STUDIES ON THE STRUCTURE OF KERATIN

I. THE ANALYSIS OF FRACTIONS ISOLATED FROM WOOL OXIDIZED WITH PERACETIC ACID

by

C. EARLAND AND C. S. KNIGHT

Department of Textile Industries, The Technical College, Bradford (England)

Towards a number of reagents, wool keratin does not behave as a homogeneous substance, since, although the bulk of the fibre may be dissolved, a resistant residue is left[14, 9, 15, 13, 1, 17]. The relationship of this residue to the histological structure of the fibre has not been established completely[6, 10], and since chemical fractionation often involves drastic conditions, *e.g.* treatment with formaldehyde[14] at 140° C or reaction with sodium sulphide solution for one month[15], the keratin may undergo extensive modification.

The mildest method for fractionating wool keratin is oxidative fission of the cystine residues with peracetic acid, followed by extraction with weak alkali, *e.g.* ammonia solution[2]. 10% of the wool is insoluble in alkali, and from the ammonia solution a fraction representing 60% of the original wool is precipitated by acidifying. Performic acid is specific for oxidizing cystine residues in insulin[19], and of the free amino acids is known to attack only cystine, methionine and tryptophane[22]. Since peracetic acid has been shown to exhibit the same specificity towards wool[4], fractions isolated from wool using this reagent have suffered little chemical modification.

Previous analyses on the two fractions isolated from wool as described have shown that in their carbon, hydrogen and nitrogen contents they closely resemble whole wool, but their sulphur contents are considerably lower[5]. These results have not established whether the compositions of the fractions really differ, and in the present paper it is shown that significant differences do exist. Thus wool keratin cannot be regarded as a chemically homogeneous protein, and this may have a bearing on the failure of previous attempts to correlate the amino acid content of whole wool with a definite sequence of amino acids in the peptide chain[16].

Although it has been found that wool after oxidation with peracetic acid is incapable of forming an ammonium salt, it is considered that this may be due to the formation of strong salt linkages such as $-SO_3^- \cdots {}^+NH_3-$, rather than the failure of the reagent to oxidize the cystine residues to sulphonic acid groups[3].

EXPERIMENTAL

Virgin wool of 64s quality was scoured twice in a warm solution of soap and ammonia, followed by thorough washing in water. After removal of weathered tip-ends, the wool was extracted with ether and ethyl alcohol.

Keratin fractions. The wool was reacted with a 2% aqueous solution of peracetic acid (100%

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excess) for 30 hours at 18° C, followed by extraction with 0.2 *N* NH_4OH to leave a residue of β-keratose. α-Keratose was precipitated from the ammonia solution by the addition of 2 *N* HCl. Both fractions were washed with water until the washings were neutral to litmus. It is convenient to designate these fractions as α- and β-keratose, as this corresponds to the probable configuration of their main peptide chains[11].

Analyses of fractions. Prior to analysis, samples were dried at 110° C. Microanalyses for carbon, hydrogen and nitrogen were determined by Weiler & Strauss of Oxford, England. Since microanalyses on wool gave only 2.0% S, compared with the accepted value of 3.6%, macro-oxidation to sulphate using a mixture of KOH and Na_2O_2 was adopted, and was found to be satisfactory. Previously published micro sulphur analyses on whole wool have also varied from the accepted value[21].

Statistical treatment of results. To determine if there was a significant difference in the analyses of the two fractions for each element, the *t*-value (ratio of the difference between the means to standard error of the difference between the means) was calculated. From tables of the probability levels (*P*) of the *t*-distribution, *P* was found for 18 degrees of freedom. If *P* is less than 0.05 the observed difference between the means is significant, and highly significant if it is less than 0.01.

RESULTS

The elementary composition of α- and β-keratose

Analyses of ten different preparations of α- and β-keratose for carbon, hydrogen, nitrogen and sulphur are given in Table I, and a statistical summary of the results in Table II.

TABLE I

THE COMPOSITION OF α- AND β-KERATOSE

Preparation	*% Carbon*		*% Hydrogen*		*% Nitrogen*		*% Sulphur*	
	α	*β*	*α*	*β*	*α*	*β*	*α*	*β*
1	47.6	46.9	6.23	6.77	15.3	15.8	2.88	2.75
2	47.7	46.2	6.18	6.72	15.7	15.8	2.96	2.79
3	46.1	45.8	6.84	7.24	15.6	16.3	2.95	2.73
4	46.9	45.7	6.97	7.10	16.0	14.6	3.00	2.80
5	45.9	46.0	6.28	6.50	16.2	15.7	2.72	2.73
6	47.0	45.3	6.81	6.46	15.9	14.9	2.76	2.79
7	46.9	45.8	6.82	6.92	17.0	16.8	2.88	2.74
8	47.2	45.7	6.66	6.85	16.9	17.1	2.80	2.72
9	47.7	46.3	6.84	7.13	16.4	16.1	3.07	2.45
10	47.8	46.2	6.84	7.41	16.1	16.3	2.93	2.65

