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Improved bioavailability of curcumin in ovalbumin-dextran nanogels prepared by Maillard reaction

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ABSTRACT

In this work, ovalbumin-dextran nanogels were fabricated via the Maillard reaction followed by a heat-gelation process and their potential to improve curcumin bioavailability was investigated using an *in vitro* gastrointestinal tract. The result of electrophoresis, conjugation efficiency and yield, and far-UV circular dichroism verified the covalent attachment of dextran to ovalbumin by the Maillard reaction. The conjugates were then heated at the isoelectric point of ovalbumin to give stable nanogels. The transmission electron microscopy observation suggested that nanogels displayed a spherical structure. Curcumin was loaded into nanogels by a pH-driven method and the incorporation of curcumin affected the morphology of nanogels marginally. The transformation of curcumin in nanogels was significantly higher than that in ovalbumin nanoparticle. On the other hand, the bioaccessibility was similar for curcumin in nanogels and nanoparticle. The ovalbumin-dextran nanogels hold the potential as delivery systems for curcumin to fortify functional foods and beverages.

1. Introduction

Curcumin is the main bioactive ingredient in turmeric extract and has been drawing substantial attention recently owing to its beneficial effects on human health, ranging from anti-inflammatory, antioxidant, antiviral to antitumor (Feng, Wu, Wang, & Liu, 2016). The oral bioavailability of curcumin is quite limited because it has a low solubility in gastrointestinal fluids and readily undergoes chemical or metabolic degradation (Wang, Vongsuiuut, Adhikari, & Barrow, 2015). In this case, specially designed colloidal delivery systems are required to improve the dispersibility, stability, food compatibility, and bioavailability of curcumin before it can be incorporated into a food matrix (Aditya et al., 2015; McClements, Li, & Xiao, 2015).

Protein–polysaccharide nanocomplex has been extensively used for the delivery of drugs and nutrients in the past decades (Peinado, Lesmes, Andres, & McClements, 2010). In such

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vehicles, proteins serve as a cargo space for lipophilic compounds, while the shells composed of hydrophilic segments protect proteins from enzymatic hydrolysis in the stomach and ensure controlled release in the intestinal phase (Zhou, Wang, Hu, & Luo, 2016). In general, two methods have been applied to prepare this type of colloids: (i) electrostatic complexation of heat-denatured protein with anionic/cationic polysaccharides; (ii) heating of initially formed protein–polysaccharide complex to promote protein gelation (Peinado et al., 2010). However, nanocomplex readily undergoes aggregation by pH adjustment or an increase in ionic strength since it is primarily stabilized by electrostatic repulsion rather than steric repulsion.

Improvement of stability of protein and protein-coated colloidal particles has been achieved by employing the Maillard reaction to form conjugates with high-molecular mass nonionic polysaccharides such as dextran and maltodextrin (Davidov-Pardo, Joye, Espinal-Ruiz, & McClements, 2015). The Maillard reaction is initiated by the condensation of an amino group of protein with a carbonyl group of dextran to produce a Schiff base, which subsequently cyclizes to an *N*-substituted glycosylamine and goes through Amadori rearrangement to give a more stable Amadori rearrangement product (Oliver, Melton, & Stanley, 2006). In practice, the Maillard reaction is limited to this stage to avoid the generation of poorly characterized products. The protein moiety of glycated protein can be adsorbed onto hydrophobic surface, whereas the polysaccharide provides strong steric repulsion (Davidov-Pardo et al., 2015).

The glycated proteins are amphiphilic copolymers which hold great potential to give nanogels with a structure of protein core and polysaccharide shell through self-assembly under proper conditions. Li, Yu, Yao, and Jiang (2008) successfully prepared a lysozyme-dextran nanogel encapsulating ibuprofen via Maillard reaction followed by a heat-gelation process at the pI of lysozyme. It has been demonstrated that the electrostatic repulsion is weakest whereas the hydrophobic interaction is the strongest at the isoelectric point (pI) of protein. Moreover, high temperature promotes the hydrophobic interaction and disulfide bond formation/interchange, which leads to protein aggregation and gel formation (Dai et al., 2015; Feng et al., 2015). The same technique has also been applied to construct BSA-dextran (Li & Yao, 2009), soy β-conglycinin-dextran (Feng et al., 2015), and whey protein isolate-dextran nanogels (Dai et al., 2015). These nanogels generally display spherical shapes with smooth surface and are stable against pH and salt owing to the steric hindrance of dextran chains at the particle exterior, which could be utilized as promising carriers for hydrophobic nutraceuticals and drugs in physiological conditions.

