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THE MORPHOLOGY AND CHEMICAL STRUCTURE OF WOOL

J. H. BRADBURY
Chemistry Department, Australian National University, Canberra, A.C.T., Australia

Abstract—The morphological structure of wool is described and related to some of the physical properties of wool. The main features of the chemical composition is given of the various morphological compounds, viz. cuticle (epicuticle, exocuticle and endocuticle), cell membrane complex and cortex (orthocortex, paracortex, macrofibrils and microfibrils). The structure and chemistry of microfibrils, matrix and protofibrils is still a matter of controversy. Sequences are available for members of two families of high-sulphur proteins, one high-glycine-tyrosine protein and partial sequences for several families of low-sulphur proteins. It is likely that the microfibrils consist largely but not entirely of low-sulphur protein.

There has been a continuing effort over many years to elucidate the structure and chemistry of keratin fibres. The details of the structure which are still controversial are those high resolution features which concern the structure of microfibrils, protofibrils, matrix and the cell membrane complex. At the same time chemical studies on the fibre and on morphological components of fibres have revealed the broad outlines of the chemistry of keratin fibres and allowed some correlations to be made between structure and chemistry.[1,2]

It is clear from many different studies on various proteins, both globular and fibrous, that the ultimate description of the system at high resolution involves the bringing together of both structural and chemical information. This endeavour is not as highly developed with keratin fibres, as with other fibrous proteins (muscle, collagen), because of the very great complexity and resistance to disruption of keratin fibres.

A further correlation to be made is that between structure and chemistry on the one hand and the function of the protein on the other, such as has been observed with various enzymes.[3] With keratin fibres we are involved with correlations between structure and chemistry and the physical properties of the fibre such as extensibility, frictional properties, etc. In this paper I propose to deal with structure and chemistry and their interrelationships, with some mention of the physical properties of wool fibres.

CELLULAR STRUCTURE OF WOOL

A fine wool fibre as sketched in Fig. 1 consists of flattened, overlapping cuticle cells (10% of the fibre) which cover the surface, the interior or cortex consists of long, polyhedral cortical cells. The size and shape of separated cuticle and cortical cells are shown in Figs. 2 and 3. There is a third type of cellular component which occurs only in coarse keratin fibres as a central core called the medulla, see Fig. 4. It is completely absent in fine wool fibres, hence is not shown in Fig. 1.

CELL MEMBRANE COMPLEX

The different cells are fused together by a cell membrane complex (see Fig. 5), which is formed from the two plasma cell membranes of the adjacent cells in the hair follicle and another material (intercellular cement) that is introduced during the process of hardening of the fibre in the follicle. The cell membrane complex of Merino wool constitutes about 3.3% of the total weight of the fibre

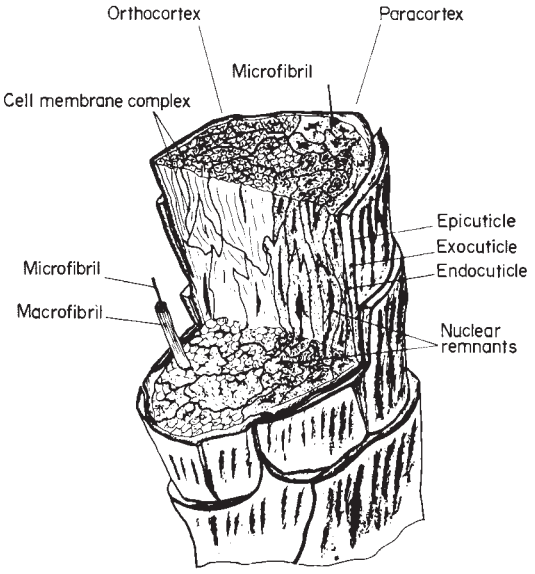

Fig. 1. Sketch of a broken section of a fine wool fibre (diameter ~20 nm) showing the major morphological components.[4]

