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Preparation, characterization and in vitro release of β-galactosidase loaded polyelectrolyte nanoparticles

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ABSTRACT

Improving encapsulation efficacy (EE) and bioavailability of β-galactosidase (β-gal) is always a challenge due to its fragility. In this work, β-gal loaded β-chitosan (CS) nanoparticles (NPs) were successfully prepared based on ionic gelation technique and electrostatic attraction for improving its EE and in vitro releasing capacity. The particle size of β-gal loaded low and high molecular weight (LMW and HMW) β-CS NPs reached 584.37 and 652.46 nm, with Zeta-potential (ZP) of 26.37 and 16.46 mV under the optimal conditions, respectively. In vitro release study conducted at pH 4.5 and 7.4 showed that β-gal loaded LMW and HMW β-CS NPs with EE of 68.32 and 58.64% sustained the release of the β-gal over 12 h. The β-gal incorporated into β-CS NPs was confirmed with the results of physicochemical and structural properties of β-gal loaded β-CS NPs, and prepared NPs had hardly any cytotoxicity in the range of 0.1–1.0 mg/mL. The results indicated that β-gal loaded β-CS NPs could serve as non-toxic delivery carriers for the treatment of lactose intolerance.

1. Introduction

Lactose intolerance, a very common disorder, is unable to digest lactose into glucose and galactose because of low levels of lactase [1]. It is estimated that 75% of adults worldwide is subjected to lactose maldigestion because of lactase deficiency, leading to undesired clinical symptoms such as abdominal bloating and pain, flatulence, diarrhea and nausea [2,3]. The diagnosis or even the prescription of lactose intolerance guides consumers to refrain from consuming containing-lactose milk and foods. The use of exogenous β-galactosidase (β-gal), produced on the brush border of small intestine, was regarded as the best treatment of lactose intolerance through digesting lactose [4–6]. In particular, the intake of digestive supplements containing β-gal is a successful and effective way to alleviate the symptoms of lactose intolerance [7]. However, commercial β-gal supplements had low utilization efficacy because of rapid inactivation, and only a small amount of the molecules remains available following oral administration, due to insufficient gastric residence time, low permeability and/or adsorption capacity within the gut, as well as their instability in the gastrointestinal tract (because of low pH, enzyme activity, presence of other nutrients and interfering agents) [8]. Therefore, selecting a proper method is critical and urgent for preventing β-gal from digestion and extending its releasing time in stomach and gut.

Microencapsulation, a frequently-used nutrients carrier technique applied in food and pharmaceutical industry, is increasingly attracted considerable attention and has been employed to protect bioactive substances against surrounding conditions due to large surface areas [9]. Moreover, encapsulated materials used must be non-cytotoxicity, good biodegradability and biocompatibility. Considering the different interactions (encapsulation, crosslinking and attaching) between entrapped bioactive compounds and polymeric system, microencapsulation can also control the release of bioactive compounds at a given time and targeted location in the digestion system. Various encapsulation methods reported in previous studies include (1) physical methods (spray drying [10], fluid bed coating [11], extrusion spheronization [12], centrifugal extrusion, and supercritical fluids technique [13]); (2) physicochemical methods (spray cooling [14], hot melt coating, ionic gelation [15], solvent evaporation extraction and coacervation); and (3) chemical methods (interfacial polycondensation, polymerization [16] and interfacial crosslinking) [17]. Among this, ionic gelation technique has attracted increasingly interest due to easy to handle, highly

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compatible, non-toxic, organic solvent free, convenient and controllable [18,19]. The complexation between positively charged β-chitosan (CS) in acidic solution and anionic crosslinking agent (sodium tripolyphosphate, TPP), creates active compounds loaded microbeads [20].

Beta-CS, originated from the deacetylation of β-chitin that exists mainly in squid pens [21], has been applied in multiple fields, such as food, pharmaceutical, medicine, water treatment and agriculture due to its excellent properties, including simple preparation, higher affinity toward solvents, and better biological activity than α-CS obtained from crab and shrimp shells wastes [22]. Up to now, no previous study has evaluated the encapsulation of β-gal using polyelectrolyte β-CS NPs. Generally, small particle size of encapsulators penetrates more effectively into the target cells, thus improving the encapsulation efficacy (EE) of target substances [23]. To better understand the EE of β-gal, β-gal loaded LMW and HMW β-CS NPs were synthesized in this work. Therefore, it was hypothesized that β-gal loaded LMW β-CS NPs exhibited better encapsulation and releasing properties than that of high Mw ones.

For achieving this goal, β-gal loaded LMW and HMW β-CS NPs was prepared to analyze the particle size, PDI and Zeta-potential (ZP) of β-gal loaded β-CS NPs. Structural analysis of β-gal loaded LMW and HMW β-CS NPs were characterized using Transmission electron microscope (TEM), Fourier transform infrared spectrometer (FT-IR) and X-ray diffraction (XRD) and differential scanning calorimetry (DSC) analysis. This study would provide new prospect of β-gal loaded β-CS NPs as potential treatment for lactose intolerance.

