Carbohydrate Polymers 128 (2015) 179–187

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Carboxymethylated hyperbranched polysaccharide: Synthesis, solution properties, and fabrication of hydrogel

Yongzhen Tao[a,*], Ruquan Zhang[b], Wei Yang[a], Hongtao Liu[a], Hongjun Yang[a], Qinghua Zhao[a]

[a] Key Laboratory of Green Processing and Functional Textiles of New Textile Materials, Ministry of Education, Wuhan Textile University, Wuhan 430073, China
[b] College of Mathematics and Computer Science, Wuhan Textile University, Wuhan 430073, China

ARTICLE INFO

Article history:
Received 22 February 2015
Received in revised form 25 March 2015
Accepted 13 April 2015
Available online 20 April 2015

Keywords:
Carboxymethylated hyperbranched polysaccharide
Silk fibroin
Chain conformation
Hydrogel

ABSTRACT

The periphery of hyperbranched polysaccharides has many end groups that can be functioned and used as sites to interact with their surroundings. A water-insoluble hyperbranched β-D-glucan, coded as TM3a, extracted from sclerotia of an edible fungus (*Pleurotus tuber-regium*), was fractionated and modified chemically to obtain carboxymethylated derivatives (CTM3a). The solution properties of the carboxymethylated polysaccharides were studied systematically in phosphate buffer saline at 37 °C. The results indicated that the carboxymethylated glucans still kept hyperbranched structure after carboxymethylation, and existed as a swollen sphere-like chain conformation. The introduction of carboxymethylated groups permitted the formation of hydrogels through crosslinking CTM3a and silk fibroin with carbodiimide chemistry. The resultant hydrogels with porous and interconnected structure displayed good mechanical and swelling properties. This work provides some valuable and fundamental information of the natural hyperbranched polysaccharide from mushroom for further application in biomedical devices and tissue engineering.

1. Introduction

Recently, bio-inspired hydrogels mimicking the extracellular matrix (ECM) environment have emerged as the predominant materials in the biomedical field. Polysaccharide-based hydrogels are quite promising in biomedical and tissue engineering application, as like the ECM, they have biocompatibility, biodegradability, cellular interactions, and high water content to afford a favorable microenvironment for cell growth and differentiation (Diolosà et al., 2014; Khan & Ahmad, 2013; Sun et al., 2011). Fungal polysaccharides exhibit significant antiviral, antitumor, and immune activities, and has been used popularly as a functional food and a source for the development of drugs (Aida, Shuhaimi, Yazid, & Maaruf, 2009; Thiele, Ma, Bruekers, Ma, & Huck, 2014; Yang & Zhang, 2009). We deduce that fungous polysaccharide hydrogels have promising superiority due to their bioactivities *in vivo* and/or *in vitro* as well as the popular characteristics as a polysaccharide. *Pleurotus tuber-regium* sclerotia are edible and extremely rich in non-starch polysaccharides, and are used as folk medicine to treat asthma, stroke, and breast cancer as well as to promote the development of fetus (Wong & Cheung, 2008; Wu, Hu, Huang, & Jiang, 2013; Zoberi, 1973). In our previous work (Tao, Yan, & Xu, 2009; Tao, Zhang, Yan, & Wu, 2007), a hyperbranched polysaccharide (TM3a) was extracted from the sclerotia of *P. tuber-regium* by hot water, and chemical structure and solution properties of the TM3a hyperbranched polysaccharides have been studied in detail. The macromolecules with hyperbranched architecture have attracted much attention from the viewpoint of materials (Gao & Yan, 2004; Jeong, Mackay, Vestberg, & Hawker, 2001; Satoh et al., 2008). The presence of a large number of end-groups at the periphery of the hyperbranched macromolecules results in high reactivity of functionalization (Tirelli, 2007). We hypothesized that the TM3a hyperbranched polysaccharide could be chemical modified to obtain carboxymethylated derivatives and subsequently to fabricate hydrogels. The resultant hydrogels may exhibit biological activities and can encapsulate chemical clues or drug molecules within inner cavities as well as the macropores of hydrogels due to the presence of the hyperbranched structure that is otherwise difficult to achieve in conventional linear polysaccharides.

