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Multilevel structural responses of β-conglycinin and glycinin under acidic or alkaline heat treatment

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ABSTRACT

The structural responses of soy protein isolate under various processing conditions are of practically important for broadening its functionalities. In this work, multilevel structural responses of β-conglycinin and glycinin under acidic or alkaline heat treatment were investigated. Our results suggested that heat treatment under acidic (i.e., pH 2.5) or alkaline (i.e., pH 8.5) conditions induced multilevel structural responses of β-conglycinin and glycinin: under acidic heat treatment, both β-conglycinin and glycinin underwent hydrolysis and experienced disruption and reorganization in ordered secondary structure. This process was accompanied with changes in tertiary structure where previously buried regions were exposed to the aqueous phase to different extent. Small-angle x-ray scattering (SAXS) results indicated that the protein conformations evolved from globular ones to elongated ellipsoids, with a more remarkable elongation effect in glycinin than β-conglycinin. Upon solvent evaporation, β-conglycinin favored aggregation with a low "lateral to vertical ratio", glycinin tended to aggregate in a high "lateral to vertical ratio" style. In contrast, alkaline heat treatment did not induce hydrolysis but disturbed the secondary structure instead. The protein monomers maintained the globular conformation and assembled into irregular large aggregates during solvent evaporation. Under either treatment, glycinin responded more sensitively than β-conglycinin at all structure levels. The observed multi-level structural responses can be used to guide the rational modification of soy protein isolate with controlled conformation.

1. Introduction

Due to its nutritional value and versatile functional properties, soy protein isolate (SPI) has been widely applied in meat, beverage and nutraceutical products (Jideani, 2011). Functional properties of SPI in food applications have involved water absorption, thickening, gelling, emulsifying and foaming activities (Moure, Sineiro, Dominguez, & Parajo, 2006). These properties vary with protein component, protein concentration, ionic strength, dielectric constant of the medium, interactions with other macromolecules, as well as processing conditions such as pH, temperature, pressure, enzymatic treatment, etc. (Cavallieri, Garcez, Takeuchi, & da Cunha, 2010; Howard & Udenigwe, 2013; Kim, Weller, Hanna, & Gennadios, 2002). In recent years, research efforts in broadening functionalities of SPI (Chen, Zhao, Sun, Ren, & Cui, 2013; Fernandez-Avila, Escriu, & Trujillo, 2015; Gan, Cheng, & Easa, 2008; Shen & Tang, 2012) have increased the need of clarifying its multilevel structural responses under various processing conditions.

As the two major components of SPI (Iwabuchi & Yamauchi, 1987), glycinin is a hexameric 11S globulin consisting of acidic and basic subunits, and β-conglycinin is a 7S trimeric glycoprotein composed of α, α′ and β subunits. They contribute distinctively to macroscale properties of SPI. Functional properties, such as heat denaturation, surface hydrophobicity, emulsifying capacity, aggregation and gelation, were found to be sensitive to the 7S/11S ratio (Marcano, Varela, & Fiszman, 2015; Maruyama et al., 2002; Miriani, Keerati-u-rai, Corredig, Iametti, & Bonomi, 2011; Salleh et al., 2002; Xu, Liu, & Zhang, 2015). To be specific, it was reported that 11S mainly contributed to the heat stability, coagulation or gelation of native SPI (Tang, Wu, Chen, & Yang, 2006). The on-set gelation of native SPI and the viscoelasticity of the corresponding gels depended upon the relative ratio of 11S to 7S (Tang et al., 2006), and the emulsifying capacity of 11S differed from 7S due to their different subunits with different portions of extended regions (Maruyama et al., 2002). Furthermore, interactions between two components were also found to play a role in regulating overall functionalities. The existence of 7S, as reported by Utsumi, Damodaran, and Kinsella (1984) and Guo et al. (2012), inhibited the dissociation and aggregation of 11S subunits. The B subunit of 11S and β subunit of 7S underwent interaction under heat treatment (Wang, Li, Jiang, Qi, &

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Zhou, 2014), and the formation of soluble complexes thereafter greatly impacted the gelling properties. Therefore, it is worth distinguishing the individual contribution of each SPI fraction when probing the structure and the related functionalities of SPI under specific processing conditions.

Heat treatment is a common processing method for SPI based products to achieve desired solubility, emulsifying or textural properties (Damodaran, 1988; Iwabuchi, Watanabe, & Yamauchi, 1991). Under heat treatment, protein subunits underwent dissociation or aggregation processes, and the kinetics of both pathways were highly sensitive to medium pH (Sokolova, Kealley, Hanley, Rekas, & Gilbert, 2010). Currently, the combined impacts of heat treatment under defined pH values on the conformation and functional properties of each SPI fraction have not been clearly delineated. Among the few available research articles, it was reported that 7S and 11S showed different aggregation behaviors under heat treatment (60–100 °C) at pH 7.0, where the former formed soluble aggregates while the latter ended up with insoluble aggregates (Guo et al., 2012). During heat denaturation at pH 7.6, the disulfide bridge linking acidic and basic polypeptides of 11S was broken and 11S dissociated partly into the 7S form under heat treatment at pH 3.8 (Lakemond, de Jongh, Hessing, Gruppen, & Voragen, 2000). The combination of pH-shifting (incubated at pH 1.5 followed by refolding at pH 7.0) and mild heat treatment (50 or 60 °C) enforced the conversion of sulphydryl groups into disulfides, thus strengthened the disulfide-mediated aggregation of SPI (Jiang, Xiong, & Chen, 2010). All of these available literatures only revealed changes on limited structural levels. Up to now, the structural responses of β-conglycinin and glycinin under acidic or alkaline incubation condition combined with heat treatment have not been fully clarified. Limited attempts have been made to clarify their multi-level structural changes, in terms of secondary, tertiary structure as well as self-assembled morphology at macroscale.

