Interface-related attributes of the Maillard reaction-born glycoproteins

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Abstract

Interfacial behavior of proteins which is a chief parameter to their emulsifying and foaming properties can be tailored through the Maillard reaction. The reaction can increase protein solubility at isoelectric point and ought to be controlled for example by high pressure processing to suppress melanoidins formation. Branched and long saccharides bring considerable steric hindrance which is associated with their site of conjugation to proteins. Conjugation with high molecular weight polysaccharides (such as 440 kDa dextran) may indeed increase the thickness of globular proteins interfacial film up to approximately 25 nm. However, an overly long saccharide can shield protein charge and slow down the electrophoretic mobility of conjugate. Maillard conjugation may decrease the diffusion rate of proteins to interface, allowing further unfolding at interface. As well, it can increase desorption iteration of proteins from interface. In addition to tempering proteins adsorption to interface, Maillard conjugation influences the rheology of protein membranes. Oligosaccharides (especially at higher glycation degrees) decrease the elastic modulus and Huggins constant of protein film; whereas, monosaccharides yield a more elastic interface. Accordingly, glycation of random coil proteins has been exploited

to stiffen the corresponding interfacial membrane. Partial hydrolysis of proteins accompanied with anti-solvent-triggered nanoparticulation either before or after conjugation is a feasible way to enhance their emulsifying activity.

Keyword

Maltodextrin; Whey protein; Soy protein; Interface thickness; Conformation; Hydrophobicity; Nanoparticle.

1. Introduction

Most vegetable (as the greatest source of protein on earth) and microbial proteins have finite functionality (Sun-Waterhouse et al., 2014); as well, some food proteins are labile under high temperature and extreme flow conditions and presence of organic solvents (Oliveira et al., 2016). Glycoconjugation may ameliorate the techno-functional properties of proteins; for example, the surface area stabilization potency of proteins can be enhanced by glycoconjugation, resulting in formation of smaller droplets in emulsions (Xu et al., 2012). Reducing the toxicity and antinutritional characteristics of some proteins are amongst the other purposes of modification (Sun-Waterhouse et al., 2014). It has been for instance indicated that glycosylation of soybean glycinin (11S) with lactose decreased the antigenicity and allergenicity of the protein due to changes in the protein's secondary structure, that is, the content of β-sheet and random coil structures decreased while that of α-helix and β-turns increased with increasing modification degree (Bu et al., 2015). Bioavailability of bioactives may as well be tuned via protein modification. An exemplar emergent is the complex coacervation core micelles formed via the self-assembly of gelatin/dextran conjugates (produced through the Maillard reaction) with hydrophilic tea polyphenols (Fig. 1). Gelatin and tea polyphenols comprised micelle core; while, dextran segments composed micelle shell. The self-assembled micelles improved tea polyphenol delivery and showed a comparable or even better antitumor activity against human breast carcinoma cells than free tea polyphenol (Zhou et al., 2012).

2. Overview of the Maillard reaction for protein modification

Proteins are modified by various chemical, physical (e.g. pulsed electric field and high hydrostatic pressure) and enzymatic (e.g. transglutaminase-induced crosslinking) methods.

Chemical modification procedures are generally easy to be carried out for many commercial biomaterials in large scale with economic feasibility and low labor costs (Nakai and Modler, 1996). The covalently formed glycoconjugates of proteins through chemical reactions have sometimes been called neoglycoproteins (Nakamura et al., 2000). Neoglycoproteins can be produced by different methods, including amidination (amidines as the final product), guanidination (guanidine derivative as the final product) (Stowell and Lee, 1980), amination (using either sodium borohydride or sodium cyanoborohydride as the reductant) (Pedrosa et al., 2000), amidation (especially with carbodiimide as cross-linking agent) (Kobayashi et al., 2001), cross-linking by bifunctional reagents (especially with glutaraldehyde, formaldehyde and glyceraldehydes) (Gerrard et al., 2003), coupling with glycoside diazonium salt or isothiocyanate (Stowell and Lee, 1980) and the Maillard reaction.

There are concerns about the hazardous and toxic chemicals used traditionally for proteins glycosylation. For example, glutaraldehyde and formaldehyde cross-linked protein conjugates are required to be extensively dialyzed to remove non-reacted species owing to the documented cytotoxicity of the aldehyde molecules (Gough et al., 2002). There exists therefore a trend toward green and environment-friendly methods that avoid synthetic chemical reagents and organic solvents. Maillard reaction is a spontaneous process that can happen by only food-grade substances through mild, safe and solvent-less procedures (Chevalier et al., 2001; Oliver et al., 2006). Thus it may be considered a consumer friendly method of proteins modification.

Maillard reaction is amongst the chemical techniques that have received much attention in recent years. It is a non-enzymatic process arising from the heat-catalyzed covalent condensation of a carbonyl-containing compound and a deprotonated amino group. The former is usually a

reducing sugar or fat breakdown product (Gerrard, 2002), while the latter is especially the ε-amino group of lysine, but also the α-amino groups of peptide chains (Ames, 1992; Dickinson, 2008). In addition to lysine, the indole group of tryptophan, guanidine group of arginine and imidazole group of histidine also involve in the Maillard reaction (Ames, 1992; Sun-Waterhouse et al., 2014). Association of protein and saccharide via covalent bonding grants an essentially persistent interaction compared to other protein-polysaccharide complexation methods (Dickinson, 2008) such as electrostatic and hydrophobic coupling. Partially de-proteinized sugar beet pectin (DSBP)/bovine serum albumin (BSA) conjugates obtained by the Maillard reaction showed superior emulsifying property to DSBP/BSA electrostatic complex, due probably to different binding patterns between the protein and SBP (Chen et al., 2016).

