EUROPEAN SCIENCE FOUNDATION

ESF Network on Silk: Properties and Production

3rd Annual Workshop on:
"Assembly, Structure and Properties of Fibrous Proteins with Emphasis on Silk"

Villa Olmo, Como (Italy), 19-21 October 2001

PROGRAM
PARTICIPANTS
ABSTRACTS

ESF SILK WORKSHOP, Como, October 19-21, 2001

"Assembly, Structure, and Properties of Fibrous Proteins, with Emphasis on Silk"

Thursday, Oct. 18, 2001

20.30 Dinner at Villa Olmo
21.30 Informal get-together

Friday, Oct. 19, 2001

9.30-10.00	*Welcome*: Stazione Sperimentale per la Seta, Prof. B. Marcandalli The Italian silk industry – An overview
10.00-11.00	*E. ATKINS – P. SIKORSKI* Transformation of liquid crystalline pre-silk to metastable Silk I and to the insoluble and stable beta-Silk II studied by molecular modeling techniques

11.00-11.30 Coffee break

11.30-12.00	*D. KNIGHT* Are silks liquid crystal elastomers?
12.00-12.30	*B. MEIER* NMR of liquid and solid silk proteins: waht can we learn about structure

12.30-14.30 Lunch

14.30-15.00	*C. RIEKEL* Local structure in polymeric materials investigated by X-ray microdiffraction
15.00-15.30	*N.POUCHKINA-STANTCHEVA* Identification of novel spider silk genes
15.30-16.00	*J.P. RIGUEIRO* Influence of water on the tensile properties of Bombyx mori and Attacus atlas Silk

16.00-16.30 Coffee break

20.00 Dinner

Saturday, Oct. 20, 2001

9.30-10.00	*H. ZAHN* Keratins: role of stress-supporting disulfide bonds during permanent setting, Supercontraction and alpha-beta transition
10.00-11.00	*F. SEHNAL* Unusual fibroin structure in the waxmoth, Galleria mellonella: Implications for fiber formation

11.00-11.30 Coffee break

11.30-12.00	*K.-H. GUEHRS* Biotechnology of silk proteins – Recent contributions from a German silk collaboration
12.00-12.30	*A. CHINALI* Reconsidering the molecular size of the spider dragline silk protein MaSp1: possible biological meanings

12.30-14.30 Lunch

14.30-15.00	*K. GARDNER* Wanderings through nature
15.00-16.00	*ROUND TABLE*

16.00-16.30 Coffee break

16.30-17.00	*Concluding remarks* The ESF Silk Network: outcomes and future perspectives
17.00-18.00	Meeting of the Stearing Committee

20.00 Dinner

Sunday, Oct. 21, 2001

10.30-12.00	Visit to the Silk Museum

12.30 Lunch

ESF Silk Workshop, Como, Oct. 19-21, 2001
List of participants

Name	Institute or Company	E-mail
Edward Atkins	Physics Department University of Bristol, Bristol, UK	E.Atkins@bristol.ac.uk
Alessandro Berlin	Stazione Sperimentale per la Seta, Milano, Italy	laboratory@ssiseta.it
Gérard Chavancy	INRA/UNS, La Mulatiere, Lyon, France	chavancy@pop.univ-lyon1.fr
Alberto Chinali	Institut fuer Molekulare Biotechnologie, Jena, Germany	chinali@imb-jena.de
Gian Maria Colonna	Stazione Sperimentale per la Seta, Milano, Italy	colonna@ssiseta.it
Giuliano Freddi	Stazione Sperimentale per la Seta, Milano, Italy	freddi@ssiseta.it
Kenn Gardner	DuPont	gardner@ill.fr
Karl-Heinz Guehrs	Institut fuer Molekulare Biotechnologie, Jena, Germany	kguehrs@imb-jena.de
Monika Hronsky	Lab. For Physical Chemistry, Zurich, Switzerland	moni@nmr.phys.chem.ethz.ch
David Knight	Oxford University, Dept. Of Zoology, UK	Knight@tegdown.u-net.com
Maria Rosaria Massafra	Stazione Sperimentale per la Seta, Milano, Italy	massafra@ssiseta.it
Beat Meier	Lab. For Physical Chemistry, Zurich, Switzerland	beme@nmr.phys.chem.ethz.ch
N.Pouchkina-Stantcheva	University of York, UK	nps2@york.ac.uk
Christian Riekel	ESRF, Grenoble, France	Riekel@esrf.fr
J-Perez Rigueiro	Dep. De Ciencia de Materiales, Univ. Politec. de Madrid, Spain	jperez@mater.upm.es
Frantisek Sehnal	Entomology Institute, Czech Republic	sehnal@entu.cas.cz
Pawel Sikorski	Polymer Physics Group University of Bristol, UK	P.sikorski@bristol.ac.uk
Fritz Vollrath	Department of Zoology, Oxford University, UK	fritz.Vollrath@zoo.ox.ac.uk
Helmuth Zahn	DWI, Aachen, Germany	Zahn@dwi.rwth-aachen.de
Nicholas Walker	CRL Ltd, UK	nwalker@crl.co.uk

