Chapter 18
Material Properties of Honeybee Silk

Abstract Colourless honeybee silk, ~3 μm diameter, is produced through a spinneret at the tip of the labium-hypopharynx. Successive generations of brood apply silk to the cell walls, making the cells smaller, as silk is deposited in the old brood combs. X-ray diffraction data show that honeybee silk contains ά-helical proteins ordered into coiled-coil structures, with an axial periodicity of about 28 nm, and form a four-stranded array parallel to the fibre axis. Honeybee fibroin is crystalline, but, when hydrated, is only half as stiff as when dry, although they are equal in strength. The fibroin is hygroscopic and highly distensible when solvated because of its molecular conformation. The mechanical properties of silk are independent of temperature. Lithium thiocyanate and urea virtually eliminate the yield point of honeybee silk tested both dry and in distilled water, and values for stress in the slope of the solvent-related curves is reduced. The solvents act directly on hydrogen bonds and then the silks behave as unconnected bends during tensile deformation. The components, hierarchical structure and the conditions of their production all affect the mechanical properties of natural silks. The amino acid sequence in honeybee silk protein provides an explanation of why the coiled-coil packing is atypically tight, and the most abundant core residue is the small amino acid, alanine. An atomistic simulation for the unfolding behaviour of ά-helical protein shows that two discrete transition states correspond to two fracture mechanisms. Six honeybee silk genes have now been identified, using a combination of genomic and proteomic techniques.

18.1 Introduction

The honeybee nest contains areas for the storage of nectar and pollen and the rearing of brood. While wax is the basic building material for the nest, with continued use the combs become modified by the addition of silk and propolis (Hepburn and Kurstjens 1988). Thus, much of the honeybee nest gradually changes from a single phase (wax) to a two-phase or composite (wax/silk)

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material. Some of the material properties of the individual phases of the honeybee nest have now been characterized (Hepburn et al. 1979; Hepburn 1986; Hepburn and Kurstjens 1988; Kurstjens et al. 1985, 1990); but, particularly important recent studies on the molecular structure of honeybee silk (Sutherland et al. 2006, et seq.) necessitate a review of the composition and properties of honeybee α-helical silk (Fig. 18.1), the elastic element in all honeybee combs. This Chapter is largely based on a recent review of honeybee silk (Hepburn et al. 2013).

"Silk" is a functional term used to describe protein fibres spun by honeybees, many different kinds of insects and other invertebrate animals (Fig. 18.1). The spinning of silk by honeybees does not involve either rotating or twisting fibres, as is done in commercial fibre production, but refers to the process of making an insoluble filament from an aqueous protein solution (Sutherland 2010a). In the case of honeybees, just before pupation, the larvae cover the waxen walls of their cells with silk (Huber 1814; Arnhart 1906), paying out the fibres randomly so that, by the end of spinning, the walls are covered by thin sheets of silk in which the individual fibrils are readily discernible (Jay 1964; Zhang et al. 2010a).

Jay (1964) observed that fibres were formed when the honeybee spinneret was drawn away from the cell wall. In contrast, films were formed when the spinneret was dragged over the cell wall, presumably because the substrate stabilized the thin film. Jay (1964) reported that silk is generated from the labial gland as the larvae perform random head movements in all directions, within the cell. Inasmuch as this behaviour may last up to 48 h, it ensures that in the final product (the cocoon), the fibres form a randomised and mechanically, planar isotropic structure. The colourless silk, about 3 μm in diameter (Zhang et al. 2010a), is produced through a slit-like spinneret located at the tip of the combined labium-hypopharynx.

The inference that the silk proteins are highly organized in the gland lumen before the larvae actually begin spinning (Flower and Kenchington 1967), has recently been supported by Silva-Zacarin et al. (2003). These authors showed that silk formation begins during the middle of the 5th instar and finishes at the end of this developmental stage. This process begins in the distal secretory portion of the gland, going towards the proximal secretory portion, and from the periphery to the center of the gland lumen. The silk proteins are released from the secretory cells as a homogeneous substance that polymerizes in the lumen to form compact birefringent tactoids. Secondly, water absorption from the lumen secretion, carried out by secretory and duct cells, promotes the aggregation of the tactoids that form a spiral-shaped filament with a zigzag pattern. This pattern is also the result of silk compression in the gland lumen, and represents a high concentration of macromolecularly, well-oriented silk proteins.

