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Improvement of heat-induced fibril assembly of soy β-conglycinin (7S Globulins) at pH 2.0 through electrostatic screening

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ABSTRACT

Protein fibril assembly at acidic conditions has recently attracted increasing interests in the fields of biochemical and food science, however, the obtained knowledge about the assembly of the proteins with complex structure (e.g. plant 7S/11S globulins) is still limited. This study investigated the influence of NaCl addition (0–300 mM) on the fibril assembly of soy 7S globulins (β-conglycinin), induced by heating at 80 °C and pH 2.0, with the aim of confirming the improvement of their fibril assembly by electrostatic screening. The formed fibril formation was investigated by using Th T fluorescence and atomic force microscopy (AFM) techniques. Heat-induced structure changes were traced by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and circular dichroism spectroscopy (CD). The results indicated that the whole fibril assembly experienced protein dissociation/hydrolysis and subsequent fibrillization process, and increasing NaCl concentration progressively increased the amount of formed fibrils, and even fibril length. AFM analyses confirmed that the increase in NaCl concentration shortened the time at which the short fibrils were visually observed, and the morphology of formed fibrils, including height of fibrils, width at half-height, and coil periodicity, slightly varied with the added NaCl concentration. The CD analyses indicated that increasing NaCl concentration greatly favored formation of β-type secondary structure, as well as extensive disruption of tertiary and/or quaternary conformations. These results confirmed that the electrostatic screening greatly improved the heat-induced fibril assembly process of soy β-conglycinin at pH 2.0. The improvement of fibril formation was largely attributed to increased extent of conformational changes at higher ionic strength. These findings would provide important information about the fibril assembly of plant oligomeric globulins at acidic pH that have important implications for the development of protein protein-based fibrillar gels.

1. Introduction

There are growing interests in the fibril assemblies from many food globular proteins, such as β-lactoglobulin (β-LG, a major whey protein), bovine serum albumin (BSA) and ovalbumin, due to their potential applications in the food industry (i.e. thickening agents in food matrixes) (van der Linden, 2010; van der Linden & Venema, 2007). The fibrillization of those important proteins can be induced by heat treatment at acidic (i.e. pH 2.0) or basic environment. During heat treatment, the fibril aggregation will follow thermal denaturation. Once the protein concentration exceeds the critical gelling point, the fibril aggregation will result in the formation of fibrillar gels (Gasal, Clark, Pudney, & Ross-Murphy, 2002; van der Linden & Venema, 2007). Those fibrillar gels are usually transparent, isotropic, and exhibiting unique features such as high thermal stability and gel strength (at a comparable protein concentration; relative to traditional heat-set gels).

To date, there has emerged a rich variety of articles for addressing the assembly kinetics and microstructure of heat-induced food protein fibrils, including whey proteins (i.e. β-LG; Arnaudov, de Vries, Ippel, & van Mierlo, 2003; Gosal et al., 2004; Arnaudov & de Vries, 2006; Bolder, Vasbinder, Sagis, & van der Linden, 2007; Akkermans et al., 2008; Oboroceanu, Wang, Brodkorb, Magner, & Auty, 2010), BSA (Veerman, Sagis, Heck, & van der Linden, 2003; Vetri et al., 2011), lysozyme (Misha et al., 2007), and legume proteins (i.e. 7S or 11S globulins from soy and kidney beans; Akkermans et al., 2007; Tang & Wang, 2010; Tang, Zhang, Wen, & Huang, 2010). Among those fibril-forming proteins, β-LG is one of the most investigated food proteins. The underlying mechanism for the fibril assembly is still not fully understood. Arnaudov et al. (2003) studied the β-LG fibrillization behavior at low pH and ionic strength by in situ light scattering (LS) and proton nuclear magnetic resonance (^{1}H NMR). Based on their results of LS and ^{1}H NMR, they proposed a multistep fibrillization model which involved initial linear aggregates formation and then slow consolidation of existing fibrils (Arnaudov et al., 2003).

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The fibril assembly of the proteins involves acidic hydrolysis of the polypeptides and conformational changes. By tracing the cleaved peptide of β-LG using reversed-phase HPLC, and mass spectrometry (MALDI-TOF), Akkermans et al. (2008) found that the peptides, released from cleavage of the peptide bonds before or after aspartic acid residues of β-LG, shared the similarity for facilitating β-type structure formation. The secondary structure changes from β-LG hydrolysis were then characterized by in situ attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) which confirmed the reduced α-helix and increased β-sheet during fibrillization (Oboroceanu et al., 2010). The phenomenon of peptide hydrolysis occurring also in the fibril assembly of lysozyme (Misha et al., 2007) and 7S/11S globulins from kidney and soy beans (Tang & Wang, 2010; Tang et al., 2010) seems to be a general process during protein fibrillization, which was confirmed by Lara, Adamcik, Jordens, and Mezzenga (2011). In our previous works, we additionally pointed out the fibril assembly of 7S/11S globulins was accompanied by considerable changes in tertiary and/or quaternary conformations (Tang & Wang, 2010; Tang et al., 2010).

