CONTROL OF THE GELATION PROCESS OF SILK FIBROIN SOLUTION

by

Yu LIU[a] and Ying ZHANG[b]*

[a] National Engineering Laboratory for Modern Silk, College of Textile and Clothing Engineering, Soochow University, Suzhou, China
[b] Inorganic Materials Department, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou, China

In the present study, silk fibroin solution was controlled through a simple shearing to accomplish the steady and rapid gelation process and the conformational transition. Antheraea yamamai silk fibroin was formed into hydrogels quicker than Bombyx mori silk fibroin on the same condition. Comparing with Bombyx mori silk fibroin, the rapid gelation of Antheraea yamamai silk fibroin was concerned with its alternate polyalanine-containing units which are tended to form the α-helix structures spontaneously. The entropic cost during the conformational transition to β-sheet is less than that from random coil to β-sheet.
Key words: *silk fibroin, gelation, shearing rate*

Introduction

Hydrogels, both synthetic and natural, are a class of highly hydrated polymeric materials, which maintain a distinct 3-D porous structure with mechanical and structural properties similar to many natural tissues and extra-cellular matrices [1]. As an important form of biomaterial, hydrogels have been of interest for tissue engineering and drug delivery [2].

Silk fibroin (SF) is natural structural proteins excreted from domestic (*Bombyx mori*) and wild silkworms. The *Bombyx mori* silk fibroin (BSF) has been widely used in the textile industry and tissue engineering scaffolds due to its impressive mechanical properties, biocompatibility and biodegradation properties [3]. As one of the species of wild silkworms, *Antheraea yamamai* silk fibroin (ASF) is characterized by more ala, arg and his and less gly than BSF. It also contains the tripeptide sequence arg-gly-asp (RGD), which is known to be the receptor of cell integrins and to mediate special interactions between mammalian cells [4]. Based on these features, interest has arisen in the use of ASF as an advanced biomedical application material. It is possible for ASF to be used as tissue engineering scaffolds and bioactive controlled release carriers if the sol-gel transformation can be completed rapidly under mild conditions.

It is generally believed that silk fibroin (SF) in aqueous solution undergoes self-assembly into a *β*-sheet structure and forms hydrogels *in vitro*. However, a very long time may be required for spontaneous gelation of SF, which limited its clinical application. SF hydrogels can also be produced by physical stimulants through hydrophobic/hydrophilic interactions [5], such as low pH, high temperatures, high ionic strength or ultrasonication *in vitro* [6], but harsh conditions may alter cell function and affect cell viability potentially [7]. Sever-

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* Corresponding author; e-mail: yingzhang@suda.edu.cn

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al chemical additives also were used to shorten the gelation time of SF, but the resultant hydrogels may suffer from the inherent toxicity of the cross-linking additives [8]. In present work, a simple physical method for control of SF gelation process was developed by shearing under mild condition, avoiding the complications and inherent toxicity caused by some unpurified or remained cross-linking additives to cells or cytokines. The experimental results showed that the shearing rate plays an indispensable and crucial role in sol-gel transformation process of SF, and gelation rate of the ASF was quicker than that of BSF with the shearing rate increased.

