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Incorporated glucosamine adversely affects the emulsifying properties of whey protein isolate polymerized by transglutaminase

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ABSTRACT

Glucosamine (GlcN) and microbial transglutaminase (Tgase) are used separately or together to improve the emulsifying properties of whey protein isolate (WPI). However, little is known about how the emulsifying properties change when GlcN residues are incorporated into WPI cross-linked by Tgase. We used Tgase as a biocatalyst to cross-link WPI in the presence of GlcN in a liquid system for 12 h at a moderate temperature (25°C). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analyses indicated that protein polymerization and GlcN conjugation occurred simultaneously, phenomena also supported by the loss of free amines (9.4–20.5%). Addition of 5 U Tgase/g protein improved the emulsifying properties of moderately cross-linked WPI polymers. The Tgase-treated WPI polymers had a larger particle size (~2.6-fold) than native WPI, which may have reduced coalescence and flocculation in an oil-in-water emulsion system. However, the incorporation of GlcN residues into WPI, predominantly via enzymatic glycation, partly inhibited the cross-links between the WPI molecules catalyzed by Tgase, reducing the size of the WPI polymers 0.81- to 0.86-fold). Consequently, WPI+GlcN conjugates provided less stability to the emulsion. Moreover, high NaCl concentration (0.2 *M*) decreased the emulsifying properties of the WPI+GlcN conjugates by neutralizing negative electric charges in the glycoconjugates. However, the improved emulsifying properties of WPI cross-linked by Tgase may be useful in food processing at higher NaCl concentrations due to the formation of the thicker steric barrier at the oil-water interface.

Key words: cross-linked whey protein isolate, glucosamine, microbial transglutaminase, whey protein isolate and glucosamine conjugates

INTRODUCTION

Whey protein isolate (**WPI**) is widely used in food processing to promote emulsification, stabilization, foaming, and gelation in a variety of foodstuffs (Sarbon et al., 2015). Although native WPI have many beneficial properties, researchers have attempted to develop new WPI-based ingredients with wider applications by enhancing the functionalities of WPI with physicochemical or enzymatic treatments.

As a naturally occurring reaction, glycation can improve the functional properties of proteins and even impart novel functionality. Glycation can improve the foaming properties of β-lactoglobulin and bovine sodium caseinate, broadening their applications as foaming agents, mainly in acidic foods. The emulsifying properties and thermal stability of some other proteins have also been upgraded via the Maillard reaction (Hrynets et al., 2013).

Maillard-type glycation is highly accelerated by heat treatment and is usually employed to modify proteins in a dry state (Hrynets et al., 2013). However, the dry-state process has limited the application of glycation in the food industry. Furthermore, the dry-state reaction is difficult to control and has a long reaction time (Hrynets et al., 2013). Glycation in a liquid state could overcome these problems and would be more viable for the food industry. Furthermore, methods that add enzymatic treatments have been proposed to increase glycoconjugation at a lower temperature in a liquid state and limit the formation of advanced glycation end products (Hrynets et al., 2013). Of the potential glycation enzymes, transglutaminase (**Tgase**) is widely available, high yielding, and inexpensive (Hrynets et al., 2013). In general, Tgase can catalyze 2 typical reactions: (A) protein cross-linking and (B) incorporation of

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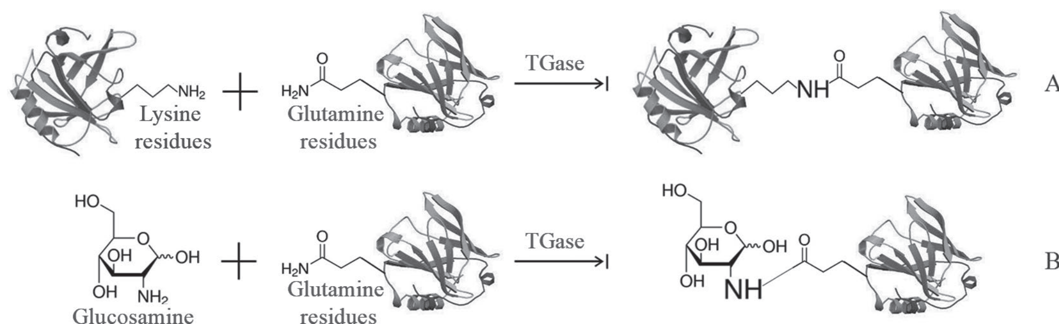

Figure 1. Two typical reactions catalyzed by transglutaminase (Tgase). Color version available online.

aminosaccharides into proteins through an acyl transfer reaction in protein substrates (Figure 1).