In general, Maillard reaction comprises three stages. At the initial stage, condensation of an amino group and an aldose leads to formation of a Schiff base with water rescue. Subsequently, the corresponding N-glycosylamine is formed from the cyclization of the Schiff base. In essence, glycation is based on the irreversible Amadori rearrangement steps to produce the corresponding Amadori product from N-glycosylamine (Ames, 1992). No color changes are observed at the initial stage (Wu et al., 2014b). The intermediate stage includes different routes such as, dehydrations, enolizations, isomerizations, cyclizations, rearrangements, oxidations, further condensations and etc., which result in the formation of various poorly distinguished compositions (Liu et al., 2012). This stage causes yellowing and strong absorption in the near-ultraviolet region (Wu et al., 2014b). Finally, at the last stage, melanoidins which are water-insoluble, extremely colored nitrogenous polymeric compositions are generated. Since these compounds are harmful, the Maillard reaction should be performed at controlled conditions to

eschew final stage (Álvarez et al., 2012; Hiller and Lorenzen, 2010). By performing the Maillard reaction under high pressure (400 MPa) and at an initial pH ≤8.0, degradation of the Amadori rearrangement products is retarded, and thereby the intermediate and advanced stages of the reaction are suppressed (Moreno et al., 2003). In a mixed solution of whey protein isolate and glucose or trehalose at pH 6, 7 and 9, high pressure-high temperature processing reduced browning at all pH values compared to conventional high temperature treatment. After high pressure-high temperature treatment at pH 7.0, viscosity of mixed protein/sugar solutions remained unchanged. These findings can be exploited for improving the quality of high-protein beverages, for which browning and high viscosities are undesirable (Ruiz et al., 2016).

Maillard reaction products (MRPs) are not a single type and include diverse glycoforms. Quantities and types of these products (and consequently glycated protein characteristics) are affected by reaction parameters, such as the weight ratio of amino group to reducing sugar, reaction time, water activity (relative humidity), amino acid and reducing sugar characteristics, pH value, reaction temperature (Oliver et al., 2006) and finally the degree of the reaction progress. Amino groups deprotonation is a prerequisite for the nucleophilic attack of amines to aldoses. Because the pK_b of the ε-amino group of lysine is about 10.5, the reaction intensity increases at higher pH values (Trofimova and de Jongh, 2004). In contrast to flexible proteins, globular proteins ought to be denatured (i.e. require longer heating times) to unfold and expose their reactive amino groups (Oliver et al., 2006). The reaction also takes longer at higher molar ratios of saccharides to protein, as well as, for higher molecular weight saccharides, owing to their lower reaction kinetics (Wooster and Augustin, 2007a; Nakamura et al., 2000) and higher glass-transition temperatures (Dunlap and Côté, 2005). Therefore, exploiting higher molecular

weight saccharides would be more expensive from the industrial point of view. Conjugation with monosaccharides and oligosaccharides may be of interest, which allows production of glycated proteins with a higher yield under milder reaction conditions (Corzo-Martinez et al., 2012b).

Water activity plays an important role in the Maillard reaction and optimum a_w ranges from 0.5 to 0.8. At higher water activities the reaction rate decreases since the concentration of the reactants is lower; therefore, the reaction is fairly self-inhibitory as water is generated as the by-product of some steps (Labuza and Baisier, 1992; Van Boekel, 2001; Li et al., 2016). Heating a lyophilized mixture of protein and carbohydrate at an appropriate relative humidity for a certain period of time is a convenient procedure to perform the reaction (Trofimova and de Jongh, 2004). The dry method needs less space and time and final product exhibits long shelf-life and more feasibility for handling and storage compared to the wet (in solution) version of the reaction (Oliver et al., 2006). Furthermore, the dry method has a higher reproducibility and less adverse effect on the protein structural entirety than the wet method (Kim and Shin, 2016; Trofimova and de Jongh, 2004). However, the requirement for solid reactants, as well as, controlled humidity in the reaction chamber may lead to inappropriateness of the dry method for industrial scale (Perusko et al., 2015). Moreover, wet version of the reaction allows microwave (Nooshkam and Madadlou, 2016a), ultrasound (Perusko et al., 2015), pulsed electric field (Guan et al., 2010) and high hydrostatic pressure (Xu et al., 2010) assisted processes. Nevertheless, water and buffer elimination for recovering reaction products is a disadvantage of the wet method (Oliveira et al., 2016).

3. Emulsification potency of proteins as affected by the Maillard reaction

Glycation is a well-known process to address the insufficient solubility and emulsifying properties of proteins at pI (Zhang et al., 2012). Soy whey protein isolate (SWPI)/fenugreek gum conjugates were entirely soluble (>90%) over the pH range of 3−8 and presented better emulsifying properties at pH 4 compared to SWPI and SWPI/fenugreek gum mixture (Kasran et al., 2013). Likewise, increased solubility at the pI conferred superior interfacial properties (lower surface tension, higher viscoelastic properties) and foam characteristics (foam expansion and drainage time) to β‑lactoglobulin/acacia gum Maillard conjugate (prepared at pH 4.2) compared to heat-treated protein (Schmitt et al., 2005). Indeed, glycoproteins anchor into non-aqueous domains by the hydrophobic patches of the protein moiety and form a coherent elastic layer, whereas the saccharide segment, which is solvated, protrudes into the aqueous phase (Kasran et al., 2013), causing steric stabilization. Enzymatic hydrolysis with either dextranase or trypsin of a β-lactoglubolin/dextran conjugate adsorbed onto polystyrene latex beads indicated that the thickness of the adsorbed layer decreased to that of β-lactoglobulin alone (about 3 nm) by dextranase digestion; while, trypsin hydrolysis slightly decreased the interfacial thickness. This finding confirms that the protein moiety of the conjugate is located in the interior side of the membrane film (Wooster and Augustin, 2006; Wooster and Augustin, 2007b).

