Food Chemistry 261 (2018) 283–291

Contents lists available at ScienceDirect

Food Chemistry

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

Improved chemical stability and cellular antioxidant activity of resveratrol in zein nanoparticle with bovine serum albumin-caffeic acid conjugate

Yuting Fan[a], Yuexiang Liu[a], Luyu Gao[a], Yuzhu Zhang[b], Jiang Yi[a,*]

[a] College of Chemistry and Environmental Engineering, Shenzhen University, Shenzhen 518060, China
[b] Western Regional Research Center, ARS, USDA, Albany, CA 94710, United States

ARTICLE INFO

Chemical compounds studied in this article:
Resveratrol (PubChem CID: 445154)
Caffeic acid (PubChem CID: 689043)
DCFH-DA (PubChem CID: 104913)
ABAP (PubChem CID: 1969)
L-ascorbic acid (PubChem CID: 54670067)

Keywords:
Resveratrol
Zein
BSA
Caffeic acid
Nanoparticle
CAA

ABSTRACT

In this study, bovine serum albumin (BSA)-caffeic acid (CA) conjugate was prepared with free radical-induced grafting method. The CA to BSA ratio of the conjugate was 115.7 mg/g. *In vitro* antioxidant activity assays suggested that BSA-CA conjugates had stronger antioxidant activity than BSA. Resveratrol-loaded zein encapsulated with BSA and BSA-CA conjugate core-shell nanoparticles were prepared with antisolvent method. Particle sizes were 206.3 nm, and 217.2 nm for BSA and BSA-CA, respectively. The encapsulation efficiencies (EEs) were 85.3% and 86.5% for zein-BSA and zein-BSA-CA nanoparticles, respectively. SEM results indicated that both nanoparticles were spherical with mean diameter approximately 200 nm and smooth surfaces. Both thermal and UV light stability of resveratrol was significantly improved after nanoencapsulation. BSA-CA conjugate showed remarkably greater protection than BSA against resveratrol degradation. Cellular antioxidant activity (CAA) study confirmed that resveratrol in both zein-BSA and zein-BSA-CA nanoparticles had significant higher antioxidant activities than resveratrol alone.

1. Introduction

Resveratrol (trans-3,4′,5-trihydroxystilbene), a naturally occurring polyphenol mainly existed in grapes and red wine, has been shown to have a lot of health-promoting effects, such as, antioxidant activity, anti-inflammatory, antiaging and antidiabetogenic property (Baur et al., 2006), and anti-amyloidogenic activity (Marambaud, Zhao, & Davies, 2005). Recent studies also showed that resveratrol may prevent diet-induced obesity, increase mitochondrial function, physical stamina, glucose tolerance in mice (Park et al., 2012), and protect against and ameliorate the symptoms of metabolic diseases. Resveratrol has attracted a lot of attention not just from food science, but also cosmetics, and pharmaceutical industries. However, resveratrol is insoluble and unstable in aqueous phases. The development of a liquid formulation to stabilize resveratrol, protect it from degradation, enhance its water dispersibility, and achieve specific location release is a great challenge. Nanoparticle-based delivery system with mesoporous silica, zein, and zein-polyphenol conjugates have been shown to improve its oral bioavailability and anti-inflammatory effects recently (Juère et al., 2017; Liu et al., 2018; Penalva et al., 2015).

Due to the cost-effective and generally recognized as safe (GRAS) properties, protein-based nanoparticles are ideal delivery systems and have been widely used for liable oxidized nutraceutical encapsulation. β-Lactoglobulin, α-lactalbumin, soybean protein, gliadin, and zein have been used (Arroyo-Maya et al., 2012; Fan, Zhang, Yokoyama, & Yi, 2017; Joye, Davidov-Pardo, & McClements, 2015; Livney, 2010; Pujara, Jambhrunkar, Wong, McGuckin, & Popat, 2017). Among these proteins, zein is a class of proline-rich corn (*Zea mays*) proteins. Almost 75% amino acid residues of zein are hydrophobic, resulting in its unique aqueous-alcohol solubility, and film-forming property (Lawton, 2002). The low solubility in aqueous phase but good solubility in alcohol makes it particularly suitable for encapsulating and delivering functional bioactives that have poor aqueous dispersibility but high ethanol dispersibility (Patel & Velikov, 2014). Recently, zein has been used for nanoencapsulation of hydrophobic nutraceuticals, including curcumin, vitamin D3, essential oil, and resveratrol. Nevertheless, the use of zein nanoparticles as a nano-delivery system may be limited due to their low stability and aggregation in aqueous systems (Hu & McClements, 2014) as a result of high surface hydrophobicity and close-to-neutral-pH isoelectric point (6.2) (Shukla & Cheryan, 2001).

