Digest Journal of Nanomaterials and Biostructures Vol. 9, No. 3, July - September 2014, p. 951 - 957

EFFECT OF IONIC STRENGTH ON FORMATION AND MICROSTRUCTURE OF SELF-ASSEMBLY GLOBULIN NANOFIBRILS AND GELS

Y. H. ZHANG[a], L.H. HUANG[b*], S. X. TING[a], S.Y. WANG[a], Y. S. LIN[a]
[a]*Guangdong Key Lab. of Agricultural Product processing, Sericulture & Agri-food Research Institute GAAS, Guangzhou 510610, P.R. China.*
[b]*Department of Food, Guangzhou City Polytechnic, 510405, P.R. China.*

Self-assembly of globulin fibrils as nanoscale material exhibited unique properties. To gain insight into the effect of various electrostatic screening on the aggregates and gels structure of rice bran globulin(RBG), a kinetic process of rice bran globulin(RBG) turbidity and gelation were performed. The absorbance of solutions and required time of gelation are all reveal significant differences at various ionic strengths. AFM images showed the assembling morphology of RBG fibrils, a string beads transformed to branched cluster, when electrostatic repulsive forces attenuated gradually with increasing ionic strength. The gel strength increased with increasing ionic strength and protein concentration. A simulated phase diagram had been drawn, according to the actual state and color of solution and gel. A dense granular structure of gel network was observed, and a more intensive structure present at low ionic strength. A hypothetical pathway for possible RBG gel formation was promoted understanding of nano-structure.

(Received April 21, 2014; Accepted July 11, 2014)

Keywords: Rice bran globulin, Nanoscale fibrils, Ionic strength, Microstructure, Electrostatic screening

1. Introduction

The widely used plant protein is increasing rapidly attribute to health benefits, low cost, good functionalities, and textural properties. Self-assembly of globulin nanofibrils may be as interesting materials in food processing and bionanotechnology. They have exhibited some unique characteristics, when added them to protein solutions could modify physicochemical properties of the system [1,2]. Assembling proteins can be defined as fibrils, and has attracted more attention in recent decade years. These linear protein fibrils have good potential as nanomaterials for application in beverage, dessert, and even wound healing [3-6].

The formation of fibrils and other polypeptide aggregates highly depends on the equilibrium of attractive forces (mainly hydrophobic bonds) among thermally unfolded molecules and repulsive forces [7,8]. Generally, almost all salts examined can promote aggregation strongly. At low ionic strength and pH, electrostatic repulsive force predominate the aggregates, and induced linear fibrils formation [5]. The electrostatic repulsive forces attenuated gradually at the high ionic strength, and the preponderant intermolecular hydrophobic forces induced clustered aggregates formation. The gels in low pH and low ionic strength have many advantages, e.g., semitransparent, more elastics, and low gel critical concentration [9]. The transparent gel imply that the linear fibrils length seems to less than the wavelength of visible light, resulting in the gels have a highly light-transmissive [10,11].

Evidence has shown that the aggregates are highly sensitive to the salt ions. The electrostatic screening could affect the structure and morphology of protein assembly. Even though

*Corresponding author: hlh@gcp.edu.cn

the ionic strengths are commonly played a pivotal role in the formation of fibrils and other polypeptide aggregates, the various electrostatic screening effects on aggregates structure and gel properties worth to further study. There is a growing demand for the use rice protein or rice bran protein as a functional ingredient attribute to less allergenic and workability in clinical Nutrition products[12]. Thus, the RBG fibrils and gels structural details redounded to facilitate the application of protein-based ingredients.

2. Materials and 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 working solutions were prepared with deionized water.

2. 1 Preparation of RBG Fibrils

The 2.0 wt% RBG solutions at various ionic strength (0, 100, 200, 300, 400, and 500 mM) were set to pH 2.0, and then the solutions were put into closed test tubes. These tubes were heated at 90℃ for 2 h, and rapid cooling to ice water for further experiments.

2.2 The kinetics of turbidity

The turbidity of RBG dispersions were examined by a UV-vis spectrometer at wavelength of 400 nm. The solutions of 0.8 wt% RBG in 75% ethanol at 0, 100, 200, 300, 400, and 500 mM NaCl and set to pH 2.0. After a fully stirred, the RBG dispersions was sealed in a 4.0 ml tube and thermostated at 50℃ for heating 120 min. Every 5 min the absorbance of the solutions were measured. The ionic strengths of the added salts in the solutions were calculated by the equation: $I=1/2\sum^{n}{}_{,i=1}c_i z_i^2$, where c_i is the molar concentration of NaCl, z_i is the charge number of the ion.

