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Effect of wool keratin proteins and peptides on hair water sorption kinetics

C. Barba · M. Martí · A. Roddick-Lanzilotta · A. Manich · J. Carilla · J. L. Parra · L. Coderch

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Abstract Most beauty care products and treatments primarily affect the cuticle layers of hair fibers. The resulting damage has an adverse effect on hair water absorption. Water changes a wide variety of properties of human hair and is therefore of fundamental interest. Wool proteins are mild, natural, biodegradable, and sustainably produced with multiple functionalities and potential for use in the personal care and detergent market. In this study, the effect on hair water sorption of two types of keratin samples obtained from wool is investigated. Modifications of hair water sorption due to a bleaching treatment have been demonstrated, with lower values of water sorption capacity and an increase of the fibers permeability. Applications of keratin peptides and proteins to bleached hair improved the water sorption properties of the fibers and reduced their permeability.

Keywords Desorption · Diffusion · Hair · Thermogravimetric balance · Water sorption · Wool proteins

Introduction

Human hair is a keratinized fiber which is divided into three structural zones: medulla, cortex, and cuticle [1]. From a cosmetic point of view, it is the cuticle that gives hair a healthy or unhealthy look. Most beauty care products and treatments primarily affect the cuticle layers. Permanent waving, straightening or relaxing, and bleaching during hair coloring processes are major causes of hair damage [2, 3]. Deleterious effects on the hair attributed to these damaging treatments include poor manageability, dryness, brittleness, loss of shine, and decreased strength (fiber breakage).

Reactive cosmetic treatment of hair often alters the fiber structure. The resulting damage has an adverse effect on water absorption by hair at ambient humidity and leads to an increase in swelling or to liquid retention on wetting [4]. Water changes a wide variety of properties of human hair, and is therefore of fundamental interest to its cosmetic performance [5].

There is a growing consumer trend toward natural actives that have the potential to maintain hair with a healthy, youthful appearance [6]. It has been well-known for some time, e.g., that proteins and protein hydrolysates are very beneficial to the hair, imparting increased moisturisation, enhancing softness, and flexibility [7]. Hydrolyzed protein and its derivatives have been widely incorporated in hair-care and skin-care products due to their effectiveness in imparting properties, such as smoothness, luster, softness and elasticity, and their protective efficacy [8, 9].

Wool proteins are mild, natural, biodegradable, and sustainable with multiple functionalities, and have potential for use in the personal care and detergent markets. In this study, the effect on hair of two keratin proteins isolated from wool are investigated, an intact keratin intermediate filament protein extract (K-protein) and a low molecular weight keratin peptide from intermediate filament proteins (K-peptide). Due to the fact that it is isolated intact and in its natural state, the K-protein has the ability to form cohesive films which may have important implications in improvement of hair properties [10]. The cosmetic

C. Barba (✉) · M. Martí · A. Manich · J. Carilla · J. L. Parra · L. Coderch
IQAC (CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain
e-mail: cbaesl@iiqab.csic.es; cbaesl@iqac.csic.es

A. Roddick-Lanzilotta
Agresearch, Private Bag 4749, Christchurch, New Zealand

$$\text{ProteinS-SO}_3^- + \text{OH}^- + \text{HairSH} \leftrightarrow \text{ProteinS-SHair} + \text{SO}_3^{2-} + \text{H}_2\text{O}$$

Fig. 1 Chemical reaction of *S*-sulphonated form of the keratins

effectiveness of the wool peptide on skin has been previously demonstrated [11, 12]. Both keratins have cystine present in the active *S*-sulphonated form. This unique chemistry enables the keratin peptide and protein to reform disulphide bonds in damaged hair and directly affect hair properties (Fig. 1).

