CHROMATOGRAPHIC STUDIES ON THE AMINO ACID COMPOSITION OF KAGHANI WOOL FIBRES

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The amino acids of a representative sample of Kaghani wool fibres have been determined quantitatively by using ion-exchange and paper chromatographic techniques. The fineness or diameter of true, heterotypical and medullated types of wool has been measured and the relationships between the characteristics investigated.

Introduction

Kaghani wool is composed of three types of fibres, true, heterotypical and medullated. The gross chemical characteristics of Kaghani wool fibres have been reported earlier.[1] The present study is concerned with the amino-acid composition of these three types of fibres.

In the present investigation slight modifications of the shorter (0.9×50 cm) columns for the determination of the basic amino acids in wool have been introduced and also a set of solvent systems which would give a clearer separation of amino acids by buffered filter paper chromatography has been devised, particularly with regard to obtaining quantitative results of acceptable precision.

Experimental

The raw wool was scoured and then sorted as laid down in the literature.[2,3] It was dried at 105°C for 5 hr before analysis.

Ion-Exchange Column Chromatography.—The amino acids in wool were determined quantitatively by employing ion-exchange column chromatography procedures according to the methods recommended by Spackman, Stein and Moore.[4,5]

(i) *Preparation of the Ion-Exchange Columns.*—Three columns, one long (0.9×100 cm) and two short (0.9×50 cm), were prepared in this study. The washed resin (Amberlite CG-120 type II) was poured into the longer column until it settled to a height of 60–70 cm. The shorter column was packed with the resin until it settled to a height of 10–15 cm.

(ii) *Preparation of Wool Hydrolysates.*—For this purpose acid as well as alkali rehydrolysates were used. The acid hydrolysate was prepared by treating 25 mg wool with 0.5 ml 6N HCl in a Pyrex glass test tube. The hydrolysate was dried as mentioned in the literature[8] and was finally taken up in 1 ml pH 3.25 buffer.

(iii) *Operation of the Columns.*—The longer column was charged with the acid hydrolysate of wool by allowing the hydrolysate to enter the resin bed under gravity. The pH 3.25 buffer was then run in the column by pouring slowly through a small funnel touching the side of the column. Two-millilitre fractions were collected in a series of 5-ml graduated cylinders and each of these fractions was then transferred to a series of 5- or 10-ml conical flasks. The rate of flow was about 8 ml/hr. The shift in the buffer solution from pH 3.25 to 4.25 was made when the first six amino acids were collected (Figs. 1–3). Eight more amino acids were eluted using the pH 4.25 buffer solution.

For the determination of the basic amino acids the two shorter columns (0.9×50 cm) were used. First of all the alkaline hydrolysate was poured into one column for the determination of tryptophane. The hydrolysate solution was allowed to enter the resin bed under gravity. The pH 5 buffer was run and the effluent fractions collected in the usual manner. The shift of the buffer solution was made from pH 5 to 6.8 when the isolation of tryptophane was complete. The column was stopped and the volumes of effluents were noted. The second shorter column was then used by charging it with acid hydrolysate. Buffer pH was run in the column. The effluent fractions were collected in the usual manner. The shift of the buffer solution was made from pH 5 to pH 6.8 when all the tryptophane was recovered. The pH 6.8 buffer solution was run until histidine and lysine were eluted. The pH 6.5 buffer solution was then employed for the elution of ammonia and arginine. The pattern of the separation of various amino acids is shown graphically in Figs. 4–6. The rate of flow in the shorter column

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was about 12 ml/hr. In Figs. 4–6 the peak A comprises all the amino acids emerging before tyrosine.

(iv) *Procedure.*—The preparation of ninhydrin reagent and analysis of effluent fractions was carried out as described in the literature. Lange's photoelectric colorimeter model VI was employed for measuring colour. The standard curve for *l*-leucine is shown in Fig. 7.