Zein colloidal particle aggregation can be reduced by encapsulating the particles with biopolymers. Sodium caseinate and β-lactoglobulin were used as stabilizer (Chen, Zheng, McClements, & Xiao, 2014; Davidov-Pardo, Pérez-Ciordia, Marín-Arroyo, & McClements, 2015).

* Corresponding author.
E-mail address: yijiangjnu@gmail.com (J. Yi).

https://doi.org/10.1016/j.foodchem.2018.04.055
Received 24 November 2017; Received in revised form 23 March 2018; Accepted 16 April 2018
Available online 17 April 2018

Even though the dispersity of zein nanoparticles can be ameliorated, the protection of encapsulated hydrophobic nutraceuticals from degradation is still limited, especially at harsh conditions such as high temperature and UV light. Protein-polyphenols conjugates have been used as stabilizers for delivery of nutraceuticals due to its great emulsifying activity and antioxidant activity. Four main approaches were used for fabrication of protein-polyphenol conjugates so far, such as activated ester (N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl))-mediated method, enzyme-mediated strategy, alkaline treatment, and free radical induced grafting approach (Liu, Ma, Gao, & McClements, 2017). However, activated ester-mediated method has the possibility to produce harmful products. Furthermore, organic solvents are also used and cause potential risk. Enzyme-mediated strategy is strictly restricted due to the high costs of enzyme. Alkaline treatment may cause the oxidation and degradation of polyphenols. Compared to other three approaches, free radical method is a much safe, green, and facile approach and is widely used recently to obtain protein-polyphenol conjugates. Free radical-induced conjugation approach is employing an H_2O_2/L-ascorbic acid redox pair, as radical initiator, to functionalize proteins with phenolic acids (polyphenols) in a single-step.

Bovine serum albumin (BSA), which is also a component of whey protein showed excellent gelation activity, surface activity, and nutraceutical binding ability (Livney, 2010; Matsudomi, Rector, & Kinsella, 1991). However, no protein-polyphenol conjugate based on BSA has been reported.

In this study, BSA-caffeic acid (CA) conjugate, prepared with free radical-induced grafting method, was characterized with SDS-PAGE, and far CD, and used as an emulsifier to stabilize resveratrol-loaded zein nanoparticle. The physical state and the interaction among resveratrol, zein, and BSA-CA conjugate was investigated with ATR-FTIR, and XRD. Thermal and UV light stability of free resveratrol, resveratrol in zein nanoparticles with or without BSA and BSA-CA conjugate was analyzed. Cellular antioxidant activity (CAA) of resveratrol was also evaluated with Caco-2 cell models.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA) (≥98%), resveratrol (≥98%), L-ascorbic acid, hydrogen peroxide (30%, w/w), 2,2′-azobis (2-amidinopropane) dihydrochloride (ABAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), fluorescein, caffeic acid (CA), gallic acid, Trolox, and 2′,7′-dichlorofluorescin diacetate (DCFH-DA) were obtained from Sigma-Aldrich (St. Louis, MO). All other analytical grade chemicals and reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) (containing 4.5 g/L D-glucose and GlutaMAX™), penicillin and streptomycin (100×), fetal bovine serum (FBS), TrypLE™ Select, Hanks' balanced salt solution (HBSS), and phosphate buffer solution (PBS)(10×) were purchased from GIBCO (Grand Island, NY, U.S.). Caco-2, a human epithelial colon adenocarcinoma cell line, was purchased from the American Type Culture Collection (Manassas, VA, U.S.).

