Electrophoretic Analysis of Proteins of Chemical Treated Human

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Abstract— *Strong oxidising agents for bleaching and chemical treatments for regeneration of dry and damaged hair are most common factors that cause changes in protein structure and composition. Composition changes in fibrillar proteins - α keratins and globular proteins - KAP's (keratin-associated proteins) were investigated by protein analysis of the hair fiber using SDS-PAGE. The most significant changes in obtained electrophoregrams from SDS-PAGE analysis of bleached hair were observed in matrix protein fractions (KAP's), which contain a high concentration of cystine. Constructed electrophoregram from SDS-PAGE gel of hair treated with protein treatment indicates a new protein fraction with a molecular weight of about 2000 Da, probably due on hydrolyzed keratin as an ingredient of protein treatments.*

Keywords— bleaching, hair protein treatments, SDS-PAGE.

I. INTRODUCTION

Hair is a filamentous biomaterial composed of fibers that extend above the surface of the scalp. Human hair is comprised of approximately 80% of protein, in which two large families, the intermediate filament protein family (α-keratins) and the keratin-associated protein family (KAP's), are present. The structure of human hairs is determined by proteins as key components, which contain different amounts of disulfide bonds. For instance, the keratins are known as low-sulfur proteins consisting of at least 4—9 distinct fragments of type I acidic (40—50 kDa) and 4—6 of type II neutral/basic (55—65 kDa). On the other hand, KAP's contain high concentration of sulfur and classified into high-sulfur proteins (10—20 kDa), ultra-high-sulfur proteins (10—20 kDa), and high-glycine/tyrosine proteins (6—9 kDa) [1-3].

The main biological function of hair is protection of the body against coldness and wetness [4]. But, it also symbolizes the beauty, which is real challenge for cosmetic industry in recent decades. The development of instrumental analytical and biochemical methods effects on scientific approach for detailed study of physical and chemical properties of hair. According to the literature, the investigation of protein composition of hair fiber allows explanation of structural changes in the keratin fractions [5]. These changes are mainly caused by strong oxidizing agents for bleaching, which caused oxidative degradation of pigment melanin. The primary purpose of bleaching is to lighten the hair with hydrogen peroxide as the principal oxidizing agent and salts of persulfate as accelerators [6, 7].

On the other hand, protein treatments are used to decrease friction and detangle, minimize frizz and regenerate dry and damaged hair. There are many types of treatments with different deposition, adherence and wash out capacity which will lead to different performances of the treatment. The ideal treatment is capable of restore the hydrophobicity of the fiber and neutralizes the static electricity. Depending on the capacity of entering the fiber, they may reach the cuticle surface or the inner part of the cortex [8].

Recently, X-ray diffraction [9], IR and solid state NMR [10, 11] have been successfully applied to investigate the structural changes of protein in hair fiber. Electrophoretic analysis of hair proteins has been used for forensic identification of human hair [12], clinical investigation of genetic variability [13], and quality control of wool important for textile industry [14]. Furthermore, electrophoresis has been used to study of S-carboxymethylated low sulfur and high sulfur proteins isolated from chemical untreated hair [15].

In this study, the effects that occur in protein composition of human hair caused by various chemical treatments (bleaching and protein regeneration treatment) were investigated using SDS-PAGE. The results for chemically treated hair obtained from this method are also indicators for the quality of the hair products.

II. EXPERIMENTAL

2.1 Materials

Organic solvents, methanol, ethanol and acetic acid were supplied by Merck (Darmstadt, Germany) with purity ≥ 99.9 %. Tris–HCl (p.a), thiourea (p.a) and urea (p.a) from Merck (Darmstadt, Germany), 2-mercaptoethanol (p.a) and SDS were obtained from Sigma–Aldrich (Steinheim, Germany). Coomassie Brilliant Blue R-250 were purchased from Merck (Darmstadt, Germany). Amersham Low Molecular Weight (LMW) calibration proteins were used as a standard. In all cases double deionized water (ddH_2O) was used.

