

independent structural component . . . and not simply the unobscured rim of a normal SO disk". Several other similar systems are known, and O'Connell *et al.* cite the examples of NGC6028, NGC2859 and IC5285. "There is no reason to suppose that radiation from these rings is anything other than direct starlight", and in the case of Hoag's object the radiation certainly cannot be reflected light from the core, since the ring is "at least" as luminous as the core.

The interpretation which seems to be favoured by O'Connell *et al.* involves gravitational encounters between galaxies which could initiate star formation in "trapped" rings; they point out that a pair of galaxies near Hoag's object on the sky could, if they are at the same distance as the object, have passed by it 10^6 to 10^8 yr ago, just right to account for the apparent age of the stars in the ring.

Merokeratins

from Peter Speakman

Wool is a mixture of about 100 different proteins, but Haylett's group in South Africa have been able to purify several proteins with a high cysteine content and to show homologies in their amino acid sequences. Argument is now over whether these proteins have evolved from a repeating penta- or deca-peptide (Swart and Parris, *Nature*, **249**, 580; 1974). Lindley and Cranston have pointed out that because of the heterogeneity of proteins a new experimental approach is needed to supplement sequence studies in order to study the pattern of disulphide crosslinks between proteins as they are arranged together in the fibre (*Biochem. J.*, **139**, 515; 1974).

Protein fragments which are very similar in molecular weight, dimensions and α -helix content have been prepared from mammalian epidermal keratin and from reduced wool by partial proteolysis, the method which was used to prepare the meromyosins and more recently, myosin subfragments 1 and 2. A sharp low-angle X-ray diffraction pattern from oriented films of the wool fragment showed that it could aggregate in a specific way into long, thin assemblies. These fragments from epidermis and reduced wool may be in the native conformation; and so studies of their internal structure and dimensions in solution and investigations of their specific aggregates, are beginning to give information about the conformation of the protein chains within the keratin macromolecular components, and about the mutual positions of the macromolecules in the fibre or tissue. Analogous studies of dissolved muscle proteins and their modes of aggrega-

tion, and of tropocollagen and its polymorphic aggregates have contributed to a very detailed understanding of muscle and connective tissue structure.

The small-angle X-ray diffraction pattern published from the CSIRO Division of Protein Chemistry in Melbourne shows that a wool keratin fragment can be made to form specific aggregates which are about 200 Å in diameter and greater than 2,000 Å long, with a longitudinal repeat of 160 Å (Suzuki *et al.*, *J. molec. Biol.*, **73**, 275; 1973). The method of preparing the fragment is interesting and relevant. Wool proteins are dissolved by reduction in a solution of a denaturing agent (8M urea). The thiol groups are blocked by reaction with iodoacetate ions, and the urea is removed by dialysis. The proteins (SCMKA) with a smaller proportion of cysteine residues and a higher α -helix content than the original wool are separated by fractional precipitation and purified by chromatography.

It is clear that some refolding of the SCMKA proteins, into conformations which are not completely flexible, takes place when the urea is removed, because a fairly large protein fragment (as well as small peptides) is produced when the SCMKA proteins are treated briefly with chymotrypsin. The fragment has a molecular weight of 41,000, a length of 170–200 Å measured in solution or 160 Å in the electron microscope, and a diameter of 20 Å. It has a higher α -helix content (80%) than the original SCMKA proteins (50%) and it is believed to consist of three chains (Crewther and Harrap, *J. biol. Chem.*, **242**, 4310; 1967; Dobb *et al.*, *J. Textile Inst.*, **64**, 374; 1973). A similar wool fragment has been prepared without using a denaturing agent (Hilburn *et al.*, *Biochim. biophys. Acta*, **214**, 245; 1970; Dilley *et al.*, Abstracts, British Biophysical Society Meeting, Leeds, April 1971). If wool is reduced at pH 10 (which itself may cause some denaturation) and treated with trypsin, a rod-shaped fragment, 200 Å long and 20 Å wide, molecular weight 55,000, with a higher α -helix content (64%) and a lower cysteine content than the original wool, can be separated from the digest.

An analogous fragment prepared by partial proteolysis of epidermal keratin protein is reported from Matoltsy's laboratory (Skerrow *et al.*, *J. biol. Chem.*, **248**, 4820; 1973). It is not necessary to reduce disulphide crosslinks in order to extract protein from mammalian epidermis but a high or low pH is needed (in this case pH 2.6), which may again cause some denaturation. The solution of epidermal protein in acid buffer is added dropwise to a trypsin solution maintained at pH 8.8, and a protein fragment can be separated from the digest, length 200 Å, molecular weight 46,000, which is 83% α -helical.

Polyacrylamide gel electrophoresis in denaturing conditions showed that the fragment consisted of three chains with approximately similar molecular weights.

The protein chains of SCMKA were denatured during the preparation, and therefore information about the structure of wool can only be inferred from the structure of the SCMKA proteolytic fragment, and from the mutual arrangement of the fragments in the specific aggregate, if partial or exact renaturation occurred when the denaturant was removed. Similarly, the other wool fragment and the epidermal protein fragment have been exposed to pH values which might cause denaturation. On the other hand, the similarity between the three fragments strongly suggests that undenatured or accurately renatured parts of the keratin structure have been prepared in these experiments. Their compositions and α -helix contents imply that they originate in the wool protofibril and the epidermal protofilament, but the low yield in each preparation (for example, less than 10% in the case of the wool fragment prepared without a denaturing agent) means that they may not represent the whole of these structures.

This work will encourage attempts to dissolve native proteins from epidermis or reduced wool without the use of proteolytic enzymes or denaturing agents, and the study of their structure in solution. Fairly extreme conditions of pH or temperature dissolve large quantities of keratin proteins but they may cause denaturation. Milder conditions are less likely to cause denaturation, but smaller amounts of protein dissolve and it may not be clear whether they are minor components or partly soluble major components. In both cases, even if the protein has a definite conformation in solution this will not necessarily be identical to the conformation adopted by the same protein in the fibre or tissue. A similar problem arose in collagen research in the 1950s. Acid or neutral buffer solutions will only dissolve small amounts of tropocollagen from calf skin, for example, but it was possible to show that the conformation of the protein chains in dissolved tropocollagen is the same as in intact connective tissue. The length of the tropocollagen molecule measured in solution was shown to be consistent with the dimensions of one of its specific aggregates (SLS) in the electron microscope; and the mutual arrangement of the tropocollagen molecules in another specific aggregate (quarter-stagger) was deciphered, and shown to be very similar to the arrangement in connective tissue. Experiments with the native fibrous protein in solution, and research with intact tissue dramatically confirmed each other.