Time required for gelation of the RBG dispersions were measured by observing the appearance of test tubes. The solutions of 4.0% RBG at various ionic strength(0-500 mM), and kept these solutions at 4℃, 10℃, 30℃, and 50℃, the gelation state was recommend.

2.3 AFM micrograph analysis

RBG samples solutions were diluted to 25 μg/mL, and a droplet spread on a freshly cleaved mica disk, 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 sample.

2.4 Dynamic oscillatory measurements

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 85℃ for 2 h and cooling to 25℃ at -2℃/min. Oscillatory measurements were performed after the cooling step. A strain sweep has been carried out at stress and frequency of 0.1 Pa and 0.1 Hz, respectively.

2.5 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 ^{o}C for 2h. The test bottles were taken out immediately and rapid cooling to 4 ^{o}C. The RBG gels are approximately simulated the actual gels complexion by computer.

3. Results and discussion

The fibrils formation is associated with viscosity increase, and form a similar gel network[13]. The absorbance of turbidity can be can be monitored by a turbidity-time plot. Fig. 1 shows the turbidity kinetics of RBG solutions at various ionic strengths. Sigmoidal curves with lag, growth, and plateau phases are observed. The plateau value of absorbance as the final turbidity was record. Interestingly, the turbidity exhibits a concave curve with increasing ionic strength. The lowest turbidity value is present in 100 mM NaCl concentration. Under the concentration range of NaCl 300 mM, the turbidity of solutions is clearly lower than without NaCl solutions. This result in the formation of more transparent solutions, may be due to the linear fibrils is smaller than the wavelength of visible light[14,15]. The turbidity values of RBG solutions rise again with increasing ionic strength because of the linear fibrils gradually become loose clusters of short fibrils.

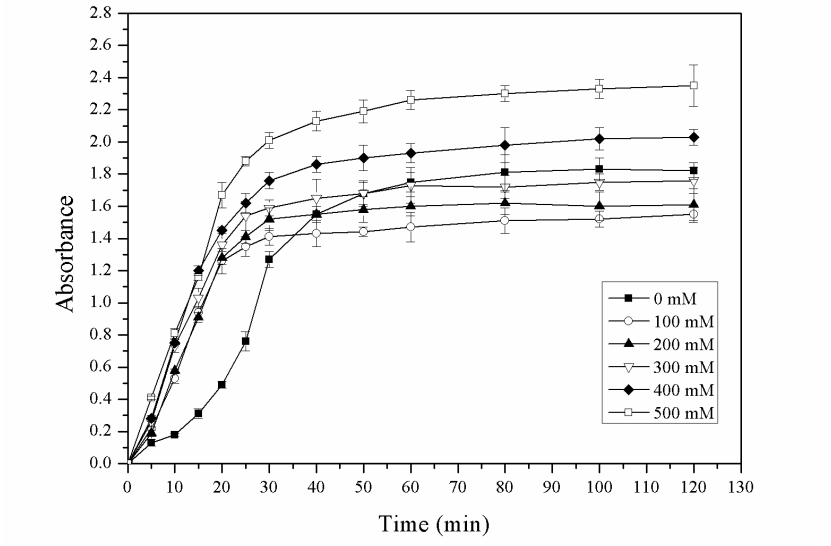

Fig.1. The kinetics of RBG turbidity at various NaCl concentrations.

The present NaCl attenuate the electrostatic repulsion, and greatly affect the kinetics of the gelation formation[16]. Table 1 shows time required for gelation. 4.0 wt% RBG in 75% ethanol solution, at 0 mM, 100 mM, 200 mM, 300 mM, 400 mM, and 500 mM NaCl to investigate the time required for the gelation. At lower temperatures, it is difficult to form a gel without NaCl. No gelation was observed in the solutions at 0 mM NaCl at least 10 hours. At the concentration range of NaCl between 100 mM and 500 mM, clear gels were observed though the time required for the gelation ranged from less than two min to 260 min. However, the formed gel was weak and unstable. These results show that the NaCl can promote RBG gelation occurs, and the rate of formed gelation is dependent upon the NaCl concentration as well as the temperature.

