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Improving the stability of wheat gliadin nanoparticles – Effect of gum arabic addition

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ABSTRACT

Wheat gliadin nanoparticles were prepared by antisolvent precipitation, and gum arabic (GA) was added to improve the stability of the nanoparticles. Effect of pH, gliadin/GA ratio and gliadin concentration on the gliadin-GA particles were studied by turbidimetric measurement. Spinodal lines for the GA-gliadin-solvent system were built under different pH. The influences of gliadin/GA ratio and their concentrations were represented in the diagram, thus the conditions for preparing stable composite were defined. The stability of GA-coated gliadin nanoparticles (Gliadin/GA = 1:3) was further studied compared with uncoated gliadin particles. GA-coated nanoparticles had a relatively good stability at pH 4.0–7.0 and remained a relatively low particle size with the elevated ionic strengths. They also had good thermal stability at 80 °C. Moreover, the interactions between gliadin particles and GA under different pH were further investigated. Hydrogen bonding was found to be the predominant force at pH 5.0, while hydrophobic force took charge of the formation of complex at pH 7.0. The findings are of great importance for extending the current knowledge about gliadin nanoparticles coupled with polysaccharides, thus providing valuable information for the development of gliadin-polysaccharide nanoparticles as potential nano-delivery systems.

1. Introduction

In recent years, intensive attention has been focused on the development of colloidal delivery systems to encapsulate, protect and release bioactive substances, such as pharmaceuticals and nutraceuticals (McClements, 2015). Protein nanoparticles represent a potential system since they are generally regarded as safe (GRAS) delivery devices, due to their exceptional characteristics, namely biodegradability, high nutritional value, abundant renewable sources and extraordinary binding capacity of drugs or nutraceuticals (Elzoghby, Samy, & Elgindy, 2012). Compared with animal proteins, hydrophobic plant proteins such as zein and gliadin have the capability of yielding sustained drug release. Due to their high hydrophobicity, these nanoparticles may not need further chemical or physical treatment to harden them thus avoiding toxic chemical crosslinkers. They are less expensive than animal proteins and also possess functional groups which can be easily used either to adsorb or to covalently couple molecules capable of modifying the targeting properties of nanoparticles. Moreover, plant proteins reduce the risk of spreading diseases such as bovine spongiform encephalitis (mad cow disease).

Wheat gliadin is a group of proteins extracted from wheat gluten by 70% ethanol. It is made of single chain polypeptides with an average molecular weight of 25–100 kDa linked by intramolecular disulphide bonds. Wheat gliadin has remarkably low solubility in aqueous solution except at extreme pH. This low water solubility has been attributed to the presence of disulphide bonds and to the cooperative hydrophobic interactions which cause the protein chains to assume a folded shape. Gliadin nanoparticles were shown to be suitable controlled release systems for hydrophobic and amphiphilic drugs. Duclairoir et al. (1999) prepared gliadin nanoparticles as carriers for all-trans-retinoic acid (RA) by a desolvation method. The nanoparticles showed good stability during several weeks in PBS or aqueous medium with the maximum payload of 76.4 μg RA per mg nanoparticles. Arangoa, Campanero, Renedo, Ponchel, and Irache (2001) observed that gliadin nanoparticles dramatically increased the carbazole oral bioavailability up to 49% and provided sustained release properties related to the bioadhesive capacity of gliadin nanoparticles with

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the stomach mucosa after oral administration. Umamaheshwari, Ramteke, and Jain (2004) developed mucoadhesive gliadin nanoparticles bearing amoxicillin for eradicating *Helicobacter pylori* in stomach and the results showed that the nanoparticles eradicated *Helicobacter pylori* from the GI tract more effectively than free amoxicillin because of the prolonged GI residence time attributed to mucoadhesion.

However, gliadin nanoparticles prepared by antisolvent precipitation are susceptible to the effects of pH, heating and salt, resulting in aggregation and instability (Joye, Nelis, & McClements, 2015). Recently, several studies have shown that protein-polysaccharide interaction can be used to improve the stability of protein nanoparticles to environmental stresses. Liang et al. (2015) investigated self-assembled zein-sodium carboxymethyl cellulose nanoparticles as an effective drug carrier and transporter. Hu and McClements (2015) found that zein nanoparticles coated with pectin could easily be redispersed in water. The increased steric and electrostatic repulsion among particles improved the stability to aggregation. Gum arabic (GA), due to its higher water solubility and lower viscosity than other polysaccharides (McNamee, O'Riordan, & O'Sullivan, 1998), has been studied to stabilize several proteins (Weinbreck, Tromp, & de Kruif, 2004; Ye, Flanagan, & Singh, 2006). When the protein and polysaccharide carry opposite charges, electrostatic attraction causes the formation of complexes, which may be stable or unstable, and the unstable complex results in phase separation (Chang, Leung, Lin, & Hsu, 2006; Michon, Konate, Cuvelier, & Launay, 2002; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). Phase diagram is often used to characterize the phase behavior of protein-polysaccharide composites. Moreover, the impacts of various factors on the existing system could be reflected by determining the boundary of phase separation. Sharafbafi, Tosh, Alexander, and Corredig (2014) constructed a binary phase diagram to investigate the phase behavior between β-glucan and protein in milk, finding that the phase separation was driven by depletion interactions. However, there is a great discrepancy between gliadin and other proteins due to the lower water-solubility.

