Aust. J. Biol. Sci., 1981, **34**, 515–26

Structural Studies on the Microfibrillar Proteins of Wool: Characterization of the α-Helix-Rich Particle Produced by Chymotryptic Digestion

E. F. Woods and L. C. Gruen

Division of Protein Chemistry, CSIRO, Parkville, Vic. 3052.

Abstract

The α-helix-rich particle produced by chymotryptic digestion of the reduced and alkylated microfibrillar proteins of wool was characterized by physicochemical methods. The preparations were homogeneous with respect to size and the particle molecular weight was found to be 50 200 ± 2 000. Hydrodynamic methods indicated a length of about 20 nm for the particle. The properties of the particle, derived from two methods of isolation of the microfibrillar proteins, were identical and were also independent of the type of wool used. From a consideration of the molecular weight in denaturing solvents and from cross-linking experiments with dimethyl suberimidate a four-chain structure, consisting of a pair of double-stranded α-helices, is proposed for the particle.

Introduction

The idea that the microfibrils of hard mammalian keratins are precise arrays of the low-sulfur proteins (Fraser *et al.* 1972) has been studied by the isolation of microfibrils from developing fibres and confirmed by showing that these are composed of low-sulfur proteins (Jones 1976). The solubilized microfibrillar proteins have α-helical contents of 50–55% (Harrap 1963) and are composed of subunits in the molecular weight range 45 000 to 58 000 (Woods 1979). The conformation of these proteins in their native state is not known but there is substantial evidence that it is the low-sulfur proteins that are responsible for the α-type X-ray pattern of α-keratins. The α-helical sections of the low-sulfur proteins are not straight but are distorted into coiled-coils. The number of strands in the coiled-coil ropes is uncertain (Fraser *et al.* 1972).

Important information regarding the structure of fibrous proteins has been obtained by the use of proteolytic enzymes. The least-ordered parts of the molecule are more susceptible to proteolysis and this property has been used to obtain fractions enriched in specific types of secondary structure such as the α-helix. Crewther and Harrap (1967) isolated a helix-rich fraction (>80% α-helix) by proteolysis of *S*-carboxymethylkerateine A (SCMKA) from wool with pronase P. Hydrodynamic measurements indicated a molecular weight of 41 000, a length of 17·2–20·4 nm and a diameter of ≃2 nm. A similar-sized particle was produced by proteolysis with α-chymotrypsin (Crewther and Dowling 1971; Dobb *et al.* 1973). X-ray diffraction on oriented films of the α-chymotryptic particle gave a typical α-pattern which indicated a coiled-coil α-helical conformation (Suzuki *et al.* 1973) and it was concluded (Crewther 1976) that the particle dimensions were consistent with a three polypeptide chain structure. The isolation of subunits, two of which have been

sequenced (Crewther *et al.* 1978; Gough *et al.* 1978), of molecular weights between 12 000 and 13 000 supported a three-chain particle.

Speakman and collaborators (Campbell *et al.* 1976; Lotay and Speakman 1977; Ahmadi and Speakman 1978; Ahmadi *et al.* 1980) have prepared a highly α-helical, rod-shaped particle, which they termed merokeratin A_1, by limited tryptic digestion of reduced wool. From molecular weight studies in the ultracentrifuge and sodium dodecyl sulfate polyacrylamide gel electrophoresis they were unable to say whether there were three or four polypeptide chains in the particle. From results of cross-linking with dimethyl suberimidate by the method of Davies and Stark (1970) they concluded that the α-helical particle had a molecular weight of 57 000 and consisted of four polypeptide chains.

In this paper we report a more extensive study of the size homogeneity, molecular weight and physical properties of the α-helical fragment produced by the digestion of SCMKA with α-chymotrypsin. Two methods of preparation of the SCMKA as well as the type of wool have been investigated in order to compare our results with those from Speakman's laboratory.

Materials and Methods

Preparation of SCMKA

SCMKA was prepared from Merino wool by reduction with 2-mercaptoethanol in the presence of 8 M urea and *S*-carboxymethylation with Tris-iodoacetate as described by O'Donnell and Thompson (1964). The steps in the purification involved dialysis, precipitation with zinc acetate at pH 5·8 (Gillespie 1957), solution in 0·02 M sodium citrate at pH 8·0, precipitation at pH 4·4 in the presence of 0·5 M KCl, solution in sodium tetraborate and finally precipitation with acetone in the presence of ammonium sulfate (Dowling and Crewther 1974). The proteins were finally dialysed against 0·01 M sodium tetraborate, pH 9·2. SCMKA was prepared also by the plasmolysis procedure of Harrap and Gillespie (1963) employing 0·5 or 0·8 M sodium thioglycollate at pH 10·5 or 0·5 M 2-mercapto-ethanol at pH 11. The plasmolytic extract was precipitated at pH 5·7, the precipitate re-dissolved in 0·1 M reducing agent at pH 8·5 and alkylated. The protein was purified as before.

