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Chitosan/β-lactoglobulin core–shell nanoparticles as nutraceutical carriers

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Abstract

Chitosan (CS)/β-lactoglobulin (βlg) core–shell nanoparticles (CS-βlg nanoparticle) were successfully prepared with the aim of developing a biocompatible carrier for the oral administration of nutraceuticals. The effects of pH and initial concentrations ($C_{\beta lg}$) of native and denatured βlg on the properties of the nanoparticles were investigated. Uniform nanoparticles were prepared by ionic gelation with sodium tripolyphosphate (TPP). The surface charge of the particles was positive, with a zeta potential of 20–60 mV. βlg loading efficiency (LE) spanned a broad range (1–60%); and was highly sensitive to formulation pH. This adsorption can be mainly attributed to electrostatic, hydrophobic interactions and hydrogen bonding between βlg and CS. Brilliant blue (BB) release experiments showed that the nanoparticles prepared with native βlg had favorable properties to resist acid and pepsin degradation in simulated gastric conditions unlike those prepared with denatured βlg or denatured βlg crosslinked with Ca^{2+}. When transferred to simulated intestinal conditions, the βlg shells of the nanoparticles were degraded by pancreatin.

Keywords: Chitosan; β-lactoglobulin; Nanoparticle; In vitro test

1. Introduction

The emergence of bioactive food compounds (nutraceuticals) with health benefits provides an excellent opportunity to improve public health. The incorporation of bioactive compounds—such as peptide, vitamin—into food systems as a potentially simple means of modulating the risks of diseases therefore holds much promise for the scientific community as it tries to develop innovative functional foods that may have physiological benefits or reduce the risks of diseases [1]. However, the effectiveness of such products in preventing diseases relies on preserving the bioavailability of the active ingredients. This represents undoubtedly a great challenge given that a large proportion of these molecules remain poorly available by oral administration due to too short gastric residence time of the dosage form; a low permeability and/or solubility within the gut; and also a lack of stability in the environmental conditions encountered in food processes (temperature, oxygen, light) or in the gastro-intestinal tract (pH, enzymes, presence of other nutrients), which limits their activity and potential health benefits [2]. Encapsulation systems can be used to overcome these limitations. This has been a daunting task for the pharmaceutical industry in the past decades. The growing interest in the effective and selective delivery of bioactive agents to the site of action has led to the development of new encapsulation materials. While many synthetic polymers have been created for delivery systems, they cannot be used in food applications that require edible compounds generally recognized as safe (GRAS).

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Chitosan (CS), a copolymer derived from chitin (an abundant natural polymer), has been widely used in numerous biomedical applications, including drug delivery systems, because it is biodegradable, biocompatible, and mucoadhesive [3–8]. Among these delivery systems, CS nanoparticles have been paid much attention in the recent years because of their special feature of adhering to the mucosal surface and transiently opening the tight junction between epithelial cells. It has also been shown that a small particle size can dramatically prolong residence time of the formulation in the gastrointestinal tract, because of an important decrease on the influence of intestinal clearance mechanisms and the high increase on the surface to interact with the biological support [9,10]. Latest reports also indicate that entire CS nanoparticles can be uptaken by human cells [11,12], significantly enhancing the bioavailability of the encapsulated bioactive molecules. CS nanoparticles have been investigated as carriers for DNA, the anticancer drug doxorubicin, and macromolecules like insulin [13–18]. However, compared to pharmaceutical applications, little work has been done on the encapsulation properties of CS nanoparticle for the oral administration of nutraceuticals in healthy food. More recently, CS has been identified as a versatile biopolymer for a broad range of health, food applications because of its safety and nontoxicity in the human beings. In 1992, Japan's Health Department approved chitin and its derivatives as functional food ingredients [19–21]. These properties make CS a good candidate for the development of nutraceutical delivery systems for food applications. However, for oral administration, CS matrix are not stable at low pH values, and rapid dissociation and degradation occurred at pH 1.0 [22], which could lead to destruction of sensitive nutraceuticals in the stomach circumstances. To overcome the drawbacks, the CS nanoparticles could be surface coated to enable the protection in the gastric tract.

β-lactoglobulin (βlg), the major whey protein in the milk of ruminant, is a small globular protein widely used as food ingredient because of its nutritional value and its ability to form gels [23,24], emulsions [25,26], and gelled emulsion [27,28]. Another important functional property of whey proteins is their ability to form cold induced gel matrices by adding cations to a preheated denatured protein suspension [29,30], which results in the formation of a network crosslinked via Ca^{2+} with carboxylate groups on denatured βlg at ambient temperature [31]. In addition to its functional characteristics, it is known to be resistant to degradation of the pepsin in the stomach in its native structure [32] or adsorbed at an interface [27]. Hydrolysis of whey proteins by pancreatin enzymes generate bioactive peptides that may exert a number of physiological effects in vivo, e.g. on the gastrointestinal, cardiovascular, endocrine, immune and nervous systems [33,34]. Due to its attractive techno-functional properties and large availability, βlg is an interesting candidate to coat CS nanoparticle to allow a protection when subject to the gastric fluids.

The purpose of this work was to elaborate a CS-βlg core–shell nanoparticle in order to develop a biocompatible carrier for oral administration of the sensitive nutraceuticals, where the shell allows protection in the stomach circumstances, and the core improves retention of nutrition within the intestine wall, and also permits controlled release. And on the other hand, to understand release pattern from these nanoparticular matrices into the gastrointestinal tract, which is expected to play a determining role in the subsequent absorption of the nutraceuticals. The ability of βlg to coat CS nanoparticules will be studied in its globular native form and compared to its denatured and cross linking form.

2. Experimental

2.1. Materials

CS was provided by Marinard Biotech. (Quebec, Canada). Degree of deacetylation was 90.2%, determined by colloidal titration. The viscosity (1.0%, 25 °C) was 58 cps, measured with a Brookfield Viscometer. β-lactoglobulin was obtained from Davisco International, Inc. (Le Sueur, Minn., USA). It contained 98.2% protein measured by semi-micro kjeldahl method (AOAC 1984) using N-factor 6.38 [35]. Sodium tripolyphosphate (TPP), brilliant blue G250, CSase from streptomyces griseus and lysozyme from chicken egg white (48,800 u/mg) were purchased from Sigma Chemical Co. (St. Louis, MO). Pepsin 1:60000, from porcine stomach mucosa, crystallized and lyophilized; pancreatin 5×, from hog pancreas were supplied by Sigma Chemical Co. (St. Louis, MO) and ICN Nutritional Biochemicals (Cleveland, OH), respectively. All other chemicals were reagent grade.

2.2. Preparation of native and denatured βlg solutions

Native and denatured βlg solutions were applied to produce CS-βlg nanoparticles. Native βlg solution was prepared by hydrating βlg in deionized water with agitation at room temperature for 1 h. The solution was allowed to rest for 2 h before further treatment in order to permit a good protein hydration [27]. The above prepared solution was adjusted to pH 2.4 and heated at 80 °C for 30 min to allow denaturation of βlg [24]. The denatured βlg solution was then cooled at room temperature for further application.