After spinning, the larvae smear a small amount of material from the Malpighian tubules onto the hardened silk layers, and faeces are also excreted between silk layers (Jay 1964). Subsequently, the larvae produce a colourless pollen-free substance and then a yellow pollen-bearing one (from the anus), both of which are applied in turn to the silk base (Verlich 1930; Jay 1964). Nothing further is known

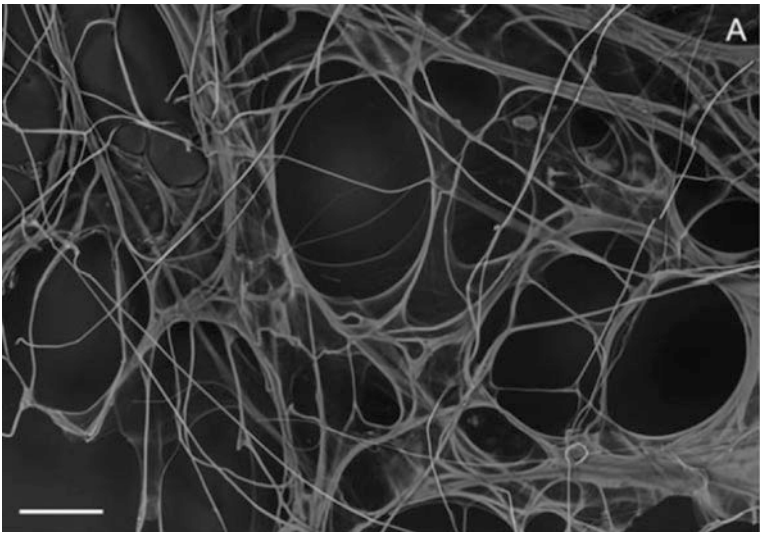

Fig. 18.1 Scanning electron photomicrograph of ά-helical silk fibres produced by *A. mellifera* larvae. Scale bar is 100 μm. Late final instar honeybee larvae were induced to spin silk within plastic tubes, and the clean silk removed before the larvae added any further material (with kind permission of the publishers, from Sutherland et al. 2011a, b)

of these four substances, but they invite the analogy of sizing in paper manufacture.

Successive generations of brood apply more silk to the cell walls so they become smaller, and the mass ratio of silk to wax greater (Chauvin 1962). Thus, old brood combs are heavily impregnated with silk (Fig. 18.2) which is inseparable from the wax except by fairly drastic chemical and/or heat treatments. The development and maturation of brood comb proceeds from a single-phase material (pure white wax), to a coloured, fibre-reinforced, two-phase composite (wax and silk) (Hepburn and Kurstjens 1988; Zhang et al. 2010a). The physical significance of these observations can be illustrated by comparing the properties of the native fibroin, wax-free sheets of silk, silk-free wax, propolis and the final wax-silk composite (Kurstjens et al. 1985; cf. Chap. 4).

18.2 Honeybee Silk: An ά-Helical Protein

Fifty years ago, the crystallographer, KM Rudall (Fig. 18.3), demonstrated in his X-ray fibre diffraction data that silk threads, drawn from honeybee silk glands contain ά-helical proteins assembled into ordered coiled-coil structures, and that their meridional reflections suggested an axial periodicity of about 28 nm (Rudall 1962, 1965). The patterns from honeybee silk fibres were considered most

Fig. 18.2 Longitudinal section of an old, dewaxed comb from *A. m. capensis* showing the layers of silk inside the base and the walls of cells (Hepburn et al. 2007)

Fig. 18.3 KM Rudall, a New Zealander, worked for many years at the then Astbury Department of Biophysics, University of Leeds. He was one of the pioneering crystallographers and molecular biologists who made special and important contributions to the study of the molecular conformations of fibrous proteins fibres, including honeybee silk

consistent with a four-strand coiled-coil structure and a tighter than expected super-helix radius of about 0.52 nm (Atkins 1967). In contrast, the dominant molecular structure in silk of other hymenopteran species is the extended ß-sheet configuration (Warwicker 1960; Sutherland et al. 2007).