The determination of a water sorption isotherm by isothermally applying discrete, cumulative humidity changes comprises a dynamic and static aspect from which diffusion coefficients and equilibrium water contents are obtained [13]. Time/absorption isotherms provide a complete description of the absorption phenomena under particular conditions including initial regain of the sample, and absorption at varying temperature and relative humidity [5]. Several equations have been proposed for modeling of sorption isotherms, each of these models has some merit for determining equilibrium moisture content. Among the proposed models suggested by different researchers, the Guggenheim–Anderson–de Boer (GAB) equation has been successfully applied [14].

The main aim of this study is to apply two different *S*-sulfonated wool keratins, K-protein, and K-peptide to untreated hair fibers and to hair fibers subjected to a bleaching treatment. Sorption isotherms of untreated and treated hair samples are evaluated. The effectiveness of these keratin ingredients at restoring the water sorption characteristics of the fibers is determined.

Materials and methods

Materials

Ammonium Persulfate was supplied by Amresco (Ohio), Hydrogen Peroxide 30% by Merck (Germany), Keratin peptide [Keratec Prosina™, *MW* <1000D (SDS-PAGE)] and Keratin protein [Keratec IFP™, *MW* of 55kD (SDS-PAGE)] by Keratec Limited (New Zealand). Natural red hair tresses (20 cm in length) were purchased from De Meo Brothers Inc (New York).

Hair treatments

Hair was chemically damaged by bleaching. Hair was placed in a *bleaching solution* (9% H_2O_2, 1% ammonium persulfate, pH 8.3) for 3 h on a rocking table; then it was rinsed with water and air dried. For comparison, tresses of virgin hair were kept as the control.

Bleached (B) and untreated (UT) hair samples were treated with a 1% aqueous solution of the keratin peptide (UT-Kpep, B-Kpep) or the keratin protein (UT-Kpro, B-Kpro) for 1 h, rinsed with water and air dried at room temperature. Treatments were performed twice a day for 5 days.

Hair diameters

Hair diameters were found using an optic microscope (Axiophot Zeiss), under ×100 of magnification. Thirty diameter measurements were performed for each fiber, which were examined by an image analysis program (DPxViewPro).

Sorption experiments

Absorption and desorption curves were obtained using a thermogravimetric balance equipped with a controlled humidity chamber (Q5000SA Sorption Analyzer, TA Instruments, New Castle, USA). The mass of the keratin samples analyzed ranged between 6 and 9 mg. All experiments were conducted at 25 °C and a total gas flow of 200 mL min^{-1} and followed the same measuring procedure:

1. Initial drying: Temperature 60 °C and 0% relative humidity (RH) overnight.
2. Pre-stabilization: Temperature 25 °C, 0% RH and then initial adsorption kinetics at 5% RH.
3. Adsorption curve: The sample previously stabilized at 5% RH is subjected to progressively increasing by steps of 10% RH up to a maximum of 95% RH. The samples remained at each step until its mass reached equilibrium (defined by a change in mass of less than 0.02%/min for 10 min).
4. Desorption curve: The sample stabilized at 95% RH after the adsorption experiment is exposed to progressively decreasing steps of 10% RH down to a minimum of 5% RH. The samples remained at each stage until its mass reached equilibrium (defined by a change in mass of less than 0.02%/min for 10 min).

The high reproducibility of these measurements was established previously in the validation study of this instrument, in which three replicates on a single sample gave consistent sorption isotherms. Due to the long time taken to measure a complete isotherm (2.5 days), and the reproducibility of the data generated, only one measurement was performed on each sample.

Sorption isotherms are generally described by mathematical models based on empirical and/or theoretical criteria, which can be easily found in the literature. One of the most commonly used equations is the Guggenheim–Anderson–de Boer (GAB) model. This model has a theoretical background and its parameters provide a physical

meaning related to the sorption process, as compared to empirical models. The GAB model is based on the monolayer moisture concept and provides the value of the monolayer moisture content of the material [15]. The GAB model has found wide applicability in hydrophilic polymer [16, 17] and food [18] systems, and has considerable theoretical justification [19]. Thus, in this study, sorption isotherm data were treated according to GAB model following protocols described previously [20, 21]. The goodness of fit was evaluated by the determination coefficient (R^2).

