THE ROYAL SOCIETY

Published online 28 February 2002

Silk: molecular organization and control of assembly

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The interface between the science and engineering of biology and materials is an area of growing interest. One of the goals of this field is to utilize biological synthesis and processing of polymers as a route to gain insight into topics such as molecular recognition, self-assembly and the formation of materials with well-defined architectures. The biological processes involved in polymer synthesis and assembly can offer important information on fundamental interactions involved in the formation of complex material architectures, as well as practical knowledge into new and important materials related to biomaterial uses and tissue engineering needs. Classic approaches in biology, including genetic engineering, controlled microbial physiology and enzymatic synthesis, are prototypical methods used to control polymer structure and chemistry, including stereoselectivity and regioselectivity, to degrees unattainable using traditional synthetic chemistry. This type of control can lead to detailed and systematic studies of the formation of the structural hierarchy in materials and the subsequent biological responses to these materials.

Keywords: silk; redox; copolymers

1. FIBROUS PROTEINS AS COMPLEX BLOCK COPOLYMERS

An important feature of biological systems is the role played by fibrous proteins in a wide range of structural needs. Classic examples include silks (spider and silkworm) as fibre-forming molecules, very strong yet flexible materials (e.g. orb webs, cocoons), and collagens, as key structural elements in all tissues, influencing mechanical properties as well as biological responses. Fibrous proteins can be distilled into their smaller repeats or blocks to provide a rational approach to the systematic study of the relationships between the primary sequence of monomers (amino acids) and self-assembly. This approach is feasible because of the repetitive nature of the primary sequence in these types of fibrous proteins. The result of this block sequence architecture is the formation of highly ordered domains, organized through intermolecular interactions as the materials undergo self-assembly. This hierarchy can be probed in a systematic way using the biological controls of polymer synthesis. The self-assembly path includes a series of material-length scales leading to liquid crystalline mesophases and eventually to macroscopic structures.

An understanding of the assembly of materials at different length scales (both biological and synthetic) can be understood in terms of transfer or 'communication' of information across these length scales, which are hierarchically organized. Biological systems are relatively well elucidated in terms of how genetic templates (information storage in biology, DNA, RNA) guide the formation of well-defined protein sequences, including the fibrous proteins of interest here. This level of communication occurs within the confines of the cell membrane and can be manipulated in precise ways with the tools of genetic engineering available to most laboratories. However, once these proteins escape the influence of the cell membrane there is little understanding of how communication occurs between molecules to promote assembly at longer length scales, leading to mesophases and eventually macroscopic materials (e.g. tissue structures or orb webs). These processes are not random and the information content is contained within the proteins to drive the process, with significant influence due to the surrounding environment. An understanding of these structures at intermediate-length scales, and the communication modes that foster the organization of the polymers, are the aims of our present work. This level of understanding will lead to insight into how to program polymers at the primary sequence level to interact with a specific environment, leading to predictable architectures and patterns at various length scales. The controlled assembly of complex polymers into materials with microstructural domain textures and defined nanoscale morphologies is a key step in engineering targeted structural and functional features. The chemical and physical nature of these domains and self-assembled morphologies determine the mechanical, electronic, thermal and biological properties and provide avenues for the adaptation of the properties of these versatile materials to specific processing and performance constraints. One of the main objectives of the current studies is to understand how to 'control' or 'direct' the process of morphology development in polymeric materials using external influences or molecular 'triggers'. Silk proteins provide a suitable model system with which to explore this feature.

The use of site-specific chemical triggers to influence conformational polymorphism and precipitation or crystallization (through regulation of solubility) provides

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additional control over the state of the polymer during the transition from a processable liquid (or soft solid) to a strong solid material. By incorporating triggers into polymorphic sequences the conformation of individual sequence motifs can be controlled, directing the protein-based polymers along different selected self-assembly pathways. As we move towards more specialized polymeric systems for different applications (e.g. electronics, optics, structural, biomaterials) more demanding specifications will be required. This will necessitate more powerful options to control polymer morphology. A working knowledge of how to control the development of specific phases, and the evolution of individual self-assembled structures amid a complex of coupled processes, can be gained using proteins, and generalized to other polymers.

2. SILKS

Silks, as one group of fibrous proteins, are produced by a wide range of insects and spiders and can consist of helical, β-sheet (the chain axis is parallel to the fibre axis) or cross-β-sheet (the chain axis is perpendicular to the fibre axis) secondary structures, depending on the organism (McGrath & Kaplan 1997). Spider dragline silk from *Nephila clavipes*, and cocoon silk fibroin from the silkworm *Bombyx mori*, are characterized by a β-pleated-sheet secondary structure with extended polypeptide chains in which the carbonyl oxygens and amide hydrogens are at near right angles to the long axis of the chain. Hydrogen bonds form between the carbonyl oxygens and amide hydrogens of neighbouring chains thus forming a pleated structure along the backbone of the peptide chain. The chain–chain interactions in silks include extensive hydrogen bonding (intra- and interchain) as well as van der Waals interactions for stacked sheets due to the predominance of short side-chain amino acids such as glycine, alanine and serine.

