

Effect of Salts on Gelation Time of Silk Sericin Solution

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Abstract

Sericin undergoes gelation by the structural transition from random coil to β -sheet transition. In the present study, the gelation time of sericin solution was investigated in the presence of NaCl, KCl and CaCl₂. The addition of salts delayed the gelation time, and CaCl₂ had the most pronounced effect, which delayed about 8 h at maximum. The gelation time increased with the concentration of salt. The transition of secondary structure of sericin was retarded in the presence of salt. The effect of salts on the gelation time of sericin might be due to the solvation effect of relevant cation

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Introduction

There were various efforts to use sericin in polymeric fields (Kundu *et al.*, 2008; Nishida *et al.*, 2011). However, sericin has some drawbacks that hinder the use in the field. One of the problems is that sericin cannot be prepared in high concentration. It does not mean that it is impossible to prepare a highly concentrated sericin solution. The problem rises after the preparation of sericin solution because highly concentrated sericin solution undergoes gelation very fast. On our experience, the gelation took place within 1 h when the concentration exceeds 5% (w/v). Once gel is formed, further fabrication of sericin would be difficult, and therefore, the concentration of sericin solution should be kept low. The low concentration of sericin solution causes another problem that it may not satisfy a proper viscosity for some fabrication method (Oh *et al.*, 2007)

The aim of present study is to find a way to delay the gelation time of sericin solution. It would be enough to maintain the sol state before fabrication is finished. We added NaCl, KCl and CaCl₂ to the sericin solution because these salts have been known to alter the gelation time of silk fibroin (Kim *et al.*, 2004). The gelation time was measured after the addition of salt and the structural transition was also investigated.

Materials and Methods

Materials

Silkworm cocoons were kindly provided by Heung Jin Co. Ltd. All chemicals were purchased from Sigma-Aldrich (USA).

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Table 1. Gelation time of sericin solution (2.91 wt%) in the presence various salts (1.0 M)

	0.5h	1h	3h	4h	6h
Sericin	X	○ ^a			
+NaCl	X	X	△ ^b	○	
+KCl	X	X	△	○	
+CaCl ₂	X	X	X	X	○

a: gel, b: gel-like

Preparation of sericin solution

SS was extracted by boiling 25 g of *Bombyx mori* silkworm cocoons with 1 L of distilled water using an autoclave for 1 h at 120°C. The extracted solution was filtered with a nonwoven filter in order to remove the remaining cocoons. The solution was concentrated with a rotary evaporator, and the final concentration was 2.91 % (w/v)

Measurement of gelation time of sericin solution

In each 20 mL vial, 10 mL of sericin solution were added. Predetermined amounts of salts (NaCl, KCl and CaCl₂) were added into the sericin solution, and stood in the laboratory at room temperature. The vial was tilt to 45 degree after predetermined intervals, and gelation time was determined when the surface line of solution did not change any longer.

Secondary structure analysis

The circular dichroism (CD, J-700, JASCO, Japan) spectra were obtained at the wavelength of 190-240 nm. The sericin solution was diluted with distilled water until a clear spectrum was obtained. In the case of sericin gel, the gel was spread gently on the surface of the quartz cell.

Results and Discussion

In general, the gelation time of sericin is affected by the initial concentration. When the concentration of sericin is below 1% (w/v), sericin tends to agglomerate rather than forming a gel. If the sericin concentration is higher than 1% (w/v), gel will be

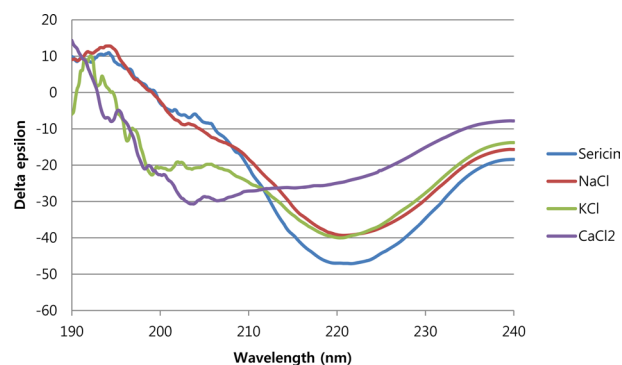


Fig. 1. CD spectra of sericin solution or gel in the presence of various salts (1.0 M) after 2 h of incubation.

formed, and the gelation time will be shorten with the increase of concentration. In the present study, the concentration of sericin was adjusted to 2.91% (w/v) by concentrating the original solution. Table 1 shows the gelation time of sericin solution in the presence of 1 M of various salts. The gelation time of neat sericin solution was 1 h, but it took 4 h in the presence of NaCl and KCl. When CaCl₂ was added, the gelation time was 6 h.

