

THE COMPOSITION OF KERATINS

THE AMINO ACID COMPOSITION OF HAIR, WOOL, HORN, AND OTHER EUKERATINS

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Previous investigations on the amino acid composition of keratins have indicated that this class of proteins may be subdivided into two groups: eukeratins and pseudokeratins (1). Both are characterized by their insolubility in aqueous and organic solvents and by a relatively high degree of resistance towards enzymatic hydrolysis, but the eukeratins appear to be differentiated by a constant proportion of histidine, lysine, and arginine which are present in the molecular ratios of approximately 1:4:12. The constancy of this ratio in proteins of widely different biological origin led to the view (*cf.* (2)) that the basic amino acids may be of some special importance in the development of tissue proteins. It is possible that these three amino acids have a directive influence on protein synthesis in the organism. Thus the recognized similarities in the gross structure of the eukeratins might depend upon the proportions of histidine, lysine, and arginine, while the variations in the individual keratins are the result of changes in the amounts of the non-basic amino acids.

In contrast to the relative constancy of arginine, histidine, and lysine, the variability in the amount of cystine present in eukeratins was early demonstrated by Buchtala (3) who isolated 14 to 15 per cent of cystine from human hair, 8 per cent from horse hair, 3 per cent from horse's hoofs, etc. Comparative biochemical investigations on the amino acid composition of eukeratins are lacking and it was deemed of interest to extend our knowledge of these proteins.

EXPERIMENTAL

The preparation of the tissues for analysis was carried out by the methods described previously (4) except that the animal hairs were not subjected to digestion by pepsin and trypsin.

All chemical analyses were repeated two or more times. Total nitrogen was determined by the macro-Kjeldahl method with Cu and Se as catalysts and sulfur by the Parr bomb method. The basic amino acids were isolated from 2.5 gm. of protein by silver precipitation (5) and the purity of the resulting flavianates, nitranilates, and picrates was checked by analyses and melting points. Cystine was determined by Miller and du Vigneaud's procedure (6); tyrosine and tryptophane were estimated by a modification of Folin's method (*cf.* (5)). Phenylalanine was determined by the dinitrobenzene procedure (5) and glycine was isolated by a modification of the method of Bergmann and Fox (*cf.* (5)).

It should be recognized that the methods used for the estimation of the eight amino acids are not of equal precision. The advantages and drawbacks of the above procedures have been discussed recently (5). However, it is the writer's opinion that all are of value in a comparative investigation although they may not be entirely reliable as a means of estimating absolute amounts of amino acids.

Results

The analytical data are summarized in Table I which also includes determinations made by Calverly (7), Hess (8), and Block and Lewis (9). The earlier results of the author have been confirmed in practically all instances. In each case, however, the more recent analysis is reported.

Arginine, Histidine, and Lysine—Of the eight amino acids investigated, arginine, which can be determined with a relatively high degree of accuracy, is the most constant and was, therefore, chosen as the base-line for the calculation of the molecular ratios. The outstanding fact apparent from Table I is the relative constancy of histidine, lysine, and arginine to each other. These three amino acids are in the ratio 1:4:12 or 1:5:12 in all cases, except for goat hair which yields somewhat more lysine.

