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Synthesis of Silica using Silk Sericin without Hydrolysis of Tetraethyl Orthosilicate

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Abstract

In this study, the effect of sericin on synthesis of the silica was investigated. Using the mixture of sericin solution and tetraethyl orthosilicate (TEOS), it was confirmed that silica could be synthesized in the presence of sericin, which was verified by thermal gravimetric analysis (TGA), Fourier-transformed infrared spectrometer (FT-IR) and nuclear magnetic resonance spectrometer (NMR) analysis. The TGA and FT-IR data revealed that silica-sericin complex was formed as a final product. Based on the TGA result, the content of silica and sericin in the complex would be 87 and 13%, respectively. The degree of silica condensation was higher than the natural biosilica. It could be concluded that sericin can induce the synthesis of silica directly from TEOS, which is similar to silicatein from marine sponges.

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Introduction

Silica is the second abundant element in the biosphere and many organisms utilize it as a structural component (Rupcich *et al.*, 2003). Diatom is the one of the example, which synthesizes silica and protects their cell structure with it (Fuhrmann *et al.*, 2004). Marine sponges also synthesize silica and their spicules are made of it (Cha *et al.*, 1999). These organisms condense silicic acid into silica, and the process is mediated by several proteins.

Silaffin is a protein from in diatoms and is known to facilitate the silica synthesis (Kröger *et al.*, 2001). Moreover, it can control the morphology of the synthesized silica (Sumper *et al.*, 2006). On the other hand, silicatein which derives from marine sponges is an enzyme that catalyzes the silica synthesis directly from the silica precursor (Shimizu *et al.*, 1998). In both proteins, serine plays an important role in the silica synthesis. Silaffin has high content of serine which is phosphorylated by post-translational modification (Kröger *et al.*, 2002). In the case of silicatein, serine is located at the active center (Cha *et al.*, 2000). This gave us an idea to use sericin for the silica synthesis because sericin has also high content of serine.

Sericin is a minor protein that is secreted by the silkworm. It bonds two brins of fibroin fiber together and make able to maintain the shape of cocoon. Sericin is usually discarded by the degumming process but could be used as new source of biopolymer, because it is easy to extract and able to get in

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large quantities. Currently sericin is used as an ingredient of cosmetics (Kim *et al.*, 2009) and new application are found in pharmaceutical, polymeric, and biomedical field (Zhaorigetu *et al.*, 2001; Kwak *et al.*, 2013; Oh *et al.*, 2011). Previously, sericin has been used to induce the biomineralization of hydroxy apatite (Takeuchi et al, 2008). In this study, we used sericin in order to synthesize the silica. The synthesis of silica was verified with various analytical methods.

Materials and Methods

Materials

Silk cocoon was obtained from Hung Jing Co., LTD. (Seoul, Korea). All other chemicals were purchased from Sigma-Aldrich LTD. (Yongin, Korea).

Preparation of hot-water extracted sericin solution

Silk cocoons were boiled with distilled water using an autoclave at 120ºC for 1 h. The solution was filtered with a nonwoven filter in order to remove the remaining silk fibers. The solution was freshly made every time before the experiment. The final concentration of sericin was 1 %(w/v).

Synthesis of silica using sericin

The synthesis of silica was performed by mixing tetraethyl orthosilicate (TEOS) and sericin solution. More precisely, 500 μL of TEOS and 500 μL of sericin solution were added in a Eppendrof tube, and it was shaken vigorously with an orbital shaker. The tube was incubated at room temperature without further stirring. After 1 wk, 400 μL of ethanol was added to the mixture and centrifuged at 10,000g for 1 min in order to precipitate the reactant. The precipitate was further washed with ethanol 3 times. The collected precipitate was dried in a vacuum chamber for 36 h in order to remove residual ethanol.

Analysis of precipitate

The formation of silica was verified by thermal gravimetric analysis (TGA, Q-5000 IR, TA-Instrument, USA), Fourier transformed-infrared spectrometer (FT-IR, MIDAC, Japan) and ^{29}Si nuclear magnetic resonance spectrometer (NMR, AVANCE, Bruker, Germany). The heating rate of TGA was 10˚C/min and the data in the range of 100-600˚C were collected under nitrogen gas purging. In the case of FT-IR, the spectrum was obtained from KBr method after 24 scans and the resolution was 4 cm^{-1}. Solid-state ^{29}Si MAS NMR spectra were acquired on a DSX-400 NMR spectrometer (Bruker, Germany) operating at 79.5 MHz. Detailed conditions were as follows: spinning rate, 3.5 kHz; pulse length, 4.2 μs; recycle delay, 30 s. Field emission scanning electron microscope (FE-SEM, SUP-RA55 VP, Carl Zeiss, Germany) was employed to observe the microscopical morphology of the precipitate.

