Carbohydrate Polymers 102 (2014) 151–158

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Nanocrystalline chitin thin films

Chao Wang, Alan R. Esker*

Department of Chemistry, Virginia Tech, Blacksburg, VA 24061, United States

ARTICLE INFO

Article history:
Received 21 August 2013
Received in revised form 26 October 2013
Accepted 31 October 2013

Keywords:
Chitin
Thin film
Chitinase
Bovine serum albumin
QCM-D
AFM

ABSTRACT

Elucidating the interactions between crystalline chitin and various biomacromolecules is of fundamental importance for designing and fabricating chitin-based biomaterials. This work highlights a simple method to prepare ultrathin films of chitin nanocrystals (chitin NC) by spincoating chitin NCs from a colloidal suspension onto a gold surface modified by an amine-terminated self-assembled monolayer. Atomic force microscopy confirmed that chitin NC films are reasonably smooth and homogeneous, and quartz crystal microbalance with dissipation monitoring (QCM-D) solvent exchange experiments demonstrated that chitin NC films have twice as much water as amorphous regenerated chitin (RChitin) films of similar thickness. QCM-D data also showed that chitinase-catalyzed hydrolysis of chitin NC films was much slower than that of RChitin films. Chitinase not only degraded, but also caused the swelling of the chitin nanocrystals. BSA adsorption studies demonstrated that chitin NC films have high protein loading capacity, and thus show potential applications for enzyme immobilization.

1. Introduction

As the second most abundant biopolymer in nature, chitin is widely distributed in the exoskeletons of crustaceans (e.g. shrimps, crabs and lobsters) and insects, the cell walls of fungi, and the beaks of cephalopods (e.g. octopi and squid) (Rinaudo, 2006). In nature, chitin occurs as ordered crystalline microfibrils which associate with other materials, such as proteins, lipids, polysaccharides, calcium carbonate, and pigments to form natural composites (Goodrich & Winter, 2007). Two polymorphs of chitin (α and β) have been reported, differing with respect to the locations and quantity of hydrogen bonds (Muzzarelli, 2012; Zeng, He, Li, & Wang, 2012). α-Chitin is the most abundant and stable form, and it is derived from crustacean tendons and shells, yeast and fungal cell walls, as well as insect cuticles (Muzzarelli, 2011; Muzzarelli et al., 2012). β-Chitin is less abundant and stable than α-chitin, and it is found in squid pens and tubeworms (Blackwell, Parker, & Rudall, 1965; Rudall & Kenchington, 1973). Compared to α-chitin, β-chitin is more susceptible to swelling. β-Chitin can be reversibly swelled by water, alcohols or amines, and irreversibly swelled by strongly acidic or basic solutions (Saito, Putaux, Okano, Gaill, & Chanzy, 1997; Saito, Okano, Gaill, Chanzy, & Putaux, 2000). β-Chitin could also be converted to thermodynamically stable α-chitin via dissolution or extensive swelling under strongly acidic or basic conditions (Noishiki et al., 2003; Saito et al., 1997). Due to its biocompatibility, biodegradability, affinity for proteins, antimicrobial and gel-forming properties, chitin is widely used in the medical and pharmaceutical area, such as wound-dressing materials (Jayakumar, Prabaharan, Sudheesh Kumar, Nair, & Tamura, 2011; Kumar et al., 2010; Muzzarelli, 2009), drug carriers (Mi et al., 2003; Rejinold, Chennazhi, Tamura, Nair, & Rangasamy, 2011), tissue engineering scaffolds (Freier, Montenegro, Shan Koh, & Shoichet, 2005; Noh et al., 2006), enzyme and cell immobilization supports (Krajewska, 2004; Muzzarelli, 1980), and biosensors (Ohashi & Karube, 1995; Ohashi & Koriyama, 1992).

