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Effects of ultra violet radiation on the soluble proteins of human hair

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ABSTRACT

Exposure of hair fibers from healthy volunteers to Ultra Violet Radiation (UVR) under laboratory conditions enhanced protein elution from the hair tresses into a buffer solution (pH 10.5). At the same time the UVR decreased the intensity of tryptophan fluorescence in the eluted proteins. After mechanical homogenization of these hair samples, the increase of soluble protein was registered for UVR treated hair as well as the rise in sulfhydryl group content of these proteins.

Analysis of soluble proteins from hair samples homogenized before and after protein elution has shown that mainly proteins rich in sulfhydryl groups were eluted and as a result sulfhydryl content of proteins in hair shaft decreased. The hypothesis concerning the effects of environmental factors on the properties of hair shaft proteins was examined, the proximal and distal parts of normal hair (0–5 cm and 15–20 cm from hair root) were compared. In the distal parts there was a higher quantity of soluble proteins registered after homogenization, with decreased sulfhydryl group content and tryptophan fluorescence. It could be supposed that this difference results from the steady rupture of cystine in sulfur bridges and tryptophan under exposure to environmental factors (mainly, UVR), followed by elution of the resulting peptides.

1. Introduction

Keratins, components of the hair fibers, are sensitive to UVR exposure [1]. The primary sites of keratin photodegradation in the wavelength range 250–300 nm are the amino acids tryptophan, tyrosine, phenylalanine, cysteine/cystine [2]. UV light absorption and the resulting excitation of electron to higher energy states is followed by a number of processes influenced by the solution pH, temperature and protein structure.

The photooxidation can induce further protein oxidation mediated by reactive oxygen species (peroxy radicals, singlet oxygen) including the Fenton reaction.

Photodegradation of cystine results in the rupture of disulfide bridges, either an R–S bond or the S–S bond [2,3]. Fission of disulfide bonds is known to influence the mechanical properties of hair fiber [4,5] and the fragile property of cuticles [6].

Tryptophan photodegradation [7–9] is well known for a number of proteins [10,11] including keratins [12,13]. Tryptophan photodestruction in hair keratins influences the fluorescence of tryptophan and of the resulting products (n-formylkynurenine, kynurenine) [1,14–16].

Disruption of hair keratins can not only be induced by UV irradiation but also by perming and bleaching which enhances protein elution from hair fiber as well as improves solubilization of some proteins in the hair cortex [1,17,18]. Since the hair growth lasts 2–6 years, it is obvious that it is repeatedly exposed to UV irradiation that can affect the properties of keratins.

The aim of our study was to compare eluted proteins and soluble proteins in the hair of healthy subjects between UV irradiated and non-irradiated samples and also between distal and proximal hair fragments. Since little is known about the composition and properties of eluted and soluble hair proteins we have chosen mild experimental conditions, where chemical disintegration of hair structure does not take place.

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2. Materials and methods

2.1. Objects

We studied non-dyed dark-brown and black hair tresses of healthy Caucasian subjects. Hair samples from 9 individuals were also irradiated with UV light. Hair tresses, from 8 further people, each about 20 cm long, were divided into proximal (0–5 cm from hair root) and distal (15–20 cm from hair root) fragments.

2.2. Laboratory apparatus and reagents

The optical absorption of the solutions at 240 nm and that of the colored products was quantified by spectrophotometry employing Shimadzu-1800UV spectrophotometer using a 1 cm quartz cell. Fluorescence spectra of EP and SP_e samples were obtained with a Hitachi F-4000 spectrofluorometer. We used a 0.5 × 0.5 cm quartz cell.

Tris and sodium carbonate salts were purchased from Chimmed (Russia) and the protein kit (biuret method) was supplied by RANDOX, UK. Bovin serum albumin (BSA), human serum albumin (HSA), Bowman–Birk inhibitor from soybeans, DTNB (5,5′-dithiobis(2-nitrobenzoic acid)), l-tryptophan and GSH (glutathione as a reduced form) were purchased from Sigma–Aldrich, aprotinin was supplied by "Inghiprol" PO "Belmedpreparaty", Byelorussia.

