Carbohydrate Polymers 121 (2015) 10–17

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Effect of net surface charge on physical properties of the cellulose nanoparticles and their efficacy for oral protein delivery

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ARTICLE INFO

Article history:
Received 17 August 2014
Received in revised form 5 December 2014
Accepted 21 December 2014
Available online 27 December 2014

Keywords:
Cellulose nanoparticles
Surface charge
Cellular uptake
Oral delivery
Protein carrier

ABSTRACT

Both net positively and negatively charged cellulose-based nanoparticles were prepared from oppositely charged carboxymethylcellulose (CMC) and quaternized cellulose (QC). Effect of surface charge on efficacy of cellulose nanoparticles for delivering both positively and negatively charged proteins was investigated. Lysozyme (LYS) and bovine serum albumin (BSA), which possess positive and negative charge at physiological pH respectively, were used as models. The results revealed that high encapsulation efficiency (67.7% and 85.1%) could be achieved when negatively charged protein was encapsulated in positively charged nanoparticles, or positively charged protein was encapsulated in negatively charged nanoparticles. Proteins encapsulated in optimal cellulose nanoparticles could be sustainably released and no obvious protein denaturation was detected. Both net positively and negatively charged nanoparticles exhibited low cytotoxicity due to cellulose's good biocompatibility. Not only net positively charged nanoparticles demonstrated high cellular uptake efficiency, but also net negatively charged nanoparticles showed somewhat efficient cellular uptake.

1. Introduction

Compared with other routes of administration, oral delivery still remains as the most favorable and preferred route for drugs administration (Ramineni, Cunningham, Dziubla, & Puleo, 2013; Win & Feng, 2005). The oral route has attractive advantages such as convenience, low costs, and high patient compliance, which can avoid injections and decrease risk of infection (Win & Feng, 2005). However, the oral protein drugs usually exhibit the low level of bioavailability (Chen et al., 2008). The major challenges include the poor protein absorption and internalization through the gastrointestinal epithelium, as well as the rapid hydrolytic and enzymatic protein degradation by the gastrointestinal fluids (Harush-Frenkel, Rozentur, Benita, & Altschuler, 2008; Sandri et al., 2007; Thanou et al., 2000).

In order to overcome the above obstacles and improve the gastrointestinal uptake of protein drugs, different delivery systems have been investigated (Sadeghi et al., 2008). An effective approach is to entrap proteins within polysaccharide nanoparticles, which could protect proteins from degradation in the gastrointestinal fluids and deliver them to the target sites for release, and improve their permeation across the gastrointestinal epithelium (des Rieux, Fievez, Garinot, Schneider, & Préat, 2006; Jadhav & Singhal, 2014; Li et al., 2011; Mo, Jiang, Di, Tai, & Gu, 2014; Pan et al., 2002). Polysaccharides are considered as highly safe, biocompatible, and biodegradable natural biomaterials. Moreover, most of polysaccharides have hydrophilic groups such as hydroxyl, carboxyl, and amino groups, which could form non-covalent bonds with biological tissues such as intestinal mucosa to facilitate protein drug absorption (Liu, Jiao, Wang, Zhou, & Zhang, 2008; Song, Zhou, Li, Guo, & Zhang, 2008).

Cellulose is one of the most widely used natural substances and has become one of the most important commercial biopolymeric raw materials (Song et al., 2011). Microcrystalline cellulose and cellulose derivatives, such as carboxymethyl cellulose (CMC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), and hydroxypropylmethyl cellulose (HPMC), are recognized as the natural materials with good tolerance by the body (Mahmoud et al., 2010), and have been routinely used in medical and pharmaceutical applications such as controlled delivery systems (Kamel, Ali, Jahangir, Shah, & El-Gendy, 2008). In our previous works (Song, Zhou, & Chen, 2012; Song, Zhou, van Drunen Littel-van den Hurk, & Chen, 2014), the polyelectrolyte complex nanoparticles were prepared by mixing negatively charged carboxymethyl cellulose (CMC) and positively charged quaternized cellulose (QC) in an aqueous medium. The novel CMC–QC nanoparticles have been proven to be a promising delivery system of negatively charged proteins and DNA vaccines. The surface charge is very important for such systems,

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http://dx.doi.org/10.1016/j.carbpol.2014.12.019
0144-8617/

involving an encapsulation efficacy, cytoxicity, and cellular uptake (Song et al., 2010). Thus it is hypothesized that CMC–QC nanoparticle surface charge can be modulated to encapsulate protein drugs of different isoelectric points (pI) and controlled release them into small intestine for improved absorption. The test of this hypothesis relies on a fundamental understanding of the protein delivery properties of cellulose nanoparticles as impacted by surface charge.

In this work, both the net negatively charged and positively charged CMC–QC nanoparticles were prepared by changing the ratio of anionic-to-cationic polymers. Two proteins, lysozyme (LYS, pI 11.4) and bovine serum albumin (BSA, pI 4.8), with opposite charges in physical condition were selected as models. It was expected that these two types of proteins could be encapsulated in both negatively charged and positively charged CMC–QC nanoparticles. The challenge was to encapsulate the cationic protein LYS molecule into the positively charged nanoparticles, while encapsulate the anionic protein BSA molecule into the negatively charged nanoparticles. To overcome this challenge, we attempted to mask the positively charged LYS by complex LYS with the polyanion CMC, while masking the negatively charged BSA by complex BSA with the polycation QC. The effects of surface charge on encapsulation efficacy, release, cytoxicity, and cellular uptake of cellulose nanoparticles were systematically investigated. These studies provided the important information of scientific value for designing the cellulose-based nanoparticles to be used as the delivery vectors efficiently and specifically.