Enzyme Digestion of SCMKA

The helix-rich particle was prepared by digestion with α-chymotrypsin for 3½–4 h at 37°C as described by Crewther and Dowling (1971). The digestion was terminated by precipitation at pH 4·0 in the presence of 0·5 M KCl. The precipitate was dissolved at pH 9 and precipitated twice at pH 4·0 and finally dialysed against 0·05 M KCl–0·05 M sodium tetraborate. Further purification was carried out by gel filtration on Sepharose CL-6B.

Physical Methods

Sedimentation experiments were carried out with a Beckman Model E ultracentrifuge equipped with both Rayleigh interference and schlieren optics. Sedimentation velocity experiments were performed at 60 000 rpm and the sedimentation coefficients were corrected to the density and viscosity of water at 20°C. Diffusion coefficients were measured in the ultracentrifuge at 5 000 rpm by applying the conventional reduced height–area method to the spreading of a preformed boundary in a synthetic boundary cell.

Molecular weights were measured by the low-speed equilibrium method (Richards *et al.* 1968) and by the meniscus-depletion method of Yphantis (1964). A six-channel centre piece was employed for the conventional equilibrium runs with column heights of 2 mm and the methods used to evaluate the molecular weights have been given in a previous paper (Woods 1979). For the meniscus-depletion experiments regular double-sector cells were used with column heights of 3 mm. The computer program of Roark and Yphantis (1969) was used to calculate the number-, weight- and *z*-average molecular weights, $M_n(r)$, $M_w(r)$ and $M_z(r)$ respectively, at regular radial distances r along the cell.

The apparent partial specific volume of the helical fragment was determined from the densities of the dialysed protein and its diffusate. The densities were measured at $20 \pm 0{\cdot}02$°C with a precision density-meter DMA-02C (Anton Parr, Graz) and protein concentrations were determined from the dry weights of the solution and its diffusate as described by Woods (1979). The absorbance and specific refractive index increment were determined on the same solutions as used for the partial specific volume measurements and are given in Table 1. These values were used for determination of the protein concentration for physical measurements.

Optical rotatory dispersion (ORD) measurements were made with a Perkin Elmer spectropolarimeter Model 141 at wavelengths of 365, 405, 436, 546 and 578 nm. Values of b_0 from the Moffitt equation were determined by a least-squares linear fit to the data using a λ_0 value of 212 nm (Moffitt 1956). To calculate the percentage α-helix the value of $b_0 = -630$ deg. cm^2 dmole^{-1} for an infinite helix was used (Chen *et al.* 1974). Measurements of ORD at lower wavelengths and of circular dichroism (CD) were made with a Jasco instrument ORD/UV5 with a modified CD attachment in cells of path length 0·1 or 1·0 mm. The CD data were expressed in terms of the mean residue ellipticity, $[\theta]$, in deg. cm^2 dmole^{-1}. The percentage α-helix was calculated from both the mean residue rotation at 233 nm, $[m']_{233}$, and $[\theta]_{222}$, assuming that only α-helix and unordered forms were present, since there is no evidence for the existence of other structures in reduced and alkylated low-sulfur wool proteins. Equations (10) and (16) of Chen *et al.* (1974) were applied and the values for 100% α-helix were taken to be those for infinite helices, ($[\theta]_H = -39\,500$ deg. cm^2 dmole^{-1}, $[m']_H = -18\,700$ deg. cm^2 dmole^{-1}). The value for the unordered coil form was taken to be that in 6 M guanidine hydrochloride, $[\theta]_R = -900$ deg. cm^2 dmole^{-1}, $[m']_R = -2\,300$ deg. cm^2 dmole^{-1}.

Intrinsic viscosities were measured at $20 \pm 0{\cdot}02$°C by means of a Schotte–Gerate AVS/N automatic viscosity measuring instrument with Ostwald microviscometers which had flow times for water of 70 s.

The Stokes' radius was determined from gel-filtration elution volumes, using the Pharmacia calibration kit proteins as standards, plotted according to the method of Laurent and Killander (1964).

Polyacrylamide electrophoresis was carried out in slab gels measuring 140 by 100 by 1·5 mm (Bio-Rad apparatus Model 220). The procedure of Davis (1964) was used to prepare the gels at pH 8·9 and 8 M urea was incorporated when required. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) was performed by the procedure of Laemmli (1970). For SDS gradient gel electrophoresis Gradipore variable cross-linkage gradient gels were used with 0·05% (w/v) SDS, 0·025 M sodium phosphate buffer, pH 7. In all methods electrophoresis was performed until the bromphenol blue tracking dye had almost reached the bottom of the separating gel. Gels were stained with Coomassie Blue G 250 (I.C.I.) and destained with 5% (v/v) methanol–10% (v/v) acetic acid. Gels were scanned with a Shimadzu dual wavelength TLC scanner at 590 nm. The proteins in the Pharmacia low-molecular weight calibration kit were used as molecular weight standards.

