International Journal of Biological Macromolecules 89 (2016) 592–598

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Keratin made micro-tubes: The paradoxical thermal behavior of cortex and cuticle

Daniel Istrate[a], Meriem Er Rafik[b], Crisan Popescu[c,*], Dan E. Demco[d], Larisa Tsarkova[d], Franz-Josef Wortmann[e]

[a] DSM Resolve, Urmonderbaan 22, 6160 MD Geleen, The Netherlands
[b] Institut Charles Sadron, CNRS, 23 rue du Loess, 67034 Strasbourg Cedex, France
[c] KAO Germany GmbH, Pfungstaedterstr. 98-100, 64297 Darmstadt, Germany
[d] DWI—Leibniz-Institute for Interactive Materials, Forckenbeckstr. 50, 52056 Aachen, Germany
[e] School of Materials, University of Manchester, Manchester M13 9PL, United Kingdom

ARTICLE INFO

Article history:
Received 7 April 2016
Received in revised form 10 May 2016
Accepted 10 May 2016
Available online 11 May 2016

Keywords:
Keratin fibre
Cuticle
Thermal stability
Micro-tubes

ABSTRACT

Keratin micro-tubes were obtained by heating medullated keratin fibres to temperatures above 230 °C under nitrogen atmosphere, when, as documented by microscopy, the cortex (the core of the fibre) melts from the medulla outwards, followed by pyrolysis of the material through the remaining solid cuticle (shell) layer. The resulted hollow tubes from fibres void of cortical material keep the external cuticle structure, as shown by AFM investigation, and the moisture sorption properties of the initial keratin fibre. Despite similar amino-acid compositions of cuticle and cortex the two morphological components differ significantly in their thermal behaviour, which appears to be a "cortex-cuticle thermal stability paradox".

1. Introduction

The hair is a filamentous appendage of the skin of vertebrates, which serves to protect the body against environmental influences. It is made of filamentous proteins, the hard alpha-keratins [1–3]. The keratin fibres have a composite structure, with a core-shell as well as filament-matrix arrangement on various levels of organisation, from the cortex wrapped by cuticle down to the intermediate filament (IF) surrounded by intermediate filament associated proteins (IFAP), also referred to as keratin associated protein (KAP) [1–3].

The keratin fibres exhibit a relatively high thermal stability; the fibre properties remain almost intact until 200 °C [4–6]. The ability of keratins to preserve their properties up to high temperatures, apart from the obvious benefit for body protection, is of interest for the potential to design thermally highly stable proteins [6].

DSC investigations of keratin fibres in the dry state show an endothermic effect, sometime a doublet, which at 10 K/min heating rate ranges from 230 to 260 °C [4,7,8]. The endothermic effect is generally attributed to the thermal denaturation of the α-helix which makes the crystalline part of the keratin fibre [5,6,8,9].

Cuticle is formed of four layers with different cross-link densities due to their contents of disulphide and iso-dipeptide bonds, namely from the outside, epi-cuticle, a-layer, *exo*-, and *endo*-cuticle, respectively [3]. Because the volume fraction of the cuticle is of the order of 10% of the total keratin fibre and because it has a similar chemical structure as the cortex, cuticle contributions are often neglected when hair properties are determined [10]. Our results show, however, that the cuticle has to be understood as a different component of the fibre with very individual properties, which require more investigation.

2. Experimental

As alpha-keratin material we used the commercial, European, brown hair purchased from Kerling Int. Haar Fabrik. L-amino-acids of analytical grade were purchased from Merck.

Preparation of cuticle: snippets of hair were mechanically abraded as described in methodology published elsewhere for isolating cuticle from cortex [11]. Microscopic evidence showed that the separation of cuticle was successful.

The heating experiments were carried out on a DSC-7 (PerkinElmer), using closed aluminium pans, whose lids were

* Corresponding author.
E-mail address: crisan.popescu@kao.com (C. Popescu).

http://dx.doi.org/10.1016/j.ijbiomac.2016.05.035
0141-8130/

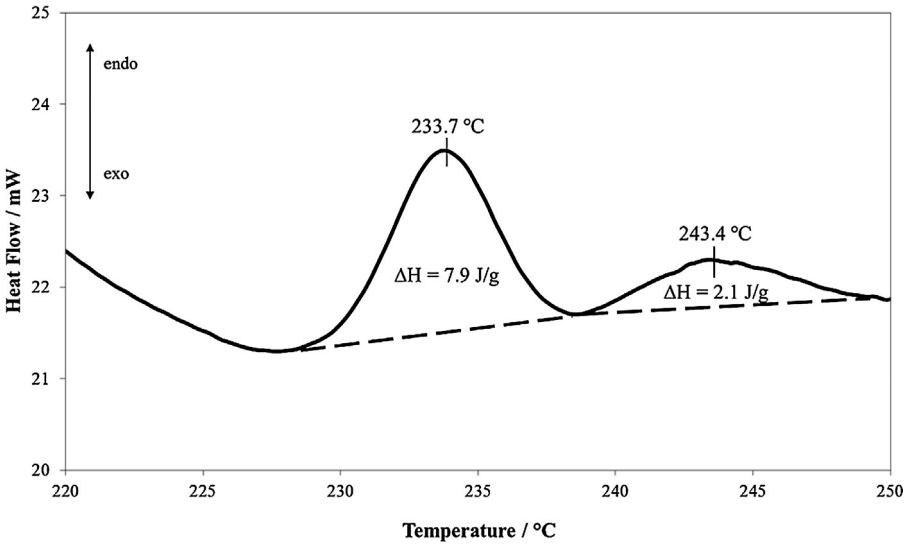

Fig. 1. DSC trace of hair heated with a rate of 10 K min^{-1}. The temperatures of the peaks and the estimated values of enthalpy are shown on the curve. The dashed lines are the baselines drawn for evaluating the areas of the peaks (enthalpy).

