A NEW APPROACH TO THE FRACTIONATION OF WOOL KERATIN

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SUMMARY

The isolation of two components, in which the cystine linkages remain intact, from wool keratin following an acid treatment is described. These components, which probably represent sub-units of the keratin structure, differ markedly from each other in amino acid composition and resemble fractions obtained from wool by procedures involving fission of the disulphide link. A tentative model from wool keratin based on these findings, in which segments of a helical structure, corresponding to one component, alternate along the fibre axis with non-helical structures, corresponding to the other component, is described.

INTRODUCTION

Progress in the elucidation of the chemical structure of wool keratin has been hampered by the fact that it consists of more than one histological component and probably more than one protein. Some form of degradation to smaller units is often necessary for the study of such insoluble fibrous proteins and, in the case of wool keratin, has involved the preliminary fission of the disulphide linkages. Subsequent fractionation of the wool gives rise to pairs of fractions of high- and low-sulphur content, which also differ in the content of other amino acids. When oxidised wool, for example, is treated with ammonium hydroxide (0.1 *N*), 90% goes into solution. α-Keratose (low-sulphur content) is precipitated on acidification and γ-keratose (high-sulphur content) by addition of ethanol to the mother liquor[1,2]. Similarly, extraction of wool with alkaline thioglycollate solution gives "low-sulphur" and "high-sulphur" proteins[3]. These various fractions have been examined for homogeneity by a variety of methods such as dialysis, ultra-centrifugation, electrophoresis, chromatography of enzymic digests and fractional precipitation[4–8]. The fractions are not homogeneous, each may contain four, five, or more closely related proteins that are difficult to separate from each other. These differences in physical and chemical properties are reflected in differences in amino acid composition, since different preparations of high- or low-sulphur fractions show small but distinct differences in composition, and protein-like polypeptides with different compositions have been isolated from some fractions.

The general amino acid pattern, however, clearly distinguishes the proteins of low-sulphur content from those of high-sulphur content.

Following a preliminary acid treatment, it has now been found possible to isolate

two Components* (I and III) from wool. They form a pair of fractions of low- and high-sulphur content similar to the pairs described above, showing a remarkable similarity to them in amino acid composition and other properties, a fact to which attention was first drawn by the present author[9]. Details of their isolation and properties, their relation to other fractions described above, and experiments on their oxidation, carried out in order to relate them directly to the keratoses, are described in the present paper. The Components are probably sub-units of the wool structure, and a tentative model for wool keratin based on these findings is described.

METHODS

Isolation of components form wool

The wool used in these experiments was Australian 64sA quality in the form of dry-combed tops. It was extracted with light petroleum in a Soxhlet apparatus, rinsed in distilled water, and air-dried before use.

Wool (7.5 g) was treated with 2 *N* hydrochloric acid (750 ml) in an oven maintained at 65° for 7 h (occasionally 16 h), filtered on sintered glass, washed with distilled water, and drained at the pump. It was then stirred vigorously in 500 ml of water while sodium hydroxide (40%, w/v) was added drop by drop as required to bring the pH to 10.5 and maintain it at this value (thymolphthalein as external indicator). Stirring was continued until the wool was disintegrated into cortical cells and fibre fragments (1–4 h) and the residue was then centrifuged off.

The alkaline extract of the wool was acidified with acetic acid, when a protein-like precipitate (Component I) settled out. The entire acidified solution was dialysed for 24 h against distilled water which was changed at intervals. Component I was collected from the contents of the dialysis sac by centrifuging. The supernatant solution was concentrated *in vacuo* below 40° to a volume of about 20 ml and 15 times its volume of ethanol added. After standing for several hours the precipitate (Component III) was collected by centrifuging. All the precipitates were washed with ethanol and ether and dried in vacuo before analysis.

Rearrangement of the cystine linkages of peptides can occur under certain conditions of treatment with hydrochloric acid[10,11]; in one experiment therefore the wool (7.5 g) was treated for 46 h at 37° with 10 *N* sulphuric acid (750 ml) containing thioglycollic acid (0.5 μl/ml), a reagent which does not cause this rearrangement. The wool was then extracted with alkali and Components I and III isolated from the extract. No significant rearrangement of the cystine linkages would therefore seem to occur in the present experiments.