Table 1 Time required for gelation of 4.0% RBG in 90% ethanol at various ionic strengths

Ionic strength(mM)	Time required for gelation 4℃	10℃	30℃	50℃
0	No gel	No gel	No gel	No gel
100	No gel	200 min	170 min	120 min
200	260 min	180 min	120 min	70 min
300	120 min	50 min	30 min	8 min
400	30 min	12 min	8±1 min	5 min
500	8 min	4±1 min	2±0.5 min	2 min

The data in the table are presented as mean±standard deviation.

Fig. 2 shows AFM height images of RBG fibrils in 100 mM and 500 mM NaCl concentrations. The two NaCl concentrations had been chosen due to significant difference in the morphological details. At low NaCl concentrations(0 or 100 mM), the fibrils exhibited string beads clearly, the contour length of fibrils were about 100-200 nm and width around 20 nm(Fig. 2 A). However, at 200 mM or above NaCl concentrations, the fibrils gradually transformed to branched cluster, and even the convolute fibrils entanglement had more prominent in 500 mM NaCl concentration. The contour length of the fibrils increased with increasing NaCl concentration, and 400-500 nm fibrils universally observed in the higher ionic strength. The interesting phenomenon attribute to electrostatic repulsive forces attenuated gradually with increasing NaCl concentrations. The results elucidated the preponderant intermolecular hydrophobic forces induced clustered aggregates formation at lower electrostatic repulsion.

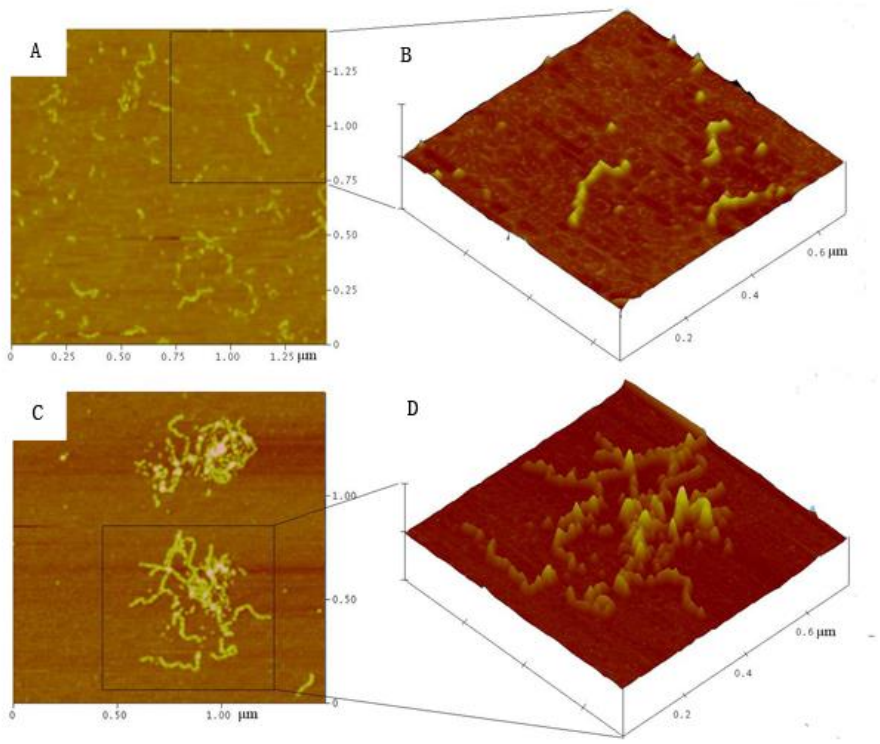

Fig.2. Tapping mode AFM micrographs of RBG fibrils at various NaCl concentrations (A, B) 100 mM (C, D) 500 mM.

Three dimensional AFM micrographs(Fig. 2B and D) exhibit a worm-like structure, and the length of each knobble around 30 nm or more. The fibrils structure may be affected by magnitude of electrostatic forces and intramolecular interaction. Similarly, the periodic knobble occurs in soy β-conglycinin and BSA, the proteins have a helical structure[17,18]. It is implies that the RBG fibrils may be have the similar helical structure.

