Fundamental DSC investigations of α-keratinous materials as basis for the interpretation of specific effects of chemical, cosmetic treatments on human hair

F.-J. WORTMANN, G. SENDELBACH, and C. POPESCU,
*University of Manchester, School of Materials, P.O. Box 88, Manchester
M60 1QD, UK (F.-J.W.), Wella Service GmbH, Berliner Allee 65,
D-64274 Darmstadt, Germany (G.S.), and DWI at RWTH Aachen
University, Pauwelsstrasse 8, D-52074 Aachen, Germany (C.P.).*

Synopsis

α-keratinous materials can be considered as two-phase, filament/matrix composites, in which partly crystalline, α-helical intermediate filaments (IF) are embedded in an amorphous matrix of IF-associated proteins (IFAP). Differential Scanning Calorimetry (DSC) of keratins in water was found to be especially suited to analyze various aspects of the thermal stability of these main morphological components. Results and considerations are reviewed, which were gained by applying the principles derived from fundamental investigations to the specific effects of oxidation (bleaching) and reduction (perm-waving). Properties and interactions of the main morphological components of human hair are considered that are specifically related to the various aspects of their thermal stability. The overall view of the results shows that the course of the thermal unfolding of the α-helix in the IFs is independent of the chemical history of hair. The matrix properties are the primary factor controlling the kinetics of the onset of the denaturation process in the IF/IFAP-composite.

INTRODUCTION

Hair, such as animal or human hair, grows in various types on all parts of the body and from cavities in the skin, called follicles, which are embedded up to 3 mm deep in the *dermis* and extend to the surface of the skin through the *epidermis* and the *stratum corneum* (1). During its growth the hair fiber, in the same way as other α-keratin materials, such as hoof, horn, and quill, develops complex morphological fine structures (2). Figure 1 shows a graphical representation of the structure of a wool fiber, as possibly the best investigated α-keratin hair fiber (3,4).

A hair fiber is constituted of cells that differentiate during hair growth to form, namely, the fiber core (cortex) and an outer protective layer (cuticle). The interface between the
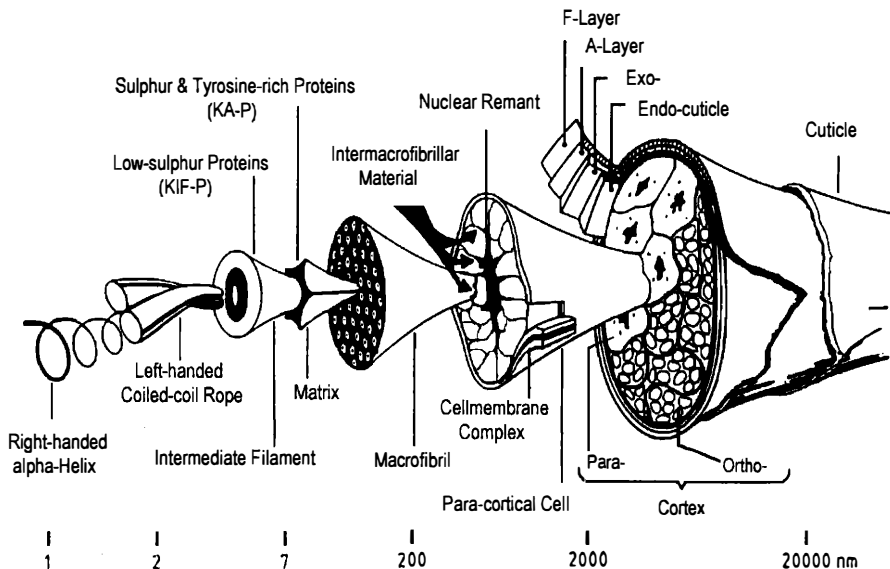
Figure 1. Graphical representation of the morphological components of a Merino wool fibre (Adapted by Zahn *et al.* (4) from a drawing by R. D. B. Fraser 1972).

cells, the cell membrane complex (cmc), is a continuous phase of proteins, lipoproteins, and adjacent membrane lipids. During hair growth, the cortex cells assume a spindle-like shape (length: approx. 100 µm, largest diameter: approx 5 µm). The cells express proteins that form as major cell component axially oriented, partial α-helical interme-diate filaments (IF, diameter about 10 nm) embedded in an amorphous matrix of IF-associated proteins (IFAP). The IFAPs are highly cross-linked through cystine, which forms mainly inter-chain disulphide bonds. The cells that were originally at the pe-riphery of the hair root form thin, flat cuticle cells that each have a layered structure of amorphous proteins. These cells form a protective layer of overlapping cells around the cortex.

