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Ultrafine Structure of α-Keratin

THE equivocal evidence for the structure of the α-keratin microfibril as revealed in transverse section has been discussed[1,2], and it is clear that other methods of approach to the important problem of the arrangement of protein molecules within the microfibril must be used together with sectioning techniques. Again, there is little experimental evidence about the length of the individual protein molecules in the microfibrils and their possible mode of longitudinal polymerization. Thus, in this communication we present a technique for dispersing the microfibrils, prior to negative staining, which is proving useful in the examination of lateral and longitudinal arrangement of protein molecules in keratin.

Fractionation methods applied to fibrous keratin give rise to two main fractions[3]. One fraction is rich in sulphur compared with the original fibre, has a molecular weight of 22,000 and exists in buffer solutions as a random coil[4]. The other fraction is low in sulphur compared with the original fibre, may have a molecular weight of 100,000, and can be regenerated as oriented films which give an X-ray diffraction photograph largely of the α type but with traces of the cross and parallel β configurations[5]. Harrap and Gillespie[6] have examined extensively the fractionation of wool keratin after alkaline reduction and have shown that treatment by sodium thioglycollate at 4° C for 18 h at *p*H 10·5 extracts a protein enriched in the high sulphur fraction and leaves a fibrous residue enriched in the low sulphur protein. This residue has been shown to give a disoriented X-ray diffraction pattern of the α type[7]. As high sulphur content proteins have been tentatively identified with the matrix which surrounds the microfibrils, it was thought that this type of extraction might give a relatively clean preparation of microfibrils which, because of the reduction of disulphide bonds, might be induced to break up into smaller units by the application of ultrasonic irradiation.

The technique finally adopted was to reduce Merino 64's wool, which had been purified by washing with ether, alcohol, and distilled water, with 0·1 M thioglycollic acid in 0·1 M disodium hydrogen phosphate adjusted to *p*H 10·0 or 10·5 with sodium hydroxide. The fibrous residue was then transferred to an intracellular physiological buffer[8] containing potassium, magnesium, chloride, and phosphate ions at *p*H 7·0, and irradiated with ultrasonics at 15 c/s and 20 W cm^{-2} for 5 h. The temperature in the water-cooled cell remained at 12° C throughout the irradiation. The dispersed material was centrifuged at 500*g* for 5 min, the supernatant liquid discarded, the sediment washed with buffer and recentrifuged and, finally, resuspended in 1 per cent sodium phosphotungstate at *p*H 5·6 and irradiated with ultrasonics for a further period of about 1 h. Drops of the dispersed material were then placed on 'holey' carbon film on specimen supporting grids and examined in a Siemens Elmiskop I electron microscope after desiccation.

It may be noted that, as in the case of transverse sections[1], optimum contrast in the specimen is found at a slightly under-focused setting of the objective lens with respect to true focus as defined by the absence of Fresnel diffraction at the edge of a hole. Because of this the structure of the carbon film is out of focus and tends to distort the appearance of the specimen. Fig. 1 shows a typical area of the preparation and it is evident that the microfibrils have been dispersed into long filaments or protofibrils; indeed there was no evidence of intact microfibrils remaining in the preparation. Microdensitometer traces with a long slit have been made on the negatively stained filaments and reveal widths between 12 Å and 40 Å; however, a width of approximately 20 Å predominates. In the region indicated by the arrows there are three filaments about 12 Å, 18 Å and 40 Å in width, but their identities are not maintained for any great length. In Fig. 1 and other micrographs, some of the 20 Å wide filaments appear to be composed of still smaller filaments twisted together, but it is not yet possible to say how many of these smaller filaments are present because of the background noise.

Fig. 1. Merino wool reduced, dispersed by ultrasonic irradiation, and stained with phosphotungstic acid (×275,000)

It may be tentatively suggested that the smallest filaments seen here are single protein molecules, and that the predominant 20 Å wide filaments are two- or three-strand ropes. Nevertheless, other arrangements of the molecules cannot be excluded. From these results it is difficult to say whether or not any one arrangement is maintained for a great distance along the microfibril, and there is no indication of a unique structure for the microfibril. Further work is being carried out.

Finally, it should be noted that, in an independent investigation, Dobb[9] has dispersed the microfibrils using a different technique and again finds predominant evidence of 20 Å wide protofibrils.

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D. J. JOHNSON
P. T. SPEAKMAN

Textile Physics and Textile Chemistry Laboratories,
Department of Textile Industries,
University of Leeds.

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