Cross-linking with dimethyl suberimidate (Davies and Stark 1970) was carried out in 0·2 M triethanolamine, 0·17 M Na_2HPO_4 adjusted to pH 10 with H_3PO_4. The protein concentration was 1 mg/ml and solid dimethyl suberimidate dihydrochloride (Pierce Chemical Co.) was added in two portions at intervals of 15 min to a final concentration of 2 mg/ml. Higher ratios of cross-linker to protein gave similar results. Before electrophoresis SDS was added to 5 mg/ml, the samples were heated in a boiling water-bath for 2 min and then dialysed against the running buffer for gradient gel electrophoresis.

Results and Discussion

Gel Filtration of the Helix-rich Acid Precipitate on Sepharose CL-6B

The helix-rich fragment prepared by acid precipitation of a chymotryptic digest of SCMKA from wool was heterogeneous when examined by the meniscus-depletion sedimentation equilibrium method, with molecular weights ranging from 35 000 to 60 000. Gel filtration on Sepharose CL-6B (Fig. 1) exhibited a major peak with minor components which eluted before and after the main component as was also found by Suzuki *et al.* (1973). The major peak tube was examined by the meniscus-

depletion sedimentation equilibrium method which gave a molecular weight of 50 000 with little evidence of heterogeneity. During the course of this study the peak tubes from many such gel-filtration experiments from different preparations were examined by the meniscus-depletion method. The molecular weight was either constant down the solution column or showed a slight decrease due to non-ideality. The tubes around the peak position (fraction II) were pooled, the protein concentrated by acid precipitation and re-run as indicated in Fig. 1. No material eluted after the major peak but there was still some asymmetry in the leading edge and this is attributed to self-association of the major component. The tubes around the rechromatographed major peak were pooled and concentrated and used for further characterization of the helical fragment. Purification by gel filtration gave material of 5–10% higher α-helix content than the parent chymotryptic fragment. It is this purified material which is subsequently referred to as the helix-rich particle.

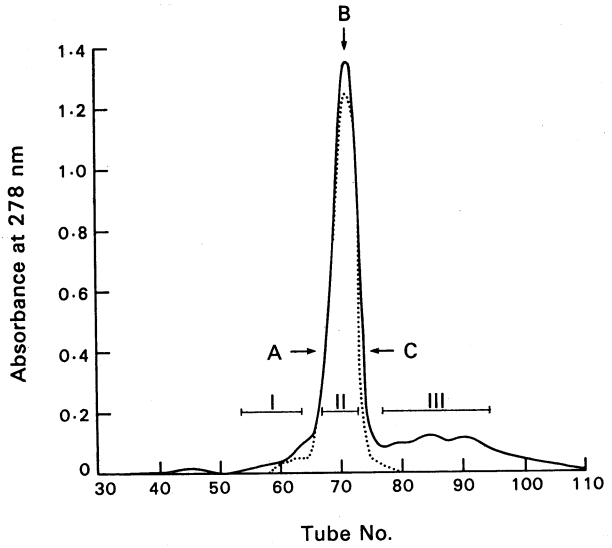

Fig. 1. Gel filtration of the chymotryptic digest of SCMKA on Sepharose CL-6B in 0·05 M sodium tetraborate–0·05 M KCl, column size 2·5 by 113 cm, fraction size 5 ml. Tubes were pooled to give fractions I, II and III. Fraction II was re-run (dotted curve) and arrows indicate samples A, B and C taken for sedimentation equilibrium molecular weight measurements (see text).

The components eluting before and after the major component (Fig. 1) during the initial chromatography were also examined. The protein from the leading edge (fraction I) had the same helical content as the major peak but was heterogeneous and of higher molecular weight; values ranging from 54 000 to 90 000 were obtained from the top to the base of the solution column in a meniscus-depletion experiment at 20 000 rpm. This material is assumed to be a self-association product since the amount increases on storage and the SDS gel electrophoresis pattern is the same as that of the major component.

The material eluting after the major peak (fraction III) was heterogeneous and of much lower molecular weight (9 000–20 000) and lower α-helix content (approx. 40% compared with >80% for the main peak).

Characterization and Homogeneity of the α-Helical Particle

The physicochemical properties of the α-helical particle are summarized in Figs. 2 and 3 and Table 1. The method of preparation of the SCMKA (i.e. the urea–mercaptoethanol or plasmolysis methods) from which the helical particle was derived had no

significant effect on its physicochemical properties. This suggests that if urea is used to extract proteins from wool the original α-helical structure is reformed upon removal of the urea. A similar-sized α-helical particle was also obtained when Lincoln wool (as used by Campbell *et al.* 1976) was used as the source of the low-sulfur proteins.

