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ORIGINAL PAPER

A Dynamic Light Scattering Study on the Complex Assembly of Glycinin Soy Globulin in Aqueous Solutions

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Abstract In this work, the complex assembly of one of the major storage proteins in soybean, glycinin, was analyzed using dynamic light scattering, from the hydrodynamic diameter of assembled forms in solution. The protein concentration and temperature were maintained constant at 10^{-1}% w/w and 20 °C, respectively, and the pH was 7.6, 7.0 and 3.0. By analyzing the intensity and volume size distributions, a complex equilibrium between self-assembled forms could be determined. At pH 7.6 and an ionic strength of 0.5 M, where the self-assembly of glycinin has been widely reported in the literature, the DLS technique revealed an equilibrium between different assembled forms, that shifted towards the 11S form. At a lower ionic strength for pH 3.0 or 7.0, the 7S form predominated. The hydrodynamic diameter evolved differently upon heating, depending on pH and ionic strength. For pH 7 ($I = 0.05$) and 7.6 ($I = 0.5$) a significant increase in d_H was observed at a temperatures of 55 and 70 °C, respectively, which were significantly lower than the denaturation onset temperatures as determined by DSC. No changes in d_H nor a transition endotherm were observed at pH 3.

Keywords Globulin · Soy · Dynamic light scattering · Structure

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Abbreviations

DLS	Dynamic light scattering
SBS	Sodium bisulfite
SDS	Sodium dodecyl sulfate

Introduction

In the food industry, vegetable proteins play an important role in the formation and stabilization of various foods in which they are used as an interesting alternative to proteins of animal origin. Soybean proteins are one of the most important vegetable protein sources due to their functional properties and high nutritional value. Soy proteins are employed in food products because of their high nutritional value and their ability to stabilize food dispersions (emulsions, foam, gels, etc.), as well as their ability to improve texture, water absorption and act as a thickening agent.

The storage protein, glycinin, represents one of the two major proteins of soybeans seeds, that account for about 50–90% of the total storage proteins [1, 2]. These two major proteins (glycinin and β-conglycinin) are present in complex molecular assemblies, like many food proteins, as a result of the balance between hydrophobic, electrostatic/ionic and van der Waals interactions. The equilibrium between different supermolecular structures of glycinin and β-conglycinin is modulated by pH and ionic strength [3].

The most accepted model for glycinin is characterized by a hexameric structure organized in a closely packed molecular conformation with a molecular mass of 300–380 kDa and an isoelectric point of 4.6. The quaternary structure consists of six AB subunits of 54–64 kDa, where the A and B polypeptides are linked together by a

disulfide bond, stabilized by electrostatic and hydrophobic interactions. Each subunit has the generalized structure A-SS-B, where A represents an acidic polypeptide (A_{1a}, A_{1b}, A_2, A_3, A_4 and A_5) of 34–44 kDa and B is a basic polypeptide (B_{1a}, B_{1b}, B_2, B_3 and B_4) of about 20 kDa [4–7]. The isoelectric point of the basic polypeptide ranges between 8.0 and 8.5 and of the acidic one between 4.7 and 5.4. A dimer structure of two identical monomers each containing six polypeptides was proposed for the glycinin molecule.

Depending on solubility, pH and ionic strength, glycinin can disassemble into different forms [7S ($(AB)_3$) and/or 3S form (AB)]. It could also occur the assembly of glycinin into the 15S form [dimer of 11S, $(AB)_{12}$] [8]. This behavior of the soy glycinin has been deduced from experimental techniques such as optical rotary dispersion, fast protein liquid chromatography, ultracentrifugation experiments or gel filtration.

Light scattering techniques have been widely applied in the study of the mass, shape and aggregation behavior of proteins in aqueous solutions. Typically, the molecular weight is measured using static light scattering. A more rapid approach is the estimation from dynamic light scattering. In DLS, the time-dependent scattered intensity following from the fluctuations of the local concentrations of the particles is studied, and it is used to estimate the hydrodynamic diameter of a protein [9, 10]. One of the advantages of dynamic light scattering is the possibility of analyzing samples containing broad distributions of species of widely different molecular masses, and detecting very small amounts of higher molecular mass species. Moreover, self-assembled protein structures are not disturbed during the measurements as they are in chromatographic separations or sedimentation studies by the presence of sugars.

DLS has been recently applied to clarify the self-assembly mechanism of proteins involved in Alzheimer's disease [10] which was not revealed by conventional methods.

The impact of different supermolecular assemblies of soy protein on its performance in food products (i.e., its functional properties) has been extensively reported in the literature [11]. Thus, a useful method to characterize the predominant self-assembled structures of soy glycinin protein upon different conditions of pH and ionic strength would be of great advantage to predict the performance of soy proteins in foods.