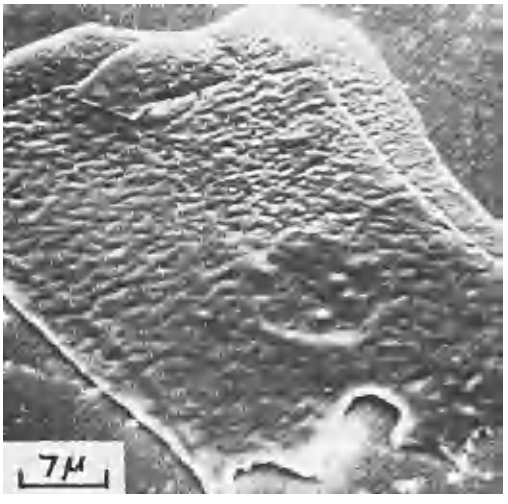

Fig. 2. Scanning electron micrograph of a cuticle cell separated from human hair.[5] The thickness of the cells is 0.3–0.6 nm[2].

Fig. 3. Light micrograph of cortical cells separated from Merino wool.[6]

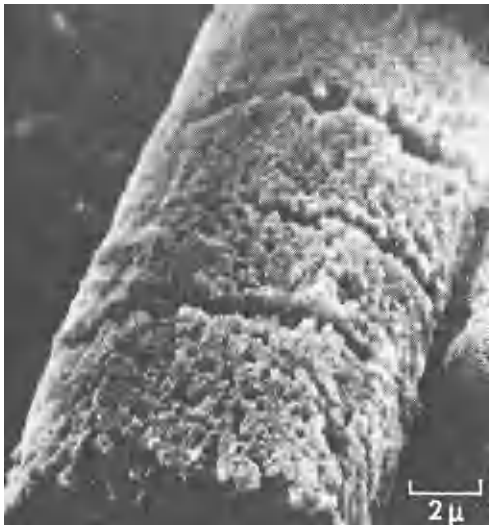

Fig. 4. Scanning electron micrograph of medullary cells from kangaroo hair. Each cell is separated from its neighbour by the transverse cracks shown and the porous appearance of the surface is characteristic of the open texture of the medulla.[7]

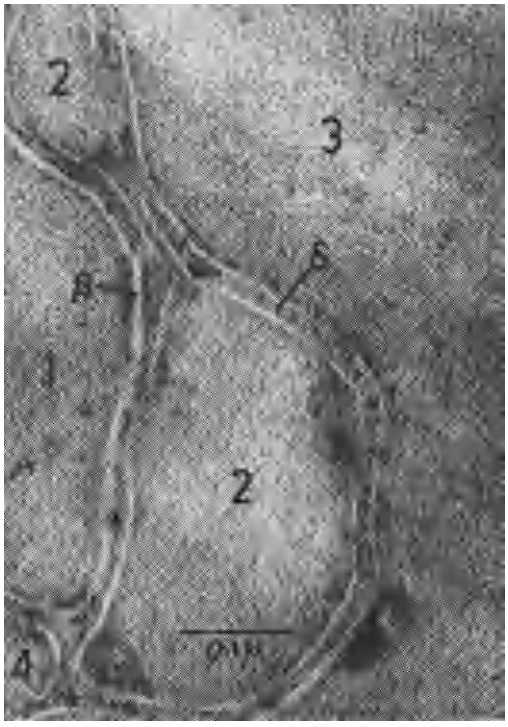

Fig. 5. Electron micrograph of a stained cross section of a Lincoln wool fibre showing the two components labelled β and δ (intercellular cement) and of the cell membrane complex which separates four cortical cells (labelled 1–4).[8]

and is made up of about 0.8% lipid, 1% of a readily extractable protein and 1.5% of a highly resistant membrane.[9,10] The distribution of these materials within the β and δ components of cell membrane complex (see Fig. 5), is a matter of speculation.[2,9]

CUTICLE

The cuticle cells consist of three major components labelled in Fig. 1 (1) epicuticle (an exterior resistant membrane) about 30 Å thick[2], (2) exocuticle which consists of two segments, the outermost of which is called the "a layer" (Fig. 6) and (3) endocuticle. The epicuticle membrane is the externally placed part of the resistant cell membrane which surrounds each cuticle cell completely; inside the fibre this resistant membrane forms part of the cell membrane complex. This membrane consists of a small amount of lipid and proteins which are rich in lysine[12,13] and is notable for its chemical inertness,[14,15] and its hydrophobic nature, which causes the low degree of wettability of wool fibres.[16,17] These properties would be consistent with the presence of ϵ-(γ-glutamyl)lysine cross links.[10]