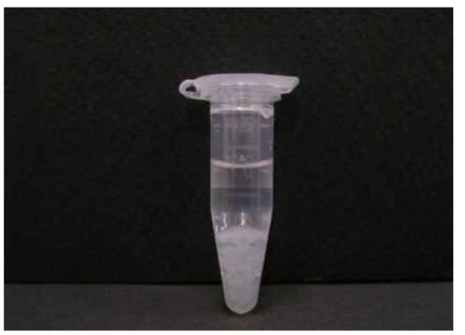
Results and Discussion

We prepared sericin solution by the hot-water extraction method. Sericin solution was added to TEOS and left in the chamber without stirring for 1 wk at room temperature. The TEOS and the sericin solution did not mix each other (Fig. 1a), and a white precipitate was formed only at the interface between the TEOS and the sericin solution. In order to improve the precipitation, we stirred the reaction tube vigorously. A dispersion of the two liquid was formed in the sericin layer and maintained through the incubation time. The white precipitate was grown in the sericin solution layer during 1 wk of incubation (Fig. 1b). Fig. 1c shows the final precipitate obtained after washing and drying.

In order to verify the synthesis of silica, we first performed TGA analysis. While sericin decomposes over 200ºC, the weight loss of precipitates was about 15% even at 600ºC indicating the formation of silica (Fig. 2). In the case of the precipitate, there was a significant loss of weight between 200~400ºC which was the same temperature range that the thermal degradation of sericin occurs. Therefore, the precipitate might be a complex of silica and sericin. Based on the TGA results, the weight percent of silica and sericin in the complex would be 87 and 13%, respectively.

The formation of silica-sericin complex could be also verified by the ATR-FTIR results (Fig. 3). The asymmetric stretch, symmetric stretch and bending vibration of Si-O-Si at 1100, 800

(a)



(b)

(c)

Fig. 1. Optical images of TEOS and sericin solution mixture before mixing (a) and after 1 wk of incubation (b). The final precipitate after washing and drying is shown in (c).

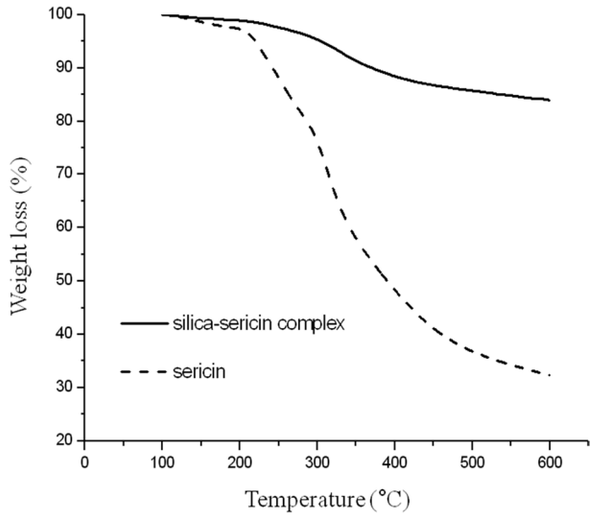


Fig. 2. TGA curves of silica-sericin complex and sericin.

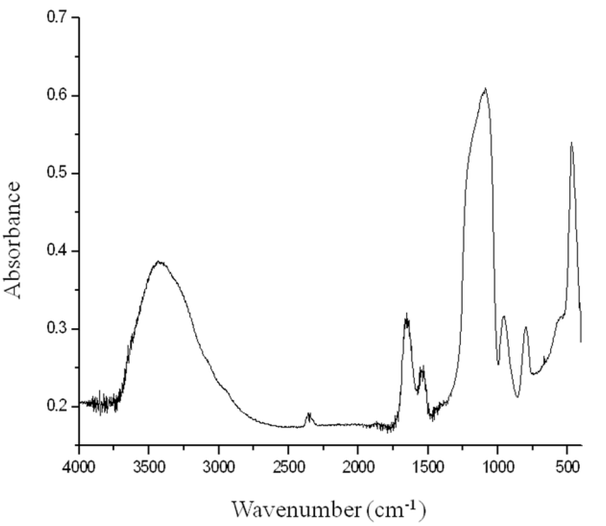


Fig. 3. FT-IR spectrum of silica-sericin complex.

and 450 cm^{-1}, respectively, could be observed in the ATR-FTIR spectrum (Siuzdak *et al.*, 1999). At the same time, a characteristic peak of amide I (1650 cm^{-1}) and amide II (1530 cm^{-1}) could be observed (Teramoto *et al.*, 2005). This result also indicates that the precipitate is a complex of silica and sericin.

