A Cystine-Rich Protein Fraction from Oxidized α -Keratin

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A soluble fraction of α -keratin was obtained on fission of disulphide bonds. The fraction was soluble in the oxidizing solution and would normally be lost when such procedures are used for isolating keratose fractions. This fraction, which constituted 6% by weight of keratin, was rich in cystine, and about 30% of the fraction had a mol.wt. of less than 20000.

Extensive studies have been made of the polypeptide chains isolated from α -keratin, and these studies have shown that α -keratin can be divided into two major fractions: one a cystine-poor fraction (α -keratose, SCMK-A), the other a cystine-rich fraction (γ -keratose, SCMK-B). Both fractions can be extracted from keratin after fission of the cystine disulphide bonds that cross-link the polypeptide chains. These fractions have been shown to be heterogeneous (Crewther *et al.*, 1965) and this heterogeneity has complicated studies of the primary structure of these fractions and of α -keratin. Dietary changes have been shown to affect the cystine content of the cystine-rich fraction of wool keratin, mainly by changing the amounts of polypeptides synthesized that are very rich in cystine (Gillespie *et al.*, 1969).

Structural studies on oxidized and reduced α -keratins have concentrated on the proteins that are insoluble after fission of the disulphide bonds. Such studies have led to the generally accepted view of keratin structure that suggests a two-phase synthesis of proteins that make up the keratin fibre. Briefly the assembly of keratin from cystine-poor and cystine-rich proteins is thought to occur in two steps: (a) the synthesis of cystine-poor proteins low in the follicle and (b) the synthesis of cystine-rich proteins at a different site, later and higher in the follicle. The enrichment of cystine-poor proteins with cystine-rich proteins occurs before keratinization, and the latter proteins are thought to cement the more crystalline cystine-poor proteins together. A comprehensive and detailed review of keratin synthesis and keratinization is given by Fraser *et al.* (1972).

The cystine-poor and cystine-rich polypeptide fractions form the major portion (90%) of the keratin fibre, but examination of the literature shows that there is a fraction of keratin produced during oxidation or reduction and alkylation that has received little attention.

Corfield *et al.* (1958) found that, by using 1.6% peracetic acid to oxidize wool, 1% of the nitrogen

and 25% of the sulphur was unaccounted for as cysteic acid. Thompson & O'Donnell (1959) have shown that the loss of nitrogen from keratin is associated with the extraction of a cystine-rich fraction. Alkylation of reduced wool with methyl iodide resulted in the loss of 30% of the sulphur (Zahn & Biela, 1968). Ward (1967) found that wool, when treated at pH 2.5–2.6 with 2-mercapto-ethanol, loses 2% (by weight) of soluble protein, of which one-sixth has a molecular weight of less than 10000. The data of Sweetman & Maclaren (1966) and Maclaren & Sweetman (1966) show that 0.5–15% (by weight) of wool fibre dissolves under their reducing conditions. In a later study 30% of the fibre was extracted with boiling water, whereas milder conditions (water at 20°C) extracted approx. 8% (Maclaren *et al.*, 1968). By using the amino acid-analysis data for wool keratin and *S*-carboxy-methylated keratin (Maclaren *et al.*, 1968), the approximate amino acid composition of this soluble fraction when calculated (cf. Table 1, column 6) shows that there is about one half-cystine residue per four amino acid residues in the soluble fraction. Studies by Buchanan (1969), Kulkarni (1969) and Rajkotwala (1970) showed that soluble cystine-rich keratin fragments are produced on fission of disulphide bonds of keratin. The amino acid compositions of these fractions are given in Table 1. Steinert & Rogers (1973), in a study of hair-follicle proteins, showed that differences existed in the amino acid compositions of the high-sulphur proteins from these sources. Steinert & Rogers's (1973) data show that, when compared with SCMK-B proteins from hair roots, the SCMK-B proteins from hair fibre are enriched for cystine, threonine, serine and proline.

In the present work a study was made of the peptides from human hair solubilized by using acidic oxidizing conditions (Thompson & O'Donnell, 1959). A portion of this soluble material diffused through a dialysis membrane (mol.wt. < 20000). Both the non-diffusible and diffusible portions were found to contain large amounts of half-cystine

Table 1. *Amino acid analysis of soluble fractions and diffusates from oxidized and reduced keratin*

Serine and threonine values are uncorrected for losses during hydrolysis. The various fractions were: 1, soluble fraction from oxidized hair (human) keratin before dialysis; 2, diffusate from oxidized hair (human) keratin; 3, soluble fraction from oxidized wool keratin before dialysis (Buchanan, 1969); 4, diffusate from oxidized wool (Buchanan, 1969); 5, soluble fraction from reduced hair (human) (Rajkotwala, 1970); 6, derived from the data of Maclaren & Sweetman (1966); 7, difference between hair and hair-follicle high-sulphur proteins (Steinert & Rogers, 1973); 8, soluble fraction from oxidized wool (Kulkarni, 1969).

