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Silk structure studied with nuclear magnetic resonance

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Abbreviations: CP-MAS, cross polarization magic angle spinning; DD-MAS, dipolar decoupling magic angle spinning; CSA, chemical shift anisotropy; DARR, ^{13}C–^{13}C correlation spectra with dipolar assisted rotational resonance; WISE, ^{1}H–^{13}C wideline separation; HETCOR, heteronuclear correlation; TPPM, two pulse phase modulation; CRAMPS, combined rotation and multiple pulse spectroscopy; FSLG, frequency-switched Lee-Goldburg; DQ/SQ, double-quantum/single-quantum; INADEQUATE, incredible natural abundance double quantum transfer experiment; DOQSY, double quantum spectroscopy; DECODER, direction exchange with correlation for orientation-distribution evaluation and reconstruction; REDOR, rotational-echo double resonance; HR-MAS, high-resolution magic angle spinning; HSQC, heteronuclear single quantum correlation; INEPT, insensitive nuclei enhanced by polarization; MD, molecular dynamics; MM, molecular mechanics; Silk I, state of silk fibroin before spinning; Silk II, state of silk fibroin after spinning; MaSp1, dragline silk spidroin 1; MaSp2, dragline silk spidroin 2.

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1. Introduction

Recently, considerable attention has been paid to silks by a range of scientists from textile engineers to polymer chemists and biomedical researchers. The application of native silk and recombinant silk as biomaterials is a particularly active area. Silk is an attractive biomaterial because of its excellent mechanical properties, that is, the combination of strength and toughness not found in today's man-made materials together with its excellent biocompatibility. These appealing physical properties originate from the silk structure and therefore, structural analysis will be the key to developing silk as a viable biomaterial. The well-developed X-ray diffraction (XRD) technique was expected to clarify the atomic level structure, but the analysis cannot be used for this purpose because it is difficult to obtain the single crystals that are required to solve the complete molecular structure of silk. NMR is a very effective method to obtain such information because it does not rely on single crystals or long-range periodicity. Solution NMR spectroscopy can be used to clarify the atomic level solution structure of proteins and peptides through the determination of the NMR parameters, such as chemical shifts, NOEs, spin-coupling constants, deuterium exchange rates. These can be applied to clarify the silk protein structure in the aqueous solution stored within the silkworm or spider silk producing glands. For silk samples in the solid-state (fibers, powders or films), solid-state NMR can be used. Solid-state NMR is a very effective tool and has been extensively used for the structural characterization of silk fibers.

For example, the structure of *Bombyx mori* (*B. mori*) silk fibroin before spinning (Silk I), which was in controversy for a long time, was determined to be a repeated type II β-turn structure with a combination of results from several solid-state NMR experiments, that is, quantitative use of ^{13}C chemical shifts, REDOR and spin-diffusion NMR together with appropriate stable isotope-labeled model peptides and stable isotope-labeled silk fibroin itself [1]. This structure can also explain the X-ray powder pattern of Silk I. In contrast to the relatively homogeneous Silk I structure, the structure of *B. mori* silk fibroin after spinning (Silk II) is very heterogeneous and this structure was also obtained from solid state NMR studies [2]. Based on these structures, it was necessary to revise the simple anti-parallel β-sheet structure reported by Marsh et al. [3] on the basis of the limited X-ray diffraction data more than half a century ago. The determination of both silk structures, Silk I and Silk II, is the key to understanding why the silkworm can produce such impressive fibers from the aqueous solution of silk fibroin.

There are several silks with different structural characteristics and NMR, particularly solid-state NMR, can give considerable information on their molecular structure that can then be used for the molecular design of recombinant silk for use as biomaterials and also for further advancing the use of native silk itself. In addition to silkworm silks, spider silks are also very interesting materials because being extremely strong and elastic gives them unrivaled toughness. Thus, the structural analysis of spider silks with solid state NMR will be important to understanding the molecular origin of the silk's impressive mechanical properties. A number of solid-state NMR techniques have been applied to the structural analysis of spider silk [4].

Asakura and Zhao have previously reviewed work on silk NMR [5] covering journal articles published till 2001. In this review of the structural analysis of silks, we will primarily cover journal articles that have appeared in the literature from 2002 to the present.

In Section 2 we review a wide variety of solid-state NMR techniques used for silk work including several new techniques that have recently been applied to determine the structure of silk. The works about *B. mori* silk fibroin structure before and after spinning were published from Asakura's group and these are reviewed in Section 3. Section 4 is a basic study of the *B. mori* silk model peptide structure with Silk II form and also shows an example of how such a basic study on lamella structure is useful in developing the molecular design principles for new biomaterials. Section 5 is the structural analysis of another silk, *Samia cynthia ricini* (*S. c. ricini*) whose primary structure consists of alternate polyalanine (PLA) and Gly-rich regions. The structural analyses of other wild silks, *Antheraea pernyi* (*A. pernyi*) and *Anaphe* silks are described in Section 6. Active research on the structure of spider silk studied with recently developed NMR techniques is covered together with model peptide studies in Section 7. Finally, Section 8 describes some basic information on PLA oligomers which are crystalline domains in spider silk and many wild silk fibroins.

2. NMR methods used for silk study

A wide variety of solid-state NMR techniques have recently been applied to determine the structures of silks in the solid state together with conventional solution NMR used for silk studies in solution.

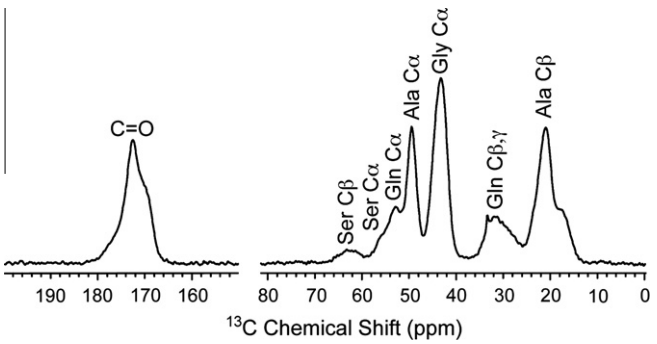

Fig. 1. ^{13}C CP-MAS NMR spectrum at 18.8 T of major ampullate silk from the *N. clavipes* spider [4].