The exocuticle is the major part of the cuticle cell (64% in Merino wool) and contains a very large amount of cystine, which causes an average of 1 cross link per five amino acid residues.[18] It also has a low content of polar residues and these two factors together may be the source of the well known barrier to diffusion of dyes and acids into wool fibres,[19–21] although another possible source is the cell membrane complex.[9,18]

The endocuticle is the innermost part of the cuticle and consists of the debris derived from the cytoplasm of the once living cuticle cell.[8,22] This material contains a relatively small amount of cystine and is similar in

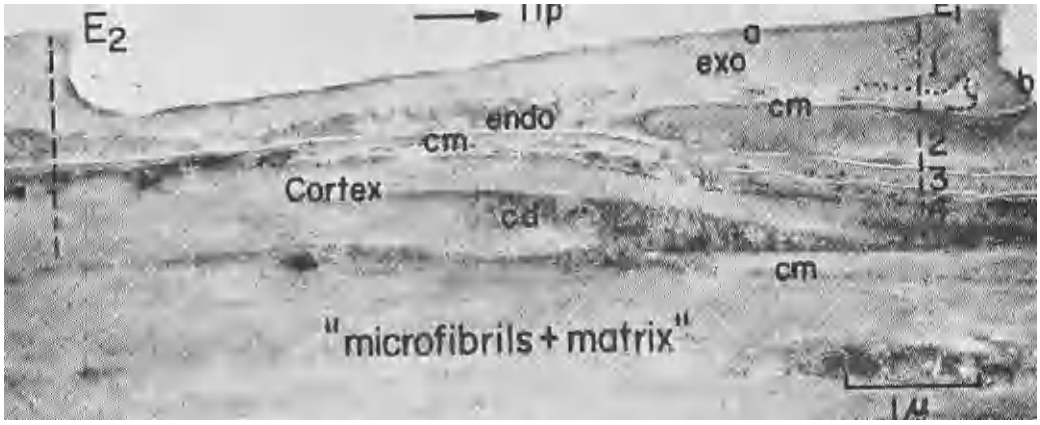

Fig. 6. Longitudinal section of a stained Lincon 36's wool fibre.[11] At E_1 there are four overlapping cuticle cells labelled 1–4, separated from one another and the underlying cortex by cell membranes labelled cm; cd represents material between the macrofibrils in the cortex.

composition to the cellular debris material present in the nuclear remnants of cortical cells.[18,23]

The arrangement of the cuticle cells on Merino wool is shown in Fig. 7 and on human hair in Fig. 8. The large variations in scale patterns between different fibres can be used as an aid in their identification.[24] The detailed structure at the scale edge is shown at E_1 and E_2 in Fig. 6. The scale edges always point from the root toward the tip of the fibre, thus the coefficient of friction in the root to tip direction is less than that in the tip to root direction. This directional frictional effect serves a function in nature as a self-cleaning mechanism in an assembly of fibres on an animal.[25] It is also the source of the felting shrinkage of assemblies of wool fibres, since they tend to move closer together when a force is applied, but are unable to return to their original positions after removal of the felting force because of the directional friction effect. This has great practical importance in the preparation of felts. Prevention of felting shrinkage of woollen garments can be achieved by partial dissolution of the scale edges or by covering the scale edges with a layer of polymer.[26,27]

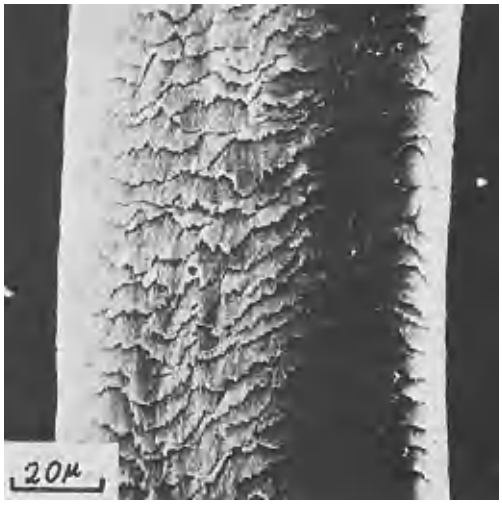

Fig. 8. Scanning electron micrograph of human hair.[5]