2.3. Hair UV exposure

The hair fibers were irradiated using UV lamp BLM-12, 15 W (Medicor, Budapest) at a distance of 15 cm, at room temperature and 60 ± 10% relative humidity. Approximately 10 mg of hair were spread out in dishes of 3.5 cm in diameter. Number of quanta on the distance of 15 cm from the lamp was close to $2.0 \cdot 10^{15}$ cm^{-2} s^{-1} (i.e. 1.6 mW cm^{-2}) in the wavelengths of tryptophan absorption (230–300 nm, mainly in the line 254 nm).

The fluorescence of standard tryptophan solution (2.5 μM, pH 7.4) in a vial (19 mm in diameter, 10 mm height) decreased by 50% after 30 min of exposure.

2.4. Protein elution

5 mg of hair, cut into pieces 1 cm pieces, was put into 1 ml of buffer solution (0.1 M carbonate, pH 10.5) and incubated with mild shaking at room temperature for 30 min. Then the extract was collected and centrifuged at 900*g* for 20 min. Eluted protein (EP) concentration and tryptophan fluorescence were assayed in the supernatant.

2.5. Hair homogenization

5 mg of hair, cut into 1 cm pieces was put into a Potter glass homogenizer with 1 ml of carbonate buffer solution and disintegrated into the homogenous suspension. After centrifugation for 30 min at 900*g* the supernatant was used for assay of soluble protein (SP), of SH-groups and of tryptophan fluorescence. If hair homogenization followed protein elution, the resulting protein fraction was marked as SP_e.

2.6. Protein assay

In the probes containing EP, SP or SP_e the protein concentration was assayed by light absorption at a wavelength of 240 nm using BSA as a standard to calculate the amount of protein. If indicated, the biuret test was also used.

2.7. SH content of soluble proteins (SP and SP_e)

Aliquots of supernatants, containing SP/SP_e were mixed with Tris-HCl buffer (0.4 M, pH 8.9), then DTNB (6.25 mM) was added and the absorption at 412 nm was read [19]. GSH solution was used as a standard. Finally the SH content of SP/SP_e was calculated (μmol/g).

2.8. Gel-filtration

Gel filtration was performed on a high-pressure column (Smartline, "Knauer", Germany) packed with Biofox 17 SEC (8 × 300 mm, V = 15 ml) in 0.05 M phosphate buffer at pH 7.4 and with a flow rate of 0.5 ml/min. The absorption at 280 nm was controlled. Bowman–Birk inhibitor (MW 8000 Da), human serum albumin (MW 66,500 Da) and aprotinin (MW 6500 Da) were used as standard proteins.

2.9. Fluorescence spectroscopy of eluted and soluble proteins (EP, SP, SP_e)

Fluorescence spectra of EP and SP_e samples were obtained in a Hitachi F-4000 spectrofluorometer. The excitation wavelength was 290 nm. The emission wavelength range was 300–550 nm and we used a 0.5 × 0.5 cm quartz cell.

2.10. Statistics

The results are presented as mean value ± standard deviation. The software used was STATISTICA 6.0. Significant differences in the mean values were evaluated by the Mann–Whitney or Wilcoxon tests. A p value below 0.05 was considered significant.

3. Results

3.1. Effects of UV irradiation of hair fibers on quantity of EP, SP, SP_e

To collect various protein fractions, we followed a previously described approach [20] based on mild protein elution (to prepare EP) and mechanical homogenization (to prepare SP and SP_e) of hair samples in a carbonate buffer solution (pH 10.5). This approach was applied to both UV irradiated and non irradiated hair of each subject as described in Section 2.

The first raw indicates if the proteins were eluted from hair before homogenization. UV irradiation significantly increased the quantity of EP (Table 1). The same effect was shown for SP and SP_e even if it was less pronounced these fractions it was less pronounced than for EP: increase by 20–35% (SP and SP_e correspondingly) vs 140% (in case of EP). Protein concentration was determined by absorption at 240 nm. In SP_e samples protein concentration was also measured by the biuret method which showed the same result – increase by 23%.

So, if UV irradiation of hair increased the quantity of soluble proteins within the fibers, some of these modified peptides were

Table 1
Quantitative changes in protein fractions EP, SP and SP_e after UV irradiation of tresses of 9 subjects for 6 h. The data are presented as percentage of control (control – values for non-irradiated hair).

