Thermal Analysis of *Ortho*- and *Para*-Cortical Cells Isolated from Wool Fibers

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ABSTRACT: The nature of the bimodal denaturation endotherm, which has been observed for some α-keratin materials, was investigated in order to evaluate the validity of the two conflicting theories to interpret the phenomenon. *Ortho*- and *para*-cortical cells were isolated from Merino wool and subjected to measurements by differential scanning calorimetry in water at elevated temperatures (80–170°C). The results show for the isolated cell fractions denaturation peaks at 138°C (*ortho*) and 144°C (*para*) that are with respect to their location and temperature difference in good agreement with the results obtained for the whole fiber material. The denaturation enthalpy of the *para*- (21 J/g) was found to be higher than of the *ortho*-cortical cells (17 J/g) in contrast to expectations from electron diffraction studies. The fact that both of these values are higher than for the whole fiber (15 J/g) is attributed to the removal of the cuticle prior and to the enzymatic digestion of some further material during cell separation. The results give unequivocal evidence for the validity of the *ortho*/*para*-hypothesis for the interpretation of the endothermic denaturation doublet of keratins.
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Key words: differential scanning calorimetry; α-keratin; bimodal denaturation endotherm; *ortho*/*para*-cortex

INTRODUCTION

In a previous article,[1] the authors investigated the denaturation performance for a variety of hard α-keratin materials, hereinafter, for ease of semantics, referred to as keratins. The study was conducted by applying differential scanning calorimetry (DSC) for temperatures up to 180°C in excess water and under conditions of equilibrium water vapour pressure (high pressure DSC, or HPDSC). Namely, for Merino wool, the traces showed a pronounced tendency to develop a bimodal denaturation endotherm in the range of 140°C with peaks that were about 4–5°C apart, at a heating rate of 5°C/min.

This endothermic doublet has been observed by various colleagues for a variety of keratins and measuring conditions[2–8] and mainly interpreted by two principally different theories.

Spei and his coworkers conducted DSC studies in the dry state on a wide variety of keratins,[9] as well as on isolated low-sulfur and high-sulfur proteins from Lincoln wool.[4,10] With respect to the endothermic doublet that they observed for various keratins, they concluded that the low-temperature peak around 230–240°C has to be attributed to helix denaturation, while the high temperature peak ($\approx$ 250–260°C) originates in the matrix from cystine pyrolysis.[11] Recently, Cao et al.[6,12] conducted DSC studies on Merino wool in

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silicon oil, with the intention to preserve a certain amount of water in the fibers during the measurement. This results in a shift of the endotherms to lower temperatures compared to the dry state. Similarly as Spei, they interpreted the lower temperature peak of the doublet at ≈ 170°C (dT/dt = 5°C/min) as originating from melting or rather denaturation of the α-helical, crystalline material of wool keratin. The broad, higher temperature endotherm beyond around 185°C they considered as coming from the thermal degradation of other histological components.[6] This interpretation of the endotherm doublet will be, hereinafter, referred to as the "helix/matrix" hypothesis.

Haly and Snaith[3] used differential thermal analysis (DTA) to examine the performance of wool samples sealed into glass containers with various amounts of water. They observed a phase transition, often a doublet, that shifted with water content from ≈ 230°C for dry wool to ≈ 140°C for wool in excess water. Crighton and Hole[5,13] applied high-pressure differential thermal analysis (HPDTA) to study the denaturation transition temperature of the helical material of various keratins in water, again appearing in some cases as a doublet, at temperatures around 140°C. Haly and Snaith,[3] as well as Crighton and Hole,[5,13] attributed the doublet as originating from differences in the transition performance of the α-helical material in the *ortho*- and *para*-cortical cells.

