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Electrophoretic analysis of alkylated proteins of human hair from various ethnic groups

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Synopsis

The general appearance of hair is influenced by many factors, among which the genetic inheritance (ethnic groups) plays a primary role. The structural modification between a blond hair and a curly black hair could be due to either a different genetic protein expression and/or to a different post-translational arrangement during the hair maturation. One approach for addressing such an issue has been to compare the composition of the hair proteins from various human races according to a procedure previously developed by Marshall and Gillespie (1,2).

In agreement with the results of these authors, the electrophoresis of Caucasian brown hair shows two groups of proteins: the low-sulfur proteins (LSP), with a MW ranging from 75 Kd to 35 Kd, and the high-sulfur proteins (HSP), with MW ranging from 30 Kd to 14 Kd. The constitutive S-polypeptides of HSP and LSP show the same molecular weights and mobilities for the different samples (European, African, Indian, and Asiatic hairs), but the intensity of HSP was found rather constant, whereas the LSP displayed a very marked difference. The yield in solubilized proteins increases from 0.3% for a Belgian blond hair sample to 19.5% for one sample of French dark brown hair. The darker the hair, the larger the yield in proteins. The variability of intensity of the bands of LSP was found to correlate with the amount of extracted proteins. This study shows that HSP and LSP behave differently with regard to the reducing buffer. These polypeptides undergo modification after synthesis, and the formation of cross-links in the hair structure might be involved. This study shows that the hair sulfur-containing proteins are identical whatever their racial origin but that the relationship between HSP and LSP varies according to hair color, suggesting different ways of post-translational modification.

INTRODUCTION

Skin appendages, such as hair, nail, or wool, are composites containing keratin filaments embedded in an intracellular matrix (3). Hair proteins can be extracted by reducing agents and then derived to more stable derivatives by S-carboxymethylation (SCM). Several approaches to separate SCM proteins have been reported. Using Sephadex chromatography (4) or analytical electrophoresis (1,5), the alkylated proteins were separated into two major groups: low-sulfur proteins (LSP) and high-sulfur proteins (HSP). From a number of physicochemical studies reviewed by Fraser and Mac Rae (6), it has been concluded that LSP are mostly fibrillar, whereas HSP derive from the hair matrix. Nutritional deficiencies (7), chemical treatments (2), weathering (8), or genetic disorders (9) may induce modification of distribution of SCM proteins which lead to

abnormalities in the hair structure. But few data (10) have been reported about the composition of proteins from the hair of humans belonging to different ethnic groups. The purpose of this study was to characterize sulfur protein electrophoretic patterns of hair from individuals of various ethnic groups. Our investigations suggest that the variability observed is related to hair color independently of the donor's racial origin.

MATERIALS AND METHODS

HAIR SAMPLES

Natural tresses free from previous cosmetic treatments from European, African, Indian, and Asiatic subjects were washed with 10% sodium lauryl sulfate (SDS), rinsed with tap water, and air dried. Hairs were cut in the middle part of the tresses (3 to 4 cm length at about 1.5 cm from the proximal root part of hair). To control a possible effect of weathering, a natural Caucasian brown hair tress was exposed for three months under daylight; this tress was mounted on a specimen rack and directly exposed to climatic conditions. The total solar energy delivered was about 150 KJ/cm^2. The effect of normal weathering was studied on 1 cm of the proximal root part of hair and 1 cm of the distal end of natural Caucasian hair (hair length about 10 cm).

EXTRACTION AND ALKYLATION PROCEDURE

Three milligrams of hair were delipidized by immersion in successive baths of petroleum ether and ethanol and subsequently rinsed with water. Fibers were cut into small pieces. Soluble proteins were extracted with 300 µl 0.05 M Tris HCl, pH 9.3, containing 8 M urea and 0.05 M dithiothreitol for 18 hours at room temperature and then treated with 6 µCi of 2-(^{14}C)iodoacetic acid (specific activity 57 µCi/mmole, Amersham, U.K.) according to Marshall and Gillespie (1).

ELECTROPHORESIS OF SCM PROTEINS

SDS-PAGE was performed in the system described by Laemmli (11) but using a stepwise separating acrylamide gel (10–15%) as reported in (1).

For the 2-D electrophoresis analysis, the first-dimension separation was carried out in 8 M urea at pH 8.9 in a glass tube using the method of Davis (12). The gel rod was then equilibrated in 0.06 M Tris HCl buffer, pH 7.0, containing 2.3% SDS before being placed on the top of the polyacrylamide slab (11).

