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Emulsifying activity of bovine β-lactoglobulin conjugated with hexoses through the Maillard reaction

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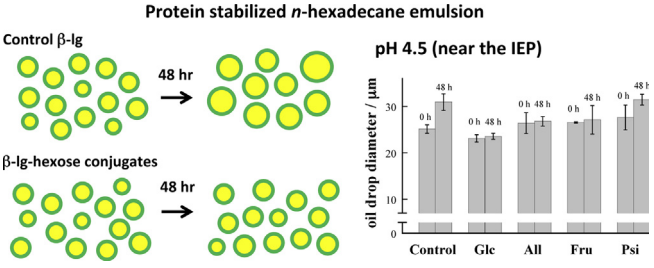
HIGHLIGHTS

- Glucose and allose resulted in a higher degree of β-lactoglobulin glycation.
- Browning and aggregation were greater for hexoses with an *S*-configuration at C3.
- Glycation shifted the IEP for oil drops in β-lactoglobulin emulsions to lower pH.
- Hexose conjugates improved emulsion stability at pH near the IEP of β-lactoglobulin.

GRAPHICAL ABSTRACT



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ABSTRACT

Four different hexoses, D-glucose (Glc), D-allose (All), D-fructose (Fru), and D-psicose (Psi), were used to prepare protein-sugar conjugates with bovine β-lactoglobulin (β-lg) via the Maillard reaction by dry-heating. Browning and protein aggregation developed during dry-heating in the following order: Glc ≈ Fru < All ≤ Psi, suggesting that the degradation rate of Amadori/Heyns compounds was influenced by the stereochemistry at C3 position of hexoses. Interfacial tension between *n*-hexadecane and aqueous solution of the prepared conjugates was similar to that of control β-lg. Employing the conjugates and control β-lg as an emulsifier, *n*-hexadecane was homogenized to prepared O/W emulsions under various pH and ionic strength conditions. Improved dispersion stability was confirmed for the conjugates compared to control β-lg if pH was around the isoelectric point of β-lg. When pH was away from the isoelectric point, the conjugates could disperse smaller size drops than control β-lg in high ionic strength medium. It was thought that non-electrostatic effects such as protection by mechanically strengthened adsorbed films formed by glycated β-lg became the dominant factor controlling the emulsification activity.

1. Introduction

Proteins, due to their amphiphilic nature, are surface-active and widely employed as emulsifiers in the food, cosmetic, and pharmaceutical industries. The emulsifying activity of proteins arises from their adsorption onto an oil–water interface to form a protecting barrier around dispersed small oil drops. The colloidal stability of oil drops in protein-based oil-in-water (O/W) emulsions depends on various factors, such as the protein species, protein concentration, pH, ionic strength, and oil content, each of which can influence the size of the oil drops and the physicochemical properties of adsorbed protein films [1–4]. In our recent work, the effects of these factors on the coalescence stability of O/W emulsions homogenized with three different proteins – bovine serum albumin (BSA),

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β-lactoglobulin (β-lg), and β-casein (β-ca) – have been estimated by means of drop size analysis and centrifugal experiments [5].

To improve the functional properties of proteins, the Maillard reaction – a non-enzymatic chemical reaction between a carbonyl compound (usually a reducing sugar) and protein amino groups – has been studied over the past few decades. The Maillard reaction leads to the formation of protein–sugar conjugates, as well as partial denaturation, owing to the exposure of buried amino acid residues in protein molecules that may provoke improved interfacial properties and potent antioxidant activity. Maillard reaction products (MRPs), therefore, are expected to be effective as foaming, emulsifying, and antioxidant agents in the food industry [6,7]. Carbonyl compounds that have been conjugated with proteins commonly include monosaccharides (aldoses and ketoses), disaccharides (lactose), and polysaccharides (dextran and maltodextrin).

Focusing on glycation by monosaccharides, the reaction conditions and the functional properties of MRPs have been examined for various proteins including BSA [8–10], β-lg [11–17], ovalbumin [17–19], α-lactoalbumin [20], casein [21–23], and shrimp protein hydrolysate [24]. In our previous work on the glycation of BSA [10], the reaction kinetics of BSA with D-glucose (Glc), D-allose (All), and a fatty acylated Glc (6-*O*-octanoyl D-glucose (GlcC8)), and the emulsifying activity of the BSA-sugar conjugates were investigated. We found no significant improvement in stability for the emulsions of BSA-Glc and BSA-All, whereas BSA-GlcC8 effectively lowered oil–water interfacial tension and increased emulsifying activity. On the other hand, glycation of β-lg with Glc has been reported to have improved the foaming properties, suggesting increased adsorption to the air/water interface [12,14,17]. By contrast, the effects of glycation on emulsifying activity have not been clarified, although some investigators have reported that β-lg-Glc increased emulsion stability as compared with native β-lg [15]. Concerning the emulsion stability of β-lg glycated with polysaccharides via the Maillard reaction, β-lg-polysaccharide conjugates effectively prevented aggregation between oil drops more than native β-lg [25,26].