The Maillard reaction may increase the emulsifying activity of proteins via influences on their conformation. After adsorption at interface, the protein moiety must be able to change conformation to maintain the balance between the degrees of hydrophobicity and hydrophilicity (Amid et al., 2013) and to suggest rheological properties (Trofimova and de Jongh, 2004). The flexibility of tertiary and/or quaternary structures plays a significant role in this regard. The higher emulsifying activity of kidney bean vicilin (phaseolin) conjugated with glucose was found

related to an increase in flexibility of protein quaternary conformation (Tang et al., 2011). Partial unfolding and enhanced flexibility of protein due to the Maillard reaction has also been accounted for the increased stability of corresponding glycoprotein foams to gravitational drainage (Medrano et al., 2009). Dry conjugated ovalbumin (OVA)/sodium carboxymethyl cellulose (CMC, a non-surface-active hydrophilic polysaccharide) complexes were of significantly higher emulsifying and foaming properties than OVA-CMC mixture or OVA alone. Nevertheless, the foaming ability, on the contrary to emulsifying, decreased with the increasing substitution degree (i.e. average numbers of carboxymethyl groups per repeating unit) of CMC which was ascribed to the increased viscosity of the conjugate solution (An et al. 2014). In fact, excess attachment of saccharide chains may also decrease the emulsifying potency of the conjugate since the protein moiety is no longer capable to adsorb to oil-water interface (Li et al., 2016). On the other hand, protein at high contents may gel during conjugation (Oliver et al., 2006).

4. Factors influencing emulsifying properties of the conjugates

Emulsifying properties of the Maillard reaction-born conjugates are affected by conjugation degree, structure of the conjugating reductant and net charge (Oliver et al., 2006). Limited conjugation of β‑lactoglobulin with fructose oligosaccharide (FOS) to avoid changes in protein structure resulted in a lower surface pressure than native protein (Trofimova and de Jongh, 2004). On the other hand, conjugation of BSA with a sugar ester (i.e. 6-O-octanoyl-D-glucose) significantly decreased the interfacial tension and caused a great improvement in emulsifying activity of the protein (Rangsansarid et al., 2008). Likewise, controlled Maillard reaction of proteins with anionic saccharides improves functional properties of proteins similar to

succinylation (Oliver et al., 2006) and phosphorylation (Kester and Richardson, 1984). Branched saccharides can also provide better emulsifying properties compared to straight counterparts, which is attributed to the greater steric hindrance brought about by the former (Kim and Shin, 2016; Wong et al., 2011). Relatively large polysaccharide moieties (>5000--10000 Da) are required to introduce adequate steric repulsion that could dominate attractive interactions between oil droplets under low pH and high electrolyte concentrations (Dickinson, 2009; Dickinson, 2008). A lower steric layer density (1 saccharide tail per 9.5 nm^2 corresponding to 1.6 maltodextrins attached per β‑lactoglobulin molecule compared to 1 saccharide tail per 7.5 nm^2 corresponding to 2 maltodextrins attached per β‑lactoglobulin molecule) was needed to stabilize emulsions against flocculation when a higher molecular weight (1900 Da) maltodextrin was implemented for β--lactoglobulin conjugation rather than a lower molecular weight (900 Da) maltodextrin (Wooster and Augustin, 2007b).

In addition to boosting steric hindrance, prolongation of the saccharide chain length decreases the adverse browning and protein polymerization phenomena (Oliver et al., 2006; Oliveira et al., 2016). Nevertheless, a lengthy saccharide chain would involve less actively in the conjugation reaction and result in significantly diminished ζ-potential (Li et al., 2016). The decrease in ζ-potential value of proteins due to the Maillard reaction (especially with large saccharides) has been explained on the basis of the electrostatic screening (shielding) of protein charge by carbohydrate layer (Li et al., 2016; Chen et al., 2014), as well as, the decreased mobility of conjugate in electric field because of the bulky hydrophilic saccharide chains (Zhang et al., 2015a). Wooster and Augustin (2006) observed that conjugation of β-lactoglobulin with a higher molecular weight dextran (440 kDa) instead of a lower molecular weight counterpart (18.5 kDa)

resulted in a lower surface coverage (surface load). Large saccharides can cause steric crowding which prohibits the formation of a saturated monolayer by proteins (Dunlap and Côté, 2005). Nonetheless, a relatively small polysaccharide (dextran 40 kDa) does not confer such a crowding phenomenon at interface and led to higher surface excess (surface load) in comparison with the oligosaccharide maltodextrin, the disaccharide maltose and the monosaccharide glucose.

5. Interfacial adsorption and film properties of the conjugates

The stabilization of transient interfaces created during emulsification and aeration practices by surface active agents is governed mainly by two factors: surfactant adsorption phenomenon to interface and physical properties of the adsorbed film. Proteins glycation affects both features. In the following, we discuss the impact of the Maillard reaction on the adsorption of proteins onto interface and physical traits of corresponding interfacial films.