2. Materials and methods

2.1. Materials and reagents

Beta-CS (purity, 99.99%) was purchased from Zhejiang Aoxing Biotechnology Co., Ltd., China. Spectra/Por® dialysis membrane (MW cut-off of 8–14 kDa) was purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA). Whatman® cellulose nitrate membrane filters (0.45 and 0.25 μm) were from Whatman GmbH (Dassel, Germany). β-gal from *Escherichia coli* (CAS: 9031-11-2), cellulase, sodium TPP were from Sigma Chemical Co. (St. Louis, MO). Glacial acetic acid, hydrochloric acid, sodium hydroxide and all other chemicals (analytical grade) were purchased from Sinopharm Chemical Reagent Co., Ltd., China. Distilled (DI) water was used in the whole study.

2.2. Preparation of LMW β-CS

LMW β-CS (MW 40 kDa) was prepared based on previous report using cellulase depolymerization [24]. A 2.5 g of HMW β-CS (MW 337 kDa) was completely dissolved in 1% acetic acid solution (pH 5.0) and stirred overnight at ambient temperature (600 r/min), a 1.5% ratio of cellulase and β-CS (w/w) was then added and reacted for 7 h at 55 °C. The 4 N NaOH was further added for adjusting the pH of mixture solution until a final pH > 10 for precipitation. The sediment was collected and washed using DI water to remove other residues, and then was freeze-dried for later use.

2.3. Preparation of β-gal loaded β-CS NPs

Beta-gal loaded β-CS NPs were prepared based on the ionic gelation and electrostatic attraction of β-CS with sodium TPP anions [25]. Briefly, 0.5 mg/mL LMW and HMW β-CS were respectively dissolved in aqueous acetic acid (0.1 mg/mL), stirred overnight at ambient temperature, and filtered through a 0.45 μm filter membrane. The pH of the resulting solution was adjusted to pH 4.7–4.8 by adding 0.1 N NaOH. The 0.5 mg/mL sodium TPP was dissolved in DI water and filtered using a 0.25 μm filter membrane. To prepare β-gal loaded LMW and HMW β-CS NPs, 15 mL of LMW and HMW β-CS solution placed in a 100 mL round-bottom flask was precooled at 4 °C for 10 min, the flask was stirred at 600 r/min, and 1 mL of 3, 2.5, 2, 1 and 0.5 mg/mL β-gal solution containing BSA (1 mg/mL) prepared using 0.08 M phosphate buffer (PB) at pH 7.7, was added into the LMW and HMW β-CS solution under the stirring condition, respectively. Then, 3 mL of 4 °C sodium TPP solution was quickly added into the above solution. For the preparation of β-CS NPs. The process of preparation was the same except that β-gal and BSA solution was not added. The reaction was carried out for 15 min and the resulting suspension was subjected to centrifugation for collecting the precipitate for further analysis.

2.4. Physicochemical analysis of β-gal loaded β-CS NPs

Beta-gal loaded LMW and HMW β-CS NPs solutions was centrifuged for 20 min at 12,000 r/min and then freeze-dried. The obtained sediment was re-suspended in DI water and measured in terms of particle size, PDI and ZP by using DLS particle size analyzer (Malvern, Nano ZS-90, Worcestershire, UK). The MM of β-CS before and after depolymerization was calculated in our previous method [21]. The supernatant of β-gal (0.5–3.0 mg/mL) loaded LMW and HMW β-CS NPs solutions was examined UV–vis spectrophotometry (Model UV-1800, Thermo Fisher Scientific, Inc., MA, USA). EE of β-gal (0.5–3.0 mg/mL) encapsulated in β-CS NPs was determined using the following equation:

$$\text{EE}\ (\%) = \frac{\text{Amount of β-gal used-amount of unbonded β-gal}}{\text{Amount of β-gal in the formulation}} \times 100 \quad (1)$$

Yields were calculated as:

$$\text{Yields}\ (\%) = \frac{\text{Amount of recoveried NPs}}{\text{Total amont of β-CS, sodium TPP and β-gal}} \times 100 \quad (2)$$

2.5. Structural characterization

2.5.1. FT-IR analysis

Samples were analyzed using FT-IR spectroscopy (Perkin Elmer Spectrum RX I, USA). Samples were directly placed in the sample holder. Spectral scanning was taken in the wavelength region between 4000 and 500 cm^{-1} at a resolution of 4 cm^{-1} with scan speed of 2 mm/s [26,27].

2.5.2. XRD analysis

A D8 ADVANCE XRD (BRUKER-AXS Co., Germany) was applied to detect the crystallinity of samples and their patterns based on the wide-angle X-ray diffraction (WAXD) analysis. 2θ was scanned from 5° to 50° at a coating time of 2 s with an angle step width of 0.05°. The crystallinity index (CrI_{peak}) was calculated as [27].

$$CrI_{peak} = \frac{(I_{110} - I_{am})}{I_{110}} \quad (3)$$

where I_{110} was the maximum intensity (arbitrary unit) of the (110) lattice diffraction pattern at $2\theta = 20°$ and I_{am} was the intensity of amorphous diffraction in the same unit at $2\theta = 16°$.

2.5.3. DSC analysis

Approximate 6 mg of sample was weighed, placed onto an aluminum cup, and sealed with an empty cup used as reference. The samples were heated up to 650 °C at a heating rate of 10 °C/min under air flow of 23 mL/min. At least three runs for each sample were conducted and the mean values were reported.

2.6. In vitro release studies

Schematic diagram of making β-galactosidase loaded β-CS NPs used for in vitro digestion is shown in Scheme 1. EE of β-gal was estimated by