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Inhibition or improvement for acidic subunits fibril aggregation formation from β-conglycinin, glycinin and basic subunits

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ABSTRACT

It was difficult to form nice nano-fibril structure from glycinin, but in this study, we found a new method to solve the problem. The acidic subunits isolated from glycinin could form thinner, longer and more flexible fibril aggregation compared with glycinin or β-conglycinin fibril aggregation at pH2.0 and 95 °C after heating 20 h β-Conglycinin, glycinin and basic subunits were respectively mixed with acidic subunits heating at 95 °C and pH2.0 to gain insight into the influence on the formation of acidic subunits fibrils. The different soy proteins interaction was probably the main reasons for inhibition or improvement the acidic subunits fibrils formation. The morphology and kinetics of fibril formation and the change of secondary structure were analyzed by transmission electron microscopy (TEM), fluorescent dye Thioflavin T (Th T) and far-UV circular dichroism (CD) spectroscopy. These results showed that β-conglycinin improved but glycinin and its basic subunits inhibited or destroyed the formation of acidic subunits fibrils. Glycinin and its basic subunits with higher surface hydrophobicity produced more aggregation, and fast aggregate was disastrous for the formation of acidic subunits fibrils. Furthermore, CD results indicated that basic subunits probably inhibited the α-helix transition into β-strands of acidic subunits.

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1. Introduction

Amyloid fibrils of proteins have been deemed as an important ingredient in food products since they enhance viscosity and form gels at lower protein concentration than other random aggregates (Loveday et al., 2009; Graveland-Bikker and de-Kruif, 2006). These fibril aggregates possess substantial β-sheet structures which exhibited Congo red birefringence and increased thioflavine T fluorescence intensity (Chiti et al., 1999). Most of globular proteins are readily to form fibril aggregates at low pH and high temperature for promoting the protein unfolding. For example, β-lactoglobulin aggregated into long semi-flexible fibrils after heating 20 h at 85 °C and pH2.0 (Akkermans et al., 2008). Pea protein formed fibril aggregates after incubating for 20 h at 85 °C and pH2.0 (Munialo et al., 2014). There are many factors, such as protein concentration (Kroes-Nijboer et al., 2009), ionic strength (Arnaudov and de Vries, 2006), pH (Loveday et al., 2010b), shear treatment (Akkermansa et al., 2008) and temperature (Loveday et al., 2012), affecting the kinetics of fibril formation and the morphology of fibrils. Moreover, the protein fibril aggregates are influenced via self-structure of proteins. Amino acid composition also has great influence on the formation of protein fibril aggregates (Sabaté et al., 2010).

β-conglycinin (7S globulin) and glycinin (11S globulin) are major globular proteins in soy proteins. Native β-conglycinin comprises three major subunits: α (67 kDa), α′ (71 kDa) and β (50 kDa). Native glycinin consists of two polypeptides, which are acidic subunits and basic subunits linked by disulfide bridges. The pI of acidic subunits and basic subunits, respectively, are 5.0 and 8.0. Some studies had been focused on fibril aggregates formed from soy proteins. For instance, Akkernans et al. (2007) suggested that soy protein isolate (SPI) had higher ability to form fibril than glycinin when heated at pH2.0 and 85 °C, and the main features of glycinin fibril aggregates were micrometer-sized, slightly branched and curved. Tang and Wang (2010) confirmed that soy β-conglycinin exhibited a higher

Abbreviations: 7S, β-conglycinin; 11S, glycinin; TEM, Transmission electron microscopy; CD, cirulardichroism; Th T, Thioflavin T; ANS, 1-anilino-8-naphthalene sulfonate; -SH, Free sulfhydryl; ANOVA, analysis of variance; So, suface hydrophobicity.

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capacity to form amyloid fibrils than glycinin at pH2.0 and at 85 °C for heating various times, and glycinin fibril aggregates had higher width and lower coil periodicity value. Wang et al. (2011) found that α, α′ and β subunits of β-conglycinin showed different fibrillation kinetics and morphologies, and the results were associated with their amino acid composition and typical sequence of peptides. These reports indicated that the ability of forming fibril aggregate maybe closely related to the protein components and structures. But little researches has been paid to study the relationship of mutual interaction between two kinds of protein on fibril aggregates formation.

The main objectives in the study are to increase the ability of glycinin to form fibril aggregation and to improve the morphology of glycinin fibril aggregation for forming long, thin and flexible fibril structure through isolating acidic subunits. At the same time, the effects of different soy protein components on the conversion of acidic subunits into fibrils structure were also examined. Transmission electron microscopy (TEM), cirulardichroism (CD), and binding to amyloid dyes Thioflavin T (Th T) are all used to achieve the objectives and to analyze the reasons.

2. Materials and methods

2.1. Materials

Soybeans (High protein contents) were purchased from Soybean Research Institute of Northeast Agricultural University, China. Thioflavin T (Th T) powder and 1-anilino-8-naphthalene sulfonate (ANS) powder were purchased from Sigma-Aldrich. All other reagents and chemicals were of analytical grade and purchased from the local market.

