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DNA interaction, antitumor and antimicrobial activities of three-dimensional chitosan ring produced from the body segments of a diplopod

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ABSTRACT

Commercially available chitins and the chitin isolated from mushrooms, insect cuticles, shells of shrimp, crab and crayfish reported in the literature are in forms of powder, flake or granule. Three-dimensional chitins have been only known from the sponges but still three-dimensional chitosan has not been reported yet. In this study, we produced three-dimensional chitin and chitosan rings from the body segments of a diplopod species (*Julus terrestris*). Obtained chitin and chitosan rings were characterized (by FT-IR, SEM, TGA, XRD, dilute solution viscometry and EA) and compared with commercial chitin and chitosan. The interactions with plasmid DNA was studied at varying concentrations of chitosan (0.04, 0.4 and 4 mg/mL). Antitumor activity tests were conducted (L929 and HeLa), low cytotoxicity and high antiproliferative activity was observed. Antimicrobial activities of *J. terrestris* chitosan were investigated on twelve microorganisms and maximum inhibition (15.6 ± 1.154 mm) was recorded for common human pathogen *Staphylococcus aureus*.

1. Introduction

Chitin isolates from various sources, commercially or otherwise, are generally available in powdered, flakes or granular forms (Ehrlich et al., 2010). Chitin isolates with two- or three-dimensional structures from sponges were reported only recently (Brunner et al., 2009; Ehrlich et al., 2013, 2007, 2010; Wysokowski et al., 2013a; Wysokowski et al., 2013b). Yet literature reports on chitin and chitosan analogous to the raw material are very scarce.

Chitin and chitosan, thanks to their nontoxic, biocompatible, antimicrobial and antitumor properties (Rinaudo, 2006), has become attractive biopolymers for medicine, pharmacy, bioengineering and water treatment (Anitha et al., 2014; Park & Kim, 2010; Synowiecki & Al-Khateeb, 2003; Wysokowski, Petrenko, Stelling et al., 2015; Zhao, Park & Muzzarelli, 2010). Two or three-dimensional chitin isolates have been successfully used in tissue engineering (Ehrlich et al., 2010) and also were suggested for filtering systems and composite production (Wysokowski et al., 2013a; Wysokowski et al., 2013b) and biomimetics (Bazhenov et al., 2015; Wysokowski, Petrenko, Motylenko et al., 2015; Wysokowski, Petrenko, Stelling et al., 2015). Production of three-dimensional chitin/chitosan from the source organism is relatively new area and more research is therefore needed to reveal the potential regarding numerous source organisms.

Diplopoda is one of the largest classes of Arthropoda and has as many as 80.000 species (Sierwald & Bond, 2007). Diplopods in the orders of Juliformia and Polydesmida have segmented cylindrical exoskeleton made up of chitin (Blower, 1951; Cong, Xia & Yang, 2009).

The aim of this study was to produce three-dimensional chitin and chitosan from cylindrical body segments of a diplopod and to characterize the products with FTIR spectroscopy, SEM, TGA, XRD, molecular weight determination and elemental analysis. In addition, DNA interaction studies, antitumor and antimicrobial activity of chitosan were conducted.

2. Materials and methods

2.1. Sample collection

Diplopods (*Julus terrestris*), collected from the private garden of the first author (Bakırdagı, Develi, Kayseri, 29.01.2015), were

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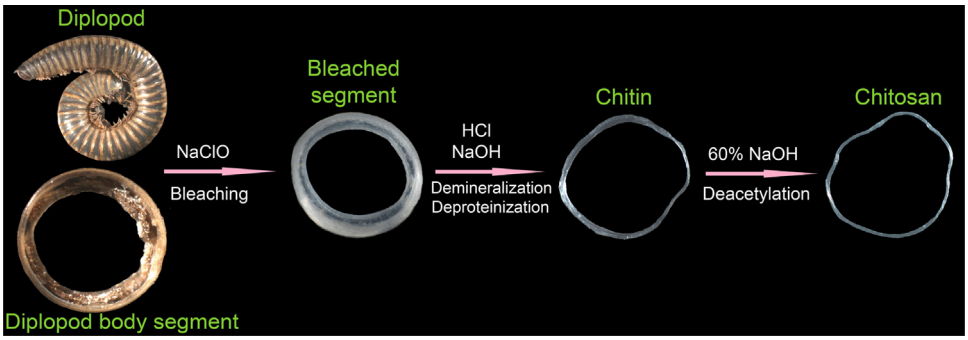

Fig. 1. The procedure for production of three-dimensional chitin and chitosan rings from the body segments of diplopod *Julus terrestris*.

cleaned with distilled water to remove any dirt or earth. Then, the samples were oven-dried at 50 °C for 2 days.

