THE ISOLATION AND PROPERTIES OF SOME SOLUBLE PROTEINS FROM WOOL

III. THE HETEROGENEITY OF THE LOW-SULPHUR WOOL PROTEIN SCMKA2*

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Summary

The low-sulphur wool protein SCMKA2, which gives only single peaks on electrophoresis and ultracentrifugation over a wide range of pH and concentration, has been divided arbitrarily by ammonium sulphate precipitation into two fractions. Amino acid analysis has shown significant differences between the fractions in some amino acid residues, particularly of *S*-carboxymethyl cysteine, glycine, phenylalanine, tyrosine, and valine. Both fractions have been shown to be heterogeneous by chromatography on DEAE-cellulose and elution with buffer containing 8M urea. One fraction, that which is less soluble in ammonium sulphate, has the lowest sulphur content of any wool protein so far separated. From this fraction a subfraction was prepared by column chromatography which differed significantly in amino acid composition from either of the ammonium sulphate fractions. These data are consistent with the hypothesis that there are a number of low-sulphur proteins in wool.

I. INTRODUCTION

As a result of the fractionation studies of Gillespie and Lennox (1955) and of Gillespie (1960) and of the chromatographic and peptide-mapping studies of O'Donnell and Thompson (1961) and Thompson and O'Donnell (1962) there is a growing realization that the protein of low-sulphur content isolated from wool may not be homogeneous. The evidence from the study of wool by X-ray and electron-microscopy techniques suggests that there is considerable regularity in the structure of the microfibrils, but the recent studies of Filshie and Rogers (1961) and Fraser, MacRae, and Rogers (1962) have shown that these structural elements (generally regarded as the location of the low-sulphur proteins) probably consist of 11 protofibrils. The X-ray evidence is consistent with the idea that each protofibril consists of three strands of α-helices which, in turn, on the basis of a repeating unit of 198 Å, can be subdivided into three similar but not identical particles. Furthermore these workers suggest that, in addition to the low-sulphur protein chains in the 11 protofibrils, there are also "interstitial" chains within the structure. If the protofibrils are not alike or even if the component parts of the strands are not the same and give rise to different proteins on solubilization then considerable heterogeneity might be expected in the isolated proteins.

A low-sulphur kerateine was isolated by Gillespie and Lennox (1955) which, after alkylation with iodoacetate and the removal of a small quantity of high-sulphur protein by zinc precipitation (Gillespie 1957), gave a material, SCMKA2,

* A scheme of nomenclature defining SCMKA2 and other wool proteins is given by Gillespie (1960, p. 102).

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which showed many of the properties of a pure protein. This purified low-sulphur protein has been extensively studied in this Laboratory (Gillespie *et al.* 1960). Although it gave single boundaries during electrophoresis and ultracentrifugation in the pH range 7–11 and at protein concentrations from 0·25 to 1·0%, the question of its homogeneity has not been settled. Because it aggregates in solution in this pH range and is insoluble at the isoelectric point, it has not been possible to apply the various critical electrophoretic and ultracentrifugal tests for homogeneity.

The evidence from other sources has varied. For example, O'Donnell, Thompson, and Inglis (1962) recently found *N*-acetyl groups in a low-sulphur wool *S*-carboxymethyl kerateine, which, if all were *N*-terminal, would be equivalent to a minimum molecular weight of about 25,000. Previously Thompson (1957) and Bradbury (1958) found *N*- and *C*- terminal end groups in small amounts, which in the case of the *N*-terminal residues were made up of six different amino acid residues, totalling to give 1 mole in 62,000 g of protein. Although the interpretation is complicated by the lack of a certain molecular weight (published values range from 10,000 (Harrap 1957) up to 50,000 (O'Donnell and Woods 1956)), these end-group results suggest heterogeneity. Solubility tests gave equivocal results, for, whereas the constant solvent solubility test deviated from ideality, the specific property and variable solvent tests gave no evidence of heterogeneity (Gillespie 1957).