Glucosamine (**GlcN**) is a naturally occurring aminosaccharide that has higher reactivity in the Maillard reaction than glucose and can be incorporated into proteins by Tgase (Arbia et al., 2013; Hrynets et al., 2014). Because GlcN possesses both amino and hydrophilic hydroxyl groups, it is a promising compound for improving the functionality of proteins (Jiang and Zhao, 2010; Hrynets et al., 2013,2014). The emulsifying activity of proteins is increased when they are treated with Tgase (Song and Zhao, 2014), but other studies have shown that using Tgase to improve cross-linking and incorporate GlcN residues into protein also improves emulsifying properties (Hrynets et al., 2014). However, the influence of GlcN on the protein cross-links induced by Tgase, and thus on the emulsifying properties of the protein, has been scarcely documented.

We planned this study to elucidate the influence of GlcN on the emulsifying properties of WPI treated with Tgase. In this work, WPI were to be treated with Tgase and GlcN. We analyzed the physicochemical properties of the treated WPI using matrix-assisted laser desorption/ionization time-of-flight (**MALDI-TOF**) mass spectrometry and dynamic light scattering (**DLS**). We also assessed differences in the performance of WPI as emulsifiers after different treatments in emulsifying activity and stability. We analyzed the microstructure of oil-in-water emulsions prepared with treated WPI using confocal laser scanning microscopy. Understanding the fundamental reactions between Tgase and WPI in the presence of GlcN will enable us to optimize the formula used to improve the qualities of food products.

MATERIALS AND METHODS

WPI Treated with GlcN and Tgase

Whey protein isolate (5%) was mixed with GlcN in ratios of 1:0, 1:1, and 1:3 (wt/wt) in 20 mL of deionized water. Depending on the addition of Tgase (0 and 5 U of Tgase/g of WPI), we prepared 6 different reaction mixtures in triplicate, according to a total factorial design. All samples were incubated using a Hy-4 shaker (Zhiboru Equipment Manufacture Co., Ltd., Changzhou, Jiangsu, China) under constant agitation for 12 h at 25°C. All samples were then heated at 85°C for 5 min, inactivating the Tgase in the samples that contained it. After the samples were cooled with running tap water for 1 h, they were dialyzed against deionized water at 4°C for 16 h, with 4 changes of water to remove free GlcN. Samples that contained neither GlcN nor Tgase served as controls.

Free Amines Analysis

Free amine content in proteins was analyzed using methods described previously (Feng et al., 2015b). Briefly, WPI samples were diluted with SDS solution (1%) to 0.2 mg/mL in 0.1 M $Na_2B_4O_7$ (pH 9.3). One milliliter of the 0.2 mg/mL sample was then mixed with 50 μL of 0.03 M 2,4,6-trinitrobenzenesulfonic acid (TNBS) and incubated for 1 h at 37°C. The whole process was protected from light. Absorbance was measured at 420 nm after reaction with TNBS. Free amine content was expressed as micromoles of free amines per milligram of protein using a standard curve constructed from glycine.

Evaluation of Glycoconjugation by MALDI-TOF-MS

The WPI samples treated with GlcN and Tgase were diluted to 0.5 mg/mL with 50 mM NH_4HCO_3. The, the WPI samples (3 mL) were subjected to overnight proteolytic digestion at 37°C with 150 μL of trypsin (0.5 mg/mL in 50 mM NH_4HCO_3). The hydrolysate was filtered through a 0.45-μm filter (3 kDa; Amicon Ultra; Millipore Corporation, Bedford, MA) by centrifugation (3,000 × g, 20 min). Then, the filtrate at the bottom of the tube was collected and lyophilized.

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