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Elaboration of curcumin-loaded rice bran albumin nanoparticles formulation with increased *in vitro* bioactivity and *in vivo* bioavailability

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ABSTRACT

Curcumin, a yellow pigment present in the spice turmeric (*Curcuma longa*), has been linked with various bioactivities, but its optimum potential is limited by its lack of dispersibility in aqueous solvents and poor oral bioavailability. Here, we employed a protein-based nanoparticle approach to improve bioactivity and bioavailability. Curcumin was encapsulated with 95.94% efficiency in biodegradable nanoparticulate formulation based on rice bran albumin (RBA). The mean particle diameter and ζ-potential of curcumin-loaded RBA nanoparticles (Cur-RBA-NPs) were 120 nm and −36.3 mV, respectively. The *in vitro* bioactivity and *in vivo* bioavailability of Cur-RBA-NPs were evaluated. The results indicated that the *in vitro* bioactivities (antioxidant activity, anti-inflammatory activity, and anti-proliferative activity on tumor cells) of Cur-RBA-NPs were superior to those of free curcumin, respectively. Moreover, Cur-RBA-NPs significantly enhanced the bioavailability of curcumin in rats as compared with free curcumin. Besides, the results clearly indicated the promise of RBA-based nanoparticles for oral delivery of poorly bioavailable molecules like curcumin.

1. Introduction

It has been well documented that many active compounds in natural foods, such as fruits and vegetables, possess bioactivities that can help prevent and cure diseases (Yen, Wu, Tzeng, Lin, & Lin, 2010). Curcumin, a yellow pigment present in the turmeric (*Curcuma longa*), is commonly used as food spice in curry as well as a medicine for the treatment of various diseases. It has been extensively studied for its pharmacological activities that include antioxidant, anti-inflammatory, anticancer, antiulcer, immunomodulatory, wound healing, neuroprotective and anti-aging effects (Pari, Tewas, & Eckel, 2008). Despite a series of bioactivities that curcumin possesses, it has low bioavailability due to its poor aqueous dispersibility (Yen et al., 2010). Several clinical trials have demonstrated that although a high dose of curcumin (3600–12000 mg/day) in patients with colorectal cancer could achieve efficient chemopreventive effect (Garcea et al., 2004; Sharma et al., 2001; Steward & Gescher, 2008), the large dose of curcumin and frequent administrations involved showed an increase of side effects and lower compliance from the users (Yen et al., 2010).

Delivery systems have been used to enhance the effectiveness of drug and food materials and to decrease the dosage required. Nanonization is one of the drug/food delivery processes that can help overcome a material's poor aqueous solubility, dissolution, and/or bioavailability (Yen et al., 2010). Nanoparticles are stable colloidal particles with a size ranges from 10 to 1000 nm. Many studies have demonstrated that reduction in particle size of the active ingredient to nanoparticle size can improve its efficacy, solubility and bioavailability (Kesisoglou, Panmai, & Wu, 2007). Recently, the application of protein-based delivery systems for the

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encapsulation of hydrophobic bioactives have gained increasing interest (Z.-L. Wan, Guo, & Yang, 2015). So far, drug or hydrophobic bioactives-loaded nanoparticles have been synthesized successfully from various proteins, including both water-soluble (bovine or human serum albumin, BSA (Fang et al., 2011) or HSA (Wartlick, Spänkuchschmitt, Strebhardt, Kreuter, & Langer, 2004), β-lactoglobulin (βLG) (Teng, Li, & Wang, 2014) and insoluble proteins (zein (Zhong & Jin, 2009), gliadin (Ezpeleta et al., 1996) and barley protein (J. Yang, Zhou, & Chen, 2013) etc.). These nanoscaled systems exhibited various advantages, such as improved solubility, controlled release property, and enhanced bioavailability of encapsulated nutraceuticals (Z.-L. Wan et al., 2015). Additionally, they exhibited low toxicity due to superior biocompatibility and nutritional value (Teng et al., 2014).

Rice bran protein has been found to be of high quality and of great importance for food and pharmaceutical applications. It is a plant protein that can be derived from rice bran, an abundant and cheap agricultural byproduct. The protein content in rice bran is approximately 10–15% and it consists of 37% water-soluble, 31% salt-soluble, 2% alcohol-soluble, and 27% alkali-soluble storage proteins. Its unique properties as being hypoallergenic and having anti-cancer activity make it a superior cereal protein that may find a wide range of applications (Fabian & Ju, 2011).

Albumin from rice accounts for about 2–6% of the total seed proteins and about 35% of the rice bran (Adebiyi, Adebiyi, Hasegawa, Ogawa, & Muramoto, 2009). Like other albumins, they are readily soluble in water due to the presence of sufficient net charge and the lack of any extensive disulfide cross-linking or aggregation (Hamada, 1997). Among the storage proteins in rice, albumins are reported to have the highest biological value being most readily absorbed and utilized by the body (Mawal, Mawal, & Ranjekar, 1987). In addition, Wei et al. stated that a 16-kDa rice albumin exhibited antioxidant activity and rice albumin was more potent than other rice proteins in preventing Cu^{2+} induced low-density lipoprotein (LDL) oxidation similar to serum albumins (Wei, Nguyen, Kim, & Sok, 2007). Similar antioxidant activity to that of serum albumin was observed with rice albumin because their N-terminal amino acid sequences are homologous (Masayuki Nakase et al., 1996). Recently, Ina et al. reported that rice albumin suppressed the elevation of blood glucose and plasma insulin levels after oral glucose loading (Ina et al., 2016).