The experiments were performed at temperature 90℃, in the beginning, G″ might greater than G', after a crossover, where G' > G″, gels are formed (data not show). G' increased drastically in the cooling stage, the final gel strength is dependent upon the pH and protein concentration. Fig. 3 shows the G' versus protein concentration fro the RBG gel of pH2.0 at various ionic strengths. The G' value is obtained in the linear regime, where G' as a function of strain. The gel strength appears to increased with increasing NaCl concentration at the same RBG concentration. An important point is a slope of the linear regression curves that depend on the concentration range can be observed in Figure 3. The relation G' versus concentration have good fitting with the fractal model, which due to highly regression coefficients[19]. The results suggesting that the mesostructure of RBG gels at various ionic strengths can be explained explicitly. The RBG gels are becoming more compact as the electrostatic repulsion decreases. The clustered aggregates can promote form a stronger three-dimensional network of gels than that of fibrils[20,21].

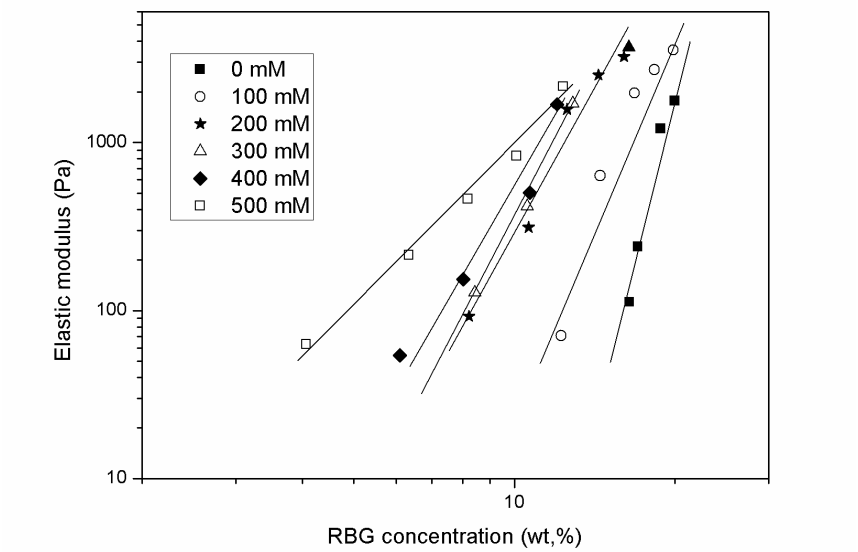

Fig. 3. G' versus protein concentration for RBG gel at various NaCl concentrations.

The effects of various NaCl concentrations on the actual states of gels can be exhibited by a stimulant illustration. The state and color of cubes represent the actual RBG gel cases(Fig. 4). The stimulant illustration of viscous solutions or gels, as a behavior of special ionic strength (0-500 mM) and protein concentration (2.0-20.0 wt%). The results indicate the critical concentration of RBG gels are also highly depended upon ionic strength. Generally, the heat-induced gel, can be defines as a fine-stranded or particulate gel. At low pH and ionic strength, RBG gel reveals a translucent light white color, suggesting it is belong to a fine gel. However, the gel color gradually changed from transparent to turbid, even opaque, as the protein concentration or ionic strength increased. The phenomena imply that an increase in NaCl concentration leads to linear fibrils become clustered aggregates gradually.