So, honeybee silk is an ά-helical fibroin (Rudall 1962), the micelles or crystallites of which form a four-stranded array of coiled-coils parallel to the fibre axis (Atkins 1967). Honeybee fibroin is crystalline, relative to other insect silks (Lucas and Rudall 1968); but hydrated fibres are only half as stiff as dry ones, although they are equal in strength (Hepburn et al. 1979). The fibroin is hygroscopic, and when solvated, is highly distensible, largely owing to its molecular conformation (Lucas and Rudall 1968). These properties of the fibroin are largely suppressed by the cocoon-spinning larvae because the silk is pressed into the wax of the cell wall, possibly aided by the anal secretions, and this immediately water-proofs and checks the silk fibroin against solvation. Thus, it is also likely that inter-micellar friction is enhanced (Warwicker 1960), and the possibility of conformational change restricted (Rudall 1962), effects which are consistent with good stiffness and reduced distensibility (Hepburn et al. 1979). That the silk fibres are spun and randomly arranged in the cell wall overcomes the basic anisotropy of the material, because dewaxed sheets of cocoon silk are planar isotropic on tensile deformation.

18.3 Behaviour of Silk at Different Temperatures

Natural variations in the temperature of honeybee nests invite a consideration of silk behaviour at varied thermal regimes. The independence of the mechanical properties of *A. m. scutellata* silk sheets, when deformed in tension at a fixed rate at different temperatures, is illustrated in Table 18.1.

Sheets of silk maintain the same relative strength and distensibility between 25 and 45 °C, and staunch the plastic flow, and ultimate collapse of wax, at higher temperatures. Consequently changes in stiffness or the energy to fracture the sheet, an index of its relative workability, were not observed. The tensile properties of silk sheets over this range of temperatures are in sharp contrast to those of pure wax (Hepburn et al. 1983), propolis (Hepburn and Kurstjens 1984) and the wax-silk composite of brood combs (cf. Chap. 4). In addition to crystal structure, white comb wax is also affected by the presence of a protein fraction (Kurstjens et al. 1985, 1990). This material is present, quite apart from silk, in both wax scales and in newly constructed combs. In both cases, this partially characterized protein (Kurstjens et al. 1990) is positively associated with enhanced stiffness in both scales and combs. Nothing is known of the molecular behaviour of this protein or how it might contribute to the stiffness of wax. To have assigned this protein to the elastic fraction is somewhat gratuitous.

Table 18.1 Tensile mechanical properties of dewaxed *A. m. scutellata* worker honeybee cocoon silk (Hepburn and Kurstjens 1988)

Temperature °C	Relative tensile strength (Nmm-1)	Breaking strain Percentage (%)	Relative stiffness Nmm-1	Work MJm-3
25	32 ± 16	98	33 ± 14	29 ± 20
30	32 ± 18	81	40 ± 13	28 ± 23
35	26 ± 10	85	31 ± 8	22 ± 14
40	39 ± 17	105	37 ± 14	38 ± 22
45	43 ± 20	106	41 ± 14	48 ± 30

For each value, n = 10

18.4 Relative Crystallinity

Lucas et al. (1960) estimated the relative crystallinity of moth fibroins by calculating short side chain—long side chain ratios. When Hepburn et al. (1979) did the same for honeybee silk, the result suggested that this silk was anomalous because crystalline fibroins generally have a high glycine content and honeybee silk has a very low one, but is nevertheless, relatively crystalline (Atkins 1967). These authors subsequently turned to cellulose, because one feature of cellulose is that the degree of crystallinity is reflected in the sensitivity of its fibres to solution effects. Water can penetrate amorphous regions in a capillary manner thus diminishing the interactions between crystallites; or, alternatively, compete for potential hydrogen-bonding sites within the fibre (Wainwright et al. 1976).

In the work on cellulose it was assumed that hydration loosened the interaction between neighbouring crystalline regions, so reducing stiffness. It was further assumed that the elastic modulus of the dry cellulose approached that of crystalline cellulose. If this were indeed so, then the ratio of modulus wet to modulus dry provides an approximate index of the degree of crystallinity; a ratio of 1 indicating complete and lesser values of progressively less crystallinity. When honeybee silk was examined for hydration sensitivity, expressed as the ratio of the elastic modulus of wet to that of dry fibre, a value of 0.53 showed that this fibre is rather crystalline, a result consistent with other forms of measurement. Tensile stress-strain curves for wet and dry $\acute{\alpha}$-helical honeybee silk are shown in Fig. 18.4. Both wet and dry honeybee silks are characterized initially by linear regions, which terminate in marked yield points at about 0.1 and 0.3 strain respectively. A yield point is defined as a marked decrease in the slope of the stress-strain curve, which occurs over a very small region of strain and, for an $\acute{\alpha}$-helical structure, is associated with the onset of a transconformational change from the $\acute{\alpha}$ to the parallel-β state (Rudall 1962, 1965).

