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Peptide structure: Its effect on penetration into human hair

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Synopsis

The influence of the peptide structure on its penetration inside hair was studied, together with the effect of hair bleaching (oxidation). For that reason, the outcome of positioning a charged sequence (KAKAK) either at the N or C terminal on hair penetration has been studied for peptides with 17 residues each. It was observed that the penetration of these peptides into hair was driven by electrostatic interactions, where the position of the charged group at the peptide structure was of major importance. The penetration was only achieved for damaged hair due to its higher negative charge at the membrane surface. It was also observed that the peptides were able to restore the original tensile strength of bleached hair. Consequently, the knowledge of hair surface properties is of extreme importance when designing peptides directed for hair treatment.

INTRODUCTION

The desire for products that improve the look and feel of hair has created a huge industry for hair care which is constantly in quest of new products and finishing treatments. Beauty care technology has advanced the cleaning, protection and restoration of desirable hair and skin properties by altering its surface. Therefore, the characterization of hair structure and the knowledge of physical and mechanical properties of hair are essential (1,2).

Human hair is a fibrous tissue, comprised of keratin. The main morphological component is the fibrous cortex (about 80%) surrounded by the multicellular flat cuticle sheath (about 15%), with the additional feature of a central medulla for some types, especially coarser fibres. These main morphological components consist of distinct chemical constituents. In the amorphous cuticle, the outer exocuticle layer is composed mainly of high-sulphur proteins and is therefore rich in disulphide cross-link bonds, leading to its high mechanical properties (tough and resilient layer) and creating a trans-cellular barrier to the penetration of various compounds to the hair structure (3–6). Because of this, penetration of chemicals into hair occurs mainly through intercellular diffusion. Unaltered human hair has an isoelectric point near 3.67. Hence, under most pH conditions the surface of hair carries a negative charge. For this reason, most conditioning

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polymers are cationic, since electrostatic interactions are believed to play a determining role in the adsorption mechanism (7).

The exposure of the hair to sun, wind and modern hair styling products and techniques (e.g. shampooing, combing, blow-drying, bleaching, colouring and shaping of hair with wave preparations) imparts significant and unwanted damage to the cuticle and cortex of the hair shaft. This damage results in a loss in body, lustre, and smooth texture. Such damage is also reflected in poor wet and dry combability, increased electrostatic charging, reduced tensile strength and breaking of the hair and in the poor appearance of hair styles. The main objectives of hair care product development are then to inhibit or reduce the damage caused by the factors described previously and to create an improved feel of the hair (8). The cosmetic industry also takes an interest in the penetration of several substances into hair fibres in order to improve the knowledge of diffusion processes in hair (9,10). To study the diffusion of molecules into hair, fluorescence dyes are often used and in this case the penetration can be investigated by conventional fluorescent microscopy.

The use of protein materials in the formulation of modern hair care products to provide shine, strength, softness, smoothness and good combing properties is well known and began more than 50 years ago. Several patents disclose compositions capable of permeating into shampooed hair to impart hair with moistness and to provide excellent finishing effects by including proteins or proteins hydrolizates, like for example a water-soluble compound derived from a vegetable protein derivative (11), non-naturally occurring keratin proteins (12), a mixture of a hydrolysed protein and an amino acid with aliphatic side chain (13) and several hydrolysed proteins (14,15).

The present study describes how the structure of two peptides, which differ only in their conformation from the N to the C-terminal, influences in their penetration inside human hair. It was verified that their penetration inside hair was structure dependent and it was essentially driven by electrostatic charging. These peptides were capable of restoring the tensile strength of bleached hair, thus compensating for its damage, which could represent a new methodology for hair treatment.

EXPERIMENTAL DETAILS/MATERIAL AND METHODS

HAIR SAMPLES AND PEPTIDE STRUCTURES

European virgin white hair samples were received from IMHAIR Ltd. (Italy). The peptide structures were synthesized by JPT Peptide Technologies GmbH (Berlin, Germany). The two synthesized peptides, with 17 amino acids each, were:

C-term: LLLLL LCLCL LLKAK AK

N-term: KAKAK LLLCL CLLLL LL

where L, C, K and A is the one-letter code to the amino acids Leucine, Cysteine, Lysine and Alanine. All the peptides were covalently linked by the N-terminal to a fluorescent dye, (5(6)-carboxytetramethylrhodamine, succinimidyl ester) *i.e.* 5(6)-TAMRA, with spectral properties of Abs_{max} = 544 nm and Em_{max} = 572 nm, to facilitate the analysis of peptide penetration. The peptides molecular weight was 2292.67 g/mol and they were supplied as a lyophilized material. They were analysed by HPLC and MS, and their

purity was over 70% (HPLC, 220 nm, C18, linear gradient). All other chemicals used were of analytical grade.