Generally, the pH of food products ranges from pH 3.0 to 7.0, and the ionic strength varies from 0.02 to 0.2 [11], whereas the majority of soy protein studies have been carried out at pH 7.6 at an ionic strength of 0.5, as it is known that soy proteins are soluble under these conditions.

Thus, the aim of this work was to evaluate the potential of dynamic light scattering analysis to assess the self assembly of soy glycinin under conditions (pH, ionic strength) more representative of food systems in comparison to conditions frequently used in the literature (pH 7.6, $I = 0.5$), corroborating the existence of correlations between the results from DLS with those obtained using different experimental techniques cited in the literature (optical rotary dispersion, fast protein liquid chromatography, ultracentrifugation or gel filtration). Additionally we used dynamic light scattering to probe conformational changes during glycinin heat denaturation.

Materials and Methods

Materials

11S globulin was isolated according to Nagano et al. [12] with slight modifications (Fig. 1), as indicated in Pizones et al. [5]. Proteins were extracted from defatted soybean meal making a slurry with 15-fold volumes of water adjusted to pH 7.5 with 2 N NaOH. This slurry was centrifuged at 10,000 rpm for 15 min. Dry sodium bisulfite was then added to the supernatant (0.98 g SBS/l), the pH was adjusted to 6.4 with 3 N HCl, and the mixture was kept at 4 °C overnight. This preparation was centrifuged (10,000 rpm for 15 min), obtaining two fractions:

(1) The supernatant, which turned out to be the 7S globulin fraction.
(2) The insoluble fraction, which turned out to be the 11S globulin fraction, was washed once again with SBS (0.98 g SBS/l), the pH was adjusted to 6.4 with 3 N HCl, and the mixture was kept at 4 °C overnight. This preparation was centrifuged (10,000 rpm for 15 min). The insoluble fraction of 11S globulin was, finally, dissolved in water at pH 8.0 and lyophilized.

The protein content of 11S fraction, determined by Kjeldahl method, was 97.9% (using a nitrogen to protein conversion factor of 6.25).

Solutions of soy glycinin globulin were prepared freshly at pH 3.0, 7.0 and 7.6 (ionic strength: 0.1, 0.05 and 0.5 M, respectively) at a protein concentration of 10^{-1}% w/w. At pH 3.0 and 7.0, the solubility of soy glycinin was higher than 85% [13]. The condition pH 7.6 and ionic strength 0.5 was included for comparison with the literature studies, because the majority of soy protein studies have been carried out in this experimental condition.

Analytical-grade Tris–HCl [$(CH_2OH)_3CNH_2$/$(CH_2OH)_3CNH_3Cl$] for buffered solutions at pH 7.0 was used as supplied by Sigma (>95%) without further purification, potassium phosphate buffer solutions at pH 7.6 (35 mM potassium phosphate with 0.4 M NaCl) and sodium citrate buffer solutions at pH 3.0 were prepared as supplied by

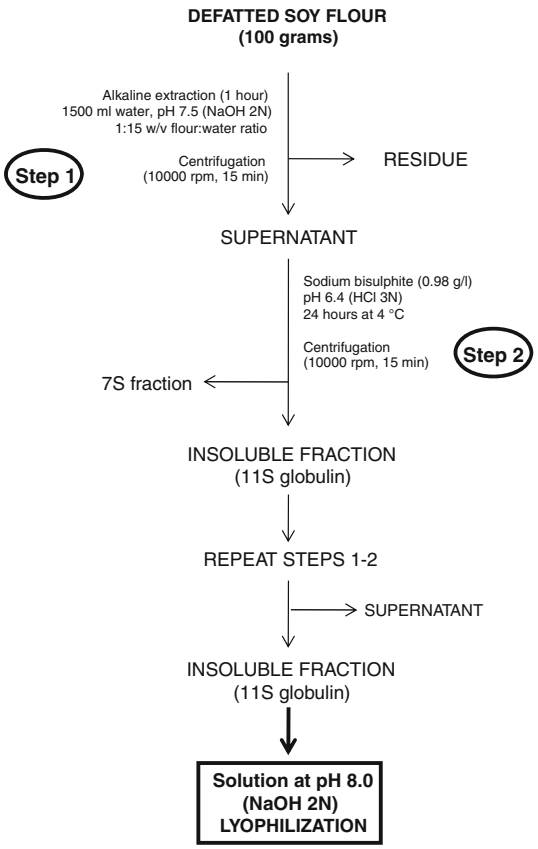

Fig. 1 Experimental protocol for isolation of 11S globulin

Merck (Darmstadt, Germany). Sodium azide was added (0.05% w/w) as an antimicrobial agent. Samples were filtered through a 0.45 and 0.22 μm microfilter (Whatman International Ltd., England) before use, to remove any particles from the deionized water or buffers.

