ウマの体毛と蹄のアミノ酸組成に関する研究

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Studies on the Amino Acid Compositions of the Equine Body Hair and the Hoof

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ABSTRACT. The amino acid analysis was carried out in order to clarify the differences in keratin between the body hair and the hoof of horse. A distinctive mark was recognized in the content of amino acid residues between the body hair and the hoof. The contents of Asx, Gly, Ala, Ile, Leu, Tyr and Phe were higher and those of Thr, Ser, Pro and Cys were lower in the hoof than in the hair for all samples examined. Of these amino acids, the difference in the content of Cys was most remarkable, implying that Cys plays an important role in determining the kinds of keratin. Moreover, a comparative analysis of specific amino acids between the body hair and the hoof of 7 horse races implied that the blood relationship might be partly reflected in the amino acid composition. Although several components were fractionated by gel filtration in the keratin solubilized with performic acid, only a wide "smear region" was visualized in the range of molecular weight from 100 kD to 30 kD with SDS gel electrophoresis.—KEY WORDS: horse hair, horse hoof, horse race, keratin.

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Keratin is widely present in the vertebrate skin, epidermis, feather, hair, nail, claw, hoof, and horn. Insoluble property of keratin in various inorganic and organic solvents prevented its analysis by biochemical methods. However, the solubilization of keratin has realized by the cleavage of disulphide bonds, by reduction [7, 9, 11] or oxidation [9]. While the feather keratin is composed of many electrophoretically different components whose molecular weight are around 10,400 daltons [10, 13], the hair and the horn keratins have three diagnostic components, designated as low-sulphur, high-sulphur, and high-glycine and high-tyrosine keratins [3, 4, 23]. The complete amino acid sequence of a feather keratin has been confirmed by direct protein sequencing [19, 20] and sequencing of the DNA of keratin genes [2]. Recently, Gregg *et al.* [8] described the complete amino acid sequence of the embryonic chick feather. However, with regard to the equine body hair and the hoof, the amino acid composition [12, 17] and lipid composition [18] of the unfractionated keratin have only been known in relation to laminitis. Recently, Samata [21, 22] has carried out the biochemical analysis of keratin in equine body hair and hoof, in which amino acid analysis and fractionation by gel chromatography were attempted.

In this study, we analysed the amino acid composition of the body hair and the hoof of 7 equine races. Basing on the comparison in the amino acid compositions, we have tried to clarify the differences in the keratin between the body hair and the hoof among the races.

MATERIALS AND METHODS

Age, body colour and immediate origins of 7 horse races examined are shown in Table 1. Samples used for the analysis contained 3 heads of male horses of each race.

Samples of the body hair were collected from several parts of horse trunks in sum-

Table 1. Horse samples examined

Breed	Sex	Age (Year)	Body colour	Main origin
Throughbred	M	4–6	Bay Chestnut	Wrangler Lunch, Nagano
Anglo-Arab	M	5–6	Bay Chestnut	〃
Arab	M	4 and 12	Chestnut Pinto	Baji Koen, Tokyo
Quarter horse	M	5–6	Chestnut Dun	Wrangler Lunch, Nagano
Pony	M	6–7	Pinto	Kodomono Kuni, Yokohama
Kiso Pony	F	8–9	Bay	Iwana En, Nagano
Percheron	M	4–5	Bay Chestnut	Baji Koen, Tokyo

mer time. The root of the body hair was excluded prior to the analysis. Samples of the hoof were taken from the outer layer (stratum externum) of the hoof wall of fore and hind legs. Samples of the human hair and the nail were collected from healthy 3 students. The sample was washed repeatedly with ethanol and distilled water and then dried. Each sample (1 mg) was hydrolysed in 2m*l* of 6N HCl in a sealed, evacuated tube at 110°C for 24 hr. Hydrolysates were concentrated on the rotary evaporator and analysed on an ATTO MLC-703S automatic amino acid analyser (ATTO Co., Japan).

The unfractionated keratin was treated with performic acid (90% formic acid: hydrogen peroxide/2:1) at 4°C for 6 hr, followed by being stirred in dilute NH_4OH (pH10.0) at 4°C for 2 hr.

The solubilized keratin was loaded on the 100×1.5cm, Bio-Gel A 1.5m column (Bio-Rad Lab. Japan) preequilibrated with 0.01M NH_4OH, pH8.4.

