



Evidence of α -helical coiled coils and β -sheets in hornet silk



Tsunenori Kameda^a, Takashi Nemoto^b, Tetsuya Ogawa^b, Masatoshi Tosaka^c, Hiroki Kurata^b, Andreas K. Schaper^{d,*}

^a National Institute of Agrobiological Sciences (NIAS), Tsukuba, Ibaraki 305-8634, Japan

^b Laboratory of Electron Microscopy and Crystal Chemistry, Advanced Research Center for Beam Science, Institute for Chemical Research, Kyoto University, Uji, Kyoto-fu 611-0011, Japan

^c Laboratory of Polymer Controlled Synthesis, Institute for Chemical Research, Kyoto University, Uji, Kyoto-fu 611-0011, Japan

^d Center for Materials Sciences, EM&Mlab, Philipps University of Marburg, 35032 Marburg, Germany

ARTICLE INFO

Article history:

Received 30 September 2013

Received in revised form 10 December 2013

Accepted 11 December 2013

Available online 15 December 2013

Keywords:

α -Helical coiled coils

β -Sheet structure

Pitch and periodicity

Cryo-electron and X-ray diffraction

Giant hornet silk

ABSTRACT

α -Helical coiled coil and β -sheet complexes are essential structural building elements of silk proteins produced by different species of the *Hymenoptera*. Beside X-ray scattering at wide and small angles we applied cryo-electron diffraction and microscopy to demonstrate the presence and the details of such structures in silk of the giant hornet *Vespa mandarinia japonica*. Our studies on the assembly of the fibrous silk proteins and their internal organization in relation to the primary chain structure suggest a 172 Å pitch supercoil consisting of four intertwined alanine-rich α -helical strands. The axial periodicity may adopt even multiples of the pitch value. Coiled coil motifs form the largest portion of the hornet silk structure and are aligned nearly parallel to the cocoon fiber axis in the same way as the membrane-like parts of the cocoon are molecularly orientated in the spinning direction. Supercoils were found to be associated with β -crystals, predominantly localized in the L-serine-rich chain sequences terminating each of the four predominant silk proteins. Such β -sheet blocks are considered resulting from transformation of random coil molecular sequences due to the action of elongational forces during the spinning process.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Natural silk is anything but a uniform material. The evolution of silk-producing animals goes hand in hand with the development of different silk structures with properties appropriate for each species. The principal structure of proteins, α -helix and β -sheet (Eisenberg, 2003), are also found in silk. Axial β -sheets and collagen-type helices are dominant in the fibrous silk proteins of *Lepidoptera* (silkworms) and *Araneae* (spiders) whereas α -helical coiled coils and β -sheet elements are present in silk from *Hymenoptera*, particularly of the *Aculeata* group including the *Apoidea* (bees) and *Vespoidea* (ants, wasps, hornets).

The primary protein structure and the resulting properties of silk from *Vespa* hornets have been studied in detail using expressed sequence tag (EST) analysis, high-resolution ¹³C solid-state NMR, and mechanical deformation experiment by Sezutsu et al. (2007), Kameda et al. (2010), and Kameda (2012). The 33–46 kDa sized hornet silk proteins are characterized by an alternating hydrophilic and hydrophobic amino acid pattern but do not show regular amino acid repeat sequences with the exception of a short sequence in one of the five identified proteins. This is in striking contrast to the

highly repetitive protein structure in silk from both silkworms and spiders. As a result of the lack of such sequence repeats, α -helices and α -helical coiled coils appear as the typical secondary and tertiary structure.

According to the fundamental analysis by Crick (1952, 1953) and Pauling and Corey (1953), most of the coiled coil-forming α -helices are characterized by a heptad residue repeat sequence $(abcdefg)_n$ with hydrophobic side chains in positions *a* and *d*. One turn of a single α -helical strand within a multi-stranded supercoil has a predicted height of about 5.1 Å against ~5.4 Å of a straight single α -helix strand. That is the result of the inclination of the twisted helix axes at crossing angles up to ~20° depending on the constitution of the primary α -helix and on the number of strands in the supercoil. Structures forming a supercoil of polypeptide chains with five and more strands have been proposed (Lupas and Gruber, 2005; Moutevelis and Woolfson, 2009).