Electrophoresis

Proteins were analyzed by SDS-PAGE-electrophoresis using a Bio-Rad Mini Protean II dual slab cell system (Bio-Rad Laboratories, Hercules, California) in dissociating conditions (2% SDS) according to the procedure of Laemmli [14]. Samples were diluted with distilled water to 1% and then they were dissolved (1:4) in the sample buffer (pH 6.8, 0.5 M Tris–HCl, glycerol with SDS and bromophenol blue (0.05% w/v) and heated at 95 °C for 5 min prior to loading, in the presence or absence of β-mercaptoethanol (Mallinckrodt) as a reducing agent. Lanes were loaded with 40 μg of protein/well.

The separating and stacking gels contained 10 and 4% polyacrylamide, respectively. The running gel consisted of a mixed Tris–HCl (0.4 M) glycine with SDS in distilled water solution to pH 8.3. Electrophoresis was performed at a constant voltage of 200 V and a constant intensity of 500 mA.

The protein bands were stained with a Coomassie brilliant blue solution (0.25%) in 45% methanol, 10% acetic acid for 24 h, and they were fixed in a solution with a mixture 1:1 of methanol–glacial acetic acid (20%) [15].

A broad range of SDS-PAGE molecular mass standard proteins from Bio-Rad was used including Myosin (200 kDa), β-galactosidase (116.25 kDa), phosphorylase b (97.4 kDa), serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21.5 kDa) and lysozyme (14.4 kDa).

The duration of this procedure was approximately 45 min.

Dynamic Light Scattering

The hydrodynamic diameter (d_H) was determined by dynamic light scattering (DLS) using a Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, United Kingdom) which has a measurement range of 0.6 nm to 6 μm according to the manufacturer. The Nano-ZS instrument incorporates noninvasive backscattering (NIBS) optics and measurements were carried out at a fixed scattering angle of 173°.

Samples were contained in a disposable polystyrene cuvette, that was introduced into the apparatus and illuminated with a He–Ne laser beam (633 nm) and five measurements were taken for each system and then the average hydrodynamic diameter was obtained. All of the DLS measurements were performed at 20.0 ± 0.1 °C. Particle size could be reproduced to ± 0.2 nm.

This technique measures the time-dependent fluctuations in the scattering intensity arising from particles undergoing random Brownian motion. Analysis of these intensity fluctuations yields the diffusion coefficient of the particle. The measured diffusion coefficient can be used to calculate d_H using the Stokes–Einstein Eq 1 [16]:

$$d_H = \kappa T / 3\pi\eta D \quad (1)$$

where D is the translational diffusion coefficient; κ is the Boltzmann's constant; T is the absolute temperature and η is the viscosity.

Samples were also heated inside the DLS equipment raising the temperature from 20 to 90 °C at intervals of 5 °C maintaining each temperature for 5 min. The autocorrelation function at each temperature was obtained in this equipment and then the size of particle/aggregate.

Two approaches were used to obtain size information: (i) the cumulative method was used to find the mean average ($Z_{average}$) or the size (diameter) of a particle that corresponded to the mean of the intensity distribution and also to know the polydispersity index as an indicator of the degree of aggregation; (ii) CONTIN was used to analyze the data for the percentile distribution of particle/aggregate

sizes. The size distribution obtained is a plot of the relative intensity of light scattered by particles in various size classes and it is therefore known as an intensity size distribution. Through Mie theory, with the use of the input parameter of sample refractive index, it is possible to convert the intensity size distributions into volume and number distributions [17, 18]. The assay was performed in triplicate.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was used to determine the onset denaturation temperature (T_{onset}) from the intersection with the baseline of the extrapolated leading edge of the heating curve; the peak temperature (T_p) indicating the apparent denaturation temperature, that was determined from the maximum heat flow; and the total calorimetric apparent enthalpy change (ΔH), calculated from the peak area using a straight baseline between the onset and final temperatures of the thermal transition. A DSC 822 Mettler Toledo Calorimeter (Schwerzenbach, Switzerland) was used to analyze the thermal transition of soy glycinin (10% w/w). The instrument was calibrated with indium (156.6 °C), lead (327.5 °C) and zinc (419.6 °C). Thermograms were evaluated using the Mettler Stare program. The thermal parameters were determined by heating 60 µl of the sample in 160-µl capacity pans from 0 to 110 °C at 10 °C/min. An empty pan was used as the reference. The average value of at least two replicates is reported.

Results and Discussion

Electrophoresis

Electrophoresis of glycinin, in Fig. 2 (lane 2) showed two major bands, one at $M_m = 57$ kDa and another at about 30 kDa. The band at 57 kDa corresponded to the AB subunits, because exposing the native glycinin to β-mercaptoethanol, prior to electrophoresis, resulted in the breakdown of this band into two major bands corresponding to acidic and basic polypeptides (lane 3). Acidic polypeptides showed two major bands with a molecular mass at around 42 kDa (A_3) and at 37 kDa (A_{1a}, A_{1b}, A_2 and A_5); basic polypeptides showed bands at approximately 20 kDa (B_{1a}, B_{1b}, B_2, B_3 and B_4 polypeptides) (lane 3), as it was also shown by Mo et al. [19].

