Materials Science and Engineering C 70 (2017) 552–563

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

In situ chitin isolation from body parts of a centipede and lysozyme adsorption studies

Esra Bulut [a], Idris Sargin [a], Ozlem Arslan [b], Mehmet Odabasi [b], Bahar Akyuz [a], Murat Kaya [a,*]

[a] Department of Biotechnology and Molecular Biology, Faculty of Science and Letters Aksaray University, 68100 Aksaray, Turkey
[b] Faculty of Science and Letters, Department of Chemistry, Aksaray University, 68100 Aksaray, Turkey

ARTICLE INFO

Article history:
Received 14 June 2016
Received in revised form 29 July 2016
Accepted 20 August 2016
Available online 21 August 2016

Keywords:
Biopolymer
Biomaterial
3-D chitin isolation
Metal immobilization
Protein adsorption

ABSTRACT

Isolation of structurally intact chitin samples for biotechnological applications has gained much recent attention. So far, three-dimensional chitin isolates have been obtained from only diplopods and sponges. In this study, three-dimensional chitin isolates were obtained from the body parts of centipede *Scolopendra* sp. (antennae, head, forcipule, collum, trunk, trunk legs and last pair of legs) without leading to structural failure. FT-IR spectra of chitin isolates confirmed that chitin samples are in α allomorph. TGA, XRD and SEM analyses and lysozyme adsorption studies revealed that each chitin isolate had different thermal stability, crystallinity and surface characteristics. Among the chitin isolates, Cu(II)-immobilized forcipule chitin showed the highest affinity for lysozyme (54.1 mg/g), whereas chitin from last pair of legs exhibited the lowest affinity (3.7 mg/g). This study demonstrated that structurally intact chitin isolates can be obtained from the body parts of centipede *Scolopendra* sp. (antennae, head, forcipule, collum, trunk, trunk legs and last pair of legs) by using a simple chemical procedure. Also, it gives a biotechnological perspective to the organisms in the group of Chilipoda.

1. Introduction

Commercial chitin has been isolated from sea-food waste which includes the shells of shrimp and crab, crayfish and squid pens. It has already established that chitin is found in organisms as three different forms; alpha, beta and gamma. It is available in dust form, flakes or granules [1]. Once chitin is obtained, casting of chitin into films requires drastic chemical and physical treatments. Chemically or physically treated chitin can lose some characteristics such as thermal stability, crystallinity and surface morphology. In recent years, there have been attempts to isolate chitin structures intact from the matrix to test their performance in specific applications including chondrocyte cell culture medium [1], uranium adsorption [2] and hydrothermal zirconium deposition [3]. However, the reports on 3-D chitin isolation remained limited to just two classes; Diplopoda [4] and Demonspongiae [1–3]. Still chitin structures in many other organisms remain unexploited. Exploration and isolation of new three-dimensional (3-D) chitin templates from new sources can provide some useful insights to the applications of chitin.

Organisms in the class of Chilipoda have segmented body parts and its exoskeleton consists of chitin [5]. Many studies have been conducted to learn about systematic (at morphological and molecular level) [6], diversity and distribution [7], ecology, evolution [8], genetics [9], physiology and anatomy [10] of centipedes until now. But in present study it was attempted to provide a more general perspective to the centipedes as a source for 3-D chitin production. The genus *Scolopendra* Linnaeus 1758 has more than 100 species worldwide and live in warm and tropical areas including central, northern and southern America, Africa, Asia, Australia and Europe [11].

Depending on its location and function, properties of chitin can vary greatly through the body of a single organism. Chitin is usually found inter-mixed with proteins, pigments or minerals, so a chemical isolation process including demineralization, deproteinization and bleaching is employed [12]. Our previous studies showed that chitin characteristics are not uniform in the body parts of honeybee (*Apis mellifera*) [13], American cockroach (*Periplaneta americana*) [14] and butterfly (*Argynnis pandora*) [15]. All the chitin isolates were in dust form and exhibited different properties. None of the chitin isolates was investigated regarding their interactions with biological molecules.

Chitin-based materials have been widely used as a matrix for protein separation, purification and adsorption [16,17]. Due to electron donating groups such as hydroxyl, acetylamino and amine groups, chitin and its derivatives show affinity for metal ions. Metal ion immobilization can enhance the interaction of chitin with proteins, which is desired for applications in immobilized-metal ion affinity chromatography [18].

Unlike our previous studies, in the current study chitin specimens from the body parts of a centipede *Scolopendra* sp. (antennae, head, forcipule, collum, trunk, trunk legs and last pair of legs) were isolated by keeping their 3-D forms, and then their protein (lysozyme) adsorption capacity was determined. Also, physicochemical differences in chitin isolates were discussed based on the results of FT-IR, TGA, XRD and

* Corresponding author.
E-mail address: muratkaya3806@yahoo.com (M. Kaya).

http://dx.doi.org/10.1016/j.msec.2016.08.048
0928-4931/

Table 1
Chitin content (%) and weight losses in amount of samples from body parts of centipede *Scolopendra* sp. in chitin isolation procedure.