PEPTIDE SURFACE CHARGE ANALYSIS

The peptide surface charge analysis was obtained by using the PyMOL v0.99 (16). PyMOL (16) is an open source molecular graphics system designed for real time visualization and rapid generation of high-quality molecular graphics images and animations.

HAIR PRE-TREATMENT

The pre-treatment process was carried out using all the samples simultaneously, such that they received exactly the same extent of processing. Hair was either washed in distilled water at 50°C for one hour (samples labelled as W) or bleached, at 50°C in 0.1 M Na_2CO_3/$NaHCO_3$ pH 9 buffer, 10% H_2O_2, also for one hour (samples labelled as B).

PREPARATION OF PEPTIDE SOLUTIONS

A 0.2 g/L peptide solution was prepared with the addition of a surfactant, 1.8 g/L of dipalmitoyl phosphatidylcholine, in a small volume of ethanol to dissolve both the peptides and phosphatidylcholine. Distilled water was added and ethanol was allowed to evaporate at room temperature or in a water bath at about 40°C. The peptide solution was refilled with distilled water to the desired level (the final volume).

HAIR TREATMENT WITH THE PEPTIDES

The hair samples were treated with a solution of these peptides, using a bath ratio of 1/100 (w/v) and the control sample was washed or bleached hair. The treatment was performed at 37°C, 100 rpm of stirring, for 5 hours. After the treatment, hair samples were well washed under running water and washed with a commercial shampoo, rubbing up with fingers for about 1 minute. After shampoo washing, hair tresses were well washed with distilled water and allowed to air dry.

TENSILE STRENGTH MEASUREMENTS

The method used broadly follows the guidelines laid down in ASTM D1445-95 for the tensile testing of fibres. The measurements were performed with an Instron 4505 tensile tester with a maximum load cell capacity of 2.5 N. For each measurement, 20 hairs were taken randomly from the tress. Each hair was individually mounted in the tensile jig by means of a paper device that was previously slashed using a fixed gauge length of 20 mm, and pulled under controlled conditions, at a rate of 1mm/min, until breakage occurred. For each hair, records of applied load against extension were taken and using an average mean diameter of 75 μm, the data were converted to stress (load/unit area) against strain (% extension).

FLUORESCENCE MICROSCOPY

The hair fibres were embedded into an epoxy resin and transversal cuts of the fibres with 10 μm were prepared using a microtome. Fibres cross-sections were analyzed by a transmission optic microscope (Olympus BH2) with a magnification of 40×.

RESULTS AND DISCUSSION

The hair surface tends to be negatively charged at neutral and/or alkaline pHs. When hair is damaged, either by chemical or mechanical factors, its negative charge increases, increasing as well its friction and adhesion properties (7,8). In this particular study the peptides were formulated together with a lipid, phosphatidylcholine, which was added to attain a peptide formulation compatible with a water environment. Due to the large size of the Leucine side chain, the synthesized peptides tend to acquire an alpha helix structure in water. The interaction with phospholipids could further stabilize this alpha helix structure (17). However, the KAKAK sequence positioned at the C or N terminus, despite the large size of the amino acid Lysine, might induce a different structure for these two peptides, due to both charge and interaction with the hydrophobic parts of phospholipids. However, we do not know for sure the exact structure of these peptides in water.

The peptide sequences were visualized by a molecular modelling program to identify the major differences in their structure (Figure 1). The molecular modelling program allows for creating the structure based on the amino acids sequence, which only differs in the position of the charged group (KAKAK), which is at the C-terminus or at the N-terminus, respectively, in the C-term or N-term peptides.

Figure 1 shows the structures of C-term and N-term peptides in vacuum. Besides illustrating the amphipathic nature of the helix, it also shows a much narrower spatial distribution of the positively charged side chains in the C-term peptide. These peptides tend to be therefore both amphipathic and cationic. Amphipathicity increases their affinity for biological membranes, while the positive charge increases their specificity toward negatively charged membranes, as those of hair (17,18). The total net charge of these peptides was found to be +3. Accordingly to Sharadadevi *et al.* (19), helices with