Fig. 7. Scanning electron micrograph of a Merino fibre.[5]

ORTHOCORTEX AND PARACORTEX

Reference to Fig. 1 shows that the cortex in fine wool fibres is divided into two sections called the orthocortex and paracortex.[28,29] In Fig. 9 it is seen that in a stained cross section the orthocortex has a different appearance from the paracortex. This results from the clear delineation of the macrofibrils by the intermacrofibrillar material which surrounds each macrofibril in orthocortex, whereas in the paracortex most of this non-keratinous material is concentrated in a few large nuclear remnants. Another important structural difference concerns the differing arrangement of the microfibrils in the matrix, as shown in Figs. 10 and 11. These two basic differences in structure of orthocortex and paracortex are also associated with differences in amino acid composition between the cortices. The various analyses are summarised by Bradbury[2]. Essentially paracortex contains more cystine (25%) than orthocortex and orthocortex contains more tyrosine (42%), phenylalanine (23%) and glycine (18%) than paracortex.

The combination of the more compact nature of the paracortex as compared with the orthocortex (see Figs. 10

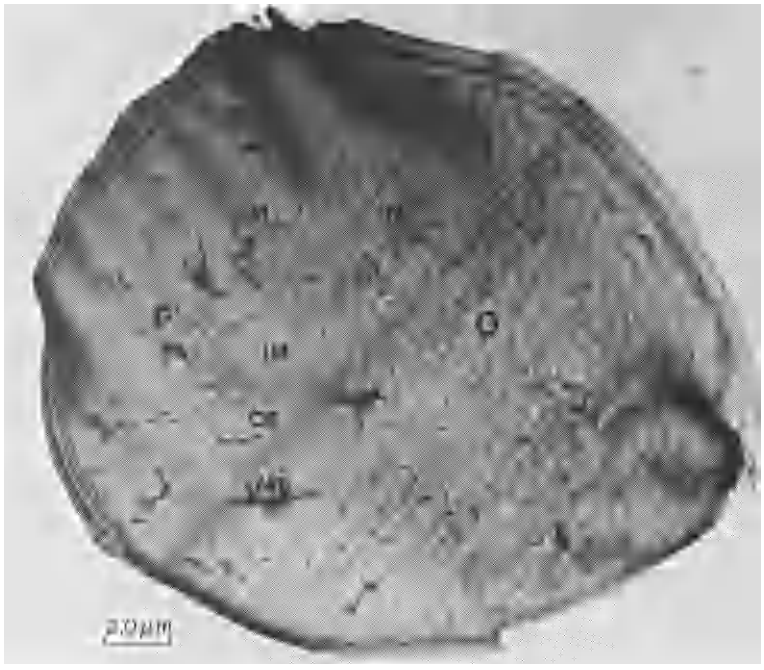

Fig. 9. Electron micrograph of a cross-section of a Merino wool fibre stained with phosphotungstic acid showing orthocortex (O), paracortex (P), macrofibrils (M), nuclear remnants (NR), cell membrane complex (CB) and intermacrofibrillar material (IM).[30]

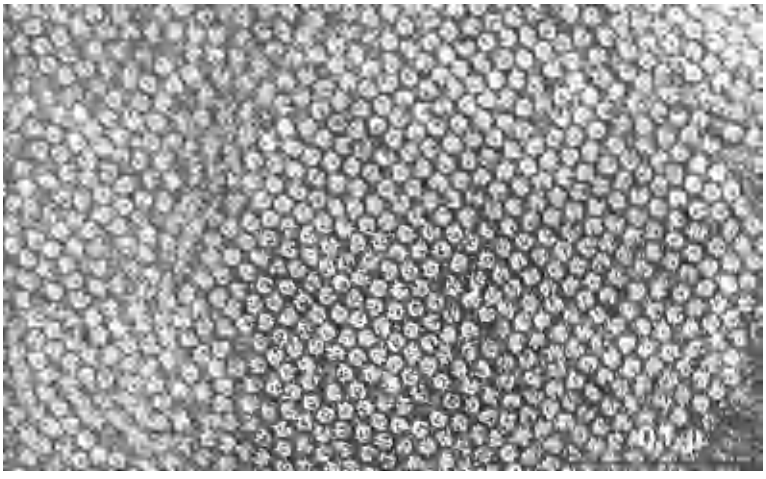

Fig. 10. Part of a cross-section of a stained paracortical cell showing the regular arrangement of microfibrils separated by heavily stained matrix.[31]