Protein elution	Eluted protein (EP), (%)	Soluble protein (%)	
		SP_e	SP
–	–	–	120 ± 19*
+	242 ± 84*	135 ± 40*	

* $p < 0.05$ significant difference from control (Wilcoxon-test).

small enough to be eluted from the hair. As follows from the data in Table 1, these "small" peptides were influenced by UVR to a greater extent than the fraction of soluble protein. Nevertheless, there was no significant difference between the effects of UVR on the quantity of SP and that of SP_e.

Our data showed that mean EP content in non irradiated hair was 19 ± 8 mg/g of hair weight while SP content was 300 ± 100 mg/g so small peptides of the EP did not significantly contribute to SP and SP_e quantity.

3.2. Gel-filtration of SP sample

We examined the elution profiles of SP samples obtained from UV irradiated and non-irradiated hair tresses of the same subject.

One can see a peak corresponding to mixture of peptides with MW less than 8 kDa in both cases. For SP from irradiated hair the peak area is greater, mainly because of the peptide fraction with a MW range of 8–6 kDa. No peaks were registered in the V_o volume (about 670 kDa) and in even at the 18 min point (where HSA was eluted).

So the majority of SP under our experimental conditions consisted of low-molecular peptides (with MW < 8 kDa).

3.3. SH-content of soluble proteins

UV irradiation of hair fibers significantly affected the SH-content of soluble proteins. After UV irradiation it increased from 39 ± 6 to 85 ± 15 nmol/g in the SP fraction and from 45 ± 21 to 67 ± 18 nmol/g in the SPe fraction (in both cases $p < 0.05$). If the protein elution was performed before homogenization of irradiated hair (SP_e fraction), SH content was lower than in the SP fraction ($p < 0.05$). The difference between the SP and SP_e fractions could be explained by an easier elution of products rich in SH-groups. No difference in SH content was found between SP and SP_e in non-irradiated hair fibers.

The evaluation of the eluted thiols with Ellman's reagent showed significant difference resulting from the UV irradiation: 18 ± 12 nmol of SH per 1 mg of hair weight was eluted before and 230 ± 103 nmol of SH per 1 mg of hair weight – after irradiation ($p < 0.001$). At the same time, the quantity of EP raised only by 2–4 times.

In order to examine the effects of UVR on cystine/cysteine and protein elution in dynamics, hair fibers were irradiated with UV light for 6 h. In two hours intervals part of the irradiated sample was taken away for subsequent analysis of EP, SP and SP_e every two hours. The data were compared with those for non-irradiated hair (Fig. 2).

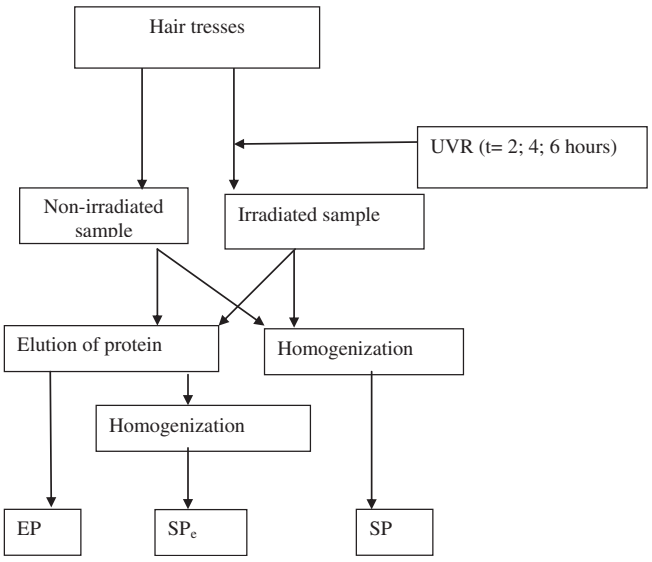

Fig. 2. Experimental scheme for the study into the effects of UV irradiation time on the proteins of hair tresses. Hair tresses from each volunteer were divided in two parts one of which was then irradiated and the other served as a control. EP – eluted proteins, SP – soluble proteins (obtained after homogenization of hair tresses without preliminary protein elution), SP_e – soluble proteins (obtained after homogenization of hair tresses after protein elution).