DETECTION OF PROTEINS

Gels were revealed by the fluorographic method of Laskey and Mills (13). Autoradiography was performed at −80°C using an X-Omatt Kodak film.

ESTIMATION OF EXTRACTED PROTEINS

Protein amounts were determined by the colorimetric method of Bradford (14) using a Biorad standard kit.

AMINO ACID ANALYSIS

Hair samples were hydrolyzed *in vacuo* with constant boiling HCl at 108°C for 22 h and freeze dried. The hydrolysate was adjusted to pH 7.8 and shaken with air to oxidize cystine. The content of amino acids was estimated with a modified Beckman (− 120C) amino acid analyzer.

RESULTS

ELECTROPHORETIC PATTERNS OF ALKYLATED PROTEINS EXTRACTED FROM A EUROPEAN BROWN HAIR

The SDS-PAGE electrophoretic pattern of SCM proteins extracted from Caucasian brown hair is shown in Figure 1b. It displays two major groups of bands: The first group corresponds to the LSP and consists of five polypeptides with molecular weights ranging from 75 Kd to 35 Kd. The second group contains the HSP which are resolved in eight bands ranging from 30 Kd to 14 Kd. The 2-D analysis (Figure 1a) shows six spots of LSP proteins and eight spots of HSP. At pH 8.9, HSP are characterized by a higher mobility than LSP. Figures 1a and 1b will serve as reference patterns.

ELECTROPHORETIC PATTERNS OF SCM PROTEINS FROM WEATHERING HAIRS

The brown hair exposed (as reference) to prolonged daylight exposure shows a marked decrease of 87% of solubilized protein yield and only exhibits HSP after electrophoretic analysis (Figure 2a).

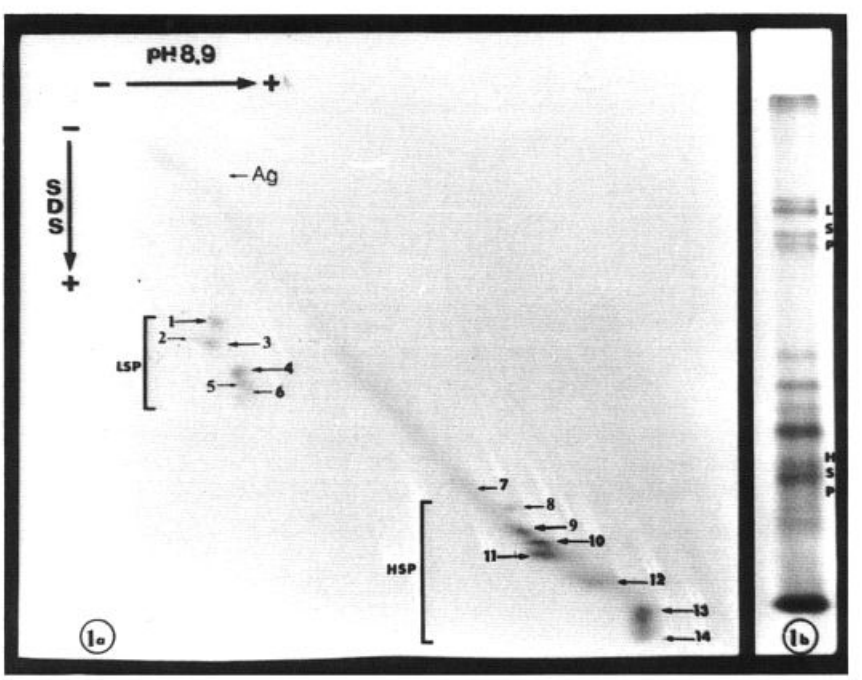

Figure 1. Autoradiographies of two-dimensional electrophoresis (1a) and SDS-PAGE (1b) of alkylated proteins extracted from European brown hair.