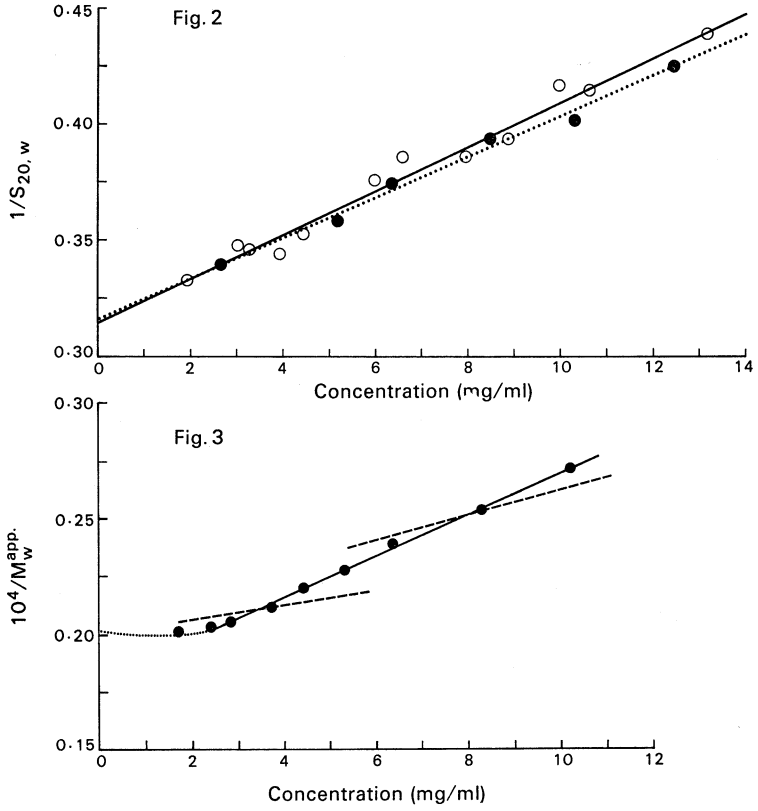

Fig. 2. Sedimentation coefficient as a function of initial concentration for the helix-rich particle in 0·05 M sodium tetraborate–0·05 M KCl. ● Helix-rich particle from urea-mercaptoethanol SCMKA (broken line). ○ Helix-rich particle from plasmolysis SCMKA (full line).

Fig. 3. Sedimentation equilibrium of helix-rich particle (from urea–mercaptoethanol SCMKA). Reciprocal of apparent weight-average molecular weight over the whole cell as a function of concentration for low-speed equilibrium runs (10 000 and 12 000 rpm). The extrapolation to zero concentration (dotted line) is uncertain due to curvature in the plot at low concentrations. The broken lines show trends of the reciprocal of point-average molecular weight with concentration for two individual cell channels.

The plot of $1/M_w$ versus c from low-speed sedimentation equilibrium (Fig. 3) shows curvature at low concentrations and the extrapolation to infinite dilution is uncertain. We believe that this arises from association and that dissociation occurs on dilution. In Fig. 3 the values of $1/M_w$ (r) for points along the centrifuge cell are also shown for two individual experiments and these indicate heterogeneity since they do not coincide with the plot for the cell-average values as they should for a homogeneous protein. This heterogeneity seems to be mainly a result of self-associ-

ation since in meniscus-depletion experiments at much lower initial protein concentrations (0·2 mg/ml) and higher speeds (26000 rpm) a constant value of 50200±2000 was observed. The presence of species with molecular weight as low as 37000 could be detected if higher speeds (32000 rpm) and higher initial concentrations (1·5 mg/ml) were used in the meniscus-depletion experiments. It therefore became important to determine whether the particle dissociated to a value of less than 50000. The protein was submitted to a further gel filtration on Sepharose CL-6B and fractions (indicated by A, B, C in Fig. 1) were taken for measurement of molecular weights by the meniscus-depletion method. The values obtained for the molecular weights at A, B and C were 51700, 50100 and 48300 respectively. Only sample C from the trailing side of the peak showed a decrease in molecular weight towards the top of the solution column to ≃41000. Thus we conclude that the main species of the helix-rich particle from the chymotryptic digestion of SCMKA has a molecular weight of 50200±2000, does not dissociate further at high dilution and the slight heterogeneity we have observed arises as a result of the enzymic digestion. The subunit patterns on SDS gel electrophoresis for samples A, B and C were identical.

Table 1. Physicochemical properties of the helix-rich particle

All measurements made in 0·05 M KCl–0·05 M sodium tetraborate pH 9·2 except where otherwise stated

Parameter	Method of preparation of SCMKA	
	Urea-mercaptoethanol	Plasmolysis
Sedimentation constant ($S^0_{20,w}$)	3·16 S	3·18 S
Diffusion coefficient, $D_{20,w}$ ($cm^2\ sec^{-1}$)	$5·0\times10^{-7}$	
$[\eta]$ ($ml\ g^{-1}$)	10·3	9·7
Partial specific volume ($ml\ g^{-1}$)	0·708[A]	
	0·726[B]	
Refractive index increment ($ml\ g^{-1}$)	0·180	
Absorbance at 278 nm ($E^{1\%}_{1cm}$)	5·2±0·1	5·2±0·1
b_0 (deg. $cm^2\ dmole^{-1}$)	−535	−525
$[m']_{233}$ (deg. $cm^2\ dmole^{-1}$)	−15400	−15200
$[\theta]_{222}$ (deg. $cm^2\ dmole^{-1}$)	−33300	−32900
α-Helix(%)[C]	83	82
Molecular weight		
Svedberg equation	52600	
Sedimentation equilibrium, low speed	50500[D]	
Sedimentation equilibrium, meniscus depletion	50200	50400
Stokes' radius (nm) from gel filtration	5·5	5·5
Stokes' radius (nm) from S or D	4·2	4·2

[A] Buffers 0·1 M KCl–0·01 M sodium tetraborate or 0·1 M KCl–0·01 M Na_2HPO_4, pH 9·2.
[B] Buffer 0·05 M (NaH_2PO_4–Na_2HPO_4), pH 7.
[C] Mean of the values calculated from the three optical methods.
[D] Extrapolation to zero concentration uncertain due to curvature in plot of $1/M_w$ versus c at low concentrations (see text and Fig. 3).

