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Glycosylated α-lactalbumin-based nanocomplex for curcumin: Physicochemical stability and DPPH-scavenging activity

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ABSTRACT

Low stability at high salt concentrations, iso-electric point, and high temperature restricted the application of proteins as stabilizers in nutraceutical encapsulation. Protein-polysaccharide conjugates made with Maillard reactions may be better alternatives. In this study, the characteristics of curcumin nanocomplexes prepared with both α-lactalbumin (ALA) and ALA-dextran conjugates as well as the effects of encapsulations on the physicochemical stability and the antioxidant activity of curcumin were evaluated. Curcumin aqueous dispersibility was boosted with nanoencapsulation. The sizes of nanoparticles coated with ALA and ALA-dextran conjugates were 63.5 ± 1.2 and 68.1 ± 2.9, respectively. Encapsulation efficiency (EE) and loading amount (LA) was not influenced with glycosylation. Stability under environmental stresses has significantly improved with ALA-dextran conjugates, compared to ALA. The oxidative stability of curcumin was also remarkably increased, especially with ALA-dextran conjugates at 95 °C. Curcumin coated in ALA and ALA-dextran conjugates had higher DPPH-scavenging activity, compared to free curcumin. ALA-dextran conjugates based nanocarrier may be an excellent delivery system for curcumin to expand the application in a wide range of food products.

1. Introduction

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, is a golden-yellow lipophilic bioactive polyphenol, which is isolated from turmeric (*Curcuma longa*), a plant of the ginger family (Anand, Kunnumakkara, Newman, & Aggarwal, 2007). Two other curcuminoids (demethoxycurcumin and bis-demethoxycurcumin) were also found in turmeric but at much lower concentration. Recent extensive studies on the biological activity of curcumins have indicated their potentials to improve human health due to their antioxidative, anticarcinogenic, antiviral, and anti-inflammatory activities (Joe, Vijaykumar, & Lokesh, 2004; Kunchandy & Rao, 1990; Kuo, Huang, & Lin, 1996). Increasing the bioavailability of curcumin is likely to make this promising natural nutraceuticals more ideal as therapeutic agents for treatment of human diseases (Anand et al., 2007).

Delivery of curcumin by dietary supplements is restricted by its low solubility in aqueous solutions, high instability especially at neutral and mild alkaline pH, and poor bioavailability for human body (Anand et al., 2007; Kaminaga et al., 2003; Wang et al., 1997). The aqueous solubility of curcumin is at most 3.0×10^{-5} μmol/mL (11 ng/mL) (Kaminaga et al., 2003). Approximately 90% curcumin was decomposed within 30 min in pH 7.2 phosphate buffer at 37 °C (Wang et al., 1997). Only a trace amount of curcumin was found in blood plasma, while most of the orally ingested curcumin was found in feces (Sharma et al., 2001).

Many proteins are natural, nontoxic, easily available, and cost-effective emulsifiers with high nutritional values and superior bioavailability. The structural properties and functionalities make them appropriate as carriers for protecting and delivering various nutrients, especially hydrophobic nutraceuticals (Livney, 2010; Yi, Fan, Yokoyama, Zhang, & Zhao, 2016; Yi, Lam, Yokoyama, Cheng, & Zhong, 2014a). The stability and aqueous solubility of curcumin was significantly improved in the presence of proteins, including casein (Sneharani, Singh, & Appu Rao, 2009), recombinant human H-chain ferritin (Chen et al., 2014), bovine serum albumin (BSA) (Bourassa, Kanakis, Tarantilis, Pollissiou, & Tajmir-Riahi, 2010; Yang,

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Wu, Li, Zhou, & Wang, 2013), soy protein (Chen, Li, & Tang, 2015; Tapal & Tiku, 2012), and β-lactoglobulin (BLG) (Sneharani, Karakkat, Singh, & Rao, 2010). Reports showed that the complexation between curcumin and protein was mainly through hydrophobic interactions, hydrogen bonds interaction could also be involved under some conditions (Li, Ma, & Ngadi, 2013; Sahu, Kasoju, & Bora, 2008; Sneharani et al., 2010).

Nevertheless, the net charge nanoparticles of a protein is low when the pH is close to its iso-electric point, leading to aggregation and instability and restricting the application in food systems (Yi, Lam, Yokoyama, Cheng, & Zhong, 2014b). High ionic strength and thermal treatment may also result in flocculation and aggregation (McClements, 2004). The stability of proteins and nano-delivery systems by protein encapsulation at certain environmental conditions can be enhanced with protein-polysaccharide conjugates prepared with Maillard reactions (Markman & Livney, 2012). The Maillard reactions are a naturally occurring nonenzymatic reactions in which the reducing-end carbonyl groups of reducing sugars are covalently combined with amine residues of proteins (Oliver, Melton, & Stanley, 2006). Heat treatment can appreciably accelerate the combination (Martins, Jongen, & van Boekel, 2000) and the reaction rate is inversely correlated with the saccharide size (ter Haar, Schols, & Gruppen, 2011). Whey protein is resistant to aggregation under heat treatment after binding with a sufficient number of maltodextrins (Liu & Zhong, 2012). Casein-dextran conjugates have successfully been used to protect zein nanoparticles against aggregation under high salt conditions (Davidov-Pardo, Joye, Espinal-Ruiz, & McClements, 2015). Nanoparticles loaded with β-carotene coated with BLG-dextran conjugate revealed better pH stability (Yi et al., 2014b). Lesmes et al. recently reported that the stability of nanoemulsion was significantly improved in gastrointestinal digestion conditions when the lipolysis were controlled (Lesmes & McClements, 2012). Our study also showed that the release of encapsulated β-carotene in nanoparticles made with BLG-dextran conjugate can be significantly controlled (Yi et al., 2014b). The steric hindrance provided by the polysaccharides moieties outside the nanoparticles and the increases in the viscosity of aqueous phase around nanoparticles were the main reasons for this (Livney, 2010). Thus, we assume that alpha-lactalbumin (ALA) and dextran glycosylation conjugates will improve the stability against severe environmental conditions and the antioxidant activity of curcumin.