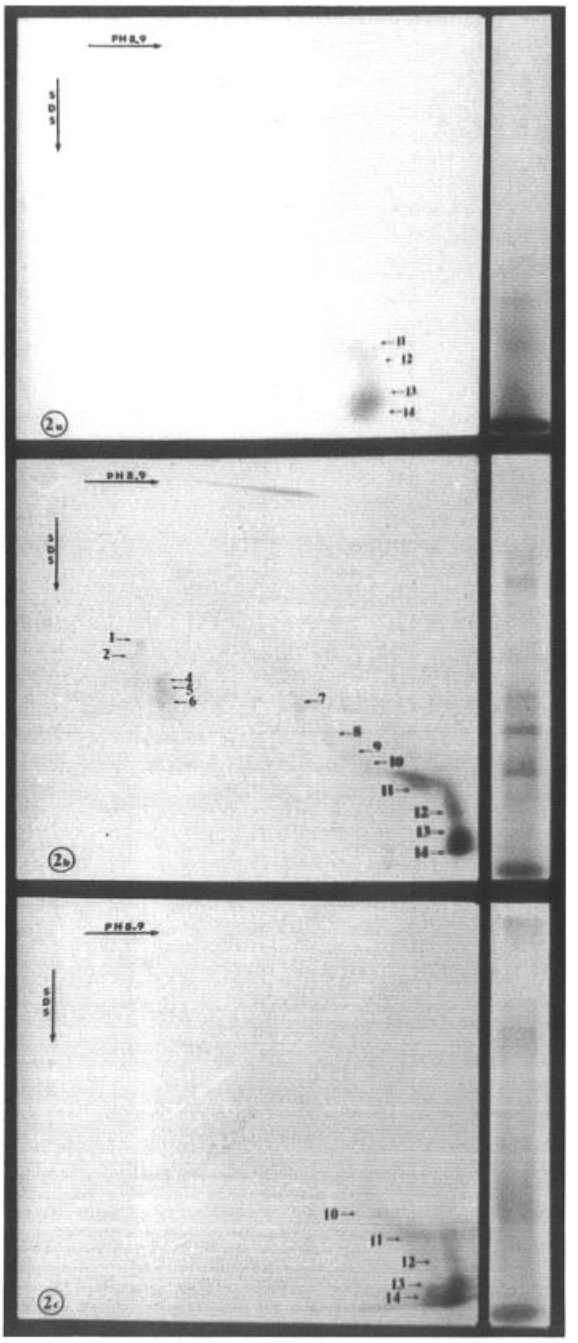

Figure 2. Autoradiographies of mono- and bidimensional electrophoresis of alkylated proteins extracted from prolonged weathered hair (a), proximal root part (b), and distal end part (c) of Caucasian brown hair.

The protein extracted from the proximal part of normally exposed brown hair has a better solubility (12.6%) than the median (7.2%) and distal (8%) part of hair (Table I). The electrophoretic pattern of the proximal part (Figure 2b) shows the same profile as

Table I
Classification, Protein Extraction Yields, and Electrophoretic Intensity of LSP/HSP of Weathered Hair Samples

Sample	Hair color	% Extracted proteins	Intensity of LSP and HSP	Classification (see text)
Daylight exposed	Brown	0.9	no LSP	a
Proximal root	Brown	12.6	LSP < HSP	c
Distal end	Brown	8.0	LSP <<<< HSP	b

the median part of hair, while the distal part of hair presents a marked decrease in LSP intensity (Figure 2c).

ELECTROPHORETIC PATTERNS OF ALKYLATED PROTEINS FROM DIFFERENT ETHNIC GROUPS

The electrophoretic patterns of alkylated proteins from hair samples belonging to individuals from different ethnic groups are shown in Figures 3a–3d. With regard to the ethnic groups, no modifications of MW or mobilities of the polypeptides were detected. Nevertheless, LSP presented different labeled intensity in each sample, while the inten-