5.1. Proteins adsorption to interface as influenced by the Maillard conjugation

Adsorption process of proteins can be divided into: i) movement of proteins from the bulk onto the interface (e.g. via diffusion), ii) adsorption to the interface and iii) protein aggregation and rearrangement on the interface. Proteins adsorption kinetic can be controlled by each of these three stages (Wu et al., 2014a). A first order equation was exploited by Medrano et al. (2009) to adjust the experimental surface tension data with two exponential components: the adsorption (k_a) and rearrangement (k_r) constants. It was found that the main parameter that contributes to reduction of surface tension by native, heat-denatured and glycated β-lactoglobulin is the adsorption kinetic of protein onto surface (not the rearrangement process), manifested as the much greater value of k_a (about 100-fold) than k_r.

Dynamic of adsorption can be investigated by measuring surface pressure (π) and protein diffusion rate onto the interface (apparent diffusion constant, K_{dif}) (Corzo-Martinez et al., 2012a,b). An inverse relationship between K_{dif} and surface pressure has been observed (Corzo-Martinez et al., 2012b). For example, a higher K_{dif} for the nanoparticles of β-conglycinin prepared through the desolvation method than nanoparticles of β-conglycinin-dextran conjugate and β-conglycinin-dextran conjugate hydrolysates allowed faster adsorption and prevented proteins unfolding at interface which in turn led to a lower surface pressure value. The lower molecular weight of β-conglycinin nanoparticles could also contribute to the lower surface pressure since the likelihood of emulsifier desorption from interface increases with decreasing size (Wu et al., 2014a).

Different proteins may have dissimilar adsorption kinetics to the surface; nevertheless, intermolecular interactions of proteins typically result in about 20-25 mN/m reduction in surface tension during adsorption. This reduction takes place beyond the surface load of 1 mg/m^2 (Trofimova and de Jongh, 2004). At bulk concentrations below 0.001 mg/mL, lag time in the surface pressure evolution vs. time plot was observed for native β-lactoglobulin, which resulted in a surface pressure about zero. Maillard conjugation of β-lactoglobulin with lactose by either wet or dry method did not influence the kinetic of adsorption at that very low concentration (0.001 mg/mL) (Gauthier et al., 2001; Fechner et al., 2007). However, at high enough concentrations, the kinetic of the adsorption of protein molecules can be increased by controlled Maillard reaction. Corzo-Martinez et al. (2012b) observed that K_{dif} (diffusion rate) of β-lactoglobulin at pH 5.0 (protein's pI) increased by glycation with galactose in comparison with the control heated protein, owing to increase in protein solubility. The increased K_{dif}

consequently resulted in improved foaming capacity (Patino et al., 2008). In addition to improving solubility, increase in surface adsorption by Maillard conjugation may also be caused by protein unfolding and increase in surface hydrophobicity (Li et al., 2016; Zhang et al., 2012). However, an excessive glycation treatment may disparage the surface activity of proteins. Dickinson and Semenova (1992) demonstrated that the adsorption of BSA at the oil-water interface (i.e. interfacial activity) decreased by attachment of more than 1 mol dextran to BSA. Likewise, a plethora of reports confirm that a higher protein proportion in the Maillard-born conjugate provides a higher surface pressure increment (Wu et al., 2014a; Kim and Shin, 2016).

Adsorption process of emulsifiers is a dynamic equilibrium between adsorption and desorption states; it was found by measuring the changes in the magnitude of the storage modulus (G′) of interfacial membrane (Fig. 2) that conjugation of soy peptides with different saccharides increased desorption iterations because of decrease in the count of hydrophobic interactions at interface among the peptides. The longer the saccharide chain for mono, di and oligosugars, the lower the glycation degree was and thus less diminishment in conjugate hydrophobicity happened. Nonetheless, surface hydrophobicity was not the only parameter that contributed to desorption iteration, as the dextran (a polysaccharide) conjugate which had a lower hydrophobicity, also showed a lower desorption iteration than maltodextrin conjugate. This discrepancy was attributed to the formation of multilayer interfacial film by the polysaccharide conjugate (Li et al., 2016). Hydrophobicity of proteins may also decrease upon the Maillard reaction due to formation of hydrophobically-driven folded aggregates (Medrano et al., 2009). The blockage of specific sites in protein (or peptide) molecules that bind with a certain molecular probe used for hydrophobicity measurements may as well contribute to decrease in

hydrophobicity. The latter happens as the cationic amino acid residues (from arginine and lysine) are consumed (or shielded) during the Maillard reaction; thereby failing to interact with 8-anilino-1-naphthalene sulfonate (ANS), a very common probe used for hydrophobicity measurement (Gasymov and Glasgow, 2007).

The adsorption process of Maillard reaction-born conjugates are affected by reaction version and degree of glycation. Wet Maillard conjugation of β-lactoglobulin with lactose (with lower glycation degree) caused more surface adsorption than the dry method. Non-covalent polymerization of unfolded β-lactoglobulin molecules accounts for the higher surface pressure in the wet method. In contrast, the dry method preserved the native-like structure of β-lactoglobulin (Gauthier et al., 2001).