Body parts	Dry weight (g)	Remaining amount of sample after each treatment (g)			Chitin percentage (%)
		demineralization	deproteinization	depigmentation	
Antennae	0.403	0.302	0.039	0.037	9.18
Head	1.172	0.792	0.158	0.152	13.02
Forcipule	0.936	0.582	0.121	0.115	12.31
Collum	1.744	1.438	0.191	0.187	10.77
Trunk	34.15	31.23	3.324	3.319	9.72
Trunk legs	7.916	6.189	1.059	1.051	13.28
Last pair of legs	2.461	1.452	0.398	0.391	15.91

SEM analyses. Variations were observed in lysozyme adsorption capacity of Cu(II)-immobilized among the chitin isolates.

2. Material and methods

2.1. Materials

HCl, NaOH and $Cu(NO_3)_2$ were purchased from Merck. NaClO solution (4%) was purchased from a local supplier. Lysozyme (hen egg white, EC 3.2.1.17), *N*,*N*,*N*,*N*′-tetramethylethylenediamine and 2-hydroxyethylmethacrylate was supplied by Fluka. Ammonium persulphate and *N*,*N*′-methylene-*bis*-acrylamide were obtained from Sigma-Aldrich.

Centipedes (*Scolopendra* sp) were collected from Aksaray University Campus (in the spring, 2015), washed with distilled water and dissected into seven different body parts; i.e., antennae, head, forcipule, collum, trunk, trunk legs and last pair of legs. Following the dissection, the each body part was washed with distilled water, oven-dried at 50 °C for 48 h and weighted.

2.2. Chitin isolation procedure

2.2.1. Demineralization

To remove the mineral content, each body part was refluxed with 2 M HCl solution at 40 °C for 4 h, recovered by filtration and washed with distilled water. After drying at 60 °C for 48 h, each sample was weighed.

2.2.2. Deproteinization

Proteins present in the samples were removed by treatment of sodium 1 M of NaOH solution at 50 °C. At the end of 24 h treatment, the samples were washed with distilled water, oven-dried at 60 °C for 48 h and mass of each sample was recorded.

2.2.3. Bleaching

To remove the pigments, the samples were incubated in 0.4% NaClO solution for 30 min at room temperature. Following the bleaching, chitin isolates were extensively washed with distilled water and oven-dried at 50 °C for 48 h. Finally, chitin isolates were weighted and chitin content of each body part was determined.

2.3. Characterization

2.3.1. FT-IR

Fourier transform-infrared bands of the centipede *Scolopendra* sp. chitins were recorded on a Perkin Elmer ATR FT-IR Spectrometer over the frequency range of 4000–625 cm^{-1}.

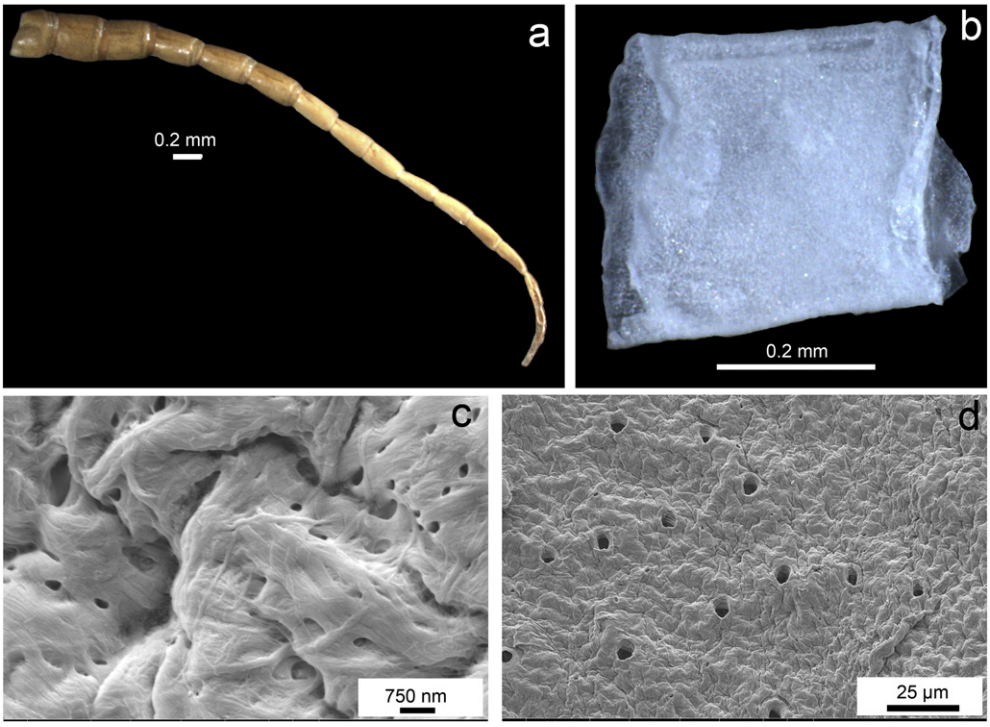

Fig. 1. The original structure of antennae (a); isolated chitin by keeping the original shape and structure examined by light microscopy (b) and SEM (c, d).