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Research Paper

Purification and Characterisation of κ-Carrageenan Oligosaccharides Prepared by κ-Carrageenase from *Thalassospira* sp. Fjfst-332

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ABSTRACT

κ-Carrageenan oligosaccharides (KCOs) are promising agents for treating inflammatory diseases. However, the lack of purification and structural elucidation of KCOs has limited structure-function evaluation. In this study, using a system coupling medium pressure liquid chromatography (MPLC) with an evaporative light scattering detector (ELSD), four types of KCOs were separated. The total yield of the four KCO powders was ~5.02% after purification (KCOs/κ-carrageenan, w/w). Their structural identities were characterised by ESI-MS, CID-MS/MS and NMR, as κ-neocarrabiose (α-DA-1,3-G4Srα/β), κ-neocarratetraose (α-DA-1,3-β-G4S-1,4-α-DA-1,3-G4Srα/β), κ-neocarrahexaose (α-DA-1,3-β-G4S-1,4-α-DA-1,3-β-G4S-1,4-α-DA-1,3-G4Srα/β) and heterozygous κ/ι-neocarrahexaose (α-DA/DA2S-1,3-β-G4S-1,4-α-DA-1,3-β-G4S-1,4-α-DA-1,3-G4Srα/β). KCOs showed no cytotoxicity in RAW264.7 macrophages, and the anti-inflammatory activity was closely correlated with the degree of polymerisation and the number of sulfated groups. κ/ι-Neocarrahexaose exhibited the highest inhibition of ROS (Reactive Oxygen Species) production in LPS-induced RAW264.7 macrophages. The MPLC-ELSD system provides a platform for large-scale fabrication of purified KCOs and affords a route to these compounds that may regulate immune defense.

1. Introduction

Carrageenan oligosaccharides (COs), the degraded carbohydrates of carrageenans, are composed of an alternating backbone of β-1,3-D-galactose (G unit) and α-1,4-D-galactose (D unit). Based on the position and number of sulfate ester units (S) and the presence of 3,6-anhydrogro-bridges (A) on D units, COs are classified into 13 types denominated by Greek letter prefixes, including κ-(DA-G4S), ι-(DA2S-G4S) and λ-(D2S6S-G2S) (Necas and Bartosikova, 2013). The functional activities of COs are closely related to the degree of polymerisation (Dp), as well as the number and position of sulfate units. However, elucidating the relationship between biological activity and structure remains challenging because large-scale fabrication the purification has not been achieved.

Inflammation is regarded as a major risk factor for the pathogenesis of cancer and various other chronic diseases. Immune cells such as macrophages regulate inflammation and host defences through secretion of cytokines including NO, interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and reactive oxygen species (ROS) (Kofler, Nickel, & Weis, 2005). However, excessive expression of macrophages results in tissue damage and cell death. Therefore, modulation of macrophage activation is an effective method for preventing inflammatory responses (Alvarez-Suarez et al., 2017). COs are potential pharmaceutical agents possessing multiple bioactivities including antiviral, anti-tumour, antioxidant and immunoregulatory activities (Wang et al., 2011; Yuan et al., 2005). In particular, the immunomodulation and antitumor activities of mixed κ-carrageenan oligosaccharides (KCOs) have been investigated in S180-bearing mice, and they exert their antitumor effects by promoting the immune system (Yuan, Song, Li, Li, & Dai, 2006). The immunomodulatory function of mixed KCOs has also been studied in lipopolysaccharide (LPS)-activated microglial cells, and their biological function is closely correlated with structure, especially the sulfate groups (Yao, Xu, & Wu, 2014). However, the immunomodulatory functions of KCOs with varying Dp are

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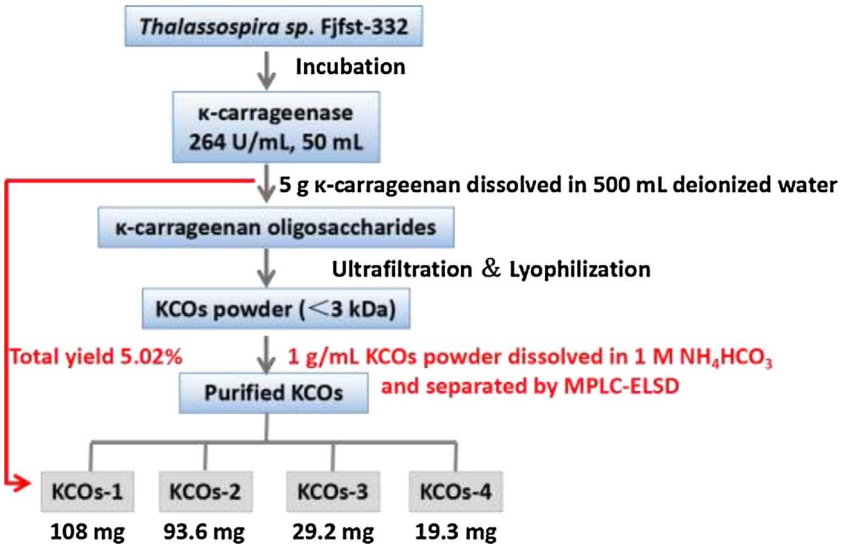

Fig. 1. Schematic diagram of KCOs enzymatic hydrolysis preparation and purification.