In our previous work, we have successfully fabricated two nanoparticles by using two soybean albumins [Kunitz trypsin inhibitor (KTI) and Bowman-Birk inhibitor (BBI)] to improve bioaccessibility and bioavailability of curcumin (Liu, Cheng, & Yang, 2017; Liu et al., 2016). However, KTI, as a major antinutritional factor in soybean, must be treated by heating in the presence of reducing agents before being used to prepare nanodelivery carrier. In addition, although BBI is an excellent carrier protein for curcumin, only small quantities of it can be found in soybean, and it is costly to isolate and purify. Therefore, in this work, efforts have been made from the following aspects: first, rice bran albumin (RBA) was efficiently isolated from defatted rice bran by using an improved method based on Adebiyi et al. (2009); second, a nanoparticulate delivery carrier has been developed and characterized by the use of RBA; third, the *in vitro* bioactivity (antioxidant activity, anti-inflammatory activity and anti-proliferative activity on tumor cells) and the *in vivo* bioavailability of nanoparticulate curcumin in rats have been investigated.

2. Material and methods

2.1. Materials

Defatted rice bran was kindly provided by Liyungang Jinhong Biological Technology Co., Ltd. (Liyungang, China). Curcumin was purchased from Sigma-Aldrich Co. (Shanghai, China). Ethanol was purchased from Nanjing Chemical Industry (Nanjing, China). All other chemicals used were of analytical grade.

2.2. Isolation of albumin from rice bran

Rice bran albumin (RBA) extraction was carried out at room temperature (25 °C) by adapting the method of Adebiyi et al. (2009) with some modification. Defatted rice bran (DRB) (100 g) was extracted by using Ultra homogenizer (IKA, Germany) with 400 mL of distilled water for 4 h and centrifuged at 8000*g* for 15 min to obtain supernatant (albumin extract). Then the albumin extract was dialysed against Millipore pure water (15 MΩ) for three days in refrigerator (4 °C) and freeze-dried in a freeze-dryer (DELTA 1–24 LSC, Christ, Germany). The yield of RBA is about 3.18%. The RBA was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (Laemmli, 1970). The protein content of RBA was determined by the micro-Kjeldahl method, the result indicated that the protein content of RBA was 95.16% (w/w).

2.3. Dynamic interfacial tension measurements

The dynamic interfacial tension of RBA solutions [0.1% (w/v), pH 7.0, 25 °C] at the oil-water interface (purified corn germ oil was used) was determined by an optical contact angle meter as described elsewhere (Z. L. Wan et al., 2014).

2.4. Formulation and characterization of curcumin-loaded RBA nanoparticles (Cur-RBA-NPs)

2.4.1. Formulation of Cur-RBA-NPs

To obtain curcumin-loaded RBA, 0.1 mL stock solution of curcumin (4 mg mL^{-1} in ethanol) was added into 2.9 mL of RBA solutions (1, 2, 3, 4, 5 mg mL^{-1}) in successive titrations with magnetic stirring. The mixtures were centrifuged at 10000*g*, 25 °C for 20 min to pellet the unbound curcumin, and the supernatants containing curcumin nanocomplexes were preserved in a light-resistant container at 4 °C for determination. As contrasts, RBA without curcumin and curcumin without RBA in the same PBS solution with homologous concentration were also prepared.

2.4.2. Encapsulation efficiency (EE) and loading capacity (LC)

The EE (%) of curcumin in the curcumin-loaded RBA was estimated as the percentage of curcumin encapsulated in the proteins by the following equation: EE (%) = 100 − [amount of free curcumin (mg)/total amount of added curcumin (mg)] × 100, where the amount of free curcumin is determined from the precipitate obtained by centrifugation. The precipitate was extracted in 5 mL of ethanol with mild stirring for 5 min under magnetically stirred conditions and then centrifuged at 10 000*g* for 15 min at 25 °C to remove the protein aggregates. The supernatant was subjected to spectrophotometric analysis at 426 nm with a GENESYS 10S UV–Vis spectrophotometer (Thermo Scientific, USA), and the curcumin concentration was determined by using an established standard curve of curcumin (R^2 = 0.9965). The LC of the samples was calculated with the following equation: LC (%) = mass of encapsulated curcumin/total mass of RBA.

2.4.3. Particle size and ζ-potential measurements

The mean particle size and ζ-potential of the nanocomplexes were determined according to our previous publication (Z.-L. Wan, Wang, Yang, Wang, & Wang, 2016) by using a Nanosizer ZS instrument (Malvern Instruments Ltd, Worcestershire, UK).