STRUCTURAL TRANSFORMATIONS IN SILK. A MOLECULAR MODEL FOR LIQUID-CRYSTALLINE PRE-SILK AND ITS RELATIONSHIP TO SILK II AND SILK I

Pawel Sikorski & Edward Atkins
Physics Department, University of Bristol, Tyndall Avenue, BRISTOL BS8 1TL, UK

A molecular model has been deduced for the liquid-crystalline phase of silk (PRE-SILK) present in the gland adjacent to the to spinneret. The model is focused on the domains of the silk molecule that have the consensus repetitive sequence [AGAGSG] for Bombyx mori and $[A]_n$ for Tussah and many spider silks, and when spun into fibers forms the SILK II crystalline regions in silk.
The anhydrous SILK II crystalline structure(s) have been established in detail for silks. We have taken these SILK II structures and using a computer modeling procedure steadily forced water molecules into the crystalline lattice, hydrating the structure in a set of equilibrium phases until it has the properties and patterns of behavior of PRE-SILK. The hydration level needed was found to be 30% w/w (1.5 water molecules per peptide). If this model is allowed to dehydrate under conditions similar to that found in extensional flow, the PRE-SILK model transforms into the original SILK II anhydrous crystalline structure. If the PRE-SILK structure was allowed to partially dehydrate, at 2% w/w water content, a structure that exhibits similar properties to SILK I forms. This structure will be discussed in relation to anhydrous SILK I structures published in the literature.

Reconsidering the molecular size of the spider dragline silk protein MaSp1: possible biological meanings.

Alberto Chinali, Karl-Heinz Gührs and Frank Grosse.
Institut für Molekulare Biotechnologie, Abt. Biochemie. Beutenbergstrasse 11, 07743 Jena, Germany.

In the past decade increasingly more data has been accumulating on spider silk and in particular on the dragline silk of the *Nephila* spiders, due to its notable physical properties. For one of the (at least) two protein components of this silk, Major Ampullate Spidroin 1 (MaSp1), different and discordant data on the molecular size have been published.

Here we present preliminary RNA and DNA data that shed a new light on the actual size of this protein. Our data points to the presence of a mRNA of ca. 17kb; the protein encoded by this messenger would present a molecular size that is almost double than the one more commonly observed by protein analysis of either silk or gland content.

We discuss possible hypothesis that might explain this data, how they could be experimentally confirmed and the significance they might hold in the biology of *Nephila* spiders and in the biosynthesis of this silk.

Biotechnology of silk proteins – Recent contributions from a German silk collaboration

Karl-Heinz Gührs and Frank Grosse

Institut für Molekulare Biotechnologie e.V.
Beutenbergstr. 11
D-07745 Jena
http://www.imb-jena.de
kguehrs@imb-jena.de

Abstract:
A brief summary of the studies of silk proteins in the Jena labs is given. Analyses have dealed with the structure of silk genes and silk proteins as well as with recombinant production of silks. Recent data of recombinant expression of silk proteins are shown and discussed in terms of promise and limitations of microbial systems.

The results of a collaboration of the lab with plant biotechnologists to overcome the limitations of bacterial expression are presented.

In the last part, potential methods to evaluate models of silk protein structure in solution will be proposed. These tools could be suited to study the process of association of recombinantly engineered silk proteins.

On the way to characterising spider silk material in the major ampulate gland of N. Edilis using synchrotron radiation circular dichroism.

John M. Kenney[1], Cedric Dicko[1,2] and Fritz Vollrath[1,2].