One Dimensional Buffered Filter Paper Chromatography.—The preparation of the buffers, solvent systems, standard amino acid solutions, hydroly-

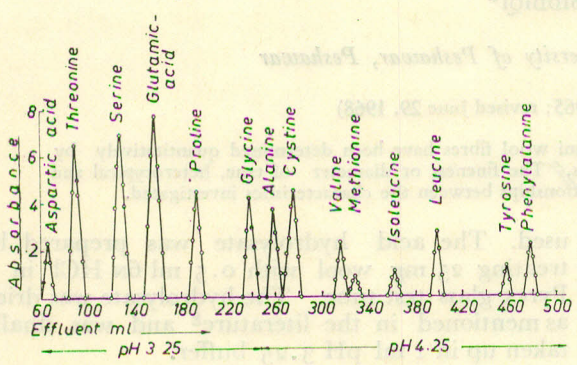

Fig. 1.—Separation of amino acids from the true type of Kaghani wool fibres.

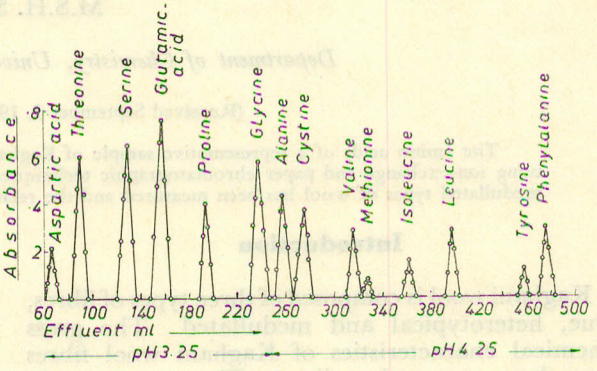

Fig. 2.—Separation of amino acids from the heterotypical type of Kaghani wool fibres.

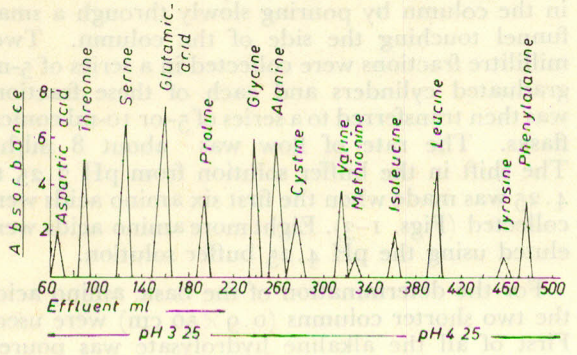

Fig. 3.—Separation of amino acids from the medullated type of Kaghani wool fibres.

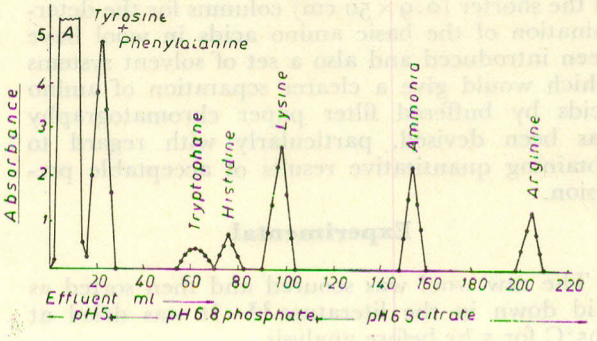

Fig. 4.—Separation of basic amino acids from the true type of Kaghani wool fibres.

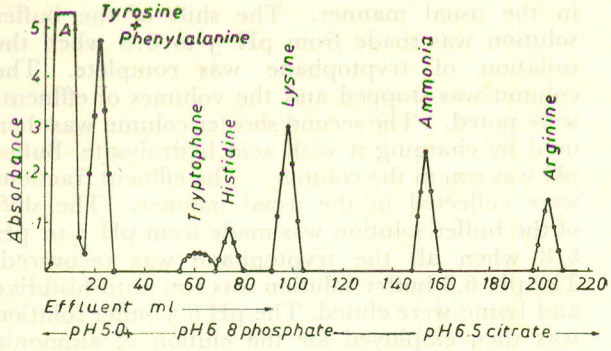

Fig. 5.—Separation of basic amino acids from the true type of Kaghani wool fibres.

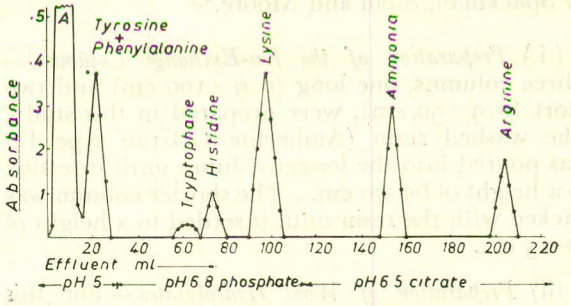

Fig. 6.—Separation of basie amino acids from the medullated type of Kaghani wool fibres.

sates and colour reagents were carried out as recommended in the literature.[6–8] An Elphor photoelectric densitometer was used to estimate maximum colour density of the spot.

