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Synthesis and self-assembly of octenyl succinic anhydride modified short glucan chains based amphiphilic biopolymer: Micelles, ultrasmall micelles, vesicles, and lutein encapsulation/release

Ranran Chang, Jie Yang, Shengju Ge, Mei Zhao, Caifeng Liang, Liu Xiong, Qingjie Sun*

College of Food Science and Engineering, Qingdao Agricultural University, Qingdao, Shandong Province, 266109, China

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ABSTRACT

The amphiphilic polymers are widely used to prepare nanocarriers by self-assembly with crucial implications for biomedical, biotechnological, and food applications. An amphiphilic polymer of octenyl succinic anhydride modified short glucan chains (OSA-SGC) with various degrees of substitution (DS) were first synthesized in this work. OSA-SGC polymer spontaneously formed vesicles, micelles, and ultrasmall micelles in a phosphate buffer solution. The properties of self-assembled nanoparticles were studied using fluorescence spectroscopy, dynamic light scattering (DLS), and transmission electron microscopy (TEM). The critical aggregation concentrations (CAC) of OSA-SGC polymer were in the range of 0.0316–0.1260 mg/mL and decreased with increasing DS. Lutein, a hydrophobic functional ingredients, was incorporated into the OSA-SGC micelles and vesicles. Lutein-loaded nanoparticles exhibited high encapsulation efficiency (~85%) and loading content (~8%). Thus, the study suggested that self-aggregated nanoparticles of OSA-SGC polymer could have potential applications in the health food, cosmetics, and pharmaceutical fields because they can aid in the delivery of hydrophobic functional ingredients or drugs.

1. Introduction

In recent years, amphiphilic polymers have proven to be one of the most important classes of specialty polymeric materials that can self-assemble into various functional, well-defined micro/nano-structures (e.g., micelles, vesicles, bilayer membranes, tubes), with crucial implications for biomedical, biotechnological, and food applications (Antonietti & Förster, 2003; Kwon & Kataoka, 2012; Palivan et al., 2016). Among the various kind of amphiphilic polymers, water-soluble polymers with hydrophobic element grafted on side chains have received special attention. Amphiphilic polymers prepared via synthesized organic compounds can spontaneously self-assemble and arise various self-aggregates. Zhu, Liu & Du (2013) have reported that self-assembled amphiphilic poly(2-hydroxy-3-phenoxypropyl acrylate) polymers can form vesicles, as well as all kinds of micelles. Wang, Agrawal, et al. (2013) have found that the aggregation of amphiphilic poly(ethylene glycol)-substituted polyethoxysiloxane polymers in water can form various silica particles, including hollow nanospheres, and very small particles. These reports provided a simple and effective way to prepare micelles and vesicles for potential applications in drug delivery, biosensors, and nanoreactors. However, nanoparticles prepared via synthetic organic polymers have some disadvantages, including complicated preparation processes, the use of toxic reagents, and poor biocompatibility.

To date, there are only very few reports of amphiphilic nanoparticles being self-assembled by natural biomolecules. A naturally occurring amphiphilic protein, beta-casein, could produce a new type of proteinaceous micelle (Kamiya et al., 2009). However, nanoparticles prepared via natural protein lack stability in aqueous solution. Therefore, many studies have focused on the hydrophobic modification of natural biopolymers. Amphiphilic poly(lactic acid)-grafted-chitosan polymer self-assembled into spherical nanoparticles in order to amphotericin B delivery (Zhou, Wang, Jian, & Song, 2013). Dextrin substituted with hexadecanethiol also self-assembled into colloidally stable, spherical nanoparticles (Gonçalves, Martins, & Gama, 2007).

* Corresponding author. College of Food Science and Engineering, Qingdao Agricultural University, 266109, 700 Changcheng Road, Chengyang District, Qingdao, China.
E-mail address: phdsun@163.com (Q. Sun).

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Starch, a major dietary source of carbohydrates, exhibits biodegradability, non-toxicity, and biocompatibility. Starch is known to be a good candidate material for the preparation of nanocarriers (Zhang et al., 2013). The micelles of octenyl succinic anhydride (OSA) modify starches with various M_w values in aqueous solution, as previously reported by Zhu, Li, Chen, and Li (2013). However, they did not characterize various nanostructures under different conditions or evaluate drug release profiles *in vitro*.

Short glucan chain (SGC) is a low-molecular-weight hydrophilic linear polymer with a degree of polymerization (DP) of *ca.* 20 that is prepared via enzymatic debranching amylopectin (Gelders, Vanderstukken, Goesaert, & Delcour, 2004). We have prepared hydrophilic starch nanoparticles based on SGC via recrystallisation (Jiang, Campbell, Blanco, & Jane, 2010; Sun, Li, Dai, Ji, & Xiong, 2014). However, the amphiphilic modification of SGC with OSA has not yet been reported. In this work, we first hydrophobically modified SGC by introducing an octenyl succinic (OS) group to obtain OSA-SGC with various degrees of substitution (DS). Then, we characterized the properties of self-assembling OSA-SGC nanoparticles by employing fluorescence spectroscopy, dynamic light scattering (DLS), and transmission electron microscopy (TEM). Furthermore, lutein was exploited to evaluate the encapsulation efficacy (EE%) and release of lutein-loaded OSA-SGC nanoparticles. Lutein was served for as a model functional ingredients due to limited bioavailability resulting from its inherently poor water solubility, although lutein is a powerful antioxidant and plays an important role in the prevention of excessive ultraviolet light exposure, stroke, lung cancer, and vision loss (Johnson, 2004). The fabricated amphiphilic OSA-SGC nanoparticles could have potential applications in hydrophobic functional ingredients or drug encapsulation in the biotechnological and pharmaceutical fields.