2.2. Preparation of BSA-CA conjugate

BSA-CA conjugate was fabricated with free radical-induced methods as described previously (Spizzirri et al., 2009) with slight modifications. In brief, 0.5 g BSA was fully dissolved in 50 mL ultrapure water, and 0.5 mL of 10.0 M H_2O_2 and 0.25 g L-ascorbic acid were added gradually. The mixture was then stirred at 25 °C under atmospheric air for 2 h. After that, 0.25 g CA was added to the mixture and continued to stir for 24 h. The free unreacted CA in reaction solution was then removed by dialysis against ultrapure water for two days with about 10 times water changes at 4 °C. The dialysis bags had a 3 kDa molecular weight cutoffs for proteins. UV–vis analysis results confirmed that no free CA or free L-ascorbic acid was detectable in the dialyzed conjugate solutions. The BSA-CA conjugate solution was lyophilized (Labconco, MO) and stored at -20 °C for further use.

2.3. Evaluation of phenolic groups by Folin–Ciocalteu reagent

The CA amount in BSA-CA conjugate was monitored with a forementioned method (Hu et al., 2016). Briefly, 0.5 mL of BSA-CA conjugate was mixed with 1 mL of Folin–Ciocalteu reagent for 5 min in the dark, then 2 mL of 20% sodium carbonate (Na_2CO_3) was added. The reacting substances was then mixed and kept for 1 h at 25 °C. The absorbance was obtained with a UV–vis spectrometer (UV2600, Shimadzu, Japan) at 747 nm. The conjugating amounts of BSA-CA conjugates were presented as milligrams of gallic acid equivalent per gram of BSA-CA conjugate.

2.4. Characterization of BSA-CA conjugate

2.4.1. SDS-PAGE

Aliquots of BSA or BSA-CA conjugates (protein content 5 mg/mL) were boiled in SDS sample buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 0.1% bromophenol blue, 10% glycerol) containing 100 mM β-mercaptoethanol for 5 min and separated by electrophoresis on 4–20% gels. The electrophoresis was performed at 120 V. After running, the gel was stained using the Colloidal Coomassie Brilliant Blue G-250 Staining protocol (Fan et al., 2017). The stained gel was scanned with a Chemilmager™ 4400 (Alpha Innotech, CA, U.S.).

2.4.2. Circular dichroism (CD)

CD spectra were collected at 20 °C with a J-815CD spectrometer (Jasco, Tokyo) in the wavelength ranging from 195 to 260 nm with 2.0 mm quartz cuvettes. BSA, and BSA-CA conjugates were dissolved with a 10 mM phosphate buffer (PB, pH 7.0) at a protein concentration of 0.2 mg/mL. Scanning was performed at 50 nm/min. Ten scans were averaged and the CD spectra were displayed as mean residue ellipticity (degrees cm^2/dmol).

2.4.3. DPPH radical scavenging activity

DPPH radical scavenging activity of BSA, BSA-CA conjugate, and CA was analyzed as described previously (Yi, Lam, Yokoyama, Cheng, & Zhong, 2015). The absorbance was measured at 517 nm.

2.4.4. Ferric reducing power

The reducing power of BSA, BSA-CA conjugate, and CA was measured as described previously in our published paper with slight modifications (Yi et al., 2015). The absorbance was measured at 700 nm.

2.4.5. Oxygen radicals antioxidant capacity (ORAC)

The antioxidant capacity of BSA, BSA-CA conjugate, and CA was analyzed with oxygen radicals antioxidant capacity assay as described previously (Zulueta, Esteve, & Frígola, 2009). In each well, 50 μL of fluorescein (70 nM) and 50 μL (5 μg/mL) of sample, control (PB, pH 7.0), or standard (trolox, 20 μM) were mixed. The mixture was kept at 37 °C for 15 min, and then 25 μL of AAPH (221 mM) was added. After that, 96-well plate was put into a fluorescence microplate reader (Synergy HTX, BioTek, Vermont, USA). The fluorescence was detected at 535 nm every 5 min for 90 min at 37 °C with the excitation at 485 nm. The ORAC values of BSA, BSA-CA conjugate, and CA were presented as μM Trolox equivalents (μM TE).

$$\text{ORAC}\ (\mu\text{MTE}) = \frac{C_t \times (AUC_s - AUC_b) \times k}{(AUC_t - AUC_b)} \quad (1)$$

where C_t is the concentration (μM) of Trolox (20 μM), k is the sample dilution factor, and AUC is the area below the fluorescence decay curve of the sample, blank, and Trolox, respectively.