Measurements of the Diameter of the Wool Fibre.— The diameter of true, heterotypical and medullated fibres was determined as reported earlier.[1,2]

Results

The amino acid composition of true, heterotypical, and medullated fibres are given in Table 2. The values represent the average of eight separate determinations. Figure 8 shows typical standard curves and Fig. 9 typical chromatograms.

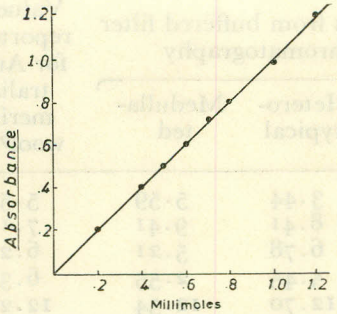

Fig. 7.—Standard curve for L-leucine.

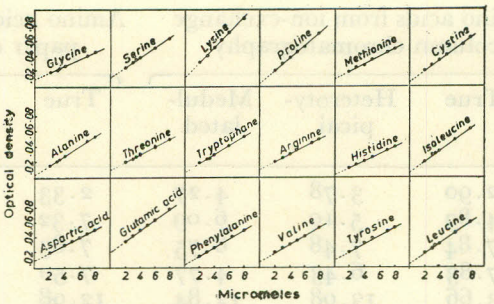

Fig. 8.—Typical standard curves for amino acid.

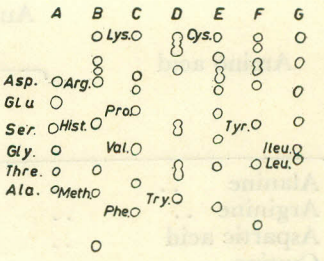

Fig. 9.—Typical amino acid chromatograms from wool. The letters A,B,C,D,E,F and G refer to the solvent system described in Table 1.

TABLE 1.—SOLVENT SYSTEMS AND CONDITIONS EMPLOYED IN THE QUANTITATIVE DETERMINATIONS OF AMINO ACIDS BY THE ONE-DIMENSIONAL BUFFERED FILTER PAPER CHROMATOGRAPHY.

Solvent sytems	Composition of solvent	Millimolarity of standard amino acid solution	Wool in the hydrolysate solutions (mg/ml)	pH of hydrolysate	Running time (hr)	Amino acid determined
A	96 ml phenol*, 4 ml 2-butanol, 100 ml buffer (pH 12.0)	2,3,3.5, 4,6,8	2.5,5	6.5–7.5	20–24	Aspartic acid, glutamic acid, serine, glycine, threonine, alanine
B	96 ml *m*-cresol*, 4 ml 2-butanol, 100 ml buffer (pH 8.4)	2,3,4,5, 7,8	12.5 25	7.8	40	Arginine, histidine, methionine
C	98 ml 2,4–lutidine*, 2 ml phenol*, 100 ml buffer (pH 6.2)	2,3,4,5, 6,8	2.5 5	6.5–7.5	36	Lysine, proline, valine and phenylalanine
D	98 ml 2,4,6–collidine*, 2 ml phenol*, 100 ml buffer (pH 9.)	2,3,3.5, 4,6,8	25	7.0–7.5	36	Tryptophane
E	100 ml phenol*, 100 ml buffer pH 1	2,3,4,5, 7,8	12.5 25	pH not adjusted	24	Cystine
F	99 ml *p*-cresol*, 1 ml phenol*, 100 ml buffer (pH 9.0)	2,3,4,5, 7,8	2.5 5	6.5–7.5	36	Tyrosine
G	50 ml 2-methyl-2-butanol*, 50 ml methyl ethyl ketone, 0.5 ml 90% formic acid 10 ml water,	2,3.3.5,4, 6,8	2.5 5	6.5–7.5	18	Leucine, isoleucine

*Distilled.