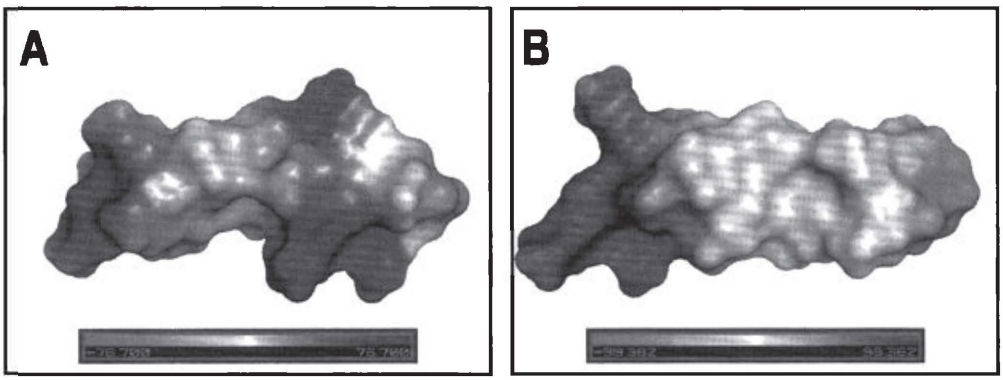

Figure 1. Surface charge analysis for the C-term (A) and N-term (B) peptides, from PyMol v0.99. Red denotes the negatively charged C-terminus while blue denotes the positively charged side chains.

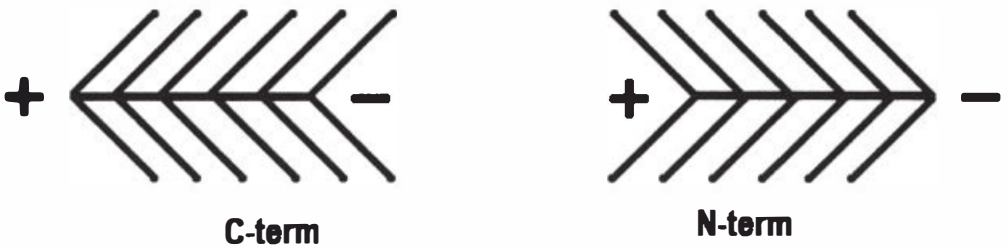

Figure 2. Schematic representation of the position of the positive and negative charges on the peptides.

net positive charge occur more frequently and net positive charge appears to favour higher hydrophobic moment, compared to net negative charge.

Observing the peptide structure it is possible to deduce some performances of these two peptides in solution. The distribution of the positive and the negative charges are important for understanding its penetration, since they allow for an orientation of the peptide at the hair surface, like a screw (Figure 2). The presence of a negative charge at the C-terminus suggests that the C-terminus will point away from the anionic surface and that adsorption will occur in a specific orientation driven by electrostatic complementarity's (17,20). The positive charges on the N-term peptide are located at the larger part of its structure, while C-term peptide shows a much more uniform size. Since hair surface is negatively charged, the electrostatic interaction between the peptide and hair surface will allow for the cationic part of the peptide to orientate into the hair surface. Because in the C-term peptide this cationic part is thinner, the penetration of the peptide inside the hair negatively charged membrane would be easier.

To corroborate these assumptions, the visualization of the penetration of the peptides inside hair structure was carried out either by visual inspection (Figure 3) or by fluorescence microscopy on hair transversal cuts (Figure 4). The penetration of the peptides inside hair structure was first seen visually and notably the C-term peptide with the bleaching pre-treatment was more coloured than the others (Figure 3).

These results were also confirmed by fluorescence microscopy. For only water washed

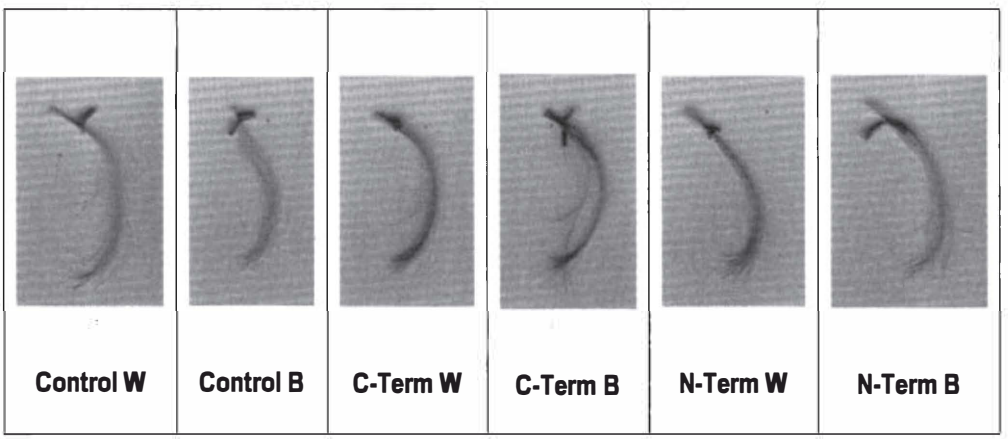

Figure 3. Hair samples after treatment with the coloured peptides, comparing with the control samples. W refers to water washed samples while B refers to bleached samples.