TABLE II

STATISTICAL SUMMARY OF RESULTS PRESENTED IN TABLE I

	Carbon		*Hydrogen*		*Nitrogen*		*Sulphur*	
	α	*β*	*α*	*β*	*α*	*β*	*α*	*β*
Mean value* %	47.08	45.99	6.65	6.91	16.11	15.94	2.90	2.72
Standard deviation(%)	0.67	0.44	0.30	0.31	0.54	0.77	0.11	0.10
Standard error (%)	0.21	0.14	0.09	0.10	0.17	0.24	0.04	0.03
t	4.32		2.59		0.57		3.78	
P (18 d.f.)	< 0.001		0.025		> 0.5		< 0.005	

* The oxygen contents of α- and β-keratose, by difference, are 27.23 ± 0.51 and 28.45 ± 0.51% respectively.

Attempted preparation of ammonium salt of α-keratose

It is seen from Table II that the carbon, hydrogen and sulphur contents of α- and β-keratose show significant differences. On the other hand the nitrogen contents of the two fractions are identical. The latter result is somewhat surprising, since if free sul-

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phonic acid groups are produced by oxidation of the cystine residues ($-S-S- + 5O + + H_2O \rightarrow 2-SO_3H$), it would be expected that β-keratose would be an ammonium salt and would contain more nitrogen than α-keratose, which is precipitated from acid solution (free sulphonic acid). It is seen from Table III, however, that α-keratose does not have an increased nitrogen content after solution in aqueous ammonia (17% w/w) followed by removal of the solvent under vacuum.

TABLE III

THE NITROGEN CONTENT OF α-KERATOSE AFTER SOLUTION IN AMMONIUM HYDROXIDE

α-keratose precipitated from NH_4OH soln. by acid (% N)	*α-keratose isolated from NH_4OH soln. by evaporation (% N)*
16.3	16.3
16.0	16.2
16.2	16.4
16.0	16.4
14.0*	16.4
16.3	15.8
Mean 16.16	16.25

* omitted from mean.

Based on the sulphur content of these materials, if the ammonium salt of the sulphonic acid were formed, it should contain 1.2% more nitrogen than the free acid. As the α-keratose was precipitated from ammonia solution by acidifying at pH 2, which is the approximate pK value for a sulphonic acid, if the ammonium salt were formed, the nitrogen contents given in Table III should differ by 0.6%. Since treatment of cysteic acid with ammonia under the same conditions produced an increase in nitrogen content from 8.45% (calc. for $C_3H_7O_5NS$; N, 8.3%) to 14.80% (calc. for ammonium salt, $C_3H_{10}O_5N_2S$; N, 15.1%), it must be concluded that keratin oxidized with peracetic acid does not form an ammonium salt under normal conditions.

DISCUSSION

Two main points emerge from the analytical data presented in this work. (1) Fractions obtained from wool by oxidizing with peracetic acid, followed by extraction with ammonia, differ significantly in their carbon, hydrogen and sulphur contents from each other. (2) Both α- and β-keratose are incapable of forming ammonium salts under conditions where such a salt is formed with cysteic acid.

The solubility of proteins and synthetic polypeptides is often dependent on their configuration, the folded α-form being more readily soluble than the extended β-form. Thus, water-soluble silk is thought to be in an α-configuration[7], and the copolymer of DL-β-phenylalanine and DL-leucine is soluble in benzene or carbon tetrachloride only in the α-form[8]. On the other hand, differences in solubility may be due to chemical factors such as the presence of cross-links or hydrophilic or hydrophobic groups.

The configuration of the ammonia-insoluble fraction of wool oxidized with peracetic acid is the subject of considerable controversy[11, 18, 12], the consensus of evidence being that part at least of this fraction is in the β-form. The ammonia-soluble fraction is indisputably in the α-form.

Thus, although it is very probable that the two fractions differ in configuration, this

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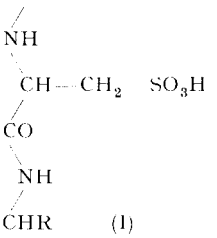
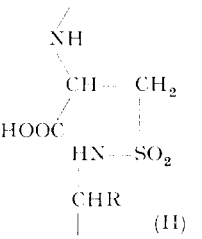
work shows that they also differ in composition. The insolubility of β-keratose cannot therefore arise from a simple $\alpha \rightarrow \beta$ transformation of the soluble fraction, and the differences in the solubility of these materials may well be due to differences in their chemical structure.