The absorption/desorption curve of each step have also been fitted to a kinetic model which enables numerical values of this rate using the following kinetic model [5]:

$$R(t) = \frac{B\,t^C}{A^C + t^C}. \quad (1)$$

Being $R(t)$ the regain of the sample at time t, B the regain at the equilibrium (R_∞), A coincides with the time of half absorption ($t_{1/2}$), and c a power coefficient of each step.

The application of the non-linear regression procedures produces the best estimates of the model parameters giving B, A, and c, which enables us to calculate the asymptotic regain at the equilibrium R_∞, and the half absorption time $t_{1/2}$ and rate $v_{1/2}$. The nonlinear regression needs unbiased initial estimators of the model parameters which are given by the linear regression between $t/R(t)$ and t through the straight line $t/R(t) = \alpha + \beta t$, where α/β and $1/\beta$ are the initial estimators of A and B, respectively [5].

The diffusion coefficient has been obtained using calculations based on the solutions of Fick's diffusion equation applied to cylindrical geometry [22]. A simplified version of this solution is given in Eq. 2.

$$Ct\,/\,Ceq = 4(D\,t/\pi\,r^2)^{1/2} \quad (2)$$

where Ct is the concentration of the diffusant at time t, Ceq is the concentration at equilibrium, D is the diffusion coefficient, r is the fiber radius, and t is the time.

The numerical data from the sorption (or desorption) experiment can be converted into a plot of (Ct/Ceq) vs $(t/r^2)^{1/2}$. The initial part of this plot (for $(Ct/Ceq) < \sim 0.5$) will be linear in its slope (calculated by linear regression) is related to the diffusion coefficient as

$$D = (\pi/16)(\text{slope})^2\ \text{cm}^2\ \text{s}^{-1}. \quad (3)$$

Results and discussion

Temperature dependent isotherms for water absorption and desorption of human hair were determined. The hair sample was subjected to a common cosmetic treatment (bleaching) which is based on an oxidation process that affects the keratin structure of the hair [23]. Then both virgin and bleached hair was treated with keratin proteins or peptides.

Sorption isotherms of virgin and bleached hair before and after keratin treatments were evaluated and are shown in Fig. 2. Differences can be seen between the sorption isotherms of untreated and bleached hair samples. Evaluation of the moisture absorbed and desorbed at different

