Structural studies on the microfibrillar proteins of wool

Interaction between α -helical segments and reassembly of a four-chain structure

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The α -helix-rich particle of M_r 50 200, derived by limited α -chymotryptic digestion of the solubilized microfibrillar proteins from wool α -keratin, consists mainly of polypeptide-chain segments of M_r 12 500 (fraction ChC) and 25 000 (fraction ChB). The 12 500- M_r segments are of two types (I and II), which are derived from different polypeptide chains of the microfibrillar complex. Each of these type-I and type-II segments partially self-associates in benign solvents to form either dimers or tetramers. When mixed, the two segments show changes in physical properties (α -helix content, difference spectra and molecular weight) indicative of complex-formation. The maximum changes occur when the two segments are mixed in an equimolar ratio. Complexes isolated after rapid dialysis of mixtures from 8 M-urea solution were examined by various methods. A tetrameric structure is the main product formed in all cases, and the maximum amount of tetramer is obtained from equimolar mixtures of the type-I and type-II polypeptides. When urea is removed by dialysis from the unfractionated 12 500- M_r segments (fraction ChC) or from the α -helix-rich particle itself, a similar complex of M_r 50 000 is formed. The physical properties of these reconstituted entities (α -helix content, molecular weight, thermal stability and exposure of tyrosine residues) are similar to those of the original α -helix-rich particle. Cross-linking experiments with dimethyl suberimidate are in agreement with a four-chain complex for the reassembled structures. A pair of double-stranded α -helices is proposed for the particle, and is considered to be an integral part of the microfibrillar complex in wool α -keratin.

Crick (1953) suggested that the α -helices in wool and hair were present as coiled-coils and grouped into rope-like structures with three strands. Pauling & Corey (1953) also suggested that seven-strand cables might be present. The possibility that two-strand ropes may be present has also been considered (Fraser *et al.*, 1964, 1965), but the presently available interpretation of the X-ray-diffraction data does not permit an unequivocal choice to be made between the two-strand and three-strand rope models (Fraser & MacRae, 1973).

It has been proposed (Steinert *et al.*, 1978) that the keratin filaments of epidermis and related tissues such as hair or wool and intermediate filaments of other types of cells are structurally homologous proteins. A three-strand coiled-coil has been proposed as the building block for epidermal and related filaments (Steinert, 1978*a,b*; Steinert *et al.*, 1978), on the basis of the properties of the α -helix-enriched particles obtained by limited proteolysis of the filaments. An analogous three-chain structure has also been proposed for the hard α -keratin from

similar studies of the highly α -helical fragments isolated from the microfibrillar proteins of wool (Crewther *et al.*, 1968; Crewther & Dowling, 1971). However, more-recent studies on the proteolytic fragments from wool (Campbell *et al.*, 1976; Lotay & Speakman, 1977; Ahmadi & Speakman, 1978; Ahmadi *et al.*, 1980) have cast doubt on a three-chain structure, and instead have favoured a four-chain structure. From a study of models of the helical portions of wool α -keratin of known sequence, McLachlan (1978) has concluded that the two-strand rope is more likely than a three-strand rope for wool.

After a further investigation of the α -helix-rich particle prepared by chymotryptic digestion of the reduced and alkylated low-sulphur proteins of wool, Woods & Gruen (1981) have proposed a pair of two-strand α -helical coiled-coils for the particle. This particle (M_r 50 200) gives two main groups of polypeptide chains of M_r 12 500 and 25 000 when submitted to gel filtration in 8 M-urea (Crewther & Dowling, 1971). The chains of M_r 12 500, when

Scheme 1. Schematic preparation of the fragments obtained by partial chymotryptic digestion of low-sulphur wool proteins (SCMKA)

fractionated by ion-exchange chromatography on DEAE-cellulose in 8 M-urea, were shown (Scheme 1) to consist of two types of α -helical segments, type-I and type-II segments, with major differences in peptide 'maps' and amino acid compositions (Crewther & Dowling, 1971). One polypeptide segment of each type has been isolated and sequenced (Crewther *et al.*, 1978a; Gough *et al.*, 1978). Type-I and type-II polypeptides were shown by Crewther & Dowling (1971) to interact and produce an increase in α -helix content. In the present work these studies are extended with the use of a range of physicochemical techniques. Some preliminary reports have been presented (Gruen, 1979; Crewther *et al.*, 1980a).