More recently, Zhang et al. (2010b) reported on the microstructures and mechanical properties of honeybee, *A. m. ligustica*, and silkworm, *Bombyx mori*, silks which were examined by environment scanning electron microscopy (ESEM), scanning probe microscopy (SPM), tensile tests, and nanoindentation.

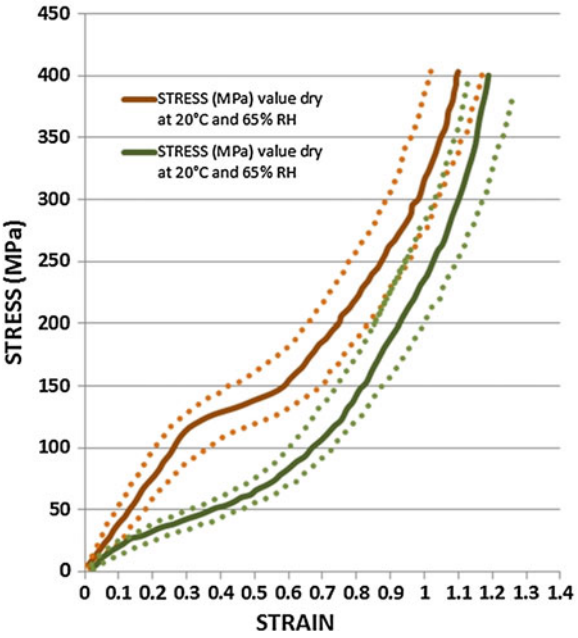

Fig. 18.4 Generalized tensile stress-strain curve for ά-helical silk of *A. m. scutellata* tested dry and then wet (Hepburn et al. 1979)

They concluded that honeybee silk, unlike silkworm silk, is a single fibre with a circular cross-section, which has a much finer, smoother texture than silkworm silk. Honeybee silk exhibits a distinctly linear and brittle elastic mechanical behaviour. Moreover, nanoindentation measurements showed that honeybee silk is much less anisotropic than silkworm silk (Zhang et al. 2010b). The ratio of the longitudinal modulus to the transverse modulus of honeybee silk is 2.0, whereas that of silkworm silk is 18.9. It is probable that the different structural and mechanical properties of honeybee and silkworm silks are likely the result of their specific biological functions (Zhang et al. 2010b).

18.5 Solvent Effects on Silk

A large amount of empirical information on the effects of solvents has accumulated over the past 100 years from the wool, leather and silk industries. A few of these solvents have been studied in considerable detail, and their effects well documented in the general chemical literature. Of these solvents, Hepburn et al. (1979) selected lithium thiocyanate, urea and formamide as high affinity hydrogen bond competitors. Specimens of honeybee silk were tested in these solutions to assess the possible role of distilled water having more than capillary sorptive effects on the general tensile behaviour of the fibres. In the case of honeybee silk, lithium thiocyanate and urea virtually eliminate the marked yield point

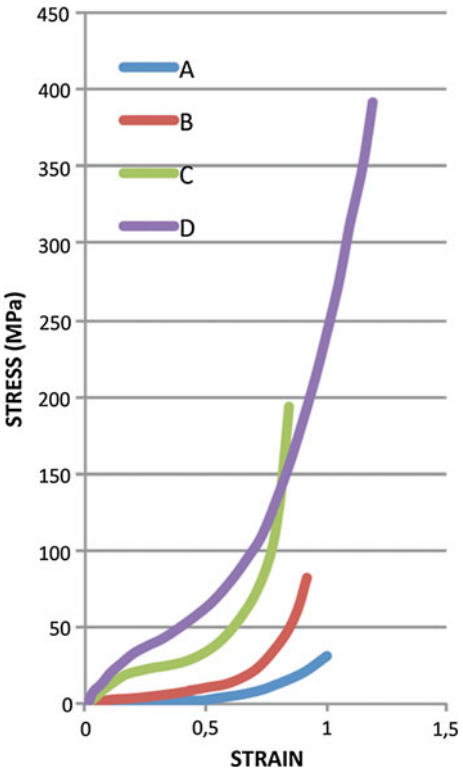

Fig. 18.5 Stress-strain curves to failure of *A. m. scutellata* silk at 20 °C in various hydrogen bond-disruptive solutions: $A = 7$ M urea; $B = 4$ M lithium thiocyanate; $C = 7$ M formamide; $D =$ distilled water (Hepburn et al. 1979)

