Int. J. Indust. Entomol. Vol. 4, No. 1, 2002, pp. 1~4

[Review]

International Journal of *Industrial Entomology*

Functional Silk Proteins: Molecular Structure and Application to Biomaterials

Makoto Demura, Joo Hong Yeo[1,*], Kwang Gill Lee[1] and Yong Woo Lee[1]

Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan.
[1]Department of Sericulture and Entomology, National Institute of Agricultural Science and Technology, RDA, Suwon 441-100, Korea.

(Received 4 January 2002; Accepted 20 January 2002)

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 based on the detailed findings. Novel method for determination of the crystalline structure of silk proteins in an atomic level using nuclear magnetic resonance (NMR) was reviewed. Recent application of silks to biomaterials and prospects for future were discussed.

Key words : Silk protein, Biomaterial, NMR, Crystal structure, Enzyme-immobilization

Introduction

The silk fibers are widely being 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 the crystallization as a protein. Thus, it is possible to make non-fabric materials from the silk proteins such as a film, gel, powder and solution. Another is a molecular recognition for the specific primary structure of the silk proteins including the wild type. In this review, usefulness of the crystallization as a biomaterial, the molecular structure from new insights, recent application of silks and prospects for future will be discussed.

*To whom correspondence should be addressed.
Department of Sericulture and Entomology, National Institute of Agricultural Science and Technology, RDA, Suwon 441-100, Korea. Tel: +82-31-290-8513; E-mail: y610525@rda.go.kr

Useful Crystallization of Silk

We have already studied application of silk fibroin and sericin to biomaterials such as an enzyme-immobilization support (Fig. 1). This new biomaterial was designed based on the property of structural transition. In general, entrapping method for an enzyme-immobilization needs a soluble support and chemical reagents for covalent crosslinking to complete insoluble supports. Contrary, the silk

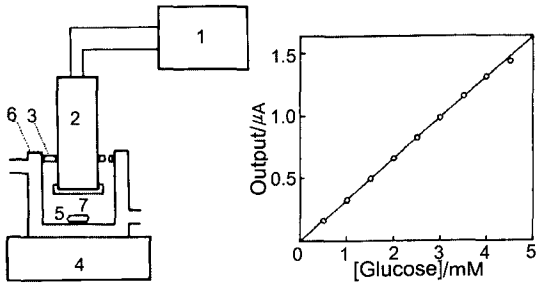

Fig. 1. Application of silk fibroin membrane to biosensor. Glucose oxidase was immobilized in the *B. mori* silk fibroin membrane (7). The enzyme reaction in the fibroin membrane was monitored by oxygen electrode (2). Linear response of the sensor as a function of glucose was obtained. Because of no leakage of enzyme from the membrane and no inactivation, the output amplitude was stabilized for long period (more than 6 months).

protein as a support can entrap enzymes with only self-structural transition which makes weak hydrogen-bond networks, i.e., antiparallel β-sheet structure, resulting formation of non-covalent cross-linkings in the silk proteins. Thus, enzyme activity in the silk is not affected by the chemical modification. Various types of silk supports such as films, powder, gel and coating of silk and sericin on non-woven fabrics have been developed. (Demura *et al.*, 1989a, 1989b, 1991, 1992; Demura and Asakura, 1989, 1991; Asakura *et al.*, 1991, 1993). Figure 1 shows the simple biosensor system which was applied silk fibroin film immobilized glucose oxidase. The linearity of the enzyme reaction indicates a good efficiency of silk fibroin as a biosensor.

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 β-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 numbers and quality of diffraction data (Marsh *et al.*, 1955). 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.

Structural refinement of the silk fibroin at the atomic level has been studied using nuclear magnetic resonance (NMR) spectroscopy (Asakura *et al.*, 1993; Demura *et al.*, 1998; van Beek *et al.*, 2000). 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

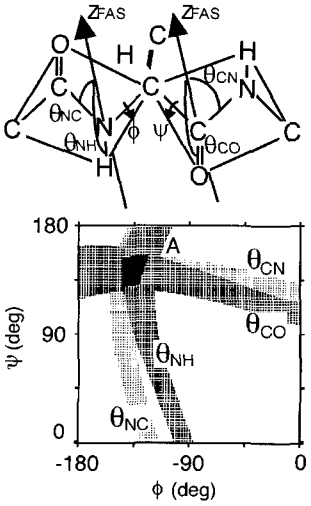

Fig. 2. Conformational space (ϕ, ψ) of Ala residue of the silk fibroin determined from the angular constraints, θs, measured by the angular dependent solid state NMR. The silk fibroin samples labeled with ^{15}N and ^{13}C labeled amino acids were used.

