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Peptide-polysaccharide conjugates with adjustable hydrophilicity/hydrophobicity as green and pH sensitive emulsifiers

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ABSTRACT

In this study, we used deamidated zein peptide-polysaccharide conjugates as emulsifiers to produce oil in water emulsions. Zein is insoluble in water. Its asparagine and glutamine residues changed to aspartic acid and glutamic acid residues and also zein was degraded during deamidation reaction in alkaline condition. Longer deamidation reaction produced more carboxyl groups and smaller peptides. The peptides with average molecular weights of 7.3, 5.5 and 4.0 kDa were obtained after 36, 96 and 144 h of the deamidation reaction, respectively. Dextran (M_w 16 kDa) and maltodextrin (M_w 3.0 kDa) were separately conjugated to the N-terminals of the peptides *via* Maillard reaction. The hydrophilicity/hydrophobicity of the peptide-polysaccharide conjugates can be adjusted by deamidation reaction time, number of conjugated polysaccharide molecules, polysaccharide molecular weight, and medium pH. The conjugates, in which about 65.4–90.6% of the peptides were conjugated with the polysaccharide, had prominent emulsification ability at acidic condition. The emulsions produced and stored at pH 4.0 and 4.5 were long-term stable. When the emulsions were stored at pH 7.4, at which the carboxyl groups were significantly deprotonated, demulsification happened. The oil layer appearance time changed from 2 days to more than 70 days; the time decreased with the increase of the conjugate hydrophilicity. This study demonstrates that the emulsions produced from the conjugates have pH sensitivity and adjustable stability. The deamidated zein peptide-polysaccharide conjugates, which synchronously possess natural moieties, adjustable hydrophilicity/hydrophobicity, smaller molecular weights and simpler structures compared with protein-polysaccharide conjugates, are excellent and green emulsifiers.

1. Introduction

Emulsifiers are widely used in pharmaceutics and food industries (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012; McClements, 2012; McClements, Decker, & Weiss, 2007; Xu, Yin, Li, & Yao, 2015). Synthetic surfactants, composed of a hydrophilic moiety and a hydrophobic moiety, diffuse rapidly to oil-water interface to reduce the surface tension and stabilize the emulsion droplets (Bouyer et al., 2012; Garti, 1999; Yang, Leser, Sher, & McClements, 2013). Various synthetic surfactants with different hydrophile-lipophile balance (HLB) values, thus with different emulsifying abilities and emulsion stabilities, can be obtained by changing the hydrophilic and hydrophobic structures (McClements & Xiao, 2014; Piorkowski & McClements, 2014). The disadvantage of synthetic emulsifiers is their potential toxicity (Kralova & Sjöblom, 2009). Very recently, Chassaing et al. reported that relatively low concentrations of two commonly used dietary emulsifiers, polysorbate-80, derived from polyethoxylated sorbitan and oleic acid, and carboxymethyl cellulose, a cellulose derivative, impact the mouse gut microbiota promoting colitis and metabolic syndrome (Chassaing et al., 2015). Proteins can also be used as emulsifiers. Many proteins, such as milk protein and soy protein, are nourishing, nontoxic and low cost, and also possess excellent emulsifying capacity (Day, 2013; McClements, 2004; Singh & Sarkar, 2011). On the other side, the emulsions stabilized by proteins are sensitive to environmental conditions, such as pH, ionic strength, and thermal processing (Delahaije, Gruppen, Giuseppin, & Wierenga, 2015; Dickinson, 2010). To solve this problem, protein-polysaccharide covalent conjugates and protein/polysaccharide electrostatic complexes have been exploited as emulsifiers to produce long-term stable emulsions (Bouyer et al., 2012). Another problem for protein emulsifiers is that proteins diffuse slowly to oil-

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water interfaces, and their emulsifying ability and emulsion stability are difficult to control compared with synthetic surfactants, because proteins are natural macromolecules with complex structures (Lam & Nickerson, 2013; Qian & McClements, 2011). Therefore, it is of great interest to develop novel emulsifiers, which combine the advantages of proteins and synthetic surfactants together, that is, synchronously possess natural moieties, adjustable hydrophilicity/hydrophobicity, relatively smaller molecular weights and simpler structures. As we know, peptide generally possesses simpler structure and smaller molecular weight compared with protein, and the peptide with desired hydrophilicity/hydrophobicity, component, and structure can be synthesized chemically and biologically. However, the synthesis and purification of peptide are costly and time-consuming. By now, no investigation on the peptide with adjustable emulsifying ability and emulsion stability was reported.