Paper chromatograms of the hydrochloric acid solution from the treatment of the wool showed a strong glycine spot, a weaker serine spot, traces of other amino acids and spots probably due to peptides. Non-dialysable peptide material amounted to only a few milligrams from 7.5 g wool. Ammonia present was probably derived from the deamidation of asparagine and glutamine residues.

Oxidation of the components

The Components were oxidised with two reagents, performic acid (for both Com-

* The use of the term Component to describe these polypeptides does not necessarily imply that no alteration has taken place during their isolation.

ponents) and peracetic acid (for Component I). Oxidation with performic acid was carried out by the method of SANGER[12], four times the stated quantity of formic acid being used in oxidising Component I. Excess reagents and water were finally removed in a vacuum desiccator over sodium hydroxide at room temperature. Oxidation with peracetic acid was carried out by the method of CORFIELD, ROBSON AND SKINNER[2], Component I being filtered off on sintered glass at the end of the reaction.

The oxidised protein was treated with 100 times its weight of 0.1 *N* ammonium hydroxide at room temperature. Solution took place leaving no residue comparable to that of β-keratose which is obtained from oxidised wool. The time of treatment with ammonium hydroxide was limited to 1 h to minimise side reactions such as hydrolysis which might occur. Isolation of the keratoses from the solution followed the method of CORFIELD *et al.*[2].

Estimation of amino acids

The proteins and polypeptides were hydrolysed for 24 h with excess of 5 *N* HCl, which was removed by distillation *in vacuo*. The residue was dissolved in water and made up to a known volume. Aliquots were then analysed for amino acids by the method of MOORE, SPACKMAN AND STEIN[13]. Tryptophan was not determined, and no value was recorded for methionine as the low height of the peak, with the amount of material applied to the column, precluded its accurate evaluation. These omissions, however, will not effect the overall amino acid analysis. Throughout the paper, amino acid-nitrogen is given as a percentage of the total nitrogen. Within the limits of error for single analyses (about 3%), the sum of the values for the individual amino acids is 100%.

Nitrogen analyses were carried out by the micro-Kjeldahl method. Ammonia-nitrogen was estimated by distillation of an aliquot of the hydrolysate with sodium hydroxide and titration of the absorbed ammonia. The values for ammonia-nitrogen do not correspond to the amide-nitrogen, since traces of ammonia which may be present in reagents are strongly retained by these peptides.

Estimation of N-terminal residues

N-terminal residues were determined by SANGER'S method[14]. The 2,4-dinitro phenyl derivative of Component I was prepared as previously described[15], the reaction being carried out for 24 h. The DNP-derivative of Component III was prepared by treatment with dinitrofluorobenzene in aqueous sodium bicarbonate solution[16] at 40° with vigorous stirring for 1 h. The reaction mixture was extracted with ether, acidified with hydrochloric acid and the precipitated DNP-peptide isolated by centrifuging, washed with water and dried *in vacuo*. The isolated DNP-derivatives were hydrolysed with 5 *N* HCl for 8 h, the DNP-amino acids extracted with ether, separated and estimated on paper chromatograms[17].

RESULTS

Amino acid composition of the components

Differences between Components I and III: Table I shows the amino acid composition of the various Components isolated from wool keratin. Components I and III show striking differences. Component I has an amino acid pattern whose predominant