Table 1. Developing applications and basic researches of silk proteins in the many fields

Fields	Applications
Non-textile	
Protein engineering	Silk production from non-insect cell*, Gene targeting*
Enzyme immobilization	Biosensor*, Bioreactor*, Biocatalyst-immobilized materials*
Medical materials	Suture, Gauze, Mask, Controlled-release drugs*, Sanitary napkin*, Bandage*, Anti-blood coagulant*, catheter*, Artificial tendon*, Contact lenses*, Artificial skin*, Osteoadhesive*, Artificial blood vessel*, Soft gel*
Foods	Confectionery, Food additives, Health foods*, Health drink*, Controlled-release sweetener*
Hair liquids	Setting liquid*, Hair treatment, Shampoo, Rinse, Hair cream*, Wave liquid*
Cosmetics	Pack, Skin cream, Facial cleansing, Rouge, Powder cake, Foundation, Conditioner, Puff, Bathing powder
Textile	
Industrial product	Textile treatment*, Sieve, Polisher, Sewing machine thread, Speaker vibrator*, Freshness preservation*, Paint, Fish line*, River purifier*
Clothes	Textile, Knit, Accessories, Felt*, Non-woven fabrics, Bedclothes, Quilt, Inner cotton, Sheet
Interior	Thick curtain, Carpet, Sofa clothes, Shoji paper, Table clothes, Lamp shade, Wallpaper
Industrial art object	String, Wrapper, Doll, Name card, Ornaments, Artificial tortoiseshell*, Impression*, Pictorial art, Musical instrument string

There are two categories, textile and non-textile. The applications putted to practical uses and basic researches (asterisk) are listed.

field direction in order 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 (ϕ, ψ) within the reduced conformational space of Ala and Gly residues were determined as (-140°, 142°) and (-139°, 135°), respectively, within experimental error (±5°). These results were in good agreement with the data from *X*-ray fiber diffraction. Using a similar NMR method, the torsion angles of other minor amino acids, Ser, Tyr and Val within the Gly-X sequence were first determined with the ^{13}C- and ^{15}N-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 (Table 1). There are two categories, textile and non-textile. 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 (Yeo *et al.*, 1999; Kang *et al.*, 2000; Madyarov *et al.*, 2001). 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 (Minoura *et al.*, 1995). 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 (Higuchi *et al.*, 1999), an artificial skin (Yeo *et al.*, 2000, 2001) and a soft oral dosage form for elderly patients with the silk gels (Hanawa *et al.*, 1995) have been reported. The hydrolyzed fibroin peptides, their derivatives and sericin peptides expressed in a bacteria (Tsujimoto *et al.*, 2001) are also interesting application.

Recently it was found that the *B. mori* fibroin forms molecular complex consisting of heavy (H)-chain, light (L)-chain and P25 (Inoue *et al.*, 2000). In 2000, the complete primary structure of *B. mori* fibroin H-chain (5,263 residues) has been finally reported (Zhou *et al.*, 2000). In addition, it has been proposed that the silk I structure attributed to pre-crystalline state before fiber formation forms a repeated β-turn structure (Asakura *et al.*, 2001). 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 use of silkworm as a host cell in the insect biotechnology (Wu *et al.*, 2001; Demura *et al.*, 2002, Arcidiacono *et al.*, 2002). In order to expand use of the silk proteins to various fields, it is important to characterize the molecular structure from the new insights, to improve, and to find new functions of the silk proteins.

References

Arcidiacono, S., C. M. Mello, M. Butler, E. Welsh, J. W. Soares, A. Allen, D. Ziegler, T. Laue and S. Chase (2002) Aqueous processing and fiber spinning of recombinant spider silks. *Macromolecules* **35**, 1262 - 1266.

Asakura, T., M. Demura and H. Ogawa (1991) K. Matsushita and M. Imanari, NMR imaging of diffusion of small organic molecules in silk fibroin gel, *Macromolecules* **24**, 620-622.

Asakura, T., M. Kitaguchi, M. Demura, H. Sakai and K. Komatsu (1992) Immobilization of glucose oxidase on non-woven fabrics with *Bombyx mori* silk fibroin gel. *J. Appl. Polym. Sci.* **46**, 49-53.

Asakura, T., J. H. Yeo, M. Demura, T. Itoh, T. Fujito, M. Imanari, L. K. Nicholson and T. A. Cross (1993) Structural analysis of uniaxially aligned polymers using solid state ^{15}N NMR. *Macromolecules* **26**, 6660-6663.

Asakura, T., J. Ashida, T. Yamane, T. Kameda, Y. Nakazawa, K. Ohgo and K. Komatsu (2001) A repeated b-turn structure in poly(Ala-Gly) as a model for silk I of *Bombyx mori* silk fibroin studied with two-dimensional spin-diffusion NMR under off magic angle spinning and rotational echo double resonance. *J. Mol. Biol.* **306**, 291-305.

Demura, M. and T. Asakura (1989) Immobilization of glucose oxidase with *Bombyx mori* silk fibroin by only stretching treatment and its application to glucose sensor. *Biotechnol. Bioeng.* **33**, 598-603.

Demura, M., T. Asakura and T. Kuroo (1989a) Immobilization of biocatalysts with *Bombyx mori* silk fibroin by several kinds of physical treatment and its application to glucose sensor. *Biosensors* **4**, 361-372.