TABLE I
Amino Acid Composition of Some Eukeratins

| Protein | Nitrogen per cent | Sulfur per cent | Histidine per cent | Lysine per cent | Arginine per cent | Cysteine per cent | Tyrosine per cent | Tryptophane per cent | Phenylalanine per cent | Glycine per cent | Molecular ratios | | | | | | | |
|------------------------|----------------------|--------------------|-----------------------|--------------------|----------------------|----------------------|----------------------|-------------------------|---------------------------|---------------------|------------------|-----------|----------|--------|----------|--------|----------|----------|
| | | | | | | | | | | | Glutamine | Glutamate | Arginine | Lysine | Arginine | Lysine | Cysteine | Tyrosine |
| Human hair | 15.4 | 5.0 | 0.6 | 2.5 | 8.0 | 15.5 | 3.0 | 0.7 | 2.6 | 4.3 | 1 | 4 | 12 | 34 | 4 | 1 | 4 | 15 |
| Chimpanzee hair (5)† | 16.7 | 4.3 | 0.6 | 2.0 | 8.1 | 15.5 | 3.3 | 1.4 | 1 | 4 | 12 | 33 | 5 | 2 | 7 | 22 | 7 | 38 |
| Goat hair | 16.2 | 3.1 | 0.7 | 3.2 | 8.1 | 8.9 | 3.0 | 0.9 | 4.6 | 6.3 | 1 | 6 | 12 | 19 | 4 | 1 | 7 | 22 |
| Cow " (5) | 15.3 | 3.7 | 0.7 | 2.0 | 7.5 | 13.4 | 3.3 | 1.4 | 3.9 | 10.3 | 1 | 4 | 12 | 27 | 5 | 2 | 7 | 38 |
| Lamb wool | 15.4 | 3.6 | 0.7 | 2.5 | 8.7 | 13.1 | 4.5 | 0.7 | 4.0 | 6.5 | 1 | 4 | 12 | 26 | 6 | 1 | 6 | 21 |
| Camel " | 15.1 | 3.1 | 0.6 | 2.7 | 8.6 | 11.0 | 3.1 | 0.8 | 4.1 | 9.2 | 1 | 4 | 12 | 22 | 4 | 1 | 6 | 30 |
| Cattle horn | 16.1 | 2.6 | 0.6 | 2.4 | 8.6 | 8.2 | 3.7 | 0.7 | 4.0 | 9.8 | 1 | 4 | 12 | 17 | 5 | 1 | 6 | 32 |
| Rhinoceros horn | 15.6 | 2.3 | 0.6 | 2.6 | 8.2 | 8.7 | 8.6 | 1.7 | 5.0 | 7.4 | 1 | 5 | 12 | 18 | 12 | 2 | 8 | 25 |
| Finger nails (7) | 14.9 | 3.8 | 0.5 | 2.6 | 8.5 | 12.0 | 3.0 | 1.1 | 2.5 | 1 | 1 | 4 | 12 | 25 | 4 | 1 | 4 | 1 |
| Porcupine quills | 15.8 | 3.0 | 0.6 | 2.6 | 7.6 | 9.4 | 3.3 | 0.9 | 3.6 | 5.7 | 1 | 5 | 12 | 18 | 5 | 1 | 6 | 21 |
| Echidna quills | 15.2 | 3.8 | 0.5 | 1.8 | 6.8 | 11.9 | 9.1 | 2.2 | 6.8 | 1 | 4 | 12 | 30 | 15 | 3 | 13 | 13 | |
| Hen feathers | 15.5 | 2.3 | 0.3 | 1.6 | 6.0 | 6.8 | 2.2 | 0.7 | 5.3 | 9.5 | 1 | 4 | 12 | 17 | 4 | 1 | 11 | 44 |
| Snake skin | 15.2 | 2.2 | 0.4 | 1.9 | 5.4 | 6.6 | 5.2 | 0.9 | 3.9 | 13.1 | 1 | 5 | 12 | 21 | 11 | 2 | 9 | 67 |
| Egg shell membrane (4) | 16.6 | 3.8 | 0.9 | 3.7 | 9.9 | 12.7 | 2.5 | 2.6 | 2.0 | 1 | 5 | 12 | 22 | 3 | 3 | 3 | 3 | |

* The cysteine values have been calculated as cysteine.

† Bibliographic reference number.

This increased amount of lysine does not appear to be the result of an analytical error but probably represents an actual difference in the composition of this tissue protein. The relatively small amount of histidine isolated from feathers may be due to analytical difficulties.

Sulfur and Cystine—In confirmation of the older literature, the cystine (3) and sulfur (10) contents of the eukeratins are variable. Primate hair (human and chimpanzee) contains more cystine than any other keratin (*cf.* (9)). The ratio of cystine to arginine in cow hair and echidna spines is almost as great as in primate hair, although the actual amounts of cystine in these proteins are less. Goat hair, cattle horn, rhinoceros horn, porcupine quills, and hen feathers yield comparatively small amounts of cystine. It is known that health and disease (8), as well as diet, can influence the cystine and sulfur content of hair (9, 11, 12), but the old claims of von Bibra (10) and of Fourcroy and Vauquelin (13) that red, blond, and white hair contains more sulfur than black hair are probably erroneous (*cf.* (9)).