It has been known that the gelation of sericin occurs by the structural transition of sericin secondary structure from random coil to β -sheet structure (Padamwar and Pawar, 2004). Fig. 1 shows the CD spectra of sericin solution or gel after 2 h of incubation. In the CD spectra, random coil structure exhibits negative peak around 195 nm and very low ellipticity above 210 nm. On the other hand, positive peak around 195 nm and negative around 210-220 nm will be assigned as β -sheet structure (Greenfield, 2006). It could be clearly seen in Fig. 2 that the addition of salt affected the structural transition of sericin. While the neat sericin solution exhibited a typical β -sheet structure, the β -sheet structure was not fully developed in the salt added sericin solutions. When CaCl₂ was added, the negative peak at 220 nm was not fully developed, indicating low β -sheet content compare to others. Indeed, after 2 h of incubation, the neat sericin solution was in a gel state, while NaCl and KCl added solutions were a gel-like state. The gel-like state means that the surface of solution was maintained at the beginning of tilting the vial but finally corrupted at the end of tilting. Conversely, the CaCl₂ added solution was still in sol state after 2 h of incubation.

Table 2 and 3 shows the effect of salt concentration on the gelation time of sericin. The gelation time of sericin solution was delayed with the increase of salt concentration. The gelation times of KCl added sericin solution were as same as NaCl

Table 2. Effect of NaCl concentration on the gelation time of sericin solution (2.91 wt%)

	0.5h	1h	2h	4h
Sericin	X	○ ^a		
0.1 M	X	△ ^b	○	
1.0 M	X	X	△	○
2.0 M	X	X	△	○

a: gel, b: gel-like

Table 3. Effect of CaCl₂ concentration on the gelation time of sericin solution (2.91 wt%)

	0.5h	1h	4h	6h	8h
Sericin	X	○ ^a			
0.1 M	X	X	△ ^b	○	
1.0 M	X	X	△	○	
2.0 M	X	X	△	△	○

a: gel, b: gel-like

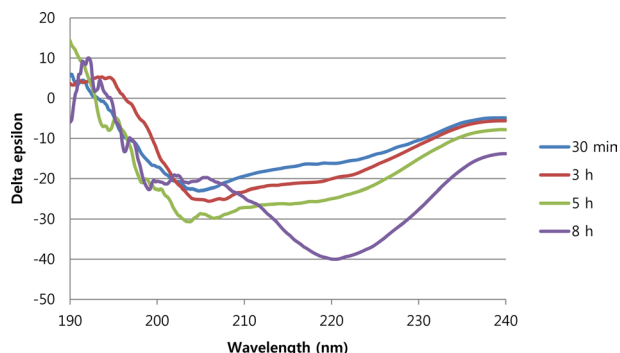


Fig. 2. CD spectra of sericin solution or gel in the presence of CaCl₂ (1.0 M) after different time of incubation.

added one. In the case of NaCl or KCl added sericin solution, the gelation time was 4 h when 2 M of each salt were added. However, when 2 M of CaCl₂ was added to sericin solution, the gelation could be prevented up to 8 h.

Fig. 2 shows the time course change of secondary structure of sericin solution when 1 M of CaCl₂ was added. As the incubation

time increased, the intensity of negative peak at 220 nm was increased indicating the development of β -sheet structure.

It could be summarized that the gelation time of sericin could be prevented by adding salt to the solution. The CaCl₂ was most effective for the delay of gelation time of sericin solution. This is unexpected result when we consider the effect of CaCl₂ in fibroin gelation. In the case of fibroin, gelation is promoted in the presence of CaCl₂ due to divalent character of Ca²⁺ which could act as an ion bridge (Kim *et al.*, 2004). Here, the effect of CaCl₂ could be explained by the solvation effect of Ca²⁺. According to the Hoffmeister series, Ca²⁺ has much higher salting-in effect compared to Na⁺ or K⁺. Therefore, Ca²⁺ helps sericin to be solvated even at high concentration. Our result shows that the gelation of sericin could be prevented up to 8 h which would be enough time for further fabrication of sericin i.e. blending, interpenetrating network formation, and post-modification, etc.

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