In view of the interaction between the type-I and type-II polypeptides, we report experiments on the isolation of the product of this interaction. The reassembly of a macromolecular complex from its subunits represents a most useful technique for examining the roles of the individual components. The potential of the method is only fully attained when the product is shown to be structurally and functionally identical with the native starting material. Since the helix-rich particle shows no biological activity, a variety of physicochemical criteria need to be employed to determine whether the product formed is the same as the particle from which the subunit polypeptides were derived.

Materials and methods

Preparation of helix-enriched segments

The production of the various fragments used in this study is shown schematically in Scheme 1.

The microfibrillar proteins of wool (low-sulphur proteins; S-carboxymethylkeratine A or SCMKA)

were prepared as described by Woods & Gruen (1981), and the procedure of Crewther & Dowling (1971) was followed for the preparation of the chymotryptic-digest α -helix-rich particle, which was further purified by gel filtration. The type-I and type-II polypeptides were separated in urea-containing buffers by gel filtration and DEAE-cellulose chromatography as described by Crewther & Dowling (1971) and Crewther *et al.* (1978a). Fractions from the columns were concentrated by ultrafiltration, exhaustively dialysed against deionized water and finally against the appropriate buffer.

The sodium dodecyl sulphate/polyacrylamide-gel and alkaline-urea/polyacrylamide-gel electrophoretic patterns are given in Fig. 1. Even though the sequence molecular weights are close, namely 12 300 for type-I polypeptides (Gough *et al.*, 1978) and 12 900 for type-II polypeptides (Crewther *et al.*, 1978a), type-I polypeptides run ahead of type-II polypeptides on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The order is reversed on alkaline-urea/polyacrylamide-gel electrophoresis, as expected from the relative elution positions from DEAE-cellulose in 8 M-urea.

Analytical methods

Protein concentrations in the helical particle were determined from the refractive-index increment in 0.05 M-KCl/0.05 M-sodium tetraborate ($0.180 \text{ ml} \cdot \text{g}^{-1}$) or from the $A_{1\text{cm}}^{1\%}$ at 278 nm (5.2) (Woods & Gruen, 1981). The type-I and type-II polypeptides have $A_{1\text{cm}}^{1\%}$ values of 4.0 and 5.4 respectively, assuming that the refractive-index increments are the same for the constituent polypeptides as for the parent helical particle.

Electrophoresis was performed in polyacryl-

Fig. 1. Gel electrophoresis of the helix-rich particle and of its polypeptide chains

For experimental details see the text. (a)–(f) Sodium dodecyl sulphate/15%-(w/v)-polyacrylamide-gel electrophoresis: (a) helix-rich particle; (b) fraction ChA; (c) fraction ChB; (d) fraction ChC; (e) total type-I polypeptides; (f) total type-II polypeptides. (g)–(n) 8M-urea/Tris/glycine-(pH 8.9)/10%-(w/v)-polyacrylamide-gel electrophoresis: (g) helix-rich particle; (h) fraction ChA; (i) fraction ChB; (j) fraction ChC; (k) total type-I polypeptides; (l) total type-II polypeptides; (m) type-II polypeptides, main peak (Fig. 2b); (n) type-II polypeptides, tetramer peak (Fig. 2b).

amide-gel slabs measuring 140 mm × 100 mm × 1.5 mm (Bio-Rad model 220 apparatus) as described previously (Woods & Gruen, 1981). In order to determine the proportions of components, gels were scanned, the charts were photocopied, and the peaks were excised from the paper and weighed. Experiments on the separated type-I and type-II polypeptide chains indicated that the Coomassie Blue uptake was comparable for both polypeptides and that it was a linear function of the amount of polypeptide over the entire concentration range examined.

Physicochemical measurements

Sedimentation experiments, c.d., o.r.d. and intrinsic-viscosity measurements were performed as described elsewhere (Woods & Gruen, 1981). Molecular weights were measured by the meniscus-depletion technique of Yphantis (1964).

