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Structure and properties of regenerated *Antheraea pernyi* silk fibroin in aqueous solution

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Abstract

Antheraea pernyi silk fibroin fibers were dissolved by aqueous lithium thiocyanate to obtain regenerated *A. pernyi* silk fibroin solution. By means of circular dichroism, ^{13}C NMR and Raman spectroscopy, the molecular conformation of regenerated *A. pernyi* silk fibroin in aqueous solution was investigated. The relationship of environmental factors and sol–gel transformation behavior of regenerated *A. pernyi* silk fibroin was also studied. The molecular conformations of regenerated *A. pernyi* silk fibroin mainly were α-helix and random coil in solution. There also existed a little β-sheet conformation. It was obviously different with *Bombyx mori* silk fibroin, whose molecular conformation in solution was only random coil but no α-helix existence. With the increase of temperature and solution concentration and with the decrease of solution pH value, the gelation velocity of regenerated *A. pernyi* silk fibroin solution increased. Especially, it showed that *A. pernyi* silk fibroin was more sensitive to temperature than *B. mori* silk fibroin during the sol–gel transformation. The velocity increased obviously when the temperature was above 30 °C. During the sol–gel transformation, the molecular conformation of regenerated *A. pernyi* silk fibroin changed from random coil to β-sheet structure. The results of these studies provided important insight into the preparation of new biomaterials by silk fibroin protein.

Keywords: *Antheraea pernyi*; Silk fibroin; Molecular conformation; Sol–gel

1. Introduction

Silkworm silk fibroin is a kind of natural biopolymer produced by two species of silkworms, domestic (*Bombyx mori*) and wild, and has been used as a source of textile material for thousands of years. In non-textile field, *B. mori* silk protein has been used in cosmetic and food additive [1–3]. The surgical suture made by *B. mori* silk has been largely applied in clinic [4]. Silk fibroin, non-toxic and non-irritating, has good biocompatibility and is beneficial for cells of human being and many kinds of mammals to adhere and proliferate [5–14]. So in recent years it has been widely studied as biomedical materials, such as wound cover materials, controlled drug release carriers, tissue engineering scaffolds and repair materials for skins, bones, ligaments, etc.

Antheraea pernyi silkworm is the most familiar species among wild silkworms. In contrast to that of *B. mori* silk fibroin, the amino acid composition of *A. pernyi* silk fibroin is characterized by more Ala, Asp and Arg and less Gly. The abundance of alkaline amino acids (Arg and His) and the presence of tripeptide sequence Arg-Gly-Asp (RGD), which is known to exhibit special interactions with mammal cells, are favorable for cells to adhere [15–17]. The above properties of *A. pernyi* silk fibroin attract us to take it as raw material to prepare biomedical materials with better properties than those made by *B. mori* silk fibroin.

Silk fibroin, which is collected from the full-grown silkworm's posterior silk gland, can only meet the small quantity requirement in laboratory, but cannot meet the demand of industrial production. Natural *A. pernyi* silk fibroin fibers, with high crystallinity and hard to be biodegraded, cannot be directly used to prepare the biodegradable materials such as tissue engineering scaffolds and controlled drug release carriers. Therefore, it is necessary to dissolve the *A. pernyi* fibers firstly to obtain

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regenerated *A. pernyi* silk fibroin aqueous solution. After that we may prepare the biomaterials with required configuration, structure and function with the aqueous solution, such as porous material, gel, film, fiber, powder, etc. The structure and properties of regenerated *A. pernyi* silk fibroin in aqueous solution are obviously very important for the preparation of above biomaterials because they have undoubtedly great influence on the preparation process and the structure and properties of the prepared materials. Furthermore, the studies on the structure and properties of silk fibroin in aqueous solution are also helpful to explore and comprehend how the silkworms make use of the liquid silk fibroin in silk gland as raw materials to spin silk fibers [18].

A. pernyi silk fibroin fibers were dissolved by aqueous lithium thiocyanate to obtain regenerated *A. pernyi* silk fibroin solution. The molecular conformation of regenerated *A. pernyi* silk fibroin in aqueous solution and the sol–gel transformation behavior were studied in this paper.