TABLE I

THE AMINO ACID COMPOSITION OF WOOL COMPONENTS

Component	*I*	*I*	*I*	*I*	*I*	*I*	*I*	*III*	*III*	*III*	*III*	*III*	*Residue*
Time of acid treatment	*7 h*	*7 h*	*16 h*	*16 h*	*7 h*	*7 h*	*(H_2SO_4)*	*7 h*	*16 h*	*7 h*	*7 h*	*(H_2SO_4)*	*7 h*
Extraction number	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*1*	*1*	*1*	*2*	*1*	
*Experiment type**	*c*	*c*	*c*	*c*	*e*	*e*	*g*	*a*	*f*	*e*	*e*	*g*	*b*
Alanine	6.85	5.78	5.85	4.97	5.86	5.47	6.03	3.23	3.79	3.39	2.87	3.02	4.17
Ammonia	3.74	4.93	3.39	2.31	3.77	4.65	2.88	3.22	2.68	4.12	5.34	4.98	5.02
Arginine	17.90	23.50	21.40	21.52	20.16	22.30	18.78	16.65	19.15	13.75	15.22	16.60	21.80
Aspartic acid	7.16	7.06	7.87	6.38	6.67	6.36	7.59	4.05	4.35	4.73	3.57	3.02	3.63
Cystine	3.56	3.41	4.33	2.90	4.31	3.93	4.82	8.35	12.68	8.93	8.72	12.56	9.86
Glutamic acid	11.70	11.53	14.58	13.20	11.05	11.20	13.55	6.30	8.35	7.77	9.61	5.73	9.22
Glycine	4.87	3.58	2.98	2.16	4.33	3.62	2.73	9.25	6.43	10.93	7.36	6.13	4.00
Histidine	2.11	1.68	1.51	2.40	2.62	1.76	0.67	2.09	1.77	1.21	0.72	1.25	0.66
Isoleucine	3.09	3.50	3.58	3.34	3.10	2.88	3.35	3.00	3.33	3.37	2.89	2.93	2.19
Leucine	8.39	8.00	9.96	7.24	7.11	7.27	8.98	4.35	3.43	4.63	3.26	3.21	4.48
Lysine	5.92	5.04	7.14	5.48	6.77	6.17	6.16	3.30	4.38	3.31	2.25	3.48	4.23
Phenylalanine	2.19	2.16	2.32	1.37	2.35	2.13	1.88	2.49	1.78	2.17	1.41	1.58	0.68
Proline	3.75	3.88	3.30	4.07	2.92	3.54	2.41	5.18	6.77	6.23	8.78	6.27	6.23
Serine	6.48	6.67	5.40	6.03	6.12	5.83	5.12	13.20	12.38	11.22	12.65	8.12	7.66
Threonine	4.35	4.43	3.97	4.80	3.42	4.12	3.41	6.14	6.96	7.08	8.22	5.77	6.03
Tyrosine	2.90	2.57	2.65	1.96	2.95	2.58	2.36	3.50	2.56	3.53	1.85	2.24	1.58
Valine	4.65	4.54	5.42	4.74	5.13	4.63	4.74	4.22	5.45	5.35	4.58	4.03	5.32

* *cf.* Table IV and text.

characteristics are high contents of glutamic and aspartic acids, leucine, alanine, arginine and lysine and relatively low contents of cystine, proline, serine and threonine. Component III has high contents of cystine, proline, serine, threonine and glycine and low contents of glutamic and aspartic acids, alanine, arginine and lysine. Other differences in amino acid composition between the Components occur but are not so marked. All the specimens of Component I prepared under different conditions have the same general amino acid pattern, as have all the specimens of Component III.

Table II gives the amino acid composition of some representative fractions of high- and low-sulphur content obtained from wool by processes involving reduction or oxidation of the disulphide linkages. Comparison of Table I with Table II shows that Component I resembles the fractions of low-sulphur content and Component III resembles the fractions of high-sulphur content. Such a close resemblance in amino acid composition between these different fractions isolated in such varied ways cannot be fortuitous and by itself indicates a close resemblance in actual structure.

TABLE II

COMPOSITION OF KERATOSES AND WOOL PROTEIN FRACTIONS

	*α-Keratose**	*γ-Keratose**	*Low-sulphur** protein*	*High-sulphur*** protein*
Alanine	4.83	2.58	4.89	2.38
Ammonia	10.25	11.05	7.69	9.17
Arginine	20.8	19.0	22.65	16.88
Aspartic acid	6.25	1.79	6.73	1.58
Cysteic acid or S-carboxy methyl cysteine	3.72	14.5	3.71	14.16
Glutamic acid	10.9	5.87	10.37	6.77
Glycine	5.16	4.97	5.00	5.22
Histidine	1.24	1.57	1.62	0.78
Isoleucine	2.49	2.14	2.75	2.07
Leucine	7.30	2.55	7.20	2.44
Lysine	4.60	1.03	5.92	0.80
Phenylalanine	1.94	1.15	2.07	1.19
Proline	2.69	9.85	2.55	10.78
Serine	6.70	9.70	6.01	9.40
Threonine	3.45	7.46	3.46	6.14
Tyrosine	2.44	1.41	2.61	1.52
Valine	3.98	4.15	4.06	4.19

* Ref. 2.
** SCMKA2 (ref. 8).
*** Zinc supernatant high-sulphur protein fraction (ref. 8).