In the context of mechanical or thermal investigations this complex structure can be simplified as a two-phase, filament/matrix composite, as originally proposed by Feughel-man (5). In this model the partly crystalline, α-helical intermediate filaments (IF) can be identified as the filamentous phase. The matrix in consequence contains as major component the IF-associated proteins (IFAP) (6) and also summarily the rest of the morphological components, such as cuticle, cell membrane complex, etc. (4). DSC of keratins in water was found (7) to be especially suited to analyze various aspects of the thermal stability of these main morphological components, since the helical segments in the IFs undergo denaturation between 110°C and 160°C in water, depending on the type of keratin, and well removed from the temperature range of pyrolysis (8,9).

Various aspects of this transition of fundamental as well as of practical relevance are reviewed, specifically considering the current theory on the interaction of filaments and matrix during denaturation and its relation to the effects of cosmetic, chemical treat-ments, namely, oxidation (bleaching) and reduction (perm-waving).

DSC OF DIFFERENT α-KERATINS IN WATER

EXPERIMENTAL

All DSC-experiments were performed on a power-compensated instrument (DSC-7, Perkin Elmer), using pressure-resistant (25 bar) stainless steel, large-volume capsules (Perkin Elmer). Prior to the measurement samples were stored under constant, ambient room conditions (approx. 22°C, 55%RH) to ensure invariant water contents. Under these conditions a given material (5–7 mg) was weighed into the sample container, 50 µL of water was added and the container was sealed. An empty container without the O-ring rubber seal was used as reference. Temperature range and heating rate were generally 70–180°C and 5°C/min, respectively. Analyses were conducted between 9- and 18-fold, depending on the complexity of the DSC-curve. Amino acid analyses were performed by the ion-exchange method on an LC6000 Amino Acid Analyzer (Biotronic). The amino acid referred to in what follows is the double amino acid cystine, containing a disulfide crosslink. Its contents in the various keratins is reported by referring to the mono amino acid, and specifically to the molar fraction of cysteine residues (Cys-R, [mol%]). The expectation value for the 95% confidence limits of the Cys-R values is ±5%.

RESULTS

Figure 2 shows typical DSC curves for mohair (hair of the Angora goat) and Merino (sheep) wool in water. As further materials rhinoceros horn (RH), porcupine quill (PQ), finger nail (FN), echidna quill (EQ), human hair (HH), and afro hair (NH) were investigated (7).

Usually keratins show a single-peak structure for the denaturation, though double peaks were frequently observed for wool, as was to be expected from the literature (8,10). This tendency, though considerably less pronounced, was also observed for finger nails, horse hair, and rhinoceros horn. The statistical analysis of the data for the denaturation enthalpies for the various keratins supports the conclusion that for all materials the
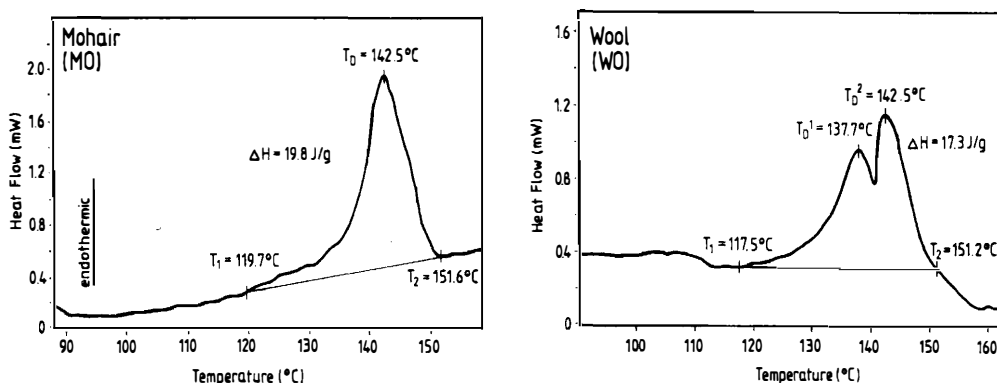