Information on the shape of the particle in solution was obtained from the hydrodynamic measurements (see Table 1). The β-function of Scheraga and Mandelkern (1953) calculated from M, $S^0_{20,w}$ and $[\eta]$ is $2·26\times10^6$. This corresponds to an equivalent prolate ellipsoid of axial ratio ≃6, an effective hydrodynamic volume of 1·45 ml/g and a hydration of 0·74 ml/g. From the equations given by Yang (1961)

the length of the equivalent ellipsoid is $\simeq 20$ nm. An upper limit to the axial ratio and length may be estimated from $[\eta]$ assuming no hydration and this gives a value of 11 for the axial ratio and 23·5 nm for the length of the equivalent prolate ellipsoid. An interesting observation is that the Stokes' radius determined by gel filtration is larger than that determined from sedimentation or diffusion (Table 1). This indicates that the frictional coefficients in gel permeation and free diffusion are not identical. Further discussion on the dimensions of the helical particle is deferred until we have considered its subunit structure.

Heterogeneity of the helical particle with respect to charge was investigated by electrophoresis on 7·5% (w/v) polyacrylamide gels at pH 8·9. There was some charge heterogeneity as shown in Fig. 4*a* but the diffuse pattern showed no clean separation of components. Further attempts at fractionation, on the basis of charge, by ion-exchange chromatography on DEAE-cellulose at pH 7·8 with a salt gradient from 0 to 1·0 M NaCl, failed to resolve any components.

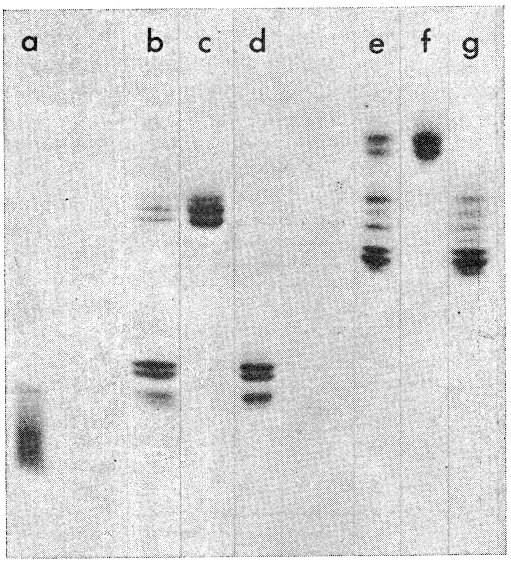

Fig. 4. Gel electrophoresis of helix-rich particle and its polypeptide chains. (*a*) Helix-rich particle, 7·5% (w/v) polyacrylamide, Tris-glycine, pH 8·9; (*b*)–(*d*) SDS, 15% (w/v) polyacrylamide: (*b*) helix-rich particle; (*c*) ChB fraction; (*d*) ChC fraction. (*e*)–(*g*) 8 M urea, 10% (w/v) polyacrylamide, Tris-glycine, pH 8·9: (*e*) helix-rich particle; (*f*) ChB fraction; (*g*) ChC fraction.

Behaviour of Helical Fragment in Dissociating Solvents

Previously gel filtration of the helix-rich particle on Sephadex G75 in 8 M urea (Crewther and Dowling 1971) showed two major peaks with molecular weights 25000 (ChB, $\simeq 35\%$) and 12500 (ChC, $\simeq 55\%$) and a small peak of higher molecular weight (ChA). We have found similar results by high-performance liquid chromatography on an I-250 (Waters Associates) column in 8 M urea.

The SDS gel electrophoresis pattern (Fig. 4*b*) is characterized by two groups of closely spaced bands with a minor slower component. The two main groups of bands correspond to ChB and ChC respectively (Figs 4*c*, 4*d*). The minor slower-moving band on SDS gels corresponds to a molecular weight of $\simeq 38000$. Estimation of the average proportions (assuming equal dye binding to all components) of components from 15 gels and five preparations gave $\simeq 66\%$ of ChC, 31% of ChB and $\simeq 3\%$ of ChA. The subunit composition did not appear to be influenced by the method of preparation of the SCMKA from which the helical particle was derived,

or by the type of wool (Lincoln or Merino) used to prepare the SCMKA. The results of gel electrophoresis at pH 8·9 in 8 M urea are shown in Figs 4*e*–4*g*. The larger number of bands seen for ChC under these conditions is consistent with the elution profile on DEAE-cellulose chromatography in 8 M urea (Crewther *et al.* 1978) where at least eight components were resolved.

