International Journal of Biological Macromolecules 91 (2016) 609–614

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Preparation and sustainable release of modified konjac glucomannan/chitosan nanospheres

Congjiao Shi [1], Pei Zhu [1], Na Chen, Xiaozhou Ye*, Yun Wang*, Shaobo Xiao

State Key Laboratory of Agricultural Microbiology, College of Science, Huazhong Agricultural University, Wuhan 430070, PR China

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form 12 May 2016
Accepted 18 May 2016
Available online 3 June 2016

Keywords:
Konjac glucomannan
Chitosan
Nanospheres
Ovalbumin
Sustained release

ABSTRACT

Biodegradable and biocompatible polymer nanospheres are useful materials for controlled drug delivery. In the present study, novel composite nanospheres were prepared from carboxymethyl konjac glucomannan (CKGM) and 2-hydroxypropyl trimethyl ammonium chloride chitosan (HACC) as a vaccine delivery vehicle by electrostatic complexation in a neutral aqueous solution without the use of chemical crosslinkers. By altering the CKGM and HACC concentrations, the average CKGM/HACC particle size could be tuned from approximately 600 nm to 1460 nm and the zeta potential from 39 mV to 50 mV. Furthermore, using ovalbumin (OVA) as a model molecule for vaccines, various parameters were determined to affect the CKGM/HACC nanosphere encapsulation efficiency and *in vitro* controlled release properties. Under optimum conditions, the OVA encapsulation efficiency of CKGM/HACC nanospheres was 71.8%, while sustained and continuous *in vitro* OVA release over a period of more than 24 h was observed. Therefore, CKGM/HACC nanospheres are novel drug delivery carriers with great potential for medical applications.

1. Introduction

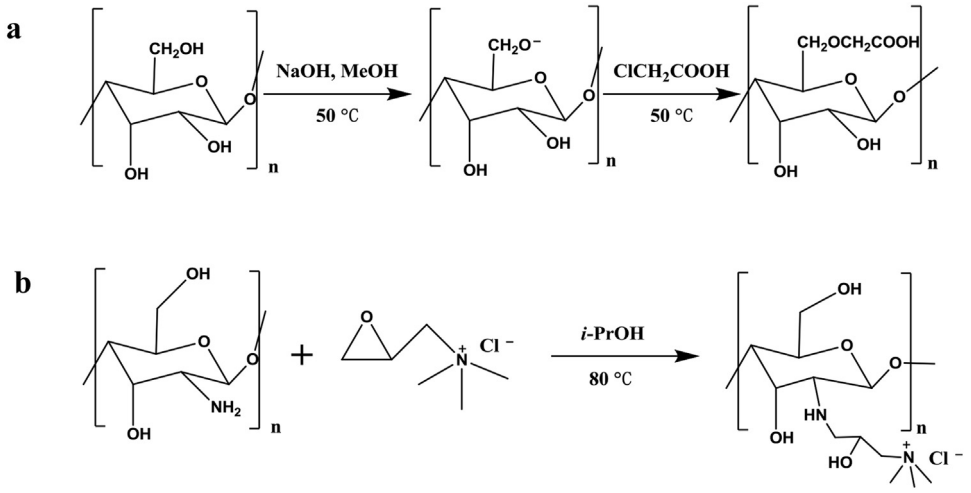
Biodegradable nano-size polymers, especially nanoparticles and nanocapsules of polysaccharides, are a class of novel carriers for sustained drug release [1,2]. Due to their good biocompatibility, ultra-fine particle size, low toxicity, and efficient utilization of drugs, they are ideal carriers for entrapping biologically active macromolecular drugs, such as polypeptides, proteins, nucleic acids, and vaccine vectors [3–6]. Oppositely charged polysaccharides can be mixed in aqueous solutions and form polyelectrolyte complexes (PECs) without the use of chemical covalent crosslinkers [7,8]. Owing to their biodegradability, non-toxicity, and sensitivity to stimuli, polyelectrolyte complexes of polysaccharides have attracted wide attentions and have been investigated in regard to drug encapsulation and delivery [9,10].

Konjac glucomannan (KGM), a major active ingredient in konjac tuber, is a natural polysaccharide macromolecule of D-glucose and D-mannose that is linked through a β-l,4-glycosidic bond [11,12]. As a natural and renewable polymer resource with excellent biocompatibility, biodegradability and biological activity, KGM has good application prospects in the biomedical field [13,14]. Carboxymethylation of konjac glucomannan produces a negatively charged polymer (CKGM) with increased water solubility, swelling rate and stability compared to KGM [15,16]. Due to these improved properties in combination with its excellent biological activity, CKGM nanoparticles have been prepared and used as a drug delivery vehicle. For instance, Li et al. prepared cholesterol-modified CKGM amphiphilic nanomicelles that had a maximum etoposide encapsulation rate of 39.4% and steady drug release for 23 h [17]. Zhang et al. immobilized asparaginase on nanospheres, and the immobilized enzyme retained its activity, while showing improved thermal stability and tolerance to acidic and alkaline environments [18].