Amino acid	Amino acid composition (residues/1000 residues)							
	1	2	3	4	5	6	7	8
Fraction ...	1	2	3	4	5	6	7	8
CyS	188	393	77	231	166	235	411	108
Asp	46	30	45	156	52	—	—	79
Thr	90	83	93	43	57	89	205	61
Ser	102	85	168	83	127	139	161	97
Glu	116	57	65	88	116	—	—	102
Pro	89	91	104	41	—	155	223	56
Gly	102	63	153	239	129	129	—	125
Ala	37	24	46	51	56	2	—	47
Val	59	45	50	18	46	23	—	57
Ile	22	15	17	5	127	29	—	30
Leu	40	29	50	13	34	—	—	90
Tyr	1	4	—	—	—	58	—	—
Phe	20	22	28	—	24	44	—	41
Lys	11	8	12	—	10	—	—	24
His	12	8	13	—	9	27	—	9
Arg	65	43	80	31	47	62	—	73
Met	—	—	—	—	—	8	—	—

residues. Furthermore the evidence suggests that low-molecular-weight cystine peptides play a part in the final steps of keratinization.

Experimental

Human hair (7g) was oxidized with 200ml of performic acid [100-volume H_2O_2 /98% formic acid (1:39, v/v)] for 18h at 4°C. The solubilized protein was isolated from the solution by rapid dialysis against water (3 × 2 litres) followed by freeze-drying of both the non-diffusible material and the diffusate. The weights of protein in each fraction were 4.8% and 1.6% respectively. Samples of each protein fraction were hydrolysed in 6M-HCl *in vacuo* at 110°C for 18h. Samples were freed from HCl by drying over KOH *in vacuo*, and analysed on a Beckman 120B amino acid analyser modified for one-column operation. Calculation of the results of the analyses was carried out with the program described by Buchanan (1977). Other samples (10mg) of each fraction were separated by paper electrophoresis

at pH 1.85 and 3.6 (Buchanan & Corfield, 1971). Mobilities of cysteic acid peptides were measured relative to cysteic acid.

Results

The amount of the soluble fraction of hair keratin released by oxidative fission is about 6%. The molecular-weight distribution of the peptides in the soluble fraction is not known, although about one-third of the soluble fraction has a mol.wt. of less than 20000, i.e. it readily diffuses through dialysis membrane. The amino acid analysis of the soluble fraction shows that on average there is about one half-cystine residue per five amino acid residues, and in the diffusate the frequency is one half-cystine residue per 2.5 amino acids. Paper electrophoresis at pH 1.85 of the diffusate showed that the main bands on the electrophoretogram are cysteincysteic acid, cysteic acid, aspartic acid and glutamic acid. Another band present on the electrophoretogram, which was ninhydrin-negative, was detected with Bromocresol Blue stain (Buchanan & Corfield, 1971). The mobility of this band relative to cysteic acid was 1.58 and for *N*-acetylcysteic acid it was 1.58.

Discussion

The evidence presented shows that there is a fraction (6%) of α -keratin that is readily soluble after fission of the disulphide bonds, that is enriched for cystine, as cysteic acid in the analysis, and that also contains about 30% low-molecular-weight peptides. Paper electrophoresis of the low-molecular-weight peptides shows the presence of amino acids (cysteic acid, aspartic acid and glutamic acid), dipeptides of cysteic acid and possibly *N*-acetylcysteic acid. Lindley & Haylett (1967), Asquith & Shaw (1968), Haylett & Swart (1969) and Buchanan & Corfield (1971) have shown that the sequence Cys-Cys occurs frequently in keratin. In this report the dipeptide cysteinylcysteine was released from human hair by oxidative fission of the disulphide bonds. It might be argued that peptide bond hydrolysis gave rise to cysteinylcysteine, but under the conditions used in this work little peptide bond hydrolysis could take place. Liberation of this dipeptide suggests that it originally formed a cross-link in the native keratin, possibly between two polypeptide chains. Further evidence to support this claim was presented by Buchanan & Corfield (1971), who found dipeptides of cysteic acid, blocked at the *N*- and *C*-termini, present in a partial acid hydrolysate of wool keratin.

The amino acid analysis of the various keratin fractions (Table 1) shows that these fractions contain various amounts of half-cystine residues. It is evident from the amino acid analysis of the diffusate

of the soluble fraction (Table 1, column 2) that this fraction contains the greater proportion of half-cystine residues. Ward (1967) showed that, by using mild reducing conditions, small peptides (mol.wt. <10000) were liberated from wool on cleavage of the disulphide bonds. Rajkotwala (1970), in a study of the presence of abnormal amino acids in human hair, showed the presence of homocysteine in the hair of patients suffering from homocystinuria. After oxidation of the disulphide bonds with performic acid it was found that 6.6% of the hair dissolved. The amino acid composition of this fraction is shown in Table 1 (column 5). Kulkarni (1969) showed that wool keratin, when oxidized with performic acid, liberated a cysteic acid-rich fraction (Table 1, column 8). These facts and the results presented provide proof for the presence of small cystine-containing peptides in keratin attached to the main-chain polypeptides through disulphide bonds. There is no information as to the function of these small molecules in keratinization, although it has already been suggested that small cystine peptides blocked at their *N*- and *C*-termini may function as polymerizing agents (Buchanan & Corfield, 1971), i.e. cross-linking one polypeptide chain to another through an extended disulphide bond network. The release of soluble peptides from oxidized or reduced keratins suggests that these cystine-rich peptides form part of the cystine enrichment process that takes place in the hair and wool follicle and that precedes keratinization.

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