Figure 2. DSC traces for mohair (MO) and merino wool (WO) under standard conditions (Adapted from Ref. 7). The relevant parameters are graphically defined.

enthalpy is largely material invariant (7). Assigning the enthalpy exclusively to the denaturation of the helical material, leads to the conclusion that thus also the helix content for these materials is largely the same, estimated to be 25–30%, in accordance with literature data (8).

In contrast, the denaturation temperature T_D increases strongly with the cross-link density of the matrix, which is effected by the double amino acid cystine linking two protein chains. The results are summarized in Figure 3. Regression analysis shows that for the majority of the keratins a linear model well describes the interrelation between T_D and Cys-R. The solid line in Figure 3 is based on all T_D-values, except those for rhino horn, which due to its low amount of matrix material appears to be an exception. Using a log(Cys-R)-relationship the values for RH may be readily included (7).

From Figure 3 it can be concluded that, though the denaturation enthalpy and the temperature range in which the denaturation takes place are material invariant, the thermal stability of the helical structures, i.e. the denaturation temperature, is controlled by the amount and the cross-link density of the surrounding non-helical matrix material.

This view is supported by investigations of the double peak structure of the DSC-curves, consistently observed namely for Merino wool. It was shown that the effect is due to the occurrence of two cell types in the fiber, *ortho*- and *para*-cortical cells, which differ significantly in the sulfur content of their IFAPs, consistent with the observed bimo-dality of the denaturation temperature (11). Increasing the heating rate, leads to an increase in T_D. The size of the effects is, however, quite different for the two cell types (12).

EFFECTS OF OXIDATION AND REDUCTION

Against this fundamental background, DSC was found to be especially suitable to assess various aspects of the changes filaments and matrix undergo through chemical, cosmetic
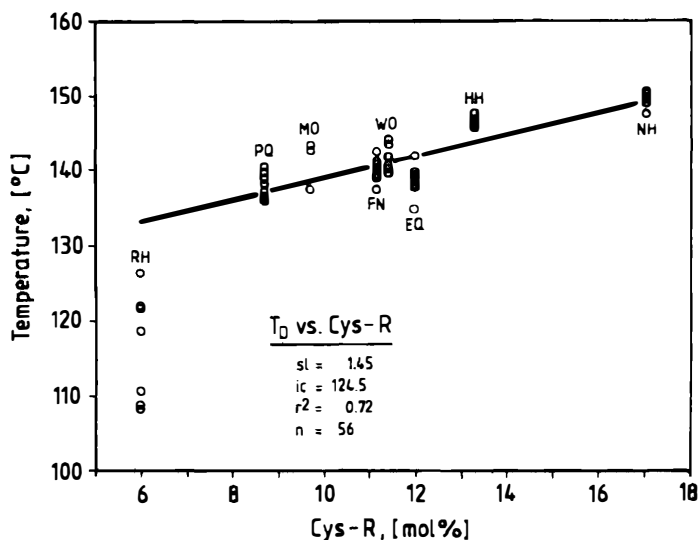

Figure 3. Denaturation temperature T_D for single peak endotherms against Cys-R content. The solid line describes the linear regression through all data, except for rhinoceros horn (RH). The parameters for the linear regression are given (Adapted from Ref. 7).

processes. In this context European human hair was investigated that had undergone either multiple oxidative, bleaching or reductive, perm-waving treatments (13).

For both types of treatments, enthalpy decreases by following apparent first-order kinetics with respect to the number of treatments and treatment times. Figure 4 compares the dependencies between denaturation temperature and enthalpy for the two types of processes. The linear relationship for bleached hair in Figure 4A indicates that the oxidative treatment leads to largely homogeneous damage in IFs and IFAPs. For the reductive treatment, enthalpy drops much faster than denaturation temperature, giving evidence that the reductive damage, in comparison, is more pronounced in the helical segments of the IFs compared to the surrounding, highly sulfur cross-linked matrix.