2. Materials and methods

2.1. Materials

Waxy corn starch (approximately 2% amylose and 98% amylopectin) was purchased from Tianjin Tingfung Starch Development Co., Ltd. Pullulanase (E.C.3.2.1.41, 6.17×10^{-4} kat/g) was gotten from Novozymes Investment Co. Ltd. Octenyl succinic anhydride was purchased from Sigma-Aldrich Chemicals. All other reagents used were of analytical grade.

2.2. Preparation of SGC

The SGC was obtained following the method described by Sun et al. (2014). Briefly, waxy corn starch slurry was gelatinized, cooled to 58 °C, and debranched via pullulanase for 6 h. The hydrolysate was centrifuged (3000 rpm, 2 min), and the supernatant was heated to fully inactivate the enzyme. Then, the SGC was precipitated using excess absolute alcohol, washed three times with distilled water until neutral, and then freeze dried.

The molecular weight distributions of the SGC were analyzed using a high-performance size-exclusion chromatograph (HPSEC) (Jiang et al., 2010). The SGC had a bimodal distribution, and the high and low DP values were 36.15 and 10.52, respectively. The weight ratio of the two fractions was 0.37 and the average DP of the SGC was 16.78.

2.3. Synthesis of OSA-SGC

OSA-SGC was prepared following the method described by Liu et al. (2008) with some modifications. In brief, 5% (w/v) SGC solution was incubated at 121 °C for 30 min in an oil bath. Then, the solution was maintained between pH 8 and 9 by adding 0.1 mol/L of NaOH. Different quantity of OSA (25, 50, or 100%, based on the weight of SGC) were added to the mixture in order to obtain different DS values. The mixture was stirred for 6 or 10 h at 50 °C. At the end of reaction, the pH was adjusted to 6.5 using 0.1 mol/L HCl aqueous solution to stop the reaction. The obtained OSA-SGC was washed with absolute ethanol and finally freeze-dried. The samples were denoted as $OSA_{0.25,6h}$-SGC, $OSA_{0.5,6h}$-SGC, and $OSA_{1.0,6h}$-SGC, with the addition of 25, 50, and 100% OSA for 6 h, respectively. Similarly, $OSA_{1.0,10h}$-SGC referred to the SGC modified with 100% OSA for 10 h.

2.4. Preparation of amphiphilic OSA-SGC nanoparticles

OSA-SGC nanoparticles were prepared according to Sebastian, Dhara, and Chattopadhyay (2014). In brief, OSA-SGC (10, 50, and 100 mg) was dispersed in 10 mL phosphate buffered solution (PBS) (pH 7.4) by stirring at room temperature. The solution was heated to 37 °C in a constant-temperature heating magnetic stirrer and formed a transparent liquid after 6 h. Then, it was allowed to cool to an ambient temperature to obtain nanoparticles, keeping it in an airtight container for further analysis. For researching the effect of pH and salt concentration on self-assembled nanoparticles (Ghosh, Das, Chatterjee, & Nandi, 2016; Kaewsaiha, Matsumoto, & Matsuoka, 2004), the $OSA_{1.0,6h}$-SGC polymer (10 mg) was dispersed in 10 mL PBS solution with the desired pH values (5.5, 9.2) and salt concentrations (0.1, 0.8 M NaCl) and self-assembled into nanoparticles according to the above-mentioned procedures. Pictures were taken to illustrate the state of self-aggregated nanoparticle dispersions of 0.1% $OSA_{1.0,6h}$-SGC polymer at the desired pH values (5.5, 7.4, and 9.2).

2.5. Fourier transform infrared (FTIR) spectroscopy

The chemical structures of SGC and OSA-SGC were confirmed using FTIR spectra (Jasco Inc., Easton, MD, USA). The background obtained from the scan of KBr was automatically deducted from the sample spectra. A total of 32 scans at a resolution of 4 cm^{-1} were accumulated to obtain a single spectrum with rapid-scan software in OMNIC 8.0. The wavelength region was between 4000 and 400 cm^{-1}.

2.6. Determination of DS

DS is the average number of hydroxyl groups substituted per glucose unit. The DS of OSA-SGC was measured via ^1H NMR, with slight modifications (Tizzotti, Sweedman, Tang, Schaefer, & Gilbert, 2011). Approximately 20 mg of OSA-SGC was dissolved in 0.5 mL DMSO-d_6 containing 0.5% (w/w) LiBr at 80 °C. TFA-d_1 (20 mg) was added directly to the mixture just before the ^1H NMR measurement, and the entire mixture was transferred to a ^1H NMR tube. The ^1H NMR spectra was recorded at 25 °C using an Avance III HD 500 MHz superconducting FT NMR spectrometer (Bruker Biospin, Rheinstetten, Germany).

The DS was calculated via the following equation:

$$DS = \frac{I_{0.89}}{3\left(I_{\alpha-1,6} + I_{\alpha-1,4} + I_{r-e}\right)} \quad (1)$$

where $I_{0.89}$ is the ^1H NMR integral of the signal of the CH_3 group of OSA in DMSO-d_6, and $I_{r\text{-}e}$ corresponds to the reducing chain ends. $I_{r\text{-}e}$ is the ^1H NMR integrals of the internal α and β reducing chain ends at approximately 4.28 and 4.91 ppm in SGC. The number of α-1,6 linkages ($I_{\alpha\text{-}1,6}$) is negligible in SGC. $I_{\alpha\text{-}1,4}$ is the ^1H NMR integrals of the internal α-1,4 at approximately 5.11 ppm (Tizzotti et al., 2011).