Recently, the application of ovalbumin, which is the main component of egg white protein, for bioactive compounds vehiculization has gained interest (Sponton, Perez, Carrara, & Santiago, 2015). In a previous study, conjugates of ovalbumin with 10 kDa dextran were developed via Maillard reaction and well characterized (Choi, Kim, Park, & Moon, 2005). However, information on ovalbumin-polysaccharide nanogels especially those loaded with lipophilic nutraceuticals is still limited at present (Li & Gu, 2014). In this work, ovalbumin–dextran conjugates with 10, 40, 70 and 150 kDa dextran were synthesized through the Maillard reaction and characterized by a combination of methods including electrophoresis, conjugation efficiency and yield, and circular dichroism (CD) analysis. Then, the ovalbumin–dextran nanogels were fabricated by a heat-gelation process and characterized in terms of pH stability, storage stability and redispersibility. At last, curcumin was incorporated into nanogels via a pH-driven method, and influence of nanogel encapsulation on the transformation and bioaccessibility of curcumin was evaluated in an *in vitro* gastrointestinal tract (GIT). The results of this work show the potential of designing nanogels to improve the oral bioavailability of lipophilic nutraceuticals, which may be useful for incorporating curcumin into functional foods.

2. Material and methods

2.1. Material

Dextran (10, 40, 70, and 150 kDa) was purchased from Aladdin Co., Ltd. (Shanghai, China). Curcumin (≥94%, curcuminoid content) and bile salts (from microbiology) were purchased from Sigma-Aldrich Corp. (St. Louis, NJ, USA). Ovalbumin (from egg white, purity >80%), pepsin (activity 3000–3500 U mg^{-1}) and pancreatin consisting of protease (activity 285 U mg^{-1}), amylase (activity 288 U mg^{-1}), and lipase (activity 56 U mg^{-1}) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). All other chemicals used were of analytical grade.

2.2. Maillard conjugate formation

Ovalbumin (1.00 w/v %) and dextran (1.00 w/v %) with desired molecular weight (10, 40, 70 or 150 kDa) were individually solubilized overnight at 4 °C in phosphate buffered saline (PBS, 10 mM, pH 7.0). The completely solubilized and hydrated samples were subsequently mixed at a 1:1 mass ratio under constant stirring for 30 min at room temperature, which led to a final ovalbumin concentration of 0.5 w/v %. The mixtures were lyophilized and the powder was heated at 60 °C for 48 h under a 79% relative humidity in a desiccator containing saturated KBr solution. After conjugation, the samples were ground using a mortar and pestle and kept at –20 °C before use.

2.3. Maillard conjugation characterization

2.3.1. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out according to the method of Laemmli (1970) with an 8% acrylamide separating gel and a 5% stacking gel. Five μL of conjugation solution (with an ovalbumin concentration of 5 mg/mL) was mixed with 5 μL of ultrapure water and 10 μL of 2 × SDS sample buffer (100 mM Tris, pH 6.8, 4% SDS, 0.2% bromophenol blue, 20% glycerol, and 200 mM 2-mercaptoethanol) and then heated for 5 min at 100 °C. Fifteen μL of each sample was loaded to designated well for the electrophoresis at 100 mV with a Tris-HEPES-SDS running buffer (100 mM Tris, 100 mM HEPES, 3 mM SDS, pH 8.0). The gels were stained for protein and carbohydrate with Coomassie brilliant blue R-250 and periodate–Schiff solution (Cerri & Sasso-Cerri, 2003), respectively.