2.2 Preparation of hair samples

Human hair samples were collected from one woman to minimize the experimental variations that may be caused by age or gender. The hair samples were washed with ethanol (75 %) prior to extraction to remove any surface contaminants. External lipids were then removed by using a mixture of chloroform/methanol (2:1 v/v) for 24 h. Hair samples were exposed to the influence of bleaching reagent (bleach by Revlon with 12% H_2O_2) for 45 min, washed with ddH_2O and air drayed. After bleaching, hair was treated by protein treatments (Fiberplex and Fiberforce by Schwarzkopf and Keratin treatment by Revlon) during 30 minutes.

2.3 Extraction of Hair Proteins

Hair was cut into small pieces with a length of approximately 1-2 mm, and 5 mg hair was mixed with 1 mL solution containing 25 mM Tris–HCl, pH 8.5, 2.6 M thiourea, 5 M urea and 600 mM 2-mercaptoethanol (2-ME) at 50°C for 72 h. After protein extraction procedure, the mixture was centrifuged at 12000×**g** for 15 min at room temperature. The obtained supernatant was used as a hair protein fraction for determination of total proteins and electrophoretic analysis.

2.4 SDS-PAGE analysis

Extracted hair proteins have been analyzed by SDS-PAGE electrophoresis. For analyses 0.9 ml of obtained supernatant was mixed with 0.1 ml 10 % SDS solution and boiled for 3 min. Then 12 µL of this mixture was applied. Electrophoresis was performed on 4-22 % gradient gel at 260 V for 2 h in 0.025 M Tris, 0.2 M glycine containing 0.1% (w/v) SDS. Gels were stained with 0.1% Coomassie Brilliant Blue R-250, and distained with a mixture of acetic acid and methanol (20:80, v/v). Obtained gels were analyzed by Gel Pro software.

III. RESULTS AND DISCUSSION

Analysis of the obtained SDS-PAGE gel from chemical untreated hair indicated appearing of intense protein bands with molecular weight in the range of 40 - 45 kDa (Fig. 1 A1 and A2). This protein band corresponded to the theoretical molecular weight of keratin of type I acid proteins. In the area around 50 kDa another fraction was identified, that was consistent with the molecular weight of keratin type II neutral/base proteins.

In the obtained gel, less intense low molecular weight protein bands in the range of about 30 kDa and 10-20 kDa were also observed (Fig 1). These two distinct groups of proteins correspond to UHS (ultra high sulfur proteins) and HS (high sulfur proteins), but there was no evidence of HTP (high tyrosine proteins) [15].

The low intensity of protein fractions with a molecular weight below 30 kDa is probably due to the exposure of hair on UV irradiation and high temperature drying and styling. These factors can cause significant changes in low molecular weight fractions especially when hair is exposed for a long period (2 to 3 years) and when the samples are taken from the tips of the hair. On the other hand, low intensity may simply be due to low concentration of these proteins.

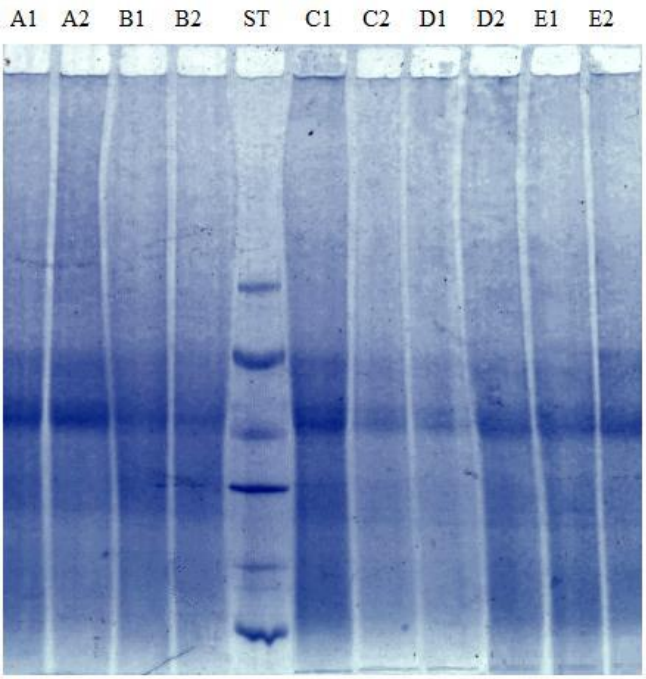

FIGURE 1. SDS-PAGE of extracted human hair protein from: A - chemical untreated hair, B - bleached hair, C - hair treated with Fiberplex by Schwarzkopf, D - hair treated with Fiberforce by Schwarzkopf and E - hair treated with Keratin treatment by Revlon (lanes 1 and 2, duplicate samples of the same hair type)