Variations in composition of single components: In addition to the striking differences between Components I and III, there are small but distinct differences in composition between specimens of the same Component obtained under slightly different conditions. These differences seem to be systematic and are more marked with regard to certain amino acids than others. Thus, the differences between specimens of Component I obtained by a first and second extraction with alkali (the second extraction involving more concentrated alkali) are in the same sense whether the preliminary acid treatment is carried out for 7 h or 16 h (Table I, columns 1, 2, 3, 4) and similar differences are observed between the specimens of Component III obtained by a first

and second extraction (Table I, columns 5, 6, 10, 11). The Component obtained by a first extraction has higher contents of alanine, aspartic acid, cystine, glycine, lysine, phenylalanine and tyrosine and lower contents of arginine, proline and threonine than the Component obtained during the second extraction. The differences for other amino acids may be small enough to be somewhat obscured by the variation between experiments. Thus, although the specimens of Component I and Component III obtained by successive extractions differ somewhat in composition, the relationship of Component I to Component III tends to remain rather more constant. These relatively small differences in composition between the specimens of a single Component probably represent minor differences in structure, compared with the broad differences in structure between Components I and III. An explanation based on the suggested keratin model is given later.

The N-terminal residues of the components

The N-terminal residue of Components I and III are given in Table III. The values have not been corrected for decomposition of the DNP-amino acid during hydrolysis, and no allowance has been made for the weight of the DNP groups introduced into the peptides. The average chain weight of Components I and III (Table III) decreases as the time for acid treatment involved in their isolation increases, but are of the same same order of magnitude as those found for α- and γ- keratoses by the SANGER method (59000 and 27100, respectively[18]).

The end-groups of the Components given in Table III, like those of intact wool, or of proteins isolated by the thioglycollate extraction, may represent points at which a limited hydrolysis of peptide bonds has occurred during the treatment[19], rather than representing the number of discrete polypeptide chains. The predominance of serine, threonine and glycine as N-terminal residues in the Components supports this conclusion, as it is known that the amino groups of serine and threonine are readily liberated from peptide linkages during acid hydrolysis[20].

The same end-groups are present in predominant amounts in α- and γ-keratoses

TABLE III

NUMBER OF MOLES OF N-TERMINAL RESIDUES IN 10^6 g COMPONENT

	Component I		*Component III*	
Time of acid treatment	*7 h*	*16 h*	*7 h*	*16 h*
Terminal Amino Acid				
Leucine Isoleucine	Trace	Trace	3.96	Trace
Valine	Trace	Trace	1.39	Trace
Alanine	Trace	1.86	4.56	3.19
Glycine	0.47	1.25	6.63	5.73
Threonine	2.32	9.83	12.60	27.90
Serine	6.37	17.95	14.45	54.80
Glutamic acid	0.58	3.96	3.37	3.35
Aspartic acid	0.63	2.54	2.38	0.86
Total	10.37	37.39	49.34	95.77
Average chain weight	96 500	26 700	20 300	10 500

and in the "low-sulphur protein" extracted by the thioglycollate procedure[19]. The close resemblance between the Components and the keratoses and thioglycollate proteins thus extends to the N-terminal residues as well as to the amino acid composition.

Yields of components

Yields of the Components obtained in typical experiments are recorded in Table IV. Component III was only isolated in relatively low yield, but some is probably lost during dialysis. It may also be more readily hydrolysed than Component I during the acid treatment (*cf.* also p. 12). An appreciable amount of Component I is dissolved during the first extraction (Expt. a). Further extraction of the residue under similar conditions only gave small additional quantities of Components (Expt. d); larger amounts could be extracted by adding additional alkali during stirring (Expt. b), or by repeating the extraction with stronger alkali (Expt. c). Extraction with a stronger alkaline solution in one step gave a yield of Components which was roughly equal to the total yield obtained on repeated extraction with stronger alkali (Expts. b and e). The yield of the Components is thus roughly constant whatever the extraction procedure with alkali.

The residue from these extraction procedures (originally described as Component II) amounts to about 20% of the original wool, but by further treatment with acid and alkali it can again be split into Components I and III. The amino acid composition of this residue approaches that of Component III, presumably because treatment with the acid and alkaline solutions preferentially dissolves Component I (*cf.* p. 10).