Pioneering work on helix-type structures in silk of *Hymenoptera* was done by Atkins (1967) and Lucas and Rudall (1968) in the last century. Our knowledge of coiled coils in the *k-m-e-f* (keratin-myosin-epidermin-fibrinogen) family is based in large part on the fundamental X-ray scattering studies by McArthur, Astbury, Fraser and others using both keratin (McArthur, 1943; Astbury, 1943; Bear and Rugo, 1951; Crick, 1952; Huggins, 1957; Fraser and MacRae, 1958; Fraser et al., 1964) and myosin (Cohen and Holmes, 1963; Elliott et al., 1968). The helical nature of fibrous

* Corresponding author. Fax: +49 6421 28 23383.

E-mail address: schaper@staff.uni-marburg.de (A.K. Schaper).

proteins and their characteristics were well described by Fraser and MacRae (1973a) and Squire (1981). The striking diversity in conformations of coiled coils and their remarkable properties have stimulated silk research in recent time (Parry et al., 2008). Different coiled coil structures are now biochemically designed *de novo* using crystallographic principles (Papapostolou et al., 2007), or they are produced by recombinant synthesis using fermentation in *Escherichia coli* (Sutherland et al., 2011).

This paper investigates silk from the giant hornet *Vespa mandarinia japonica* using electron diffraction and imaging as well as X-ray scattering techniques. The investigated silk was spun by hornet larvae who wrap themselves into a silky cocoon to protect their metamorphosis inside the cells of the comb-like nest which is fabricated by the adult hornet. Hornet silk's properties, especially its mechanical stability, the dielectric and the thermal behavior (Ishay et al., 1994), are unique and so is its hierarchical physical fine structure. We provide a structural model of the oriented α -helically coiled coil fiber proteins and discuss the functional contribution of β -sheet regions.

2. Materials and methods

2.1. Sample material

Nests of Japanese giant hornet *Vespa mandarinia japonica* were collected from a field in the city of Tsukuba Japan, in the autumn of 2012. Cocoons produced by the pupating larvae of the hornet were carefully gathered from the comb cells. The silk material consisted of individual cocoon fibers and of thin film portions. Within the cocoon fabric the films bridge the space between neighboring fibers like sails, or form a continuous overlay or may even completely mask the fibers. They are of the same material as the woven cocoon fibers. The samples for the ED studies were prepared either by positioning a copper grid with a supporting carbon film in front of the larva's mouth directly to collect the as-spun silk, or finished cocoon silk was fixed in a sandwich copper grid. Coating was used with a thin layer of aluminum or gold as diffraction standard. The samples prepared for the X-ray measurements were bundles of twenty silk fibers each $\sim 10 \mu\text{m}$ in diameter cut to $\sim 3\text{--}5 \text{ mm}$ length and mounted on the X-ray specimen holder in a parallel fashion.

2.2. Electron diffraction and imaging

Samples were examined by selected-area electron diffraction (SAED) and imaged in a JEM-2100F(G5) (JEOL Ltd., Tokyo, Japan) transmission electron microscope (TEM) operated at 200 keV and ultra-low temperature of liquid helium (4.2 K). Such cryo-conditions provide optimal protection of the internal ordering structure of organic materials (Schaper et al., 2006). An UltraScanTM 2 k \times 2 k pixels high-resolution slow scan CCD Camera with HCRTM phosphor scintillator (Gatan Inc., Warrendale, Pleasanton, CA) was used for image recording. Only the thinnest parts $< 1 \mu\text{m}$ thick of the silk fiber and film sample pieces were suitable for microscopy. The outer morphology of the silk cocoons was characterized by scanning electron microscopy (SEM) using a JSM-7500F cold-field emission microscope (Jeol Ltd., Tokyo, Japan) at a voltage of 2 keV under gentle beam conditions.

2.3. Wide-angle and small-angle X-ray diffraction (WAXD, SAXD)

X-ray diffraction experiments were done with a Rigaku Micro-Max-007HF High Intensity Microfocus Rotating Anode X-ray Generator (Rigaku Corporation, Shibuya-Ku, Tokyo, Japan) operating at 40 kV and 30 mA, and equipped with a Dectris 100 K Pilatus recording system (DECTRIS Ltd., Baden, Switzerland). Silver

behenate (Eastman Kodak Comp., Rochester, New York, USA) was used as reference.

