ULTRA-HIGH-SULPHUR PROTEINS IN THE HAIRS OF THE ARTIODACTYLA

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Abstract

Wool follicles are potentially able to synthesize specific high-sulphur proteins in which about 30% of the amino acid residues are half-cystine (Gillespie and Reis 1966). The amount of these proteins incorporated into the fibre is related to the availability of sulphur-containing amino acids for metabolism in the sheep. There is a linear relation between the sulphur content of a wool fibre and its content of these proteins (Broad, Gillespie, and Reis 1970).

Similar proteins have been found in the hairs of animals from four families (Suidae 1; Camelidae 3; Cervidae 1; and Bovidae 7) of the order Artiodactyla in amounts which bear a linear relationship to the sulphur content of the hair. This suggests that sheep are not unique in this respect and that other Artiodactyla have a similar cysteine-regulated biosynthesis of certain of their hair proteins.

I. Introduction

It is a characteristic property of wool that it has a very variable composition, particularly in its content of cystine (Reis 1965). Recent studies have shown that the variable cystine content of wool is due entirely to variations in the content of proteins which are exceptionally rich in cystine (Broad, Gillespie, and Reis 1970). These proteins, in which about 30% of the residues are half-cystine, have been called ultra-high-sulphur (UHS) proteins to distinguish them from other high-sulphur proteins which contain less cystine and which are invariant in amount in wool (Broad and Gillespie 1970). There is a linear relation between the sulphur content of wool and its content of UHS proteins, the latter being present in amounts too small to be detected in wool with less than 3% sulphur content, but accounting for about 10% of the weight of wool of 4·4% sulphur content (Gillespie, Broad, and Reis 1969). The rate of synthesis of these proteins in the follicle is regulated by the availability for metabolism of sulphur-containing amino acids. High levels of sulphur-containing amino acids with consequent high levels of synthesis result from low wool growth rates or high intake rates of such amino acids or a combination of these factors (Gillespie and Reis 1966).

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It is of considerable interest to know whether sheep are unique in this respect or whether the follicles of other animals are also able to make UHS proteins in a cysteine-regulated biosynthetic process. A search has therefore been made in the hairs of 11 animals from four families of the order Artiodactyla for proteins which are similar in electrophoretic mobility to the UHS proteins of Merino wool. The amount of UHS protein found in each hair has been plotted against the sulphur content of each hair and the presence of a linear relationship between these parameters sought. The presence of a linear relation, similar to that already found for wool would provide evidence for the existence of cysteine-regulated biosynthetic processes in the follicles of Artiodactyla generally.

II. Materials and Methods

(a) *Preparation of High-sulphur Proteins and Fractions Derived from Them which are Enriched in UHS Proteins*

High-sulphur proteins in the *S*-carboxymethyl form were prepared by standard procedures from each of the hairs listed in Table 1 (Darskus and Gillespie 1971). These proteins were than fractionally precipitated with ammonium sulphate (1% protein concn., 0·1M sodium acetate, pH 6, 1·6M ammonium sulphate) and after stirring for 2 hr at room temperature the supernatant was recovered by centrifugation. The supernatant was titrated to pH 2·9 with HCl and the precipitated UHS proteins collected by centrifugation, dissolved in sodium acetate solution, dialysed, and freeze-dried.

(b) *Moving-boundary Electrophoresis*

Moving-boundary electrophoresis was carried out in a glycine–NaOH buffer of ionic strength 0·1 at pH 10·0 with protein concentrations between 1·1 and 1·3%. Under these conditions the UHS proteins of Merino wool are partially resolved as a fast-moving peak numbered D2 (Gillespie and Reis 1966). Using standard procedures (Broad, Gillespie, and Reis 1970), mobilities were calculated from the descending boundaries but the proportion of components was estimated from the relative areas under peaks in the better-resolved, ascending-boundary patterns.

(c) *Amino Acid Analysis*

The proteins were hydrolysed in 6M HCl–0·5 mM thioglycollic acid *in vacuo* ($\leqslant$ 0·01 torr) for 22 hr at 108°C. The HCl was removed by freeze-drying and the hydrolysate stored at −20°C until analysed. In spite of the precautions taken, difficulty was experienced in reproducibly getting good recoveries of *S*-carboxymethylcysteine (CMCys).

