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The sulfated polysaccharide from a marine red microalga as a platform for the incorporation of zinc ions

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ABSTRACT

The cell-wall sulfated polysaccharide of the marine red microalga *Porphyridium* sp. is a high molecular weight biopolymer that has potential for use as a platform for metal complexation for various applications. This paper describes the structural and rheological characterization and antibacterial activity of the polysaccharide in combination with Zn^{2+} (Zn-PS). SAXS and rheology studies indicate that with the addition of $ZnCl_2$ to the sulfated polysaccharide the only change was the increase in viscosity in the entangled regime. The antibacterial activity of Zn-PS solutions was more potent than that of the native polysaccharide against Gram-negative and Gram-positive bacteria. The synergy between the bioactivities of Zn^{2+} (which is a key player in wound healing and is active against variety of pathogens) and the unique bioactivities of the polysaccharide (e.g., anti-inflammatory) indicates promising potential for the development of novel products for the pharmaceutical and cosmetics industries.

1. Introduction

A variety of polysaccharides are currently under development as novel antibacterial agents in light of their inherent biocompatibility. Such polysaccharides do not suffer from the disadvantages of many currently available antimicrobial products, namely, bacterial resistance, high toxicity to humans, short shelf life or high production costs (Stratton, Howarter, Allison, Applegate, & Youngblood, 2010). Among the polysaccharides with intrinsic antimicrobial activity are the natural polysaccharides chitin and chitosan (and its derivatives) and a plethora of synthetic polymers (Benhabiles et al., 2012; Muñoz-Bonilla & Fernández-García, 2012; Xia, Liu, Zhang, & Chen, 2011). The antibacterial activity of these materials can be promoted by chemical modification or through the incorporation of metal salts (Muñoz-Bonilla & Fernández-García, 2012). For example, the antimicrobial activity of chitosan-metal (Zn, Cu or Fe) complexes against Gram-positive and Gram-negative bacteria and fungi was shown to be superior to that of free chitosan and metal salts (Wang, Du, Fan, Liu, & Hu, 2005). Similarly, it was shown that the anti-viral activity of a carrageenan–zinc acetate combination was synergistically superior to that of carrageenan alone (Fernandez-Romero et al., 2012). Yet there is still a need for novel biocompatable antimicrobials materials.

A sulfated polysaccharide produced by the marine red microalga *Porphyridium* sp. has been the subject of intensive study in the Arad (Malis) laboratory for a number of years and is already in use in many applications (Arad (Malis) & Levy-Ontman, 2010, 2013). Nonetheless, the structure, chemical composition and physical properties of the sulfated polysaccharide are not fully known, partially due to its structural complexity and to the absence of known carbohydrolases that can degrade it (Arad (Malis) & Levy-Ontman, 2010). However, it is known that the polysaccharide is composed of about 10 different sugars, of which the main monosaccharides are xylose, glucose and galactose. In addition, disaccharide (Geresh, Dubinsky, Arad (Malis), Christiaen, & Glaser, 1990; Jaseja et al., 1989) and tetrasaccharide (Geresh et al., 2009) have been isolated and characterized. It is also known that the sulfated polysaccharide has a molecular mass of $2–7\times10^6$ Da and that it is negatively charged due to the presence of glucuronic acid and half-ester sulfate groups (Geresh & Arad (Malis), 1991).

X-ray diffraction studies have revealed that in solution the polysaccharide consists of a single two-fold helical structure with

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a pitch of 1.6 nm. Thus, it has been suggested that in solution the polysaccharide chains adopt a helical, wormlike chain cylinder conformation consisting of helical segments joined by more flexible moieties (Eteshola, Karpasas, Arad (Malis), & Gottlieb, 1998). The polysaccharide structure and conformation have, however, not been investigated by small angle X-ray scattering (SAXS), a technique that is commonly used to characterize the structural features of polymers in solution—a lacuna that we address in the current study.

The external part of the sulfated polysaccharide that encapsulates the algal cells dissolves from the cell surface into the medium, thereby increasing the viscosity of the medium (Ramus & Groves, 1972). Solutions of the polysaccharide tend to be highly viscous at low polymer concentrations. Such solutions are stable over a wide range of temperatures (30–160 °C), pH values (2–9), and salinities (Arad (Malis) & Levy-Ontman, 2013). It has thus been suggested that polysaccharide serves as a buffer layer around the cells, protecting them from extreme environmental conditions, or as a physical barrier, preventing attack from bacteria, viruses, and fungi (Arad (Malis), 1988).

In contrast to the paucity of information on the physical characterization of the *Porphyridium* sp. sulfated polysaccharide, a considerable number of studies have been devoted to its biological functions. The polysaccharide has been shown to possess a variety of bioactivities, among them antiviral (Arad (Malis), Ginzberg, & Huleihel, 2006; Huheihel, Ishanu, Tal, & Arad (Malis), 2002; Huleihel, Ishanu, Tal, & Arad (Malis), 2001), antioxidant (Tannin-Spitz, Bergman, van-Moppes, Grossman, & Arad (Malis), 2005), anti-inflammatory and soothing activities (Matsui, Muizzuddin, Arad (Malis), & Marenus, 2003), and it can also act as biolubricant (Arad (Malis), Rapoport et al., 2006; Gourdon et al., 2008).