characteristic of honeybee silk tested both dry and in distilled water. Secondly, the entire slope of the solvent-related curves is markedly reduced, as are the associated values of stress, point for point, along the curves (cf. Figs. 18.4 and 18.5).

These differences can be explained in the following way. An aqueous environment facilitates microfibrillar lubrication, as evidenced by decreasing values of the elastic modulus, and in increasing total extensibility in honeybee silk. On the other hand, organic solvents drastically reduced modulus and stress in honeybee silk, and virtually eliminated the transition from linearity to non-linearity in these curves. We suggest that, in these cases, the solvents are in fact directly acting on hydrogen bonds, so that during tensile deformation, the silks essentially behave as loose collections of unconnected bends (like a bowl of cooked spaghetti or noodles), which require only very small loads to unfold them.

Loose fibres of honeybee silk placed in a 7 M solution of formamide or urea and in a 4 M solution of lithium thiocyanate, showed no change in length, but were remarkably rubbery to the touch and very easily distended. This distensibility was reversible over the ranges examined, 100–200 % (ε_1 = 0.69 − 1.1), and the silk highly reminiscent of solvated resilin (Andersen and Weis-Fogh 1964) and other rubber networks with moderate cross-linking. However, there are basic differences between solvated fibroins and rubber networks; the integrity of the former lies in

Table 18.2 Properties of the proteins of *A. mellifera* ά-helical silk compared with other insects silks (with kind permission of the publishers, from Sutherland et al. 2006)

Species	Protein name	Number of amino acids	Percent of cDNA library clones
Bumblebee	BBF1	327	4
	BBF2	313	14
	BBF3	332	20
	BBF4	357	32
Bulldog ant	BAF1	422	16
	BAF2	411	30
	BAF3	394	26
	BAF4	441	24
Weaver ant	WAF1	391	35
	WAF2	400	22
	WAF3	395	13
	WAF4	443	17
Honeybee	AmelF1	333	6d
	AmelF2	309	7d
	AmelF3	335	11d
	AmelF4	342	7d
Bumblebee	BBSA1	>501	3
Honeybee	AmelSAl	578	13d

the secondary hydrogen bonding topology of the structure, while in the latter, bonding is usually of the sulphydryl covalent type. Thus, we conclude that solvation of honeybee silk in lithium thiocyanate, urea and formamide, and even distilled water, disrupts, the crystalline organization of the fibroin by directly reducing hydrogen bonding in the structure. Properties of the proteins of the ά-helical honeybee silk are shown in Table 18.2.

18.6 Honeybee Silk: An ά-Helical Silk and a Coiled-Coil Protein

It appears to be a general property of natural silks that the components, hierarchical structure and the conditions of their production all affect their mechanical properties (Vollrath and Knight 2001; Shao and Vollrath 2002). It is therefore not surprising that the discovery of the amino acid sequence in honeybee silk protein provided an explanation of why the coiled-coil packing was atypically tight: while the core of coiled-coils usually contains large hydrophobic residues such as leucine and isoleucine, in coiled-coil silk the most abundant core residue is the small amino acid, alanine (Sutherland 2007).

