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Original Research Paper

Auricularia auricular polysaccharide-low molecular weight chitosan polyelectrolyte complex nanoparticles: Preparation and characterization

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ABSTRACT

Novel polyelectrolyte complex nanoparticles (AAP/LCS NPs) were prepared in this study and these were produced by mixing negatively charged auricularia auricular polysaccharide (AAP) with positively charged low molecular weight chitosan (LCS) in an aqueous medium. The AAP was extracted and purified from auricularia auricular, and then characterized by micrOTOF-Q mass spectrometry, UV/Vis spectrophotometry, moisture analyzer and SEM. The yield, moisture, and total sugar content of the AAP were 4.5%, 6.2% and 90.12% (w/w), respectively. The AAP sample was water-soluble and exhibited white flocculence. The characteristics of AAP/LCS NPs, such as the particle size, zeta potential, morphology, FT-IR spectra, DSC were investigated. The results obtained revealed that the AAP/LCS NPs had a spherical shape with a diameter of 223 nm and a smooth surface, and the results of the FT-IR spectra and DSC investigations indicated that there was an electrostatic interaction between the two polyelectrolyte polymers. Bovine serum albumin (BSA, pI = 4.8) and bovine hemoglobin (BHb, pI = 6.8) were used as model drugs to investigate the loading and release features of the AAP/LCS NPs. The results obtained showed that the AAP/LCS NPs had a higher entrapment efficiency (92.6%) for BHb than for BSA (81.5%). The cumulative release of BSA and BHb from AAP/LCS NPs after 24 h in vitro was 95.4% and 91.9%, respectively. The in vitro release demonstrated that AAP/LCS NPs provided a sustained release matrix suitable for the delivery of protein drugs. These studies demonstrate that AAP/LCS NPs have a very promising potential as a delivery system for protein drugs.

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1. Introduction

Currently, over 160 therapeutic protein drugs have been licensed, and even more protein drugs will be approved by regulatory agencies in the next few years [1]. The physicochemical and biological properties of protein drugs are unlike those of conventional ones, particularly with regard to their molecular weight, solubility, physico-chemical stability, biological half-life, conformational stability, oral bioavailability, dose requirements, and administration [2]. Moreover, oral protein drugs usually exhibit a low level of bioavailability. The major challenges to be overcome involve poor protein absorption and internalization through the gastrointestinal epithelium, as well as the rapid hydrolysis and degradation by gastrointestinal fluids [3,4]. Hence, the design and manufacturing of delivery systems for protein is attracting much attention.

In order to overcome the above obstacles and increase the gastrointestinal uptake, some nanoparticles made from natural biodegradable polymers have been exploited and applied to protein drugs [5,6]. However, biodegradable polymer nanoparticles made from polyglycolic acid and polylactide and their copolymers are usually obtained by using organic solvents, high temperatures and sheer forces and are easily inactivated by physical and chemical denaturation. Furthermore, after the formulation has been administered, changes in the microenvironment can lead to polymer degradation which can dramatically affect the tertiary structure of the protein [7,8].

Entrapping protein drugs within polysaccharide nanoparticles is an effective way of protecting them from degradation in the gastrointestinal fluids, delivering protein drugs to the target sites for release and increasing their permeation across the gastrointestinal epithelium [9–11]. Natural polysaccharides have many promising properties, including excellent biodegradability, high biocompatibility, low toxicity, good safety, abundant availability, and their cost of production is low [12]. Moreover, most polysaccharides have hydrophilic groups, such as hydroxyl, carboxyl, and amino groups, which may form non-covalent bonds with biological tissues like the intestinal mucosa to facilitate the absorption of protein drugs [13,14].

Consequently, many references [5,15] have described how polyelectrolyte complex (PEC) nanoparticles are formed spontaneously by mixing oppositely charged polyelectrolytes in aqueous media without any chemical covalent cross-linker [16,17], and such PEC nanoparticles have bright application prospects for protein drugs. The major interactions between the two polyelectrolyte polymers are electrostatic, formation of hydrogen and hydrophobic bonds, as well as dipole–dipole association [18].

Auricularia auricular polysaccharide (AAP) is abundant in auricularia auricular, and is a significant bioactive substance, with broad physiological activity and which is attracting much attention because of its potential medical applications [19]. However, AAP as a novel natural polysaccharide has not been studied in any detail with regard to its use as a drug carrier. Hence, in this study, we used AAP as a negatively charged polyelectrolyte to form PEC with Chitosan (a cationic polyelectrolyte in acidic medium).

Based on the reasons mentioned above, the goal of this paper is to manufacture AAP/LCS NPs, composed of positively charged low molecular weight chitosan (LCS) and negatively charged AAP. Two model protein drugs with different pI values, BSA (pI = 4.8) and BHb (pI = 6.8), were used to investigate the protein loading capacity and release of AAP/LCS NPs. The physicochemical characteristics of the prepared nanoparticles were characterized by investigation of Fourier transform infrared spectra (FT-IR), particle size, zeta potential, differential scanning calorimetry (DSC) and transmission electron microscopy (TEM). The results obtained indicated that novel AAP/LCS NPs had a very promising potential as a delivery system for protein drugs.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA) and bovine hemoglobin (BHb) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Coomassie brilliant blue G-250 was obtained from Regent Chemicals Co., Ltd (Tianjin, China). Auricularia auricular was bought from a Carrefour supermarket (Shenyang, China), and it had been grown in Liaoning Province, China. Low molecular weight chitosan was purchased from Golden-Shell Pharmaceutical Co., Ltd (Zhejiang, China), and the degree of deacetylation was 90%. All other reagents and chemicals were of analytical grade.

2.2. Extraction and purification of AAP

The AAP was extracted and purified by a modified water extraction and alcohol precipitation method [20]. For this, the auricularia auricular was defatted by reflux, dried and the resulting powder was weighed and extracted, then subjected to precipitation and washing with ethanol, to obtain crude grey polysaccharide by vacuum-drying. A 2% crude polysaccharide solution was prepared by removing protein by the Sevag method (chloroform: n-butanol = 4:1). The pH was adjusted to 8.0 with ammonia, and the color was removed with hydrogen peroxide. Then, the decolorized polysaccharide solution was dialyzed against distilled water and purified AAP was obtained by concentrating and freeze-drying the dialysate.

2.3. Characterization of AAP

2.3.1. Polysaccharide content

The AAP content was measured by phenol-sulfuric acid method using D-glucose as a standard [21]. The percentage of AAP extraction yield (%) was calculated with the formula as follow:

$$Yield\,(\%) = \frac{W_2}{W_1} \times 100 \quad (1)$$

Where W_2 is the polysaccharides content of extraction, and W_1 represents the dried sample weight.

The protein of the polysaccharide was detected by UV-4802 Double Beam UV/Vis Spectrophotometer (Unico, Shanghai, China). The moisture content of the AAP was obtained by a moisture analyzer (Shuangquan, Shanghai, China).