The approximate constancy of the yields of Components under varying conditions

TABLE IV

THE YIELDS OF COMPONENTS ISOLATED FROM WOOL KERATIN AS A PERCENTAGE OF THE ORIGINAL WOOL

Experiment	*No. of extraction*	*Extraction procedure*	*Component*		*Residue*
			I	*III*	
a	1	Standard	18.3	2.9	43.0
b	1	Standard + 1 ml 40% w/v NaOH	51.3	7.6	18.6
c	1	Standard	15.7	4.4	
	2	200 ml 0.2 *N* NaOH for 0.5 h	28.3	1.1	19.0
d	1	Standard	11.8	3.2	
	2	Standard	2.4	0.4	
	3	Standard	0.8	Trace	
	4	Standard	2.4	Trace	60.9
e	1	Standard	14.6	5.1	
	2	200 ml 0.05 *N* NaOH for 1 h	25.8	1.2	
	3	200 ml 0.05 *N* NaOH for 1 h	8.7	0.6	
	4	200 ml 0.5 *N* NaOH for 1 h	1.5	0.1	15.4
f*	1	Standard	30.7	2.5	
	2	Standard + 1 ml 40% w/v NaOH	17.5		
g**	1	Standard	29.6	2.3	15.4
h***	1	Standard + 1 ml 40% w/v NaOH	7.9	0.5	6.5

* 16 h treatment with acid.

** Treatment with H_2SO_4.

*** Re-treatment of residue representing 20.7% of original weight of wool from experiment similar to b.

of extraction with alkali, and the fact that all the wool can be accounted for as Components I and III (65%–70%) or their degradation products (small peptides and amino acids) are consistent with the view that wool keratin consists almost entirely of the two Components.

The extraction of the Components from acid-treated wool resembles the extraction of proteins with alkaline thioglycollate, in that the wool residue displays an increased resistance to further extraction, necessitating the use of a higher pH to extract additional protein. A residue is finally obtained which is very resistant to further extraction[21].

When wool is extracted with alkaline thioglycollate solution[22] or oxidised wool is extracted with ammonium hydroxide[2], the residue resembles the original wool in amino acid composition, whereas in the present experiments the residue differs markedly from the original wool.

In the present experiments the fraction with the fewest disulphide linkages, *i.e.*, Component I, is preferentially dissolved. When the disulphide linkages are broken, the large difference in the number of covalent disulphide linkages between the fractions no longer obtains, and no marked preferential solution of a particular fraction takes place.

A preliminary examination of the Components was made in the ultracentrifuge (Spinco model) at a speed of 56100 rev./min. Component I was dissolved in 0.1 *N* ammonium hydroxide and Component III in distilled water, a solution of approximate concentration 0.6% being employed in each case. Component I gave a small discrete peak while Component III gave a very broad, low sedimentation "peak" similar to that given by γ-keratose (*cf.* PEACOCK AND O'CALLAGHAN[23]). Even on the basis of these limited results, the resemblance between the Components and the keratoses is again evident.

Oxidation of the components

Component I on oxidation gives α-keratose and virtually no γ-keratose, while Component III gives γ-keratose and no α-keratose (Table VI). (The small amount of precipitate obtained by addition of ethanol after oxidation of Component I and treatment with ammonium hydroxide contained large amounts of ammonia (30–50% ammonia nitrogen) and differed somewhat from γ-keratose on analysis (Table V), *e.g.*, the cysteic acid content was lower.)

Table V shows the composition of the keratoses obtained from the Components. α-Keratose from Component I is similar to α-keratose from intact wool. The γ-keratose from Component III, however, shows some differences in detail from γ-keratose from intact wool; the cysteic acid and proline contents are lower while the aspartic acid and glutamic acid and glycine contents are higher. The keratose in fact resembles the Component from which it is derived.

This fact strengthens the view that the small differences in composition between the Components and the keratoses reflect differences in structural detail. Considering the fact that different specimens of the Components, keratoses, and thioglycollate-extracted proteins vary in composition over a limited range, the differences in composition between Component I on the one hand and α-keratose, or the low-sulphur proteins SCMKA and SCMKB on the other, are not marked. For certain amino acids, however, there are differences between Component III and γ-keratose or the high-sulphur protein which are greater than the variation found for a single Component or

keratose. The aspartic acid, glutamic acid and glycine contents of Component III tend to be higher than those of γ-keratose and those of proline and cystine lower. The lower contents of cystine and proline may be correlated, since CONSDEN AND GORDON[24] obtained evidence that proline residues occur near to, if not next to, cystine residues in wool.