Lucas and Rudall (1968) suggested that the pattern of coiled-coil proteins that occur in the silk gland could be to prevent agglutination of the proteins within the silk gland. Another, not incompatible, reason put forward by Sutherland et al. (2007),

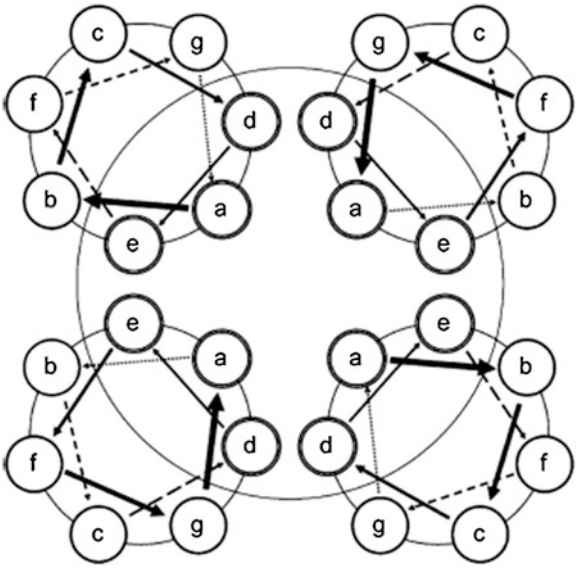

Fig. 18.6 A structural model for a coiled-coil silk as produced by *A. mellifera* honeybees. The ά-helical strands corresponding to each of the fibroins are arranged in an antiparallel tetrameric configuration (direction indicated by arrows). Three residues (**a**, **d**, **e**) from each heptad repeat are buried in the core (with kind permission of Sutherland et al. 2007)

is that it could provide a mechanism to reduce the flow viscosity of the protein solution, in order to allow the concentrated silk dope to pass through the spinneret. Obviously, the behaviour of silk must be based on its chemical composition. Sutherland et al. (2006) were able to identify the coiled-coil silk sequences from silk gland cDNA libraries of European *A. mellifera,* and determine the amino acid sequence of the coiled-coils.

Sutherland et al. (2007) confirmed that honeybee silk is formed from four coiled-coil proteins (fibroins), as originally proposed by Rudall (1962, 1965) on the basis of his X-ray diffraction data. The fibroin proteins contained extensive coiled-coil regions of conserved length, flanked by largely unstructured termini. Sutherland et al. (2007) proposed a structural model for coiled-coil silks (Fig. 18.6). The ά-helical strands corresponding to each of the fibroins are arranged in an antiparallel tetrameric configuration (direction indicated by arrows). Each fibroin contains a continuous predicted coiled-coil region of around 210 residues, flanked by 23–160 residue length N- and C-termini. The cores of the coiled-coils were unusually rich in alanine, a hydrophobic amino acid, in the '**a**' and '**d**' core positions (Fig. 18.6). Sutherland et al. (2011a, b) further provided a schematic top-down view of one strand of a coiled-coil generated from coiled-coil silk proteins such as those that occur in honeybees (Fig. 18.7).

Three residues (**a**, **d**, and **e**) from each heptad repeat are buried in the core. Most known coiled-coils contain predominantly large hydrophobic residues at these positions to maximize the hydrophobic forces stabilizing the core (Woolfson 2005). Sutherland et al. (2007) ascribed the atypical composition of the coiled-coils in bee silks as possibly due to the metabolic constraints of having to produce a continuous and copious secretion of silk during the many hours of larval spinning.