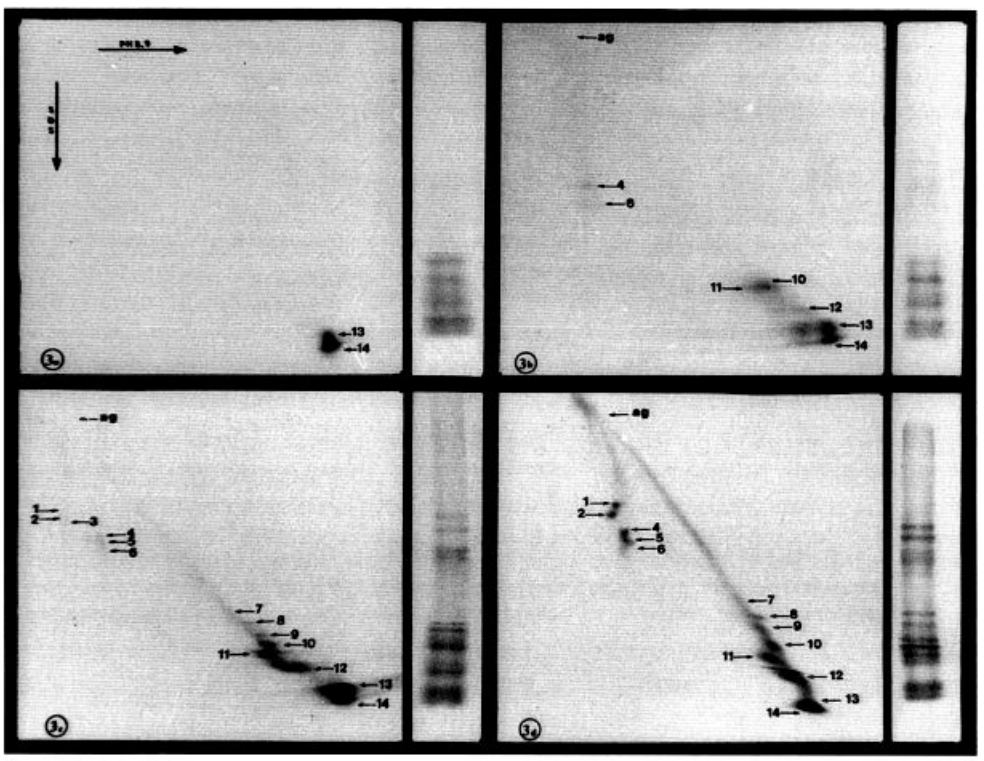

Figure 3. Autoradiographies of mono- and bidimensional electrophoresis of alkylated proteins extracted from humans belonging to various ethnic groups. a: Blond hair with undetectable LSP (class a). b: Light brown hair with hardly detectable LSP (class b). c: Dark brown hair with detectable LSP (class c). d: Black hair with very intense LSP (class d).

sities of the bands of HSP appear to be nearly constant except for blond hairs. Hair samples can be classified according to their content of LSP:

- Class a: No LSP
- Class b: Traces of LSP
- Class c: Few LSP associated to high HSP
- Class d: LSP as intense as HSP

The analysis of the proteins of curly black hair from African individuals and of Caucasian black hair reveals the presence of a 35-Kd band in the LSP group (Figure 4). This same band is also present when originating from brittle hair (in pathological cases such as trichothiodystrophy). From the data reported in Table II, it appears that the intensity of LSP is related to the quantity of solubilized proteins (which is ranging from 0.3% for a Belgian blond hair to 19.5% for a Caucasian brown hair) and that a relationship between protein amounts and hair color may be obtained. From these data we arbitrarily define four classes corresponding to the previous classes a, b, c, and d, but no modification in amino-acid composition was observed (Table III).

DISCUSSION

The electrophoretic analysis of SCM hair proteins shows a typical pattern, characterized by heterogeneity in size (from 10 to 70 Kd) and mobility at pH 8.9, according to the observations of Marshall and Gillespie (1,7). Nevertheless, these patterns can be modified when proteins are extracted from hair exposed to the influences of environment. Indeed, the daylight exposure experiment indicates that only HSP are detectable and that the yield of proteins solubilized by reducing agents is diminished. These observations are not surprising: weathering has been described to induce changes in the structure of hair proteins (8). Accordingly, to compare the protein composition as a function of ethnic groups required a prior determination of the influence of weathering on hair. Proteins extracted from the distal part of normal exposed hair display the same pattern as a prolonged weather-exposed hair, but the use of the median part as described in our study provides the same pattern as the proximal part of a non-exposed hair. Therefore,

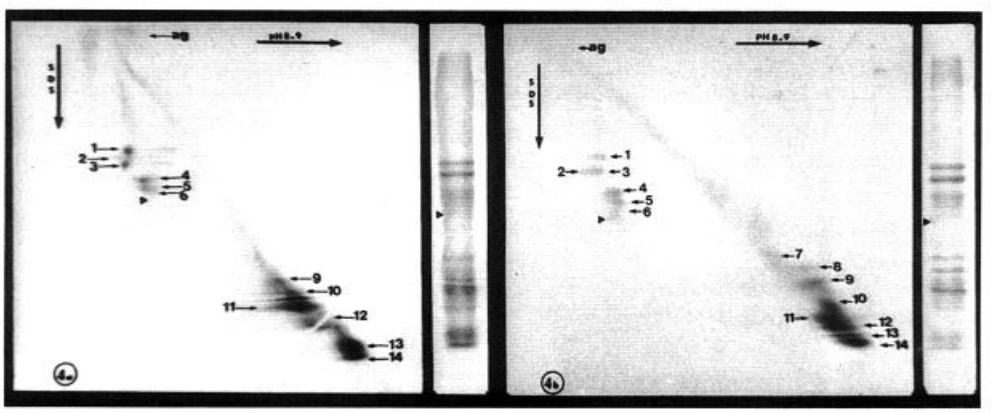

Figure 4. Autoradiographies of mono- and bidimensional electrophoresis of alkylated proteins extracted from black hair of African Negro (a) and Caucasian (b) individuals.