One of the objectives of this study was to clarify the emulsifying activity of β-lg-monosaccharide conjugates. β-lg – a globular protein containing 15 lysine residues with a monomer molecular weight of 18 kDa – is a major component of whey protein, a waste product of cheese production. This protein can provide beneficial nutritional and functional properties as a food additive. Consequently, improving the emulsifying activity of β-lg by conjugation to monosaccharide via a simple method such as the Maillard reaction may present an interesting possibility for the food industry.

In the present study, emulsifying activity was analyzed for four different D-type monosaccharides – Glc, All, D-fructose (Fru), and D-psicose (Psi) – conjugated to β-lg via the Maillard reaction. All and Psi are the C-3 epimers of Glc and Fru, respectively. All and Psi are both rare sugars that have recently been attracting attention because of their biological activities and potential utilization in functional foods and agricultural chemicals [18–20,27,28]. A second aim of this study was to see how the stereochemistry of sugar molecules influences the Maillard reaction in order to determine adequate conditions to prepare MRPs with better functional properties. The progress of the Maillard reaction was explored from brown color development and amino group consumption during dry-heating. Gel permeation chromatography was also applied to determine the extent of protein aggregation. To estimate the emulsifying activity of MRPs, O/W emulsions were prepared using the β-lg-sugar conjugates, and oil droplet size and volume of oil released after centrifugation were examined for various pH and ionic strength conditions.

2. Experimental

2.1. Materials

Lyophilized powder of β-lg from bovine milk (≥90% by PAGE, product no. L0130) was purchased from Sigma Chemical (St. Louis, MO) and used without further purification. Glc and Fru were obtained from Tokyo Chemical Industry (Tokyo, Japan). All and Psi were supplied by the Rare Sugar Research Center, Kagawa University. To quantify free amino groups in β-lg, *o*-phthaldialdehyde (OPA) purchased from Wako Chemical (Osaka, Japan) was used. Buffer solutions were prepared using citric acid monohydrate (pH 2.5–3.5), sodium acetate (pH 4.5–5.5), sodium dihydrogen phosphate and disodium hydrogen phosphate (pH 6–7) (all guaranteed grade reagents of Wako Chemical); HEPES (pH 8) (Dojindo, Kumamoto, Japan); and glycine (pH 9–10) (Nacalai Tesque, Kyoto, Japan). The ionic strength of buffer was adjusted by adding guaranteed grade sodium chloride from Wako Chemical. Water used in this study was purified by a Barnstead E-pure purification system (Dubuque, Ia). *n*-Hexadecane from Wako Chemical was used as the oil to prepare emulsions.

2.2. Preparation of β-lg-sugar conjugates

Each monosaccharide and β-lg were dissolved (both 0.5% (w/v)) in 10 mM Na-phosphate buffer (pH 7) and freeze-dried. The molar ratio between the monosaccharide and amino group of β-lg was ca. 6:1. The lyophilized mixture was placed in a desiccator above a saturated aqueous solution of $MgCl_2$ (31% relative humidity). The Maillard reaction proceeded by dry-heating at 50 °C under reduced pressure for a given period. After incubation, the samples were dissolved in pure water and ultra-filtrated through a cellulose acetate membrane (molecular weight cut-off 20 kDa, Advantec, Tokyo) to remove remaining monosaccharide and small molecular by-products. The sample solution was then freeze-dried to obtain β-lg-monosaccharide conjugate and stored in a freezer at ca. −18 °C. Control β-lg powder was also produced by the same procedure without any sugar addition.

To monitor the development of brown color by dry-heating, an aqueous solution (1 g/L) of the incubated sample before ultrafiltration was prepared and absorbance at 420 nm was measured by a spectrophotometer (U-1800, Hitachi, Tokyo). The amount of free amino group in glycated β-lg was quantified by an OPA assay using a fluorescence spectrophotometer (FP-6300, Jasco, Tokyo); the excitation and emission wavelengths were 340 and 455 nm, respectively [29]. Native β-lg was used as the standard for the free amino group content.

2.3. Characterization of the β-lg-sugar conjugates

The hydrodynamic size of the β-lg-monosaccharide conjugates was investigated by gel permeation chromatography using a Jasco LC-2000plus HPLC system equipped with a TSKgel G3000PWXL column (10.7 mmID; 30 cm length, Tosoh, Co.) and UV–vis detector (Jasco UV-2070 plus). As the mobile phase, 0.1 M Na-phosphate buffer (pH 6.9) also containing 0.1 M NaCl was used. Sample solution (1% (w/v), 20 μL) was applied to the column at 30 °C at a flow rate of 0.5 mL/min. Elution profiles were recorded as the absorbance at 280 nm.

The oil–water interfacial tension ($\gamma_{o/w}$) between *n*-hexadecane and an aqueous solution of conjugate was explored by the pendant drop volume method using an automated computer-controlled apparatus (DVS-2000, Yamashita Giken, Japan) at 25 ± 0.01 °C. The conjugate solutions were placed in a glass syringe, and slowly pushed down to form a pendant drop at the tip of the glass syringe protruding into the *n*-hexadecane reservoir. The $\gamma_{o/w}$ was