The availability of a complete amino acid analysis of high accuracy has now made possible a more sensitive application of the specific property solubility test to this problem. This paper is concerned with the description of two fractions of SCMKA2 produced by salt fractionation, of their amino acid analysis, and of their chromatographic behaviour on DEAE-cellulose when eluted with buffer containing 8M urea.

II. Materials and Methods

(*a*) *Preparation of Protein Fractions*

SCMKA2 was prepared by the methods of Gillespie and Lennox (1955) and Gillespie (1957) except that redistilled thioglycollic acid was employed and, at the completion of alkylation, any excess iodoacetate was destroyed with a small excess of thioglycollate. The procedures used for preparation and fractionation are summarized in Table 1.

(*b*) *Amino Acid Analysis*

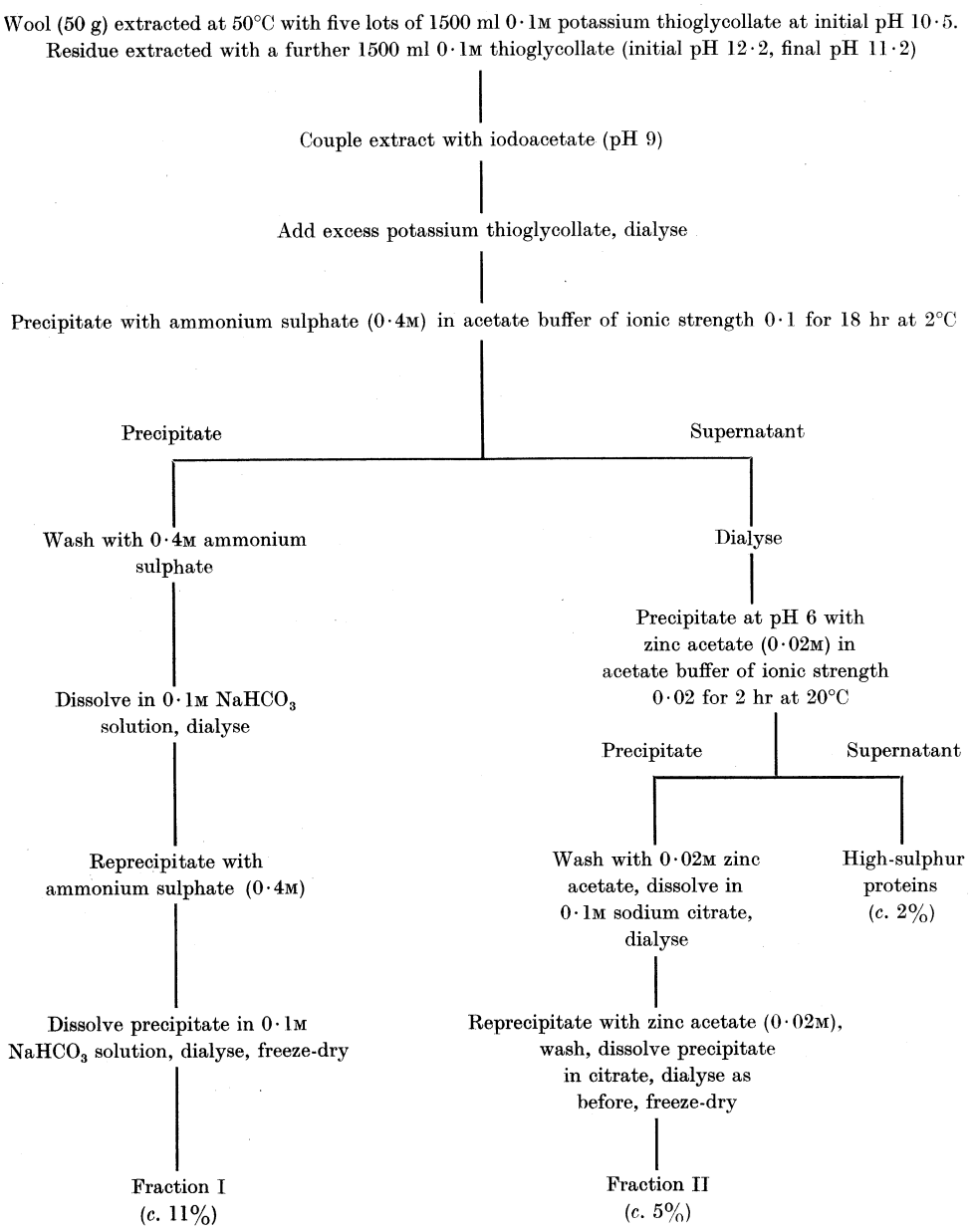
The freeze-dried protein fractions were dissolved in 1% sodium bicarbonate solution and dialysed against two changes of 0·2N NaCl, then three changes of distilled water. Each dialysis lasted 24 hr and a volume ratio of 200:1 was used. The protein solutions were then freeze-dried and hydrolysed for 18 hr under reflux with 6N HCl. The amino acids in each hydrolysate were analysed by the Moore and Stein technique with a Spinco automatic analyser.