SDS-polyacrylamide gel electrophoresis was conducted, according to the procedure of Anderson [1], followed by silver staining with the kit (Wako Pure Chemical Industries, LTD., Japan).

RESULTS

Amino acid composition of the body hair and the hoof in 7 races are shown in Tables 2 and 3 respectively. Values of amino acid residues are the averages of those obtained from 3 heads of horses of each race. In addition, Table 4 shows the ratios of each amino acid residue in the hoof to the body hair. As clearly shown in Table 4, 8 kinds of amino acids show the higher ratios and 4 kinds of amino acids show the lower ratios in the hoof. In these kinds of amino acids, the contents of Asx, Gly, Ala, Ile, Leu, Tyr, Phe and Lys are higher in the hoof and those of Thr, Ser, Pro and Cys are higher in the body hair. In particular, the content of Cys in the body hair is two- or three-fold that in the hoof. The contents of Pro, Tyr and Lys show relatively large variations between the hoof and the body hair.

According to the results shown in Table 2, some characteristic profiles are demonstrated on the levels of specific amino acids in the body hair among 7 races examined.

Characteristic features in amino acid composition are the high level of Ser, the low level of Ala, the high ratios of Ser/Thr and Gly/Ala for the body hair of Thoroughbred, Anglo-Arab and Arab. A slight difference is

Table 2. Amino acid compositions of the horse body hair (molar %)

	Race[a)]						
	Th	An	Ar	Qu	Po	Ki	Pe
Asx	6.37	6.51	6.45	5.85	6.58	6.99	7.10
Thr	5.58	5.58	5.75	5.23	6.21	6.36	6.88
Ser	11.57	11.55	10.93	10.72	9.21	9.72	9.10
Glx	12.60	12.76	11.96	12.45	11.51	12.55	11.55
Pro	8.54	8.20	8.51	8.21	8.47	8.53	8.33
Gly	7.60	7.85	7.10	7.64	6.49	6.10	6.08
Ala	5.59	5.80	5.85	6.55	5.86	6.30	5.95
Cys	7.43	6.96	7.26	7.00	7.58	7.19	7.57
Val	6.21	6.64	6.55	6.42	6.43	6.13	6.44
Met	0.40	0.22	0.17	0.19	0.28	0.35	0.15
Ile	3.65	3.91	4.10	3.84	4.05	4.14	4.22
Leu	7.78	7.79	7.91	8.58	8.24	8.30	7.92
Tyr	2.25	2.10	1.80	1.95	2.11	1.97	1.98
Phe	2.77	2.65	2.54	2.75	2.84	2.51	2.59
Lys	3.00	2.99	3.11	3.01	3.25	3.00	3.33
His	1.02	1.10	1.59	1.08	1.34	1.01	1.21
Arg	7.62	7.39	8.42	8.53	9.54	8.85	9.60
Glx/Asx	1.98	2.04	1.85	2.13	1.75	1.80	1.63
Ser/Thr	2.07	2.07	1.90	2.05	1.48	1.53	1.32
Gly/Ala	1.36	1.35	1.21	1.17	1.11	0.97	1.02
Phe/Tyr	1.23	1.26	1.41	1.41	1.35	1.27	1.31

a) Th: Throughbred, An: Anglo-Arab, Ar: Arab, Qu: Quarter-horse, Po: Pony, Ki: Kiso Pony, Pe: Percheron

noted in the contents of Cys, Glx, Gly, Arg and in the ratio of Ser/Thr among 3 races.

Some other races have characteristic composition with the high level of Thr and the low level of Gly, combined with the low ratios of Ser/Thr, Gly/Ala and Glx/Asx (Pony, Kiso Pony and Percheron).

The characteristic composition of the high levels of Ala and Leu, and the low levels of Asx and Thr is recognized in the body hair of Quarterhorse.

Table 3 shows some characteristic profiles in the hoof on the levels of specific amino acids.

Characteristic profiles are the high level of Ser, the low level of Cys, and the high ratio of Ser/Thr for the hoof of Thoroughbred, Anglo-Arab and Arab. There are some minor differences in the amounts of Cys, Gly and Ala, and in the ratio of Glx/Asx.