Endothermic doublets were also observed for keratins, which do not exhibit the classical, bilateral separation into *ortho*- and *para*-cortical cells,[1,8,11] as it can be observed microscopically for Merino wool.[14] In view of the relationship between denaturation temperature and cystine content for various keratins,[1,3,13] we generalized[1] the interpretation of the endothermic doublet by assuming that such double peak structures are observed, if in a given sample cell group differences in cystine content are pronounced enough to allow the separation of the peaks. Further evidence for this theory comes, for Merino wool as a distinctly bilateral fiber, from the observation of the phenomenon of "differential" supercontraction.[1,14] Especially in view of the later phenomenon and for reasons of simplicity of semantics, this interpretation for the endothermic doublet will, hereinafter, be referred to as "*ortho*/*para*" hypothesis.

To further evaluate the validity of the helix/matrix- or the *ortho*/*para* hypothesis, respectively, *ortho*- and *para*-cortical cells were isolated from Merino wool and investigated by HPDSC.[15,16]

EXPERIMENTAL

Removal of the Cuticle

The preparatory step for the isolation of cortical cells from wool is the removal of the cuticle layer. A suitable method to obtain cuticle free (stripped) fibers has been described by Hüsken,[17] based on the approach of Wortmann et al.[18] for the stripping of coarser keratin fibers. Wool top was cut into 2–3-mm snippets using a hand microtome. 100 mg of snippets, 2.5 g of corundum powder, and 25 mL of a water–propanol mixture (50 : 50 by volume) were introduced into a 75-mL plastic bottle with a screw cap. A few thymol crystals were added to prevent bacterial growth. The bottle was clamped into a high-frequency (≈ 2800 rev/min), ellipsoid shaker placed in a low temperature room (6°C) to avoid heating of the sample. Shaking proceeded for between 48 and 72 h, intermittently checking the progress of the cuticle removal on small subsamples in the scanning electron microscope (SEM). After achieving an adequate degree of stripping, the suspension was passed successively through metal sieves (mesh widths: 100, 40, 28, and 20 μm). Corundum and cuticle particles passed the sieves, leaving the stripped fiber snippets, that is the cortex. These cortex snippets were washed repeatedly with destilled water and dried in air.

Enzymatic Digestion

To achieve disintegration of the cortex, 3.5 g of stripped wool were treated with 70 mg Subtilisin, an unspecific serine protease. The digestion was performed in a stoppered glass flask under intermittent shaking for 72 h at 40°C in 130 mL of 0.1M phosphate buffer at pH 8. A few thymol crystals were added to prevent bacterial growth. Subsequently, the residual material was passed through metal sieves as specified above, isolating the cortical cells that had been set free from the cortex snippets. The cells were washed repeatedly with destilled water and dried. The progress of the process was monitored on small subsamples by SEM.

Cell Separation

The separation of the cells was conducted by variations of the density methods proposed by Bradbury and coworkers[19,20] and by Lundgren,[21] as-

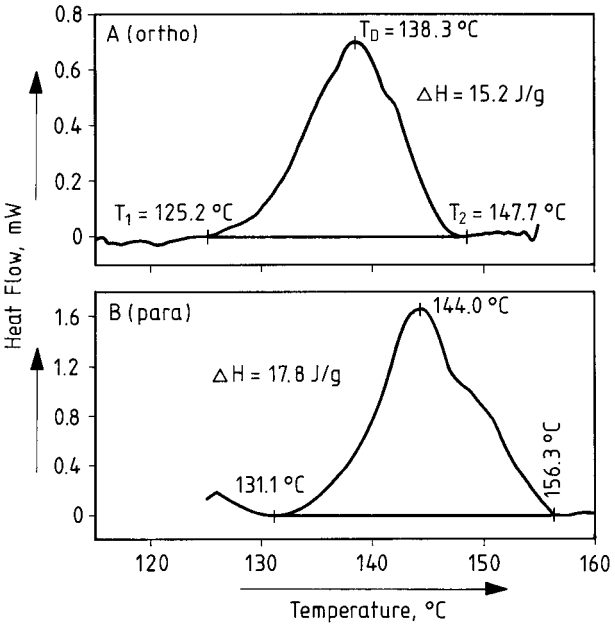

Figure 1 HPDSC traces (processed from the originals) for the (A) *ortho*- and (B) *para*-cortical cell fractions, respectively, isolated from Merino wool. Start (T_1) and end (T_2) temperatures, peak temperatures (T_D), and denaturation enthalpies (ΔH) are given.

