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Mechanism of the Gelation of Fibroin Solution

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Degummed silk was dissolved in a mixture of $CaCl_2$ and ethanol at low temperature. The concentration and pH of the centrifuged, filtered, and dialyzed silk solution were adjusted, and then it was kept in an air-conditioned room for gelation. The mechanism of the gelation and the gel structure were studied. It was found that the gel was formed due to the formation of beta-structure in the fibroin solution. The gelation was dependent on the pH of the fibroin solution.

Silk is the most valuable natural fiber in the world. It is rich in lustre, hand feeling, and warmth, but yellowing in sunlight and easy splitting into microfibrils by friction are weak points. At present, the price of one kilogram of silk yarn is about 12,000 yen, in Japan, but even so, this miracle fiber has not been losing its popularity. During processing we get a lot of silk waste, which eventually increases the price of silk yarn. Moreover, its disposal is another problem. In these circumstances, if we could find out a way to use this material properly, it will be a plus to the silk industry as a byproduct. In our laboratory, we have been working on the waste silk for recycling.

Recent experimental reports[1–4] proposed some new applications and we have been concentrating our work on the mechanism of the gelation of silk solution and on the gel structure. Preparation of silk solution is essential when we want to study the mechanism of silk spinning. It was found that fibroin solution gels when it is left alone for a few days. This phenomenon suggested the idea of preparing a jelly from the silk solution, but manufacturing food from the high-priced silk materials cannot be a feasible product. Therefore, we paid attention to the unusable waste silk coming out during the processings. For the dissolution of silk we generally use lithium bromide solution, but it is expensive and not a safe chemical in biochemical processing. To reduce the cost and assure safety we decided to use a calcium chloride solution.

Materials and Methods

Waste silk gets dirty during handling and processing. Therefore cleaning and degumming have to be done before dissolving the silk in an aqueous mixture. The waste silk was degummed using 0.5% $NaCO_3$ solution and treated twice at 100°C for 20 min, before being washed with distilled water.

The maximum amount of fibroin (about 20 g) dissolved readily in 100 ml of aqueous $CaCl_2$ when the concentration was about 40–50% (Fig. 1). But prolonged boiling of silk fibroin in such solution breaks the molecular chains. Therefore, we dissolved 16 grams of fibroin in an aqueous mixture of $CaCl_2$ and ethanol.[5] The proposed ratio of $CaCl_2$, ethanol, and water was 1 : 2 : 8 moles. Addition of ethanol increases solubility of fibroin at low temperature with less effect on the molecular weight and size. Accordingly, fibroin was dissolved in the aqueous mixture of ethanol and $CaCl_2$, then the solution was separated from foreign particles by centrifuging at $1000 \times g$ for 10 min. The regenerated fibroin solution was obtained by filtering the centrifuged solution and by dialyzing the filtered solution for 3 days using cellulose tubes. After measuring the concentration (wt%) of the fibroin and adjusting the pH of the dialyzed solution with acids and alkali, it was kept in an air-conditioned room at 20°C and at a relative humidity of 56–64%. Gelation was confirmed by the fact that the solution had a semi-solid texture which remained still when the whole content was inverted by tilting.

The structureal changes during the gelation of fibroin solution were studied, using a recording spectropolarimeter J40AS (JASCO, Japan). The conformation responsible for the gelation was studied with freeze-dried gel, using an infrared spectrophotometer IR-435 (Shimadzu Corp., Japan). The surface structure was studied with freeze-dried gel, using an electron microscope H-700 (Hitachi Corporation, Japan). For a better understanding of the gel structure the sample was exposed to CuK_α radiation from a Rigaku Geigerflex X-Ray unit. For the studies the pH of the 3% solution was adjusted to pH 3.2.

Results

Figure 2 shows the relationship between the gel setting time and the pH of 3% fibroin solutions. The initial pH of the fibroin solution was pH 7.6–7.9. The pH for the solution on the acid side was adjusted by citric acid and HCl, and the alkali side was adjusted by NaOH. It was found that the gelation occured within two days at pH around 3.0 to 4.0. It was also found that at pH below 1.5 or above 13.0 no gelation took place.