and 11) and greater degree of crosslinking by cystine of the former, cause the orthocortex to be more accessible to dyes and other reagents and more reactive chemically than the paracortex.[2] Thus, microfibrils can be separated in low yields from orthocortical cells by treatment with α-chymotrypsin (see below), but not from paracortical cells.[32,33]

The segmentation of the cortex shown in Fig. 9 occurs in fine wools which exhibit crimp, whereas coarse, straight wool fibres such as Lincoln 36's show a central core of orthocortex surrounded by paracortex. Furthermore, as shown in Fig. 12 the paracortex in fine fibres forms the inside and the orthocortex the outside of the crimp wave. They are thus wound around each other helically in phase with the crimp of the fibre, but the sense of the helix varies so that there is very little net twist in the fibre.[35]

MACROFIBRILS

Cortical cells (see Fig. 3) from both orthocortex and paracortex can be disrupted to give macrofibrils by enzymic methods[36] or by ultrasonication in formic acid.[30,37] They are generally similar in shape to cortical cells, about one tenth the length and diameter[37] and have an amino acid composition which is essentially the same as that of the cortical cells from which they are prepared.[30]

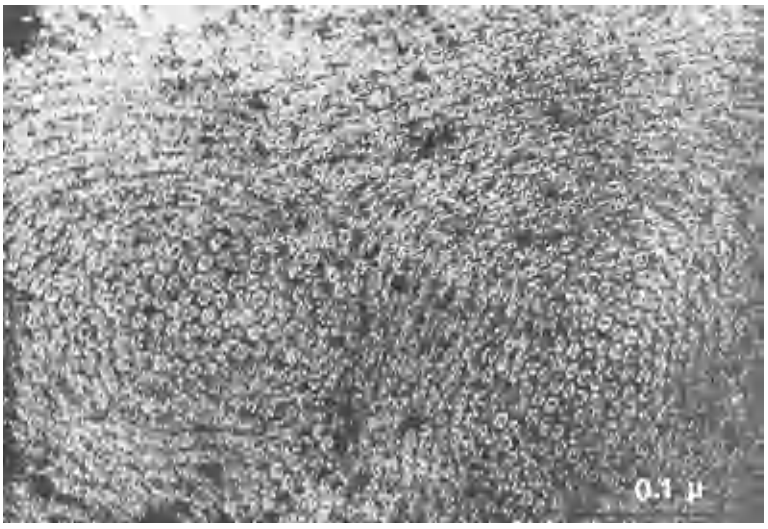

Fig. 11. Portions of two macrofibrils from a cross-section of an orthocortical cell showing the packing of microfibrils in whorls, with less matrix present than in Fig. 10.[31]

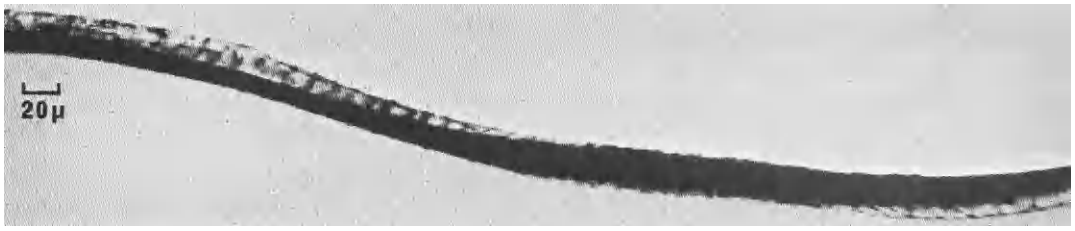

Fig. 12. Merino wool fibre stained with gold preferentially in the paracortex which forms the inside of the crimp wave.[34]