The failure of wool oxidized with peracetic acid to form an ammonium salt is in keeping with the findings of ALEXANDER, FOX AND HUDSON[3], who were unable to detect any exchange between potassium ions and H^+ or NH_4^+ ions which should have been absorbed on any free sulphonic groups in peracetic acid-oxidized wool. From this and other indirect evidence, it was concluded that the initial reaction between peracetic acid and wool is not the formation of a sulphonic acid (I), but a sulphocarboxylic imide, which on subsequent treatment with alkali hydrolyses to a sulphonamide (II).



(I)



(II)

Both mechanisms require an oxygen content of 26.6% for the final reaction product, compared with the values of 27.23 ± 0.51 and 28.45 ± 0.51 found for α- and β-keratose respectively.

More recently, WESTON[23] has found direct evidence from the infra-red absorption spectra of these fractions for the presence of sulphonic acid groups and has been unable to detect sulphonamide groups. In view of this, an alternative explanation to that given by ALEXANDER *et al.* for the failure of wool oxidized with peracetic acid to give reactions characteristic of a free sulphonic acid must be sought. Thus, it is generally accepted that the carboxyl and amino groups of wool form a salt link $-COO^- \cdots {}^+NH_3-$[20] which is such a weak bond that it is readily broken outside the pH range of 4 to 8. It seems highly probable that any strong sulphonic acid groups produced in wool by reaction with peracetic acid will form similar salt links such as $-SO_3^- \cdots {}^+NH_3-$ with basic residues. These bonds would be stable enough to prevent both the proton from exchanging with other cations and salt formation with weak alkalis. It has been suggested, for example, by WOODIN[24] that an arginine-bisulphate interaction is such a stable linkage that it would be difficult to distinguish it from a covalent bond, since the high pH necessary to suppress the salt linkage would hydrolyse labile covalent bonds.

CONCLUSIONS

1. Fractions isolated from wool after oxidation with peracetic acid differ in their carbon, hydrogen and sulphur contents. This indicates that the high molecular weight material in wool keratin held together by disulphide bonds is heterogeneous.

2. Wool oxidized with peracetic acid does not form an ammonium salt under conditions where such a salt is formed with cysteic acid.

3. The failure of wool oxidized with peracetic acid to give reactions characteristic of a sulphonic acid is probably due to the formation of strong salt links such as $-SO_3^- \cdots {}^+NH_3-$ between sulphonic acid groups and basic residues.

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SUMMARY

A number of preparations of ammonia-soluble and -insoluble fractions obtained from wool oxidized with peracetic acid has been analyzed for carbon, hydrogen, nitrogen and sulphur. Statistical examination of the results has shown that these fractions differ in carbon, hydrogen and sulphur contents, but that their nitrogen contents are identical. It is also shown by direct analysis that wool oxidized with peracetic acid does not form an ammonium salt under conditions where such a salt is formed with cysteic acid. It seems probable that strong salt links such as $-SO_3^- \cdots {}^+NH_3-$ prevent the sulphonic acid residues in oxidized wool from taking part in normal reactions.

RÉSUMÉ

Les compositions en carbone, hydrogène, azote et soufre d'un certain nombre de préparations de fractions solubles et de fractions insolubles dans l'ammoniaque, obtenues à partir de laine oxydée par l'acide peracétique, ont été déterminées. L'analyse statistique des résultats montre que ces fractions diffèrent par leurs teneurs en carbone, hydrogène et soufre, mais que leurs teneurs en azote sont identiques. Il est également démontré par analyse directe que la laine oxydée par l'acide peracétique ne forme pas de sel d'ammonium dans des conditions où l'acide cystéique en forme un. Il paraît probable que la liaison saline forte $-SO_3^- \cdots {}^+NH_3-$ empêche les résidus sulfoniques de la laine oxydée de prendre part à leurs réactions normales.

ZUSAMMENFASSUNG

Aus mit Peressigsäure oxydierter Wolle wurden eine Reihe von Präparaten von in Ammoniak löslichen und unlöslichen Fraktionen gewonnen und der Kohlenstoff-, Wasserstoff-, Stickstoff- und Schwefelgehalt analysiert. Die statistische Untersuchung der Ergebnisse hat bewiesen, dass diese Fraktionen verschiedene Mengen von Kohlenstoff, Wasserstoff und Schwefel enthalten, ihr Stickstoffgehalt jedoch identisch ist. Die direkte Analyse hat weiterhin bewiesen, dass mit Peressigsäure oxydierte Wolle kein Ammoniumsalz formt, unter Bedingungen, bei welchen Cysteinsäure ein Ammoniumsalz bildet. Es darf angenommen werden, dass die starke Salzbindung $-SO_3^- \cdots {}^+NH_3-$ die Sulfonsäurereste in oxydierter Wolle daran hindert, an normalen Reaktionen teilzunehmen.

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