5.2. Physical properties of interfacial layer as influenced by the Maillard conjugation

Flexible random-coil proteins such as casein form slightly open interfacial membranes with low rigidity, whereas, films made by globular proteins are dense with high viscoelasticity (McClements, 2005). Glycation is perceived a potent method to enhance the stiffness of casein interfacial membrane (Corzo-Martinez et al., 2012a) because results in widespread hydrogen bonding among adsorbed protein molecules at interface rather than comparatively weak hydrophobic interactions. In agreement with the observations for random coil proteins, β-lactoglobulin-galactose conjugate formed a more elastic and cohesive film at pH 5 (protein's pI) than both the native and thermally treated proteins (Corzo-Martinez et al., 2012b).

A denser interfacial layer can more effectively protect encapsulated cargo against degradation. WPI/SBP Maillard conjugates by forming a denser and thicker interface than WPI and physical

mixture of WPI/SBP provided higher β-carotene protection against degradation during processing and storage of emulsion (Xu et al., 2012). Carbohydrate molecular weight is a main parameter that determines the site of conjugation and mass of the generating conjugate; thus it dictates the influence of glycation on interfacial film thickness. For example, Wooster and Augustin (2007b) found that maltose glycation of β-lactoglobulin did not affect the interfacial film thickness; whereas, conjugation with maltodextrin increased the adsorbed layer thickness. Likewise, dextran molecular weight was a foremost factor that controlled the interfacial film thickness of conjugated β-lactoglobulin. Conjugation with 18.5 kDa dextran and 440 kDa dextran increased the thickness of the β-lactoglobulin interfacial film from 2.9 nm to 8.2 nm and 23 nm, respectively (Wooster and Augustin, 2006). It was shown that in contradiction to a higher molecular weight carbohydrate (i.e. dextran D65 (41 kDa)), which conjugated to the hydrophilic N-terminus of wheat protein, attachment of low molecular weight carbohydrates (glucose, maltodextrin and dextran D10 (6.4 kDa)) happened at the C-terminal domain of protein (Fig. 3). The large saccharide (i.e. dextran D65) could not react with the lysine residues located within the globular structure of the C-terminus. It was presumed that the N-terminal domain diffuse layer protruded into the aqueous phase and the C-terminal domain anchored onto hydrophobic phase. Accordingly, the D65 dextran which conjugated at the N-terminus formed an additional polysaccharide steric outer layer, resulting in a thicker film (Wong, 2010; Wong et al., 2011). Saccharide geometry is another prominent parameter that contributes to the impact of glycation on interfacial protein film thickness. Carbohydrates with lower molecular weights (e.g. maltodextrin 3.8 kDa) and rigid rod configuration may form thicker conjugated β-lactoglobulin interfaces than higher molecular weight carbohydrates (e.g. dextran 12 kDa) with random coil

structure (Wooster and Augustin, 2007b). Interfacial film thickness might also be influenced by solvent characteristics. Dextran-conjugated phosvitin could not efficiently adsorb onto interface at pH 7 since the competence of C-terminal domain of the protein that interacts with apolar phase was interfered by the dextran tail. It also shielded the protrusion of N-terminal region into the aqueous solution (Fig. 4A). However, at pH values close to the protein's pI, owing to the aggregation of phosvitin, the two linear chains of phosvitin and dextran turned into one. Hence, the interference effect was vanished and subsequently the interfacial layer thickness increased (Fig. 4B) (Chen et al., 2014).

Another important physical feature of interface is rheology. Interfacial rheology has been defined as the relationship between stress, deformation and rate of deformation at interface. Stresses may cause two types of interfacial deformation including shear and dilatational deformations (Patino et al., 2008). Surface dilatational modulus (E) when plotted versus surface pressure presents some insights about the extent of intermolecular interactions between components of adsorbed layer (Trofimova and de Jongh, 2004), and the amount of proteins adsorbed to interface (Wu et al., 2014a). This complex modulus is a measure of the total unit material dilatational resistance to deformation (elastic+viscous) and comprises of two parts: storage and loss components, which are the dilatational elasticity and viscosity, respectively (Corzo-Martinez et al., 2012b). Interfacial dilatational modulus depends on the compactness of the membrane film. Higher concentrations of β-conglycinin and β-conglycinin glycoconjugates formed layers with lower dilatational modulus because a more compact interfacial layer restrains the adsorption of further protein molecules to interface and prevents the adsorbed proteins from intermolecular interactions at interface (Wu et al., 2014a).

Carbohydrate molecular weight influences the elasticity and the Huggins constant (K_H) of the interfacial film. Conjugation of β-lactoglobulin with FOS (Trofimova and de Jongh, 2004) and WPI with dextran (Wooster and Augustin, 2007a) decreased the elastic modulus and Huggins constant (K_H) of protein film which was attributed to the augmented spatial adaptability of the interfacial film. This higher adaptability allows a surface layer to expand or compress more readily. In contradiction, fructose conjugation of β-lactoglobulin yielded a highly elastic interfacial film. Coupling of the small sugar fructose to protein did not significantly increase the steric hindrance, but fructose moieties only served as templates for additional hydrogen bonding or van der Waals interactions, thereby forming a more rigid surface film (Trofimova and de Jongh, 2004).

In addition to the saccharide molecular weight, glycation degree may also extremely influence the elastic rheology of interfacial film. WPIs conjugated with dextrans of different molecular weights (35 and 70 kDa) at approximately identical glycations degrees formed films with indifferent elastic moduli. However, once the glycation degree increased from ≈1 to ≈5 dextran per mole WPI, a remarkable decrease in elasticity modulus occurred for both dextran-conjugated protein samples. A higher amount of dextran in the conjugate was premised to facilitate protein unfolding (loss of secondary structure and rigidity), either by chemical attachment of dextran or by changing the solvation environment (Wooster and Augustin, 2007a). Likewise, β-lactoglobulin/acacia gum (an anionic arabinogalactan) glycoproteins obtained at a higher protein to carbohydrate ratio (2:1 compared with 1:2) delivered higher surface elasticity (E′) (Schmitt et al., 2005).