The major band of 57 kDa consisted of the principal subunits (AB) resulting from the dissociation of the quaternary structure of soy glycinin under influence of sodium dodecyl sulfate, as it was indicated by Wolf [6]. The following AB complexes are included in this band: $A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a} and A_3B_4. A small contamination with the β polypeptide from the β-conglycinin soy globulin, whose molecular mass is around 54 kDa, can also be observed in native glycinin because after the reductive treatment, it still appeared as a light band around 54 kDa (lane 3).

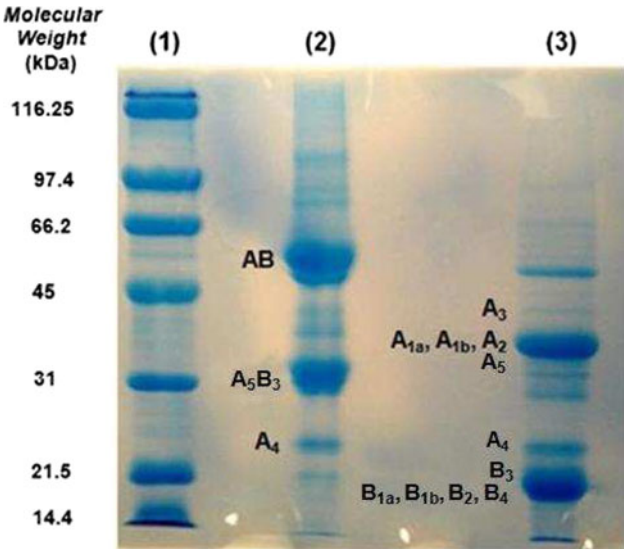

Fig. 2 SDS-PAGE of glycinin before and after reduction with β-mercaptoethanol (*lane 2* and *3*, respectively). *Lane 1* contains molecular mass standards (kDa)

Light bands above 85 kDa were also present in the native glycinin (lane 2) but they practically disappeared with the reductive treatment (lane 3). These bands were assumed to be SS-linked low molecular mass aggregates [20]. The band around 30 kDa in the native glycinin (lane 2) exhibited properties reported for A_5B_3.

A minor band at approximately 24 kDa corresponded to the A_4 acidic polypeptide, which stayed in this position although glycinin was reduced with β-mercaptoethanol; this is just because this polypeptide is apparently non-covalently bound to a basic polypeptide, as it was indicated by Staswick et al. [7].

Glycinin purity was estimated to be higher than 90% with Bio-Rad Molecular Analysis/PC Molecular Imager Software, from Bio-Rad (Bio-Rad Laboratories, Hercules, California).

Estimation of the Hydrodynamic Diameter of Self-Assembled Forms of Soy Glycinin

Soy glycinin adopts different self-assembled forms (3S, 7S, 11S, 15S and >15S) resulting from a rearrangement of the polypeptides within the glycinin complex, modulated by the ionic strength and pH [21]. It is very important to indicate that this 7S form is not the same as the other major

soy protein fraction, the β-conglycinin soy globulin. The behavior of soy glycinin has been determined from experimental techniques such as optical rotary dispersion, fast protein liquid chromatography, ultracentrifugation experiments or gel filtration. Ultracentrifugal investigations of soybean protein in alkaline solutions have been reported by Naismith [22], Wolf and Briggs [23] and Wolf et al. [24]. Naismith [22] found four resolvable fractions corresponding to 2S, 7S, 11S and 15S in ultracentrifugal experiments; Wolf and Briggs [23] and Wolf et al. [24] found the same ultracentrifugal components as Naismith, however, they also found considerable amounts of unresolved material >15S. This unresolvable material consists partly of disulfide polymers of the 7S and 11S proteins [24].

Badley et al. [25] reported that at pH 7.6 and at an ionic strength of 0.5, glycinin was mainly present in a hexameric form with a sedimentation coefficient of 11S, determined from ultracentrifugation experiments. Lowering the ionic strength to 0.01 at pH 7.6 causes glycinin to dissociate from the 11S form mainly into the 7S form [26], believed to be the trimeric form [27]. According to Lakemond et al. [11] at pH 7.6 and an ionic strength of 0.5 or 0.2, glycinin has a sedimentation coefficient of 11S. A fraction with a higher Svedberg coefficient, probably the 15S fraction as described by Wolf and Nelsen [28], was also present at an ionic strength of 0.5. At an ionic strength of 0.03, next to an 11S fraction, a 7S fraction, representing 15–25% of all protein, was observed.