III. Results and Discussion

(a) *Detection of UHS Proteins in Mixed High-sulphur Proteins of Different Hairs*

Moving-boundary electrophoresis patterns of high-sulphur proteins of 11 hairs and wool are shown in Figure 1. Peaks, indicated by arrows, of comparable mobility to peak D2 of wool (Table 1) can be seen in the patterns of the proteins from sheep, Angora goat, cattle, musk ox, bison, guanaco, and antelope. The proportion of D2-like proteins in the latter preparations, excluding guanaco (which was not well resolved), has been plotted in Figure 3 against the sulphur content of the parent keratin. It can be seen that with increasing sulphur content of a keratin the proportion of its high-sulphur protein contributed by the UHS proteins increases. The relation is similar, at least qualitatively, to that found previously for wool (Broad, Gillespie, and Reis 1970).

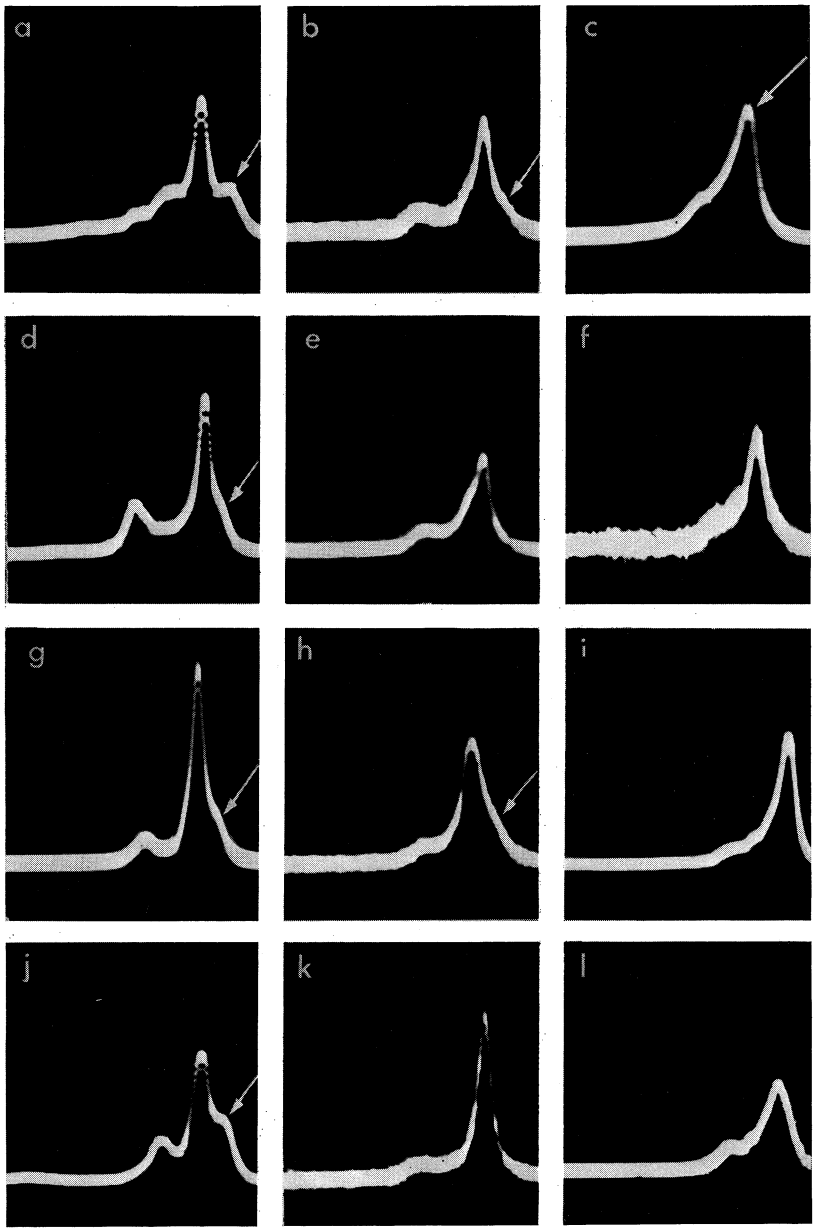

Fig. 1.—Moving-boundary electrophoresis patterns (ascending boundary) of unfractionated high-sulphur proteins from hairs and wool run at pH 10·0. (*a*) Merino sheep; (*b*) bison; (*c*) guanaco; (*d*) Angora goat; (*e*) yak; (*f*) camel; (*g*) cattle; (*h*) antelope; (*i*) llama; (*j*) musk ox; (*k*) deer; (*l*) pig. Arrows indicate the location of peaks with mobilities similar to peak D2 of Merino wool. Direction of movement from left to right. Protein run for *c.* 150 min at a voltage gradient of 7 V/cm.

