

Self-assembly of Globulin Nanofibrils at Various Ionic Strength: Microstructure and Gels

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Abstract The effects of various ionic strengths and concentrations on the structure and gel properties of rice bran globulin(RBG) at pH 2.0 were investigated using atomic force microscopy(AFM) and rheometer. AFM showed the assembling RBG fibrils translated from strand beads to branch clustered, when electrostatic repulsive forces attenuated gradually with increasing ionic strength. The percolation model $G' \sim (C-C_p)^n$ calculate the theoretical critical concentration formed RBG gels at various ionic strengths(0-500 mM), which decreased from 15.17 ± 0.63 to 2.26 ± 0.27 wt%. RBG gels according to the actual complexion had been drawn by computer, the color and state of cubes were simulated. A granular dense structure and intensive mesh like gel network was observed, and a more homogenous structure were formed at low ionic strength.

Keywords: percolation model, rice bran globulin, ionic strength, electrostatic screening, critical protein concentrations

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1. Introduction

Proteins assembly is of great interesting materials in food processing and bionanotechnology, due to they can modify structural properties of the system [1]. Proteins assembly as well as ordered linear aggregates, has attracted more attention in recent years, because of their unique characteristics. These linear protein fibrils have good potential as materials of thickening or gelling [2,3,4,5].

Above denatured temperature heating plays a trigger for unfolding protein molecule, and cause exposure of inner hydrophobic region. The linear fibrils are primarily governed by the equilibrium between attractive forces (mainly hydrophobic bonds) among thermally unfolded molecules and repulsive forces [6,7]. Electrostatic repulsive forces attenuated gradually at the high ionic strength, and the preponderant intermolecular hydrophobic forces induced clustered aggregates formation. Contrarily, at low ionic strength and pH, electrostatic repulsive force predominate the aggregates morphology, and formed linear fibrils [5]. At low pH and low ionic strength, the gels have many advantages, e.g., transparent or semitransparent, more elastics, and low gel critical concentration [8]. It is may imply that the length of linear fibrils seems to less than the wavelength of visible light, leading to the gels are transparent [9].

The electrostatic screening affected the structural and physicochemical properties of protein assembly. The effects of electrostatic interactions on the critical protein concentration (Cp) of gel can be investigated by a certain

model, and a decreasing Cp with increasing in a range of ionic strength. Furthermore, theoretical models contribute to better understanding the special gels structure.

Rice is one of the earliest cultivated crop species, and human have adapted to various components of rice. There is a growing demand for the use of less allergenic rice protein or rice bran protein as a functional ingredient in food products [10]. Thus, the RBG fibrils structural details redounded to understanding the gel properties, and facilitate the application of rice protein-based ingredients.

2. Materials & Methods

Fresh rice bran was purchased from The Rice Research Institute of Guangdong Academy of Agricultural Sciences, Guangzhou, China. Other chemicals applied in the work were of chemical grade. All solutions were prepared with deionized water.

2.1. Preparation of RBG Fibrils

The defatted rice bran were sieved through 60 mesh screen, and then first fractionated by extracting with distilled water and centrifuging. The residue from this step was extracted with 0.5 M NaCl to recover the globulin fraction (supernatant). The 2.0 wt% rice bran globulin (RBG) solutions at various ionic strength (0, 100, 200, 300, 400, and 500 mM) were set to pH 2.0 respectively, and then the solutions were put into closed test tubes. These tubes were heated at 90°C for 2 h, rapid cooling to ice water, and formed RBG fibrils.

2.2. Samples Preparation for AFM

RBG samples dispersions were diluted to 25 $\mu\text{g}/\text{mL}$, and a droplet spread on a freshly cleaved mica disk and dried overnight. AFM images were captured by using tapping mode. A Dimension 3000 microscope (Digital Instruments-Veeco, Santa Barbara, CA) were manipulated by a Nanoscope IIIa controller. 3-5 images for each samples.