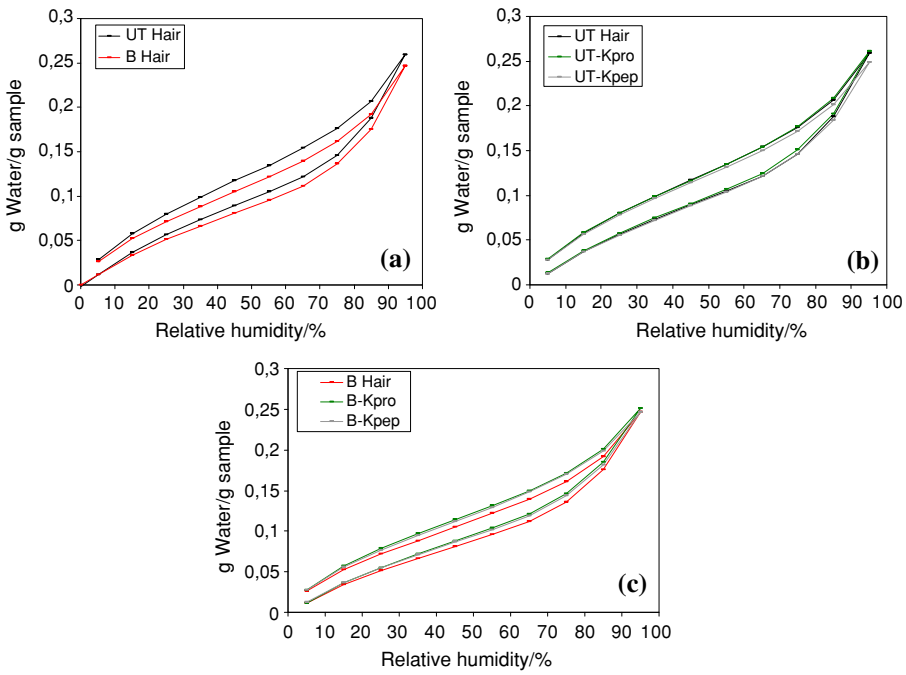

Fig. 2 Water sorption isotherms for virgin (UT) and bleached human hair with (**b** and **c**) and without (**a**) keratin treatments

relative humidity demonstrates that the bleach treatment damages the hair fibers, slightly reducing their moisture sorption capacity with respect to the untreated ones. This decrease in the moisture sorption for the bleached hair may be attributed to the chemical treatment which affects mainly the fiber surface and slightly decreases its ability to retain water.

Moisture sorption isotherms for the bleached and virgin hair after being treated with either the keratin protein or keratin peptide are also shown in Fig. 2. Very small change to the water uptake occurred for virgin fibers when treated with the two wool keratin samples (Fig. 2b, Table 1). Considering the results for the bleached hair treated with wool keratin protein and peptide (Fig. 2c), it can be seen that application of both keratin types lead to higher levels of moisture absorbed and desorbed across the range of relative humidity studied, when compared to the non-treated bleached sample (Table 1).

Therefore, from these results, it can be concluded that both wool keratin samples induce an increase on the water sorption for bleached hair fibers. This change in the moisture absorbed and desorbed, reaching values similar to that of the virgin hair sample, indicates a possible restoration of the fibers due to the keratin treatments. This is in accordance with an earlier study where an improvement of the moisture content of bleached hair due to the application of wool keratin peptide and protein was demonstrated [24]. The difference in water uptake of the virgin and bleached hair samples following keratin treatments is likely due to the higher substantivity of the keratins on the bleached fibers.

The regression of the experimental sorption data by the GAB model gives values of the monolayer capacity (*Wm*) and the energy constant (*Cg*) [25]. A good fit of the GAB model to the uptake and desorption data was achieved ($R^2 > 0.997$), the values obtained are shown in Table 1. Results show that both keratin treatments improve the water sorption characteristics of virgin and bleached fibers. There is an increase in the amount of water absorbed in the monolayer, although the bonding is weaker as indicated by

Table 1 Maximum moisture regain, GAB monolayer capacity (*Wm*), GAB energy constant (*Cg*), and GAB determination coefficient (R^2) for Virgin and Bleached human hair non-treated (UT) and submitted to the K-protein and K-peptide treatments

	Regain at 95% RH/%		*Wm*/%		*Cg*		R^2	
	Virgin	Bleached	Virgin	Bleached	Virgin	Bleached	Virgin	Bleached
UT	25.87	24.60	7.487	6.630	6.706	6.803	0.9976	0.9982
K-Peptide	25.04	24.69	7.565	8.829	6.588	5.173	0.9991	0.9996
K-Protein	26.04	25.09	7.725	7.677	6.644	6.245	0.9985	0.9979