(*c*) *Column Chromatography*

DEAE-cellulose (EK 7392) columns were prepared as described previously (Gillespie 1959) except that the imidazole–HCl buffer and the buffer–sodium

TABLE 1

PREPARATION AND FRACTIONATION OF SCMKA2



chloride eluant both contained 8M urea. This was necessary, for at pH values around 7 and 8 the low-sulphur wool proteins only partly elute with salt solutions alone. The DEAE-cellulose was discarded after each run. About 50 mg of freeze-dried protein, dissolved in freshly prepared buffer containing 8M urea, was applied to the column (2 by 10 cm) and eluted with 200 ml of buffer–8M urea with a gradient of 0 to 0·5M NaCl. 3-ml fractions were collected and analysed for protein content by means of an optical density measurement at 277 mμ. For rechromatography, appropriate tubes were pooled, dialysed, and freeze-dried before use.

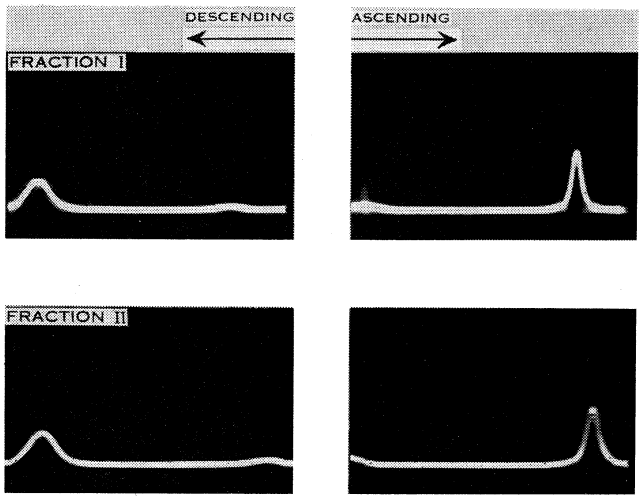


Fig. 1.—Electrophoretic patterns of fractions of SCMKA2 run at pH 11·0 in β-alanine–NaOH buffer of ionic strength 0·1.

(*d*) *Electrophoresis*

The proteins were examined by moving boundary electrophoresis at pH 11·0 in β-alanine–NaOH buffer of ionic strength 0·1. The instrument was made by LKB Produktor, Stockholm, and standard 11-cm cells were employed and a voltage gradient of 5·5 V/cm was used. Further experimental details are given by Gillespie and Lennox (1955).