3. Results and discussion

3.1. Coiled coil axial periodicity

Both the fairly sharp reflection arc on the meridian of the electron diffraction patterns in Fig. 1a,b, belonging to a spacing of 5.1 Å, and the broad equatorial intensity maximum related to a spacing of $\sim 9 \text{ Å}$, are characteristic diffraction features of oriented coiled-coil structures (Squire, 1981; Parry et al., 2008). The meridional arc at $1/5.1 \text{ Å}^{-1}$ corresponds to nearly one turn of the α -helix in a coiled-coil, whereas the pitch of the straight undistorted α -helix is $1/5.4 \text{ Å}^{-1}$ (Crick, 1952, 1953; Pauling and Corey, 1953; Brown and Trotter, 1956). According to Pauling's original prediction one turn of the α -helix comprises 3.6 residues, on average, with an axial rise per residue of 1.5 Å (Pauling et al., 1951). X-ray studies by Perutz (1951) supported this, Bamford et al. (1954) stated 1.495 Å as correct residue spacing of α -poly-L-alanine.

In Fig. 1a,b the broad first maximum on the equator covers a range of d -spacings, however the second harmonic at $1/4.6 \text{ Å}^{-1}$, seen in Fig. 1b, confines the relevant peak spacing to 2×4.6 or 9.2 Å. The occurrence of the second order equatorial peak is evidence of very regular packing and lateral size of the α -helices in the supercoil (see discussion below).

The diffraction patterns in Fig. 1 originate from a thin membrane-like part of the silk cocoon. The morphology and genesis of those films or sheets, which often appear in connection with the cocoon fibers, was described by Joseph and Ishay (2004) for silk from growing larvae of the Oriental hornet, and by Sutherland et al. (2011) for silk fibers from European wasp larvae. The SEM micrograph in Fig. 2a illustrates similar features in silk of the hornet *Vespa mandarinia japonica*. The different silk forms investigated in this study are: the individual cocoon fiber (Fig. 2b), the fibrillated silk layer (Fig. 2c), and the continuous film (Fig. 2d,e). We did not find any significant differences in the molecular orientation and ordering between these three forms.

The wide-angle X-ray diffraction (WAXD) pattern of a fiber bundle of *Vespa mandarinia* silk in Fig. 3a confirms the characteristic features observed by electron diffraction of the coiled-coil structure. In the small-angle (SAXD) range $2\theta < 5.5^\circ$, using 1 h (Fig. 3b') and 10 h (Fig. 3b) exposure time, distinct new meridional reflection arcs are resolved with the highest intensity at 3.08° relating to a spacing of 28.7 Å. Taking this value along with the additional reflections on successive layer lines shown in the intensity profile in Fig. 3c and listed in Table 1, an overall axial period (R)

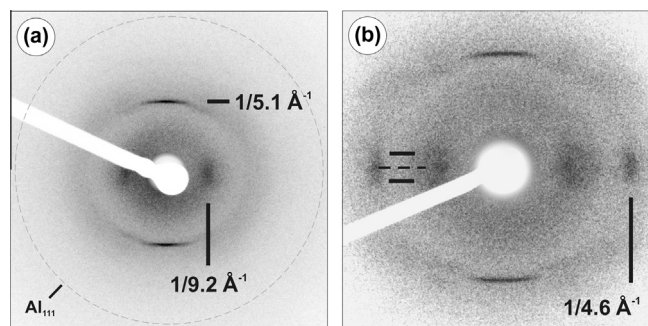


Fig. 1. ED from two different pieces of *V. mandarinia* giant hornet silk showing in (a) typical features of a coiled-coil structure with the 5.1 Å meridional arc and the broad equatorial maximum around 9.2 Å, and in (b) the additional second order equatorial maximum at $\sim 4.6 \text{ Å}$, and weak near-equatorial scattering maxima at layer line spacing $\sim 1/4.6 \text{ Å}^{-1}$ above and below the 9.2 Å peak (see line markings).

Download English Version:

<https://daneshyari.com/en/article/5914020>

Download Persian Version:

<https://daneshyari.com/article/5914020>

[Daneshyari.com](https://daneshyari.com)