Along with the type and amount of reactants, the Maillard reaction version (i.e. wet or dry) may affect the surface rheological properties of the obtained glycoproteins. Gauthier et al. (2001) reported that lactose conjugation of β‑lactoglobulin by wet method resulted in a more rigid surface (higher shear elastic constant) than dry version. Extensive conformational changes of protein in aqueous solution accompanied by dissociation of covalent unfolded β‑lactoglobulin dimers and their subsequent self-association through hydrophobic interactions have been accounted for the stronger interactions of wet conjugated proteins with the air-water surface (Gauthier et al., 2001).

6. Pre and post-conjugation processing to alter interfacial attributes

In addition to proteins, their partial hydrolysates have been used for Maillard conjugation. Hydrolysis can enhance the surface activity of proteins. Improvement of protein amphiphilicity by hydrolysis is argued as the underlying mechanism for enhanced interfacial activity of hydrolysate (Zhang et al., 2015b). Generally, a low degree of hydrolysis (2--5%) is sufficient to enhance the surface activity of protein. Extensive hydrolysis can reduce hydrophobicity and result in shorter peptides that are unable to form bulky polymeric layer around the droplets (Zhang et al., 2012, 2014). However, in a recent study *in vitro* biologically active peptides with considerable free radical scavenging activity were utilized for the Maillard conjugation with lactulose (Nooshkam and Madadlou, 2016b). Such peptides and corresponding conjugates might receive attention in formulation of health-promoting nanoemulsions stabilized by a mixture of low-molecular weight synthetic surfactants and bioactive peptides. Limited hydrolysis of soy proteins and subsequent Maillard conjugation with maltodextrin yielded ingredients with superior emulsifying (surface and interfacial) activities than non-hydrolyzed protein or non-

conjugated hydrolysate (Zhang et al., 2015b). Apart from the glycation of protein hydrolysates, hydrolysis of the conjugates is also beneficial for improving the interfacial properties. Hydrolysates (DH 2.9%) of soy protein/maltodextrin conjugate were introduced as superior ingredients to soy protein/maltodextrin conjugates and mixtures for preparing fish oil microcapsules by freeze-drying method (Zhang et al., 2014). In addition to tuning amphiphilicity, the limited hydrolysis of protein/sugar conjugates increases their flexibility which contributes to modified interfacial properties. Limited hydrolysis of β-conglycinin/dextran conjugates (DH 2.2%) that generated more flexible offspring increased protein adsorption to interface and decreased saturation surface load (Γ_{sat}) (Zhang et al., 2012).

The interfacial properties of the Maillard reaction-born conjugates can be further elaborated via hydrophobically-driven particlulaion. Nanoparticulation of partially hydrolyzed β-conglycinin/dextran conjugates through ethanolic desolvation caused a higher hydrophobicity and provided higher interfacial pressure (Fig. 5). Post-conjugation nanoparticulation and hydrolysis of β-conglycinin/dextran conjugates were also shown to increase the hydrophobic interactions and dilatational modulus of the interfacial membrane (Wu et al., 2014a). These characteristics favor the usage of amphiphilic nanoparticles in encapsulations and site-specific drug delivery systems.

7. Concluding remarks

The Maillard conjugation influences numerous characteristics of proteins including solubility at the pI and emulsifying and foaming properties. It is required to control a diverse range of parameters such as the reaction time, saccharide chain length, and saccharide-to-protein ratio for acquiring improved interfacial properties. Conjugation with branched saccharides compared to

linear counterparts can provide more steric stability to emulsion droplets. Short dextran molecules bring higher surface loads; whereas, large dextrans, which conjugate at the hydrophilic N-terminus of globular proteins form an additional steric protrusion layer, resulting in a thicker interfacial film. It is feasible to increase the elasticity of the interfacial films formed by random coil proteins through the Maillard conjugation. One may further manipulate the interfacial behavior of proteins via post and pre-conjugation nanoparticulation and limited hydrolysis.