Tyrosine and Tryptophane—The tyrosine contents of the eukeratins reported in this investigation are about 3 per cent, except in the cases of rhinoceros horn and echidna spines which yield approximately 9 per cent of this amino acid and snake skin which contains over 5 per cent. Rhinoceros horn and echidna spines are remarkably hard substances. Egg shell membrane is the only protein reported in which the content of tryptophane equals that of tyrosine. This observation of Calverley (7) has been confirmed by the author.

Phenylalanine—Variations in the amount of phenylalanine present in several eukeratins were indicated by the earlier investigations of Fischer and Dörpinghaus (14), Abderhalden and Voitinnovici (15), and Lissizin (16). The analytical results reported in Table I, all of which are those of the writer, confirm these impressions, for the values range from 2.0 per cent of phenylalanine in egg shell membrane to 6.8 per cent in echidna spines. The values of 2.5 per cent in human finger nails and of 2.6 per cent in human hair are of interest.

Glycine—The eukeratins are relatively rich in glycine. The amounts present in hen feathers and snake skin are especially high. The isolation of over 13 per cent of glycine from the latter

protein indicates some similarity to the *pseudokeratins*, gorgonin and spongin, which contain approximately 14 per cent of glycine (4). Buchtala (3) claimed that the amino acid composition of human hair and sheep wool differs principally in that the former has a considerably greater proportion of glycine. Our results do not support this opinion, for when the same method of determination was employed, human hair yielded 4.3 per cent, while wool contained approximately 6.5 per cent of glycine.

SUMMARY

1. The amounts of nitrogen, sulfur, histidine, lysine, arginine, cystine, tyrosine, tryptophane, phenylalanine, and glycine were determined in human hair, goat hair, lamb wool, camel wool, cattle horn, rhinoceros horn, finger nails, porcupine quills, echidna spines, hen feathers, and snake skin.

2. In confirmation of earlier findings, the molecular ratios of histidine to lysine to arginine in these proteins remained relatively constant. In contrast, decided variations in the actual and relative amounts of cystine, tyrosine, tryptophane, phenylalanine, and glycine were noted.

3. Similarities and differences in the amino acid composition of fourteen eukeratins are given in Table I. Some salient points are (a) the relatively high content of sulfur and cystine in human and chimpanzee hair, of phenylalanine in echidna quills, of glycine in feathers and snake skin, of tyrosine in echidna spines and rhinoceros horn, and of tryptophane in egg shell membrane and echidna spines; (b) the relatively small amounts of cystine in hen feathers and cattle horn, of tyrosine in egg shell membrane, of phenylalanine in egg shell membrane and finger nails, and of glycine in human hair.

4. The data given in this paper are interpreted as lending support to previous suggestions concerning the special function of histidine, arginine, and lysine, particularly the first two, both in the genetic development of tissue proteins and in imparting to a protein its group or general characteristics. Variations in the other amino acids are probably responsible for specific individual differences. The stress laid on the constancy of the basic amino acids should not be understood as implying that these three acids alone have a unique importance in protein structure, although

they may have in protein synthesis. The variability of the other amino acids and especially the changes in the cystine content of nails and hair in health and disease are of great significance. Further investigations on the comparative composition of the eukeratins are contemplated, for it is this way alone which will show whether or not amino acids, other than the basic amino acids, are present in the eukeratins in definite proportions.

Addendum—The discrepancies between the values reported for total sulfur and cystine in gorgonin and spongin (4) are due to the failure to correct for the large amount of ash. Similar analytical results were obtained in 1931 as shown by the data given in Column 2 of Table I (4). When the correction for ash is made, the pseudokeratins, like the eukeratins, appear to contain the greatest proportion, if not all, of their organic sulfur in the form of cystine sulfur.

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