The mean molar ellipticities from c.d. measurements were converted into α -helix contents by using

the data of Chen *et al.* (1974). To calculate the α -helix content from o.r.d. measurements, either the b_0 values from Moffitt plots or the mean residue rotation at 233 nm was used, with the values given by Chen *et al.* (1974) for infinite helices. The relevant values for the unordered forms were taken to be those in 6M-guanidinium chloride.

Difference spectra were obtained with a Cary 15 spectrophotometer, with rectangular tandem cells with 4.4 mm sector path-lengths. The base-line was measured with identical protein solutions in the reference and sample cells. Difference spectra were generated by mixing the contents of the sample-cell compartments while leaving the reference cell undisturbed. All spectroscopic measurements were made at room temperature ($20 \pm 2^\circ\text{C}$) except where otherwise stated, at protein concentrations of approx. 1 mg/ml. The numbers of buried tyrosine residues were calculated from the known tyrosine contents (tryptophan is absent) and assuming a value of $-700\text{ cm}^2 \cdot \text{mol}^{-1}$ for the change in molar

absorption produced by transfer of a phenolic group from the interior of a protein into water (Donovan, 1969).

Cross-linking experiments

Cross-linking with dimethyl suberimidate (Davies & Stark, 1970) was performed in 0.2 M-triethanolamine/0.17 M- Na_2HPO_4 adjusted to pH 10 with H_3PO_4 , as described in Woods & Gruen (1981).

Results

Molecular-weight studies of type-I and type-II polypeptides and their mixtures

When examined in benign solvents by sedimentation velocity in the ultracentrifuge, with a synthetic-boundary cell, the type-I and type-II polypeptides gave single peaks with sedimentation coefficients of 1.85S and 1.21S respectively at a concentration of 3 mg/ml. However, sedimentation equilibrium showed that they were heterogeneous in molecular size, with average molecular weights greater than the sequence values of 12300 and 12900 respectively. The weight-average molecular weight $\bar{M}_w$, of type-I polypeptides was approx. 23000. The concentration distribution in the ultracentrifuge at equilibrium could be fitted to a system in which there was 25% monomer and 75% dimer, but there were indications of higher-molecular-weight species. Type-II polypeptides showed variability in degree of association. Association to species as high as tetramer could be detected. In general, the greater the self-association, the higher was the α -helix content. The average α -helix content (f_H) of the type-I polypeptides is 0.73 and that of the type-II polypeptides is 0.30.

Gel filtration on Sepharose CL-6B was performed on the pooled type-I or pooled type-II polypeptides, and the results are shown in Fig. 2. Molecular-weight measurements on selected fractions from the column confirmed that type-I polypeptides exist as 75% dimer, about 20% monomer and a small amount of higher-molecular-weight species. The profile of type-II polypeptides showed two peaks (Fig. 2b). The peak that was eluted first was shown to be slightly heterogeneous by sedimentation equilibrium, with an $\bar{M}_w$ of 49500, and is therefore mainly tetramer. It comprised 22% of the total and was of high α -helix content ($f_H = 0.79$). The major peak tube was of high homogeneity (M_r of main component 12600, $\bar{M}_w$ 13500), and is therefore mainly the monomeric species. It had low α -helix content ($f_H = 0.14$). Summation over these two peaks leads to a predicted overall α -helix content of $f_H = 0.28$ for the type-II polypeptides, in close agreement with the experimentally observed value ($f_H = 0.30$). After storage for 18 weeks at 4°C, the fractions from the main peak were concentrated and

Fig. 2. Gel filtration of (a) type-I polypeptides and (b) type-II polypeptides on Sepharose CL-6B

The elution buffer was 0.05 M-KCl/0.05 M-sodium tetraborate, pH 9.2. The column size was 2.5 cm $\times$ 113 cm; the void volume was 216 ml, and the fraction size was 5.3 ml. For further experimental details see the text. Fractions from the main peak in (b) (as indicated by the bar) were re-run, and this is shown by the broken curve.

re-chromatographed, and gave mainly the single peak as shown by the broken curve in Fig. 2(b), indicating little further significant association. The sodium dodecyl sulphate/polyacrylamide-gel and alkaline-urea/polyacrylamide-gel electrophoresis patterns are shown in Fig. 1, where it is seen that the polypeptides of the tetramer moved slightly more slowly on the urea-containing gels than did the polypeptides of the monomer.

