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Structure–physicochemical function relationships of 7S globulins (vicilins) from red bean (*Phaseolus angularis*) with different polypeptide constituents

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ABSTRACT

Red bean 7S globulins (vicilins) with different polypeptide constituents or heterogeneity were fractionated using acidic extraction (at 0.5 M NaCl) and anion-exchange column chromatography. The physicochemical and conformational properties, including amino acid composition, net charge and/or surface hydrophobicity (H_0), protein solubility (PS), thermal and emulsifying properties, as well as secondary, tertiary and/or quaternary conformations, were evaluated. There were distinct differences in zeta potential, H_0, DSC characteristics, emulsifying activities and tertiary and/or quaternary conformations among the vicilins with different polypeptide constituents, but the PS and secondary conformation were slightly different. The PS as a function of pH and thermal stability were closely related to their surface charge and/or hydrophobicity. The emulsifying ability and the emulsion stability were closely dependent on the PS and H_0, and even the flexibility in tertiary and/or quaternary conformations. These results suggested good relationships between the physicochemical functions and conformational features of red bean vicilins, which could be of great help for further utilization of legume proteins as a potential functional ingredient.

1. Introduction

During recent decades, there is persistently increased demand for the exploitation of legume proteins for use in food and non-food formulations, due to their good nutritional and functional properties. The functionality may be more important than the nutritional property, when the protein is applied as an ingredient in a food system. Legume storage proteins usually contain 7-8S (vicilin) and 11S (legumin) globulins, and these globulins account for 70–80% of total seed proteins (Utsumi, Matsumura, & Mori, 1997). In general, the functionalities of legume storage proteins are essentially determined by their physicochemical and conformational properties. Thus, the elucidation of the structure–physicochemical functions relationships, or the dependence of the physicochemical and/or functional properties on the structure of these proteins, is still a major existing subject in recent legume protein research. The elucidation and understanding of the structure–function relationships of various legume storage proteins and globulins in particular would lead to their better utilization.

One relatively recognized strategy to carry out this kind of studies is through recombinant or protein engineering techniques. Many physicochemical and other characteristics of recombinant 7-8S globulins (vicilins) are characterized and even compared with those of native vicilins. The most investigated legume vicilins are from soy bean and mung bean (Bernardo et al., 2004; Fukuda, Prak, Fujioka, Maruyama, & Utsumi, 2007; Garcia, Adachi, Tecson-Mendoza, Bernardo, & Utsumi, 2006; Maruyama et al., 1998, 1999, 2002a, 2002b; Mendoza, Adachi, Bernardo, & Utsumi, 2001). In this aspect, it is generally indicated that the presence of N-linked glycans is not essential in the assembly and stable conformation of related vicilins, but it might contribute to their protein solubility, thermal stability, as well as surface hydrophobicity. However, limited information is available for the structure–function relationships of the vicilins, especially those concerning about the conformations. The subunit or polypeptide heterogeneity of might account for this limitation.

Another potential strategy to reveal the structure–function relationships of legume vicilins is through comparison of physicochemical and conformational properties of different purified vicilins with different polypeptide constituents. The purified vicilins with different polypeptide constituents may be from various legume sources, and also from the same variety. To date, this kind of works on the structure–function relationships

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are relatively limited, and need to be investigated in detail. By comparison of some selected physicochemical properties of native Adzuki bean (or red bean) 7S globulin (vicilin), it was found that two purified vicilins with different subunit or polypeptide compositions exhibited different thermal stability and surface hydrophobicity, but the solubility and emulsifying ability were similar (Fukuda et al., 2007). The major limitation, in this regard, is to fractionate and purify vicilin samples with low denaturation in native conformation, and with high polypeptide homogeneity.

In an our recent work, we successfully fractionated and purified several vicilin fractions with various polypeptide constituents from mung bean, using combined acidic extraction at high salt condition and ion-exchange chromatographic technique (Tang & Sun, 2010a). The physicochemical and conformational properties of various vicilin fractions, including amino acid composition, surface charge and hydrophobicity, free sulfhydryl group (SH) and disulfide bond (SS) contents, protein solubility, thermal and emulsifying properties, as well as secondary and tertiary conformations, were evaluated. It was found that these physicochemical and conformational properties varied largely with their polypeptide constituents, and there were close relationships between some physicochemical properties (e.g. emulsifying activities) and conformational characteristics. In another our work, we also characterized and compared the physicochemical and conformational properties of the major fractions of various vicilins from three legume varieties (kidney, red and mung beans), and in particular, found that at pH 7.0 and 9.0, there are close relationships between emulsifying activity index and flexibility in quaternary conformation, and between emulsion stability index and flexibility in tertiary conformation (Tang & Sun, 2010b).