Thus the minimum irradiation time was 2 h while the maximum was 6 h. The analyses of EP showed increase in protein elution as early as after 2 h of exposure to UVR, and the further growth was registered after 4 h of UV irradiation (Fig. 3). At the same time, an increase in SH content of SP was registered, but not for SP_e (Fig. 4).

The maximum in SH content of SP was reached after 4 h of UV irradiation. If the soluble proteins were determined after protein elution from hair fiber (SP_e), the difference between irradiated and non-irradiated hair was less pronounced than for SP. We suppose that the pool of soluble ("light") proteins in hair cortex is homogeneous in cystine/cysteine content and that SH-rich peptides are eluted easier. Such an effect could result from UVR-induced destruction of disulfide bridges between proteins.

3.4. Intrinsic fluorescence spectroscopy

Protein photodamage involves aromatic amino acids such as tryptophan. Intrinsic fluorescence spectra of EP had maxima at

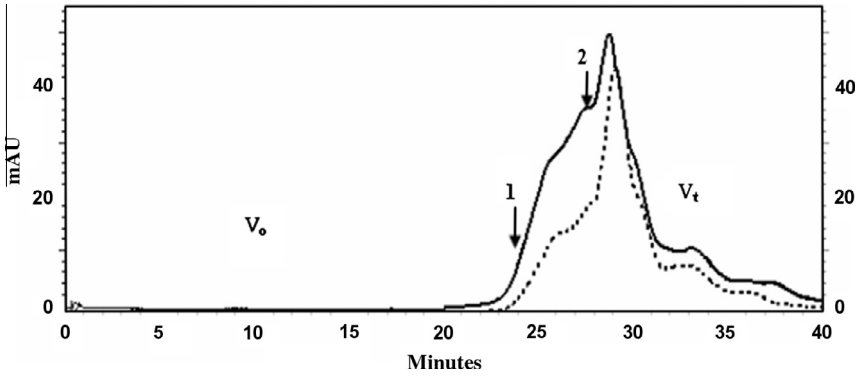

Fig. 1. Elution profile of the soluble protein (SP) from non-irradiated (dotted line) and UV-irradiated (solid line) hair. Gel filtration was performed on a column packed with Biofox 17 SEC (8 × 300 mm, V = 15 ml), in 0.05 M phosphate buffer, pH 7.4 and 0.5 ml/min flow rate. Standard proteins (arrows): (1) Bowman–Birk inhibitor (MW 8000 Da) and (2) aprotinin (MW 6500 Da).

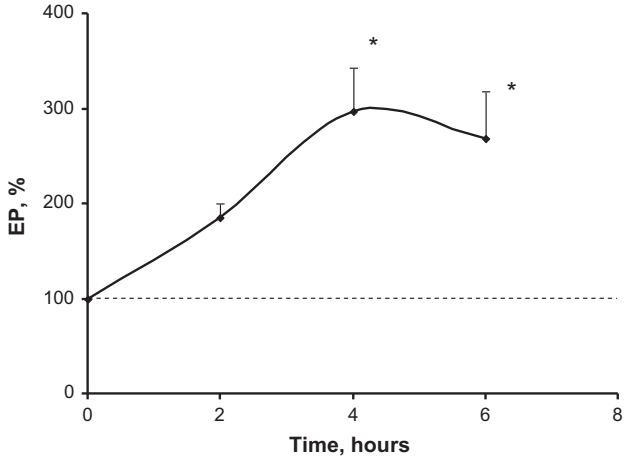

Fig. 3. Changes to the quantity of EP under exposure of hair to UVR. Data are presented as a percentage of the corresponding control (value for non-irradiated hair). At each experimental point the difference between irradiated and non-irradiated hair was significant ($p < 0.05$ according to the Mann–Whitney test, $n = 3$). * – significant growth vs values at "2 h" point ($p < 0.05$ according to the Mann–Whitney test).