When type-I and type-II polypeptides were mixed in benign solvents, an increase in molecular weight occurred over that expected for a non-interacting system. As mentioned above, different type-II polypeptides showed varied degrees of association, but when mixed with type-I polypeptides there was always an increase in molecular weight, indicative of complex-formation. The maximum effect was observed when they were mixed in equimolar proportions (Fig. 3). If they were mixed in 8 M-urea to convert them all into monomers, followed by dialysis against successive changes of benign solvent to remove the urea, the increase in molecular weight was enhanced.

Spectral changes on mixing type-I and type-II polypeptides

The u.v. difference spectrum generated by mixing the two types of helical polypeptides in aqueous

Fig. 3. Change in physical properties on mixing type-I and type-II helical polypeptides

■, Relative change in molar absorption coefficient ($\Delta\epsilon/\epsilon$) at 287 nm plotted as a function of mixture composition. The solvent was 0.05 M-sodium tetraborate, pH 9.2; appropriate volumes of type-I polypeptide (0.85 mg/ml) and type-II polypeptide (1.67 mg/ml) were combined. ●, Experimental weight-average molecular weights. ○, Molecular weights calculated for no interaction. $\bar{M}_w$ values are estimated to be accurate to within 4%, as are the molecular-weight data in Table 1.

(non-denaturing) solvent is 'positive' relative to the unmixed species. The height of the principal peak (287 nm) in the difference spectrum was found to depend on the composition of the solutions. Fig. 3 shows how the relative change ($\Delta\epsilon/\epsilon$) in molar absorption coefficient varied with the proportion of type-I and type-II polypeptides in the mixture. The maximum effect occurred when the two types of polypeptides were present in equimolar amounts, and corresponds to the complete burial of one tyrosine residue per polypeptide chain. This result was confirmed with several type-I and type-II polypeptide preparations.

O.r.d. and c.d. measurements on mixtures of type-I and type-II polypeptides also indicated an increase in α -helix content, with a maximum effect occurring when they were mixed in equimolar amounts, in agreement with the molecular-weight and u.v.-difference-spectrum results. This inter-

action occurred over a wide temperature range (10–60°C).

Reassembly of a helix-rich particle from denatured polypeptide chains

The interactions described above between mixtures of the two types of polypeptides were performed in benign solvents. Since the type-I polypeptides are largely associated to a dimer and the type-II polypeptides exist partly as tetramer, we may not have optimum conditions for the formation of the energetically most favourable complexes if, as appears likely, there are preferred interactions between different type-I and type-II species. Therefore, in order to examine the nature of the complexes formed as a result of type-I–type-II polypeptide interaction, we have denatured the starting material (helical particle, fraction ChC or type-I/type-II polypeptide mixture) by dialysis against 8 M-urea or 6 M-guanidinium chloride. In these solvents the o.r.d. and c.d. spectra were typical of a random coil, and only single polypeptide chains were present. The denaturant was removed by rapid dialysis against successive changes of water and finally 0.05 M-KCl/0.05 M-sodium tetraborate.

The renatured product was examined by gel filtration on Sepharose CL-6B, and the elution profiles are given in Figs. 4(a)–4(g). The renatured fractions ChA and ChB gave a product of high α -helix content ($f_H=0.75$ –0.80), but they were very heterogeneous (Figs. 4b and 4c), containing species from M_r 50 000 to $M_r > 100$ 000. With the exception of fractions ChA and ChB, the major product of renaturation appeared to be a species of M_r 50 000 (see first line in Table 1). The maximum amounts of refolded 50 000- M_r complex were obtained from the unfractionated polypeptides of fraction ChC or when the type-I and type-II polypeptides were mixed in an equimolar ratio. The fractions corresponding to M_r 50 000, indicated by the bars in Fig. 4, were pooled, concentrated and subjected to further examination. All the products exhibited a high degree of mass homogeneity, with a major component of M_r between 49 000 and 50 000, and with less than 5% of lower-molecular-weight material.