Chitosan (CS) is a positively charged natural polysaccharide that contains a large number of amine groups. Due to its biodegradable and biocompatible properties, chitosan is considered a promising biomaterial for applications in biomedicine, food, health care, cosmetics, and others [10,19]. In particular, chitosan has been used as a raw material for the preparation of nanosphere drug carriers that showed good drug loading and sustained release properties [20–22]. Positively-charged 2-hydroxypropyl trimethyl ammonium chloride chitosan (HACC), a water-soluble chitosan derivative [23], may be superior to chitosan, as its quaternized cationic nature enables stronger electrostatic interactions with negatively charged tumour cells when used as a drug carrier for tumour therapy [24]. In addition, HACC can potential be applied in many fields, e.g., prevention of fungal skin infections [25,26], orthopaedics [27], nanofiltration [28,29] and drug delivery [24,30],

* Corresponding authors.
E-mail addresses: xzye@mail.hzau.edu.cn (X. Ye), yunwang123@126.com (Y. Wang).
[1] Equal contribution by the first two authors.

http://dx.doi.org/10.1016/j.ijbiomac.2016.05.073
0141-8130/



Scheme 1. The synthetic route of CKGM (a) and HACC (b).

due to its biocompatibility, water-solubility, low cytotoxicity, permanent cationic charges, among others.

Negatively charged CKGM and positively charged CS may form PECs with potential drug loading and delivery applications [31]. For instance, Du et al. prepared CKGM/CS nanospheres by complex coacervation in an acidic solution that had a high encapsulation efficiency for bovine serum albumin (BSA) [32,33] and the potential for colloidal drug delivery [34]. However, despite these promising results of CKGM/CS complex nanospheres in the field of drug delivery, there have been few published articles on this topic, while the use of CKGM/HACC composite nanospheres for drug delivery, especially that of vaccines, to our knowledge has not yet been reported. Herein, we report the facile preparation of CKGM/HACC complex nanospheres in a neutral aqueous solution without chemical crosslinkers. The effects of the concentrations of CKGM and HACC on the average particle size and zeta potential of CKGM/HACC composite nanospheres were also determined. To investigate the potential application of CKGM/HACC nanospheres for vaccine loading and delivery, we used ovalbumin (OVA) as a model drug and evaluated its encapsulation and sustained release.

2. Materials and methods

2.1. Materials

KGM with a viscosity average molecular weight of approximately 100000 g/mol was purchased from Wuhan Shenshi Chemical Technology Co. (Wuhan, China). CS with a viscosity average molecular weight of approximately 8000 g/mol and a degree of deacetylation of 92% was purchased from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China). The reagent 2,3-epoxypropyl trimethyl ammonium chloride, with a degree of substitution of 61%, was prepared in-house according to published methods [35]. OVA was purchased from Aladdin Reagents Co. Ltd. (Shanghai, China). All reagents were of analytical grade.

2.2. Preparation of CKGM/HACC nanospheres

2.2.1. Degradation and carboxymethyl modification of KGM

KGM (35 g) was hydrolysed with 250 mL of a HCl-ethanol solution (v/v, 70:180) in a 500-mL three-neck flask under mechanical stirring at room temperature for 2 h. The product was subsequently washed with a 70 wt.% aqueous ethanol solution, vacuum filtered, and vacuum dried at 30 °C for 16 h to obtain acid-hydrolysed KGM (AHKGM) for the preparation of carboxymethyl-modified KGM.

AHKGM (10 g) was mixed with 20 mL of a 50 wt.% aqueous methanol solution in a three-neck flask and mechanically stirred at room temperature for 30 min until the AHKGM had completely swollen. Subsequently, 50 mL of anhydrous methanol was added to the three-neck flask and the mixture was heated to 50 °C. Then, 20 mL of a 30 wt.% aqueous NaOH solution was added to the mixture dropwise, which was followed by a 30 min reaction. After the addition of 7.5 g of monochloroacetic acid, the reaction was allowed to continue for 15 h at 50 °C under mechanical stirring. Finally, the product solution was neutralized with HCl; washed several times with 70 wt.%, 80 wt.% and 90 wt.% aqueous methanol solutions to remove impurities, vacuum filtered; and vacuum dried at 50 °C to produce CKGM. The reaction scheme was presented in Scheme 1a. The degree of carboxymethyl substitution of KGM was measured according to the literature to be 0.49 [36].

KGM, AHKGM, and CKGM compounds were swollen in 100 mL of water (1 g each), and their apparent viscosities, measured with a NDJ-79 rotary viscometer, were 9, 1.4, and 2.6 mPa·s, respectively.

2.2.2. Modification of cationic CS

CS (8.0 g) was mixed with 72 mL of isopropanol in a three-neck flask, heated to 60 °C, and mechanically stirred for 1 h. Subsequently, 80 mL of a 37 wt.% aqueous 2,3-epoxypropyl trimethyl ammonium chloride solution was added and the mixture heated to 80 °C under mechanical stirring for 14 h. The resulting product was washed with 80 wt.% isopropanol by suction filtration. The filter cake was dissolved in distilled water in a dialysis bag and dialysed for 48 h against distilled water. The dialysis solution was replaced every 4 h. The dialysed solution was then precipitated with a certain amount of acetone, suction filtered and vacuum dried at 50 °C to produce HACC. The reaction scheme was presented in Scheme 1b.The degree of 2,3-epoxypropyl trimethyl ammonium chloride substitution of CS was measured according to the literature to be 0.61 [37].

2.2.3. Preparation of blank nanospheres and drug loaded nanospheres

Blank CKGM/HACC composite nanospheres and CKGM/HACC/OVA composite nanospheres were prepared by complex coacervation.

2.2.3.1. Preparation of blank CKGM/HACC nanospheres. A series of CKGM and HACC solutions at different concentrations were prepared and mechanically stirred at room temperature. Five millilitres of a CKGM solution was added dropwise to 10 mL of a HACC