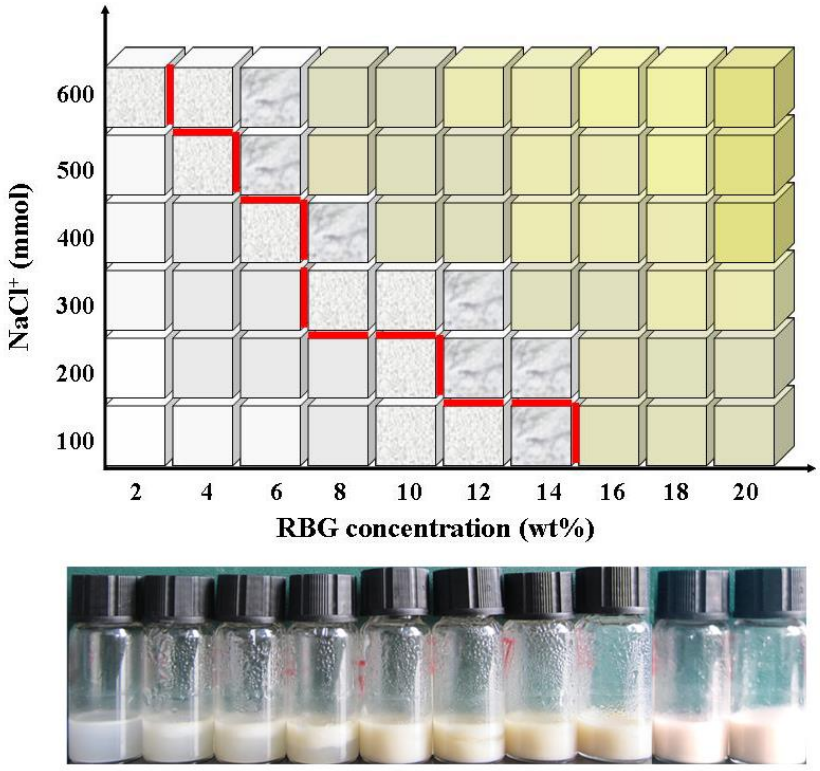

Fig. 4. Macroscopic simulated diagram exhibit the actual state and color of RBG gels. The left side of the red line is liquid, the right side is gel.

Hydrophobic interactions, electrostatic repulsive force, and covalent bonds are all affected the appearance of gel network[22]. Generally, most of the gels mesostructure were observed by scanning electron microscopy. Fig. 5 shows AFM images of RBG gels at ionic strength of 100 mM and 500 mM respectively. A droplet of samples in critical states was drip on the surface of mica without any dilution, and then air dried over the night. It is easy to observe the interaction and arrangement of protein molecule in natural state. A granular dense mesh like structure, and intensive cross-linked network was observed. Apparent mesh-like structure attribute to molecular interaction at higher electrostatic screening.

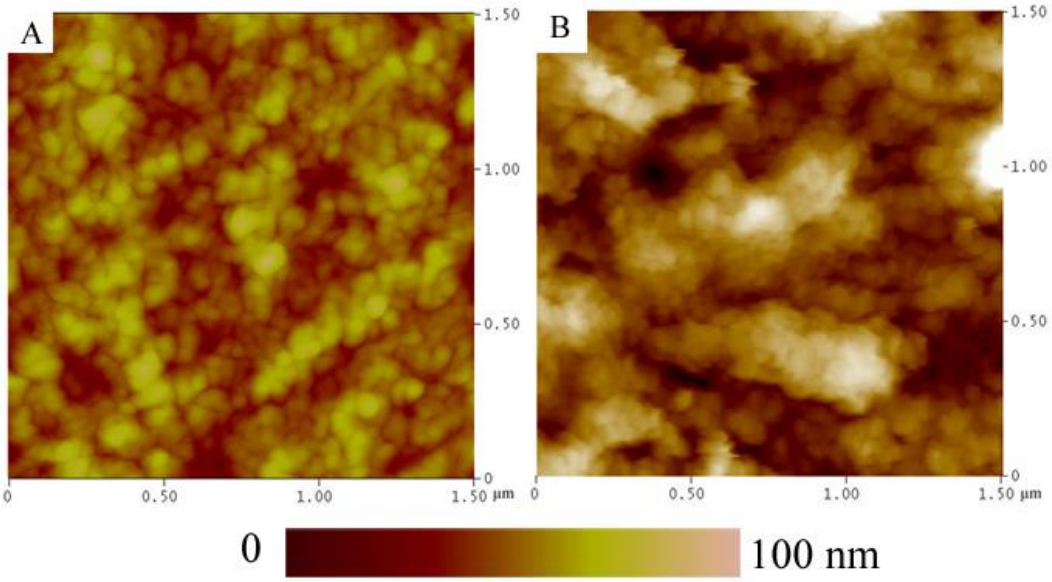

Fig. 5. AFM images of RBG gels were adjusted hue for structure.

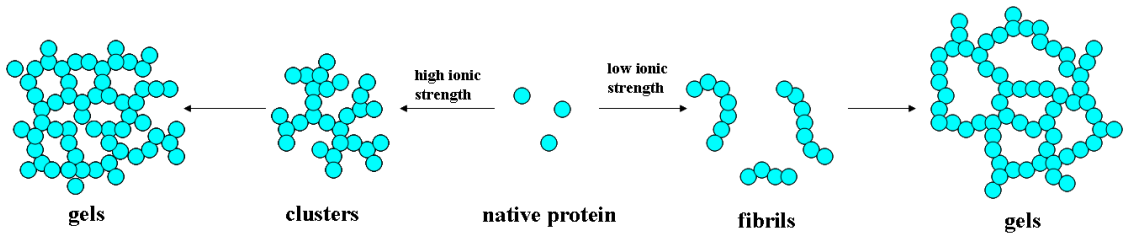

Fig. 6. Schematic illustration of gels formed process at lower(A, 100 mM)and higher(B, 500 mM) ionic strengths.