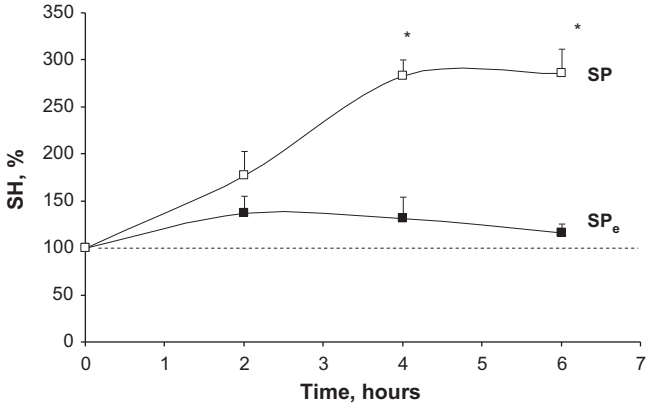

Fig. 4. Content of SH groups of the SP from UV irradiated hair (as % of the values for the corresponding non-irradiated samples). The hair was exposed to UVR for 2, 4 and 6 h. * – significant growth vs values at "2 h" point ($p < 0.05$ according to the Mann–Whitney test).

350 nm which is typical for tryptophan and/or in the area of 420 nm which is typical for, N-formylkynurenin, a product of tryptophan photooxidation. This is in good agreement with the previously published data, see Refs. [14,16].

Under UFR the fluorescence maximum at 350 nm for a 2.5 mM tryptophan solution fell with time course. Half an hour of irradiation led to a 48% decrease of fluorescence intensity for the tryptophan solution.

Comparison of the specific fluorescence intensity at 350 nm, F(350), of EP after UVR and of EP of non-irradiated hair from the same subjects showed a significant ($p < 0.005$) decrease of the value for irradiated samples. Fig. 5 demonstrates individual variance between 9 subjects in hair reaction to UVR determined as F(350)/μg/ml of protein. There was only one of nine cases where the value grew after the exposure (subject No 6).

No correlation was found between the visible hair color and changes in tryptophan fluorescence. The patient #6 was the only with curly hair but we have not found any report on the effects of hair shape on its photo degradation.

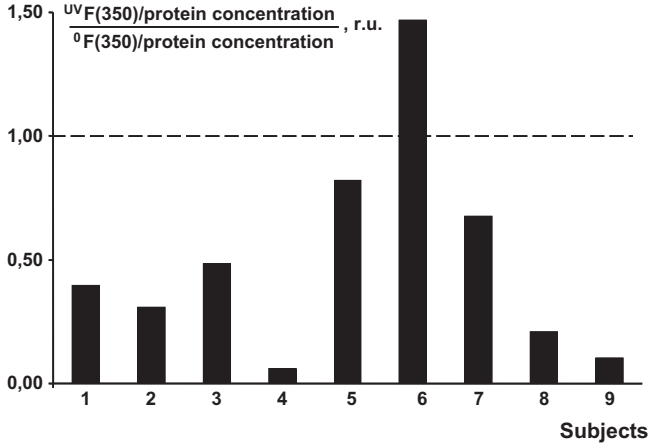

Fig. 5. Individual variance between subjects for reaction of hair to UVR determined as specific tryptophan fluorescence intensity of EP. ^{0}F(350)/protein concentration – specific fluorescence intensity of EP of non-irradiated hair. ^{UV}F(350)/protein concentration – specific fluorescence intensity of EP of irradiated hair. Excitation wavelength – 290 nm, emission wavelength – 350 nm. Significant difference of ^{0}F(350)/protein vs ^{UV}F(350)/protein is $p < 0.05$ according to the Wilcoxon test.

3.5. Comparison of distal and proximal hair fragments

Repeated exposure of hair to UV irradiation and to water (at washing) can influence the quality and/or quantity of EP and SP in the hair fiber. We assume that such changes should be detected when sections of hair are compared from a distal position with those positioned proximal to the root of the hair (if hair is long enough).

For this study eight healthy subjects with hair at least 20 cm long were invited to participate. 5-cm long hair fragments were cut from each sample: proximal (as 0–5 cm from hair root) and distal (as 15–20 cm from hair root).

The assay of EP and SP$_e$ was performed as described earlier. The results are summarized in Table 2.

According to data shown in Table 2 quantity of SP in the hair fibers grew going from proximal to distal fragments. SH-content of SP$_e$ decreased with hair length. Presumably, repeated UV irradiation destroys cystine in hair keratins and the peptides that were formed after disulfide bridges fission were washed away.