2.3. Percolation Model for Gel System

The percolation model as one of theoretical models for elasticity of gels, it is based on percolation concept [11]. The percolation model $G' \sim (C - C_p)^n$, where G' is elastic moduli, C is the native protein concentration, C_p is the critical threshold concentration, n is a scaling exponent. We determined C_p and n as a function of ionic strength by using a fitting procedure.

The G' was measured using a parallel stainless steel plates ($d=40$ mm) were carried out in a rheometer (AR 1500ex, US). About 1.5 mL RBG samples (2.0-20.0 wt%) were placed into parallel plates, and seal with mineral oil. The heating-cooling cycle program was set at 90°C for 2 h and cooling to 25°C at -2°C/min.

2.4. Turbidity Experiments

The appearance of RBG samples gel were exhibit a series of test tubes were prepared with a 3.0 ml at pH 2.0, various RBG concentrations (2.0-20.0 wt%), and ionic

strength (0-500 mM). The test tubes with hermetic lids and heated in water bath at 90 °C for 2h. The test bottles were taken out immediately and rapid cooling to 4 °C. The RBG gels are approximately simulated the actual gels complexation by computer.

3. Results and Discussion

The RBG could form linear fibrillar aggregates at pH 2.0 and 90°C. Figure 1 exhibit AFM height images of RBG fibrils in 100 mM and 500 mM NaCl concentrations. The two NaCl concentrations were chosen due to a significant difference in the fibrils morphology. In low NaCl concentrations (0 or 100 mM), linear fibrils exhibited strand beaded clearly, the contour length of fibrils were about 100-200 nm and width around 20 nm (Figure 1 A). However, at 200 mM or above NaCl concentrations, the fibrils became branch clustered gradually, and even the entanglement of the fibrils had more prominent in 500 mM NaCl solutions. The contour length of the fibrils increased with increasing ionic strength, and 400-500 nm fibrils universally present in the higher ionic strength. The interesting phenomenon attribute to electrostatic repulsive forces attenuate gradually with increasing NaCl concentrations. The result elucidated the preponderant intermolecular hydrophobic forces induced clustered aggregates formation.