2.2. Isolation of acidic and basic subunits

Soy flours were prepared by milling soybean and sieving 0.5 mm, then these flours were defatted using diethyl ether at 48 °C as the method of Soxhlet extraction. β-conglycinin and glycinin were separated from the defatted soy flours according to the method of Nagano et al. (1992) with little modifications. Acidic and basic subunits were separated from the glycinin according to methods described by Mo et al. (2006) with slight modifications. 0.5% (w/v) glycinin were prepared using 30 mM Tris buffer (pH 8.0) and 15 mM β-mercaptoethanol. The protein solution was heated at 90 °C for 30 min and then centrifuged at 10,000 g at 4 °C for 20 min. Then the precipitate was washed twice with deionized water and suspended in deionized water. The supernatant was adjusted to pH 5.0 (2 mol/L HCl) and then centrifuged at 6500 g at 4 °C for 20 min. The precipitate was washed twice with deionized water, and then prepared for the aggregates formation.

2.3. The preparation of various protein aggregates

Various soy globulin aggregates were determined by the method of Akkermans et al. (2008) with some modifications. β-conglycinin, glycinin (including acidic subunits and basic subunits) were, respectively, adjusted to pH 2.0 using 6 mol/L HCl and then centrifuged at 15,000 g for 30 min at 20 °C to remove undissolved proteins. The supernatants were accurately diluted to 1.0% (w/v) with deionized water. The concentration of protein solution was determined by Kjeldahl analysis (N × 5.71) (Akkermans et al., 2007). Then solutions were accurately adjusted to pH 2.0 using 6 mol/L HCl. To induce fibril aggregates formation, these solutions were heated at 95 °C for 20 h in a water bath. Meanwhile, 2% (w/v) β-conglycinin, glycinin, acidic subunits and basic subunits whose pH were pH2.0 were, respectively, added into 2% (w/v) acidic subunits (pH2.0) in 1:1 ratio as volume to make co-assembly fibrils heating at 95 °C for 20 h. Acidic subunits were also heated for 0 h, 10 h, 17 h 20 h at pH2.0 and 95 °C to investigate the effect of time on the formation of acidic subunits. Meanwhile, to obtain the effect of pH on acidic subunits fibril formation, acidic subunits heated at 95 °C for 20 h at different pH (pH1.5, pH2.2, pH2.5, pH3.0). After heating at requisite conditions, all samples were immediately cooled in an ice bath for further analysis.

2.4. Thioflavin T fluorescence assay

The stock solution was prepared by dispersing 8.0 mg Th T into 10 mL of phosphate NaCl buffer (10 mM phosphate and 150 mM NaCl, pH 7.0). The stock solution was filtered through a 0.2 mm syringe filter to removed undissolved Th T. The stock solution should be stored at 4 °C in a brown glass bottle covered with aluminum foil. The working solution was prepared by diluting the stock solution 50 fold in phosphate with NaCl buffer (final Th T concentration of 60 mM). In the assay, 50 μL of sample was added to 4 mL of working solution; the mixture was vortexed briefly and held at room temperature for 1 min before measuring fluorescence (Hitachi F4500 fluorescence spectrometer; Hitachi High-Technologies Corp., Tokyo, Japan). Excitation and emission wavelengths were 460 nm and 490 nm, respectively (Loveday et al., 2012; Tang and Wang, 2010).

2.5. Nonlinear regression of Thioflavin T fluorescence

According to the method of Loveday et al. (2012) with some modifications, thioflavin T fluorescence (Th T) was measured at heating time 0 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h, 16 h, 20 h for different samples. The Th T data were fitted with Equation (1), originally given by Morris et al. (2008). Note that we use this equation as an empirical curve-fitting function, in which f_t was fluorescence at time t and α, β and γ arbitrary constants:

$$f_t = \alpha - \frac{\frac{\beta}{\gamma} + \alpha}{1 + \frac{\beta}{\alpha\gamma}\exp[(\beta + \alpha\gamma)]} \tag{1}$$

The lag time (t_{lag}), time for fluorescence to increase to half of its maximal value ($t_{1/2\ max}$), and maximum rate of increase in fluorescence [$(df/dt)_{max}$], were calculated with the analytical expressions in Equations (2)–(4), which were derived from Equation (1) in the earlier work (Loveday et al., 2010a):

$$t_{1/2\max} = \frac{\ln\left[2 + \frac{\alpha\gamma}{\beta}\right]}{(\beta + \alpha\gamma)} \tag{2}$$

$$\left(\frac{df}{dt}\right)_{\max} = -\frac{\left(\frac{\beta}{\gamma} + \alpha\right)(\beta + \alpha\gamma)}{4} \tag{3}$$

$$t_{lag} = \frac{1}{\beta + \alpha\gamma}\left(\ln\left(\frac{\alpha\gamma}{\beta}\right) - 4\frac{\alpha\gamma}{\beta + \alpha\lambda} + 2\right) \tag{4}$$

2.6. Transmission electron microscopy

The microstructure of heated protein (β-conglycinin, glycinin, acidic subunits and basic subunits dispersions) were investigated with transmission electron microscopy (H-7650; Hitachi High-Technologies Corp.). The procedures were operated as followed

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