# Functional Silk Proteins: Molecular Structure and Application to Biomaterials

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## Introduction

The silk fibers are widely used as an excellent textile since old times and sericultural and related industries are currently developing in East Asia. It is remarkable that silkworms can produce strong and stiff cocoon fibers at room temperature and from aqueous solution, whereas synthetic polymers with comparable properties must be processed at higher temperature and/or from less benign solvents. Thus, it is important to study details of the fiber structure and alignment at the molecular level in order to clarify the origin of the impressive mechanical properties. On the other hand, the silk proteins consist of two major proteins, fibroin and sericin. There is currently an enormous reawakening of interest in these silk proteins as a biomaterial due to their mechanical and biological properties. One of the most favorable properties is the structural transition from solution to insoluble form, namely crystallization. Thus, it is possible to make non-fabric materials from the silk proteins such as film, gel, powder and solution. Another is a molecular recognition for the specific primary structure of the silk proteins including wild type. In this report, usefulness of the crystallization as a biomaterial, the molecular structure from new insights, recent application of silks and prospects for future will be discussed.

## Useful Crystallization of Silk

We have already studied application of silk fibroin and sericin to biomaterials such as an enzyme-immobilization (Fig. 1). This new biomaterial was designed based on the structural transition. In general, entrapping method for an enzyme-immobilization uses a soluble support and

chemical reagents for covalent cross-linking to complete insoluble supports. Contrary, the silk protein as a support can entrap enzymes with only self-structural transition which makes weak hydrogen-bond networks, i.e., antiparallel $\beta$-sheet structure. Thus, enzyme activity in the silk is not affected directly. Various types of silk supports such as films, powder, gel and coating of silk and sericin on non-woven have been developed. [1-4].

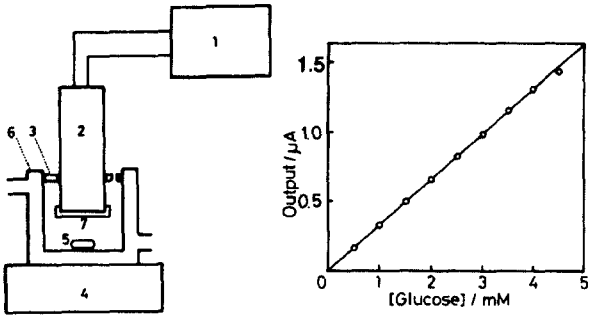


Fig. 1. Application of silk fibroin membrane to biosensor. Glucose oxidase was immobilized in the *B. mori* silk fibroin membrane. The enzyme reaction in the fibroin membrane was monitored by oxygen electrode. Linear response of the sensor as a function of glucose was obtained. Because of no leakage of enzyme from the membrane and no inactivation, the output amplitude was stabilized for long period (more than 6 months).

### Molecular Structure of the Crystalline Studied by NMR

Silk fibroin has a high content of small side chain amino acid and 60 - 80 % crystallinity based on the repetition of the sequence Gly-Ala-Gly-Ala-Gly-Ser. Antiparallel $\beta$-sheet structure of *Bombyx mori* silk fibroin (silk II) assuming simple amino acid repetition of Gly-Ala in the crystalline domain, has been first characterized by X-ray fiber diffraction in 1955 with the limited diffraction data [5]. However, there is a little information of the conformational space for Ser, Tyr and Val residues that have more bulky side chain than Gly and Ala and the non-crystalline domain because it was difficult to clear this structural information using only X-ray fiber diffraction.

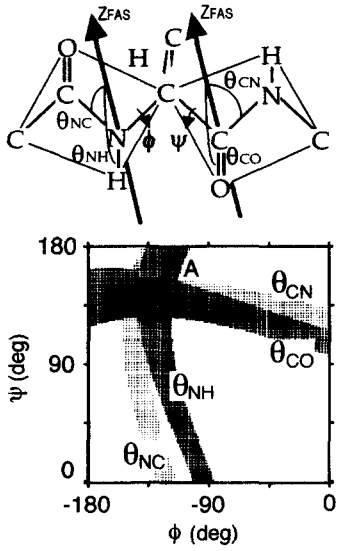


Fig. 2. Conformational space ($\Phi$,$\Psi$) of Ala residue of the silk fibroin determined from the angular constraints, $\theta$ s, measured by the angular dependent solid state NMR.