MICROFIBRILS AND MATRIX

The structure shown in Figs. 10 and 11 is that of ordered microfibrils (which give rise to the X-ray diffraction pattern of wool) embedded in a matrix of a lower degree of order. This general type of structure is observed with α-keratin from various sources—fibres, quills, etc. The diameter of the microfibrils obtained from diverse sources is approximately constant at about 72 Å,[38–41] but it has not yet been possible to determine their maximum length although values of the order of 1 μm have been obtained.[8,32,40,41] Because of the reproducibility of the X-ray diffraction pattern and the constancy of the diameter of microfibrils from various sources of α-keratin, it is clear that the microfibril is an important element of structure in α-keratins.

Small amounts of microfibrils have been separated from hair follicles and examined by electron microscopy[40–42] and recently we have been able to produce a preparation of microfibrils and aggregates of microfibrils (see Fig. 13) by treatment of orthocortical cells of Merino wool with α-chymotrypsin.[32,33] The aggregates shown are fused together with matrix material, so that there is some contamination (order of 10%) of the microfibrils with matrix. The amino acid analysis of this material is

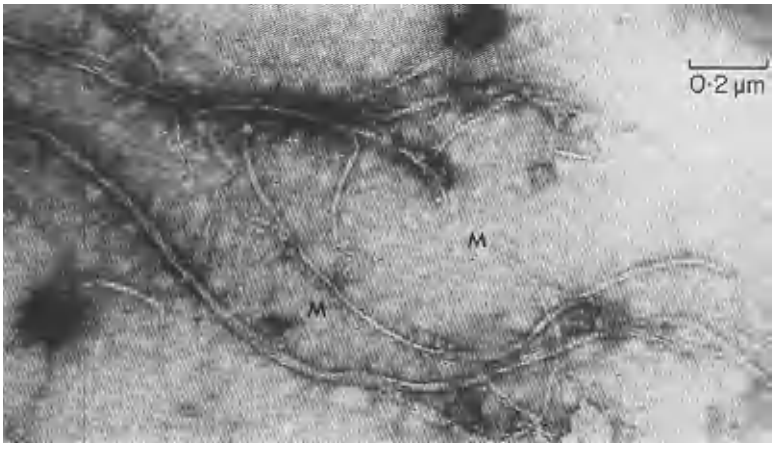

Fig. 13. Electron micrograph with negative staining of microfibrils (M) with some aggregates of microfibrils also present.[32]

intermediate between that of the low-sulphur proteins fraction (SCMKA or α-keratose) and the high-sulphur proteins fraction (SCMKB or γ-keratose) which are obtained after dissolving wool fibres in reducing or oxidising agents.[1,2] The analyses can be rationalised on the basis that the microfibrils and microfibrillar aggregates contain about two thirds of low-sulphur proteins and one third high-sulphur proteins.[33] Since this amount of high-sulphur proteins is considerably greater than the estimated degree of contamination of microfibrils by matrix proteins, it seems likely that the microfibrils contain at least some high-sulphur proteins. The simple idea that the microfibrils consist solely of low-sulphur proteins and that the matrix consists of high-sulphur proteins and the high-glycine-tyrosine proteins[1,2,43] is therefore unlikely to be correct, but more work is required to resolve this question with certainty.

The stress-strain or load-extension curves obtained for wool fibres are characterised by a Hookean region in which there is a linear relation between load and extension up to about 2% extension of the fibre. The characteristic X-ray diffraction pattern of α-keratin (α-pattern) is observed in this region. At higher loads there is a rapid increase in extension and this is designated as the yield region. In this region there is a transition in the X-ray diffraction pattern from the α-pattern to the β-pattern which is characteristic of fully extended fibres.[44–46] These experiments show that the microfibrils (which contain ordered α-helical chains, see below) can maintain their integrity up to about 2% extension, but at higher extensions the α-helical chains are progressively disrupted and extended polypeptide chains are produced. In general, the mechanical properties of fibres can be interpreted in terms of the microfibril-matrix structure of wool.[1,47,48]