The yields of keratoses obtained by oxidation of the Components were not quantitative (Table VI). This was probably due to the formation of peptides which were not precipitated by the means usually employed; low yields of oxidised chains are obtained by similar treatment of insulin[12,25]. During the oxidation of Component I with peracetic acid, a relatively large amount of material dissolved, but little high molecular weight material could be recovered. Examination of the products obtained on oxidising Component III with performic acid was made by paper chromatography but no indication of the presence of free amino acids or very small peptides was obtained. These experiments described above, furnish strong evidence that Components I and III are directly related to the α- and γ-keratoses, respectively.

TABLE V

AMINO ACID COMPOSITION OF KERATOSES PRODUCED BY OXIDATION OF THE COMPONENTS

	Component I	*α-Keratose**	*α-Keratose***	*Peptide precipitated by ethanol****	*Component III*	*γ-Keratose****
Alanine	5.85	6.67	5.53	4.70	3.39	3.65
Ammonia	4.63	4.62	4.60	—	4.12	—
Arginine	17.65	18.55	19.65	16.95	13.75	13.67
Aspartic acid	7.62	7.82	7.57	5.08	4.73	4.33
Cysteic acid (or cystine)	2.51	2.30	2.91	5.67	8.93	11.02
Glutamic acid	11.90	12.12	10.85	9.88	7.77	7.55
Glycine	5.60	3.79	3.97	5.73	10.93	10.33
Histidine	2.95	2.64	2.90	3.03	1.21	1.46
Isoleucine	3.54	3.95	3.45	3.28	3.37	2.56
Leucine	7.45	10.15	8.87	3.95	4.63	4.18
Lysine	7.23	8.13	7.82	5.23	3.31	4.22
Phenylalanine	2.72	2.42	2.41	1.99	2.17	1.99
Proline	2.63	2.46	2.48	6.83	6.23	6.63
Serine	6.38	5.53	5.53	11.47	11.22	13.46
Threonine	4.42	3.68	4.16	7.07	7.08	6.91
Tyrosine	2.98	2.57	2.55	2.45	3.53	2.47
Valine	4.64	5.68	5.18	6.81	5.35	5.62

* Peracetic acid oxidation.
** Performic acid oxidation.
*** Corrected for ammonia content.

TABLE VI

YIELDS OF KERATOSES OBTAINED ON OXIDISING THE COMPONENTS

	Oxidising agent		*Yield (%)*
Component I	Peracetic acid	α-Keratose	66.8
		Peptide precipitated by ethanol	5.2
Component I	Performic acid	α-Keratose	68.2
		Peptide precipitated by ethanol	4.1
Component III	Performic acid	γ-Keratose	48.3

DISCUSSION

There can be little doubt from the results presented above that Components I and III represent large fragments of the original wool keratin structure and may perhaps be considered as sub-units of that structure. In the broad outlines of structure they are closely related to the keratoses and the thioglycollate-extracted proteins, except that their cystine linkages remain intact. There are undoubtedly differences in detail between the keratoses and the Components; these are considered later.

In the intact fibre the two Components are united together in some way. The linkages uniting them are almost certainly not disulphide bridges since these would not be split by the procedures used in the present experiments; they are probably peptide linkages which are hydrolysed during the treatment with 2 *N* hydrochloric acid. The many similarities between the Components on the one hand, and the keratoses and thiolglycollate-extracted proteins on the other hand, would imply that a similar hydrolysis or chain fission takes place during the formation of the latter compounds from wool keratin. This hydrolysis need not necessarily be very extensive in character, the hydrolysis of one or two peptide bonds per chain could be sufficient to liberate the Components or keratoses (*cf.* BARTULOVITCH *et al.*[26]). Such a limited chain fission may well occur during the isolation of the keratoses or thioglycollate-extracted proteins, and evidence for such a cleavage of peptide bonds by performic acid has in fact been obtained[27]. About 1% of the total nitrogen of wool dissolves on treatment with peracetic acid[2], and a small proportion of wool becomes dialysable on extraction with alkaline thioglycollate[28]. These facts whould be consistent with a limited degree of chain fission.