Due to the negative charge of the polysaccharide and to its ion exchange capacity (Arad (Malis) & Levy-Ontman, 2010), it has been suggested that the polysaccharide can serve as a platform for metal incorporation with the aim to create synergistic bioactivities. For example, the complexation of copper ions to the polysaccharide was recently shown to modulate bacterial adhesion and prevent biofilm formation (Golberg et al., 2016). In the current study, we focused on the zinc-polysaccharide (Zn-PS) combination, particularly, on its physical characteristics and antibacterial activity.

Zinc was chosen because it is an essential trace element known to play key roles in cell replication and division, protein synthesis, gene expression, and the metabolism of nucleic acids. It also acts as a cofactor in numerous enzymes (Lansdown, Mirastschijski, Stubbs, Scanlon, & Ågren, 2007; Pasquet et al., 2014). During the process of wound healing there is a need for zinc ions, because many enzymes and proteins that direct the process of skin regeneration are zinc dependent (Lansdown et al., 2007; Qin, Zhu, Chen, Liang, & Wo, 2007). Applying zinc alone topically may damage or irritate the skin therefore, zinc is often added to products when applied topically for wound healing (Lansdown et al., 2007).

In addition, several studies have reported that zinc salts exhibit antibacterial activity against variety of bacteria (Atmaca, Gul, & Clcek, 1998; Lansdown et al., 2007) and antiviral activity against vaginal herpes simplex virus type 2 in vitro and in animal models (Bourne et al., 2005).

In the current study, we explored *Porphyridium* sp. sulfated polysaccharide as a platform for zinc incorporation- Zn-PS, characterize its physical properties and antibacterial activity. We believe that the synergy between zinc, with its variety of bioactivities, and the polysaccharide, with its unique structure and bioactivities, have potential for the development of novel products for the cosmetics and pharmaceutics industries.

2. Materials & methods

2.1. Algal growth and polysaccharide production

Porphyridium sp. (UTEX 637) obtained from the culture collection of the University of Texas at Austin was grown in artificial seawater (Jones, Speer, & Kury, 1963). Culture of the algal cells and isolation of the extracellular polysaccharide were performed as previously described (Cohen & Arad (Malis), 1989). Sugar content was determined using the phenol-sulfuric acid reaction (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.2. Preparation and stability measurements of Zn-PS samples

$ZnCl_2$ was dissolved in deionized water, and the solution was added to a ~1.1% polysaccharide solution to give a final concentration of 250–2000 ppm Zn^{2+}, as required for the different experiments, and a 1% w/v concentration of polysaccharide. The Zn-PS solution was gently stirred with a magnetic stirrer for 2 h at room temperature and then sterilized by autoclaving. For the stability measurements, 5-ml samples of Zn-PS (1000 ppm Zn^{2+}) were stored in polypropylene bottles under the following conditions: in the dark at 4, 22, and 42 °C and in the light at 22 and 42 °C. After 1, 4, 12 and 24 weeks the following parameters were examined: viscosity, conductivity, pH, sterility, polysaccharide content and general appearance of the Zn-PS solutions.

2.3. Viscosity

Viscosities of the polysaccharide solutions was determined using a HAAKE RotoVisco 1 (Thermo Scientific), equipped with an extended temperature cell for temperature control and a stainless steel cone-and-plate (d = 60 mm and q = 0.5°). The viscosities of the polysaccharide solutions were measured at constant room temperature as a function of the shear rate in an upward sweep from 1 s^{-1} to 1000 s^{-1}.

2.4. Small angle X-ray scattering

SAXS patterns of polysaccharide solutions were obtained with a SAXSLAB GANESHA 300-XL. Cu Kα radiation was generated by a Genix 3D Cu-source with an integrated monochromator, 3-pinhole collimation and a two-dimensional Pilatus 300 K detector. The scattering intensity was recorded in the interval $0.012 < q < 3$ Å^{-1} Measurements were performed under vacuum at ambient temperature. The scattering curves were corrected for counting time and sample absorption. The solution under study was sealed in a thin-walled quartz capillary of about 1.5 mm diameter and 0.01 mm wall thickness. Data analysis was based on fitting the scattering curve to an appropriate model by software provided by NIST (NIST SANS analysis version 7.0 on IGOR) (Kline, 2006).

2.4.1. Model fitting of small-angle scattering pattern

The form factor of a semiflexible chain with excluded volume effects (Chen, Butler, & Magid, 2006; Pedersen & Schurtenberger, 1996) is expressed by:

$$P(q,b,L,R_{cs}) = P_{exv}(q,b,L) + C(L/b)\frac{b}{15L}\left[4+\frac{7}{u}-\left(11+\frac{7}{u}\right)e^{-u}\right]\cdot\left[2\frac{J_1(q,R_{cs})}{q,R_{cs}}\right] \quad (1)$$

$$P_{exv}(q,b,L) = \left[1-w(qR_g)\right]\cdot P_{Debye}(q\cdot b\cdot L) + w(qR_g)\left[C_1(qR_g)^{-1/\nu}C_2(qR_g)^{-2/\nu}C_3(qR_g)^{-3/\nu}\right] \quad (2)$$

Details of the functions can be found in a manuscript published by Pedersen and Schurtenberger (1996).