Figure 3 shows the results of the circular dichroism analysis of clear and turbid fibroin solutions just before the gelation. The peak at 195 nm indicates that the fibroin solution contains a random coil structure. But the solution just before the gelation (observed by naked eye) transformed into a beta-structure[6] as the peak in this case

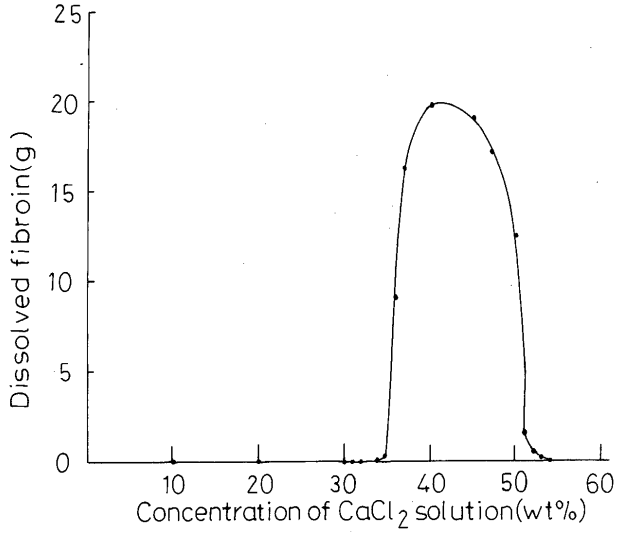


Fig. 1. Relation between the Dissolved Fibroin and the Concentration of 100 ml Calcium Chloride Solution (wt%).

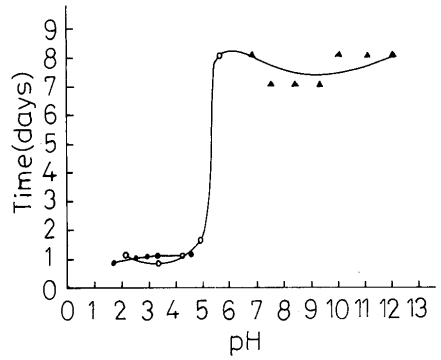


Fig. 2. Gel Setting Time at Different pHs of 3% Fibroin Solution. ○, citric acid; ●, HCl acid; ▲, NaOH.

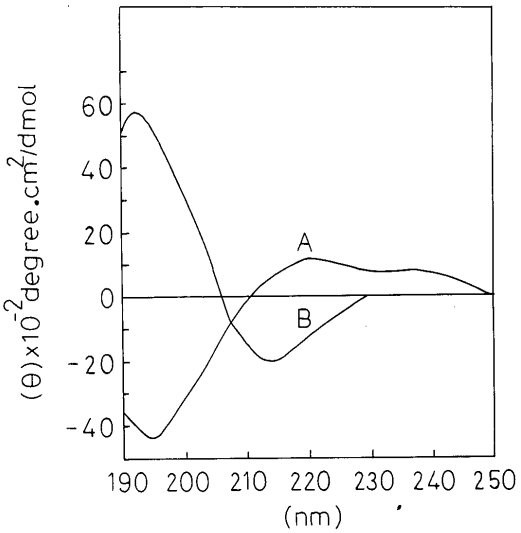


Fig. 3. Change in Circular Dichroism during Gelation.
A, in the state of solution; B, before commencing the Gelation.

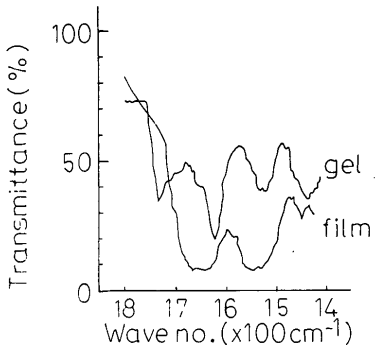


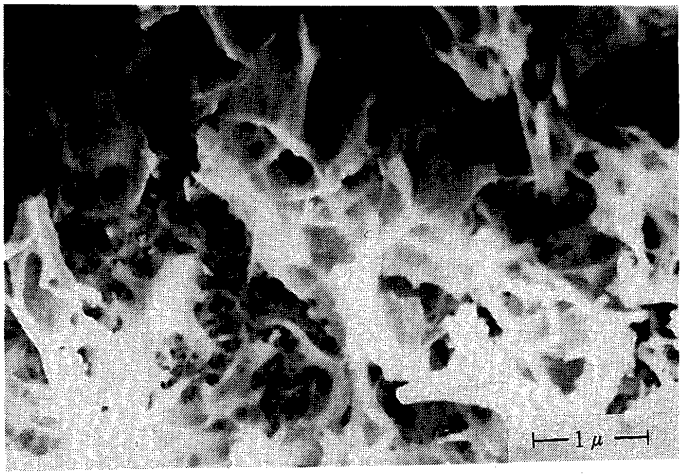
Fig. 4. Infrared Spectra of Fibroin Gel and Film.

appeared at 215 nm.