Discussion

By employing the combined techniques of modified ion-exchange (Amberlite CG-120 type II) column and buffered filter paper chromatography, a total of eighteen amino acids and also ammonia have been determined in the wool sample. In Table 2 the percentages of these amino acids as determined by these two different techniques are listed, and for the sake of comparison, the values of these amino acids as reported in the literature for Australian merino wool. Ammonia contents as observed earlier[10] have been given at the end of the table. The results

TABLE 2.—THE PERCENTAGE COMPOSITION OF AMINO ACIDS IN KAGHANI WOOL FIBRES (Oven Dried).

Amino acid	Amino acids from ion-exchange column chromatography			Amino acids from buffered filter paper chromatography			Values reported for Australian merino wool[9,10]
	True	Heterotypical	Medullated	True	Heterotypical	Medullated	
Alanine	2.90	3.78	4.27	2.33	3.44	5.59	5.5
Arginine	4.89	5.10	6.09	7.32	8.41	9.41	7.1
Aspartic acid ..	7.84	7.48	6.75	7.83	6.78	5.21	6.2
Cystine	7.83	6.43	4.77	7.50	5.45	2.55	6.3
Glutamic acid ..	13.66	13.08	12.84	12.98	12.70	12.54	12.2
Glycine ..	5.72	7.75	8.43	5.76	7.41	8.16	8.8
Histidine ..	0.65	0.78	0.98	0.80	0.87	1.14	0.8
Isoleucine ..	2.76	3.27	3.36	4.11	4.43	4.55	3.1
Leucine	5.21	5.66	7.29	7.32	8.43	9.38	7.6
Lysine	3.86	4.83	5.43	2.54	3.15	3.40	2.6
Methionine ..	0.66	0.56	0.37	0.54	0.44	0.36	0.5
Phenylalanine ..	3.19	4.11	4.79	3.05	3.35	3.60	2.8
Proline	7.94	6.61	5.82	7.05	6.56	5.50	7.5
Serine	11.02	10.46	10.34	10.82	10.40	10.16	11.5
Threonine ..	7.48	6.74	5.72	7.88	6.56	5.51	7.0
Tryptophane ..	1.39	1.28	0.90	1.16	0.93	0.64	0.9
Tyrosine	6.33	4.54	3.49	4.60	3.55	3.02	4.0
Valine	4.88	5.75	6.28	5.48	6.36	8.34	5.6
Ammonia N ..	0.80	0.91	1.18	—	—	—	1.18
Total ..	99.01	99.12	99.10	99.07	99.22	99.06	100+1.18 =101.18

TABLE 3.—THE FINENESS OF THE FIBRE AND THE RELATIVE DISTRIBUTION OF AMINO ACIDS IN WOOL.

Type	Fineness μ	Amino acids in higher proportions	Amino acids in lower proportions
True	24.6	Aspartic acid, cystine, glutamic acid, methionine, proline, serine, threonine, tryptophane, tyrosine	Alanine, arginine, glycine, histidine, isoleucine, leucine, phenylalanine, valine and ammonia
Heterotypical	37.2	Intermediate values	Intermediate values
Medullated	54.9	Alanine, arginine, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, valine and ammonia	Aspartic acid, cystine, glutamic acid, methionine, proline, serine, threonine, tryptophane and tyrosine

obtained by the two techniques do not agree in a few cases such as those for arginine and leucine. However, in the majority of the cases, the results agree very well. In the case of arginine a possible uneven splitting of guanido group during hydrolysis, which was carried out for the ion-exchange column chromatography, could have occurred. Also the relative order of the average values for the whole sample is in good agreement with the Australian report.

Table 3 shows that as the fineness of wool increases the amino acids, aspartic acid, cystine, glutamic acid, methionine, proline, serine, threonine, tryptophane and tyrosine appear to be present in excess compared with the remaining amino acids. With increases in coarseness (larger diameter of the fibres) the content of alanine, arginine, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, valine and ammonia N appear to be relatively higher. This interesting overall correlation between the fineness and the amino acid composition of wool fibres is in agreement with the earlier findings that the medulla present in coarse fibres is poor in a number of amino acids, especially cystine.[11] Thus the causal basis for the correlation between fineness and the amino acids appears largely to be due to medulla, although the possibility of variations in the composition and structure of the wool keratin cannot be precluded.

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