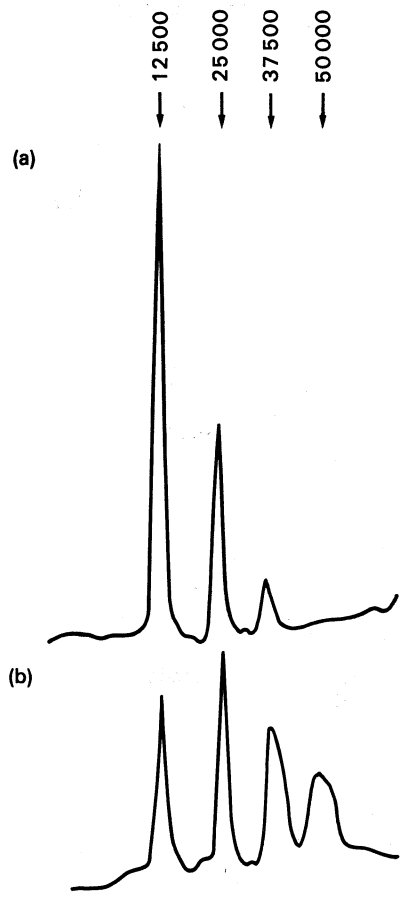

Fig. 5

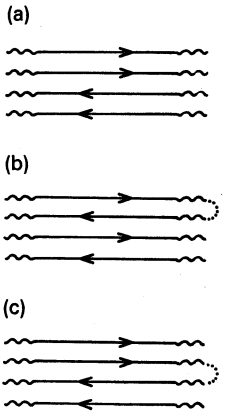

Fig. 6

Fig. 5. SDS gradient gel electrophoresis of helical particle. Densitometer scans. (*a*) control; (*b*) cross-linked with dimethyl suberimidate. Molecular weights calculated from the positions of the peaks, assuming cross-linked species are multiples of 12 500, the fastest moving band being taken as 12 500.

Fig. 6. Proposed structures for the α-helix rich particle. (*a*) a pair of α-helical coiled-coils composed of 12 500 molecular weight subunits. (*b*), (*c*) a pair of α-helical coiled-coils incorporating one 25 000 molecular weight subunit and two 12 500 subunits. The solid lines represent α-helix; the wavy lines represent non-helical regions at the ends. In (*a*) and (*c*) one pair of coiled-coils is anti-parallel to the other; in (*b*) the individual chains of each coiled-coil are antiparallel. The dotted line represents a putative linkage between two chains (see text).

Cross-linking with Dimethyl Suberimidate

The results of cross-linking the helical particle with dimethyl suberimidate are given in Fig. 5 which shows the densitometer scans of SDS gradient gels. There are three groups of bands in the control sample but resolution within the groups is not as good as with 15% (w/v) polyacrylamide SDS gels (Figs 4*b*–4*d*). The molecular weight of the most mobile component is 12 500 (Crewther *et al.* 1978; Gough *et al.* 1978). The molecular weights of the other two bands were obtained from the calibration curve of log (molecular weight) versus mobility for the standard proteins and were found to be 25 000 and 37 500. After cross-linking with dimethyl suberimidate and separation of the products by SDS gel electrophoresis four bands were now evident. The molecular weights of these four components, estimated from their mobilities, were 14 000, 26 000, 38 000 and 56 000 and we therefore assume that they correspond to monomer, dimer, trimer and tetramer respectively. This indicates that there are four chains in the helical particle in close proximity. After treatment with cross-linker the amount of monomer decreased to about 25%. The percentage of dimer remained unchanged, the trimer increased to $\simeq$25% and up to $\simeq$20% of a species corresponding to tetramer appeared. Only about 40% of the subunits cross-link to form oligomeric species. This could arise from side reactions which do not lead to cross-linking such as hydrolysis of one end of the reagent, preference for intrasubunit as opposed to intersubunit cross-linking, or the absence of ε-lysine amino groups in certain binding domains due to subunit heterogeneity as shown by the gel patterns (Figs 4*e*–4*g*). A similar degree of cross-linking was also attained with dimethyl adipimidate. The results are similar to those of Ahmadi and Speakman (1978) and lead to the conclusion that the helical particle has four polypeptide chains.