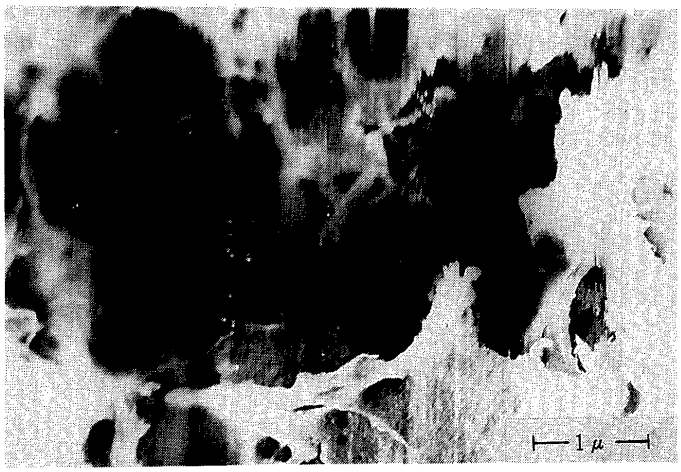
The absorption at 1630 cm^{-1} (Fig. 4) indicates the presence of beta-structure in the freeze-dried gel. There was random structure in the solution and in the film of fibroin, but a beta-structure was observed when a gel was formed. The gel contains more beta-structures[7] than that of the fibroin film.

The electron micrographs (Fig. 5) show that the freeze-dried gels have a network structure. It was found that the gel contains a large number of pores of irregular shape and size. The white portion is fibroin and the black portion is the space occupied by the water molecules.

The X-ray results are summarized in Table I. The fibroin gel showed spacing distances which were almost the same



(A)



(B)

Fig. 5. Electron Micrographs of Fibroin Gel.
A, ×200; B, ×2000.

Table Comparison of Spacing Distance (Å) of Silk Gel

Reflection	pH 3.2	pH 10.3	Shimizu's β structure
1	9.51	9.86	9.70
2	4.46	4.46	4.69
3		4.16	4.30
4	3.69	3.57	3.67
5	2.87	3.75	2.78
6	2.28	2.33	2.27
7	2.08	2.04	2.07

as those of Shimizu's beta-structure. The spacing distances for the gel at pH 3.2 had good accordance with the data reported by Shimizu.[8]

Discussion

The nearness of pH of the fibroin solution to the isoelectric point (pI = 3.8–3.9) accelerated the gelation process and thus the gel was formed within a short period. This phenomenon is similar to other proteins which aggregate easily near their respective isoelectric points. The gel

did not form below pH 1.5 or above pH 13.0 due to the repulsion among the molecules when they were in too low or high pH.

In this study, we have tried to eludicate the mechanism for the gelation and the structural characteristics of fibroin gels by circular dichroism, infrared spectroscopy, electron micrography, and X-ray diffraction.

By circular dichroism, the random coil conformation of fibroin solution was seen to be transformed into beta-structure when the gelation occured. The formation of beta-structure in the gel was confirmed by infrared spectroscopic results. The sharp peak at $1630\,cm^{-1}$ indicated that the gel had beta-oriented structure.

The mechanism of this gelation is consistent with the general principle that hydrophobic interaction is the major driving force for macromolecular interactions, and electrostatic or hydrogen bonds contributed to the stability of the interactions with the formation of beta-structure.

The electron micrograph showed that the freeze-dried gel had a network structure. It is suggested that aggregation of fibroin molecules and formation of beta-structure in the gel produced this network structure.

The X-ray diffraction studies of gels dried at 40°C showed beta-type crystalline structure. From the results of X-ray diffraction, no distinctive differences in structure could be found. Therefore, it is concluded that the network is an aggregation of fine beta-type crystals produced by hydrogen bonding in the mutual interaction process.

The gelation of silk fibroin occurred by the reduction of the movement of free fibroin molecules in solution and the formation of hydrogen bonds between the fibroin molecules. Thus networks were produced in the gel and all the results have shown that gels might have been formed when intermolecular beta-structure acted as the main source for gelation.

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