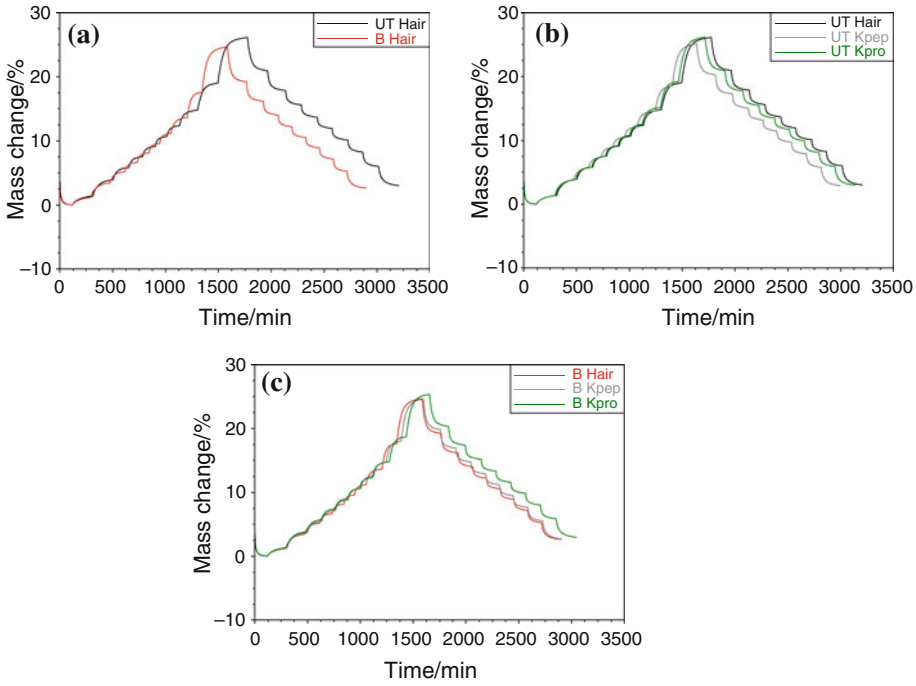

Fig. 3 Moisture uptake for virgin (UT) and bleached (B) human hair with (**b** and **c**) and without (**a**) keratin treatments as a function of time

Table 2 Fiber diameters, diffusion coefficient (D) and time of half absorption/desorption ($t_{1/2}$) for virgin and bleached hair with and without K-protein and K-peptide treatments (mean value ± SD)

	Diameter/μm		D/cm^2 s^{-1}		$t_{1/2}$/min	
	Virgin	Bleached	Virgin	Bleached	Virgin	Bleached
UT	73.8	88.6	4.29E−0.7 ± 4.4E−0.7	8.67E−0.7 ± 9.5E−0.7	24.09 ± 9.15	20.86 ± 10.36
K-Peptide	74.6	79.0	5.13E−0.7 ± 5.2E−0.7	4.74E−0.7 ± 4.8E−0.7	22.16 ± 8.84	22.80 ± 9.98
K- Protein	76.2	73.8	4.30E−0.7 ± 4.4E−0.7	5.65E−0.7 ± 5.9E−0.7	23.59 ± 9.38	22.10 ± 8.98

lower values of the energy constant. Again, this increase is much more important when the bleached hair sample is subjected to the wool keratin treatments reaching higher monolayer capacity values than that obtained for the virgin hair sample. This modification indicates an increase of water binding sites in hair which can be attributed to the presence of the keratin peptide and the keratin protein in/on the hair fibers.

The rate at which equilibrium is achieved, it is a good indicator of the sample condition. The kinetics of moisture sorption was evaluated for all hair samples studied and curves are shown in Fig. 3. Virgin hair reaches equilibrium more slowly than bleached hair (Fig. 3a). Virgin hair is more hydrophobic than bleached hair because has less anionic surface groups, thus has less driving force for water adsorption. Application of the keratins to virgin hair appears to increase the rate at which the hair reaches equilibrium indicating that the keratins themselves uptake and release water faster than undamaged virgin hair (Fig. 3b). Evaluation of the kinetics of moisture uptake and loss at each RH step used to build up the isotherm demonstrate that both keratin treatments altered the rate at which the bleached hair reached equilibrium moisture content at any given RH (Fig. 3c). When the bleached hair is subjected to either wool keratin treatment the time to reach equilibrium is increased, on both the absorption and desorption cycle. Thus, the wool keratins may limit the quantity of moisture absorbed when hair is exposed to environments with high levels of humidity over short time periods, therefore preventing styling problems associated with limp over-hydrated hair. In addition, the wool keratin samples increased the absolute amount of water absorbed into bleach-damaged hair over a wide range of moderate RH conditions which may prevent problems associated with dry hair, such as poor texture, increased tendency for split-ends, and poor tensile/bending strength [26].

