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Structural characterization and antioxidant activities of κ-carrageenan oligosaccharides degraded by different methods

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ABSTRACT

In the present study, four kinds of κ-carrageenan oligosaccharides were obtained by the degradation of parent κ-carrageenan using free radical depolymerization, mild acid hydrolysis, κ-carrageenase digestion and partial reductive hydrolysis, respectively. Their structure types were accurately and comparatively elucidated by ESI-MS and CID MS/MS. The antioxidant activities of different degraded products were investigated by four different antioxidant assays, including superoxide radical scavenging activity, hydroxyl radical scavenging activity, reducing power and DPPH radical scavenging activity. The methods of depolymerization had an influence on the antioxidant activities of κ-carrageenan oligosaccharides obtained from κ-carrageenan. These results indicated that the antioxidant activities of κ-carrageenan oligosaccharides could be related to the degree of polymerization, the content of reducing sugar and sulfate groups, and the structure of reducing terminus.

1. Introduction

Carrageenans are hydrophilic linear sulfated galactans extracted from numerous marine red seaweeds (Rhodophyta). They are major components of the matrix involved in the building of the cell-wall architecture. These polysaccharides share the same backbone structure, which consists of linear chains of 3-linked β-D-galactose (G-units) and 4-linked α-D-galactose (D-units). Carrageenans are classified according to the number and position of sulfate groups (S), and by the occurrence of 3,6-anhydro-bridges in α-linked residues (An-units). The three most commercially exploited carrageenans are kappa-(κ), iota-(ι) and lambda-(λ) carrageenans. In the food industry, carrageenans are widely utilized due to their exceptional physico-chemical properties, such as emulsifying, thickening, gelling and stabilizing abilities. These additives give textural properties and protective effects to a wide range of food products. Carrageenans are also extensively used in non-food products, such as pharmaceutical, cosmetics, printing and textile industries (Campo, Kawano, da Silva, & Carvalho, 2009; Prajapati, Maheriya, Jani, & Solanki, 2014). However, the high molecular weight (MW) and poor tissue-penetrating ability of carrageenan polysaccharides have influenced their biological action and limited their further application (Kalitnik et al., 2013). κ-Carrageenan oligosaccharides obtained via degradation of κ-carrageenan, possess significant potential for biomedical and physiological applications (Caram-Lelham, Sundelöf, & Andersson, 1995).

In recent years, the search for natural antioxidant compounds has gained considerable attention. Antioxidants are molecules which can delay or prevent the uncontrolled formation of free radicals and activated oxygen species, or inhibit their reactions with biological substrates. Reactive oxygen species (ROS) in the forms of superoxide anion ($\cdot O_2^-$), hydroxyl radical (·OH) and hydrogen peroxide (H_2O_2) are generated from the normal metabolism or exogenous factors and agents (Lee & Lee, 2006; Wang, Liu, et al., 2009; Wang, Wang, et al., 2009). The excessive production of ROS may attack macromolecules such as membrane lipids, proteins and DNA, leading to many diseases such as cancer, rheumatoid arthritis and atherosclerosis as well as in degenerative processes of aging (Sallmyr, Fan, & Rassool, 2008). Additionally, uncontrolled generation of ROS directly correlate with the

Abbreviations: G4S, 4-O-sulfate-β-D-galactopyranose; An, 3,6-anhydro-α-D-galactopyranose; ROS, reactive oxygen species; $\cdot O_2^-$, superoxide anion; ·OH, hydroxyl radical; H_2O_2, hydrogen peroxide; PMS, phenazine methosulfate; NADH, nicotinamide adenine dinucleotide-reduced; NBT, nitro blue tetrazolium; DPPH, 1,1-diphenyl-2-picrylhydrazyl; Dp, degree of polymerization.

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development of neurodegenerative and neuropsychiatric disorders like Cruetzfeldt-Jacob disease and Alzheimer's disease (Bleich et al., 2000; Chauhan & Chauhan, 2006). There are some synthetic antioxidant compounds, such as BHA (butylhydroxyanisol), BHT (butylhydroxytoluene) and TBHQ (*tert*-butylhyfroquinone), which are commonly used in the food industry as well as pharmaceutical industry. However, the commonly used synthetic antioxidants are restricted with concerns about toxicological safety. Considerable interest has arisen in developing alternative natural and highly active antioxidants (O'Sullivan, Lynch, Lynch, Buckley & Kerry, 2004; Soubra, Sarkis, Hilan, & Verger, 2007).

Marine algae represent one of the richest sources of natural antioxidants (Wijesekara, Pangestuti, & Kim, 2011). Carrageenans have been demonstrated to play an important role as potential ROS scavengers *in vitro* and antioxidants for the prevention of oxidative damage in living organisms (Yuan et al., 2006). Compared with ι- and λ-carrageenans, κ-carrageenan exhibited stronger antioxidant activities, which has attracted increasing interest in developing potential antioxidants (Abad, Relleve, Racadio, Aranilla, & de la Rosa, 2013). Antioxidant activities closely depend on the structural features such as the degree and position of sulfation, the molecular weight and the composition of the monosaccharides (Wang, Zhang, Zhang, & Li, 2008).

