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Influence of glycosylation of deamidated wheat gliadin on its interaction mechanism with resveratrol

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ABSTRACT

Gliadin is a main composition of wheat storage protein with unique characteristics. Polyphenol with health benefits tends to form complex with protein. In this study, glycosylation of deamidated wheat gliadin (gliadin) was carried out. Fluorescence quenching was applied to evaluate their binding mechanisms with resveratrol. Results showed that glycosylation could increase the solubility and decrease the surface hydrophobicity of gliadin. Both gliadin and glycosylated gliadin have strong affinity with resveratrol. The thermodynamic parameters of binding process indicated that complexation of resveratrol with gliadin was mainly driven by hydrophobic interaction, while by hydrogen bonds with glycosylated gliadin. The hydrosolubility of resveratrol was dramatically increased especially in the presence of glycosylated gliadin. This was consistent with the higher binding constant of glycosylated gliadin with resveratrol. Therefore, gliadin and glycosylated gliadin are both effective to carry resveratrol or other bioactive compounds, and their binding mechanisms are different due to structural difference.

1. Introduction

Polyphenol compounds from plant are now attracting more attention as they are proved to own prominent healthy benefits. Polyphenol has free-radical scavenging ability and was normally used as antioxidant ingredient in food (Jakobek, 2015). Resveratrol (trans-resveratrol; trans-3,5,4′-trihydroxystilbene) is a kind of polyphenol which mainly exists in grape seeds, skins and peanuts. It has been widely studied for its ability to lower the risk of cardiovascular disease, cancer and its antioxidant activity *in vitro* and *in vivo* (Rayne, Karacabey, & Mazza, 2008). However, although it has great potential in using in food and beverage, its application was limited due to its low solubility in both aqueous and lipid phase and its instability toward light and oxygen (Acharya, Sanguansri, & Augustin, 2013).

The interaction behavior between resveratrol and milk protein such as casein, β-lactoglobin and whey protein has been well characterized (Acharya et al., 2013; Hemar, Gerbeaud, Oliver, & Augustin, 2011). Other researchers studied the binding characteristics of soy protein with resveratrol (Wan, Wang, Wang, Yang, & Yuan, 2013). Most of these carriers exhibit high potential as good delivery system for resveratrol. The complexation is promising to improve the solubility of polyphenol and its stability against degradation to light and oxygen. In addition, nanocomplexes are a promising carrier to protect the bioactive compounds from the gastrointestinal tract which can improve its bioactivity (Tapal & Tiku, 2012). Interactions also exist between protein and polyphenol in food or during processing (Siebert, 1999). The interaction between polyphenol and protein mainly includes hydrophobic, hydrogen bonding and van der Waals forces through binding between phenolic groups to aromatic rings of proline of protein. Quenching study of fluorescence of protein is normally used to evaluate the binding type between protein and polyphenol. It can reflect the changes of environment polar of tryptophan and tyrosine in protein during interaction (Keppler, Stuhldreier, Temps, & Schwarz, 2014).

As researchers are searching for new kinds of protein materials, protein from plant is getting more attention for their special functional properties. Wheat gliadin is one of the constituents of wheat gluten with special viscoelastic and surface properties (Bos, Dunnewind, & van Vliet, 2003; Wang, Zhao, Yang, Jiang, & Chun, 2007; Wang, Zhao, Yang, Jiang, & Cui, 2007; Wang, Zhao, Zhao, Jiang, et al., 2007). Researchers have demonstrated its capacity to interact with anthocyanin derivatives and it was applied to delivery all-trans-retinoic acid (Mazzaracchio et al., 2011; Ezpeleta et al., 1996). Gliadin was reported to bind with

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resveratrol mainly through hydrophobic interaction, while to zein through hydrogen bonds (Joye, Davidov-Pardo, Ludescher, & McClements, 2015). However, as gliadin has low water solubility, its application in food system is greatly limited. Water soluble wheat protein can be obtained after acid deamidation. This modification led to the transformation of amide groups of side chains from asparagine and glutamines residues into the carboxyl groups, which can efficiently enhance the protein solubility (Mimouni, Raymond, Merle-Desnoyers, Azanza, & Ducastaing, 1994; Qiu, Sun, Zhao, Cui, & Zhao, 2013). Additionally, deamidated wheat gliadin has higher surface activity with its hydrophobic groups exposed and showed antioxidant ability in lipid system (Qiu, Zhao, Decker, & McClements, 2015). Therefore, modified wheat protein has great potential to be developed as food additive. In addition, proteins with more basic residues and high proline amount are relatively large and hydrophobic. These kinds of protein with flexible structure could strongly bind with polyphenols (Le Bourvellec & Renard, 2012).

As protein materials usually are sensitive to environment factors, conjugation of the protein with polysaccharides is effective to improve its solubility and stability (Wang & Ismail, 2012). This process occurred with no chemical reagent which is considered to be safe (de Oliveira, Coimbra, de Oliveira, Zuñiga, & Rojas, 2016). The controlled conjugation also helped to develop new biofunctional biopolymer as an emulsifier to apply in food industry. Deamidated wheat protein has been proved to be able to form conjugates with dextran and provide effective steric stabilization of emulsion droplets (Wong, Day, & Augustin, 2011). Therefore, the conjugate could be a potential effective new emulsifier for food industry. Meanwhile, as glycosylation can induce changes of protein conformation and surface hydrophobicity, consequently, it was supposed to cause difference in the interaction mechanism of protein with hydrophobic molecules in solution.