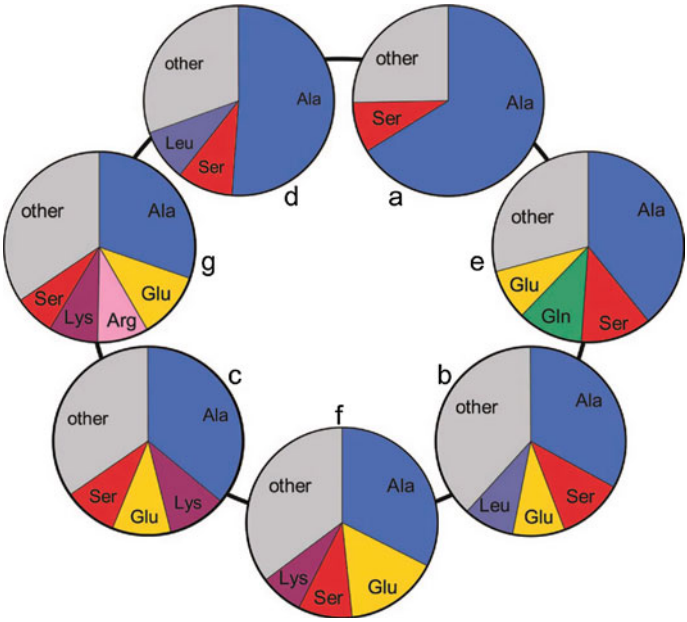

Fig. 18.7 Schematic top-down view of one strand of a coiled-coil generated from coiled-coil silk proteins. Formation of coiled-coils occurs when two strands of protein containing repeats of amino acids in the pattern HPPHPPP (where H are generally hydrophobic residues and P are generally polar residues), come together to shield the hydrophobic residues from the solvent. The heptad repeat is commonly denoted as '**a**–**g**' with '**a**' and '**d**' positions corresponding to the core residues. The relative abundance of different amino acids in each position, averaged over-all silk proteins for seven species, is shown in pie chart form (with kind permission of the publishers, from Sutherland et al. 2011a, b)

Amino acid sequence comparisons indicate that different regions of silk proteins have different levels of sequence constraint. A pairwise alignment of the closely related silk proteins from European *A. mellifera* (Sutherland et al. 2007) and *A. cerana* (Shi et al. 2008) show, on average, 3 % amino acid changes in predicted coiled-coil core positions, 8 % amino acid changes in predicted coiled-coil non-core positions, and 14 % amino acid changes in the N- and C-termini regions (Sutherland et al. 2011a, b). Thus, composition, molecular topology and amino acid content and sequence appear to be highly conserved features in the evolution of *Apis*.

18.7 Molecular Dynamics of ά-Helical Proteins

Over the past few years the molecular dynamics of ά-helical protein behaviour has gained enormous momentum, particularly with the works of Ackbarow et al. (2007, et seq.), who published highly significant work on how hierarchies, multiple

energy barriers and robustness govern the fracture mechanics of ά-helical and β-sheet protein domains. The authors point out that the fundamental fracture mechanisms of protein materials remain largely unknown, in part because of a lack of understanding of how individual protein building blocks respond to mechanical loads. As an example, they report that there is uncertainty as to whether the unfolding behaviour of ά-helical proteins consists of multiple transition state changes continuously with the pulling velocity. Ackbarow et al. (2007) reported on a direct atomistic simulation over four orders of magnitude in time scales of the unfolding behaviour of ά-helical protein, in which they found that two discrete transition states corresponded to two fracture mechanisms.

Whereas the unfolding mechanism at fast fibre extensions involves the sequential rupture of individual hydrogen bonds, unfolding at slower rates involves the simultaneous rupture of several hydrogen bonds. Ackbarow et al. (2007) derived a theory that explicitly considers the hierarchical architecture of proteins, providing a rigorous structure-property relationship. Their results provide evidence that the molecular structure of ά-helical proteins maximizes their robustness with minimal use of building materials (Ackbarow et al. 2007; Buehler and Ackbarow 2007).

Although not directly germane to the present discussion, it is of considerable interest to learn of the existence of both reconstituted honeybee and other fibres produced by recombinant techniques (Wesiman et al. 2010). The coiled-coil silk proteins of honeybees are small compared to the fibrous silk proteins of spiders and silkworms, and therefore can be produced as full length proteins by fermentation in the bacterium *Escherichia coli*. The native coiled-coil silk self-assembles within the silk gland before spinning (Flower and Kenchington 1967), and key elements of this self-assembly are replicated in reconstituted or recombinant silk, potentially allowing straightforward capture of native silk functionality in a biomaterial (Sutherland et al. 2007, 2011a, b, 2012).

Most recently, Sutherland's group described controlled micellar refolding of coiled-coil honeybee silk proteins using the detergent sodium dodecyl sulphate (SDS) (Walker et al. 2013). Their circular dichroism and dynamic light scattering experiments demonstrated that micellar SDS promotes folding of randomly coiled honeybee silk proteins into isolated α-helices, and that removal of detergent micelles, or addition of salt, leads to a coiled-coil formation. They further proposed a mechanism of protein folding:

"In the presence of micellar detergent, hydrophobic residues are associated with the detergent tail groups within the micelles, whereas hydrophilic residues are paired with the detergent head-groups on the micelle's surface. These detergent–protein interactions prevent residue–residue interactions and allow the protein to fold, according to the natural tendency of individual residues. From this condition, when hydrophobic residue–micellar interactions are reduced by lowering detergent levels to below the critical micelle concentration, or by using salt to increase detergent packing in micelles and thereby excluding the protein from the interior, the proteins fold into coiled-coils. We propose that under low SDS conditions,