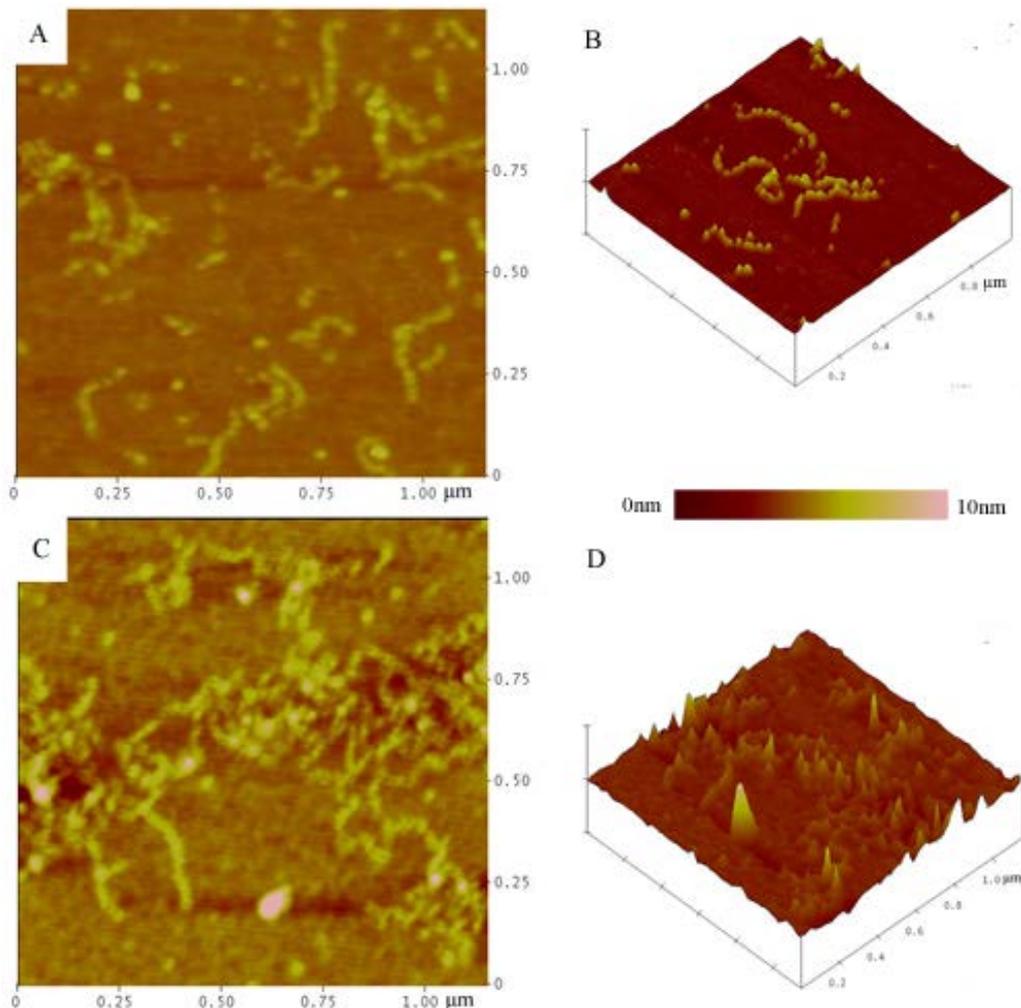


Figure 1. Tapping mode AFM photographs of RBG fibrils at various ionic strengths (A, B) 100 mM, (C, D) 500 mM

Three dimensional AFM topographies (Figure 1B and D) exhibit a structure of worm-like fibrils, and with a rolling periodicity between 20 nm and 30 nm. Similarly, RBG fibrils periodic changes occurs in soy β -conglycinin and BSA [12,13]. It implies that the RBG fibrils may be also have the similar helical structure. The periodicity of helical structure may be affected by magnitude of electrostatic forces and intramolecular interaction.

The G' as a power law according to percolation model $G' \sim (C-C_p)^n$, which can uses $(G')^{1/n}$ versus C and extrapolates these plots to $(G')^{1/n} = 0$. The modulus G' has an explicit positive correlation to the gels strengths. G' of gels can be obtained in a linear regime through strain sweep, where the value is independent of the strain amplitude (data not shown). Figure 2A shows the G' of various ionic strengths and protein concentrations. A point of interest noteworthy is that the values of G' in same ionic strength seem to exhibit a strong linear correlation in logarithmic axes.

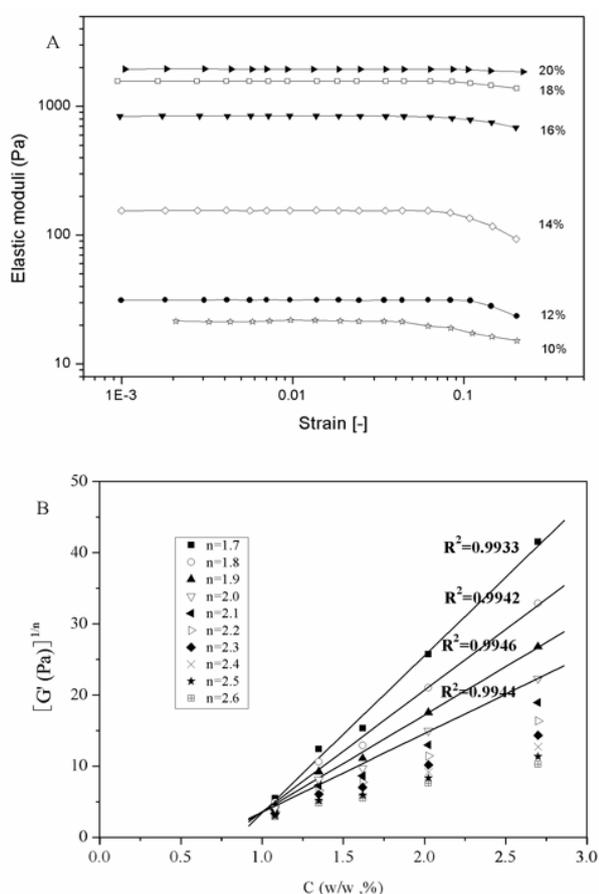


Figure 2. G' as a function of percolation model for RBG gels at pH 2.0. (A) G' versus protein concentration for RBG gels and various ionic strength. (B) $[G' \text{ (Pa)}]^{1/n}$ as a function of concentration, for n from 1.7 to 4.0

