值对丝素蛋白构象转变的影响

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摘要 模仿家蚕吐丝过程中伴随丝素蛋白自然脱水的纤维化过程研究了再生丝素蛋白在各种 pH 值的磷酸盐缓冲溶液体系中自然干燥脱水成膜后的构象转变(利用激光拉曼散射光谱及其二维相关光谱定性分析了丝素蛋白酰胺区散射峰的相关组成及结构(在此基础上利用固体核磁共振谱对丝素蛋白丙氨酸峰进行了解析拟合(从而确定了体系中与构象相关的组成含量与 pH 值的关系(结果表明酸性溶液有利于蚕丝丝素蛋白从向构象转变而中性与碱性溶液和则对丝素蛋白的构象转变影响甚小(关键词 丝素蛋白 构象转变 pH 值影响 核磁共振 拉曼光谱

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1.1 酸碱特性

<table>
<thead>
<tr>
<th>pH</th>
<th>1.9</th>
<th>1.4</th>
<th>3.0</th>
<th>6.0</th>
<th>9.0</th>
</tr>
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由于家蚕丝再生丝素蛋白溶液与腺体中天然丝素蛋白的一级及二级结构相同因此本文选用各种 pH 值的再生丝素蛋白缓冲溶液在空气中自然干燥成膜的过程来模拟蚕吐丝过程中腺体逐步丧失水分而成为固体丝纤维的过程。分别用一维和二维拉曼相关光谱及固体谱对系列再生丝素蛋白样品的构象组成进行了定性和定量分析并对蚕在吐丝过程中腺体内的 pH 值变化进行了讨论。
1. 2

Dilor LabRam-1B Raman (632.8 nm), 6 mW, 1 cm\(^{-1}\). InfinityPlus-400 13\(^{13}\)C CP-MAS NMR, C\(\beta\) 1 000 s.

2D-Pocha (Daisuke Adachi) 13\(^{13}\)C CP-MAS NMR, C\(\beta\) 1 000 s.

2

2.1

pH 5.2, Silk 1 (1 666 cm\(^{-1}\)), pH = 6.5 ~ 6.7, Silk 1 (1 670 ± 5 cm\(^{-1}\)), a, b.

pH 6.5 ~ 6.7, Silk 1, Silk 1 (1 666 cm\(^{-1}\)), 1 659 cm\(^{-1}\), 1 666 cm\(^{-1}\), 1 667 cm\(^{-1}\).

2.2

(Related to the Generalized Two-Dimensional Correlation Spectroscopy) [15, 16]. pH 5.2, 6.5 ~ 6.7, Silk 1, 1 666 cm\(^{-1}\), 1 667 cm\(^{-1}\).

2(A), 2(B), 2(C), 2(D).

Fig. 1 Raman spectra of regenerated silk-fibroins membranes with different pH values

a. Originally regenerated silk fibroin; b, c, d. regenerated silk fibroin membranes formed in buffer solutions with pH = 5.2, 6.9, 8.0, respectively.
Fig. 2 2D Raman correlation spectra of fibroin membranes formed in buffer solutions with various pH values

(A) Cross section of synchronism; (B) vertical section of synchronism; (C) cross section of asynchronism;
(D) vertical section of asynchronism.

1659, 1667, 1682 cm⁻¹. 

2.3 ¹³C CP-MAS NMR

Silk 1  ,  Cα, Cβ, δ 14.5 17.5. 

Spectroscopy
Samples with conformation-related composition content reached 34%. pH 6.9 showed 21.5% 20.4%. [Silk (B) 18.3%] (A) 5.2. Silk (C) 6.8. pH 8.0 and 5.2. When the value of (B) was 1 2 3 4, the composition content of the sample conformation-related was 4.8. (A) 1 2 3 4 and 6.2 7.2. When the value of (B) was 1 2 3 4, the composition content of the sample conformation-related was 4.8. (A) 1 2 3 4 and 6.2 7.2. Relative to the original regenerated silk protein samples, it was seen that the conformation-related composition content of the samples increased significantly when the value of (B) was 1 2 3 4, while other samples were basically similar to the original regenerated silk protein samples. The quantitative analysis results of nuclear magnetic resonance spectroscopy were consistent with the qualitative conclusions provided by Raman spectroscopy.

According to the experimental results, we believe that in a certain concentration of ions such as (B), the silk protein solution is easier to destroy the hydrogen bond network in the coiled conformation and tend to stretch, forming hydrogen bonds between the extended chains, which is conducive to the formation of Z-type folding conformation and reaching a new stable state. In addition, due to the gradual evaporation of the solvent, the segmental movement of the chain was gradually increased, which led to the generation of some transition state conformations. In the system with lower ion concentration, the hydrogen bond network in the coiled conformation of the silk protein solution can be stably maintained, and the composition content of Z-type folding conformation is relatively low, while the composition content of Z-type folding conformation is relatively high.
Influence of pH Value on the Phase Transition of Silk Fibroin

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Abstract Both ¹³C CP-MAS solid state NMR and laser Raman scattering spectroscopy were used to investigate the influence of pH values on the conformation transition of silk fibroin of Bombyx mori silkworm. The regenerated silk fibroins in the phosphate buffer solution were used to imitate the silk fibroins of the gland. A series of membranes formed in buffer solutions with different pH values were obtained by the evaporation of water at room temperature (for mimicking the gradual loss of water in spinning process). The compositions and conformation in the fibroin membranes were determined quantitatively by Raman and Generalized Two-Dimensional Correlation Raman Spectroscopy for amide I region (1600—1700 cm⁻¹) of the silk fibroins. There are four types of conformations at least existing in the fibroin membranes, including conformations of typical silk I (α-form and/or random coil), typical silk II (β-sheet), silk I transition and silk II transition, etc.. Based on the qualitative results from 2D Raman spectrum, the relationship between pH value and conformation contents in the studied systems was determined quantitatively by simulation of C₃ peak (δ 14.5—22) in ¹³C CP-MAS solid state NMR spectrum. The results show that acidic solution (pH=5.2) is more favorable than neutral (pH=6.9) and basic (pH=8.0) ones for the conformation transition from Silk I to Silk II because the silk fibroin molecular backbones are easier to extend and then to form β-sheets in acidic solution.

Keywords Silk fibroin; Conformation transition; Influence of pH value; NMR; Raman spectroscopy (Ed.; W, Z)