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Assessment of the oxidative cellular toxicity of a κ-carrageenan oxidative degradation product towards Caco-2 cells

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ABSTRACT

Carrageenan, especially degraded carrageenan (poligeenan) has been known to induce colonic inflammation for a long time. We isolated an oxidized degradation product (κ-CODP) from κ-poligeenan. To explore the toxic potential of this byproduct on human colonic epithelial cells, we examined the oxidative toxicity and pro-inflammatory effects on Caco-2 cells. κ-CODP showed cytotoxicity against Caco-2 cells with concentration higher than 40 μg/mL, and this event was accompanied by the increase of reactive oxygen species (ROS), while the addition of the antioxidant N-acetyl-L-cysteine was able to rescue the fatal effects of κ-CODP on cell growth and apoptosis. The molecular signals following κ-CODP exposure were shown as: the increased secretion of two inflammatory molecules, namely, IL-8 and TNF-α; the activation of NF-κB translocation; and the up-regulation of expression of p-JNK and p-ERK protein. It was suggested that κ-CODP may activate the MAPK signaling pathway and the pro-inflammatory transcription factor NF-κB, leading to phosphorylation of transcription factors that participate in the regulation of cell inflammatory action. These findings suggest that κ-CODP generated during food or medicine processing may influence the cell function of colonic epithelial cell by inducing apoptosis and inflammatory response through ROS production.

1. Introduction

Carrageenan, a high molecular weight sulfated polygalactose obtained from marine macroalgae, has been applied widely as an ingredient in the food industry to improve the texture of food products, by acting to thicken, stabilize or emulsify dairy products, salad dressings, infant formulas, processed meat, soymilk and other food products (Weiner, Nuber, Blakemore, Harriman, & Cohen, 2007; Şen & Erboz, 2010). The safety of carrageenan has been assigned as GRAS (Generally Regarded as Safe) status, which means it is an acceptable daily intake of "not specified", many agencies have evaluated, however, there are alleged harmful effects that oral exposure to carrageenan can produce significant mucosal ulceration of the colon, especially the degraded carrageenan (poligeenan), which is manufactured by the acid hydrolysis of carrageenan at high temperature (>80 °C) (Cohen & Ito, 2002; Tobacman, 2001; Wakabayaski, Inagaki, Fujimoto, & Fukuda, 1978). Studies also demonstrated that the colonic inflammation produced by oral administration of carrageenan or poligeenan is similar to that found in human inflammatory bowel disease, such as ulcerative colitis, Crohn's disease and coeliac disease (Hata et al., 2006). A number of these bowel disorders are characterized by accelerated epithelial cell turnover and apoptosis, leading to altered crypt/villus morphology (Ramachandran, Madesh, & Balasubramanian, 2000). Since epithelial surface is constantly exposed to antigens from the normal microbiota, food antigens and pathogenic microorganisms, unscheduled epithelial cell apoptosis may lead to the breakdown of epithelial barrier function, facilitating the invasion of pathogenic microorganisms and initiating the aggressive inflammatory immune response. In this sense, the effects of degraded κ-carrageenan on epithelial cells attract our continuous serious considerations.

During our studies, we degraded κ-carrageenan by dilute hydrochloric acid at 80 °C for 6 h, and separated the mixtures by Sephadex G25 and G15 columns. We isolated an oxidized oligosaccharide derivative (Fig. 1). This compound was the byproduct of the carrageenan acid hydrolysis process. Literature studies showed that such hydroxyaldehyde structure can enolize and autoxidize to diketone or glyoxal, with concomitant production of superoxide radical, which may possess strong oxidant activity to interact with proteins, lipids, or nucleic acids (Robertson, Fridovich, Misra, & Fridovich, 1981; Abordo, Minhas, & Thornalley, 1999). They can also amplify oxidant damage in a vicious circle to elicit a series of events resulting in the activation of p53 tumor suppressor protein, originating the activity of caspase system, and finally producing internucleosomal DNA breaks that are characteristic of apoptotic death (Suzuki & Miyata, 1999; Forman, Torres, & Fukuto, 2002).

Abbreviations: Caco-2, human colon adenocarcinoma cell line; κ-CODP, κ-carrageenan oxidative degradation production; DAPI, 4′,6-diamidino-2-phenylindole; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid; FACS, flow cytometry; FITC, fluorescein-5-isothiocianate; FSC, forward scatter; GRAS, generally regarded as safe; GSH, glutathione; IκB, inhibitor of kappa B; NAC, N-acetyl-L-cysteine; PBS, phosphate buffered saline; PDTC, pyrrolidine dithiocarbamate; PVDF, polyvinylidene fluoride; SSC, side scatter; TBST, tris-buffered saline Tween-20.

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Recently, many other glycolaldehydes were reported to increase the intracellular reactive oxygen species (ROS) production, cause growth arrest and loss of viability (Al-Enezi, Alkhalaf, & Benov, 2006).

However, little information is available on the effects of degraded carrageenan oxidative product on cells, particularly on ROS generation, apoptosis or inducing inflammatory factors, considering the wide distribution and sometimes high concentration of κ-CODP in certain foods (Shah & Huffman, 2003). Therefore, we tested the Caco-2 cell apoptosis and inflammatory cytokines production induced by scalar doses of κ-CODP incubated at different time, in order to obtain a deeper insight into its underlying mechanisms, such as ROS production, glutathione (GSH) level, gene transcription and translation of some key proteins, including p38, NF-κB, ERK and JNK.