General Discussion and Conclusions

The following experimental facts must be accounted for when considering a model for the structure of the helical particle: (1) an α-helix content of $\simeq$83% (Table 1); (2) a molecular weight of 50 200 (Table 1); (3) a length of $\simeq$20 nm deduced from hydrodynamic measurements; and (4) a tetrameric subunit structure indicated by cross-linking experiments. The only way in which all of these data could be accommodated would be to have four polypeptide chains of molecular weight 12 500 aligned laterally as a pair of two-stranded, α-helical coiled-coils. This assumes α-helices in register with an axial rise of 1·49 Å per residue. X-ray diffraction (Suzuki *et al.* 1973) on oriented films of the helical particle has shown that the coiled-coil structure is present and it is reasonable to assume that this also exists in solution. The length of the coiled-coils would be $\simeq$13 nm and each particle would have a small amount of unordered chain at each end. Such a structure would explain the hydrodynamic data which indicates a much more swollen particle than a rigid α-helix. The length of coiled-coil regions is likely to be greater in the native keratin since the potential number of residues which can take up this conformation is 103 and 100 respectively for the two types of polypeptide chains involved (Fraser *et al.* 1976) ($\simeq$94% α-helix and a potential length of 15 nm). Fragments of the α-helical muscle protein, α-tropomyosin, obtained by either chemical splitting or limited proteolysis (Woods 1977; Pato *et al.* 1981) all show a lower α-helix content than the parent protein. The molecular weight of 50 200 for the helix-rich particle excludes the

similar three subunit structure of Crewther and Dowling (1971), Crewther (1976) in favour of a four-chain particle. Their results were based on a molecular weight of 41 700. However, they did not carry out a detailed investigation of the homogeneity of their preparations and the present study (see also Suzuki *et al.* 1973) has shown that gel filtration of the acid-precipitable material is required to remove low molecular weight species.

The main difficulty with the above structure is to accommodate polypeptide chains of molecular weight 25 000 (the ChB fraction of Crewther and Dowling 1971). There is no evidence that these are caused by lanthionine, lysinoalanine or isopeptide cross-links between single chains. Work in this laboratory (Crewther 1976; Dowling, unpublished data) indicates that ChB consists of two helical segments linked end-to-end by less than 10 residues. Sequence studies (Crewther *et al.* 1980, Sparrow and Inglis 1980) on two subunits (components 8c-1 and 7c) of SCMKA are now sufficiently advanced to show that two such linked segments do not occur in these components. It is unlikely that linked helical segments occur in the other subunits because of the similarity of all the component 7's and component 8's. Thus we are left with the conclusion that the 25 000 molecular weight chain in the helical particle is formed during the preparation, possibly by enzymic re-synthesis of a peptide bond. Such a chain would have to be folded back on itself in the four-chain particle and this suggests that either the chains in the coiled-coil are anti-parallel or that the pair of double-stranded α-helices are in an anti-parallel alignment.

Suggested model structures for the helical particle are given in Fig. 6. The postulated four-chain structure composed solely of subunits of molecular weight 12 500 (ChC) is shown in Fig. 6*a* and we consider that this must be present in solution since there is insufficient of the 25 000 molecular weight subunit (ChB) for it to be included in every particle. Models which accommodate the ChB subunit are depicted in Figs 6*b* and 6*c*. In Fig. 6*b* a putative peptide bond occurs between the two chains of a coiled-coil and therefore the chains would need to be in an antiparallel alignment. In Fig. 6*c* the putative peptide bond occurs between the chains of a pair of coiled-coils which must therefore be in an antiparallel alignment.

Acknowledgments

The authors wish to thank Mr G. G. Lilley for carrying out high-performance liquid chromatography, and Misses M. J. Davis and D. H. O'Brien for technical assistance.

References

Ahmadi, B., and Speakman, P. T. (1978). Suberimidate cross-linking shows that a rod-shaped, low cystine, high helix protein prepared by limited proteolysis of reduced wool has four protein chains. *FEBS Letts.* **94**, 365–7.

Ahmadi, B., Boston, N. M., Dobb, M. G., and Speakman, P. T. (1980). Possible four-chain repeating unit in the microfibril of wool. 'Fibrous Proteins: Scientific, Industrial and Medical Aspects.' (Eds D. A. D. Parry and L. K. Creamer.) Vol. 2. pp. 161–6 (Academic Press: New York.)

Campbell, M. E., Dobb, M. G., Hilburn, M. E., Loh, P., Lotay, S. S., Speakman, P. T., Stainsby, G., and Yarwood, R. E. (1976). Studies of two, possibly undenatured, low-sulphur proteins dissolved from reduced wool. Proc. 5th Int. Wool Text. Res. Conf., 1975, Aachen. Vol. 2. pp. 243–53.

Chen, Y-H., Yang, J. T., and Chan, K. H. (1974). Determination of the helix and β-form of proteins in aqueous solution by circular dichroism. *Biochemistry* **13**, 3350–9.

Crewther, W. G., and Harrap, B. S. (1967). The preparation and properties of a helix-rich fraction obtained by partial proteolysis of low-sulfur S-carboxymethylkerateine from wool. *J. Biol. Chem.* **242**, 4310–19.

Crewther, W. G., and Dowling, L. M. (1971). The preparation and properties of large peptides from the helical regions of the low-sulphur proteins of wool. *Appl. Polymer Symp.* **18**, 1–20.

Crewther, W. G. (1976). Primary structure and chemical properties of wool. Proc. 5th Int. Wool. Text. Res. Conf. Aachen, 1975. Vol. 1. pp. 1–101.

Crewther, W. G., Inglis, A. S., and McKern, N. M. (1978). Amino acid sequences of α-helical segments from *S*-carboxymethylkerateine A. Complete sequence of a type-II segment. *Biochem. J.* **173**, 365–71.