Demura, M., T. Asakura and E. Nakamura (1989b) Immobilization of peroxidase with *Bombyx mori* silk fibroin membrane and its application to biophotosensor. *J. Biotech.* **10**, 113-120.

Demura, M. and T. Asakura (1991) Porous membrane of *Bombyx mori* silk fibroin: Structure characterization, physical properties and application to glucose oxidase- immobilization. *J. Membr. Sci.* **59**, 39-52.

Demura, M., T. Komura and T. Asakura (1991) Membrane potential of *Bombyx mori* silk fibroin membrane induced by an immobilized enzyme reaction. *Bioelectrochem. Bioenerg.* **26**, 167-175.

Demura, M., T. Takekawa, T. Asakura and A. Nishikawa (1992) Characterization of low temperature plasma-treated silk fibroin fabrics by ESCA and the use of the fabrics to an enzyme-immobilized support. *Biomaterials* **13**, 276-280.

Demura, M., M. Minami, T. Asakura and T. A. Cross (1998) Structure of *Bombyx mori* silk fibroin based on solid state NMR orientational constraints and fiber diffraction unit cell parameters. *J. Am. Chem. Soc.* **120**, 1300-1308.

Demura, M., M. Sato, S. Nagaoka, T. Asakura, N. Kamo and K. Nitta (2002) in preparation.

Hanawa, T., A. Watanabe, T. Tsuchiya, R. Ikoma, M. Hidaka and M. Sugihara (1995) New oral dosage form for elderly patients. II. Release behavior of benfotiamine from silk fibroin gel. *Chem. Pharm. Bull. (Tokyo)* **43**, 872-876.

Higuchi, A., S. Uchiyama, M. Demura,T. Asakura, C. S. Cho, T. Akaike, H. Takarada and M. Hara (1999) Enhanced CEA production associated with aspirin in a culture of CW-2 cells on some polymeric films. *Cytotechnology* **31**, 233-242.

Inoue, S., K. Tanaka, F. Arisaka, S. Kimura, K. Ohtomo and S. Mizuno (2000) Silk fibroin of *Bombyx mori* is secreted, assembling a high molecular mass elementary unit consisting of H-chain, L-chain, and P25, with a 6:6:1 molar ratio. *J. Biol. Chem.* **275**, 40517-40528.

Kang G. D., J. H. Nahm, J. S. Park, J. Y. Moon, C. S. Cho and J. H. Yeo (2000) Effects of poloxamer on the gelation of silk fibroin. *Macromol. Rapid. Commun.* **21**, 788-791.

Madyarov S. R., J. H. Yeo, K. W. Lee and Y. W. Lee (2001) A comparison of silk fibroin hydrolysates by hydrochloric acid and proteolytic enzymics. *Int. J. Indust. Entomol.* **2**, 7-13.

Marsh, R. E., R. B. Corey and L. Pauling (1955) An investigation of the structure of silk fibroin. *Biochim. Biophys. Acta* **16**, 1-35.

Minoura, N., S. Aiba, M. Higuchi, Y. Gotoh, M. Tsukada and Y. Imai (1995) Attachment and growth of fibroblast cells on silk fibroin. *Biochem. Biophys. Res. Commun.* **208**, 511-516.

Tsujimoto, K., H. Takagi, M. Takahashi, H. Yamada and S. Nakamori (2001) Cryoprotective effect of the serine-rich repetitive sequence in silk protein sericin. *J. Biochem. (Tokyo)* **129**, 979-986.

Wu, X., K. Kamei, H. Sato, S. I. Sato, R. Takano, M. Ichida, H. Mori and S. Hara (2001) High-level expression of human acidic fibroblast growth factor and basic fibroblast growth factor in silkworm (*Bombyx mori* L.) using recombinant baculovirus. *Protein Expr. Purif.* **21**, 192-200.

van Beek, J. D., L. Beaulieu, H. Schafer, M. Demura, T. Asakura and B. H. Meier (2000) Solid-state NMR determination of the secondary structure of *Samia cynthia ricini* silk. *Nature* **405**, 1077-1079.

Yeo, J. H., K. G. Lee, Y. W. Lee, J. H. Nam and S. Y. Kim (1999) Changes of silk protein compositions by solubility condition. *Analy. Sci. & Tech. (Korea)* **12**, 306-311.

Yeo, J. H., K. G. Lee, H. C. Kim, Y. L. Oh, A. J. Kim and S. Y. Kim (2000) The effect of Pva/chitosan/fibroin (PCF)-blended spongy sheets on wound healing in rats. *Biol. Pharm. Bull.* **23**, 1220-1223.

Yeo, J. H., K. G. Lee and Y. W. Lee (2001) Collagen growth effects in rats using *B. mori* fibroin. *Korean J. Seric. Sci.* **43**, 49-52.

Zhou, C. Z., F. Confalonieri, N. Medina, Y. Zivanovic, C. Esnault, T. Yang, M. Jacquet, J. Janin, M. Duguet, R. Perasso and Z. G. Li (2000) Fine organization of *Bombyx mori* fibroin heavy chain gene. *Nucleic Acids Res.* **28**, 2413-2419.