1. ISA, University of Aarhus, Aarhus, DK.
2. Zoology Dept., Oxford University, Oxford, UK.

To better understand how spider silk can be formed in the laboratory we have been characterising the of silk material in the major ampulate gland of the spider N. Edilis. We have explored the affects of environmental factors, e.g., pH, based on physiological measurements of the gland, and assayed the results using synchrotron radiation circular dichroism (SR CD). The SR generated by ASTRID at the UV1 photobiology beamline (ISA, University of Aarhus, Denmark) can provide new information on the structure and dynamics of biomolecules using CD [1]. Molecular stability and conformational changes can be studied as a function of environment as well as folding and unfolding processes under normal and abnormal physiological conditions; e.g., as a function of temperature and pH. SR CD has a number of advantages over conventional CD machines. SR CD has much more light in the UV and VUV (< 200 nm) wavelength ranges which gives 1) higher signal to noise, 2) access to new spectral features (only seen at low wavelengths) allowing more accurate structure determination, 3) higher throughput for stop-flow/continuous-flow experiments and 4) opportunities for implementing new techniques (e.g., CD microscopy). The SR CD has been used to monitor the change in molecular structure of the silk material directly extracted from the gland as a function of concentration, pH and salt. Initial data suggest that the conversion process in the gland from a soluble state to the ß-sheet enriched state that is the precursor to solid silk fibres is dependent on more than one factor. This research follows from earlier research that has already helped to shed light on the molecular mechanism underlying silk formation in the spider, indicating a relatedness to fibril formation in amyloids (e.g., a-synuclein of Parkinson's disease) and suggesting that it could be a valuable model system for exploring fibrilogenesis in amyloid diseases [3] .

[1] S.V. Hoffmann & J.M. Kenney, A UV-Visible Synchrotron Radiation Beamline for Circular Dichrotism and UV Photo-absorption, in preparation.
[2] P.H. Jensen, K. Islam, J.M. Kenney, M.S. Nielsen, J. Power & W.P. Gai, Microtubule-associated Protein 1B is a Component of Cortical Lewy Bodies and Binds Alpha-synuclein Filaments, J. Biol Chem. 275, 21500-21507 (2000)
[3] J.M. Kenney, D.P. Knight, M. Wise & F. Vollrath, Amyloidogenic Nature of Spider silk, submitted.

Local structure in polymeric materials investigated by X-ray microdiffraction

C. Riekel
European Synchrotron Radiation Facility, B.P. 220, F-38043 Grenoble Cedex

Practically all types of spider silks can nowadays be examined by X-ray microdiffraction techniques using synchrotron radiation. These techniqueshave a more general interest for the study of the local structure of polymers and biopolymers. I will show several microdiffraction techniques which are now routinely available to the user commnunity and also developing techniques such as microdiffraction with 100 nm X-ray beams. I will limit the examples to common polymeric materials such as Kevlar or polyurethane and I will not forget to show a few silk fibers.

INFLUENCE OF WATER ON THE TENSILE PROPERTIES OF *Bombyx mori* AND *Attacus atlas* SILK

J. Pérez-Rigueiro[@], M. Elices[@], J. Llorca[@], C. Viney[#]

[@] Departamento de Ciencia de Materiales. Universidad Politécnica de Madrid. ETS de Ingenieros de Caminos. Ciudad Universitaria. 28040 Madrid. Spain.

[#] Department of Chemistry. Heriot-Watt University. Edinburgh EH14 4AS. U.K.

The outstanding mechanical properties of silk depend on the presence of weak interactions (i.e. hydrogen bonds) as well as strong intraprotein covalent bonds. In contrast to the extreme stability of the covalent bonds, the strength of weak interactions depends on the environment of the chemical species. Consequently, the study of the tensile properties of silk in different environments may cast light on the contibution of these weak interactions to the mechanical behaviour of silk. This approach has been used with spider (drag line) silk by submerging silk fibres in water: the observation of significant contraction (referred to as supercontraction [1]) has been explained in terms of water acting to disrupt hydrogen bonds initially present in the material.

A similar approach has been undertaken in the present work to determine the influence of hydrogen bonds in the silk fibres of two *Lepidoptera* species: *Bombyx mori* and *Attacus atlas*. *Bombyx mori* (*Bombycidae*) fibres were tensile tested in water and the force-displacement curves were compared with curves obtained from control samples tested in air. Consistent with previous results [3], these fibres were not observed to supercontract. However, immersion in water led to a significant decrease of the elastic modulus and the proportional limit, and to an increase in the elongation. Following the results presented on spider silk, these data were interpreted as a consequence of the disruption of hydrogen bonds, so that van der Waals interactions became the dominant weak interaction in the submerged fibre. This hypothesis was the basis of a proposed shear lag model of the elastic modulus of silkworm silk that has shown a remarkable agreement with the experimental elastic modulus measured in air.