Silks from silkworms and spiders can be viewed as complex copolymers that are amenable to genetic manipulation for the detailed study of structure assembly (Winkler & Kaplan 2000). Ongoing studies are directed at gaining insight into the process of silk assembly as well as the use of silks as biomaterials. Conformational transitions using model silk peptides and genetically engineered variants of silk proteins are studied to provide insight into these relationships. These studies are conducted with key consensus sequences (blocks) from the native sequences and changes in conformation are probed in solution and at interfaces. The interactions between sequence and environment are critical in assessing these transitions. Questions regarding conformational polymorphism are a current area of focus.

Our recent studies have successfully demonstrated the design, synthesis and function of molecular-level 'triggers' to control polymer assembly using silk as a model (Valluzzi *et al.* 1999*a*; Szela *et al.* 2000; Winkler *et al.* 2000). Two trigger designs were constructed and evaluated, one chemically driven and one biochemically (enzymatically) driven—a methionine redox system and an enzymatic phosphorylation–dephosphorylation system. Genetic variants of the *N. clavipes* spider dragline-silk protein-consensus sequence, containing methionine triggers or phosphorylation triggers, were expressed from *E. coli*, purified and characterized in both the non-triggered (reduced in the case of the methionine triggers, dephosphorylated in the case of the enzymatic trigger) and triggered (oxidized in the case of the methionine trigger and phosphorylated in the case of the enzymatic trigger) states. The triggering worked to control solubility and crystallinity of normally insoluble β-sheet-forming proteins. For example, over two orders of magnitude in improvement in solubility in water was observed for the oxidized methionine-modified silk versus the reduced version of this protein.

The transition from more random coil (oxidized methionine trigger) to β-sheet crystallites (reduced methionine trigger) was confirmed, based on X-ray diffraction and IR analyses. A more gradual transition from amorphous, to silk III, to β-sheet was apparent with increasing chemical modification (reduction). (Note: silk I is poorly defined and consists of extended helices or random coil, silk II consists of β-sheets and silk III consists of threefold polyglycine II-like helices.) At moderate levels of oxidation, silks III and I (and a little silk II) were present in the methionine-modified silks, while at high levels of phosphorylation in the phosphate-triggered silk, mostly silk I or amorphous random coil was present. The methionine trigger blocks β-sheet crystallinity but some β-strand was present at moderate oxidation levels. The phosphate trigger blocks β-strand conformation but crystallization of the silk I-like conformation was evident. Based on these results the location of the trigger relative to the various motifs in the primary sequence, as well as the size and relative hydrophobicity or hydrophilicity of the change on the amino-acid side chain, may all be important in influencing sequence motif interactions and morphology control. At the next level of structural hierarchy, morphological differences were observed as a direct result of the alterations in conformation and crystallinity due to triggering and assembly. For example, banded structures were clearly observed (using TEM) for the reduced methionine-triggered silk and for the dephosphorylated silk, while such morphologies were absent in the reduced or phosphorylated versions. Changes in precipitate morphology were also observed with changes in crystallography.

The effect of a membrane mimetic environment on structure and morphology development in silk-like protein polymers is also under study (Valluzzi *et al.* 1999*b*; Wilson *et al.* 2000). The focus of these studies is on the polymorphic behaviour of the silk motifs, based on *B. mori* silkworm silk, and their assembly at interfaces. Silks provide a 'databank' of well-characterized polymorphic sequences, acting as a window onto structural transitions. The studies concentrated on the role of surface interactions as a route to understanding silk self-assembly. Surface chemistry could be used to refine conformation. Peptides with conformationally polymorphic silk-like sequences were characterized using FTIR spectroscopy, circular dichroism and electron diffraction. Polymorphs resembling the silk I, II and III crystal structures were formed (GAGAGS for the crystallizable sequence, GAGAGY for the amorphous sequence—both derived from the consensus sequence of the silkworm fibroin protein). Precipitates obtained by drying the peptide solutions on a hydrophilic surface preserved the β-strand conformation, observed using FTIR.

Some β-sheet crystallinity, characterized using parallel electron diffraction and microscopy studies, was also observed. At a hydrophobic interface, this peptide was adsorbed from solution and changed its conformation to silk I, the β-sheet precursor form, which was observed using FTIR. When dry, the peptide rearranged itself to form another β-sheet precursor conformation, a threefold extended polyglycine II helical structure. Both were verified using FTIR and electron diffraction. It was also possible to convert the β-sheet precursor form to a β-sheet by treatment with methanol. While these studies are still in progress, it is clear that environment-dependent polymorphisms with both these core sequences are possible, although the conformational freedom available in GAGAGS is broader than when tyrosine replaces the serine.

We thank the National Science Foundation (Materials and Bioengineering Programs) (NSF), National Aeronautics and Space Administration (NASA), National Institutes of Health (NIH) and the Department of Defense (DoD) for their support in the various phases of this work.

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