Alpha-lactalbumin, the second most abundant whey protein in cow's milk, has a molecular mass of 14.2 kDa with 4 disulfide bonds (Delavari, et al., 2015; Livney, 2010). ALA has been shown to have the ability to bind hydrophobic nutrients (Livney, 2010). In this study, ALA-dextran (molecular weight 5 kDa) was conjugated and used as an emulsifier to encapsulate curcumin. The characteristics of ALA-dextran conjugates were studied with SDS-PAGE and far UV CD. The physicochemical stability and antioxidant activity of curcumin complexed with ALA-dextran conjugate was compared with free curcumin and ALA-curcumin nanocomplexes.

2. Materials and methods

2.1. Materials

Alpha-lactalbumin was purchased from Davisco Foods International Inc. (Le Sueur, MN, USA) and the protein content was 94.0% on a dry basis according to supplier. Dextran (MW 5 kDa), curcumin (at least 99% purity, C1386), and HPLC-grade solvents (ethanol and acetonitrile) were purchased from Macklin (Shanghai, China) and used without further purification. 4%–20% gradient polyacrylamide gel was prepared in our lab. EZ-Run Rec Protein ladder (10–200 KDa) were purchased from Fisher Scientific (BP36021, Fair Lawn, NJ). All other chemicals used were of analytical grade without further purification. Ultrapure water was used in all experiments.

2.2. Preparation of glycation conjugates

ALA-dextran conjugates were prepared according to the method described by Liu et al. with minor changes (Liu & Zhong, 2012). A solution containing 4% ALA and 8% dextran was prepared to hydrate them together overnight in ultrapure water. The pH of the solution was adjusted to 7 and lyophilized. The powder was incubated for 48 h at 60 °C and 79% relative humidity (saturated KBr solution) in an incubator to produce glycosylated ALA. Native ALA was also incubated for 48 h at the same condition and used as control. The samples were then collected and stored at −20 °C for further use.

2.3. Characterization of Maillard-based conjugates

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

Aliquots of ALA or ALA-dextran conjugates (protein content 5 mg/mL) were heated in SDS sample buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 0.1% bromophenol blue, 10% glycerol) containing 100 mM 2-mercaptoethanol for 10 min at 96 °C and separated by electrophoresis on 4–20% gels. The electrophoresis was performed at 120 V and 12.5 μg protein in each well was added. After running, the gel was stained using the Colloidal Coomassie G-250 Staining protocol (Dyballa & Metzger, 2009). The stained gel was scanned with a Chemilmager™ 4400 (Alpha Innotech, CA, U.S.).

2.3.2. Circular dichroism (CD)

A J-815 CD spectrometer (Jasco, Tokyo) was used to analyze the conformational changes of ALA after glycosylation in the wavelength ranging from 195 to 260 nm. Native ALA, ALA control, and ALA-dextran conjugates were dissolved with 10 mM phosphate buffer (PB, pH 7.0) at protein concentration of 0.2 mg/mL. Scanning was performed at 50 nm/min at 20 °C. CD spectra were collected with 10 repetitive scans in 2.0 mm quartz cuvettes for each sample and displayed as mean residue ellipicity (degrees cm^2/dmol) using 123 for the average number of amino acid residues per molecule of ALA.

2.3.3. Degree of glycosylation

The degree of glycosylation (DG) was estimated by measuring the free amino groups in ALA and conjugates using OPA assay, using a published method with slight modifications (Vigo, Malec, Gomez, & Llosa, 1992). In brief, 80 mg OPA dissolved in 2 mL of 95% ethanol, 50 mL of sodium tetraborate buffer (0.1 M, pH 9.5), 5 mL of 20% (w/v) SDS, and 0.2 mL of 2-mercaptoethanol were fully mixed and diluted to 100 mL with distilled water. ALA or the conjugates was dissolved in distilled water with a protein concentration of 3.0 mg/mL. Then 0.1 mL of sample was mixed with 2.7 mL OPA reagents in 96-well plates and kept at room temperature for 1 min. The absorbance at 340 nm was detected with a microplate reader (Epoch, BioTek, Vermont, USA). L-leucine (0.1–3 mg/mL) was used as a standard. All assays were performed at least 3 times. The degree of glycosylation was determined as follows:

$$\begin{aligned} DG\ (\%) = &[(\text{absorbance of sample without glycosylation} \\ &- \text{absorbance of sample after glycosylation}) \\ &/\text{absorbance of sample without glycosylation}] \\ &\times 100\% \end{aligned} \quad (1)$$