In this study, gliadin nanoparticles were produced by antisolvent precipitation and then GA was added to improve the stability of the nanoparticles. The phase behavior of gliadin particles and GA system was investigated to determine the critical point of the phase separation and ensure that the composite nanoparticles are still in the smaller size range. Furthermore, the impact of pH, ionic strength and heating treatment on the stability of particles was studied to approach their capability for further applications. Finally, the interactions between gliadin particles and GA at different pH were further investigated.

2. Materials and methods

2.1. Materials

Wheat gluten was provided by Anhui Ruifuxiang Food Co. Ltd (Bozhou, China). Gum arabic (GA, Instant gum AA), was a product from the Tianjin Jebsen Specialty Chemicals Co. Ltd (Tianjin, China), with a protein content of 2.1% (dry matter basis) and a weight average molar mass (Mw) about 320,000 g/mol by size exclusion chromatography (SEC-HPLC). All other chemicals, reagents, and solvents were of analytical grade.

2.2. Preparation of gliadin nanoparticle uncoated and coated with GA

2.2.1. Extraction of gliadin

Wheat gluten of 100 g was added to 70% (v/v) ethanol (1 L) with magnetic stirring for 3 h followed by centrifugation at 8000 rpm for 30 min. The supernatant was collected and the ethanol was evaporated by a rotating evaporator. The final gliadin extract was freeze-dried [protein content 92.2% (dry matter basis); Mw 60,000 g/mol] and stored at −20 °C before use.

2.2.2. Nanoparticle preparation

Gliadin nanoparticles were prepared by a method of antisolvent precipitation. Briefly, 0.4 g gliadin was added into 10 mL 70% (v/v) ethanol with continuous magnetic stirring, then centrifuged at 4000 rpm for 10 min to eliminate the insoluble impurities. 1.0 mL of gliadin stock solution was dropped into 19.0 mL of deionized water (adjusted to pH 4.9) using a syringe while being blended on a magnetic stirrer at 660 rpm. GA was dispersed in deionized water and adjusted to pH 4.9 using 1 M HCl. An aliquot of GA stock solution was poured into the freshly prepared gliadin nanoparticles suspension at ambient temperature, with the final gliadin concentration of 2 mg/mL. The gliadin-coated or uncoated nanoparticle suspensions were stirred for an extra 2 min before analysis.

2.3. Zeta potential measurement

The zeta potential of fresh suspensions was measured by particle size and zeta potential analyzer (Brookhaven, USA). The suspensions were diluted 5 times and then pH was adjusted for the following analysis. Each trial was measured by employing triple independent suspension replicate, and each measured for three times.

2.4. Turbidity analysis

The turbidimetric titration upon acidification was measured by a UV-Vis spectrophotometer (Shimadzu, Japan) at 600 nm with plastic cuvettes (1 cm path length), from pH 6.0 to 3.0. In brief, 20 mL mixture with pH 6.0, at which samples were adjusted slightly by NaOH, was gradually acidified by the dropwise addition of HCl with a gradient of concentrations (0.05 M, 0.1 M and 0.5 M HCl), respectively. In this way, the added volume could be controlled in the range of 1.5–3% of total volume, thus the dilution effect was not significant. Titrations were carried out at 25.0 ± 1.0 °C, and pH was carefully monitored. The turbidity titration was also conducted at different gliadin/GA ratio (4:1–1:3, w/w). All measurements were made in triplicate.

2.5. Determination of phase boundary at different pH

The gliadin-GA-solvent ternary phase diagram could be conducted by previous method with several modifications (Ranganathan & Kwak, 1996). In detail, the spinodal lines were drawn by determining the phase separation points through changing the gliadin concentration (0.1%–0.3%) and the proportions of gliadin and GA (20:1–1:5). The phase separation was verified by a sharp increase in turbidity through measuring the optical density (OD_{600}) by UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan) with a 1 cm slit. The complex concentrations (w/v) were calculated by determining the amounts of gliadin suspension and GA solution that were employed. Then the effect of different pH values (3.0, 4.0, 5.0 and 6.0) on the phase boundary was also investigated. All measurements were made in duplicate.

2.6. Stability to environmental conditions

2.6.1. Effect of pH

Freshly prepared suspensions were diluted 5 times and adjusted pH values ranging from 3.0 to 7.0 with HCl or NaOH, and then the particle size was measured by a size and zeta potential analyzer