suming a density of $\rho = 1.283$ g/cm^3 for the *para*- and $\rho = 1.275$ g/cm^3 for the *ortho*-cortex.[22] To obtain a suitably discriminating solvent mixture of $\rho = 1.280$ g/cm^3, CCl_4 ($\rho = 1.594$ g/cm^3) and ethanol ($\rho = 0.794$ g/cm^3) were mixed in appropriate amounts in a thermostated glass column ($\approx$ 25 cm long, $\approx$ 4 cm wide). Approximately 30 mg of cortical cells were suspended in a small volume of the solvent and placed on top of the liquid in the column. Overnight, the cells fractionated into the *para*-cortex at the bottom and the *ortho*-cortex at the top of the column.[16] The fractions were collected separately by suction, washed with water, and air-dried. The purity of the cell fractions was checked by applying the method described by Ley and Schäfer,[23] which makes use of the differential fluorescence performance of *ortho*- and *para*-cortical cells after staining with Rhodamine B and Acid Yellow.[15]

Thermal Analysis

All analyses were conducted on a power-compensated DSC instrument (DSC-7, Perkin–Elmer), using pressure-resistant (24 bar), stainless-steel, large-volume (60 μL) capsules, applying the following standard conditions:

temperature range:	80–170°C
heating rate:	5°C/min
sample weight:	4–7 mg
protective gas:	N_2

After equilibration at constant, ambient room conditions (approximately 22°C, 55% RH, 24 h), the samples were weighed into the capsules. Fifty microliters of water were added, and the capsules sealed. As reference, an empty container without the O-ring rubber seal was used. All analyses were conducted well beyond tenfold.

The DSC instrument was calibrated prior to a series of measurements, using indium and palmitic acid, both of high purity. In accordance with conventional practice in calorimetry, endothermic effects, that is, heat absorption by the sample, are represented by an increase in the ordinate value from the baseline position.

RESULTS AND DISCUSSION

Figures 1(A) and (B) show the HPDSC traces for *ortho*- and *para*-cortical cells, respectively. For comparative purposes, Figure 2 shows the trace for Merino wool taken from the same experimental curve as Figure 3 in Wortmann and Deutz.[1] Compared to their use in Wortmann and Deutz,[1] Deutz,[15] and Deutz et al.[16] and making use of the progress in software technology, the curves were reprocessed applying suitable, nonlinear baselines (PeakFit, Jandel Scientific), which led to

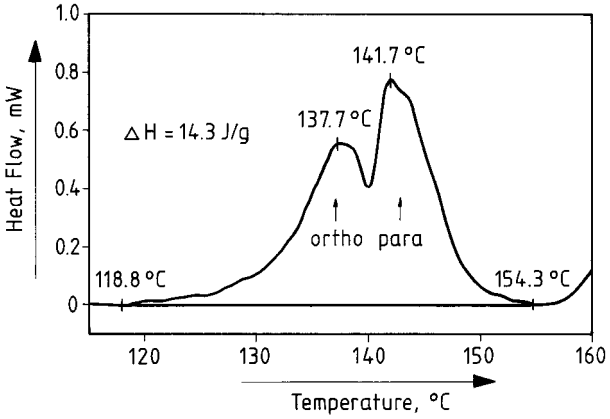

Figure 2 HPDSC trace (processed from the original) for Merino wool fabric. The allocation of the peaks to the cortical cell types is indicated.

Table I Peak Temperatures T_D and Denaturation Enthalpies ΔH for the *Ortho-* and *Para*-Cortical Cell Fractions, as well as for the Whole Fibers of the Related Merino Wool Fabric

	T_D (°C)		ΔH (J/g)	
Cell Type	Separated Cells	Whole Fibers	Separated Cells	Whole Fibers
ortho	137.9 ± 1.25	138.9 ± 0.70	17.4 ± 1.99	15.0 ± 1.10
para	143.9 ± 1.24	143.0 ± 0.78	20.6 ± 1.99	
Δ	6.0 ± 1.67	4.2 ± 0.98	3.2 ± 2.60	—

Note: The respective differences between the values, Δ, are given. Enthalpies are not corrected for the estimated water content of the materials under room conditions (≈ 14 wt %[1]). All values are given with their 95% confidence limits.

improved estimates for the characteristic parameters.