The bands of components with higher molecular weights (65-75 kDa) were also shown in electropherogram (Fig. 2). These components may represent dimeric forms of the low-sulphur proteins, that possibly obtained in incomplete reduction of the low-sulphur proteins during extraction or alternatively limited re-oxidation prior to or during electrophoresis

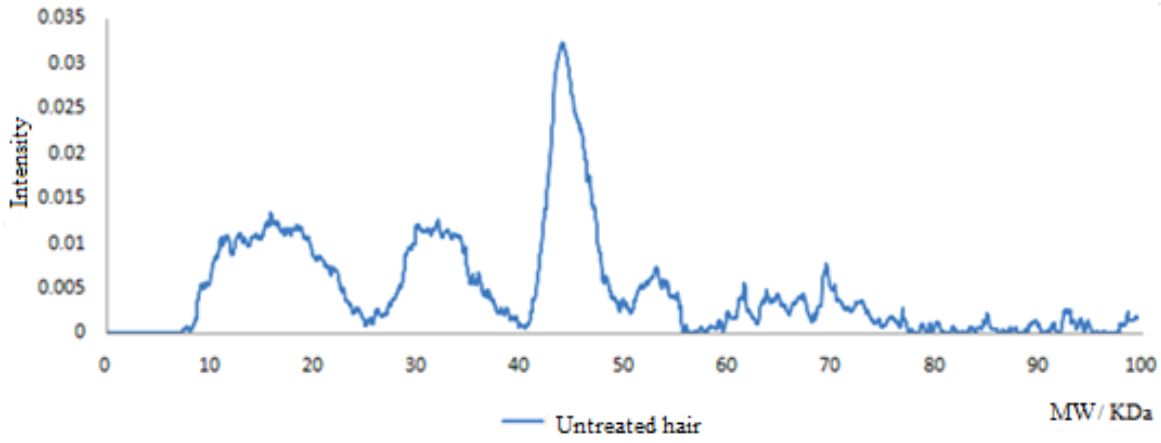

FIGURE 2. SDS-PAGE electropherograms of proteins extracted from chemically untreated hair

During bleaching, hydrogen peroxide primarily reacts with melanin and initiates a destruction of the chromophore. However, hair fiber primarily contains a large percentage of oxidizable groups (e.g., disulfide bonds of the cortical matrix and of the cuticle) and degradation of hair proteins also occur. Determination of the isolated proteins from bleached hair was achieved by SDS-PAGE analysis at the same experimental conditions. The results obtained from electropherogram (Fig. 3) exhibit that all protein fractions undergo changes, which are mostly expressed in proteins with lower molecular weight (KAP's). These changes are due to the structure of the proteins and their composition, especially the content of cystine. The cysteic acid is the final product of the oxidative cleavage of the disulfide bond during the bleaching of human hair, identified by HPLC [16]. This oxidation occurs via intermediate oxidation products (disulfide dioxides, disulfide tetroxide, cystine monoxide and cystine dioxide), extremely sensitive to alkaline hydrolysis.

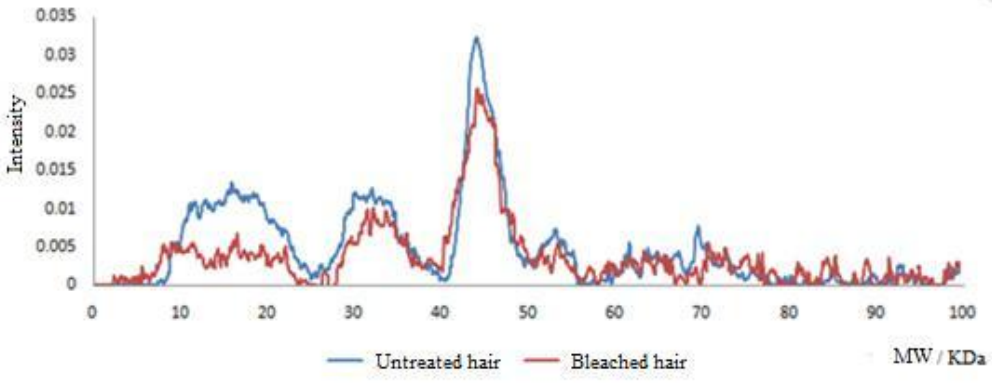

FIGURE 3. SDS-PAGE electropherograms of proteins extracted from chemically untreated and bleached hair