Table II
Classification, Protein Extraction Yields, and Electrophoretic Intensity of LSP/HSP of the Different Samples Studied

Sample	Hair color	% Extracted proteins	Intensity of LSP and HSP	Classification (see text)
French	Blond	0.6	No LSP	a
Belgian	Light blond	0.3		
German	Blond	1.2	LSP <<<< HSP	b
Italian	Light blond	0.9		
Indian	Brown	3.0		
Caucasian	Medium brown	7.2	LSP < HSP	c
South American	Black	3.6		
Indonesian	Black	3.9		
Polish	Dark brown	6.6		
Belgian	Dark brown	5.9		
Indian	Black	18.6	LSP > HSP	d
North African	Black	19.3		
African	Black	18.7		
French	Black	19.5		

these observations allowed us to select the middle part of the hair as a model in our study.

In terms of molecular weights and mobilities at pH 8.9 we found no influence of the ethnic origin of the hair. In the same way, no significant differences were found in the amino acid compositions (Table III) of hair from different racial origins or according to

Table III
Amino Acid Composition of Hair Samples (g/100 g)

Amino acid	European	Asiatic	African	South American
Cysteic acid	0.9	1.0	0.5	0.7
Aspartic acid	6.0	6.5	6.7	6.1
Threonine	7.2	7.1	7.2	7.2
Serine	10.0	9.8	10.4	9.8
Glutamic acid	14.9	15.0	15.0	14.7
Proline	7.5	7.1	6.2	7.5
Glycine	3.6	3.7	3.3	3.9
Alanine	3.1	3.1	3.4	3.4
Valine	5.1	5.2	5.2	5.2
Cystine	13.7	12.6	13.5	12.7
Methionine	0.7	0.5	0.7	0.6
Isoleucine	2.7	2.8	2.6	2.8
Leucine	6.7	6.7	7.1	6.8
Tyrosine	2.4	2.6	2.6	2.7
Phenylalanine	2.3	2.7	2.3	2.6
Lysine	2.8	3.1	3.1	2.9
Histidine	1.2	1.1	1.2	1.1
Arginine	9.2	9.3	8.9	9.1
Lanthionine	0	0	0	0

donors' ages, suggesting a chemical identity of the proteins. Nevertheless, a surprising fact is that the lighter the hair color, the lesser amount of extracted proteins. The similar electrophoretic patterns of a Caucasian black hair and of an African black hair (Figure 4) illustrate this statement. For a comparable amount of analyzed proteins, intensity of LSP increases with the yield in the protein extraction. The 35-Kd LSP polypeptide is yield-detectable only when this LSP group is very intense. Intermediate patterns (LSP-detectable but less intense that HSP) are obtained with medium brown hair. The highest yields of extracted proteins were obtained from dark brown and black hair samples, and the lowest yields were obtained with blond hair. These data suggest that the low-sulfur and high-sulfur proteins from black and blond hair would behave differently in the reducing buffer and that these proteins are more cross-linked in the blond hair sample than in the black hair sample. Furthermore, this cross-linking is via bonds that are not reducible.

The analysis of blond hair proteins provides results similar to that of the weathered Caucasian brown hair. Light and oxygen are well known to significantly affect the protein structure, by promoting the formation of photo-induced cross-links (8). The role of melanin photoprotection is well recognized and thus the less efficient protection of the complex eumelanin-pheomelanin in blond hair might explain the increase in new types of bonds (15). The chemical analysis of weathered hair (16,17) revealed that the sulfur content changed very little but that the cystine content presented a marked loss. Crewther (18) showed that the solubility of weathered hair in urea-bisulfite medium decreased. It can be therefore suggested that in such a case other types of cross-links are initiated to compensate for the loss in disulfide bridges. In blond hair, as in weathered hair, the proteins are non- or hardly extractible, showing that non-reducible cross-links are present but that the exact nature of these bonds remains unknown.

In conclusion, we summarize the following results: First, no major differences were found in the expression of the SCM polypeptides of the hair with regard to its racial origin. Second, it is of interest to note that the lowest protein recoveries were from those hair samples with the least pigment (pheomelanin). In the case of weathered hair, this result could be due to photochemical cross-linking or other factors. Sulfur protein composition in light hair and in weathered hair samples show the same electrophoretic pattern changes, suggesting that both of these types of hair contain more non-reducible cross-links than darker hair samples. The differences between dark and light hair seem to be related to the number of reducible cross-links, indicating that the formation of these cross-links may be either under genetic control or in conjunction with pigment production.

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