2. Experimental methods

2.1. Materials

Wood cellulose (spruce bleached sulphate pulp) was kindly provided by Alberta-Pacific Forest Industries Inc. (Alberta, Canada), and the molecular weight was determined by viscometry in LiOH/urea aqueous solution to be 40.5×10^4 g/mol (Song et al., 2012). 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), Fetal bovine serum (FBS), and Minimal Essential Medium (MEM) were purchased from Invitrogen Corp. (CA, USA). Caco-2 cells were purchased from American Type Culture Collection (ATCC, VA, USA). Sodium monochloroacetate, 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC), LYS, BSA, Coumarin-6 (COUM-6), 4′,6-diamidino-2-phenylindole (DAPI), and Alexa Fluor® 594 conjugate of Concanavalin A were purchased from Sigma-Aldrich (MO, USA). Other reagents were of analytical grade and used without further purification.

QC and CMC were prepared according to previous work (Song et al., 2012). Briefly, 50 g CHPTAC (60 wt% aq.) or 25 g solid sodium monochloroacetate was added gradually into 100 g of wood cellulose solution (1 wt% in LiOH/urea aq.), respectively, and then the mixture was stirred vigorously. The quaternization reaction was kept at 25 °C for 24 h, while the carboxymethylation reaction was kept at 55 °C for 5 h. The reaction products were dialyzed against distilled water, and freeze-dried to obtain the water-soluble QC and CMC samples.

2.2. Preparation and characterization of CMC–QC nanoparticles

LYS-encapsulated CMC–QC nanoparticles were prepared by the following method: 10 mL LYS aqueous solutions were mixed with 10 mL CMC aqueous solutions, then 10 mL QC aqueous solutions was added and followed by vigorous vortex for 30 s. As for BSA, 10 mL BSA aqueous solutions were mixed with 10 mL QC aqueous solutions, then 10 mL CMC aqueous solutions was added and mixed by vigorous vortex. Detailed preparation conditions are summarized in Table 1. The formed suspensions were kept for 30 min at room temperature before further use or characterization.

The zeta potential was measured using Zetasizer Nano-ZS ZEN1600 (Malven Instruments, UK) at 25 °C. The particle size was measured based on dynamic laser scattering (DLS, Malven Instruments, UK). The morphology of the protein-encapsulated CMC–QC nanoparticles was observed using a transmission electron microscope (TEM, Philips Morgagni 268, FEI Company, Netherlands). Freshly prepared samples were placed on a copper grid covered with Formvar film. After the deposition, the aqueous solution was blotted away with a strip of filter paper, and then examined at 80 kV.

2.3. Protein encapsulation and in vitro release

The protein encapsulation efficiency (EE) and loading capacity (LC) of CMC–QC nanoparticles was determined by isolating the nanoparticles from suspension by centrifugation at 20,000 rpm for 30 min. The concentration of remaining free protein in the clear supernatant was determined by micro-BCA protein assay. Absorbance was measured by a microplate reader (GENios, Tecan, Männedorf, Switzerland). The standard curves for proteins concentration was created using known concentrations of proteins. EE and LC were calculated as follows:

$$\text{EE}\ \ (\%) = 100 \times (W_0 - W_f)/W_0$$

$$\text{LC}\ \ (\%) = 100 \times (W_0 - W_f)/W_N$$

where, W_0 was the total weight of protein added, W_N was the total weight of nanoparticles, and W_f was the weight of free protein remained in the supernatant.

In vitro protein release profiles from CMC–QC nanoparticles were determined as follows: 5 mg lyophilized protein-loaded nanoparticles were dispersed into 20 mL HCl buffer at pH 1.2 (simulated gastric fluid), then incubated on a shaking water bath at 37 °C, 100 rpm for 2 h. At appropriate intervals, 0.4 mL supernatant was taken, separated from nanoparticles by centrifugation (20,000 rpm, 30 min) for protein determination and replaced by fresh medium. After 2 h, the nanoparticles were transferred to 20 mL phosphate buffer at pH 6.8 (simulated intestinal fluid) and incubated at 37 °C for additional 4 h. At appropriate intervals, 0.4 mL supernatant was taken, separated from nanoparticles by centrifugation (20,000 rpm, 30 min) for protein determination and replaced by fresh medium. The concentration of released protein was determined by micro-BCA protein assay.

2.4. Bioactivity of released lysozyme

The relative bioactivity of released lysozyme was determined using the decrease in optical density at 450 nm of a *Micrococcus luteus* suspension. Briefly, *M. luteus* was suspended into phosphate buffer solution (66 mM PBS, pH 6.15), and then diluted to obtain an absorbance (at 450 nm) between 0.2 and 0.6. Then 0.1 mL aliquot of appropriately diluted lysozyme sample was mixed with 2.5 mL prepared *M. luteus* suspension in a quartz cell, which was then immediately placed into a spectrophotometer. The rate of decrease of absorbance at 450 nm was monitored by a microplate reader (GENios, Tecan, Männedorf, Switzerland) during a total period of 2 min at 25 °C. The relative bioactivity of lysozyme was calculated from the linear slope of the curve (absorbance versus time) according to the technique described by van de Weert et al. (van de Weert, Hoechstetter, Hennink, & Crommelin, 2000).