Structural refinement of the silk fibroin at the atomic level has been studied using nuclear magnetic resonance (NMR) spectroscopy [6-8]. Stable isotope-labeling of *B. mori* silk fibroin was achieved biosynthetically at [$^{13}C_1$] Gly, [$^{13}C_1$] Ala, [$^{15}N$] Gly and [$^{15}N$] Ala. Orientation dependent $^{15}N$ and $^{13}C$ solid state NMR spectra of these isotope labeled silk fibroin fibers were observed when the fiber axis was arranged at various angles to the magnetic field direction to determine the specific orientations of N-H, N-$C_1$, $C_1$=O, and $C_1$-N bonds for Ala and Gly residues (Fig. 2). The best-fit torsion angles ($\Phi$,$\Psi$) within the reduced conformational space were determined as (-140 °, 142 °) and (-139 °, 135 °), respectively within experimental error (±5°). Using similar method, the torsion angles of other minor amino acids, Ser, Tyr and Val was first determined with the $^{13}C$ and $^{15}N$ labeled oriented samples. It is also possible to obtain the orientational distribution of non-crystalline domain, which may contribute to an intermolecular interaction of various substrates.

**Recent Application of Silk and Prospects for Future**

Many industrial fields for use of the silks are considered (Fig. 3). Especially the non-textile materials have been already used in cosmetics and foods. Since various materials such as film, gel and powder can be made from the silk, a medical application of silks is in progress. For example, the attachment and growth of fibroblast cells on matrices of silk fibroins from *B. mori* and *Antheraea pernyi* wild silkworm have been studied by a cell culture method [9]. The difference of cell-attachment between these fibroins was discussed from a specific interaction site containing the RGD sequence in the non-crystalline domain. Other cell culture matrices with a thin film [10], an artificial skin [11] and a soft oral dosage form for elderly patients with the silk gels [12] have been reported. The hydrolyzed fibroin peptides, their derivatives and sericin peptides expressed in bacteria [13] are also interesting.

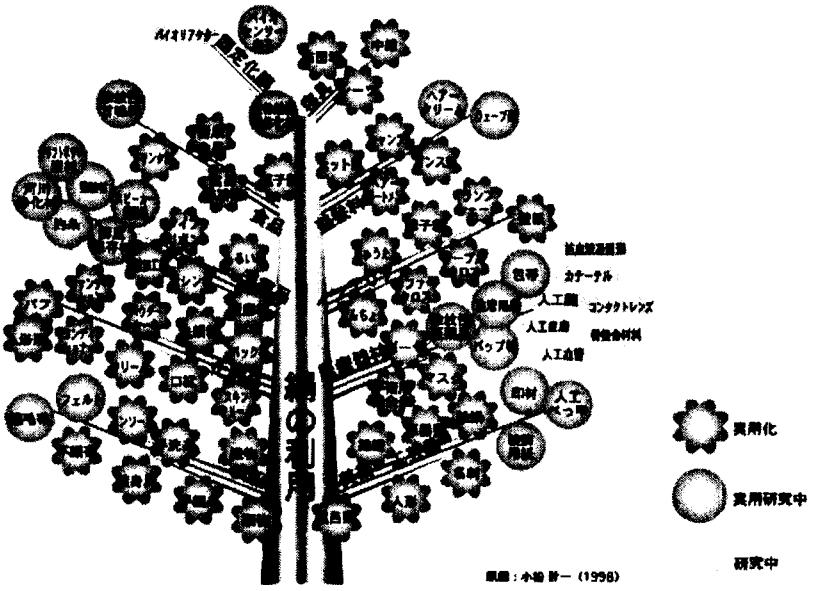

Fig. 3 Developing Uses and Researches of Silks in the Many Fields (K. Komatsu, 1998). There are two categories, textile (in fashion, industry, interior, works of art and medical treatment), and non-textile fields (cosmetics, hair liquids, foods, medical treatment and enzyme-immobilization).

Recently it was found that the *B. mori* fibroin forms molecular complex consisting of heavy (H)-chain, light (L)-chain and P25 [14]. In the last year, the complete primary structure of *B. mori* fibroin H-chain (5263 residues) has been finally reported [15]. In addition, it has been proposed that the silk I structure attributed to pre-crystalline state before fiber formation forms a repeated $\beta$-turn structure [16]. Based on these structural findings for *B. mori* silk fibroin, new application, molecular design of the silk fibroin and continuous production of silk proteins using a genetic technology will be opened in future together with the insect biotechnology with silkworm. In order to expand use of the silk proteins to various fields, it is important to characterize the molecular structure from new insights, to improve the silk proteins and to find new functions in collaboration.

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