Kiso Pony and Percheron has chracteristic composition with the high level of Asx, the low levels of Gly and aromatic amino acids, combined with the high ratio of Phe/Tyr and the low ratio of Gly/Ala.

The characteristic composition of the high level of Glx and the low levels of Asx and Ser, combined with the high ratio of Glx/Asx and the low ratio of Ser/Thr is recognized in the hoof of Pony.

A sample of Quarterhorse has the high level of Gly and the low levels of Glx, Thr and acidic amino acids.

Amino acid composition of the human hair and the nail is shown in Table 5. The data demonstrates clearly that the variations in the values for each amino acid correspond to those of the equine body hair and the hoof. The higher levels of Cys and Pro, and

Table 3. Amino acid compositions of the horse body hoof (molar %)

	Race[a)]						
	Th	An	Ar	Qu	Po	Ki	Pe
Asx	7.13	7.24	7.65	7.05	6.94	7.70	7.94
Thr	5.19	5.21	5.45	5.70	5.50	5.69	5.66
Ser	9.42	9.48	9.60	8.75	8.50	9.21	9.44
Glx	12.88	12.66	12.29	10.91	13.91	13.03	13.45
Pro	5.76	5.65	5.95	7.21	5.26	6.64	5.17
Gly	9.61	9.44	8.88	9.96	8.57	7.00	6.96
Ala	6.82	7.03	6.46	6.98	6.71	7.48	6.59
Cys	3.13	3.54	3.57	3.67	3.70	3.71	3.77
Val	6.03	6.00	6.22	6.00	6.28	6.09	6.55
Met	0.30	0.33	0.49	0.58	0.68	0.39	0.45
Ile	4.04	4.14	4.44	4.33	4.19	4.46	4.52
Leu	9.72	9.21	9.23	9.57	9.61	9.96	9.78
Tyr	3.40	3.18	3.00	3.10	3.55	2.29	2.20
Phe	3.35	3.20	2.98	3.16	3.10	2.58	2.77
Lys	3.96	4.09	4.31	3.99	4.01	4.11	4.33
His	1.16	1.26	1.20	1.22	1.30	1.20	1.26
Arg	8.11	8.35	8.47	8.45	8.19	8.46	9.06
Glx/Asx	1.81	1.75	1.65	1.55	2.00	1.69	1.69
Ser/Thr	1.82	1.82	1.76	1.73	1.55	1.62	1.67
Gly/Ala	1.41	1.34	1.37	1.43	1.28	0.94	1.06
Phe/Tyr	0.99	1.01	0.99	1.02	0.87	1.13	1.26

a) See the foot-note on Table 2

Table 4. Ratios of each amino acid residues in the hoof to the body hair of horse

	Race[a)]						
	Th	An	Ar	Qu	Po	Ki	Pe
Asx	1.12	1.11	1.16	1.21	1.05	1.10	1.12
Thr	0.93	0.93	0.95	0.97	0.89	0.89	0.82
Ser	0.81	0.82	0.88	0.91	0.92	0.73	0.82
Glx	1.01	0.95	0.99	0.88	1.21	1.04	1.16
Pro	0.67	0.69	0.70	0.88	0.62	0.78	0.62
Gly	1.26	1.20	1.25	1.30	1.32	1.15	1.14
Ala	1.22	1.21	1.10	1.07	1.15	1.19	1.10
Cys	0.42	0.58	0.49	0.52	0.49	0.52	0.50
Val	0.97	0.90	0.95	0.93	0.98	0.99	1.02
Met	0.75	0.73	2.88	3.05	2.43	1.11	3.00
Ile	1.11	1.03	1.08	1.13	1.03	1.08	1.07
Leu	1.25	1.18	1.17	1.12	1.17	1.20	1.23
Tyr	1.51	1.51	1.67	1.59	1.68	1.16	1.11
Phe	1.21	1.21	1.17	1.15	1.09	1.03	1.07
Lys	1.32	1.37	1.39	1.33	1.23	1.37	1.33
His	1.14	1.05	0.75	1.13	0.97	1.19	1.04
Arg	1.06	1.13	1.01	0.99	0.86	0.96	0.94

a) See the foot-note on Table 2

Table 5. Amino acid compositions of the human hair and nail (molar %)

	hair	nail
Asx	5.92	7.46
Thr	6.50	5.74
Ser	10.49	9.04
Glx	13.00	12.64
Pro	10.19	7.24
Gly	6.77	8.36
Ala	4.03	4.30
Cys	9.32	5.44
Val	5.14	5.35
Met	0.27	0.81
Ile	3.28	3.62
Leu	8.19	10.21
Tyr	2.15	3.35
Phe	2.24	3.04
Lys	3.41	4.68
His	1.13	1.24
Arg	7.95	7.50