Wolf et al. [24] found that at pH 3.8–2.2 the glycinin complex is present in the 7S and/or 3S form. At pH 3.8 and an ionic strength of 0.5 about half of the glycinin molecules are present in the 7S form, whereas at an ionic strength of 0.2 and 0.03 glycinin is predominantly present in the 7S form [11].

Therefore, glycinin is a freely reversible association–dissociation system containing different self-assembled forms, with varying amounts of them, reflecting the existence of an equilibrium between these that may be summed up as follows (Eq. 2):

$$3S(AB) \leftrightarrow 7S\ \left((AB)_3\right) \leftrightarrow 11S\ \left((AB)_6\right) \leftrightarrow 15S\ \left((AB)_{12}\right) \leftrightarrow >15S \quad (2)$$

The molecular masses of the different assembled forms in Eq. 2, reported in the literature, are shown in Table 1 [8]. Hydrodynamic diameters (d_H) were estimated from the correspondent molecular mass values of the self-assembled forms with the software (version 5.10) of the Zetasizer Nano-ZS instrument involving size (d_H) versus molecular mass calibration curves for globular proteins [29]. Table 1 shows d_H of A–B subunits and the different self-assembled structures of soy glycinin that will further allow the identification of species of glycinin in the DLS approach.

Table 1 Size estimation (d_H) predicted from the literature-reported molecular mass values of glycinin polypeptides and glycinin assembled forms, using the globular protein model in the software of the Zetasizer Nano-ZS instrument

	Molecular mass[a] (kDa)	d_H (nm)
Acidic polypeptide	34–44	5.4–6.0
Basic polypeptide	20	4.3
3S (AB)	58	6.8
7S ($(AB)_3$)	175	10.8
11S ($(AB)_6$)	350	14.6
15S ($(AB)_{12}$)	780	20.5

[a] from Martin et al. [8]

Size Distribution of Glycinin

The majority of studies on soy proteins assembly have been carried out at pH 7.6 (potassium phosphate buffer) and an ionic strength of 0.5 where proteins exhibit maximum solubility. Under these conditions, the 11S form has been described as predominant in ultracentrifugal investigations [25], but 3S, 7S and 15S components have also been found [28, 30].

Under these experimental conditions (pH 7.6 and $I = 0.5$ M) the maximum of the single peak in the intensity size distribution (Fig. 3a) was found at 24.4 nm, but the width of the peak (9–100 nm) indicated a high polydispersity due to the presence of different assembled forms of glycinin. The area under each peak in the DLS measured intensity particle size distribution is proportional to the relative scattering intensity of each particle family. Since the intensity of the scattered light increases with the sixth power of the particle diameter, the scatter signal due to larger particles can dominate the total signal and skew the apparent size distribution towards larger sizes. Thus, conversions of the measured intensity size distributions (Fig. 3a) into volume size distributions (Fig. 3b) were done using Mie theory. The hydrodynamic diameter (d_H) of the single peak, in the volume size distribution, was found between 7 and 40 nm, indicating that particles with a size above 40 nm are not significant in mass. The size of 7S, 11S and 15S forms of glycinin fall within 7 and 40 nm (Table 1), thus the existence of an equilibrium between them is apparent at pH 7.6 ($I = 0.5$). The peak in the volume size distribution at 13.5 nm is close to the expected size of the 11S form (Table 1) thus indicating its predominance. This result agrees with literature data [25, 28, 30]. According to Lakemond et al. [11], at pH 7.6 and an ionic strength of 0.5 or 0.2, glycinin has a sedimentation

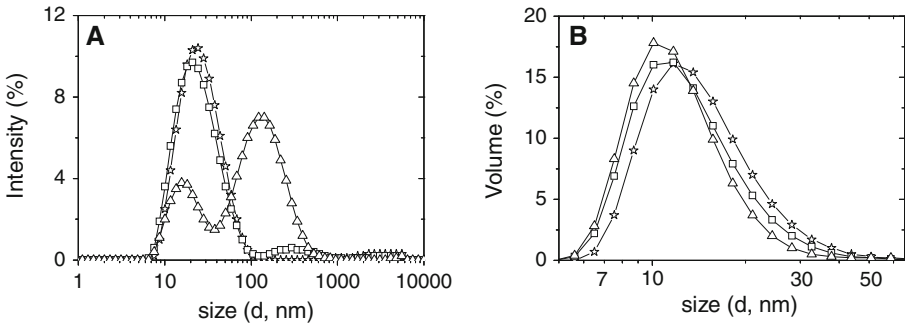

Fig. 3 Intensity (**a**) and volume (**b**) size distribution for glycinin solutions (10^{-1}% w/w) at pH 7.6 (phosphate buffer, $I = 0.5$) (*open stars*); pH 7.0 (Tris–HCl buffer, $I = 0.05$) (*open squares*) and pH 3.0 (citrate buffer, $I = 0.1$) (*open triangles*). Temperature: 20 °C