III. Results and Discussion

On electrophoresis at pH 11·0 and at a protein concentration of 0·5%, the two fractions both gave single symmetrical peaks (Fig. 1). The mobilities calculated from the descending boundaries (in cm^2 $volt^{-1}$ sec^{-1}) were $-6{\cdot}90\times10^{-5}$ (fraction I) and $-6{\cdot}95\times10^{-5}$ (fraction II), and therefore in this respect the fractions do not differ. Yet the amino acid analyses presented in Table 2 show that the two fractions contain five amino acids whose concentrations differ by more than 10% and, as the analytical error is ±3%, it means that in this respect the proteins differ significantly. It can be seen that the amount of the basic amino acid residues in each fraction are but little different and that the major differences are in the

content of *S*-carboxymethyl cysteine, glycine, phenylalanine, and tyrosine. In terms of residues in a protein of molecular weight set arbitrarily at 50,000 (this value is about the lowest obtained in 8M urea (O'Donnell and Woods 1956)) then the two proteins differ by about three in phenylalanine, valine, and tyrosine, six in *S*-carboxymethyl cysteine, and seven in glycine.

TABLE 2

AMINO ACID ANALYSIS OF SCMKA2 FRACTIONS

Amino Acid	Fraction I		Fraction II		Difference as Percentage of Fraction I
	Amino Acid Nitrogen (as % total N)	Amino Acid Content* (μmoles/g)	Amino Acid Nitrogen (as % total N)	Amino Acid Content* (μmoles/g)	
Alanine	4·66	559	4·71	565	1·1
Amide	8·02	962	8·53	1024	6·4
Arginine	20·29	609	21·38	641	5·4
Aspartic acid	6·08	730	6·25	750	2·8
S-Carboxymethyl cysteine	3·43	412	4·49	539	33·8
Glutamic acid	10·30	1236	11·05	1326	7·3
Glycine	5·29	635	4·11	493	−22·3
Histidine	1·34	53	1·35	54	—
Isoleucine	2·51	301	2·55	306	—
Leucine	6·90	828	7·06	847	2·3
Lysine	5·02	301	5·22	313	4·0
Methionine	0·34	41	0·36	43	—
Phenylalanine	1·98	238	1·52	182	−23·2
Proline	2·56	307	2·66	319	—
Serine†	6·20	744	5·81	697	−6·5
Threonine†	3·25	390	3·49	419	7·4
Tyrosine	2·47	296	1·95	234	−20·2
Valine	3·90	468	4·36	523	11·8

* Calculated on a nitrogen content in the protein of 16·8%.

† Not corrected for hydrolysis losses.

Although the *S*-carboxymethyl cysteine content of fraction I (412 μmoles/g) is the lowest yet recorded for a wool protein, this fraction may still contain sulphur-rich impurities, for a wool-root protein isolated by Rogers (1959) and presumably a precursor contains even less of this residue.

The chromatographic data in Figure 2(*a*) show that the two fractions differ slightly in ease of elution, the main peak of fraction I eluting with 0·255M NaCl and that of fraction II with 0·28M NaCl. Each pattern is rather broad and shows the presence of some minor components, fraction I being slightly richer in the more easily eluted minor components and fraction II in the more difficultly eluted components. Approximately 65% of the protein in fraction I was in the main peak and this accounted for only about 7% of wool.