KINETIC ANALYSIS

For the oxidatively treated hair the course of the denaturation was further investigated by a kinetic analysis of the DSC-curves. Oxidation was chosen, since it represents the comparatively straightforward case, where the treatment affects both morphological components to very similar extents.

The kinetic analysis, assuming *a priori* a first-order denaturation process and using the Friedman-method, is based on (14):

$$d\alpha/dt = (1 - \alpha) \cdot A \cdot \exp(-E_A/RT) \tag{1}$$

where α is the degree of conversion, determined from the DSC-curves. A and E_A are the pre-exponential factor and the activation energy in the Arrhenius law, respectively, describing the reaction rate constant. R is the gas constant.

The analysis shows (15) that the curves can be well described by this model for a wide range of the degree of conversion. Activation energy and pre-exponential factor (as lnA) each show only a comparatively small and highly correlated decrease with the number of treatments. Since the correlation between the two parameters cannot be explained by mathematical compensation effects, it is concluded that keratins may well show a genuine enthalpy-entropy compensation effect (16) for the denaturation of the α-helix in the IF/IFAP composite. This is in pronounced contrast to the change of the denaturation
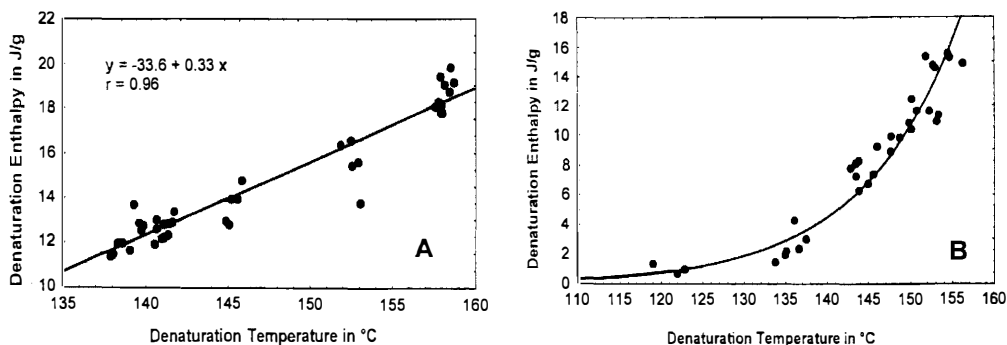

Figure 4. Denaturation enthalpy plotted vs T_D for the oxidized (A) and reductively treated samples (B). In Fig. 4A the regression line is given, while the solid line in Fig. 4B is a guide for the eye (Adapted from Ref. 13).
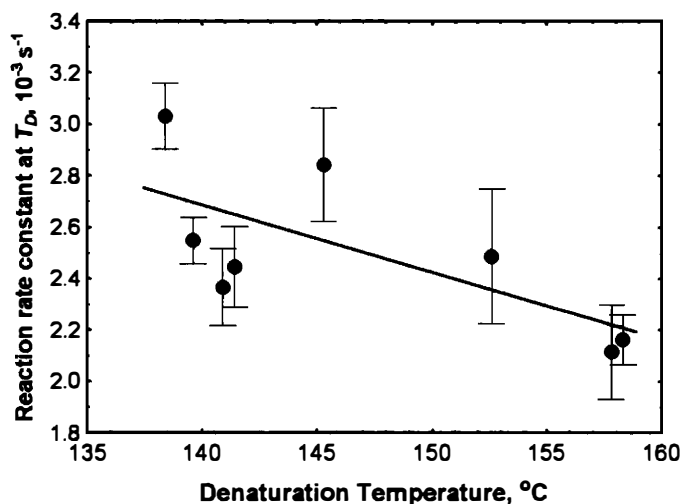
Figure 5. Reaction rate constants at the peak temperatures, $k(T_D)$, for hair samples, repeatedly treated by oxidation. The whiskers denote the standard deviations for fivefold determinations (15).

temperature from 158°C for untreated hair to 138°C after the 7th treatment and a concurrent decrease of the relative amount of native, denaturable α-helix by 40% (13).