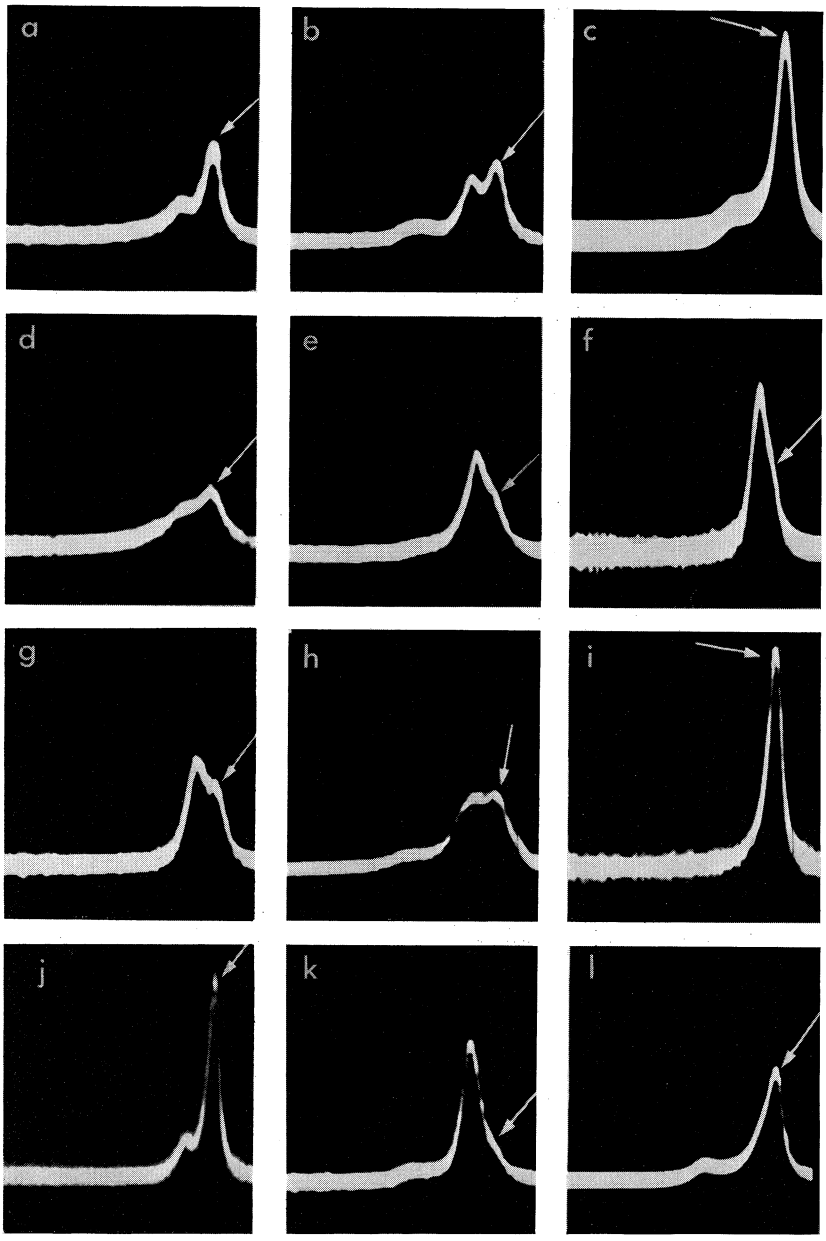

Fig. 2.—Moving-boundary electrophoresis patterns (ascending boundary) of ammonium sulphate supernatant fractions isolated from the high-sulphur proteins from hairs and wool run at pH 10·0. (*a*) Merino sheep; (*b*) bison; (*c*) guanaco; (*d*) Angora goat; (*e*) yak; (*f*) camel; (*g*) cattle; (*h*) antelope; (*i*) llama; (*j*) musk ox; (*k*) deer; (*l*) pig. Arrows indicate the location of peaks with mobilities similar to peak D2 of Merino wool. Direction of movement from left to right. Protein run for *c.* 150 min at a voltage gradient of 7 V/cm.

(*b*) *Detection of UHS Proteins in the Ammonium Sulphate Supernatant Fraction of High-sulphur Proteins*

There are a number of possible explanations for the non-detection of peak D2 in the patterns of the high-sulphur proteins from hair of yak, deer, camel, llama, and pig. The relevant proteins may be either absent or present in amounts too small to be detected. Alternatively they may be present in significant amounts but their resolution may be affected by the presence of proteins of lower mobility which are not present in the other keratins. Whatever the explanation, if UHS proteins are present in a keratin an improvement in resolution should result if, before electrophoresis, samples are enriched in UHS proteins by fractionation with ammonium sulphate.

Moving-boundary electrophoresis patterns of the proteins in such fractions, prepared from the high-sulphur proteins of the 11 hairs and wool, are shown in Figure 2. Each pattern shows a peak corresponding in mobility to peak D2 of wool (Table 1) and the proportion of protein within this peak has increased compared with