coefficient of 11S, as estimated by calibration with proteins with a known Svedberg coefficient. A fraction with a higher Svedberg coefficient, probably the 15S fraction as described by Wolf and Nelsen [28], also seems to be present at $I = 0.5$. The volume size distribution (Fig. 3b) skews towards larger sizes indicating a significant presence of 15S or >15S forms. The presence of the 15S form has not been always detected by ultracentrifugal or gel filtration methods. Wolf and Nelsen [28], in an attempt to purify and characterize the 15S globulin of soybeans, a dimer of glycinin, proved the inherent instability of this self-assembled form during gel filtration, dialysis and freeze-drying. Interconversion of 15S into 11S provided additional evidence that the 15S fraction is an aggregate of 11S, and this instability presumably accounts for the inability to achieve a higher degree of purification. Although the 15S protein was slowly converted into the 11S protein, the reverse reaction was not observed. Moreover, it appears that dimerization of the 11S protein of seeds may be a common phenomenon. Mori and Utsumi [31] isolated the 15S fraction of *Vicia faba* L. by sucrose density gradient centrifugation. On recentrifugation of their isolated 15S fraction they observed an appreciable amount of 11S (roughly one-third of the total protein), but apparently they did not investigate the possible dissociation of 15S into 11S protein [28].

Therefore, it is possible that the 15S form, due to its instability, could not be fully assessed by conventional methods such as fast protein liquid chromatography, ultracentrifugation experiments or gel filtration, where it may dissociate into the 11S form. However its existence may be revealed by a non-disturbing method like DLS.

Figure 3a shows the intensity size distribution for soy glycinin at pH 7 ($I = 0.05$). A bimodal distribution is apparent. The main peak for glycinin in buffer solution at pH 7.0 was at a diameter of around of 21 nm and the second low peak was at around 300 nm. The last peak would represent highly associated forms of glycinin. As was indicated by Rackis et al. [30] it appeared that the lowest molecular mass fractions associated into the 15S component were then capable of aggregating into high molecular mass material.

The volume size distribution (Fig. 3b) was monomodal and indicated that particles with sizes higher than 40 nm were not significant in mass. Thus most of the particles have d_H between 6 and 50 nm.

The maximum of the main peak in the volume size distribution (10.1–11.7 nm) (Fig. 3b) may be attributed mainly to the 7S form according to Table 1, reflecting its predominance. However, the 11S form was also significant in mass, but the 15S form was less significant at pH 7.6 ($I = 0.5$).

Comparing the above results it may be concluded that the equilibrium between the assembled forms of glycinin was shifted to less assembled structures at pH 7.0 ($I = 0.05$) as compared to pH 7.6 ($I = 0.5$), which can be mainly attributed to the decrease in ionic strength. At increased ionic strengths, a reduction of the thickness of the electric double layer over charged surfaces or around charged protein molecules occurs resulting in screening of charges, lowering electrostatic repulsion. So, proteins can then approach the surface closer and attractive forces could prevail over electrostatic repulsion [32–34], allowing stabilization of more assembled forms. Lowering the ionic strength to 0.01 at pH 7.6 causes glycinin to dissociate from the 11S form mainly into the 7S form [26, 27]. At $I = 0.03$, next to an 11S fraction, a 7S fraction, representing 15–25% of all protein, could be observed [11].

At pH 3.0 (citrate buffer, 0.1 M) the intensity size distribution was bimodal (Fig. 3a). The value of the maximum of the lower size peak was at 15.7 nm (Fig. 3a), and the maximum of the main higher size peak was around 100 nm. The last one revealed the presence of big aggregates of glycinin at this pH. Nevertheless, these aggregates were negligible in mass as shown by the volume size distribution (Fig. 3b). Most of the particles ranged within 6.0 and 30 nm. The maximum of the main peak in the volume size distribution (10.1–11.7 nm) may be attributed mainly to the 7S form according to Table 1, reflecting its predominance. However, the 11S form was also significant in mass, but the 15S form was not significant at this pH condition. Wolf et al. [24] found that at pH 3.8–2.2 the glycinin complex is present in the 7S and/or 3S form.

According to Rackis et al. [30] glycinin in acid solution (pH 3.0) is a freely reversible association–dissociation system containing three resolvable fractions by ultracentrifugal analysis having values of approximately 3, 7 and 11S. The relative amounts of the fractions are dependent upon pH, ionic strength and type of salt present. Low pH and low ionic strength favor dissociation primarily into the 3S and 7S fractions.

Dissociation of 11S form was suggested to be due to electrostatic repulsion among the subunits. Dissociation into subunits starts at pH 3.75 and reaches a maximum at pH 2.0 [21]. However, due to the ionic strength of citrate buffer (0.1 M) it is possible that dissociation to the lower assembled form (3S) would be restricted in the present work.