The results obtained from the SDS-PAGE analysis of hair treated with protein treatments by Schwarzkopf (Fiberplex and Fiberforce) and Revlon, (Keratin treatment) shown in Fig. 4 indicate a new protein fraction with average molecular weight of about 2000 Da. This protein fraction is due to the main component of chemical treatments which is hydrolyzed keratin, product of alkaline or enzymatic hydrolysis of keratin isolated from sheep or goat wool. Depending on the molecular weight, hydrolyzed proteins cannot diffuse into deeper layers, so they may only sorption on the surface of the hair shaft.

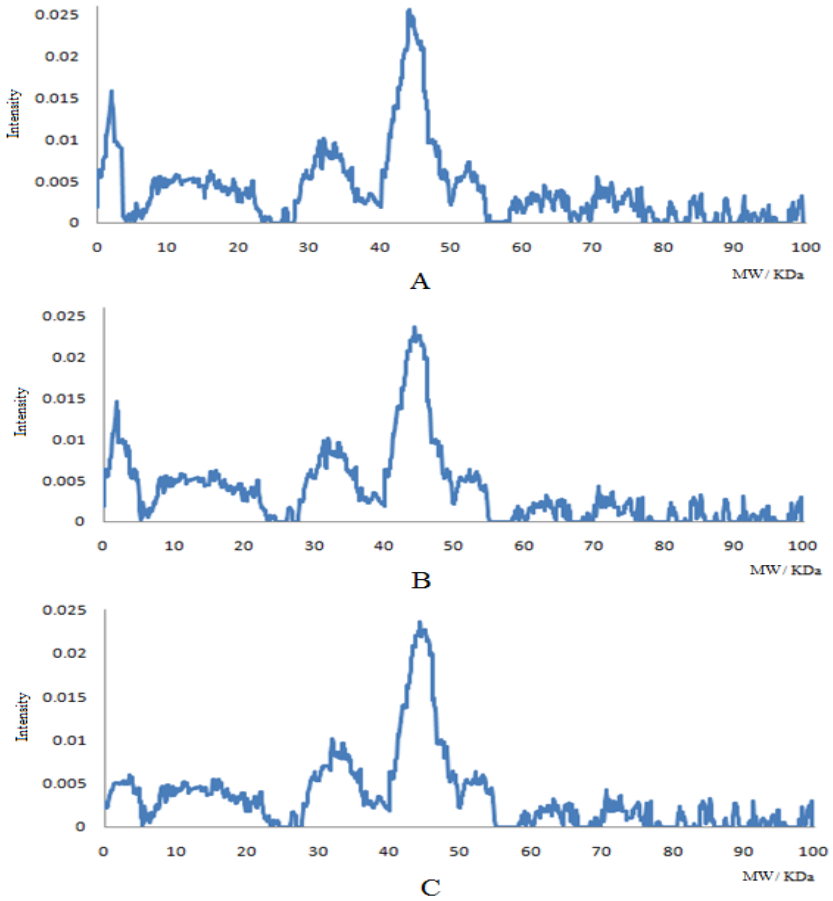

FIGURE 4. SDS-PAGE electropherograms of proteins extracted from A - hair treated with Fiberplex by Schwarzkopf, B - hair treated with Fiber force by Schwarzkopf and C - hair treated with Keratin treatment by Revlon

To provide better results, treatment is performed in several steps. In the first step, the hair is washed with high pH shampoo which affect on cuticle opening. In the next step, hydrolyzed keratin is applied and penetrates to the surface layers of the fiber through the open cuticle. The rest of the keratin that has not penetrated because of its ability to form colloids gives

a shiny and healthy look of the fiber. The final step involves washing the hair with a shampoo with pH below 5, which provides closure of the cuticle and lock in place the hydrolyzed keratin in the fiber.