TABLE 1

LIST OF THE HAIRS USED IN THIS STUDY, THEIR ORIGIN AND SULPHUR CONTENTS, AND THE ELECTROPHORETIC MOBILITY OF THE FASTEST MOVING PEAK OF THEIR HIGH-SULPHUR PROTEINS

Hair	Source*	Sulphur content (%)	$10^5 \times$ Mobility ($cm^2\ V^{-1}\ sec^{-1}$)†	
			High-sulphur protein	Supernatant fraction
Merino sheep (*Ovis aries*)	A	3·65	9·2	9·3
Angora goat (*Capra hircus*)	B	3·54	9·3	9·4
Jersey cattle (*Bos taurus*)	A	3·94	9·3	9·5
Musk ox (*Ovibos moschatus*)	C	3·62	9·1	9·2
Bison (*Bison bison*)	D	3·60	9·3	9·3
Yak (*Poephagus grunniens*)	E	3·63	8·8‡	9·3
Situtunga antelope (*Limnotragus spekei*)	D	4·11	9·4	9·4
Red deer (*Cervus elephus*)	F	3·23	8·9‡	9·3
Guanaco (*Lama huanacos*)	D	4·45	9·3	9·2
Camel (*Camelus dromedarius*)	D	3·20	8·6‡	9·4
Llama (*Lama glama*)	G	4·20	8·6‡	9·5
Pig: large white (*Sus scrofa*)	H	4·14	8·8‡	9·2

* A, commercial source; for sources B–H see Section IV.

† Calculated from the descending boundary in electrophoretic runs at pH 10·0.

‡ Peak D2 not resolved.

the comparable patterns of Figure 1. In certain cases, e.g. pig, llama, guanaco, antelope, and musk ox, D2-like material appears to be the major constituent of the ammonium sulphate supernatant fraction. The original non-detection of peak D2 can be ascribed in the cases of yak, deer, and camel to insufficient of these proteins being present and in the others to imperfect resolution.