Influence of heating on glycinin self assembly

It has been reported that the heat induced unfolding/denaturation of proteins can be followed by the measurement of their d_H by dynamic light scattering [35–38]. Real time dissociation and aggregation of 11S globulin at pH 7.6 ($I = 0.5$) upon heating is shown in Fig. 4. The predominant peak in the intensity size distribution (Fig. 4a) shifted to lower sizes upon heating as well as decreasing in height (from 20 to 50 °C). At 70 °C, a change in the intensity took place, while at 80 °C, a transition from a single peak to two peaks occurred, indicating the onset of aggregation. The volume size distribution in Fig. 4b shows that these aggregates are negligible in mass and the predominant 11S form showed a gradual shift to lower sizes by increasing temperature from 20 to 50 °C indicating the increasing predominance of 7S and 3S forms. Thus at 80 °C, as the protein began to denature, the instrument picked up the first traces of aggregation, despite representing a negligible fraction in mass, as shown in the volume size distribution (Fig. 4b). Fig. 5a exhibits the entire temperature profile and shows the average hydrodynamic diameter ($Z_{average}$) which started to increase over 70 °C. The onset denaturation temperature for 11S globulin at pH 7.6 ($I = 0.5$), as determined by DSC, was 84 °C and the peak temperature (T_p) was 98 °C. Lakemond et al. [11] determined a T_{onset} at 90 °C and a T_p at 94 °C with a DSC microcalorimeter.

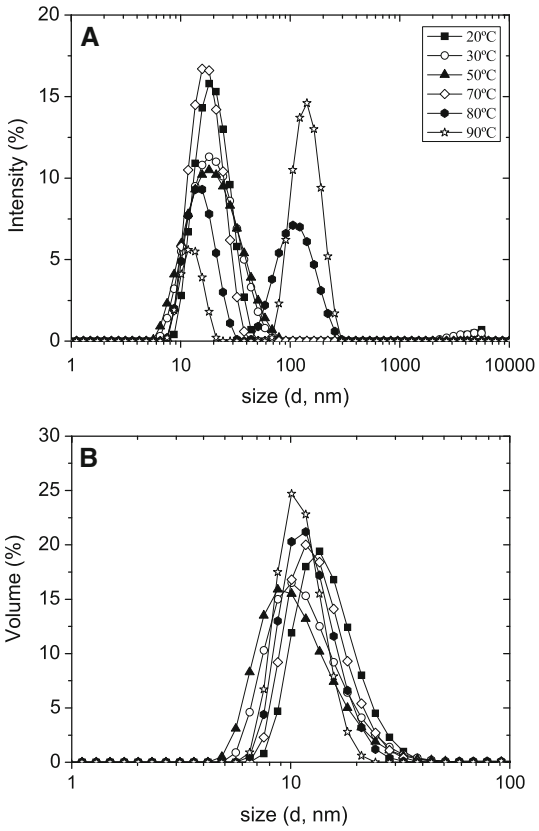

Fig. 4 Intensity (**a**) and volume (**b**) size distribution for glycinin solutions (10^{-1}% w/w) at pH 7.6 (phosphate buffer, $I = 0.5$). Temperature: (*filled squares*) 20 °C, (*open circles*) 30 °C, (*filled triangles*) 50 °C, (*open rhombus*) 70 °C, (*filled circles*) 80 °C and (*open stars*) 90 °C

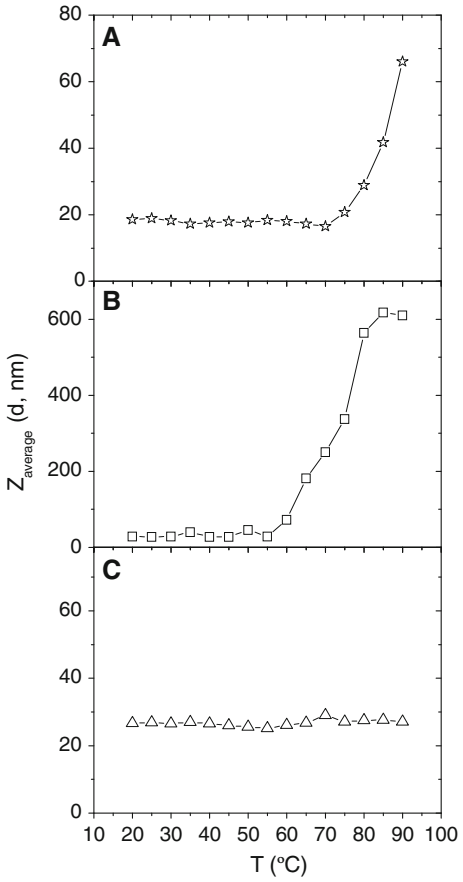

Fig. 5 $Z_{average}$ as a function of temperature for glycinin solutions (10^{-1}% w/w): **a** (*open stars*) pH 7.6, **b** (*open squares*) pH 7.0, **c** (*open triangle*) pH 3.0

