivative are showed in Fig. 3. The chemical shifts (Fig. 3a) of LMW κ-carrageenan were compared to published values (Knutsen & Grasdalen, 1992; Yu et al., 2002), showing the major signals consistent with diads G4S-DA belonging to κ-carrageenan. The relative intensities (Fig. 3b) of 3.55 and 3.46 ppm band, corresponding to H-2 of the internal (int) β-4-*O*-sulfogalactopy-ranose (β-Galp4S_{int}) and H-2 of the nonreducing end (nre)

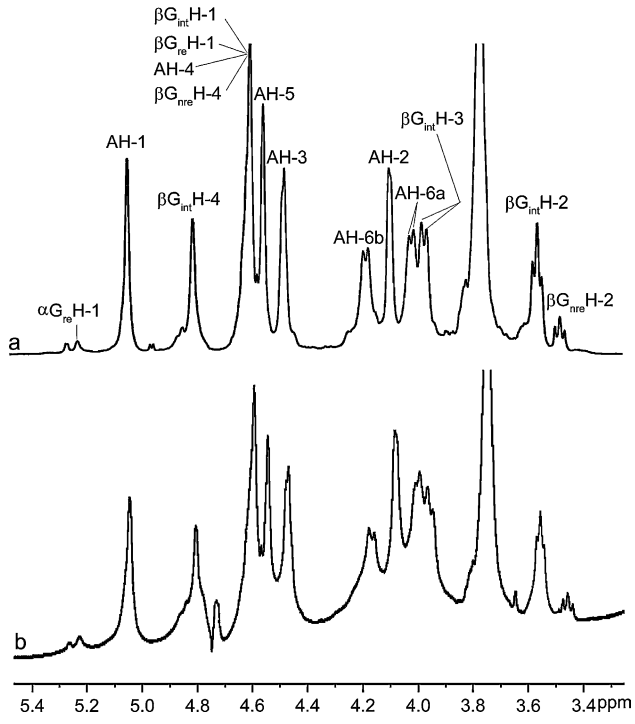

Fig. 3. ^1H NMR spectrum of LMW κ-carrageenan and its *O*-maleoyl derivative. (a) LMW κ-carrageenan; (b) product obtained from tetrabutylammonium salt after reaction for 6 h.

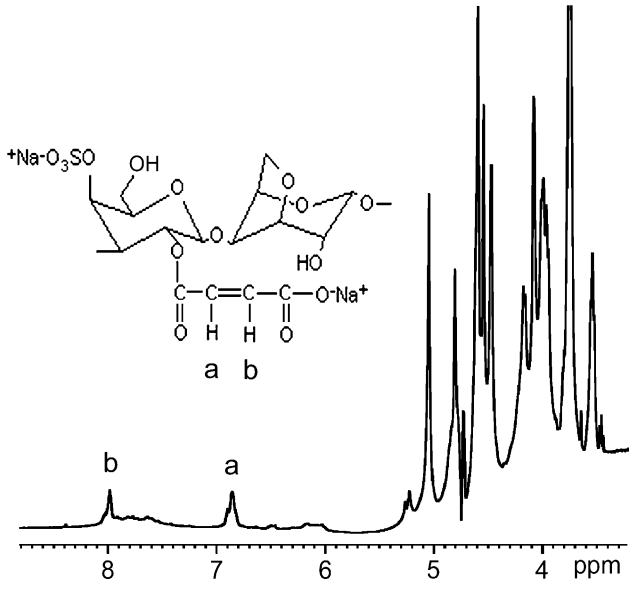

Fig. 4. Full-range ^{1}H NMR spectrum of product obtained from tetrabutylammonium salt after reaction for 6 h.

β-4-*O*-sulfogalactopyranose (β-Galp4S$_{nre}$), respectively, were decreased. The results imply that maleoylation took place at C-2 of the 3-linked unit. Maleoylate substitution at the 2-positions of 3-linked units would be expected to have most impact on the chemical shifts of the hydrogen atoms in the corresponding positions (i.e. β-Galp4S$_{int}$H-2 and β-Galp4S$_{nre}$H-2; the α-effect) and those adjacent to them (i.e. H-1 and H-3; the β-effect). So, 1.18 ppm downfield of β-Galp4S$_{int}$H-2 brought a new signal at 4.73 ppm for *O*-maleoyl LMW κ-carrageenan. It was concluded that a new peak at approx 4.6 ppm could appear due to downfield of β-Galp4S$_{nre}$ H-2. In fact, the new peak overlapped with others located in the same region. The signal of β-Galp4S$_{int}$ H-3 at 3.97 ppm become weaker due to the β-effect of 2′-maleoylation on it. This further confirmed that maleoylation took place at C-2 of the 3-linked unit. The full-range ^{1}H NMR spectra of LMW κ-carrageenan and its *O*-maleoyl derivative are given in Fig. 4. The peaks observed at about 6.9 and 8.0 ppm, we think, could be assigned to H-a and H-b in carbon-carbon double bond (C=C) of *O*-maleoyl LMW κ-carrageenan.

4. Conclusions

The preparation of *O*-maleoyl LMW κ-carrageenan has been achieved. The result showed that the rate of maleoylation of LMW κ-carrageenan from the tetrabutylammonium salt was higher than that from the tributylammonium salt. A monoester derivative with maleoyl group was formed when LMW κ-carrageenan reacted with maleic anhydride. The maleoylation took place regioselectively at C-2 of the κ-carrageenan 3-linked unit.

Acknowledgements

The authors are grateful for the financial support from Shantou University. The authors are also grateful to Dr D. -S. Zheng (College of Science, Shantou University) for useful comments and Mrs X. -F. Tian (College of Science, Shantou University) for her help in experiment.

References

Carlucci, M. T., Pujol, C. A., Ciancia, M., Noseda, M. D., Matulewicz, M. C., Damonte, E. B., & Cerezo, A. S. (1997). Antiherpetic and anticoagulant properties of carrageenans from the red seaweed *Gigartina skottsbergii* and their cyclized derivatives: correlation between structure and biological activity. *Journal of 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.

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 pp. 957.

Knutsen, S. H., & Grasdalen, H. (1992). The use of neocarrabiose oligosaccharides with different length and sulphate substitution as model compounds for 1H-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 Polmers*, *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., Fournier, J. P., & Choay, J. (1992). Selectively O-acylated glycosaminoglycan derivatives. *Carbohydrate Research*, *236*, 107–119.

Rees, D. A. (1977). *Polysaccharide Shapes*. USA: Chapman and Hall pp. 62.

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.

Yalpani, M. (1988). *Polysaccharides—Syntheses, Modifications and Structure/Property Relations*. Amsterdam: Elsvier pp. 444.

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.

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.

Yu, G., Guan, H., Ioanoviciu, A. S., Sikkander, S. A., Thanawiroon, C., Tobacman, J. K., Toida, T., & Linhardt, R. J. (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.