An alternative method was used to calculate the proportion of D2-like proteins in these high-sulphur proteins for which a direct estimate was not made in Section III(*a*). An illustrative example is given for the case of pig hair. D2-like proteins of pig hair comprise about 81% of the ammonium sulphate supernatant fraction (Fig. 2)

and, as this fraction comprises about 30% of the total high-sulphur protein, it can be calculated that D2-like proteins in this fibre represent about 24% of the high-sulphur protein. Similar calculations were made for the high-sulphur proteins of llama,

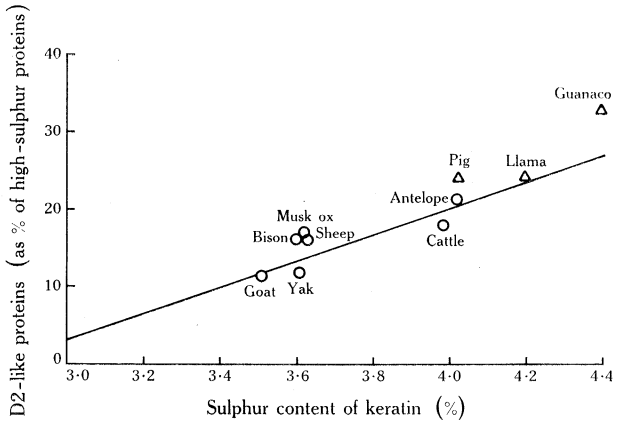

Fig. 3.—Relation between the sulphur content of a hair and the proportion of its high-sulphur protein mixture contributed by D2-like proteins. ○ Computed from the data of Figure 1. △ Computed from Figure 2 and other data. The solid line shows the relation which exists between these parameters for different samples of wool (Broad, Gillespie, and Reis 1970).

guanaco, and yak (Fig. 3) and gave results which were in reasonable agreement with those predicted from the sulphur content of the keratin, assuming a linear relationship.

TABLE 2

AMINO ACID COMPOSITIONS (AS RESIDUES PER 100 RESIDUES) OF AMMONIUM SULPHATE SUPERNATANT FRACTION OF THE HIGH-SULPHUR PROTEINS OF CERTAIN HAIRS AND A PURIFIED UHS PROTEIN FRACTION FROM MERINO WOOL

Amino acid	UHS proteins from wool	Guanaco	Antelope	Musk ox	Llama	Pig
Lys	0·89	0·46	0·76	0·85	0·45	0·77
His	1·29	0·37	0·70	0·96	0·50	0·91
Arg	6·90	6·94	7·11	5·32	6·88	6·70
CMCys	29·90	28·50	28·40	29·40	28·40	28·30
Asp	0·61	1·81	1·44	0·92	1·78	1·02
Thr	11·10	8·95	9·77	12·90	9·26	9·36
Ser	12·70	11·50	12·80	11·70	11·70	11·50
Glu	7·90	9·88	6·63	8·64	10·20	10·00
Pro	12·80	13·10	12·80	13·00	12·40	13·30
Gly	4·16	5·59	5·25	5·51	5·88	4·85
Ala	1·96	2·89	2·31	1·96	2·59	2·99
Val	4·34	4·54	4·54	3·21	3·85	4·94
Ile	1·74	2·05	2·97	1·90	1·88	1·62
Leu	1·38	1·69	2·42	1·34	2·24	1·95
Tyr	1·85	1·17	1·50	1·88	1·10	1·01
Phe	0·46	0·52	0·61	0·54	0·82	0·63

(c) *Amino Acid Composition of Certain Ammonium Sulphate Supernatant Fractions*

The amino acid compositions are given in Table 2 of those ammonium sulphate supernatant fractions which contained D2-like proteins as the major constituent

(Fig. 2). For comparison the composition is given of a preparation of UHS protein from Merino wool of similar state of purity. There is a strong resemblance between the fractions in that they all contain very small amounts of lysine, histidine, and phenylalanine and comparatively large amounts of proline, serine, threonine, CMCys, and usually glutamic acid. These five amino acids account for almost three-quarters of the residues in each protein. Some differences can be noted in the amount of CMCys in the different preparations but it is probable that these are all within experimental error.

The evidence presented here has shown that the hairs produced by certain representative species of the Artiodactyla drawn from the families Suidae, Camelidae, Cervidae, and Bovidae contain proteins which are similar to the UHS proteins of wool. They occur in amounts which are linearly related to the sulphur content of the hair. As this relation is very similar to that already found for wool (Broad, Gillespie, and Reis 1970), where it is a manifestation of a cysteine-regulated biosynthetic process, it seems reasonable to conclude tentatively that a similar process is operative in the follicles of other members of the Artiodactyla.

IV. Acknowledgments

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