Attacus atlas (*Saturniidae*) is relatively close to *B. mori* in evolutionary terms, but the amino acid composition of its silk is similar to spider (*Nephila clavipes*) drag line. Despite this similarity, no significant contraction was observed when submerging *A. atlas* silk in water. Moreover, the force-displacement curves were qualitatively similar to those obtained from *B. mori* silk: a decrease in the elastic modulus and the proportional limit but an increase in the elongation were apparent when comparing submerged fibres and control fibres tested in air. These results indicate the existance of a fundamental microstructural difference between silkworm and spider (drag line) silk, that is independent of the exact amino acid composition of silk.

[1] R.W. Work, Textile Res. J., 47, (1977), 650-662.
[2] Y. Termonia, Macromolecules, 27, (1994), 7378-7381.
[3] D.L. Kaplan, S.J. Lombardi, W.S. Muller, S.A. Fossey. In D. Byrom (ed.) Biomaterials: novel materials from biological sources, N.Y., Stockton Press, 1991, pp. 1-53.

Unusual fibroin structure in the waxmoth, Galleria mellonella: Implications for fibre formation

M. Zurovec and F. Sehnal
Institute of Entomology, Academy of Sciences, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic

Heavy chain fibroin (H-fibroin), which is the principal protein in the silk of caterpillars, is characterized by hierarchical arrangement of repetitive regions. The H-fibroin of *Bombyx mori* consists of 12 large domains that contain Gly-rich »crystalline" cores dominated by GAGAGS repeats, and are interspaced with short »amorphous" regions (Zhou et al. 2000). In *Antheraea pernyi*, each of the 80 tandemly arranged »crystalline" repeats consists of about 13 Ala residues and is flanked by one of four types of »amorphous" motifs (Sezutsu & Yukuhiro 2000). These structures are consistent with the X-ray diffraction data revealing presence of crystallites that are made form ß-pleated sheets packed at 9.3 Å in the silk of *B. mori* (type 1 silk) and at 10.6 Å in the silk of *A. pernyi* (Warwicker 1960). The silk of *Galleria mellonella*, which is unique by combining high tensile strength with high extensibility (Deny et al. 1980), exhibits intersheet packing similar to that of *A. pernyi*, but our investigations disclosed very different molecular organisation. *G. mellonella* H-fibroin consists of regular repetitions of just three motifs that are extremely homogenous in their composition and length: motif A contains 63 amino acid residues, B_1 43 residues, and B_2 18 residues. The motifs are assembled into a block $AB_1AB_1AB_1AB_2(AB_2)AB_2$ that is probably reiterated 11 times in the 500 kDa H-fibroin molecule. Only sequences SSAASAAS and SSAASAAAA in the A motif resemble the typical »crystalline" regions. Striking are the high contents of residues with long hydrophobic side chain (16%) and of proline (3.2%). Conserved spacing of the apolar and charged residues, such as the sequence VIVI preceded by PAP or AGE and followed by DD or ED (in a single case ND), indicates the importance of hydrophobicity and charge in H-fibroin cross-linking. It must be emphasized that the N-terminal and C-terminal parts of all known H-fibroins are similar, confirming their common origin. Forces driving rapid diversification and subsequent unification of the repeats are not fully understood but recombination of short DNA motifs is certainly involved. Recombination is the major obstacle for cloning and expressing *H-fibroin* genes in bacteria. From the *H-fibroin* genes identified so far, only that of *G. mellonella* can probably be considered in the schemes of bacterial silk production because it lacks cumulations of short iterated motifs that are particularly prone to recombination.

Strategic disulfide bonds in keratin fibres and their role during permanent waving and a -b **transition.**

Helmut Zahn and Franz-Josef Wortmann
Deutsches Wollforschungsinstitut an der Rheinisch-Westfälischen Technischen Hochschule Aachen e.V.

We define strategic disulfide bonds the one who are the stress supporting bonds or stress-relaxation impeding bonds in wet keratin fibres.

It has been stated by John A. Maclaren and Brian Milligan (1981) "that the cystine residues of wool have a wide range of reactivities but to divide them arbitrarily into reactive and unreactive fractions is illogical". This conclusion applies to unstrained keratin fibers. If however wet keratin fibers are stretched, the modulus of the crystalline regions in the microfibril is 5 GPa whereas the amorphous wool matrix has a modulus of 0.2 Gpa only (Postle et al., 1988). This is evidence for the assigning to the disulfide bonds in the crystalline regions to be strategic disulfide bonds. The strategic disulfide bonds play an important part during the reduction step of the permanent waving process of hair and the α-β transition in strained keratin fibres.