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Control of Gel – Sol Transition of Silk Fibroin by Metal Ions

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In silk glands of the silkworm, *Bombyx mori,* the gel – sol transition of silk fibroin occurs. At the anterior part in middle division (MA) of silk gland, the silk fibroin undergoes gel – sol transition. In order to elucidate the mechanism of this transition, the quantity of metal ions in each part of silk glands was measured by using an element analyzer. It was found that in MA region the amount of Ca^{2+} ions decreased and K^+ ions increased. We carried out dynamic viscoelastic measurements of silk fibroin aqueous solution (0.214 wt%) with various $[Ca^{2+}]$. The result indicates that in the low calcium concentration region (0 – 0.5 mM) storage modulus G' and loss modulus G'' of silk fibroin solution increased with increase in $[Ca^{2+}]$. Furthermore, values of G' and G'' were almost independent of angular frequency ω. This experimental result means that some elastic network exists in silk fibroin solution and calcium ions stabilizes this network structure. It could be concluded that physical cross-links are formed by calcium ion and the gel – sol transition is induced by metal ions.
Key words: silk fibroin, gel, transition, calcium, potassium

1. INTRODUCTION

Silkworms produce silk fiber which is a fine and lustrous filament. The liquid silk from silkworms is a highly viscous aqueous solution of two separate proteins, fibroin and sericin. Recently, various studies have been performed concerning about the physical structures and mechanism of conformational changes, fiber spinning processes of silk fibroin by using various methods[1-4]. The silk glands of the silkworm, *Bombyx Mori,* are pair of tubes. Each gland is divided into three divisions – posterior (P), middle (M) and anterior (A) – and a spinneret region (Fig. 1). Silk fibroin molecules are synthesized in the P division of the silk gland. In P division, silk fibroin is in a weak gel state. Fibroin molecules move forward into middle (M) division. The M division is further divided into three parts: posterior part in M division (MP), middle part in M division (MM) and anterior part in M division (MA). In this M division, the second silk protein, sericin, is synthesized and accumulates as a layer. Silk fibroin is surrounded by sericin and these two kinds of protein move together without mixing into anterior (A) division. In MP and MM region, silk fibroin is stored as a gel state. In MA region, silk fibroin undergoes a gel – sol transition and after that liquid state silk flows in narrow duct whose diameter is about 50 μm to spinneret. In this study, we aim to elucidate the mechanism of gel – sol transition of silk fibroin.

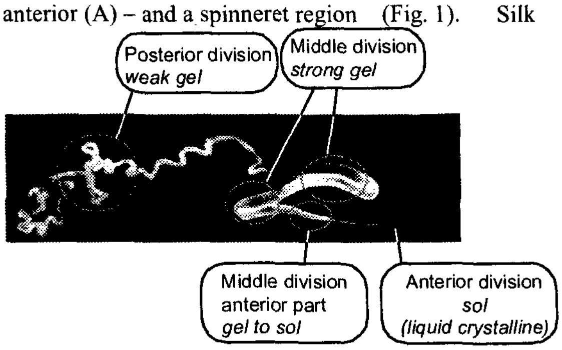

Figure 1 Photo micrograph of the silk gland of silkworm, *Bombyx mori.*

2. EXPERIMENTAL

A weak gel state silk fibroin solution was obtained from each division of silk glands of full-growth larvae of domestic silkworm, *B. Mori,* one or two days before spinning. The thin membrane of cells surrounding the gel was taken out, and the gel was immersed in distilled water for 6 hours to remove sericin. Thereafter, the gel was immersed in distilled water again, then the silk fibroin molecules gradually dissolved in water during 1 or 2 days in the refrigerator at 5 °C. The obtained silk fibroin solution was filtered.

The inorganic constitutions of fibroin in each division of silk glands were measured by using an element analyzer (JEOL JSX-3201). The ash of silk gland and liquid silk was treated by heat at 550 °C for 24 hours. Be-filtered Rh X-ray tube was used and the voltage of Rh tube was 30 kV. The measurement was performed in vacuum for 600 s for each samples.

The rheological experiments were performed using a rheomter (HAAKE Rheometer, RS1). 60mm diameter parallel plate was used. The gap length was 1mm and applied stress was 0.2 – 1.0 Pa which was under the yield stress of samples. Storage modulus G' and loss modulus G'' of samples were measured in the range of angular frequency 0.035 – 62.8 rad s^{-1} at 25 °C. The concentration of silk fibroin solution was 0.214 wt% and calcium ion was 0, 0.5, 5 and 50 mM prepared by adding calcium chloride.

3. RESULT AND DISCUSSION

The inorganic constitutions in middle division of silk gland are shown in Fig. 2. It was found that in MM and MP region the constitution of Ca^{2+} ion is highest (about 70 wt%) and K^{+} ion is 12 – 14 wt% against total inorganic elements. On the other hand, in MA region

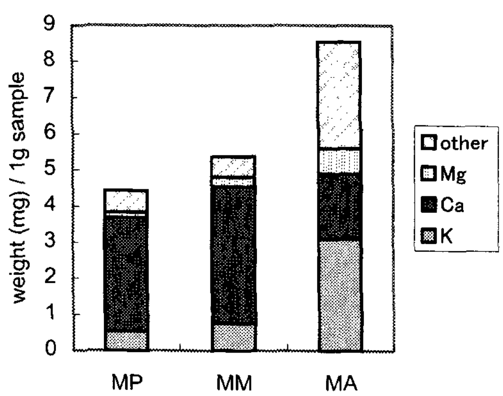

Figure 2. Inorganic elements in middle division of silk gland.

the highest inorganic constitution is K^{+} ion ; 37 wt% and Ca^{2+} ion ; 21 wt%. More over the quantity of total inorganic elements is increasing from 5.37 mg / 1 g in MM to 8.54 mg / 1 g in MA. From this result, in MA region the concentration of Ca^{2+} ion decreases and K^{+} ion increases compared with those in MM and MP regions. This inorganic constitution change suggests that Ca^{2+} ions play a role of cross-linker to form gel state and much amount of K^{+} ions deform this cross-linking.