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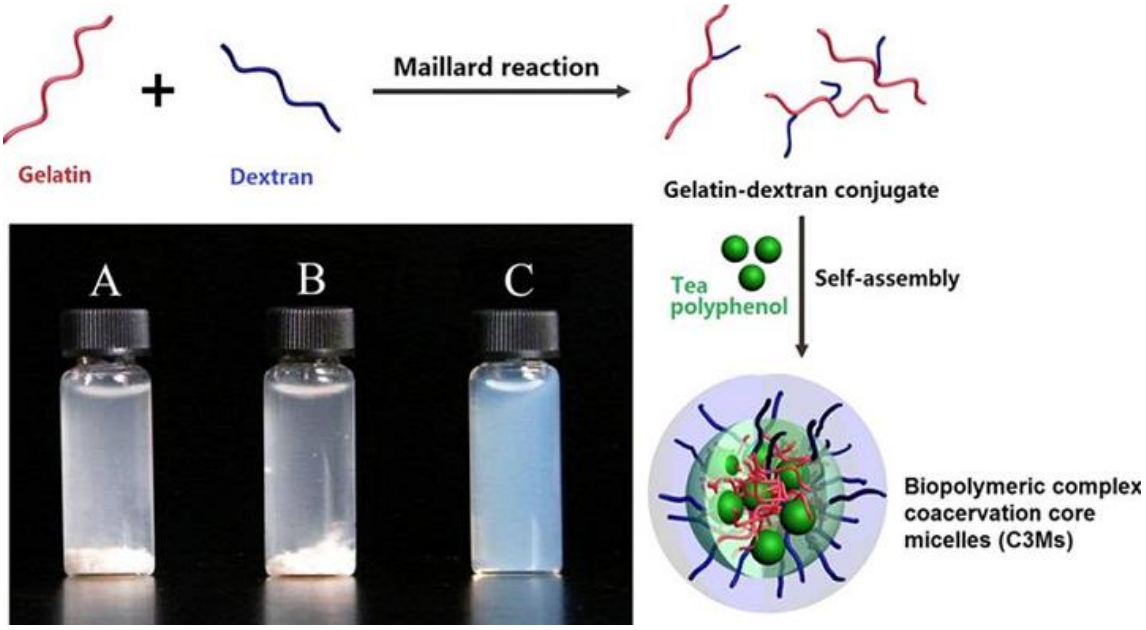

Figure 1. Schematic illustration of self-assembled complex coacervation core micelles of gelatin/dextran conjugates and tea polyphenols. Photographs of (A) gelatin/tea polyphenol physical mixture; (B) gelatin/dextran/tea polyphenol physical mixture; and (C) micelles composed of gelatin/dextran conjugate and tea polyphenols. Reprinted with permission from Zhou et al. (2012).

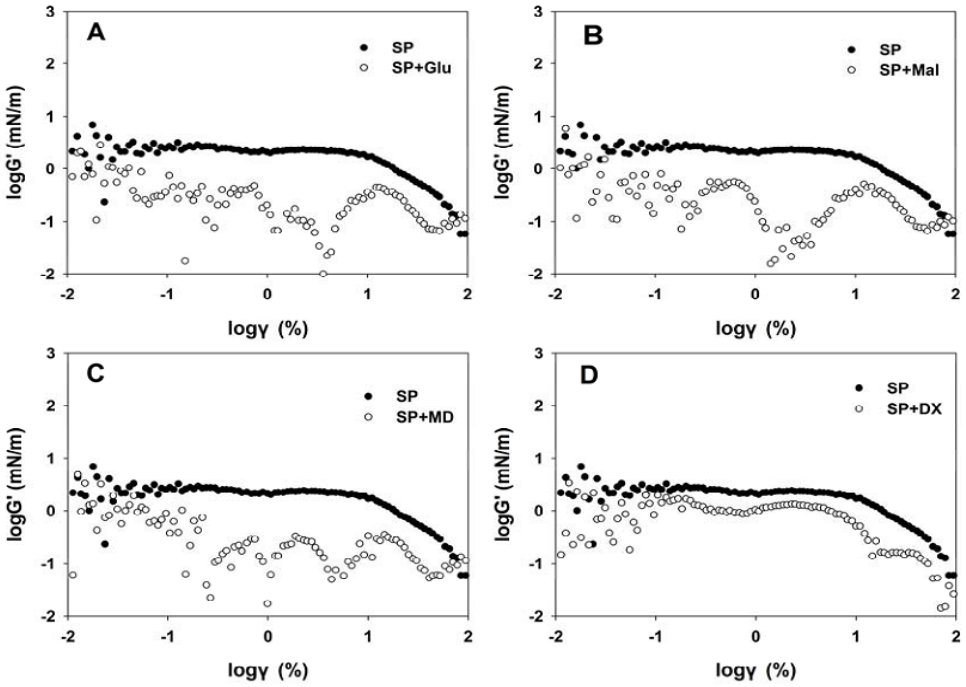

Figure 2. Strain dependence of interfacial storage modulus (G´) of (A) soy peptide-glucose, (B) soy peptide-maltose, (C) soy peptide-maltodextrin, and (D) soy peptide-dextran, stabilized at the oil/water interface. Reprinted with permission from Li et al. (2016).