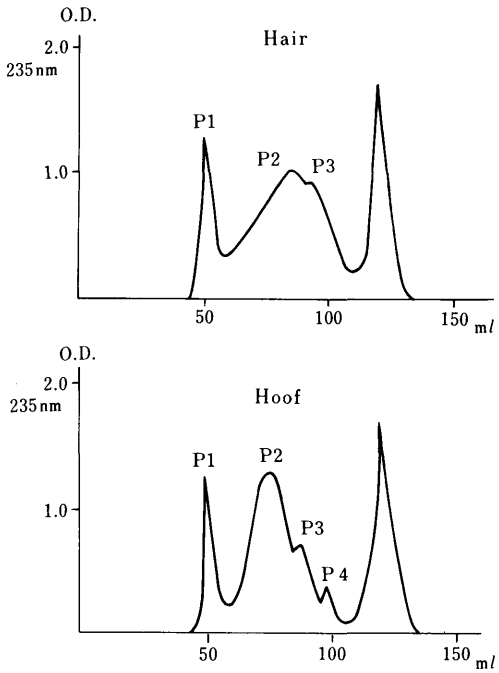

Fig. 1. Bio-Gel A 1.5m chromatogram of performic acid-oxidized components in the horse body hair and the hoof. Elution was carried out with 0.01 M NH_4HCO_3 (pH8.4).

the lower levels of Asx, Glx and Leu, in the hair than in the nail are observed in both cases.

Fig. 1 shows an elution diagram of the solubilized keratin in the horse hair and the hoof on Bio-Gel A 1.5m. Three components are fractionated at the position of Vo (P1), approx. 60–80 kD (P2) and 30–50 kD (P3) for the body hair and 4 components at the position of Vo (P1), approx. 100–150 kD (P2), 50–70 kD (P3) and 20–30 kD (P4) for the hoof. In addition, a large number of small peptides are eluted at the position smaller than 2 kD for both hair and hoof.

DISCUSSION

All the samples analysed were found to contain the high levels of Cys, Ser, Glx, Pro, Gly and Arg and the low levels of Met, Tyr, Phe and His. Thus, both the equine body hair and the hoof are most probably composed of keratin.

Comparison of amino acid values between the body hair and the hoof reveals each distinctive marks in amino acid composition. As more than 70% of the total amount of amino acids shows variations between the body hair and the hoof, regardless of the races, the different structure of the keratin may be considered in the body hair and the hoof. When amino acid composition of the hair (or the wool) is compared with the horny keratin from several mammalian species, there is the overall similarity, although significant differences are observed for a few amino acids. Compared with the hair (or the wool), the horny keratin generally contains more Gly, Leu, Tyr and Phe but less Cys, Pro, Thr and Ser [5, 6, 16, 17]. The level of Cys, in the body hair is two- or three-fold higher than that in the hoof may suggest a significant structural difference among different kind of keratins. As shown in Table 5, the content of Cys in the human hair was 60% greater than that in the nail. In addition, similar results have been described for the hair and the horny keratins of several mammalian species, for example, in the wool, there is more than twice as much Cys as in the horn and the hoof [16], there is 22% more Cys in the dog hair than in the claw [15]. There is 36 to 59% more Cys in the echidna hair than in the quill and the claw [5, 15]. Since cysteine is considered to stabilize the structure of keratin by the formation of disulphide bonds, the difference in the level of Cys may account for the degree of keratinization. Apart from the level of Cys, considerable variability has been reported in the amino acid composition of the hair and the horny keratins among different mammalian orders [14]. These results imply that variations in the amino acid compositions of keratins may reflect differences in phylogenetic relationship.