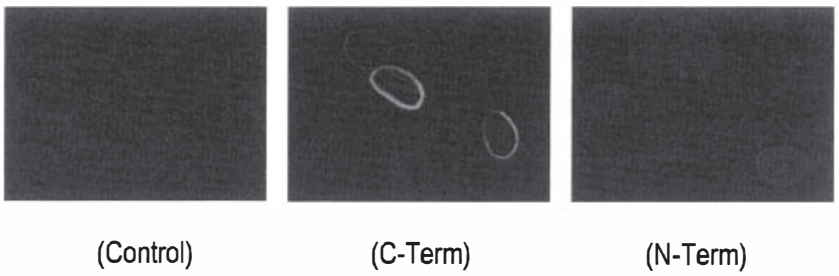

Figure 4. Flourescence micrographs of cross sections of bleached virgin white hair after treatment with the peptides.

hair no changes could be observed (data not shown), comparing to the control. Contrarily, for bleached hair, which as previously stated has the surface damaged and is therefore more negatively charged, the penetration was only attained for the C-term peptide (Figure 4). It was also observed that the peptides were layered around the hair surface and did not penetrate completely inside its structure (ring-dyeing). It is believed that if longer penetration times and higher temperatures were used, the migration of the peptides could reach the cortical cells (9).

Since the virgin hair that was only water washed lacks chemical or mechanical damages, its hydrophobic layer remains intact. Therefore, water and other substances are hardly adsorbed (or desorbed) and penetrate into the hair surface. This explains the fact that there was no penetration of the peptide structures inside hair when it was only water washed. On the other hand, the hydrophobic lipid layer of damaged hair surface may be depleted or damaged; therefore, inner cellular structures of hair, which consists of many hydrophilic molecules, such as cystines, are now exposed to water. Several authors relate the increase in the adsorption of several compounds, such as polymers or proteins hydrolysates, with hair damage (bleaching), which has been described as a "self-adjusting" system, while reporting also an increase of protein adsorption with a decrease in the molecular size of the compounds (7,9,13).

The physical characteristics of the hair fibres after treatment were also determined. The tensile strength resistance and elongation for the hair samples are presented in Figure 5.

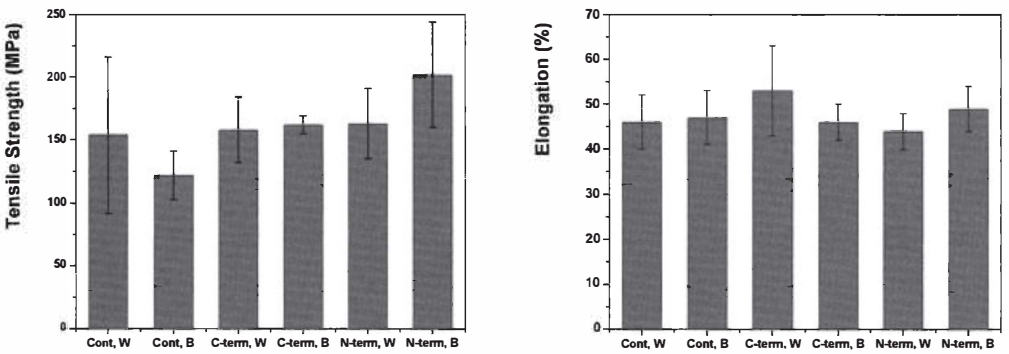

Figure 5. Tensile strength resistance (MPa) and Elongation (%) for the controls and for the hair samples after peptide treatment.

The two typical parameters used to characterize materials behaviour under a tensile load are stress and strain. The ultimate tensile strength and elongation of a variety of materials has been determined and for human hair these parameters were found to be around 193 MPa and 40%, respectively (21). Nevertheless, it is common knowledge that the determination of these parameters is quite prone to variation, depending on the method chosen, the part of the hair measured, the type of hair, among other factors. In this study there were no significant variations for the tensile strength resistance of the hair samples after the peptide treatment. It is important to relate that a mean diameter of 75 μm for hair was used, which brings an additional variation for the determination of this data. However, a trend towards restoration of part of the strength lost by over-oxidized bleached hair was observed, which as expected has a lower resistance. Elongation was also not statistically different among all the samples.

This study shows the importance of knowing the peptide structure and the possible interactions that it may exhibit with the membrane surface, in order to evaluate its penetration inside hair. It was observed that the localization of the charge at peptide structure is extremely important for enhancing the peptide penetration inside hair, which occurs mainly due to electrostatic complementarities. It was also confirmed that hair oxidation enhances peptide penetration, since it increases its negative charge at the surface.

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