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Influence of marine oligosaccharides on the response of various biological systems to UV irradiation

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ABSTRACT

Low molecular weight alginate-derived oligosaccharide (ADO) (373–571 Da) and chito-oligosaccharide (COS) (855–1671 Da) were purified from alginate and chitosan, and known as marine oligosaccharides with polyanionic and polycationic properties, respectively. We compared the effects of ADO and COS on cell regulation using several biological models (*Candida albicans*, *Escherichia coli* and *Bacillus subtilis* spore), cellular uptake determination, erythrocytes haemolysis inhibition and antioxidant capacity assay to investigate stress response under UV radiation. Our results further confirmed the anti-UVR potential of ADO and COS and their potential for commercial UVR protector application in the area of functional foods as food ingredients.

1. Introduction

Ultra-violet radiation (UVR) stress-induced cell damage has long been implicated in skin diseases (e.g. tumour) (Armstrong & Krickerb, 2001), skin disorders (e.g. photoaging and hyperpigmentation) (Krutmann, 2000), and immunological unresponsiveness (Kripke, 1984). Oxidative stress, DNA damage and cell inflammation could be caused by acute or chronic UVR exposures, which lead to these undesirable damages in organisms. Numerous studies have shown that the incidence of these damages is growing with increase in the amount of sunlight reaching the earth surface and the extent of artificial sun bathing activities. Among the therapeutic strategies against UVR induced damages, oligosaccharide, also known as a non-toxic natural substance, is raising the attentions from the general public. For example, the alginate-derived oligosaccharides (ADO) and chito-oligosaccharides (COS) have been utilized to evaluate the reactive oxygen species (ROS) scavenging effects, which can be induced by UVR and lead to cell apoptosis (Kulms, Zeise, Pöppelmann, & Schwarz, 2002).

ADO and COS have been regarded as non-toxic, -immunogenic, -carthogenic, soluble and biodegradable polymers, making them excellent candidates for a wide range of biomedical applications. ADO is a block copolymer degraded from alginate (prepared from brown algae), in which continuous β-D-mannuronic acid (M) and/or α-L-guluronic acid (G) units are joined through 1,4-O-glycoside bonds and arranged in a form of a homopolymeric (pM or pG-blocks) and/or heteropolymeric sequences (pMG blocks). COS, a linear polymer

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Abbreviations: COS, chito-oligosaccharides; ADO, alginate-derived oligosaccharides; T-AOC, total antioxidant capacity; UV, ultraviolet; UVR, ultraviolet radiation; ROS, reactive oxygen species; PDA, potato dextrose agar; BPM, extract peptone medium; OD, optical density; TBA, thiobarbituric acid; MWCO, molecular weight cut off; DA, degree of acetylation; MW, molecular weight

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composed of β-1,4-linked D-glucosamine residues with low degrees of *N*-acetylated residues, is the degradation and deacetylation products of chitosan (prepared form chitin).

The biological activities of ADO and COS, and their applications in pharmaceutical and food applications have attracted increasing attentions (Aam et al., 2010; Freitas, Rodrigues, Rocha-Santos, Gomes, & Duarte, 2012; Hayes, 2012). These two functional oligosaccharides have been used extensively both as pharmaceuticals, to inhibit tumour growth (Shen, Chen, Chan, Jeng, & Wang, 2009), to inhibit DNA and protein oxidation (Ngo, Lee, Kim, & Kim, 2009), to reduce cardiovascular and renal damage (Terakado et al., 2012) and to improve immune response (Yan, Guo, Yuan, Liu, & Zhang, 2011), and as food ingredients (Xu, Chao, & Wan, 2009; Xia, Liu, Zhang, & Chen, 2011), to maintain weight-loss, to aid glucose control for diabetic patients and to reduce serum total cholesterol. For marine living organisms, production of oligosaccharides can protect them from radiation and oxidation in the presence of appropriate cell signals and prohibit cell apoptosis, via mechanical, chemical and biological pathways. Hence, the contribution of chemical and biological properties of these oligosaccharides to physical health was deemed as the critical factor for preventing cell damage.