Polysaccharides hydrogels can be successfully formed using physically or chemically crosslinking reactions (Delair, 2012; Koop,

* Corresponding author. Tel.: +86 27 59367580; fax: +86 27 59367578.
E-mail address: yongzhentao@126.com (Y. Tao).

http://dx.doi.org/10.1016/j.carbpol.2015.04.012
0144-8617/

de Freitas, de Souza, Savi Jr., & Silveira, 2015). The applications of physical hydrogels are limited due to lack of thermal, pH, or salt stability, since they are formed through hydrogen bonding or inter-molecular electrostatic (ionic) interactions. Chemical modification and synthetic chemistry have greatly broadened the scope of the formation pathways to fabricate polysaccharide hydrogels with satisfying and tailored properties. Carboxymethylation is broadly used to functionalize the polysaccharide with introducing carboxymethyl groups to the sugar rings and improve the solubility of polysaccharide in water (Kagimura, da Cunha, Barbosa, Dekker, & Malfatti, 2015). Carbodiimide is a carboxyl and amine-reactive crosslinker with low cytotoxicity and water solubility, and it actives the coupling reaction in mild conditions (Gřundělová et al., 2015). Silk fibroin (SF) contains quantities of reactive serine, threonine, aspartic and glutanmic acid, and the hydroxyl, carboxyl, or amino groups in the hydrophilic amino acids are ready to interact or react with polar groups in a certain condition (Murphy & Kaplan, 2009). More importantly, SF has excellent structural properties, superior mechanic properties, and good biocompatibility (Rockwood et al., 2011). Thus, SF is an ideal candidate for fabricating hydrogels by the covalent attachment of carboxymethyl groups of polysaccharides to amine groups of SF with carbodiimide.

Biological activities and physical properties are related to the chemical structure and chain conformation of polysaccharides, so it is essential to understand the solution properties of polysaccharides for further biomedical and tissue engineering application. In the present project, the TM3a hyperbranched polysaccharide was modified chemically to introduce carboxymethyl groups to the glucan chains, and the chemical structure and molecular parameters of the carboxymethylated derivatives were studied. Furthermore, we attempted to fabricate hydrogels based on CTM3a and SF through amidation with carbodiimide. The chemical structure, morphology, compressive modulus, and swelling ratio were characterized to clarify the effect of CTM3a M_w on the properties of the resultant CTM3a-SF hydrogels. This work provides some fundamental information for further application of the natural hyperbranched polysaccharide from mushroom in biomedical devices and tissue engineering.

2. Experimental

2.1. Materials

The native hyperbranched polysaccharide (TM3a) and its fractions were the same as given in our previous work (Tao et al., 2007), and the preparation of the samples was described there in detail. The raw silk filament was degummed, dried and pulverized to obtain the silk fibroin (SF) powder, and the preparation and characterization of the SF powder was described in our previous work in detail (Tao, Xu, Yan, & Cao, 2012; Tao, Yan, & Xu, 2010). All the chemical reagents used here were analytically pure, and obtained from commercial sources in China.

2.2. Preparation of carboxymethylated hyperbranched polysaccharide (CTM3a)

About 900 mg sample (TM3a and its seven fractions, individually) was suspended in a mixture of 15 mL 20% NaOH and 37.5 mL isopropanol in an ice bath with stirring for 3 h. Then, a mixture of 7.89 g chloroacetic acid, 15 mL 20% NaOH and 37.5 mL isopropanol was slowly added with stirring. The reaction was continued at room temperature for 1 h, and then at 60 °C for 2 h. After the solution was cooled to room temperature, 20% CH_3COOH was added to adjust the pH to 7. Finally, the carboxymethylated samples were dialyzed using a regenerated cellulose tube (M_w cut-off 8000, USA) for 7 days against deionized water that was changed two times a day, and freeze-dried to afford spongy samples coded as CTM3a, CF4, CF5, CF6, CF7, CF8, CF9, and CF11.