As shown in Table 1, samples used in the present research cover a relatively wide range of equine races, whose blood relationships are known to be diverse. Thoroughbred, Anglo-Arab and Arab, whose blood relationship is close, show high similarity in the amino acid compositions of keratin both in the body hair and the hoof. Two separate types of Pony, Schetland and Hackney Pony are known to form a group of horse race. Samata [21] has shown the similarity in the composition of the amino acids either in the body hair or the hoof of both types of Pony, suggesting the close blood relationship between them. Kiso Pony shows similar amino acid composition to Percheron particularly in the hoof. A relatively low amount of Gly is marked in them. The amino acid composition of the body hair from the two races are similar to each other and also to that of the Pony. The considerably different composition of both the body hair and the hoof from other races is recognized in Quarterhorse, but the reason is now unclear.

In order to perform the comparative biochemical studies of keratin, it may be thought that a homogeneous component in keratin from the samples collected under the same conditions should be analysed. However, the samples used for this analysis vary each other in various respects, such as colour of the body hair and the hoof, age, sex distinction, physical and breeding conditions. In addition, the analysis was carried out concerning the unfractionated keratin, but not some specific components in it. Nevertheless, some differences in the amino acid compositions were observed among 7 races. Therefore, it may be possible to clarify the difference in the structure of the specific component in keratin among the horse races by the use of the samples collected under the restricted condition and the successful fractionation of the entire keratin.

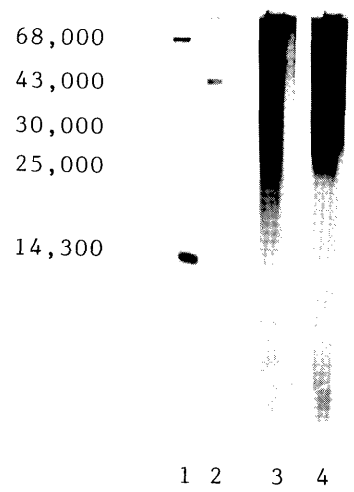

Fig. 2. Gel electrophoresis of keratin components of the horse body hair (4) and the hoof (3) on SDS-8% polyacrylamide. Bovine serum albumin (68.000), carbonic anhydrase (30.000), lysozyme (14,300) (1) and ovalbumin (43.000) and chymotrypsinogen (25.000) (2) were used as moleuclar weight standards (values in parenthesis in daltons). The proteins were visualized by silver staining using a commercial kit (Wako Pure Chemical Industries, Ltd.).

Since keratin is a relatively insoluble protein containing disulphide-bonded monomers, it requires rather severe conditions to make the protein dissolve and disaggregate. A prime requirement for its solubilization is the cleavage of disulphide bonds which can be achieved by reduction or oxidation. Investigations concerning the solubilized components have shown that the hair and the horn keratin are the mixture of at least 3 protein fractions. In our study, several components of the body hair of Arab could be fractionated from the performic acid-oxidized product by gel filtration on Bio-Gel A 1.5m. Moreover, an attempt to fractionate keratin components of the body hair and the hoof is also made on a SDS-polyacrylamide gel. As shown in Fig. 2, several protein components, ranging in molecular weights from approx. 100kD to 30kD, which cannot be well fractionated at present any more, are visualized by silver

staining. This result suggests that keratin from the equine body hair and the hoof may contain some distinctive protein components. Practically, it is shown to carry an apparent difference in the amino acid compositions for each of the keratin components in the body hair and the hoof, fractionated by gel filtration (data not shown). Furthermore, some electrophoretic investigations on the horse keratin, using other systems for separation and staining, are now in progress.

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要　　約

ウマの体毛と蹄のアミノ酸組成に関する研究：佐俣哲郎・松田基夫[1)]（麻布大学教養部，[1)]獣医学部分子生物学教室）——ウマの体毛と蹄のケラチンのアミノ酸組成を解析した．蹄の Asx, Gly, Ala, Ile, Leu, Tyr および Phe の含有量は体毛より高く，また Thr, Ser, Pro, Cys は逆に低かった．両者の Cys の含量の差異が最も著しかったことから，形成されるケラチンの種類決定に Cys が重要なことが示唆された．さらに，7品種のウマの体毛と蹄のアミノ酸組成の比較生化学的解析から，品種間における血統関係がそのアミノ酸組成に部分的に反映することが示唆された．過ギ酸で可溶化されたケラチンをゲル濾過分画したところ，いくつかの成分が検出され，SDS-ポリアクリルアミドゲル電気泳動による分画で，分子量100,000～30,000ダルトン領域に広いスメア様バンドが検出された．