IV. CONCLUSION

In this study effects of bleaching agents and protein treatments on protein composition of hair were investigated. Bleaching of human hair involved oxidative degradation of melanin and side reactions with the hair proteins as result of reaction conditions. The most significant changes in obtained electrophoregrams from SDS-PAGE analysis of bleached hair were observed in low molecular weight proteins, which contain a high concentration of cystine. These changes are probably due to oxidation of cystine to its final product - cysteic acid. On the other hand, hydrolyzed keratin is main intergradient of protein treatments and its fraction was detected on constructed electrophoregram from SDS-PAGE gel, with a molecular weight at about 2000 Da. The intensity of this band depended of the quality of cosmetic products and their efficiency for repairing dry and damaged hair.

REFERENCES

[1] N.H. Leon, "Structural aspects of keratin fibres," J. Soc. Cosmet. Chem, vol. 23, pp. 427–445, 1972.

[2] M.A. Rogers, L. Langbein, S. Praetzel-Wunder, H. Winter, and J. Schweizer, "Human hair keratin-associated proteins (KAPs)," Int. Rev. Cytol., vol. 251, pp. 209–263, 2006.

[3] D.A.D. Parry, T.A. Smith, M.A. Rogers, and J. Schweizer, "Human hair keratin-associated proteins: Sequence regularities and structural implications," J. Struct. Biol, vol. 155, pp. 361–369, 2006.

[4] G.E. Rogers, P.J. Reis, K.A. Ward, and R.C. Marshall**,** The biology of wool and hair, 1st ed, Chapman and Hall, London, New York 1989.

[5] N. Nishikawa, Y. Tanizawa, S. Tanaka, Y. Horiguchi, and T. Asakura, "Structural change of keratin protein in human hair by permanent waving treatment," Polymer (Guildf), vol. 39, pp. 3835–3840, 1998.

[6] L.J. Wolfram, D. Ph, K. Hall, B. Sc, and I. Hui, "The Mechanism of Hair Bleaching," J. Soc. Cosmet. Chem, vol. 900, pp. 875–900, 1970.

[7] C.R. Robbins, "Chemical Aspects of Bleaching Human Hair," J Soc Cosmet. Chem, vol. 22, pp. 339–348, 1971. [8]

[8] G. Dias, and M. F. Reis, "Hair cosmetics: An overview," Int J Trichol, vol. 7, pp. 2-15, 2015.

[9] R. D. B Fraser, and T. P. MacRae , "Conformation in Fibrous Proteins and Related Syntetic Poripeptides," Academic Press, pp. 469–525, 1973.

[10] H. Saitô, "Conformation-dependent 13C chemical shifts: A new means of conformational characterization as obtained by high-resolution solid-state 13C NMR," Magnetic Resonance in Chemistry, vol. 24, pp. 835-852, 1986.

[11] H. Saitô, and I. Ando, "High-resolution solid-state NMR studies of synthetic and biological macromolecules," Annual reports on NMR spectroscopy, vol. 21, pp. 209-290, 1989.

[12] R.C. Marshall, J.M. Gillespie, and V. Klement, "Methods and Future Prospects for Forensic Identification of Hairs by Electrophoresis," J. Forensic Sci, vol. 25, pp. 57-66, 1985.

[13] R.C. Marshall, and J.M. Gillespie, "Comparison of samples of human hair by two-dimensional electrophoresis," J. Forensic Sci. Soc, vol. 22, pp. 377–85, 1982.

[14] R.C. Marshall, H. Zahn, and G. Blankenburg, "Possible Identification of Specialty Fibers by Electrophoresis," Textile Research Journal, vol. 54, pp. 126- 134, 1984.

[15] R.C. Marshall, "Characterization of the proteins of human hair and nail by electrophoresis," J Invest Dermatol, vol. 80, pp. 519–524, 1983.

[16] C.R. Robbins, and C. Kelly, "Amino acid analysis of cosmetically allerted hair," J. Soc. Cosmet. Chem, vol. 20, pp. 555–564, 1969.