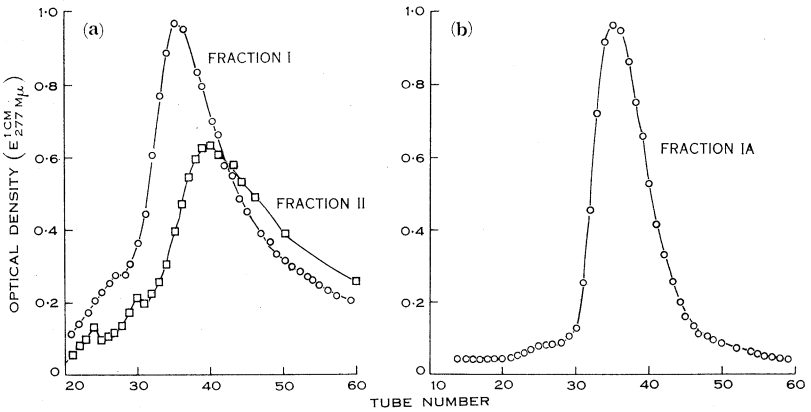


Fig. 2.—(*a*) Elution patterns of SCMKA2 fractions in imidazole–HCl buffer containing 8M urea at pH 7·0 and ionic strength 0·01, under an elution gradient of 0 to 0·5M NaCl. (*b*) Elution pattern of fraction IA.

TABLE 3

AMINO ACID ANALYSIS OF FRACTION IA

Amino Acid	Amino Acid Nitrogen* (as % total N)	Amino Acid Content‡ (μmoles/g)	Difference as Percentage of Fraction I	Difference as Percentage of Fraction II
Alanine	4·94	593	5·6	5·9
Amide	9·94	1193	—	—
Arginine	21·55	647	6·2	—
Aspartic acid	6·64	797	8·9	6·3
S-Carboxymethyl cysteine	3·52	422	3·6	−21·6
Glutamic acid	11·27	1356	9·4	2·0
Glycine	4·58	550	−14·8	11·4
Histidine	1·34	540	—	—
Isoleucine	2·64	313	4·0	—
Leucine	7·44	893	7·8	5·4
Lysine	5·78	385	15·1	10·7
Methionine	0·39	47	—	—
Phenylalanine	1·72	206	−13·1	11·8
Proline	2·25	270	−12·3	−18·2
Serine‡	5·64	677	−9·3	2·9
Threonine‡	3·13	376	−2·8	10·3
Tyrosine	2·33	280	−5·6	19·5
Valine	4·30	516	10·0	1·4

* Mean of two determinations.

† Assuming a nitrogen content in the protein of 16·8%.

‡ Not corrected for hydrolysis losses.

Because of the two main fractions fraction I comprised the larger proportion of wool, protein material from tubes Nos. 33 to 38 inclusive (fraction IA) was rechromatographed. It chromatographed in its original position as shown in Figure 2(*b*). Under these conditions of gradient elution no further resolution into minor components was obtained. Fraction IA was also subjected to amino acid analysis (Table 3) and found to differ significantly from fraction I by containing more lysine and valine and less glycine, phenylalanine, and proline, and from fraction II by containing more tyrosine, threonine, lysine, phenylalanine, and glycine and less *S*-carboxymethyl cysteine and proline.

When O'Donnell and Thompson (1961) subdivided α-keratose into three fractions of different amino acid content, they were handling a material which contained about half the low-sulphur proteins from oxidized wool. The results here presented show that an even more highly fractionated protein from reduced wool, representing less than a third of the low-sulphur protein, is also heterogeneous and can be subdivided by salting-out and chromatography. Although not figured here, the low-sulphur protein extractable from reduced wool at pH 10·5 (after alkylation it is predominantly SCMKA1) gives an elution pattern identical with fraction I. In spite of this it appears that SCMKA1, on the basis of a partial amino acid analysis (Gillespie 1960), differs significantly from either of the fractions in the present study.

Apart from the glycine- and tyrosine-rich minor constituents (Gillespie 1960), fractions of the low-sulphur *S*-carboxymethyl kerateine have a similarity in amino acid composition but differ significantly in their content of a number of amino acids. The results presented here do not enable a choice to be made between the alternative ideas that wool contains many low-sulphur proteins or a relatively few components contaminated with small amounts of minor constituents. It is therefore not at all certain that it will be possible to obtain a pure low-sulphur protein, at least in yields which make it a significant fraction of the wool fibre, although a common architecture may apply to all the components which may allow them to fit into the ordered structure of the microfibril. For this reason attention is now being concentrated on the high-sulphur proteins of Merino and other wool and hair fibres.

IV. Acknowledgments

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