Generally speaking, protein fibril assembly is affected by many factors including pH, ionic strength, protein concentration and heating conditions. Once pH, protein concentration and heating conditions are kept constant, the kinetics of fibril assembly will be strongly dependent on ionic strength. For instance, Aymard, Nicolai, and Durand (1999) investigated the aggregation kinetics of β-LG at pH 2.0 by light scattering with multiple sources (laser, neutron, and X-ray). Their small-angle scattering results exhibited a strong decrease trend for persistence length of β-LG fibrils with ionic strength increase, which was also observed by Kavanagh, Clark, and Ross-Murphy (2000) by using transmission electron microscopy (TEM). More recently, Arnaudov and de Vries (2006) even found that ionic strength increase up to 100 mM largely shifted the critical fibrillization concentration to low values, which facilitates fibril formation at low protein concentration. Similarly, Veerman, Ruis, Sagis, and van der Linden (2002) observed that increase of ionic strength from 0.01 to 0.09 M lead to the decrease of critical percolation concentration for the fibrillar β-LG gels at pH 2.0. Besides, their work also verified that fibril assembly rate of β-LG was slower at lower ionic strengths, which was initially stated by Aymard et al. (1999). The impact of ionic strength on fibril assembly is different among distinct proteins, such as human serum albumin (Juárez, López, Cambón, Taboada, & Mosquera, 2009) and kidney bean protein isolate (rich in 7S globulins; Zhang et al., 2010).

On the other hand, soy protein-based ingredients have attracted much attention from food industry due to their health benefits and processing availability. Elaboration of fibril assembly from soy protein will magnificently deepen our understanding of the structure of soy protein under different conditions, which facilitate the scientific design of soy protein-based ingredients. Based upon our previous investigation of 7S/11S globulins fibrils (Tang & Wang, 2010; Tang et al., 2010), we further investigate the influence of ionic strength on the fibril assembly of soy β-conglycinin (7S globulins) at low pH in a systematic way, which is scarcely reported yet. The main objective of this investigation is to study the influence of ionic strength (0–300 mM) on the kinetics and morphology of soy β-conglycinin fibril assembly at pH 2.0. Ionic strength impact on protein hydrolysis and conformation changes were simultaneously evaluated. To the best of our knowledge, this is the first report for addressing the improvement of fibril assembly of plant oligomeric globulins at low pH through electrostatic screening.

2. Materials and methods

2.1. Materials

Defatted soy flakes with low protein denaturation were processed by initial flash desolventization and vacuum drying at 60 °C by Shandong Yuwang Industrial & Commercial Co., Ltd. The flakes were ground in a Straub mill (Model 4E, Straub Co., Philadelphia, PA, USA) to pass through an 80 mesh sieve. The obtained flour was stored in sealed container at 4 °C until used. The protein content of the flour was 55.5 ± 0.4% (determined by Kjeldahl method with nitrogen conversion factor of 6.25; on dry basis) and nitrogen solubility index 84.0%. Congo Red, Thioflavin T (Th T), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals applied in the work were of analytical grade.

2.2. Preparation of soy β-conglycinin

The soy β-conglycinin samples were prepared according to the method described in our previous work (Tang & Wang, 2010), which is an improvement based on the work of Nagano, Hirotsuka, and Mori (1992).

2.3. Thermal aggregation experiments

Soy β-conglycinin solutions (1.0%, w/v) with various ionic strengths were prepared by dissolving the freeze-dried soy β-conglycinin samples into 0.1 M HCl solution with different NaCl concentrations (0–300 mM). The solution pH was accurately adjusted to 2.0 by using 6 M HCl. The protein concentrations in the solutions were determined by Lowry assay (Lowry, Rosembroug, Lewis, & Randall, 1951), with BSA as the standard protein. Aliquots (5 μL) of the protein solutions were poured into glass tubes with hermetic lids. The glass tubes were heated at 80 °C for specific periods of time in a temperature-controlled bath with temperature deviation of less than 1 °C. After heat treatment, the samples were immediately cooled in an ice bath and directly subject to further experiments.

2.4. Thioflavin T (Th T) fluorescence analysis

Th T stock solution was prepared by dispersing 8 mg Th T into 10 mL of phosphate buffer (pH 7.0) containing 150 mM NaCl (LeVine, 1995). The dispersion was filtered with 0.2 μm syringe filter to remove undissolved Th T. This stock solution should be stored in dark at 4 °C and covered with aluminum foil. The stock solution was diluted 50 times by the same buffer on the day of analysis to generate the working solution (Koscielska-Kasprzak & Otlewski, 2003; Nilsson, 2004). Aliquots (50 μL) of the tested samples were mixed with 5 mL Th T working solution and allowed to stand for at least 1 min. The fluorescence spectra of the mixtures were measured using a fluorescence spectrophotometer (HITACHI F-7000, Tokyo, Japan). The excitation wavelength was 460 nm (slit width = 10 nm) and the emission wavelength was 490 nm (slit width 5 nm), with a scanning speed of 240 nm/min. The excitation wavelength at 460 nm (rather than at 440–450 nm as applied in many previous studies) was chosen to minimize light scattering problems (Mishra Misha et al., 2007). The fluorescence spectrum of the Th T working solution was subtracted from the fluorescence spectra of the samples to correct the background signal.

2.5. Zeta potential (ζ) measurement

The ζ values of the proteins in the dispersions at varying NaCl concentrations (0–300 mM), as a function of pH were measured by a laser doppler velocimetry and phase analysis light scattering (M3-PALS) technique using a Malvern Zetasizer Nano ZS (ZEN 3600) instrument (Malvern Instruments Ltd., Malvern, Worcestershire, UK), in connection with a multipurpose autotitrator (model MPT-2, Malvern Instruments, Worcestershire, UK). The protein samples were prepared in deionized water containing various concentrations of NaCl (0–300 mM), and diluted to about 0.5% (w/v) with the corresponding same NaCl solutions. The diluted protein dispersions were