From the physiochemical standpoint, it is extremely important that ADO and COS are hydrosoluble and negatively and positively charged. These characteristics enable each of them to cooperate with negatively or positively charged biomacromolecules, e.g. lipids, proteins, amino acids, and genes which have labile properties that are susceptible to degradation under stressful condition during metabolic processes, or interact with specific ionic compounds in a biological system. Thus, these particular cross-linking features can be explored for cell's resistance to irradiation, and then stabilize the normal living cell system. Additionally, from the biochemical viewpoint both of them possess the individual characters of cell mucosal surfaces adhesion to form multicellular structure, which reveals how the marine oligosaccharide-signal modulates cell behavior to reduce radiation damage. Indeed, obtaining new knowledge on the capacity of marine-oligosaccharides in regulating cellular adhesiveness and migration could pave the way to advance our application of cellular anti-radiation (Janes, Calvo, & Alonso, 2001; Klausen, Gjermansen, Kreft, & Tolker-Nielsen, 2006), an important issue in the area of functional foods (Annunziata & Vecchio, 2011; Chen, Ma, Liang, Peng, & Zuo, 2011). However, their exact functional mechanism, chemical structure and promotional capacity in terms of chemical properties in the application for UVR resistance have not been done at the present time.

Recently, we described the use of a set of oligosaccharides for detecting the antioxidative and stress resistance effects with great effectiveness and (Liu et al., 2009; Wang et al., 2007). Due to the paucity of information on the UVR resistance effect of ADO and COS and its mechanism of action, we investigated their roles in protecting cells from UV irradiation with selected biological models. We also re-examined the antioxidant potential, one of the popular design objectives of functional foods (Ramadan & Al-Ghamdi, 2012), of these oligosaccharides against the stress caused by free radicals. By providing the convincing evidence on the anti-UVR property of ADO and COS, we aim at elucidating the mechanisms of their UVR resistance action.

2. Materials and methods

2.1. Materials

Alginate and chitosan were purchased from Hua-hai Pharmaceutical industry (Qingdao, China), and Qingdao Hecreat Biotech Company Ltd. (Qingdao, China), respectively. Total antioxidant capacity (T-AOC) kits were purchased from Jiancheng Bioengineering Co. Ltd., Nanjing, China. Chitosanase, alginate lyase, chicken derived erythrocytes, *Candida albicans, Escherichia coli, Bacillus subtilis,* and other cell culturing materials were obtained from Applied Microbiology Laboratory (Ocean University of China, Qingdao, China). The best grade or analytical grade chemicals and other materials were purchased commercially.

2.2. Preparation of marine-oligosaccharides

2.2.1. Preparation of ADO

Alginate lyase (30.72 U/mg) was purified by using *Vibro* sp. 510 (CCTCC# M200015) based on our modified protocol (Zhang et al., 2004). Briefly, 5 g alginate (M/G, 2.28; molecular weight (MW), 300 kDa) were added to 1000 mL of 50 mM Tris–HCl buffer (pH 7.5) with subsequent mixing with 50 units alginate lyase. The enzymolysis reaction was then kept in boiling water for 10 min after storage at 28 °C for 24 h. After that same volume of absolute ethanol was added, centrifugation was carried out at 3000 rpm (987×*g*) for 30 min (Anke GL-20G-II, Anting Scientific Instrument Factory, Shanghai, China). The precipitates and supernatant were removed and collected, separately. The suspended hydrolysates were passed through a 0.45 μm membrane filter. The filtrate was filtered for a subsequent UF treatment with molecular weight cut off (MWCO) 1 kDa (0.15 MPa, 0.6 m/s), lyophilized (FD-1A Freeze Dryer, Boyikang Laboratory Instruments Co. Ltd., Beijing, China) at 0.01 mBar for 48 h and stored at −20 °C. The oligosaccharide molecule mass was elucidated based on Micromass Q-TOF Ultima Global Mass Spectrometer (Waters, Milford, MA, USA) analysis.

2.2.2. Preparation of COS

Chitosanase was prepared to hydrolyze chitosan by using *Paecilomyces sp. JW1727* (CCTCC# 200025) with high enzyme activity (500 U/mL) (Jiang & Wang, 2003). Briefly, 100 g of chitosan (degree of acetylation (DA), ~65%; MW, 250 kDa) were suspended in a solution of chitosanase (3 U/g chitosan) in 950 mL distilled water; subsequently, 50 mL of acetic acid were added to the chitosan suspension and stirred at 50 °C for 24 h. After removing the precipitates by centrifugation at 5000 rpm (2712×*g*) for 30 min, the hydrolytic solution was separated using UF membrane with MWCO 3 kDa (0.15 MPa, 0.6 m/s). Lyophilization was carried out using the FD-1A Freeze Dryer at 0.01 mBar for 48 h. The hydrolysis mixture was analyzed with a BiflexTM II MALDI-TOF Mass Spectrometer (Bruker Daltonics Co. Ltd., Bremen, Germany).