The specific fluorescence intensity of EP and SP$_e$ for tryptophan from the proximal hair fragments was lower than the equivalent values for the distal fragments ($p < 0.05$ both for EP and SP$_e$). The specific fluorescence intensity shifts, when adjusted for protein concentration or for hair weight showed similar tendencies. However for the ratio of F(350) to hair weight the effect was more pronounced (Fig. 6). Apparently a gradual loss of tryptophan occurs from the shaft, this agrees with the data in Table 2.

4. Discussion

An analysis of weathering on the soluble proteins of the hair shaft is an effective approach to understand alterations of hair

Table 2
Quantity of EP and and SH content of SP$_e$ for distal and proximal fragments of hair.

	Hair fragment, distance from root	
	0–5 cm	15–20 cm
EP, g/g	0.031 ± 0.007	0.025 ± 0.008
SP$_e$, g/g	0.12 ± 0.02	0.17 ± 0.02*
SH, μmol/g SP$_e$	14 ± 4	8 ± 2*

* Significant difference vs proximal fragments (0–5 cm) ($p < 0.05$ according to the Wilcoxon test).

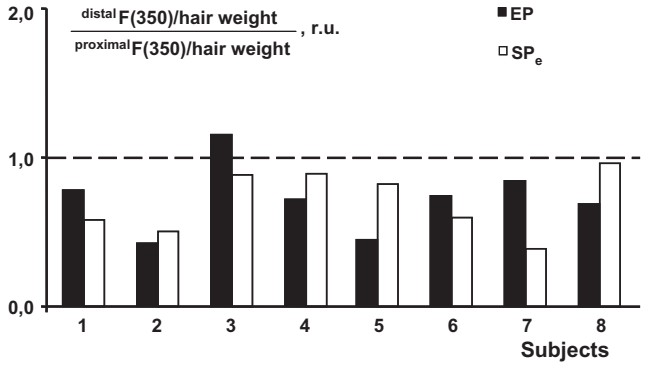

Fig. 6. Individual variance of the specific tryptophan fluorescence intensity of EP and SP_e between 8 subjects for proximal and distal hair fragments. $^{proximal}F(350)$/mg hair and $^{distal}F(350)$/mg hair are expressed on a weight basis in the sample. A significant difference of $^{proximal}F(350)$/mg hair vs $^{distal}F(350)$/mg hair is $p < 0.05$ according to the Wilcoxon test.

quality under bleaching, perming and exposure to ultraviolet irradiation [1,6,17,18,21].

The literature methods that are usually applied for extraction of hair proteins are based on their solubilization by SDS overnight at 65 °C [22] or for 5 h at 45 °C [1] with dithiothreitol at pH 9.0–9.5 and urea or 2-mercaptoethanol for 16–72 h [18,23].

Since our purpose was to detect UVR-induced changes in extracted and residual soluble peptides and proteins upon UV irradiation of hair we preferred mild experimental conditions in order to get the material unaffected by reducing agents and long incubation times. We used carbonate buffer solution (pH 10.5), and it was shown earlier that visible and UV light increase hair alkaline solubility [24].

Our data have shown growth in proteins elution by 142% (Table 1) which is consistent with data of other investigators (68% growth) [1] even though in our study the effect was significantly more prominent. The difference could result also from the parameters of the particular UV light sources and experimental protocols.

Earlier it was speculated that rise of eluted proteins after hair damage reflects the increasing quantity of soluble proteins in the shaft [18]. For this reason we studied both proteins eluted from the hair shaft fragments and soluble proteins obtained by homogenization of hair fibers.

We measured protein concentration, SH concentration and tryptophan fluorescence in the fraction of soluble proteins. Tryptophan is very sensitive to ultraviolet radiation which was the reason to analyze the intrinsic fluorescence spectra of the hair proteins in this study. The intrinsic fluorescence spectra (excitation wavelength – 290 nm) showed the fluorescence maximums near 350 nm (typical for tryptophan) and near 420 nm. The latter signal is usually considered to be a sign of the presence of tryptophan photooxidation products that were formed by UVR [13,14].