Experimental analysis

Figure 1(a) shows the relationship between SF gelation time and shearing rate at room temperature. With the increase of shearing rates, the gelation time of ASF decreased sharply. The sol-gel transformation of the ASF solution was completed within 20 hours by shearing for 90 minutes with the increasing of shearing rates. The gelation time of BSF was not obviously decreased initially, but decreased clearly when the shearing rate above 300 rpm. The results showed that the gelation rate of ASF was distinctly faster than that of BSF under the same conditions. In addition, the shearing influenced on the gelation rate of ASF more than that of BSF markedly, and could promote the completion of ASF sol-gel transition in one day. Figure 1(b) shows the relationship between viscosity of SF solution and shearing rates at room temperature. A yield stress existed in the beginning movement of SF solution. After the shearing stress was greater than the yield stress, the SF solution began to flow along the direction of the force. With the shearing rates grew, more and more SF molecules flew along the direction of shearing, resulting in the viscosity gradually reduce. The downward trend of viscosity performed significantly with the increasing of shearing rates, especially for ASF solution. Because the entangled polymer chains were stretched in the process of shearing, so the molecular orientation increased and viscosity decreased.
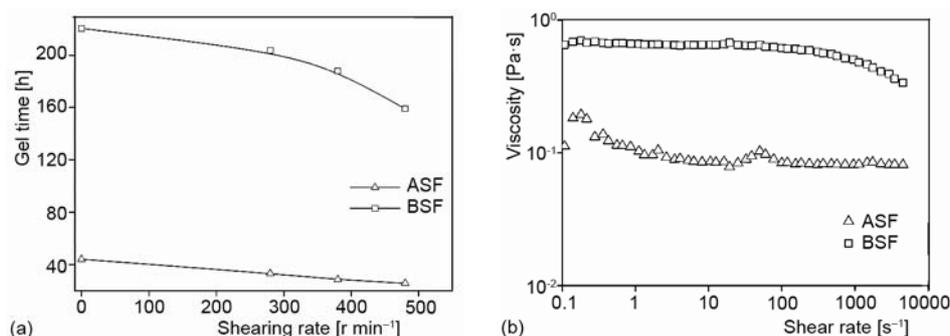

Figure 1. Effects of shear rate on the gelation time (a) and viscosity (b) of SF solution

Theoretical analysis

In the previous reports [9], the secondary structure content in initial ASF solution was quite different from that of BSF. The secondary structure of a native protein has close relations with its primary structure. It depends on the amino acid sequence and the solution environment in which the protein is dissolved [10]. The significant difference of molecular conformation between the initial ASF and BSF aqueous solutions is due to the primary structures. The heavy chain sequence of BSF is characterized by the 94% coverage of gly residues [11].

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However, the ASF is the ala-rich residues protein. The ala is uncharged and its R-groups are small, so it is inclined to form α-helix in solution, but the gly-rich region is basically in a random coil state [12]. It is possible for the significant differences of the molecular conformation between ASF and BSF in the initial solution, particularly for the α-helix content of ASF was higher than that of BSF. The thermodynamic state of the β-sheet conformation is more stable than that of the random coil and α-helix in SF protein. The conformational transition from random coil and α-helix to the β-sheet structure is needed to overcome the energy barrier and form thoroughly stretched peptide chains. The presence of the β-carbon in ala and other amino acids induces a large decrease in the conformational entropy of the unfolded state, so gly has the larger conformational entropy than ala [13]. The entropy is the mainly driving force for the hydrophobic interaction assembly [14]. The conformational entropic cost during the processes of transformation from random coil to β-sheet is larger than that from α-helix to β-sheet in SF protein. The results of this study have shown that stirring can accelerate the gelation of SF, especially for ASF. The ASF gelation could be controlled within one day. This was because stirring caused the flowing shearing force to promote the stretching of the random coil and α-helix chain segments, allowing the reformation of hydrogen bonds and formation of the β-sheet structure. The primary structures of BSF and ASF have significant differences, so the tendency to forming the β-sheet is in favor of ASF. The α-helix content in initial ASF solution was much greater than that of BSF under the same conditions [15], and the conformational entropic cost during the transition from α-helix to β-sheet is less than that from random coil to β-sheet, leading to that the sol-gel transformation of ASF was more rapid than that of BSF under the same conditions.

Conclusions

The results show that gelation rate of the SF solution can be controlled by changing shearing rate. ASF gels could be prepared quickly within one day by stirring silk fibroin solutions. Comparing with BSF, the rapid gelation of ASF through shearing was concerned with its alternate polyalanine-containing units (motifs) which are tended to form the α-helix structures spontaneously, which entropic cost during the conformational transition to β-sheet is less than that from random coil to β-sheet.

Acknowledgments

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