Fundamental knowledge of the interactions between chitin and proteins, polysaccharides, calcium carbonate, enzymes, drugs, cells and synthetic materials is not only important for elucidating biological processes associated with chitin, but also for designing novel chitin-based biomaterials. Model chitin surfaces and the development of surface characterization techniques provide a convenient way to study and quantify these interactions. Recently, our group reported a simple method to prepare homogeneous, smooth and ultrathin chitin films by spincoating trimethylsilyl chitin (TMS-Chitin) from a mixture of chloroform and tetrachloroethane onto silica or gold surfaces. These TMSChitin films were subsequently regenerated to amorphous chitin upon exposure to the vapor of a hydrochloric acid solution. The regenerated chitin thin films were used as a model surface to study the interactions between chitin and bovine serum albumin (BSA) via a quartz crystal microbalance with dissipation monitoring (QCM-D), surface plasmon resonance (SPR) and atomic force microscopy (AFM) (Kittle et al., 2012), as well as chitinases (Wang, Kittle, Qian, Roman, & Esker, 2013). However, the regenerated chitin films are only representative of amorphous chitin structures, differing in crystallinity from native chitin.

* Corresponding author at: Department of Chemistry (0212), Virginia Tech, Blacksburg, VA 24061, United States. Tel.: +1 540 231 4601; fax: +1 540 231 3255.
E-mail addresses: cwang09@vt.edu (C. Wang), aesker@vt.edu (A.R. Esker).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.10.103

Crystalline chitin thin films are expected to provide a better model to study the enzymatic degradation of native chitin, chitin–protein interactions, fungal cell wall component interactions and biomineralization.

Various cellulose model thin films (including both amorphous and crystalline) have been prepared via spincoating or the Langmuir–Blodgett techniques (Ahola, Salmi, Johansson, Laine, & Österberg, 2008; Edgar & Gray, 2003; Eriksson et al., 2005; Fält, Wågberg, Vesterlind, & Larsson, 2004; Gunnars, Wågberg, & Cohen Stuart, 2003; Habibi, Foulon, Aguié-Béghin, Molinari, & Douillard, 2007; Kontturi, Thüne, & Niemantsverdriet, 2003; Kontturi, Tammelin, & Osterberg, 2006; Maren, 2009; Schaub, Wenz, Wegner, Stein, & Klemm, 1993; Yokota, Kitaoka, Sugiyama, & Wariishi, 2007). Edgar and Gray (2003) reported the preparation of smooth cellulose nanocrystalline thin films by spincoating colloidal suspensions of cellulose nanocrystals onto mica surfaces. Habibi et al. (2007) prepared monolayers of cellulose nanocrystals via the Langmuir–Blodgett technique.

Similar to cellulose, native chitin is highly crystalline with some disordered or paracrystalline regions that arise from defects. The disordered or paracrystalline regions are preferentially hydrolyzed or oxidized under certain conditions, whereas crystalline regions remain intact. Thus, rod-like chitin nanocrystals (chitin NCs) or whiskers can be produced (Habibi, Lucia, & Rojas, 2010; Zeng et al., 2012). Chitin NCs are normally prepared by hydrolysis of chitin in hydrochloric acid solutions (Goodrich & Winter, 2007; Tzoumaki, Moschakis, & Biliaderis, 2010; Zeng et al., 2012). The sizes of the chitin NCs are greatly affected by the origin of the chitin, concentration of the hydrochloric acid solutions and the hydrolysis time, with the lengths varying over the range of 150–2200 nm, and the widths over the range of 10–50 nm (Zeng et al., 2012). Recently, Fan, Saito, and Isogai (2008) prepared chitin NCs via 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) mediated oxidation of α-chitin with NaClO as a co-oxidant. The resulting rod-like nanocrystals had high surface charges because some hydroxyl groups on the surface were oxidized to carboxylate groups, and the average nanocrystal length and width were 340 nm and 8 nm, respectively.

This work presents a simple method to prepare smooth and ultrathin nanocrystalline chitin films. The morphologies, surface roughnesses, thicknesses and water contents of these films were characterized via atomic force microscopy (AFM), ellipsometry and a quartz crystal microbalance with dissipation monitoring (QCM-D). The chitinase-catalyzed hydrolysis of these chitin films was investigated via QCM-D. The utility of these chitin films as potential enzyme immobilization supports was demonstrated through the adsorption of bovine serum albumin (BSA) onto the films in QCM-D studies.