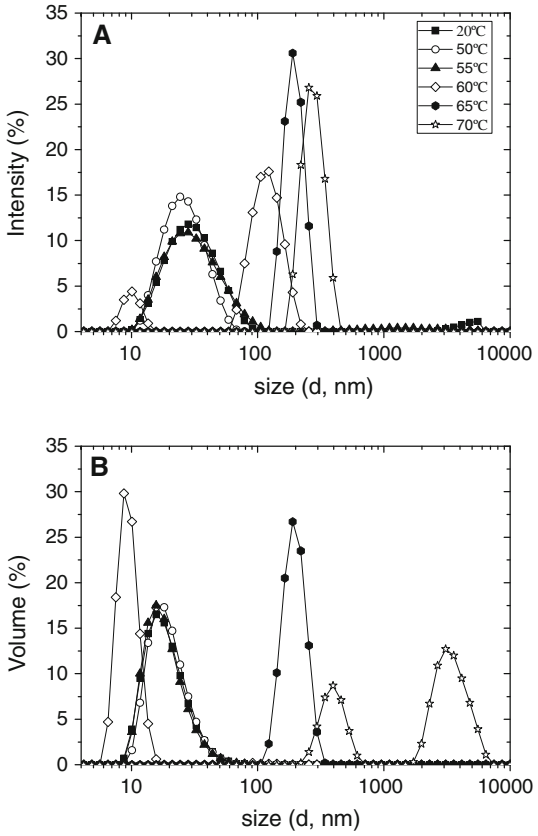

Fig. 6 Intensity (**a**) and volume (**b**) size distribution for glycinin solutions (10^{-1}% w/w) at pH 7.0 (Tris–HCl buffer, $I = 0.05$). Temperature: (*filled squares*) 20 °C, (*open circles*) 50 °C, (*filled triangles*) 55 °C, (*open rhombus*) 60 °C, (*filled hexagons*) 65 °C and (*open stars*) 70 °C

The increase in d_H was observed before the onset of protein denaturation. Hence, the DLS could detect changes in quaternary structure (self-assembly) or the onset of protein aggregation upon heating soy glycinin by determining changes in d_H.

At pH 7.0 ($I = 0.05$) the protein structure was almost stable up to 55 °C (Fig. 6). At 60 °C a transition from a single peak to two peaks occurred (Fig. 6a). Nevertheless, the peak at around 100 nm was not significant in mass as shown in Fig. 6b. The area of the lower size peak at 60 °C strongly decreased and shifted to lower sizes indicating the increasing predominance of 7S and 3S forms. Thus the oligomeric dissociation of glycinin at this experimental condition is not gradual and occurs simultaneously with some protein aggregation. Fig. 5b shows the entire temperature profile and the average hydrodynamic diameter ($Z_{average}$) which started to increase over 55 °C. The onset denaturation temperature for 11S globulin at pH 7.0 ($I = 0.05$) as determined by DSC was 84 °C and T_p was 92 °C, which resulted lower than values at higher ionic strength (pH 7.6). Renkema et al. [39] determined the T_{onset} and T_p of 11S globulin at pH 7 by micro-DSC at 80 and 88 °C respectively. As reported by Lakemond et al. [11], the transition temperatures generally decrease when the ionic strength is lowered.

At pH 3.0 ($I = 0.1$), neither the intensity size distribution (data not shown) nor the $Z_{average}$ (Fig. 5c) were modified by increasing temperature, indicating that glycinin is stable at this pH. Secondary α-helical conformation (determined by circular dichroism) was also found to be maintained upon heating 11S globulin at pH 3.0 [40]. Nevertheless, these results contradict the results from DSC showing no endothermic transition at this pH [40, 41] which has been generally attributed to total denaturation of the globulin at pH 3.0. Therefore it may be concluded that the absence of an endothermic transition in DSC experiments is due to the strong stability to dissociation/aggregation/denaturation of 11S globulin at pH 3.0 and low ionic strength.

Conclusions

The dynamic light scattering technique proved to be a good tool to characterize the different self-assembled forms of soybean glycinin in solution under different experimental conditions of pH and ionic strength, relevant to food products.

The dynamic light scattering approach revealed the existence of equilibrium between several self-assembled forms. DLS could also detect the shift of such equilibrium upon changes in pH or ionic strength. In addition, DLS data revealed that there was a significant increase in d_H even before the protein became denatured. Hence, by determining changes in d_H, DLS could detect changes in quaternary structure (self-assembly) or the onset of protein aggregation upon heating soy glycinin.

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