2.3. Solution preparation of SF in PBS

About 5 g of SF powders were dissolved in 25 mL of 9.3 M LiBr solution at 60 °C for 4 h, yielding a 20% (w/v) solution. This solution was dialyzed using a regenerated cellulose tube (M_w cut-off 8000, USA) for 3 days against deionized water that was changed three times a day. Subsequently, the resultant SF solution was dialyzed in 1 L of 20% (w/v) aqueous poly(ethylene glyco) (PEG, 6000 g/mol) for 12 h using dialysis tubing (M_w cut-off 3500, USA) in order to obtain concentrated SF solution. Finally, the concentrated SF solution was dialyzed against phosphate buffer saline (PBS, pH = 7.4) for 12 h to alter the solvent in SF solution from deionized water to PBS. After dialysis, the final solution concentration of SF in PBS was about 15% (w/v), which was determined by measuring the volume of the resultant SF solution and base on the weight of SF powder. The solution of SF in PBS was stored at 4 °C and used within 2 weeks.

2.4. Hydrogels fabrication

About 400 mg of CTM3a was dissolved in 4 mL of PBS under stirring for 12 h at room temperature. About 200 mg of 1-ethyl-3-[3-(dimethylammino)propyl]carbodiimide hydrochloride (EDC·HCl) and 300 mg of *N*-hydroxysuccinimide (NHS) were added to the CTM3a solution, and then the mixture was continuously stirred for 30 min at room temperature. Subsequently, the resultant mixture was added into 8 mL of 15% (w/v) SF in PBS solution by vigorously stirring for 5 min to prepare the pre-gel solution. About 1 mL of pre-gel solution was placed into 24-well plates, and then stayed for 24 h at 37 °C to form a hydrogel disk. The resulting CTM3a-SF hydrogels were treated with 70 vol.% MeOH aqueous for 30 min at room temperature. Then the hydrogels were washed in deionized water for more than five times, and incubated in deionized water at 37 °C for 24 h before being lyophilized and characterization.

2.5. Characterization

Infrared spectra (IR) of the CTM3a derivatives, and the dried hydrogels were recorded with FTIR spectrometer (Bruker Tensior 27, German) in the range of 4000–400 cm^{-1} using the KBr-disk method. ^{13}C NMR measurement of CTM3a derivative was analyzed on a Mercury 600 NMR spectrometer (Varian Inc., Palo, Alto, CA, USA) at 20 °C. CTM3a was dissolved in D_2O to obtain a concentration of 100 mg/mL. The elemental compositions for C and H of all the samples were determined by using an elemental analyzer (EA, FLASH 2000, ThermoFisher, USA).

Intrinsic viscosity ($[\eta]$) of the CTM3a derivatives in PBS was measured at 37 ± 0.1 °C by using an Ubbelohde capillary viscometer. The kinetic energy correction was assumed to be negligible. Huggins and Kraemer equations were used to estimate the $[\eta]$ value by extrapolating to an infinite dilution formulated as

$$\frac{\eta_{sp}}{c} = [\eta] + k'[\eta]^2 c \quad (1)$$

$$\frac{\ln \eta_r}{c} = [\eta] - \beta[\eta]^2 c \quad (2)$$

Both k' and β are constants for a given polymer at a given temperature in a given solvent; η_{sp}/c is the reduced specific viscosity; $(\ln \eta_r)/c$ is the inherent viscosity.

Weight – average molecular weight (M_w) and radius of gyration ($\langle S^2 \rangle_z^{1/2}$) of the CTM3a derivatives in PBS were measured with a laser light scattering instrument (MALLS, λ = 632.6 nm; DAWN