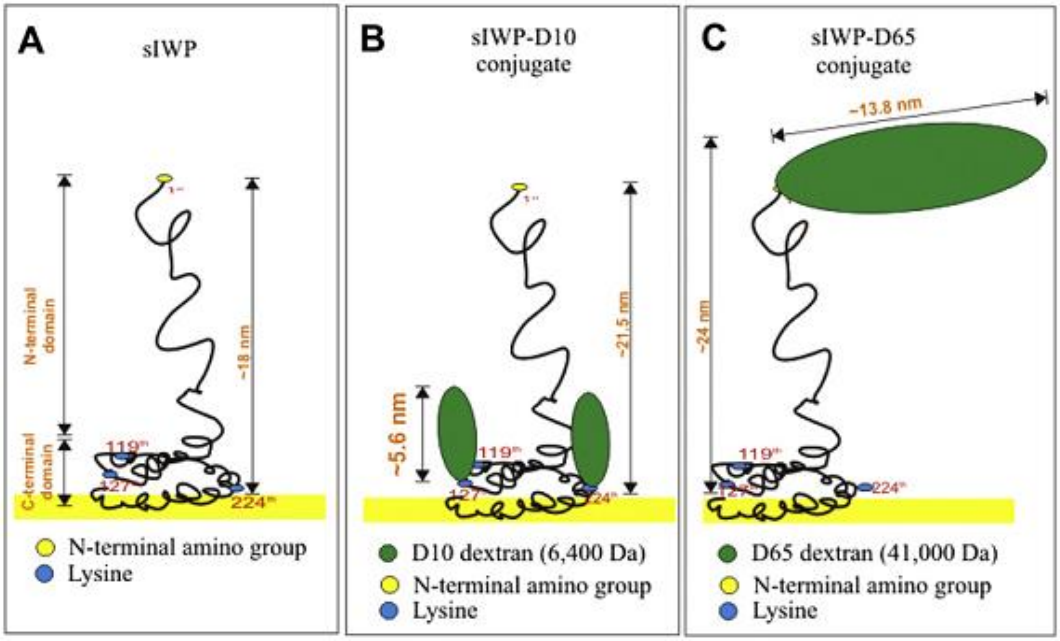

Figure 3. Schematic illustration of wheat protein (sIWP) and conjugates adsorbed at interfaces. (A) sIWP; (B) 6.4 kDa dextran (D10) conjugated onto sIWP; and (C) 41 kDa dextran (D65) conjugated onto sIWP. Reprinted with permission from Wong et al. (2011).

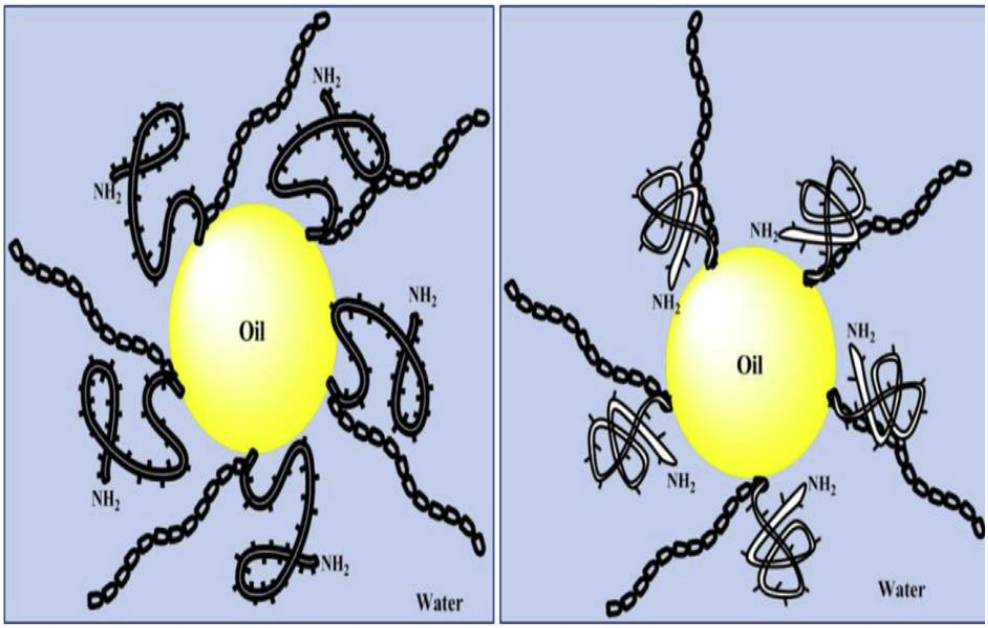

Figure 4. Schematic illustration of the adsorbed structure of phosvitin-dextran conjugate. (A) at pH 7 (B) At pH 4. Reprinted with permission from Chen et al. (2014).

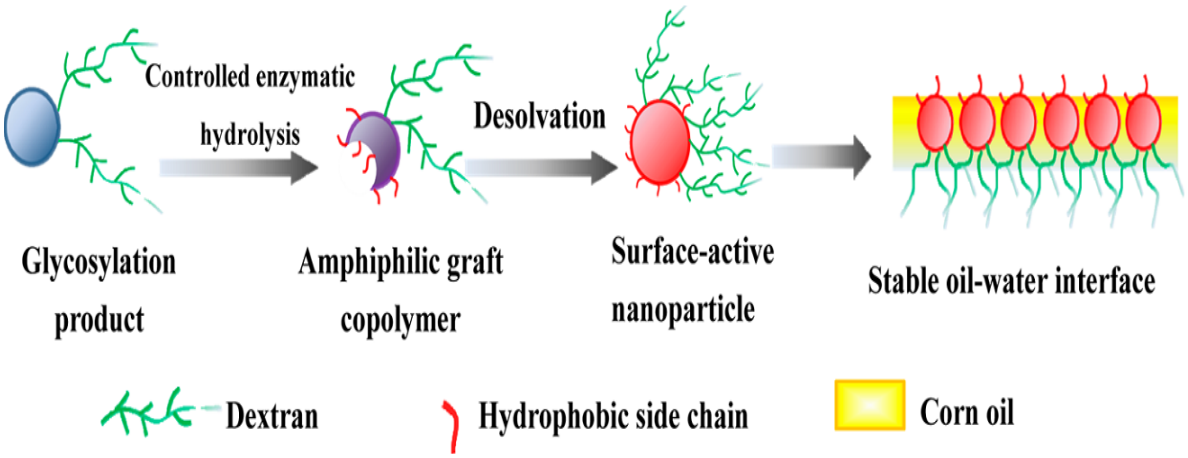

Figure 5. Schematic illustration of nanoparticles prepared from amphiphilic graft copolymer and its application in stabilizing oil−water interface. Reprinted with permission from Wu et al., (2014a).

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