The fitting procedure relies on the independency of the exponent n . The fit linear must all intersect the concentration axis at the same value. When assumed n value is close to the actual value, the R value is more closed to 1. R is a coefficient of determination for measuring goodness of fit. These points have goodness of fit with line (Figure 2B). The ranges of n were assumed from 1.5 and 4.0. In the fitting procedure, $(G')^{1/n}$ was extrapolated to zero should yield the same C_p . Afterwards,

these points will be on a straight line when n is close to the highest value. The theoretical value of C_p could calculate from percolation model $G' \sim (C-C_p)^n$. In this study, the RBG gels, the average exponent $n=1.96 \pm 0.19$, and the theoretical C_p can be obtained from the slope of straight line. The n value of gel at the range of percolation model, which assumes an isotropic force between nearest neighbors on the gel network, and suggests form a homogenous gel network [14,15]. The result expound the exponent n is a function for the gel structure. The average C_p and n values at various ionic strengths are also list in Table 1, respectively. RBG gel C_p successively decreased from 15.17 ± 0.63 to 2.26 ± 0.27 wt% when ionic strength increased from 0 to 500 mM.

Table 1. The values of critical percolation concentration and exponent n at various ionic strengths

Ionic strength (M)	n	C_p
0	1.83 ± 0.16	15.33 ± 0.41
100	2.10 ± 0.13	10.52 ± 0.18
200	2.07 ± 0.22	6.60 ± 0.33
300	1.96 ± 0.13	5.65 ± 0.27
400	2.08 ± 0.34	4.38 ± 0.35
500	1.99 ± 0.25	2.81 ± 0.23

Error bars represent standard deviations of average values.

The effects of simulated diagram on the actual complexation of gels, the color and state of cubes were drawn in Figure 3. The phase diagram of viscous solutions or gels, as a behavior of special ionic strength (0–500 mM) and protein concentration (2.0–20.0 wt%). NaCl plays an important role in the gel color and transparency attribute to the structural difference of aggregates. The structure forming RBG gels are growing in size with increasing ionic strength, and results in attenuate to electrostatic repulsion. The results of phase diagram indicate the C_p of RBG gels are also highly depended upon ionic strength. Generally, the heat-induced gel, which can be defines as a fine-stranded or particulate gel. Fine-stranded gels are transparent or semitransparent, whereas the particle gels are turbid or opaque. At low pH and ionic strength, RBG gel reveals a translucent white, which gradually changed from transparent to turbid, even opaque, as the protein concentration or ionic strength increased.

Hydrophobic interactions, electrostatic repulsive force, and covalent bonds are all affected the appearance of gel network [16]. Figure 4(A,B) shows AFM images of RBG gels (16.0 wt%) at ionic strength of 100 mM and 500 mM respectively. A droplet of samples in critical conditions was drip on the surface of mica without diluted, and then air dried over the night. In order to highlight microscopic images, the AFM images were adjusted hue for network. The images show a granular dense mesh like structure, and intensive cross-linked network was observed. Apparent gel mesh-like structure attribute to molecular interaction at higher electrostatic screening. The above mentioned processes for RBG fibrils formed gels at various ionic strengths are illustrated in Figure 4C.

4. Conclusion

Electrostatic screening affected dramatically the formation and characteristics of RBG fibrils. The fibrils reveal linear beads structure and periodic changes at lower ionic strength, whereas the voluble fibrils is prominent in higher ionic strength. The structure and morphology of fibrils at the initial period plays a key role in gels type. The percolation model $G' \sim (C-C_p)^n$ fit for RBG gels, and can calculate RBG gels critical concentrations. The present in fibrils can modify textural properties of RBG gels, which are suitable to application as a natural nanoscale gelling material.

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