In order to confirm this idea we carried out dynamic viscoelastic measurements of silk fibroin aqueous solution (0.214 wt%) with various [Ca^{2+}]. The result was shown in Fig. 3. The values of storage modulus G' of no-calcium silk fibroin solution are a few times larger than those of loss modulus G'' and both values are almost independent of angular frequency ω. This

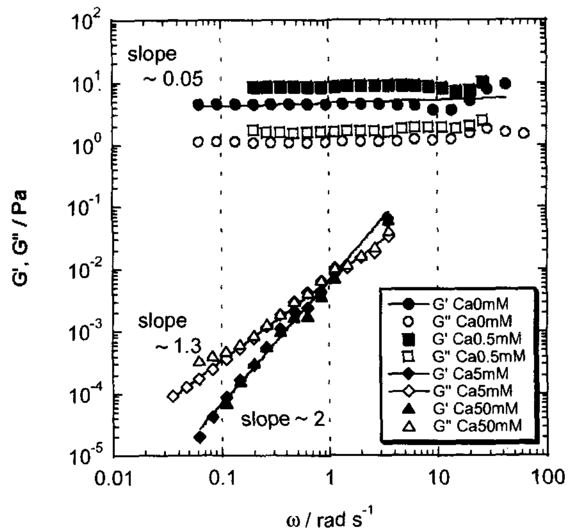

Figure 3. Frequency dependence of G' and G'' for silk fibroin solutions with various [Ca^{2+}].

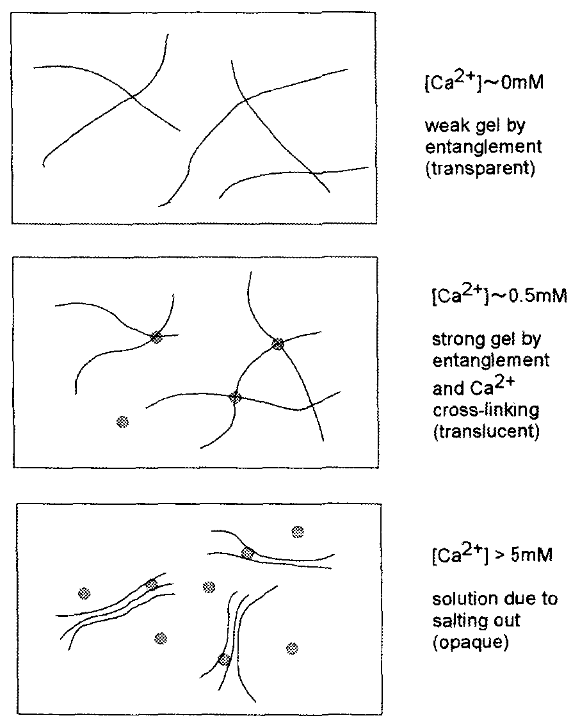

Figure 4. Schematic diagrams of silk fibroin gel and solution with various [Ca^{2+}].

means that some elastic network exists in silk fibroin solution. This network structure would be come from an entanglement. Furthermore, values of G' and G'' of silk fibroin solution with 0.5 mM [Ca^{2+}] are about two times larger than those of no-calcium system. This experimental result indicates that calcium ions stabilize silk fibroin network in the [Ca^{2+}] range 0 ~ 0.5 mM . On the other hand, in much higher calcium concentration region ([Ca^{2+}] > 5.0 mM), the values of G' and G'' depend on ω and the slope is 2.0 and 1.3, respectively. From this rheological behavior, the silk fibroin solution with high calcium concentration flows like a fluid. This would be come from a salting out effect because this high calcium concentration solution is translucent or opaque in appearance though low calcium concentration solution is transparent. Fig.4 indicates schematic diagrams of silk fibroin gel and solution with various [Ca^{2+}].

From these experimental results, physical cross-links would be formed by ionic bonding between divalent metal ions Ca^{2+} and COO^- groups of the amino acid residues in silk fibroin. As shown in Fig. 2, in MA region the quantity of total inorganic elements is increasing, especially in K^+ ion, therefore a salting-out may occur in MA. It could be concluded that a decrease in Ca^{2+} ion and an increase in total inorganic constitution in MA region lead to gel – sol transition of silk fibroin.

4. CONCLUSION

In MP and MM division of silk gland, silk fibroin molecules are stored as a gel state by physical cross-linking due to calcium ions, which plays as a cross-linker. In MA division, calcium ions are exchanged by much amount of potassium ions, then silk fibroin molecules would experience gel – sol transition. The silk worm, *B. Mori*, controls gel - sol transition skillfully *in vivo* by metal ions.

5. REFERENCES

[1] J. Magoshi, Y. Magoshi, M. A. Becker, S. Nakamura, *Polymeric Materials Encyclopedia*, Joseph C. Salamone, Ed., **1(A-B)**, 667-679 (1996).
[2] K. S. Hossain, N. Nemoto, J. Magoshi, *Langmuir* **15**, 4114-4119 (1999).
[3] S. Inoue, J. Magoshi, T. Tanaka, Y. Magoshi, M. A. Becker, *J. Polym. Science: Part B: Polymer Physics*, **38**, 1436-1439 (2000).
[4] K. S. Hossain, N. Nemoto, J. Magoshi, *NIHON REOROJI GAKKAISHI*, **27**, 129-130 (1999).

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