It is apparent from the gel-filtration profiles that complete renaturation into 50 000- M_r species is not achieved, and we have also examined the material not included in the main peak. For the helix-rich particle, the 20% of material that was eluted first (Fig. 4a) was heterogeneous in size, with $\bar{M}_w$ approx 110 000 (range 75 000–125 000) and f_H 0.62. With fraction ChC, approx. 13% was eluted after the main peak (Fig. 4d). This later-eluted material was heterogeneous with respect to molecular weight and had $f_H = 0.49$, and alkaline-urea/polyacrylamide-gel electrophoresis revealed that it contained both type-I

Fig. 4. Gel filtration on Sepharose CL-6B of the helix-rich particle and of its polypeptide chains. Conditions were as indicated in Fig. 2 legend. (a) Helix-rich particle renaturation from 8 M-urea; (b) fraction ChA; (c) fraction ChB; (d) fraction ChC; (e)–(g) type-I/type-II polypeptide mixtures, with the type-I/type-II molar ratios: (e) 1:1; (f) 1:2; (g) 2:1. The fractions indicated by the bars in each case were pooled and re-chromatographed to give the profiles indicated by the broken lines.

Table 1. Physicochemical properties of renatured helix-rich complexes

The percentage of 50 000- M_r complex was estimated from the elution profiles (Figs. 4d–4g) by determining M_r from sedimentation equilibrium on selected fractions. $T_{\frac{1}{2}}$ is the temperature required to decrease the helix content to half of its original value at 20°C. The number of buried tyrosine residues is expressed as residues per tetramer, with numbers in parentheses being the actual number of tyrosine residues present per tetramer. The mean of scans of four separate gels is used to calculate the percentage weight of type-I polypeptide in gels, s.d. = 1.5 for alkaline-urea/polyacrylamide-gel electrophoretograms and 2.1 for sodium dodecyl sulphate/polyacrylamide-gel electrophoretograms. Since the sequence-derived M_r values of type-I polypeptide (12 300) and type-II polypeptide (12 900) are similar, the percentages are close to the mole percentages. Abbreviation: N.D., not determined.

	Helix-rich particle (Woods & Gruen, 1981)	Renatured complexes				
		Helix-rich particle	Fraction ChC	Type-I + type-II polypeptides		
				1:2	1:1	2:1
% of 50 000- M_r complex in renatured product	—	78	86	73	85	60
$s_{20,w}^0$ (S)	3.18	3.18	3.29	N.D.	3.31	N.D.
$[\eta]$ (ml·g ⁻¹)	10.7	N.D.	8.3	N.D.	N.D.	N.D.
M_r	50 200	49 500	49 000	49 500	49 500	49 500
Fraction α -helix (f_H)	0.84	0.80	0.84	0.83	0.82	0.83
V_e (ml) (conditions as in Fig. 3)	375	373	372	373	372	371
Thermal stability ($T_{\frac{1}{2}}$) (°C)	54	54	51	N.D.	52	N.D.
No of buried tyrosine residues	9.2 (14)	8.9 (14)	9.0 (15)	N.D.	6.0 (15)	N.D.
% by wt. of type-I polypeptide in 50 000- M_r complex from alkaline-urea/polyacrylamide gels	41	43	45	43	46	50
% by wt. of type-I polypeptide in 50 000- M_r complex from sodium dodecyl sulphate/polyacrylamide gels	42	41	42	42	43	44

Fig. 5. Densitometer scans of gradient sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of samples cross-linked with dimethyl suberimidate

For experimental details see the text. (a) Reassembled fraction ChC, high cross-linker ratio; (b) reassembled fraction ChC, low cross-linker ratio; (c) reassembled type-I-type-II polypeptide complex (equimolar), high cross-linker ratio.

and type-II polypeptides in nearly equal proportions. For the type-I/type-II polypeptide mixtures, the composition of the material eluted after the main (M_r 50000) peak depended on the relative proportion of the two polypeptides in the starting mixture.

This material was richer in whichever type of polypeptide was in excess initially.