The synthesis of silica can be also verified by the NMR. Generally, Q^n notation (Q^1, Q^2, Q^3 and Q^4) is used to identify the environment of silicon atom, and *n* indicates the number of siloxane bonds connecting a silicon atom with other silicon atoms via oxygen bridges. In NMR, the resonance signal of Q^2, Q^3 and Q^4 appears at $\delta \approx$ -92, -101 and -110 ppm, respectively (Bertermannm *et al.*, 2003; Cong *et al.*, 1993). All 3 peaks could be found in the NMR spectrum indicating the synthesis of silica (Fig. 4). The Q^4/Q^3 ratio indicates the degree of silica condensation, and it was 2.28 which are higher than the natural biosilica (Table 1).

Fig. 5 shows the FE-SEM image of silica-sericin complex. It had a fractal structure where sphere-like particles having tens of nanometer size are agglomerated into large and irregular shapes.

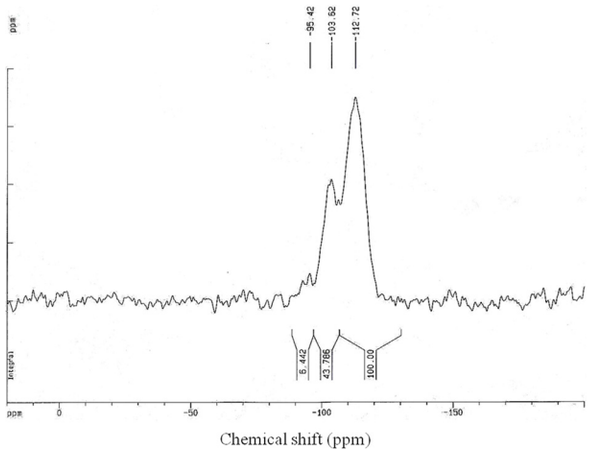


Fig. 4. Solid phase ^{29}Si MAS NMR spectrum of silica-sericin complex.

Table 1. Quantitative analysis of Q^2, Q^3, and Q^4 peaks in silica-sericin complex.

	Q^4	Q^3	Q^2	Q^4/Q^3
Natural biosilica[a]	100	53.13	3.13	1.9
Silica-sericin complex	100	43.77	24.82	2.28

a: from Bertermannm *et al.* (2003)

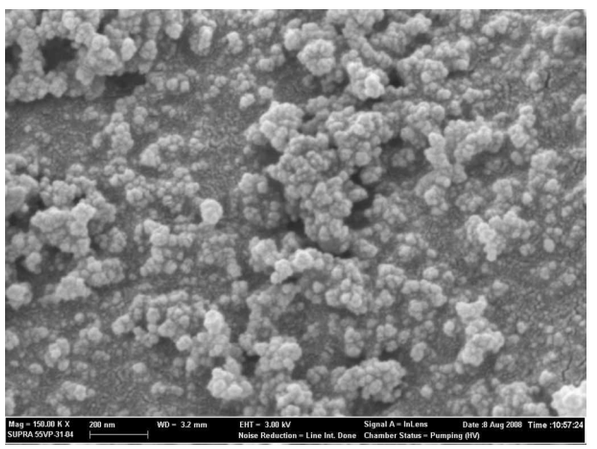


Fig. 5. FE-SEM image of silica-sericin complex. (×15000)

Generally, TEOS is hydrolyzed under acidic or basic condition to in order to synthesize silica through sol-gel process. However, the present study shows that silica could be synthesized directly from TEOS without any hydrolysis. It seems that the role of sericin during silica formation is similar to silicatein. Further application of the synthesized silica nano- and microparticles in biomedical field is under investigation. Expected area is drug delivery and tissue engineering where silicatein is currently applied (Schröder *et al.*, 2007).

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