The moisture diffusion kinetics for the hair fibers has also been evaluated. Hair diameters have been calculated as a mean value of 30 diameter measurements for each fiber as described in the experimental part. The diffusion coefficients (D) and the time of half absorption/desorption ($t_{1/2}$) have been calculated as detailed in the experimental section for each humidity step. The mean values of fiber diameter, diffusion coefficients, and time of half absorption/desorption are summarized in Table 2. The time of half absorption/desorption ($t_{1/2}$) for each RH step has also been calculated, results are shown in Fig. 4.

There is an inverse relationship between the time parameter and the diffusion coefficient, i.e., a higher time is needed to reach equilibrium for fibers with small water permeability and, therefore, a small diffusion coefficient. Results demonstrate that when the keratin samples are applied to intact virgin hair very little changes are found. However, when damaged (in this case, bleached) hair is used, treatment with either wool keratin sample appears to induce an improvement in the fiber integrity, as evidenced by an increase on the mean value of $t_{1/2}$ and increased water uptake for the majority of the RH steps (Fig. 4). In addition, there is a decrease in the apparent diffusion coefficient demonstrating a decrease of the fiber water

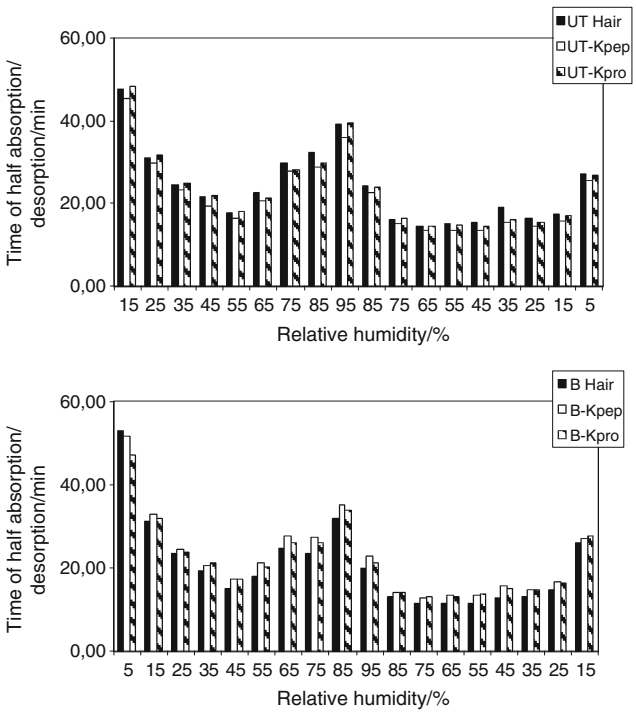

Fig. 4 Time of half absorption/desorption in min for virgin (UT) and bleached (B) human hair with and without keratin treatments as a function of time

permeability. It is important to emphasize that treatment of the bleached hair with the keratin peptide and the keratin protein decreases its water permeability reaching the same values as those of the virgin hair subjected to the keratin treatments. As described in previous study [24], the keratin protein has the ability to form a cohesive film in the hair surface; this keratin coating may act to prevent/decrease water diffusion through the hair fibers. Furthermore, the keratin peptide has been demonstrated to improve the mechanical properties of damaged fibers [24]. The overall effect of the keratin protein and peptide treatment is to restore the damaged hair to its original water permeability levels.

Conclusions

Modification of hair water sorption properties following a conventional cosmetic bleaching treatment has been demonstrated. A reduction in the fiber moisture sorption capacity and an increase in the fiber permeability were observed.

Application of the keratin peptide and protein was demonstrated to improve the moisture absorbtion/desorption capacity of bleached hair.

Keratin peptides and proteins were shown to restore the integrity of chemically damaged fibers, inducing a decrease in the fiber diffusion coefficient, which in turn indicates improved (decreased) permeability of the hair fibers.

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