Physicochemical properties of refolded complexes (Table 1)

The number of tyrosine residues in a protein that are shielded from the solvent can be estimated by measuring the change in absorption that accompanies transfer of the protein into a denaturant such as 8M-urea, as described by Wetlaufer (1962). It is apparent from Table 1 that the helix-rich particle can be denatured and refolded from 8M-urea to give almost the same extent of tyrosine shielding as was present in the parent undenatured helix-rich particle, and this is also true for renatured tetrameric fraction ChC. However, fewer tyrosine residues were shielded in the renatured tetrameric complex formed from an equimolar mixture of type-I and type-II polypeptides, although there is almost complete recovery of the α -helix (Table 1). This probably reflects either imperfect refolding after removal of denaturant or the loss of polypeptides in the selection of the type-I and type-II polypeptide pooled fractions.

The helix-rich particle has a slightly lower α -helix content after denaturation in 8M-urea and subsequent renaturation (Table 1). The thermal stabilities, however, of the original and renatured particles are very similar, and they have almost identical denaturation profiles. On the other hand, both the unfractionated chains of M_r 12500 (fraction ChC) and the equimolar mixture of type-I and type-II polypeptides refolded from 8M-urea to yield a tetrameric structure of slightly lower stability. In all cases, recooling the heated protein solutions from 65–70°C (where f_H was 0.10–0.15) to 20°C led to the recovery of about 90% of the initial α -helix content. It should be emphasized that complex-formation leads to a marked increase in stability of the α -helices. For example, the $T_{\frac{1}{2}}$ value for the type-I-type-II polypeptide complex is 52°C, whereas the corresponding value for the type-I polypeptide is 38.5°C and for the type-II polypeptide is 36.0°C.

Cross-linking experiments with dimethyl suberimidate

In a previous paper (Woods & Gruen, 1981) cross-linking experiments on the helix-rich particle were reported. The interpretation was complicated by the presence of fraction ChB (M_r 25000) in the control, and smaller amounts of trimers and higher-molecular-weight species. Therefore cross-linking experiments were performed on the reconstituted fraction ChC and on the reassembled type-I-type-II polypeptide complex, which consist solely of species of M_r 12500. The results (Fig. 5) observed with fraction ChC and with the equimolar type-I-type-II polypeptide complex were very similar. In both cases

only dimer was produced at low cross-linker ratios, whereas some trimer and tetramer in addition to the dimer was formed at higher ratios. Species higher than tetramer were not observed with further increase in cross-linker ratios.

Discussion

The results reported in this paper, on the interaction and refolding of the helical polypeptides from the proteolytic digestion of the low-sulphur proteins of wool, show that the major product is either a dimer or a tetramer. The product of the interaction is believed to be a coiled-coil, since these polypeptides possess the repeating heptapeptide sequence of the type (a-b-c-d-e-f-g)_m where residues a and d are hydrophobic, as predicted for coiled-coils (Crick, 1953). Suzuki *et al.* (1973) have shown, by X-ray-diffraction and i.r. studies, that films cast from the chymotryptic digestion product and also the product formed from the 12 500-*M_r* subunits (fraction ChC) have a coiled-coil α -helical conformation similar to that in α -keratins. The interactions between the type-I and type-II polypeptides have been analysed computationally by Parry *et al.* (1977) and Parry (1980) for two-strand and three-strand rope models, and it was shown that the stabilization from ionic interactions is maximal for an in-register parallel arrangement of chains. Parry (1980) concluded that interchain electrostatic interactions are unlikely to be a criterion by which two-strand and three-strand coiled-coil models for α -keratin can be distinguished. For two-strand ropes the most favoured combination of segment types was type-I with type-II polypeptide or type-I with type-I polypeptide. Our results show that type-I polypeptide helical segments form a stable dimer of high α -helix content and that there is a strong interaction between type-I and type-II polypeptide segments, in agreement with these predictions. Type-II polypeptide helical segments on their own possess low α -helix content and exist mainly as monomers in aqueous solution. However, approx. 20% of the type-II helical polypeptides exists as highly α -helical tetramers. It is evident that the α -helix content parallels the extent of association. Sequence analysis of other type-I and type-II polypeptide segments (Crewther *et al.*, 1978b) have shown that amino acid substitutions occur. Apparently the sequences of some of the type-II polypeptides are such that the formation of four-chain structures is energetically feasible.