Partial depolymerization by chemical or enzymatic hydrolysis to obtain a range of oligosaccharides is a common strategy for detailed structural analysis and for use in activity assays (Yang et al., 2009). There are a lot of reports published on degradation of carrageenans through different chemical and enzymatic depolymerization (Yu et al., 2002). The structures of the resulting oligosaccharides have been analyzed using NMR spectroscopy (both ^{1}H and ^{13}C NMR) and mass spectrometry (ESI-MS and MALDI-MS) (Antonopoulos, Hardouin, Favetta, Helbert, & Lafosse, 2005; Van de Velde, Knutsen, Usov, Rollema, & Cerezo, 2002). However, few examples of comparative mass spectrometric studies on the carrageenan-derived oligosaccharides degraded by different methods have been described (Gonçalves, Ducatti, Grindley, Duarte, & Noseda, 2010; Sun et al., 2014; Yu et al., 2006). Furthermore, the effects of carrageenan oligosaccharides obtained from different degradation methods on the antioxidant activities have not been reported yet.

The purpose of this study was a comparative investigation of the antioxidant activities of κ-carrageenan oligosaccharides prepared by different methods. We prepared four kinds of κ-carrageenan oligosaccharides through free radical depolymerization, mild acid hydrolysis, κ-carrageenase digestion and partial reductive hydrolysis. The accurate structures of different prepared oligosaccharides were determined by ESI-MS and CID MS/MS. Antioxidant properties were assayed *in vitro* assay systems, including superoxide radical scavenging activity, hydroxyl radical scavenging activity, reducing power and DPPH radical scavenging activity. These results could be helpful to further understand the structure–activity relationships of carrageenan-derived oligosaccharides prepared by different methods.

2. Materials and methods

2.1. Materials

Purified food-grade κ-carrageenan was purchased from Lubao (Quanzhou) Biochemistry Co., Ltd (Jinjiang, Fujian, China). Borane-4-methylmorpholine complex, phenazine methosulfate (PMS), nicotinamide adenine dinucleotide-reduced (NADH), nitro blue tetrazolium (NBT), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Other chemical reagents were of analytical grade.

2.2. Preparations of the oligosaccharides

2.2.1. Free radical depolymerization

κ-Carrageenan (4 g) was dissolved in 3% H_2O_2 solution (400 mL), and incubated at 40 °C for designated time periods (1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, and 12 h, respectively) (Wu, 2014). Aliquots of the suspension were with-drawn periodically and cooled to room temperature. The oligosaccharides were precipitated using 5 volumes of absolute ethanol. The solution was centrifuged and dialysed against deionised water using a dialysis bag with a MWCO of 3500 Da. The dialysable (outside the dialysis bag) sample was then concentrated by rotary evaporation and freeze-dried.

2.2.2. Mild acid hydrolysis

κ-Carrageenan (4 g) was dissolved in 0.1 mol/L hydrochloric acid solution (400 mL), and kept at 60 °C for the above time periods. Aliquots of the suspension were with-drawn periodically and neutralized with 1 mol/1 NaOH (Yuan et al., 2005). The solution was dialysed against deionised water using a dialysis bag with a MWCO of 3500 Da. The dialysable (outside the dialysis bag) sample was then concentrated, desalted on a Sephadex G-25 column (50 × 2.0 cm), and lyophilized.

2.2.3. Enzymatic degradation

κ-Carrageenase was prepared from the cell-free medium of *Pedobacter hainanensis* 13-Q^T grown in κ-carrageenan by our laboratory. κ-Carrageenan was degraded by κ-carrageenase according to our previously described method (Sun et al., 2014). Briefly, the κ-carrageenan solution (0.2% in 0.05 mol/L Tris–HCl buffer, pH 7.0) was incubated with an enzyme aliquot (10 μg/mL) at 40 °C for designated time periods (4 h, 8 h, 12 h, 16 h, 24 h, 28 h, 32 h, 36 h, 48 h, 52 h, 60 h, and 72 h, respectively). Aliquots of the suspension were taken out at intervals and boiled in a 100 °C water bath to stop the reactions. The products were dialysed, desalted and lyophilized as above.

2.2.4. Partial reductive hydrolysis

The reductive hydrolysis method was a modified version of the method described by Usov and Elashvili (1991). Briefly, κ-carrageenan (1 g) was dissolved in water (100 mL), and the solution was heated to 60 °C. Borane-4-methylmorpholine complex (4 g) was then added followed by 2 mol/L trifluoroacetic acid (10 mL). The mixture was incubated at 65 °C for designated time periods (1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, and 12 h, respectively). After the reaction, the acid was evaporated with water, the residue was then dissolved in small amount of water, dialysed, desalted and lyophilized.

2.3. Reducing-sugar content and sulfate content assay

The hydrolytic process of different methods was analyzed by measuring the increase of reducing-sugar ends formed by hydrolysis, using the modified dinitrosalicylic method of Dubois, Gilles, Hamilton, Rebers, and Smiths (1956). The κ-carrageenan oligosaccharides obtained by the four methods at their designated intervals were assayed for reducing sugar content. Sulfate content of different hydrolysates was measured by the barium chloride-gelatin method using K_2SO_4 as standard (Dodgson & Price, 1962).

2.4. ESI-MS and CID MS/MS analysis of κ-carrageenan hydrolysates

The κ-carrageenan oligosaccharides obtained by different methods were analyzed using a LTQ-XL ion-trap mass spectrometer equipped with an electrospray ion source and an HPLC system (Thermo-Fisher, Waltham, USA) in negative ion mode (Sun et al.,