2.2. Bleaching treatment

The samples were refluxed in NaClO solution (4%) (from a local supplier) at 70 °C for 20 min and then samples were filtered and washed with distilled water. Bleaching treatment gave white coloured separate rings.

2.3. Demineralization

To remove the mineral content, the bleached rings were treated with 1 M HCl solution (Merck) for just 10 min at room temperature and then rinsed extensively with distilled water until neutrality.

2.4. Deproteinization

Demineralized diplopod segments were refluxed in 2 M NaOH solution (Merck) at 100 °C for just 20 min to remove the proteins. Then, the segments were rinsed with distilled water until reaching the neutral pH.

2.5. Deacetylation

Chitin rings were deacetylated in 60% NaOH solution at 150 °C for 3 h. Then the chitosan rings were filtered and rinsed up to neutral pH. Finally, the ring shaped wet chitosan samples were dried at room temperature.

Commercial shrimp shell chitin (Sigma-Aldrich, pcode: 1001416772) and low molecular weight chitosan (Sigma-Aldrich, 448869-50G, CAS: 9012-76-4) were used for the identification of ring shaped chitin and chitosan from *J. terrestris*. The production of three-dimensional chitin and chitosan is illustrated in Fig. 1.

2.6. FT-IR analysis

Fourier transform infrared spectra of chitin and chitosan rings were recorded on a Perkin-Elmer FTIR spectrometer (frequency range of 4000–625 cm^{-1}).

2.7. Scanning electron microscopy (SEM)

The overall view and surface features of chitin and chitosan rings were examined on a Quanta FEG 200. The samples were gold-coated using Gatan Precision Etching Coating System (PECS).

2.8. Thermogravimetric analysis (TGA)

Thermograms of chitin and chitosan rings were recorded on an EXSTAR S11 7300 (in inert atmosphere with a temperature rate of 10 °C per min from 25 to 650 °C).

2.9. X-ray diffraction (XRD)

In XRD diffractograms of chitin two sharp peaks at around 9 and 19° and four broad peaks appearing at 12, 21, 23 and 26° are characteristics of α-chitin. On the other hand, in diffractogram of chitosan two sharp peaks are observed at around 9 and 19°. The intensities of XRD peaks enable to calculate percentage crystallinity of chitin and chitosan. The following expression is used to calculate crystallinity index:

$$CrI_{110}(\%) = [(I_{110} - I_{am})/I_{110}] \times 100 \quad (1)$$

where CrI denotes% crystallinity; I_{110} is the maximum intensity at 2θ 20°; I_{am} is the maximum intensity of the amorphous peak at 2θ 13°.

2.10. Molecular weight (MW) determination and elemental analysis (EA)

Viscosity-average molecular weight of chitosan rings were determined by an Ubbelohde Dilution Viscometer following the common procedure (Roberts & Domszy, 1982; Wang, Qin & Bo, 1991). Five different concentrations of chitosan solution were used to determine molecular weight using a solvent system (0.1 M CH_3COOH and 0.2 M sodium chloride, 1:1 v/v) at 25 °C. Each procedure was conducted triplicate. Mark–Houwink–Sakurada equation was employed to calculate MW (Jung & Zhao, 2012).

$$[\eta] = k(M_v)\alpha \quad (2)$$

where [η] denotes intrinsic viscosity, *Mv* is viscosity average molecular weight, k (1.81×10^{-3}) and α (0.93) are the constants.

An elemental analysis (FLASH-2000) was used to determine C, H and N content of the ring chitosan. Deacetylation degree (DA) of chitosan rings was calculated using the formula below (Abdou, Nagy & Elsabee, 2008):

$$DA(\%) = [(6.857 - C/N)/1.7143] \times 100 \quad (3)$$

2.11. Preparation of chitosan solutions

Chitosan rings (4 mg) were dissolved in 1 mL of acetic acid (1%, v:v) (JT Backer, USA) and the solution was agitated on a shaker for 48 h to ensure complete dissolution of chitosan. This chitosan solution was used in DNA interaction, antitumor and antimicrobial efficacy studies.