The information gained from both molecular-weight and spectroscopic data indicates that type-I and type-II polypeptides interact in aqueous buffers. This interaction appears to be maximal for equimolar amounts of the two kinds of polypeptides. The changes in the measured physical properties, such as the increase in α -helix content, increased stability of

the α -helix and burying of the tyrosine residues into a more hydrophobic environment, are interpreted as arising from the formation of a coiled-coil structure when the two types of polypeptides are mixed. It is pertinent that an analogous stabilization occurs on mixing the parent subunits, from which the helical polypeptides are derived (Crewther *et al.*, 1980b). It would seem logical to assume that this stabilization arises, at least partly, from the interaction between the ordered helical segments (type-I and type-II polypeptides) within these subunits.

The reassembly experiments on mixtures of type-I and type-II polypeptide segments, which were performed by dialysis from 8M-urea to allow maximal interaction to occur, indicated that a tetramer was the major product. The physical properties of this tetramer (Table 1) are close to those of the helix-rich particle. It has a slightly higher sedimentation coefficient, has fewer buried tyrosine residues and is less stable to heat than is the helix-rich particle, possibly reflecting our inability to reconstitute perfectly the original helix-rich particle from these polypeptides. Maximum amount of tetramer was formed when reassociation was made from a 1:1 mixture of the two types of polypeptides. However, the composition of the tetrameric complex varied slightly according to the ratio of type-I to type-II polypeptide species used in the renaturation mixture (Table 1). Our experimental results show that the complexes, on average, contain slightly more type-II than type-I polypeptide segments. The existence of coiled-coil complexes containing compositions other than equimolar cannot be excluded, since type-I polypeptide segments alone have been shown to exist as dimers, and type-II polypeptide segments alone can exist as tetramers. Thus it is readily explicable that the tetrameric complex in all cases contains slightly more type-II than type-I polypeptide chains. The tetramer formed from reassembly of the unfractionated 12 500-*M_r* segments (fraction ChC) is similar to that resulting from reassembly of the type-I and type-II polypeptide mixtures (Table 1).

Even though the helix-rich particle contains some chains of *M_r* 25 000 (fraction ChB) and some chains of *M_r* 37 500 (fraction ChA), it can be renatured to form a molecule of the same size and with physical properties identical with those of the original helix-rich particle (and also similar to those of the tetramers formed by reassociation of the 12 500-*M_r* chains). This lends support to the proposed model for the helix-rich particle as consisting of four polypeptide chains aligned laterally as a pair of two-strand α -helical coiled-coils (Woods & Gruen, 1981). A problem is to incorporate the 25 000-*M_r* chains (fraction ChC) into this model. Fraction ChB has a high α -helix content, but, on its own, refolds only to a relatively small extent as 50 000-*M_r*

particles (Fig. 4c). There is clearly insufficient fraction ChB (approx. 31%) for it to be present in every helix-rich particle, and the reconstitution experiments show that a particle of similar size (M_r 5000) and shape can be reassembled from 12 500- M_r segments alone. Thus we believe that the four polypeptide segments comprising the helix-rich particle are derived from four separate subunits. They can remain as four distinct segments or transpeptidation may form a new peptide bond between two of them, as discussed previously (Woods & Gruen, 1981). The conclusion that four laterally aligned α -helices, probably a pair of two-strand coiled-coils, form a structural unit or entity of the microfibril of wool is supported by the studies made by Ahmadi *et al.* (1980).

The molecular weights and α -helix contents of the subunits of the filaments of soft keratins (Steinert & Idler, 1975) and of the microfibrils of wool (Woods, 1979) are similar. Several models have been proposed for the structure of intermediate and related filaments. These involve a three-chain model of triple coiled-coils (Steinert *et al.*, 1978) or a four-chain model whose basic building block is a tetrameric complex comprising a pair of double coiled-coils (Ahmadi *et al.*, 1980; Woods & Gruen, 1981) or the model proposed by Fraser *et al.* (1976), which is adaptable to double or triple coiled-coils. A recent determination of the linear mass density of intermediate filaments by scanning transmission electron microscopy (Steven *et al.*, 1982) did not resolve the question of the arrangement of the protein subunits within a protofilament, and it was not possible to decide between a three-chain or a four-chain model. The evidence given in the present paper favours a four-chain model for the microfibrils of wool.

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