2. Materials and methods

2.1. Materials

κ-carrageenan oxidative degradation production used in this study was obtained by hydrochloric acid-mediated hydrolysis: 1% κ-carrageenan (Sigma) was hydrolyzed by 0.1 mol/L hydrochloric acid at 80 °C for 6 h. The hydrolysate was neutralized and concentrated by rotary evaporation. The mixture was then fractionated based on size by a Sephadex G25 and Sephadex G15 columns. Fractions separated were freeze-dried and stored at 4 °C prior to experiments. The structural characterisation was identified by UPLC-Q-TOF-MS (Waters) and NMR (Bruker).

MS analysis was performed by a Waters UPLC-ESI-Q-TOF Premier mass spectrometer operating in positive ion electrospray mode. In order to promote the accuracy and detection sensitivity, an ion pairing reagent (heptylamine) was used. The mass scan range was 300–4000 amu. The source temperature was 120 °C. The electrospray capillary voltage was set at 2.5 kV, the sampling cone voltage was set at 40 V, and the collision energy was set at 5 V. The ESI-MS spectrum present ions consisting of the oligomer attached with a number of heptylammonium ions depending on the molecule size. In our case, the precise molecular mass $[M+(C_7H_{15}NH_3^+)_3–H_2O]^+$ is 1302 (Fig. 1a).

^{1}H NMR spectra were recorded with BRUKER DRX-400 NMR spectrometer, at a probe temperature of 25 °C. Chemical shifts are expressed in ppm with reference to the external standard

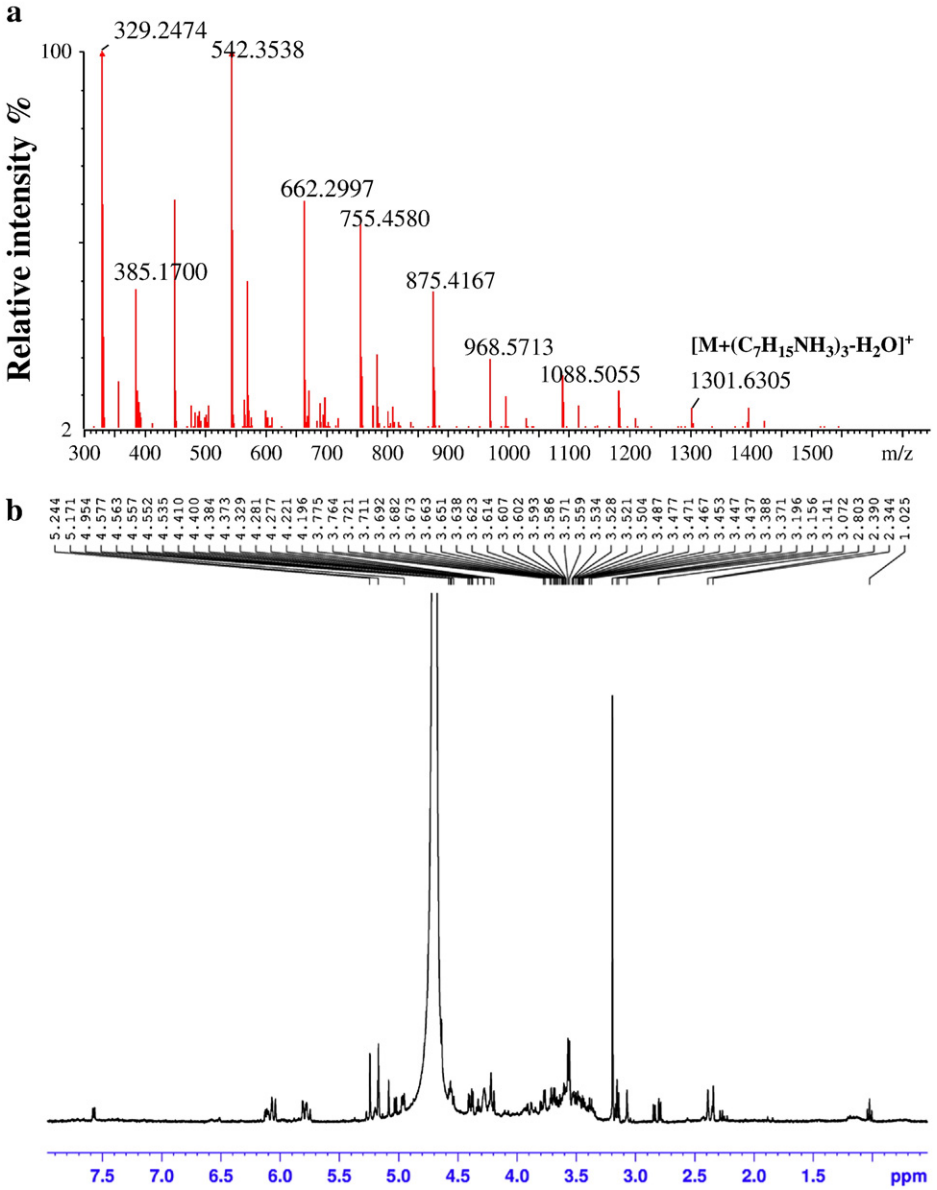

Fig. 1. (a) Positive ion-mode ESI-MS; (b) ^{1}H NMR; (c) ^{13}C NMR and (d) ^{1}H COSY spectra of the isolated oligosaccharide derivative. (e) Structure of κ-carrageenan oxidative degradation product.