On the basis of the results, a hypothetical formation pathway of RBG gels at various ionic strengths is illustrated in Fig. 6. The activation of native RBG monomers by heat denaturation at low pH, and followed assemble formation of fibrils and clusters at low and high ionic strengths respectively. These aggregates lead to form various three-dimensional structures, and ultimately affect the type of gel.

4. Conclusions

The Possible effects of NaCl concentration on earlier processes of form fibrils and clustered aggregates in this model system have been investigated, and obtain more insights into about the gels structures and common mechanism. The structure and morphology of fibrils at the

initial period plays a key role in the type of gels. The electrostatic screening affected dramatically the formation and profiles of RBG fibrils. The fibrils revealed linear string beads and periodic changes at lower ionic strength, whereas the clustered fibrils are prominent in higher ionic strength. The various structural fibrils lead to changes of gels textural properties, which can be applied as a natural nanoscale material.

Acknowledgements

This work is part of the research projects of National Science Foundation of China (NSFC, 31301603), Guangdong Natural Science Foundation (S2013040013718), The agriculture Ministry 948 Program of China (2011-G8-2-5), and Guangdong agricultural Program(2012A020602097).

References

[1] R. V. Lisa, V. Michele, M. D. Christopher, P. J. K. ACS Nano**, 7**, 10443 (2013).
[2] C. Akkermans, A.J. Goot, P. Venem, E. Linden, R. M. Boom, A. Cynthia, J. G. Atze, V. Paul, L. Erik, M.B. Remko. Food Hydrocolloid. **22**, 1315 (2008).
[3] M. Alaeiyan, A. Bahrami, M. R. Farahani, Dig. J. Nanomater. Bios. **6**, 143 (2011).
[4] C. H. Tang, Y. H. Zhang, Q. B. Wen, Q. R. Huang. J. Agr. Food Chem. **58**, 8061 (2010).
[5] C. Veerman, H. Ruis, L. M. C Sagis, E. Linden. Biomacromolecules **3**, 869 (2002).
[6] E. M. Ashley, S. Abhishek, G. Y. Yaroslava. Mater. Sci. Eng. C **32**, 2583 (2012).
[7] F. Satoru, M. Fumiko, Y. Yasushige, J. Mol. Biol. **331**, 21 (2003).
[8] A. Farahnaky, H. Askari, M. Majzoobi, G. Mesbahi, J. Food Eng. **100**, 294 (2010).
[9] B. D. Matthew, H. S. Kenneth, R. N. Rajesh. Chem. Rev. **108**, 4935 (2008).
[10] M. Weijers, F. Velde, A. Stijnman, A. Pijpekamp, R. W. Visschers, Food Hydrocolloid. **20**, 146 (2006).
[11] E. van der Linden, M. C.S. Leonard. Langmuir **17**, 5821 (2001).
[12] G. H. Lang, K. Yukari, O. K. Mayumi, K. Kazumi, Biosci. Biotech. Bioch. **77**, 126 (2013).
[13] K. Klement, K. Wieligmann, J. Meinhard, P. Hortschansky, W. Richter, and M. Fändrich, J. Mol. Biol. **373**, 1321 (2007).
[14] M. Weijers, L.M.C. Sagis, C. Veerman, B. Sperber, E. van der Linden, Food Hydrocolloid. **16**, 269 (2002).
[15] N. A. Luben, V. Renko, I. Hans, P. M. M. Carlo, Biomacromolecules 4, 1614 (2003).
[16] S. Fujiwara, F. Matsumoto, Y. Yonezawa, J. Mol. Biol. **331**, 21 (2003).
[17] C. H. Tang, S. S. Wang, Q. R. Huang, Food Res. Int. **46**, 229 (2012).
[18] C. Veerman, L. M. C. Sagis, J. Heck, E. van der Linden, Int. J. of Bio. Macromol. **31**, 139 (2003).
[19] Y. H. Zhang, C. H Tang, Q. B. Wen, X. Q. Yang, L. Li, and W. L. Deng. Food Hydrocolloid. **24**, 266 (2010).
[20] M. Nakano, J. R. Shen, K. Kamino, Biomacromolecules **8**, 1830 (2007).
[21] P. Moritz, H. Ulf, J. M. Daniel, Nano Lett. **13**, 5585 (2013).
[22] S. Ikeda, V. Morris, Biomacromolecules **3**, 382 (2002).