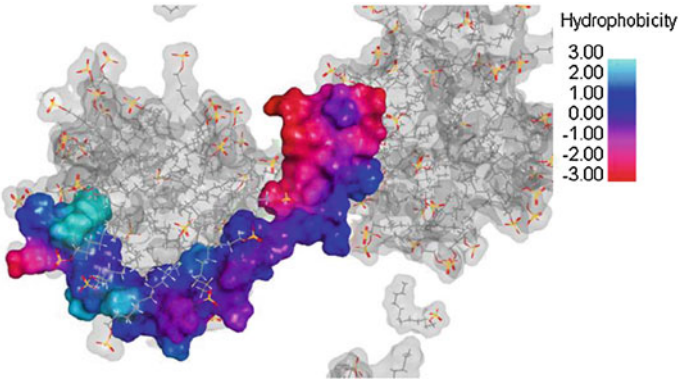

Fig. 18.8 Honeybee silk protein after 8 ns simulation. The hydrophobic residues (*blue*) are situated within the micelle, while the hydrophilic residues (*red*) form a solvent-accessible surface (unpublished, courtesy of T. Sutherland)

hydrophobic–monomeric SDS tail-group and hydrophilic–monomeric head-group interactions (low SDS conditions) or hydrophilic–micellar SDS head-group interactions (high salt conditions), stabilize a transient α-helix intermediate in coiled-coil folding. The folding pathway constitutes a new kind of micellar refolding, which may be profitably employed to refold other proteins rich in coiled-coils." Moreover, in future, this work will likely come within the gambit and purview of patents offices around the world (Sutherland et al. 2010b; Sutherland et al. 2013) (Fig. 18.8).

18.8 Genetic Basis of Honeybee ά-Helical Fibroin

Sutherland et al. (2007) published the results of some pioneering work that described a highly divergent gene cluster in honeybees that actually encodes a novel silk family. Using a combination of genomic and proteomic techniques, they identified four honeybee fibre genes; (*AmelFibroin*1-4) and two silk-associated genes (*AmelSA*1 and 2). The four fibre genes are small, each consisting of a single exon, and are clustered on a short genomic region where the open reading frames are GC-rich amid low GC intergenic regions. The genes encode similar proteins that are highly helical and are predicted to form unusually tight coiled-coils. Despite the similarity in size, structure, and composition of the encoded proteins, the genes have low primary sequence identity. Sutherland et al. (2007) proposed that the four fibre genes have arisen from gene duplication events, but have subsequently diverged significantly. The silk-associated genes encode proteins likely to act as glue (*AmelSA*1), and are involved in silk processing (*AmelSA*2). Although the silks of honeybees and silkworms both originate in larval labial glands, the silk

proteins are completely different in their primary, secondary and tertiary structures, as well as the genomic arrangement of the genes encoding them.

This implies independent evolutionary origins for these functionally related proteins. Six honeybee silk genes have been confidently identified by a combination of genomic and proteomic techniques. Five of these genes, encoding the four proteins and the *AmelSA1* glue protein, are completely novel, with no sequence similarity found to any known gene. The four *AmelFibroin* genes are physically clustered in the genome, and are each composed of a single short exon. Although they encode proteins with similar amino acid composition, helical conformation, and heptad substructure, they share little primary sequence homology. The four related, but diverged genes, may have slightly different roles in coiled-coil formation. All four proteins might be required at fixed ratios for proper silk formation, or expression of the different genes, at varying levels, might allow honeybee silk to adapt rapidly to environmental changes. Alternatively, the four proteins might be functionally equivalent with gene duplication required to support a very high level of expression.

The important and burgeoning field of genomics is concerned with the study of genes and their effects on macroscopic functions, and has led to considerable advances. However, as Ackbarow et al. (2009) noted, genomics does not illuminate material properties, nor the mechanistic relation of hierarchical multi-scale structures and their resulting properties. Elucidating the relation between structure and material properties and multi-scale behavior of protein assemblies, such as the honeybee α-helical silk, represents a grand challenge at the interface of materials science and biology (Ackbarow et al. 2009). This gap in understanding can be closed by systematically studying the material properties of hierarchical protein structures and their effects on the macroscopic properties; an approach, part of a larger effort, to study the role of materials in biology, referred to by Buehler and Keten (2008) as materiomics.

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