Though the beneficial effect of wheat protein and resveratrol for the health has been well documented, there was little information about applying wheat protein to forming complex with resveratrol, and it is critical to know the interaction mechanism. The aim of this work was therefore to evaluate the binding affinity of resveratrol with wheat gliadin and glycosylated gliadin by means of fluorescence spectroscopy. This information could help to develop soluble wheat protein as a kind of new protein material to encapsulate bioactive compounds.

2. Materials and methods

2.1. Materials

Resveratrol (purity > 98%) was purchased from Sigma Co. Wheat gluten with protein content of 71.5% (w/w, dry basis) was purchased from Lianhua Co. Ltd. (Zhoukou, China). Dextran (molecular mass 67 kDa) was purchased from Sigma Aldrich (Sydney, Australia). O-Phthaldialdehyde (OPA) of chemical grade was obtained from Sigma Aldrich (Sydney, Australia). All other chemicals used were of analytical grade.

2.2. Preparation of protein material

2.2.1. Preparation of deamidated wheat gliadin (gliadin)

Hydrochloride acid (0.2 M) was added to wheat gluten solution (10 wt%) with constant stirring in a sealed glass container at 70 °C for 2 h. The dispersion was then neutralized, dialyzed, and freeze-dried. The gliadin-rich fraction was extracted from deamidated wheat gluten by stirring with 70% ethanol for 2 h. Ethanol in the supernatant was removed by rotary evaporation (50 °C). The final deamidated gliadin was freeze-dried. The protein content of deamidated wheat gliadin prepared using this method was 80.2% measured by the Kjeldahl method (N×5.7) and the deamidation degree was 40.6% determined according to Kato, Tanaka, Lee, Matsudomi, and Kobayashi (1987).

2.2.2. Preparation of gliadin-dextran conjugates

Maillard conjugates were prepared using an approximately 1:1 mol ratio of measured available -NH_2 in the protein and reducing sugar end in the dextran according to Wong et al. (2012). The protein and dextran were first dissolved in deionized water at room temperature for 1 h then freeze-dried. The resultant protein and dextran mixtures were incubated at 60 °C and at 75% relative humidity (over saturated potassium bromide) for up to 5 days. After incubation, the samples were dispersed in deionized water for 2 h at room temperature and then freeze-dried.

2.3. OPA assay

The number of available -NH_2 groups were determined using O-phthaldialdehyde (OPA) assay (Vigo, Malec, Gomez, & Llosa, 1992). An OPA reagent was prepared by dissolving 80 mg OPA in 2 mL of absolute ethanol and mixed with 5 mL of 10% sodium dodecyl sulfate (SDS), then 0.2 mL of mercaptoethanol and made up to 100 mL with 0.1 M borax buffer (pH 9.85). The assay consisted of mixing 4 mL of the OPA reagent with 200 μL of conjugate solution and measuring the absorbance of the mixture at 340 nm after 3 min of incubation using a spectrophotometer (UV-1600, Mapada Instruments Co., Ltd., China). The OPA reagent and protein solution without OPA were used as the blank. The calibration curves were constructed with L-leucine (0.2–5 mM). The degree of conjugation was defined as follows:

$$\text{Conjugation degree}\ (\%) = (1 - \text{amine groups after glycation}/\text{amine groups before conjugation}) \times 100$$

2.4. Determination of protein solubility

Protein dispersions (10 mg/mL, dissolved in deionized water) were adjusted to a specific value within the range of pH from 3 to 9 with 1 M HCl or NaOH. The dispersions were agitated with a magnetic stirrer for 1 h at room temperature, then centrifuged at 10,000*g* for 20 min. Protein content in the supernatant were determined according to Lowry, Rosebrough, Farr, and Randall (1951). Bovine serum albumin was used as the standard. Protein solubility was expressed as percent of protein concentration in the supernatant with respect to that of protein suspension before centrifugation.

2.5. Surface hydrophobicity (H_0)

Surface hydrophobicity was determined by the hydrophobicity fluorescence probe ANS (8-Anilino-1-Napthalene Sulfonate) using an F7000 fluorescence spectrophotometer (Hitachi Co., Japan). A series of dilutions of each sample were made with 5 mM phosphate buffer (pH 7.0) to obtain a range of protein concentrations at 0.05, 0.1, 0.2, 0.5, 1.0 mg/mL. Then, 4 mL protein dispersion was mixed with 20 μL of 8 mM ANS. Fluorescence intensity (FI) was measured at the wavelengths of 390 nm (excitation) and 470 nm (emission), with a constant excitation and emission slit of 5 nm. The FI for each sample was then computed by subtracting the FI attributed to protein in buffer without ANS. The initial slope of the FI *versus* protein concentration plot was calculated by linear regression analysis and used as an index of H_0.