Relationship between the components and the histological structure of the fibre

Attempts have been made to identify the fractions of high- and low-sulphur content, such as the keratoses and thioglycollate-extracted proteins, with the ortho- and para-cortex, the two segments of supposedly different cystine content of which the cortex is made up[29]. Such attempts are probably invalidated by RYDER's[30] observations that wool fibres containing cystine labelled with ^{35}S show a uniform distribution of radioactivity across a lateral cross-section, and by the work of PEACOCK AND O'CALLAGHAN[23].

The protein of high-sulphur content has also been identified with a matrix in which are embedded fibrils of a lower sulphur content[31]. This identification is based principally on two experimental approaches, *viz.*, (a) the differential staining of cross-sections of the fibres and (b) the preferential dissolution of a fraction of high-sulphur content, *i.e.*, γ-keratose, from oxidised wool by buffer solutions. The staining approach, however, can lead to erroneous results, *e.g.*, in the assignment of differential chemical compositions to the ortho- and para-cortex[32]. It is now also doubtful whether the preferential solution of γ-keratose, on which the second method of approach is based, takes place to any marked degree[33]. Moreover, it is difficult to reconcile the present results, in which preferential solution of a low-sulphur protein (Component I) occurs, with the idea of a matrix high in sulphur.

Any model involving numerous cystine cross-linkages between the matrix and microfibrils[34], *i.e.*, between high- and low-sulphur proteins, would seem to be ruled out by the fact that the Components are not linked together by disulphide bridges.

Suggested model for keratin

The present data enable a tentative model for the structure of wool keratin to be drawn.

It now seems to be generally accepted that the structure of keratinous fibrous proteins is based at least in part on the α-helix of PAULING AND COREY[35], the principal evidence for this view being the presence of a 1.5 Å reflection in the X-ray diffraction pattern[36,37]. SZENT-GYÖRGY AND COHEN[38] have recently shown that a striking correlation exists between the α-helix content of a number of fibrous proteins and their proline content. They concluded (a) that less than 3% proline distributed statistically in a peptide chain permitted it to contain more than 50% of helix and (b) about 8% of proline deformed the backbone into a random coil. If it is justifiable to apply similar considerations to the Components of wool keratin, it seems probable that Component I has predominantly an α-helical configuration and Component III has a non-helical structure.

It is then interesting to consider the structure of Component I in the light of the LINDERSTRØM-LANG model for beef insulin[39], which has a right-handed helix (B chain) and a left-handed helix (A chain) side by side. Interaction between the leucine (and valine) residues from both chains forms a stabilising force holding the helices together. The ends of the helices are similarly stabilised by inter-chain bonding between asparagine and glutamine side-chains. The high proportions of glutamic and aspartic acids and leucine in Component I may mean that the helical structures are stabilised in a similar way.

The high proline content of Component III probably precludes it from adopting a helical configuration. The high serine content will also favour a non-helical configuration[40]. Proline residues generally facilitate a change of direction of peptide chains[41], and its high cystine content in addition suggests that Component III has a complex system of folded peptide chains with numerous cystine bridges. HARTLEY[42] has recently suggested a similar structure for the N-terminal half of the B chain of chymotrypsin. SCHROEDER[43], from a study of feather keratin (calamus), suggested that in a sequence such as X-Pr, X would tend to be an amino acid with a relatively short side chain such as serine, threonine or glycine. If this suggestion applies to Component III, it would account for its high concentration of serine and threonine.

The evidence thus suggests that wool keratin consists of helical structures (Component I) associated with non-helical structures (Component III). The fact that each Component on oxidation yields almost entirely the corresponding keratose, and practically no keratose derived from the other Component, reinforces the idea that the Components are not linked to each other by disulphide bridges. Expressed in a different way, this implies that the peptide chains in each Component are not vastly different to each other. The individual chains in each Component may be cross-linked to each other by disulphide bonds, or the disulphide bonds may be mainly intra-chain. The half-cystine residues that have been identified as occupying adjacent positions of the peptide chains[44] may denote the presence of such intra-chain links.

The idea that the Components occur in series along the chain is suggested by the fact that γ-keratose derived from Component III by oxidation shows minor differences from γ-keratose from oxidised wool and resembles the original Component in amino acid composition.