2. Materials and methods

2.1. Materials

A. pernyi silk fibers (100 g) were boiled for 30–45 min three times in 5000 ml aqueous solution of 2.5 g/l Na_2CO_3. After being rinsed and air dried at 60 °C, the degummed *A. pernyi* silk fibroin fibers would be obtained.

2.2. Solubility of A. pernyi silk fibroin fiber

Degummed *A. pernyi* silk fibroin fibers (10 g) were respectively placed in 100 ml 10 M aqueous lithium thiocyanate solution and in 100 ml melted $Ca(NO_3)_2$, stirred to dissolve at 40 and 100 °C for special times, respectively. The undissolved *A. pernyi* silk fibroin fibers were filtrated when the set time ended up and then dried at 140 °C to obtain their remained weight. The solubility could be calculated per Eq. (1):

$$\text{solubility (wt\%)} = \frac{\text{original weight (g)} - \text{remained weight (g)}}{\text{original weight (g)}} \times 100\% \quad (1)$$

2.3. Preparation of regenerated A. pernyi silk fibroin solution

A. pernyi silk fibroin fibers (10 g) were respectively placed in 100 ml 10 M aqueous lithium thiocyanate solution and in 100 ml melted $Ca(NO_3)_2$, stirred to dissolve at 40 °C for 60 min and at 100 °C for 5 h, respectively. The cooled solution was respectively dialyzed in cellulose tube against water for 4 days to obtain the regenerated *A. pernyi* silk fibroin solution. Some dialyzed solution, obtained by dissolving the fibers with lithium thiocynate, was concentrated to 7.0% (w/w) by evaporating water slowly at 15 ± 2 °C.

2.4. SDS-PAGE

The molecular weight distribution of the regenerated *A. pernyi* silk fibroin was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method described by Laemmli [19] with 7.5% acrylamide gel and 3.0% condensing gel in Tris–glycine buffer containing 0.1% SDS. The proteins were stained with Coomasie Brilliant Blue R-250.

2.5. Molecular conformation analyses of regenerated A. pernyi silk fibroin in solution

Circular dichroism spectra of the regenerated *A. pernyi* silk fibroin in aqueous solution were collected on a Jasco spectropolarimeter, model 715, with a quartz cell of 1 mm path length for far-UV, at protein concentration of 0.1 mg/ml. The bandwidth was 1.0 nm, and all the CD spectra were obtained at a scan speed of 100 nm/min with a response time of 0.25 s.

Raman spectra were recorded using a Dilor LabRam-1B spectrometer, operating at a resolution of 1 cm^{-1}. The Spectra Physics Model 164 argon ion laser was operated at 632.8 nm with about 6 mW power.

^{13}C NMR spectrum was obtained by high resolution NMR spectrometer (FT-NMR(600 MHz) AVANCE 600, Bruker, Germany) to determine the secondary structure of silk fibroin molecules in solution.

2.6. Observation of sol–gel transformation

A total of 20 ml of silk fibroin aqueous solution was placed in 50 ml flat-bottomed vials. The vials were sealed and kept at 10, 20, 25, 30, 40 and 50 °C, respectively. Gelation time was determined when the sample seemed opaque straw yellow and was difficult to fall from the inverted vial. The pH value of the silk fibroin solution was adjusted with HCl or NaOH solution.

2.7. Molecular conformation analyses of regenerated A. pernyi silk fibroin gel

Regenerated *A. pernyi* silk fibroin gel was formed from silk fibroin solution (25 °C, concentration 3%). The regenerated silk fibroin solution with concentration of 3%, as a control and the gel were respectively poured into stainless steel dishes. The dishes were placed in a freezing chamber at −70 °C and frozen for 6 h by MDF-492 Ultra Low-Temperature Freezer (Sanyo Electric, Tokyo). Then they were freeze-dried for about 48 h by Genesis 25-LE Freeze Dryer (Virtis, USA). The freeze-dried gel and the regenerated silk fibroin solids were cut into micro particles with radius less than 40 μm for XRD and FT-IR analyses.

X-ray diffraction was performed by a Rigaku D/Max-3C diffractometer with Cu Kα radiation from a source operated at 40 kV and 40 mA. Diffraction was measured in reflection mode at a scanning rate of 2° min^{-1} for $2\theta = 5$–40°. Fourier transform infrared (FT-IR) spectra were obtained with a Nicolet Avatar-IR360. The samples were prepared in KBr pellets.