Crewther, W. G., Dowling, L. M., and Inglis, A. S. (1980). Amino acid sequence data from a microfibrillar protein of α-keratin. Proc. 6th Int. Wool Text. Res. Conf. Pretoria, 1980. Vol. 2. pp. 79–91.

Davis, B. J. (1964). Disc electrophoresis. Pt. 2. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* **121**, 404–27.

Davies, G. E., and Stark, G. R. (1970). Use of dimethyl suberimidate, a cross-linking reagent, in studying the subunit structure of oligomeric proteins. *Proc. Natl Acad. Sci. USA* **66**, 651–6.

Dobb, M. G., Millward, G. R., and Crewther, W. G. (1973). Examination in the electron microscope of *S*-carboxymethylkerateine-A and the helix-rich fraction obtained from it by partial proteolysis. *J. Text. Inst.* **64**, 374–85.

Dowling, L. M., and Crewther, W. G. (1974). Isolation of components from the low-sulphur proteins of wool by fractional precipitation. *Prep. Biochem.* **4**, 203–26.

Fraser, R. D. B., MacRae, T. P., and Rogers, G. E. (1972). 'Keratins, their Composition, Structure and Biosynthesis.' Ch. 5. (Charles C. Thomas: I11.).

Fraser, R. D. B., MacRae, T. P., and Suzuki, E. (1976). Structure of the α-keratin microfibril. *J. Mol. Biol.* **108**, 435–52.

Gillespie, J. M. (1957). The fractionation of *S*-carboxymethylkerateine-2 from wool. *Aust. J. Biol. Sci.* **10**, 105–17.

Gough, K. H., Inglis, A. S., and Crewther, W. G. (1978). Amino acid sequences of α-helical segments from *S*-carboxymethylkerateine-A. Complete sequence of a type-I segment. *Biochem. J.* **173**, 373–85.

Harrap, B. S. (1963). The conformation of a soluble wool keratin derivative. *Aust. J. Biol. Sci.* **16**, 231–40.

Harrap, B. S., and Gillespie, J. M. (1963). A further study on the extraction of reduced proteins from wool. *Aust. J. Biol. Sci.* **16**, 542–56.

Jones, L. N. (1976). Studies on microfibrils from α-keratin. *Biochim. Biophys. Acta* **446**, 515–24.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (*London*) **227**, 680–5.

Laurent, T. C., and Killander, J. (1964). A theory of gel filtration and its experimental verification. *J. Chromatogr.* **14**, 317–30.

Lotay, S. S., and Speakman, P. T. (1977). Three chain merokeratin from wool may be a fragment of the microfibril component macromolecule. *Nature* (*London*) **265**, 274–6.

Moffitt, W. (1956). The optical rotatory dispersion of simple polypeptides. II. *Proc. Natl Acad. Sci. USA.* **42**, 736–46.

O'Donnell, I. J., and Thompson, E. O. P. (1964). Studies on reduced wool. IV. The isolation of a major component. *Aust. J. Biol. Sci.* **17**, 973–89.

Pato, M. D., Mak, A. S., and Smillie, L. B. (1981). Fragments of rabbit striated muscle α-tropomyosin. 1. Preparation and characterization. *J. Biol. Chem.* **256**, 593–601.

Richards, E. G., Teller, D. C., and Schachman, H. K. (1968). Ultracentrifuge studies with Rayleigh interference optics II. Low-speed sedimentation equilibrium of homogeneous systems. *Biochemistry* **7**, 1054–76.

Roark, D. E., and Yphantis, D. A. (1969). Studies of self-associating systems by equilibrium ultracentrifugation. *Ann. N.Y. Acad. Sci.* **164**, 245–78.

Scheraga, H. A., and Mandelkern, L. (1953). Consideration of the hydrodynamic properties of proteins. *J. Am. Chem. Soc.* **25**, 179–84.

Sparrow, L. G., and Inglis, A. S. (1980). Characterization of the cyanogen bromide peptides of component 7c, a major microfibrillar protein from wool. Proc. 6th Int. Wool. Text. Res. Conf., Pretoria, 1980. Vol. 2. pp. 237–46.

Suzuki, E., Crewther, W. G., Fraser, R. D. B., MacRae, T. P., and McKern, N. M. (1973). X-ray diffraction and infrared studies of an α-helical fragment from α-keratin. *J. Mol. Biol.* **73**, 275–8.

Woods, E. F. (1977). Stability of segments of rabbit α-tropopmyosin. *Aust. J. Biol. Sci.* **30**, 527–42.

Woods, E. F. (1979). Microfibrillar proteins of wool: partial specific volumes and molecular weights in denaturing solvents. *Aust. J. Biol. Sci.* **32**, 423–35.

Yang, J. T. (1961). The viscosity of macromolecules in relation to molecular conformation. *Adv. Protein Chem.* **16**, 323–400.

Yphantis, D. A. (1964). Equilibrium ultracentrifugation of dilute solutions. *Biochemistry* **3**, 297–317.

Manuscript received 9 July 1981, accepted 4 September 1981