pierced with two holes. An empty crucible was used as reference. The DSC device was calibrated using indium and palmitic acid, both of high purity.

Prior to the measurements, the hair samples were cut into 1–2 mm snippets and stored under constant, ambient room conditions (approx 22 °C, 55% relative humidity) to ensure invariant water contents. Samples of 7–10 mg were heated with a heating rate of 10 K min^{-1} under a nitrogen flow of 20 mL min^{-1}, for temperatures ranging from 50 to 300 °C. After reaching certain aimed temperatures the heating was stopped and the sample rapidly cooled. This way we were able to collect samples before, at, and after the endothermic peak recorded on DSC.

Thermo-gravimetrical analysis (TGA) of amino-acids was performed on an Iris TG209C (Netzsch). Samples of around 10 mg of pure amino-acid were placed in the alumina crucibles and heated at 10 K min^{-1} under a nitrogen flow of 20 mL min^{-1} from room temperature to 400 °C.

Thermo system FP 90 with FP82 hot stage (Mettler Toledo) and optical microscopy were used to follow the events observed at DSC under normal atmosphere.

Scanning Electron Microscopy photos were taken for gold sputter-coated snippets sampled at temperatures chosen from the DSC curve to lie before, at, and after the peak using a SEM S360 (Zeiss NTS GmbH, Oberkochen) at an acceleration voltage of 15 kV.

Amino-acid analysis was carried out on the snippets before and after the peak. Each sample was hydrolysed in 3 mL 6 N HCl at 110 °C for 24 h. The hydrolysed samples were dried in a rotary evaporator under heating. Amino acid analysis was carried out using an analyser type Alpha-Plus II, (Pharmacia).

Scanning force microscopy (SFM) measurements have been performed in Tapping mode (Bruker, ICON) using Nanoscope 8.10 software and OTESPA tips (spring constant 12–10^3 N m^{-1}, resonant frequency 278–357 kHz) under ambient conditions. The samples of the native hair and of the keratin micro tubes have been fixed on pieces of silica substrates using double-side adhesive tape (Tesafilm®).

Moisture sorption-desorption isotherms were measured on an IGA Sorp Moisture Sorptiometer Analyser (Hiden Analytical), at 25 °C, using a programme of increasing the relative humidity, RH, in steps of 10–15%, from 1% to 95% RH.

3. Results and discussion

The DSC plot in Fig. 1 shows the endothermic effects occurring at around 240 °C. The first effect is attributed to the melting of the crystalline phase, that is the unfolding of the alpha helices, and is termed as the thermal denaturation of keratin fibres [4]. The process is irreversible and kinetically controlled by the surrounding environment, which is the amorphous matrix phase, made of IFAPs, in which the alpha-helices are embedded [4–6].

The second effect, less visible in most experiments, is attributed to the pyrolysis of the fibre [4,5,9].

We sampled snippets at various temperatures up to 300 °C and examined them in the scanning electron microscope (see Fig. 2).

One notices that at temperatures beyond 230 °C the cortex seems to vanish and the original fibres converted into tubes, consisting only of cuticle. Because the diameter of the tubes matches those of original hair fibres, of around 50 μm in this particular case, we term them “micro-tubes”. At around 300 °C the micro-tubes appear to become highly brittle and disintegrate into small pieces.

The results shown in Fig. 2 suggest that the thermal stability of cortex and of cuticle differ significantly. Similar results were obtained on fibres sourced from various mammalians, indicating that this is a general property of keratin fibres [12].

More detailed information on the surface topography and heterogeneity of the keratin micro tubes was obtained with the SFM measurements. Fig. 3a shows a three-dimensional topographic image of the keratin tube surface with clearly preserved scales. The surface of the tubes shown in close up images in (Fig. 3b, c) is relatively homogeneous with sub–100 nm holes (Fig. 3b) which presumably assisted the removal of the degraded material of the cortex after pyrolysis. The area close to the scales (Fig. 3d, e) exhibits a more heterogeneous character with a noticeable lifting at the scale edge. Such lifting is clearly seen in the plot in Fig. 3f which compares an averaged topographic profile of a native hair (which has flat plateaus between the scales) with the topographic structure of the keratin micro-tubes. Another observation suggested by the analysis of the cross-sectional profiles of the SFM topography images in Fig. 3f is the shrinkage with up to 35% of the cuticle during thermal processing, asderived from the averaged step-height between the residual scales. We note that a detailed statistical analysis would be required for making more precise conclusions on cuticle shrinkage and shape deformation upon thermal treatment.