UVR exposure resulted in a decrease of the specific fluorescence intensity F(350) of proteins eluted from hair shaft, Fig. 5. However, one of nine subjects showed an increase of F(350) under UVR. Probably the destructive effect of UVR was manifested foremost in protein breakage and its subsequent elution rather than in tryptophan degradation. In our study the hair samples were irradiated at 254 nm where aromatic amino acids, disulfide bonds and melanin participate in the absorption of the light [21]. The protective effect of melanin could hardly explain the anomalous result observed for the sample #6 (Fig. 5) since it was dark-brown hair, like the other samples. But it was from a curly individual and maybe hair shape played some role here.

Exposure of hair to UVR significantly enhanced protein elution and the quantity of soluble proteins in hair fiber. Hair cortex contains an amorphous mass of keratin associated proteins (KAPs), maintaining regularity of spatial structure of keratin fibers [25]. 23 families of these proteins have been previously described, and a share of them have a significant amount of sulfur (10–20 kDa) or tyrosine (6–9 kDa) [26].

Our gel-filtration data for soluble proteins demonstrated that their molecular weight was near 6 kDa. After UVR exposure the amplitude of the peak grew and a fraction of larger protein molecules with weight near 8 kDa appeared (Fig. 1). Such an asymmetric widening of the peak could be due to the destruction of disulfide bonds between keratins and low-molecular-weight peptides rich in cystine and cysteine residues.

Fission of disulfide bridges via C–S or S–S splitting can proceed with sulfonic acid or cysteic acid as main products as shown for UVC radiation (254 nm) and UVA [21,24,27–29].

In our study the direct assessment of sulfhydryl groups with Ellman's reagent demonstrated increase in SH-content for the eluted and soluble proteins after UVR irradiation of hair. Rise of thiols could result from hair damage showing the same trend as that of the proteins released from damaged hair [30]. Another possible explanation could be the disulfide bond cleavage with formation of other then cysteic and sulfonic acids products, which can be determined with Ellman's reagent.

According to our data the loss of low-molecular SH-containing peptides occurs after protein elution. This could influence the properties of the remaining cortex (and cuticle) proteins as far as the lifetime of hair is 2–4 years and within this period UVR and water repeatedly affected them.

A natural model to test the hypothesis is the analysis of the factors of interest in distal and proximal fragments of the hair. In this work the hair at least 20 cm long that is considered representing about a year and a half of hair growth.

We evaluated the concentration of eluted and soluble proteins, content of sulfhydryl groups and fluorescence of tryptophan in soluble proteins in pieces cut 0–5 and 15–20 cm from the hair root. The distal pieces showed the growth in soluble proteins if compared with the proximal ones while SH content was decreased. Lower specific fluorescence intensity was also very typical for the proteins from distal fragments. These data are in accordance with published data found in Refs. [1,14] which demonstrated lower tryptophan content in the hair proteins found in distal parts of the hair. Taking into account both the effect of UVR exposure and the difference between distal and proximal fragments of the hair we suppose that in aqueous media elution of hair proteins with higher content of sulfhydryl groups and tryptophan residues occurs. The pool of the soluble proteins gradually loses cysteine and tryptophan as a result. Further investigations will help to understand better the mechanisms involving cysteic acid and SH-groups formation.

Cyclic hair growth hides these alterations in case of healthy subjects. However in hair with growth abnormalities, i.e. in patients with androgenetic alopecia, a significant increase of soluble (and eluted) proteins in the hair shaft was demonstrated for the occipital area [20]. Such patients with weakened hair could be in a risk group and need a particular hair protection from UVR.

5. Conclusions

The present results support that UV irradiation raises the solubility of hair proteins, presumably, due to the cleavage of disulfide bonds. The increased SH content has been registered in the fractions of the proteins eluted from hair fibers and of the soluble proteins resulting from hair homogenizing. Protein elution resulted in

the more prominent loss of thiols from irradiated hair fibers if compared with non-irradiated samples. As a result, the thiol content of the residual soluble proteins decreased after irradiation and elution of the hair. This is a possible way that could influence hair structure via cystine/cysteine modification.

The eluted and soluble proteins also showed decreased specific tryptophan fluorescence intensity so one can suppose that tryptophan photo degradation also contributes into the photomodification of hair proteins.

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