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Application of ultrasound pretreatment and glycation in regulating the heat-induced amyloid-like aggregation of β-lactoglobulin

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ABSTRACT

Ultrasonication and glycation are widely used in the food industry. In this study, we evaluated the independent and combined effects of ultrasound pretreatment (UP) and glycation on the heat-induced amyloid-like aggregation of β-lactoglobulin (β-Lg) by investigating both the fibrillation kinetics and aggregate conformations. Both UP and glycation respectively were found to accelerate the fibrillation process by promoting the denaturation and unfolding behavior of β-Lg according to both the thioflavine-T binding assay. Glycation reduced the fibrillation capacity of β-Lg by reducing the content of β-sheet secondary structures in β-Lg on the basis on circular dichroism spectral measurements. Based on the results of transmission electron microscopy, glycation derived from glucose and lactose suppressed the elongation of fibrils, and β-Lg incubated with maltodextrin was shown to form spherical particle micelles. When applied jointly, UP was found to limit the glycation process by promoting aggregation during the initial heating, which may bury a portion of the glycation sites. According to these findings, UP and glycation are promising methods that can be used independently or jointly to regulate both fibrillation kinetics and fibril conformation in the food industry.

1. Introduction

Amyloid fibrils are ordered aggregates of peptides or proteins that are fibrillar in structure, which share several specific features in common, such as β-sheet-rich structures and fluorescence emission when combined with ThT (Nilsson, 2004). In the amyloid-like aggregation of a protein, protein initially undergoes unfolding process. β-Sheet structures inside the protein will expose and connect with each other. Intermolecular β-sheets of proteins or peptides extend over the length of the fibril and arrange perpendicularly to the fibril axis driven by hydrogen bond, leading to the formation of protofibrils (Arnaudov, de Vries, Ippel, & van Mierlo, 2003; Bromley, Krebs, & Donald, 2005). Then protofibrils elongate into more stable fibrillar structure. Amyloid-like aggregation of dietary proteins is related to improvements in their functional properties. Fibrillar aggregation of whey protein was reported to improve its foaming properties and foaming stability, and relationships between viscosity and amyloid-like morphology of whey fibrils were found (Loveday, Su, Rao, Anema, & Singht, 2011; Oboroceanu, Wang, Magner, & Auty, 2014). In addition, fibrillar protein aggregates are promising building blocks for the preparation of macrostructures in food products. Microcapsules which were composed of protein fibrils had been established to incorporate limonene or release drug (Rossier-Miranda, Schroën, & Boom, 2010; Humblet-Hua, Scheltens, Van Der Linden, & Sagis, 2011).

Among the various amyloidogenic proteins, β-lactoglobulin (β-Lg) is the subject of extensive study because of its high contents in whey protein which is widely applied in food industry. Additionally, the dominant β-sheet in β-Lg was a crucial structure in fibrillation (Giurleo, He, & Talaga, 2008; Qin et al., 1998). Several factors such as heat treatment, shear force, seeding, acid pH, and addition of Ca^{2+} can cause acceleration of nucleation (Akkermans, van der Goot, Venema, van der Linden, & Boom, 2008; So et al., 2011; Loveday,

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Su, Rao, Anema, & Singh, 2011; Bhattacharya, Jain, & Mukhopadhyay, 2011). Heat treatment was shown to aid in overcoming the free-energy barrier to nucleation and accelerate the amyloid-like aggregation process to a large extent (Akkermans et al., 2008). The use of acid was found to particularly facilitate the prolongation step by providing electrostatic interactions that can assist in overcoming the hydrophobic interactions (Arnaudov et al., 2003). In addition, hydrolysis of protein generated peptides, which were more flexible than intact protein and are important bricks building fibrils at acid pH. Relation between hydrolysis and growth kinetics of β-Lg fibrils was reported (Kroes-Nijboer, Venema, Bouman & van der Linden, 2011).

Ultrasound treatment and glycation are widely used in food industry. However, the independent or combined effects of ultrasound pretreatment (UP) and glycation on the amyloid-like aggregation of proteins under conditions related to food production have rarely been reported. High-intensity ultrasound (20 kHz) has been shown to change the secondary structure of β-Lg, resulting in reduction in β-sheet and increase in α-helix and/or random coil structure (Stanic-Vucinic et al., 2012). Ultrasound treatment can also result in the unfolding of β-Lg (Chandrapala, Zisu, Kentish, & Ashokkumar, 2012), which may also change the fibrillation process. In β-Lg, the first amino group located at the N-terminal (Leu), 15 Lys and 3 Arg residues can be modified by reducing sugars during food production, especially Lys residues 47, 70, 100 and 101 (Milkovska-Stamenova & Hoffmann, 2016; Fogliano et al., 1998). Inserting hydrophilic groups into a protein by glycation has been reported to change the surface hydrophobicity, electrostatic interactions as well as increase steric hindrance, which exert various effects on its secondary structure and aggregation behavior of a protein (Liu & Zhong, 2012; Pinto et al., 2012). Compared with unglycated whey protein, reduction of β-sheet, α-helix and β-turn secondary structure were found in the maltodextrin-glycated whey protein (Liu & Zhong, 2012). Therefore, UP and glycation are presumed to affect the fibrillation process of β-Lg by changing its unfolding behavior and content of β-sheet secondary structure. In addition, UP was reported to change the subsequent glycation degree of a protein by changing its conformation. Number of glycation sites of bovine serum albumin was increased from 12 to 42 after UP (Zhang et al., 2014), whereas UP of casein were found to induce reduced formation of Maillard reaction products (MRPs) in the subsequent glycation using glucose (Bi et al., 2016). Therefore, combined application of UP and glycation may result in complex influences on fibrillation process, which deserve investigation.