Zein, a major byproduct of corn starch production, composed of α-zein (19 and 22 kDa), β-zein (14 kDa), γ-zein (16 and 27 kDa) and δ-zein (10 kDa) (Thompson & Larkins, 1989; Zhang et al., 2015). α-Zein accounts for 70–85% of the total zein fraction, and γ-zein accounts for 10–20% as the second most abundant fraction. Zein possesses superior properties of film forming, antioxidation, biodegradability and biocompatibility (Dong, Sun, & Wang, 2004; Fernandez, Torres-Giner, & Lagaron, 2009; Zhang et al., 2015). Zein is insoluble in water and is soluble in 60–85% ethanol solution (Tang et al., 2010; Wu, Luo, & Wang, 2012) because zein is rich in hydrophobic amino acid residues as well as is deficient in basic and acidic amino acid residues (Cabra, Arreguin, Vazquez-Duhalt, & Farres, 2007; Flores, Cabra, Quirasco, Farres, & Galvez, 2010; Shukla & Cheryan, 2001). Zein is absence of tryptophan and lysine (Shukla & Cheryan, 2001). More than 20% of the amino acid residues of zein are asparagine and glutamine (Hu, Peifer, Heidecker, Messing, & Rubenstein, 1982), which can change into aspartic acid and glutamic acid residues *via* deamidation reaction. The carboxyl groups of zein produced by deamidation reaction increase the solubility of zein in water (Cabra et al., 2007; Flores et al., 2010). During the deamidation reaction, degradation reaction also happens which produces zein peptides with smaller molecular weights. Tang et al. reported that the low molecular weight peptides from Alcalase-treated zein hydrolysate had strong free radical scavenging activity (Tang et al., 2010). Cabra et al. compared the enzymatic, acidic and alkaline deamidation conditions of Z19 α-zein, and they found that the alkaline deamidation condition was better for improving the emulsifying property (Cabra et al., 2007). For deamidated zein, improvement of the emulsification ability is mainly based on the electrostatic repulsive force of the deprotonated carboxyl groups (Flores et al., 2010). At acidic condition, deamidated zein becomes hydrophobic one due to protonation of the carboxyl groups and therefore cannot be an emulsifier. Because of the acidic environment in most food and beverage (Piorkowski & McClements, 2014), the applications of deamidated zein are limited.

Conjugation of polysaccharide to deamidated zein peptide can increase the hydrophilicity of the peptide. Herein, we produced deamidated zein peptide-polysaccharide conjugates and adopted following ways to adjust the hydrophilicity/hydrophobicity of the conjugates: (1) deamidation degree and degradation degree of the peptide, (2) number of conjugated polysaccharide, (3) polysaccharide molecular weight, and (4) protonation degree of the carboxyl groups. We used the conjugates with adjustable hydrophilicity/hydrophobicity as emulsifiers to produce oil in water emulsions with pH sensitivity and adjustable stability. As illustrated in Scheme 1, after deamidation reaction, the produced peptide is soluble in neutral and alkaline solutions due to the deprotonated carboxyl groups; polysaccharide is conjugated to the primary amine group in N-terminal of the peptide *via* Maillard reaction; at acidic condition, the conjugate possesses good emulsifying ability; at neutral condition, demulsification happens when the electrostatic repulsion and hydrophilicity of the conjugate are strong enough. This study proves that the deamidated zein peptide-polysaccharide conjugates are green and pH sensitive emulsifiers with adjustable hydrophilicity/hydrophobicity.

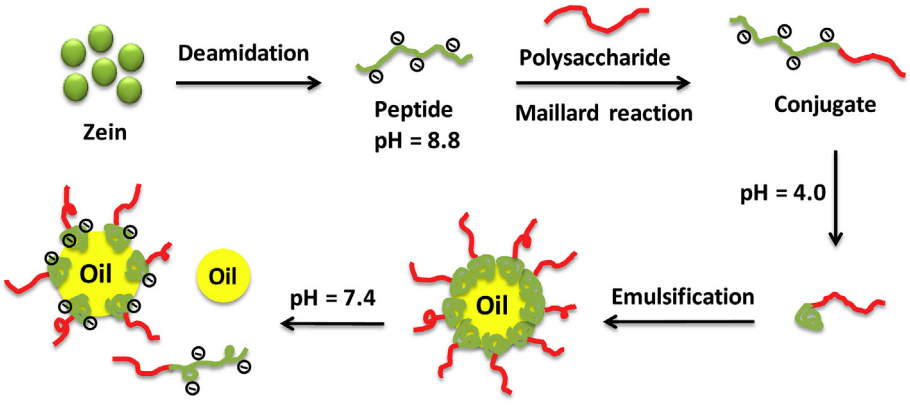
2. Experimental

2.1. Materials

Zein from maize was purchased from Sigma (Shanghai, China). Maltodextrin (MAL, DE 20–23) was supplied by DSM Nutritional Products AG (Kaiseraugst, Switzerland). Dextran (DEX, dextran-10) was purchased from Sangon Biotech Shanghai Co., Ltd. (Shanghai, China). Medium chain triglyceride (MCT) for injection was from Avic (Tieling) Pharmaceutical Co., Ltd. (Tieling, Liaoning, China). All other chemicals were from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of deamidated zein peptides

Zein was dissolved in a solution containing 0.5 M NaOH and 70% (v/v) ethanol with zein concentration of 10 mg/mL. The solution was incubated at 37 °C under stirring for 6, 36, 72, 96 or 144 h. After the incubation, the ethanol in the solution was immediately removed by rotary evaporation under vacuum. The remaining solution was changed to pH 3.1 by addition of 5 M HCl and then the solution was kept at room temperature overnight to precipitate



Scheme 1. Illustration of the preparation processes of pH sensitive deamidated zein peptide-polysaccharide conjugate and emulsion.