SUBSTRUCTURE WITHIN MICRFIBRILS (PROTOFIBRILS)

The two questions of the nature of the protofibril and of its arrangement within the microfibril are undoubtedly the most interesting and controversial unsolved problems in the field of keratin fine structure. Recent reviews[1,2] have summarised the considerable body of information obtained from X-ray diffraction studies of fibres and of electron microscopy of cross sections of fibres and of separated filamentous material. The protofibril consists of two[1,2] or three[49] α-helical polypeptide chains twisted about one another to form a rod-like structure. The diameter of the protofibril is probably about 20 Å, but the length over which its structural integrity is maintained (the coherence length) may be very small (< 100 Å) or much longer. Tropomysin, a muscle protein, consists of two α-helical chains twisted around one another and has a particular amino acid sequence, which shows that the double stranded rope is stabilised by hydrophobic interactions between non-polar side chains.[50] Sequence studies on the low-sulphur proteins from wool (which form the major component of microfibrils) are in progress and indicate the likelihood of stabilisation of the multi-strand rope by hydrophobic and ionic interactions.[49]

The arrangement of protofibrils within the microfibril has also been a subject of considerable controversy,[2] but it is agreed that the microfibril contains an outer ring of radius about 29 Å and an inner core of high electron density.[38,51–54] The core presumably contains one or more protofibrils and there are an indeterminate number arranged around the ring, the annulus between the core and ring may contain non-helical material of the low-sulphur proteins and perhaps high-sulphur proteins.

SEQUENCE STUDIES OF PROTEIN FRACTIONS

The determination of the sequence of soluble proteins from wool was delayed for years by the extreme heterogeneity of the low-sulphur and high-sulphur protein fractions of wool, whether prepared by reductive[55–57] or oxidative procedures.[58–60] The successful fractionation of high-sulphur proteins by South African workers[61] allowed the determination of the sequences of three very closely related proteins containing about 100 residues.[62–64] These molecules contain a high-sulphur fraction (residues 1–48) and a low-sulphur fraction (residues 49–99), but the regular occurrence of proline throughout the sequences would prevent the formation of appreciable stretches of α-helix. Three sequences have been obtained from another family of closely related high-sulphur proteins containing 150–171 residues.[65–69] There is a considerable amount of repetition of a ten residue sequence in each of these proteins.[68] Together, these six proteins would account for about 12% of the total weight of the fibre.[2]

Studies in various laboratories, summarised elsewhere,[2] have shown the presence of a heterogeneous group of proteins called high-glycine-tyrosine, or tyrosine-rich proteins, present in yields of ⩾ 12% in fine wool. These now appear to consist of at least two families[43] and a member of one family has been purified and sequenced.[70,71] This small 61-residue protein contains 14 residues of glycine and 11 residues of tyrosine. There are two sections in which glycine occurs in alternate residues (i.e. $(Gly-X)_3$ and $(Gly\text{-}X)_4$) which is reminiscent of the silk structure, yet there is no evidence of the occurrence of the β-pleated sheet structure in solutions or in cast films.[71] This protein amounts to at least 2% of the weight of Merino wool.

As already stated it is likely that the sequence of low-sulphur proteins will give considerable insight into the structure of protofibrils and microfibrils. Studies in progress[49] indicate there are three types of low-sulphur proteins one of which is a single species and the others consist of two families of proteins. The amino acid analysis of the three types is significantly different and single members of each type have been prepared, but no complete sequence information is available.[72] Chymotryptic or tryptic digestion of low-sulphur protein (SCMKA) produces helical fragments which are considered to be triple chains[49,73,74], rod-like with lengths of about 160 Å.

CONCLUSION

The most interesting features of the fine structure of wool fibres still to be resolved are those concerned with the nature of the microfibrils and protofibrils. It should be possible to obtain this information by (1) further electron microscopic and X-ray studies of separated microfibrils coupled with chemical studies, (2) sequence studies on low-sulphur proteins which originate from the microfibrils and (3) further X-ray diffraction studies. However, there are many other structural and chemical aspects of wool fibres which require elucidation, before it will be possible to relate in detail the structure and chemistry to the physical properties of the fibre.

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