In view of this compensation, a more comprehensive parameter to assess the changes of the denaturation process is the rate constant at the respective peak temperatures. These were determined from the individual experimental curves (15) and are graphically summarized vs. T_D in Figure 5. A slight increase of $k(T_D)$ is observed with decreasing temperatures, that is with increasing oxidative changes. This reflects the fall of the activation energy, which overrides the decrease of the pre-exponential factor, which is linked to a decrease of the activation entropy (15).

CONCLUSIONS

The results from the various facets of the investigation show that the kinetic hindrance of the unfolding of the α-helix by the matrix in the IF/IFAP-composite is in fact the primary controlling mechanism of the onset of the denaturation process. Once the temperature rise in combination with the natural composition and/or the chemical change has induced a suitable drop of the viscosity of the matrix around the IFs, their denaturation occurs along a process pathway that is largely independent of temperature and of the previous treatment.

REFERENCES

(1) A. Schwan-Jonczyk, G. Lang, T. Clausen, J. Koehler, W. Schuh, and K. D. Liebscher, "Hair Preparations," in *Ullmann's Encycl. Ind. Chem.,* 3.Ed. (Wiley-VCH, Weinheim, D, 1998).
(2) R. D. B. Fraser, T. P. MacRae, and G. E. Rogers, *Keratins: Their Composition, Structure, and Biosynthesis* (C. C. Thomas, Springfield, Il, 1982).
(3) M. Feughelman, *Mechanical Properties and Structure of Alpha-Keratin Fibres* (UNSW Press, Sydney, Australia, 1997).

(4) H. Zahn, F.-J. Wortmann, G. Wortmann, K. Schaefer, R. Hoffman, and R. Finch, "Wool," in *Ullmann's Encycl. Ind. Chem.,* 6.Ed. (Wiley-VCH, Weinheim, D, 2003).

(5) M. Feughelman, A two-phase structure for keratin fibers, *Text. Res. J.,* **29,** 223–228 (1959).

(6) D. A. D. Parry and P. Steinert, Intermediate filaments: Molecular architecture, assembly, dynamics and polymorphism, *Quarterly Rev. Biophys.,* **32**(2), 99–187 (1999).

(7) F.-J. Wortmann and H. Deutz, Characterizing keratins using high-pressure differential scanning calorimetry, *J. Appl. Polym. Sci.,* **48,** 137–150 (1993).

(8) M. Spei and R. Holzem, Thermoanalytical determination of the relative helix content of keratins, *Colloid Polym. Sci.,* **267,** 549–551 (1989).

(9) V. F. Monteiro, A. P. Maciel, and E. Longo, Thermal analysis of Caucasian human hair, *J. Thermal Anal. Calorim.* **79,** 289–293 (2005).

(10) A. R. Haly and J. W. Snaith, Differential thermal analysis of wool—The phase transition endotherm under various conditions, *Text. Res. J.,* **37,** 898–907 (1967).

(11) F. -J. Wortmann and H. Deutz, Thermal analysis of ortho- and para-cortical cells isolated from wool fibers, *J. Appl. Polym. Sci.,* **68,** 1991–1995 (1998).

(12) H. Deutz, *Thermische und mikroskopische Charakterisierung von Keratinen,* PhD-thesis, DWI at RWTH Aachen University of Technology, Aachen, Germany, 1993.

(13) F.-J. Wortmann, C. Springob, and G. Sendelbach, Investigations of cosmetically treated human hair by differential scanning calorimetry in water, *J. Cosmet. Sci.,* **53,** 219–228 (2002).

(14) M. E. Brown, *Introduction to Thermal Analysis* (Chapman and Hall, New York, 1988).

(15) F.-J. Wortmann, C. Popescu, and G. Sendelbach, Nonisothermal denaturation kinetics of human hair and the effects of oxidation, *Biopolymers* **83,** 630–635 (2006).

(16) K. Sharp, Entropy-enthalpy compensation: Fact or artefact?, *Prot. Sci.,* **10,** 661–667 (2001).