To verify these hypothesis, heat-induced amyloid-like aggregation of β-Lg with and without ultrasound treatment or reducing sugars were investigated in this work. This work may help to expand the application of ultrasonication treatments and glycation in the food industry and to develop new ways in regulating amyloid-like aggregation of dietary protein.

2. Materials and methods

2.1. Materials

Bovine milk β-Lg (≥90% identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)), glucose (Glu), lactose (Lac) and maltodextrin (Mal, dextrose equivalent 16.5–19.5), thioflavin T (ThT) and 1-anilinonaphthalene-8-sulfonate (ANS) were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Ultrasound pretreatment

A VCX 500 system (SONICS & MATERIALS, Newtown, USA) with a probe (13 mm in diameter) was used for sonication. The instrument frequency was 20 kHz, and the power output was set to deliver a maximum of 250 W. Solutions were sonicated for 0 to 60 cycles, where each cycle consisted of 30 s of sonication followed by a 30 s pause. An ice-water bath was used to keep the temperature of the sample solution below 25 °C during treatment.

2.3. Glycation preparation

Glycation was performed in phosphate buffer (25 mM, pH 7) containing 8 mg/mL protein (with and without UP) with and without 50 mM of reducing sugar. These mixtures were then transferred to 10 mL glass vials, each one containing 4 mL of solution, and the vials were sealed and placed in a water bath at 70 ± 1 °C for set times (0, 1, 3, 5, 10, 15, 30, 60, 120 and 240 min).

2.4. Surface hydrophobicity (S_0)

ANS is a small organic compound that is fluorescent only when associated with hydrophobic materials. Therefore, exogenous fluorescence of ANS implies there is a change in hydrophobic sites on the surface of a protein (Haskard & Li-Chan, 1998; Moro, Gatti, & Delorenzi, 2001). Diluted samples (290 μL, 0.02–0.1 mg/mL) were mixed with 10 μL of ANS (2 mM) in a microtiter plate. The fluorescence of mixed solution was measured using an Infinite M200 PRO microplate reader (Tecan, Grödig, Austria), with λex of 390 nm and λem at 488 nm. The fluorescence intensity of ANS was measured as a control and was subtracted. S_0 was determined as the initial slope when fluorescence intensity was plotted against protein concentration.

2.5. Dye-binding assays

ThT binding assays were performed according to Nilsson's method (2004). A stock solution of ThT was prepared by dissolving 8 mg of ThT into 10 mL of phosphate buffer (10 mM containing 150 mM NaCl, pH 7.0). The stock solution of ThT was diluted 50 fold with the same buffer to obtain a working solution. Then, 15 μL of each sample was mixed with 0.4 mL ThT working solution for 3 min before testing. Each reaction solution (250 μL) was transferred into a microtiter plate. The exogenous fluorescence of ThT was measured using an Infinite M200 PRO microplate reader (Tecan, Grödig, Austria) with λex of 440 nm and λem of 482 nm. The fluorescence intensity of ThT was measured as a control and was subtracted.

2.6. Particle size

The heated protein samples were diluted with distilled water to approximately 0.5 mg/mL and were filtered through a 0.22 μm filter before measurement. The hydrodynamic diameters of the samples were determined using a Nano Zsp-MPT-2 system (Malvern Instruments Inc., Worcestershire, UK). The measurements were performed in triplicate at 25 °C with a scattering angle of 173°, and the viscosity of the solvent was presumed to be the same as that of water.

2.7. o-Phthaldialdehyde (OPA) assay

The content of free amino groups was measured using a modified *o*-phthaldialdehyde (OPA) assay (Nielsen, Petersen, & Dambmann, 2001). The OPA reagent was prepared as follows: 40 mg of OPA and 25 mL of 10 mM sodium tetraborate were mixed with 2.5 mL of 20% (w/w) sodium dodecyl sulfate (SDS) and 100 μL of β-mercaptoethanol. The mixture was diluted to a final volume of 50 mL with distilled water. Sample solution (15 μL) was mixed with