2. Experimental

2.1. Materials

α-Chitin from shrimp shells (practical grade, >95% acetylated) was purchased from Sigma–Aldrich. The TMSChitin (degree of substitution = 2.0) was synthesized as previously described (Kittle et al., 2012; Kurita, Sugita, Kodaira, Hirakawa, & Yang, 2005). Colloidal suspensions of chitin NC were prepared through a HCl hydrolysis procedure reported by Goodrich and Winter (2007). Chitinase (from *Streptomyces griseus*, lyophilized powder, ≥200 units/g solid) is an extracellular enzyme complex (Berger & Reynold, 1958) with a molar mass of ~30 kDa and was purchased from Sigma–Aldrich. Bovine serum albumin (lyophilized powder) was purchased from Sigma–Aldrich and used as received. 11-Amino-1-undecanethiol was purchased from Fisher Scientific and used as received. Sodium phosphate monobasic monohydrate and sodium phosphate dibasic heptahydrate were purchased from Sigma–Aldrich and used as received to prepare buffer solutions (pH 6.0). Hydrochloric acid (37.3%) was purchased from Fisher Scientific. Hydrogen peroxide (30%, w/w), sulfuric acid (conc.), and ammonium hydroxide (28%, w/w) were used to clean the surfaces and were purchased from EM Science, VWR International, and Fisher Scientific, respectively. All other chemicals and solvents were obtained from Fisher Scientific and used as received. Ultrapure water with a resistivity of 18 MΩ cm and <5 ppb inorganic impurities was used in all experiments (Milli-Q Gradient A-10, Millipore).

2.2. Surface preparation

Surface cleaning. Silica coated QCM-D sensors (Q-Sense AB, QSX-303, 5 MHz) were cleaned prior to use by immersion into a 7:3 (v/v) solution of sulfuric acid:hydrogen peroxide, followed by a rinse with ultrapure water and drying with nitrogen. Gold coated QCM-D sensors (Q-Sense AB, QSX-301, 5 MHz) were cleaned prior to use by exposure to UV/ozone for 20 min, immersion into a 1:1:5 (v/v/v) solution of ammonium hydroxide:hydrogen peroxide:water at 80 °C for 1 h, a rinse with ultrapure water and drying with nitrogen.

Amorphous regenerated chitin (*RChitin*) *films*. The details of the preparation and characterization of the smooth and amorphous chitin films was previously published (Kittle et al., 2012). The TMS-Chitin was dissolved in a chloroform/tetrachloroethane (4:1, v/v) mixture to form 0.6% TMSChitin solutions by mass. These solutions were filtered through a 0.45 μm syringe filter (VWR PTFE) to remove dust particles and aggregates. The filtered solutions were spincoated onto silica coated QCM-D sensors at a spinning speed of 3000 rpm for 1 min and regenerated to amorphous chitin by exposure of these surfaces to the vapor of 10% by mass aqueous hydrochloric acid solution to obtain RChitin films with thicknesses of ~20 nm.

Nanocrystalline chitin films. A SAM-NH_2 was formed on a cleaned gold QCM-D sensor crystal after immersion in a 1 mM solution of 11-amino-1-undecanol in ethanol for 24 h. Films of chitin NC with different thicknesses were obtained by spincoating at 4000 rpm for 1 min from colloidal suspensions of chitin NCs with concentrations that ranged from 0.50% to 2.20% by mass. Films were then dried overnight at 70 °C before thicknesses were determined.

2.3. AFM measurements

All the surfaces were imaged in tapping mode with an Asylum Research AFM (MFP-3D-BIO, Asylum Research). Height images were collected under ambient conditions (22 °C, 50% humidity) using a silicon tip (OMCL-AC160TS, Olympus Corp.). The reported roughnesses were determined from the root mean square (RMS) values of 2 μm × 2 μm, 5 μm × 5 μm, or 10 μm × 10 μm scan areas. In order to observe the morphology of chitin NCs, chitin NCs were deposited onto SAM-NH_2 coated gold surfaces through spincoating of an aqueous 0.005% by mass chitin NC suspension at 4000 rpm for 1 min. The samples were then dried overnight at 70 °C before imaging.

2.4. XRD measurements

X-ray diffraction patterns of lyophilized chitin NCs, raw α-chitin and regenerated chitin powders were obtained on a Rigaku Mini-Flex II Desktop X-Ray Diffractometer. The radiation source was Cu(Kα) radiation with a wavelength of 1.54 Å. The angular scanning range was 2θ = 5–50° with 0.01° steps.