Table I summarizes the results for the peak temperatures T_D and denaturation enthalpies ΔH for the cell types. These deviate slightly from those preliminary reported in Deutz et al.[16] due to the incorporation of further results. During the investigation described in Wortmann and Deutz,[1] 18 DSC tests were conducted for the same Merino wool fabric of which 12 exhibited bimodal endotherms. Allocating the lower peak to the *ortho-* and the higher one to the *para*-cortex (see Fig. 2), the peak temperatures were determined and are summarized in Table I, together with the mean melting enthalpy for all 18 tests.

For the separated *ortho-* and *para*-cortical cells, the denaturation temperatures are 6°C apart and are located at 138 and 144°C, respectively. For the whole fibers these temperatures are shifted by about 1°C to higher temperatures for the *ortho-* (139°C) and to lower temperatures for the *para*-cortex (143°C). These small shifts of the two denaturation temperatures for the whole material are readily explained by the influence of the strong overlap of the DSC peaks on the respective peak positions. Thus, good agreement is observed between the tests on separated cortical cells and the whole fiber material (see Table I).

The denaturation enthalpies of the cell fractions (see Table I) show a difference of 3.2 J/g that, due to its confidence limits, is significant just beyond the 95% threshold. The higher value for the *para-* (20.6 J/g) compared to the *ortho*-cortex (17.4 J/g), is interesting to note in view of the expectation, based on the investigations by Dobb[24] that the *ortho*-cortex should contain about double as much α-helical material than the *para*-cortex. This observation needs further investigation. For both types of cell fractions the denaturation enthalpy is higher than for the whole fiber (15.0 J/g). This can mainly be attributed to the removal of the cuticle and possibly, furthermore, to the removal of a small fraction of nonkeratinous material during the enzymatic treatment.

It is interesting to note that the HPDSC curves of the *ortho-* as well as of the *para*-cell fraction show shoulders on the high-temperature side, indicating an underlying doublet of peaks [see Figures 1(A) and (B)]. A similar, though less obvious, effect is observed for the peak structures for the whole material in Figure 2. This observation of possible further substructures in the peaks of the endothermic doublet has been reported previously by Crighton[25] and Leroy et al.[8]

The origin of these substructures may either be considered as coming from contaminations with cell fractions of intermediate properties that were separated together with either the *ortho-* and *para*-fraction. Another interpretation that builds on experiments and considerations put forward by Crighton[25] is favored by the authors in view of the structure of the intermediate filament (IF) and its mechanical properties.[26] In this hypothesis, the substructure of a denaturation peak, be it for *ortho-* or *para-* cells, reflects differences in the denaturation performance of two types of helical segments in the IF monomer. In our view, these are the 1A, 2A, and 1B segments, on the one hand, at a lower temperature, and the 2B-segment, which is sulfur-crosslinked within the tetrameric structure,[27] on the other hand, at a higher temperature. This hypothesis is the subject of further investigations.

CONCLUSION

The bimodal endotherm observed for wool and other keratins in water, for example, by HPDSC, with peaks that are 4–6°C apart, was shown to

have its source in the presence of *ortho-* and *para*-cortical cells in Merino wool. While the structure of the intermediate filament and, thus, the arrangement of the helical material, can plausibly be assumed to be the same for both cell types, its denaturation temperature is considered[1] as being kinetically controlled by the viscosity of the amorphous matrix material, associated with, and surrounding the filaments. Due to the higher content of disulfide linkages in the *para-* cells, the denaturation temperature is higher than for the *ortho*-cells. The results are thus giving unequivocal evidence for the validity of the *ortho*/*para* hypothesis for the interpretation of the endothermic denaturation doublet of some keratins in water.

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