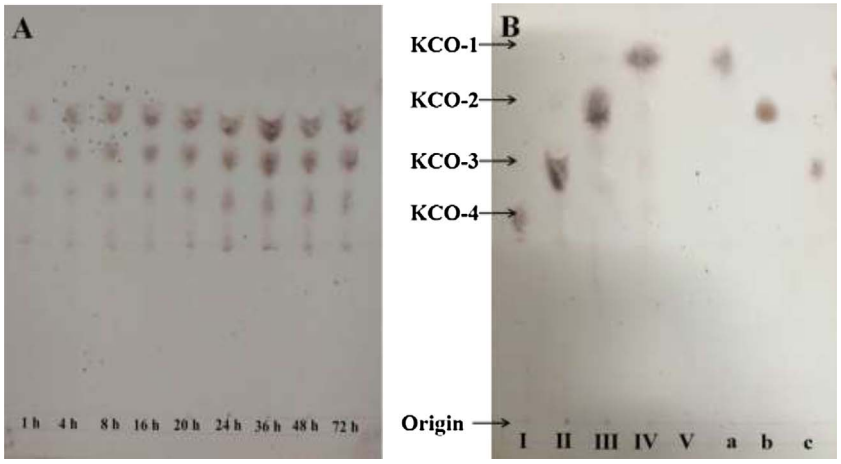

Fig. 2. TLC analysis of KCOs. (A) The components prepared through diverse enzymatic time. (B) I-V represented the components that separated by MPLC-ELSD system, a was the standards of κ-carrabiose alditols, b is the standards of κ-carratetraose alditols, c is the standards of κ-carrahexaose alditols.

not well understood, due to low yield and difficulties with purification. Thus, establishing a platform for the large-scale fabrication and purification of COs is necessary.

Column chromatographic systems consisting of a pump, detector and chromatographic column are commonly used for the separation and purification of natural products. Recently, high-pressure anion-exchange chromatography (HPAEC) (Jouanneau, Boulenguer, Mazoyer, & Helbert, 2010b), high-performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD) (Niu, Zhang, Chen, & Yan, 2015), AKTA-fast protein liquid chromatography (FPLC) (Zhang et al., 2010) and high-performance gel permeation chromatography (HPGPC) (Duan, Yu, Liu, Tian, & Mou, 2016) have been used to purify COs. However, these technologies have drawbacks including a low loading amount, and can be complex to operate. Therefore, medium-pressure liquid chromatography (MPLC), a type of flash chromatography, has been developed to separate carbohydrates. This high-resolution method can be easily automated with an autosampler for peak collection (Weber, Hamburger, Schafroth, & Potterat, 2011). Furthermore, compared with the aforementioned technologies, the loading amount can be relatively high, ranging from milligrams to hectograms, facilitating large-scale separation of naturally occurring compounds (Challal et al., 2015). However, since carbohydrates do not adsorb ultraviolet (UV) light, a phenol-sulfuric acid approach is usually used for assessment, while evaporative light scattering detection (ELSD) provides an ideal detection system for carbohydrates (Young and Scientific, 2003). MPLC systems coupled with ELSD have been used for separating lotus seed oligosaccharides but not COs (Lu et al., 2017), which have a high polarity due to the sulfate groups, and varying Dp, making the preparation CO monomers challenging. Gel filtration has been used to purify COs using a Bio-Gel P-2 column (Sun et al., 2014), a Superdex 30 pg column (Niu et al., 2015), a TSK gel GMPWxl column (Zhang et al., 2010) and a Q-Sepharose Fast Flow column (Yang et al., 2011). Compared with other columns, the Superdex 30 pg column provides advantages such as high resolution, a rigid matrix, and good retention properties (Caram-Lelham, Sundelöf, & Andersson, 1995). However, when a Superdex 30 pg column is applied in HPLC or HPGPC, the low loading amount remains a limiting factor preventing large-scale purification and fabrication.

In the present study, we used MPLC coupled with ELSD and a HiLoad Superdex 30 prep grade column to separate KCOs from the Thalassospira sp. Fjfst-332 strain (Fig. 1). Compared with traditional methods, the approach removed the need for a pre-treatment operation such as desalting and depigmentation, which simplified the processing steps. Furthermore, the method provides an ideal automated, high-resolution, rapid separation compatible with a high loading amount. ESI-MS (Electrospray Ionization Mass Spectrometry), CID-MS/MS (Collision Induced Dissociation-Tandem Mass Spectrometry) and NMR (Nuclear Magnetic Resonance) were used to characterise the structural sequence,