Red bean is a native legume in northeastern part of China. The 7S globulin (vicilin) accounts for about 80% of the total proteins in this bean, while 11S globulin (legumin) makes up only about 10% (Meng & Ma, 2001a). The functional properties including solubility (as a function of pH), emulsifying and foaming properties, thermal property, thermal aggregation and/or gelation and flow property of red bean total globulins have been reported (Meng & Ma, 2001a, 2001b, 2002a, 2002b; Meng, Ching, & Ma, 2002). However, only the thermal stability of purified 7S globulin has been reported (Meng & Ma, 2001a). Furthermore, the structure–function relationship of vicilins from red bean is little mentioned. In this paper, we applied the same fractionation and purification technique to obtain various vicilins with different polypeptide constituents from red bean, as described in our previous work (Tang & Sun, 2010a), and characterized and compared their physicochemical and conformational properties, in order to investigate their potential structure–function relationships.

2. Experimental

2.1. Materials

Red bean (*Phaseolus angularis*) seeds, cultivated in North-East area of China, were purchased from a local supermarket (Guangzhou, China). The seeds were soaked in de-ionized water for 12 h at 4 °C and de-hulled manually. The de-hulled seeds were freeze-dried, ground and defatted by Soxhlet extraction with hexane to produce the defatted flour. 5, 5′-dithio-bis-2-nitrobenzoic acid (DTNB), 2, 4, 6-trinitrophene sulphonic acid (TNBS) and 1, 8-anilinonaphthalenesulfonate (ANS) reagents were purchased from Sigma (St. Louis, MO, USA). Bovine serum albumin (BSA) was obtained from Fitzgerald Industries International Inc. (Concord, MA, USA). All other chemicals used were of analytical or better grade.

2.2. Preparation of acid- and salt- extracted red bean globulins (control)

The acid- and salt- extracted red bean globulins (control) were prepared according to the process described by Hall, McLeester, and Bliss (1977), with slight modifications. This process was applied mainly to isolate vicilin-type globulins from legume seeds. The defatted flour (5.0%, w/v) was dispersed in 0.5 M NaC1 solution containing 0.025 M HCl (pH 3.5). The resultant dispersion was gently stirred at room temperature for 2 h. The slurry was centrifuged (30,000 *g*, 20 min) at 4 °C in a CR22G centrifuge (Hitachi Co., Japan), and the supernatant was diluted with 5-fold volumes of de-ionized water (0–4 °C). Then, the obtained precipitate was collected by centrifugation at 12,000 *g* for 20 min at 4 °C. The pellet was dissolved in 0.5 M NaCl solution, and re-precipitated twice as above. The last obtained precipitate was finally dissolved in 0.5 M NaCl solutions and dialyzed against de-ionized water at 0–4 °C for 48 h, and then lyophilized to produce the crude globulins (control; denoted as RG).

2.3. Fractionation of the globulins by DEAE-Sepharose chromatography

The crude red bean globulins (RG) were further fractionated using DEAE-Sepharose fast flow column chromatography with AKTA Purifier (GE Co. Ltd, USA), according to the process described by Gueguen, Vu, and Schaeffer (1984). Elution was performed using 50 mM phosphate buffer (PBS; pH 7.0) with a gradient of 0–0.5 M NaCl, at a flow rate of 2.5 mL per minute. The eluent was collected at 10 mL per tube. The collected fractions, pooled according to the requirement, were resolved by sodium dodecyl sulfate-polyacryamide gel electrophoresis (SDS-PAGE), and also dialyzed against de-ionized water at 4 °C and further freeze-dried to produce various fractionated globulins (denoted as RG-1~RG-4).

2.4. SDS-PAGE

SDS-PAGE was performed on a discontinuous buffered system according to the method of Laemmli (1970) using 12% separating gel and 4% stacking gel. The gel was stained with 0.25% Coomassie brilliant blue (R-250) in 50% trichloroacetic acid, and destained in methanol–water solution containing 7% (v/v) acetic acid and 40% (v/v) methanol. The tested protein solution samples were prepared by directly mixing the collected fractions with electrophoretic sample buffer (2×), namely 0.25 M Tris–HCl buffer (pH 8.0) containing 2.0% (w/v) SDS, 0.1% (w/v) bromophenol blue, 50% (v/v) glycerol and 10% (v/v) β-mercaptoethanol (2-ME). The freeze-dried protein samples were prepared by dissolving in the electrophoretic sample buffer (1×).

2.5. Amino acid analysis

The powder protein samples were hydrolyzed with 6 N HCl for 24 h at 110 °C in a sealed tube. Amino acid composition was determined using automatic amino acid analyzer (Waters M510, USA), with PICO.TAG column. The determination was carried out at 38 °C, detection wavelength 254 nm and flow rate 1.0 mL per minute. Amino acid composition was reported as g/100 g protein.

2.6. Zeta potential

The zeta potential profiles of various red bean globulins as a function of pH were measured using a Zetasizer Nano ZS (Malvern Instrument Ltd., Malvern, Worcestershire, UK) in combination with a multipurpose autotitrator (model MPT-2, Malvern Instruments,