In this study, we conducted heat treatment (i.e., 80 °C) under acidic (i.e., pH = 2.5) or alkaline (i.e., pH = 8.5) conditions. We are particularly interested in the respective effects of the above-mentioned treatments upon conformational changes of 7S and 11S. In order to obtain detailed information about structural changes in 7S and 11S, samples were subjected to the small-angle X-ray scattering (SAXS) analyses. Tertiary and secondary conformational changes were monitored through tryptophan intrinsic fluorescence spectra and circular dichroism (CD), respectively. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted to detect the extent of protein hydrolysis during heat treatment. Macroscale morphological observation was then performed via atomic force microscopy (AFM). Knowledge obtained in this work will improve our understanding of structure-property relationship of glycinin and β-conglycinin and facilitate their functionality oriented structural modification process.

2. Materials and methods

2.1. Materials

Soy β-conglycinin (7S) and glycinin (11S) were prepared using the methods described previously (Feng et al., 2015; Yuan et al., 2013). The protein contents of 11S and 7S as determined by Kjeldahl method were 93.6% and 92.0%, respectively. Tris-alkali, sodium dodecyl sulphate (SDS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), 2-mercaptoethanol (2-ME), bromophenol blue, Coomassie brilliant blue R-250 were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). PageRuler™ prestained protein ladder (#26619) was purchased from ThermoFisher Scientific, Inc. (Waltham, MA, USA). Milli-Q water was used throughout the experiments.

2.2. Acidic or alkaline incubation combined with heat treatment

Two milligrams per milliliter of 7S and 11S were prepared by dissolving freeze-dried samples into deionized water. The stock solutions of both 7S and 11S were then divided into two sets, respectively. One set of them was adjusted to pH = 2.5 with 1 N HCl, the other set was adjusted to pH = 8.5 with 1 N NaOH. Sodium azide (0.02%, w/v) was added to inhibit microbial growth. For thermal treatment, protein solutions were poured into glass vials with lids and heated at 80 °C for preseted time (1 h or 5 h) in a temperature-controlled bath with temperature deviation of 0.1 °C. After heat treatment, samples were immediately cooled in an ice bath and stored at 4 °C until further use, typically within 2 days. Control groups were held at specific pH values at room temperature (20–25 °C) without heat treatment for 5 h and then stored at 4 °C until further use. Triplicate preparations were made for each sample. Sample abbreviations corresponding to specific treatments were summarized in Table 1.

2.3. Small-angle X-ray scattering (SAXS) measurement

SAXS data were collected at Bio-CAT, 18-ID beam line section, at the Advanced Photon Source, Argonne National Laboratory. Protein samples (2 mg/mL) in respective aqueous solutions were examined. The X-ray wavelength was 1.033 Å, and the experimental setup included a 3.5 m sample-to-detector length camera and another 0.3 m sample-to-detector length camera with the high-sensitivity CCD detector. A quartz capillary flow cell of 1.5 mm diameter was fitted to a brass block. This sample holder was maintained at 25 °C in all experiments. Each 2D scattered intensity distribution profile recorded by detector was integrated into the one-dimensional scattering function I(Q), as a function of the scattering vector, Q, whose amplitude is given by $Q = (4\pi / \lambda) \sin(\theta / 2)$, λ is the wavelength of the X ray beam, θ is the scattering angle. Ten curves were collected for each sample, and their averaged curves were utilized for further analysis. Identical measurement configurations were used for solvent backgrounds and sample solutions for proper background subtraction.

2.4. Small angle X-ray scattering data analysis

Kratky plots ($I(Q) * Q^2$ versus Q) were plotted to distinguish the folded globular protein from their disordered states. The scattering intensity of a folded globular protein has a Gaussian-like shape at small Q range and decays approximately as $1 / Q^4$ at high Q yielding a bell-shaped Kratky plot with a well-defined maximum. For an unfolded protein, the Kratky plot presents a plateau over a specific range of Q, followed by a monotonic increase at large Q region.

Guinier analyses for compact sphere (Guinier & Fournet, 1955) were performed by following equations to obtain the radius of gyration, R_g, and the scattering intensity at zero angle, I(0):

$$I(Q) = I(0) \exp\left(-\frac{R_g^2 Q^2}{3}\right) \quad (1)$$

The P(r) function or pair-distance distribution function (PDDF), which describes the paired-set of all distances between points within the object, was also performed according to Eq. (2) to detect conformational changes within the macromolecular structure and maximum

Table 1
Sample abbreviations corresponding to specific treatments.

Sample name	Protein fraction	pH	Heating time (h)	Sample name	Protein fraction	pH	Heating time (h)
7S-2.5-0h	7S	2.5	0	11S-2.5-0h	11S	2.5	0
7S-2.5-1h	7S	2.5	1	11S-2.5-1h	11S	2.5	1
7S-2.5-5h	7S	2.5	5	11S-2.5-5h	11S	2.5	5
7S-8.5-0h	7S	8.5	0	11S-8.5-0h	11S	8.5	0
7S-8.5-1h	7S	8.5	1	11S-8.5-1h	11S	8.5	1
7S-8.5-5h	7S	8.5	5	11S-8.5-5h	11S	8.5	5

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