Structures of Silks and Silk-like Materials Studied with Solid-state NMR, and Application to Biomaterials

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Today, development of excellent biomaterials becomes very important. For example, rapid development of cell engineering needs excellent biomaterials as scaffold.
Silk has been used as excellent textures for long times, but it has been also used as suture because of the high tensile properties and high biocompatibility. Therefore, silk will be a good candidate for such biomaterials. The process for development of silks for biomaterials is summarized in Figure 1 as a flowchart.

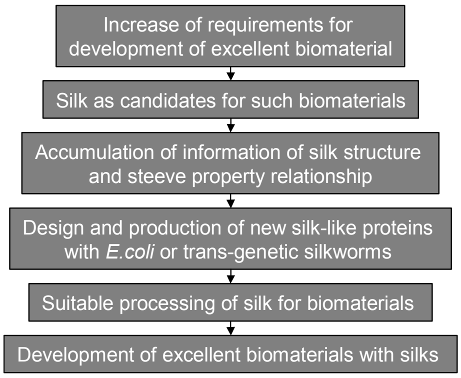

Fig.1 flowchart of development of biomaterials with silk

There are several kinds of silkworms including spiders to produce silk fibers with different physical properties. This is also suitable for biomaterials because a variety of properties are required depending on the purpose. In addition, development of biotechnology becomes possible to produce new-silk like materials which are suitable for the biomaterials. Thus, it is necessary to accumulate information on structure and property of many silks as much as possible.

Solid-state NMR is suitable to characterize silk fibers because it is difficult to prepare single crystals for structural determination with X-ray diffraction method. In addition, the structure of such silk fiber is basically β-sheet in the solid state and random coil in aqueous solution. Therefore solution NMR is not available for the structural determination.

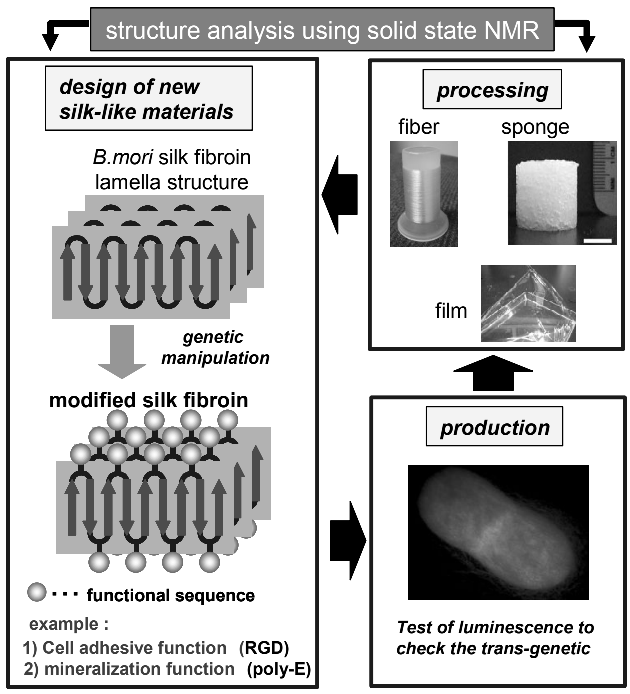

Fig.2 strategy and roadmap of silk and silk-like materials to apply the biomaterials

In this talk, the structural determination of *Bombyx mori* silk fibroin which is a well-known silk fiber will be described. In general, there are many repeated amino acid sequences in the primary structure of silk fibroins and therefore the use of model peptides including stable-isotope labeling of appropriate positions is very useful. Several solid state NMR techniques including ^{13}C chemical shift, 2D spin-diffusion NMR, RFDR, REDOR and relaxation could be used for the structural determination. Two

固体高分解能 NMR, 遺伝子組み換えカイコ, ラメラ構造, 親水性残基, 細胞接着性配列
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distinct structures of *B.mori* silk fibroin in the solid state have been proposed as Silk I (Silk structure before spinning) and Silk II (Silk structure after spinning). Our NMR studies reveal that the conformation of Silk I is a repeated type II β-turn and Silk II is a heterogeneous structure with mainly β-sheet structure.

Then, the structure determination with sold-state NMR will be also described for silk fibroins from wild silkworms such as *Samia cynthia ricini* or *Anaphe* together with silks from spiders. Especially, Alanine oligomers are model peptides of the crystalline regions of *S.c.ricini* or *Nephila clavata* (a spider) and the structural determination for a series of alanine oligomers gives very precise information on the anti-parallel and parallel β-structures.

On the basis of these structural information on silk, we can design new-silk like materials. For example, as shown in Figure 2, it was proposed by us that the model peptide, poly(Ala-Gly) for the crystalline region of *B. mori* silk fibroin with Silk II form, took lamella structure by using ^{13}C site-specific labeling peptide samples and several ^{13}C solid state NMR. The proposed lamella structure is suitable to design silk-like materials with several functional groups. In the lamella structure, the stem part contributes to the stiffness through inter-molecular hydrogen bond formation and the turn part is possible to be sites for introduction of functional groups. The production of silk-like proteins with repeated such sequences was possible with *E. coli* and transgenic silkworms using biotechnology.

Silks and silk-like materials can be used in several forms, long fiber, nonwoven-fiber, film and sponge after appropriate processing process as shown in Figure 2. Solid-state NMR can be also used to characterize the structure and to monitor the structural change in order to develop the silk-based scaffold.

The development in the hardware of solid-state NMR was performed in our laboratory to study the structure and structural change of silk and silk-like materials. Namely, we have developed a higher sensitive probehead for mass-limited sample by using small coil and rotor (1mmΦ). Figure 3 shows an example of application to investigate the properties of silk fiber using this probehead. The strength of silk fiber is an important property for suture and the structure of silk fibroin fiber should be uniform. We observed fracture of silk by our 1mmΦ rotor and it is found that the fractured fiber gives wide line with according to the variation of structure. It means in the fracture of fiber is not only with β-sheet and it cause the weakness of fiber. These approaches could not be possible with ordinary solid state NMR. Another example, the structure of biomaterial coated with silk are investigated by 1mmΦ rotor because the coated silk is very small quantities compare with standard sample.

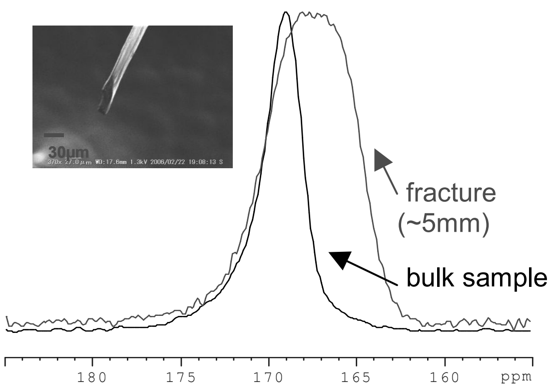

Fig.3 ^{13}C CP/MAS spectra of [1-^{13}C]Gly *s.c. ricini* fibroin fiber and fracture using 1mmΦ MAS

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