A model which would be in accordance with the present observations is a rod-like

one in which segments of helical structure alternate with segments of more random non-helical structure along the longitudinal axis The segments of helical structure may be considered as the "crystalline" portions of the fibre and the more random segments as the "amorphous" portion of the fibre. Both may form part of one and the same peptide chain. A diagrammatic representation is given in Fig. 1. This model resembles in many ways the models recently suggested for silk fibroin and particularly myosin.

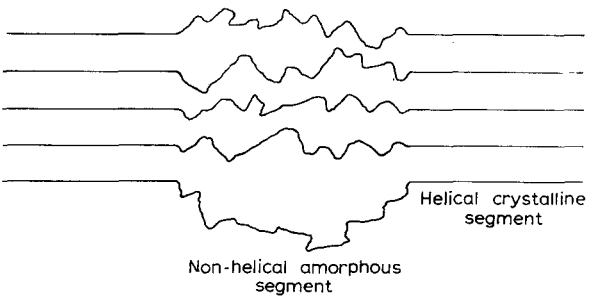

Fig. 1. Diagrammatic representation of the helical and non-helical segments of the fibre.

The isolation of relatively large sub-units of the keratin structure following acid treatment probably means that certain regions of the peptide chain are hydrolysed more rapidly than others. The peptide bonds of the α-helix are probably less accessible to hydrolytic attack than those in the more random parts of the structure[45,46]. One readily attacked region may be the junction between the helix and the more random parts[45,46].

Fission of the peptide chains at such junctions would lead to the formation of fragments similar to Components I and III. It is probable that there are a number of other points or discontinuities, most likely situated in the amorphous part of the structure, where hydrolytic attack takes place fairly readily. The minor differences in composition between, *e.g.*, the Components and the keratoses or thioglycollate-extracted proteins, and also the variations found between different specimens of a single Component, keratose, or high- or low-sulphur protein are explicable on the basis that fission occurs at such subsidiary points as well as at the main junctions between the helical and non-helical regions.

Thus, in the different procedures for isolating fragments from wool, although all the fragments corresponding to one Component or keratose, for example, will have large sections of the chain in common, certain fragments will in addition contain small sections of the chain which are not present in other fragments.

This model is of interest in relation to the changes wool undergoes on extension. On stretching, the α-keratin type X-ray diffraction pattern of wool is transformed to the β-keratin type, the change reflecting an elongation or straightening of the peptide chains[47,48]. If adjacent α-helices are joined by several disulphide linkages, it is impossible to elongate the helices without breaking the disulphide linkages, and it is generally considered that such a breaking of the linkages does not take place on stretching wool. This is a prime difficulty in applying the α-helical structure of proteins to wool[49]. On the present theory Component I with the α-helical configuration would be expected to elongate under tension and be responsible for the elastic properties of wool, while

Component III containing a large proportion of the disulphide bridges would represent a non-extensible part of the structure. The difficulty encountered in applying the α-helical theory to wool is thus largely removed.

Further work will be necessary before we have a detailed picture of the structure of wool keratin. It is believed, however, that the present model is in better agreement with many of the properties of the wool fibre than are previous models.

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CONTRACTILE RESPONSES IN THE PRESENCE OF ELECTRON DONORS AND ACCEPTORS

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SUMMARY

Serotonin, a good electron donor, was found to have little or no effect on the proboscis of Phascolosoma. The preparation, however, could be sensitized to serotonin by prior application of electron acceptors (sevron blue, acridine red or riboflavin).

Similarly, acceptors (naphthoquinone or iodine) sensitized striated muscle to a donor (iodide), producing contracture.

It is suggested that electron-transfer reactions could be involved in the contractile responses.

INTRODUCTION

Observations on electronic properties of numerous substances of biological importance, have led SZENT-GYÖRGYI to suggest that charge transfer reactions may be of widespread biological importance[1]. Many drugs are remarkably good donors[1–4] and it is also conceivable that the transfer of single electrons may be involved in muscular contraction.

On the basis of these concepts, experiments were performed on smooth and striated muscle to study the effects of combining certain electron donors with acceptors. The donors (serotonin and iodide) were chosen because of their known effects on muscle while the acceptors (sevron blue, acridine red, riboflavin, naphthoquinone and iodine) were selected purely for their acceptor properties.