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A somewhat unexpected result from the deconvolution of DSC-curves for human hair:
There is no apparent relation between cortical cell fractions and hair curliness.

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Key words:
human hair curl, ethnic differences, cortical cell fractions, differential scanning calorimetry, curve deconvolution
Abstract

A deconvolution process has been developed for curves obtained by Differential Scanning Calorimetry in water for Merino wool and the main ethnic hair types. This enables estimation of the fractions of ortho- and para-type cell groups. The results also indicate that hair may contain a further, low-sulphur sub-group of ortho-type cells. The sizes of the major cell fractions are in line with expectations from microscopical investigations. The fractions are comparable for hair types and no consistent association between cell type fractions and hair curvature is observed.

Background

Though individual hair forms can be very diverse and complex, strong geographical and ethnic and thus genetic associations are observed (1, 2, s1). Asian hair tends to be straight, European hair is generally straight to wavy, and African usually strongly curled.

Crimp (curliness) in a Merino wool fibre is associated with the bilateral arrangement (segregation) of the two primary cell types in the cortex of the hair shaft, ortho- and para-cells (o/p-hypothesis) (3, 4, 5, 6, s2, s3). The cell types differ in their arrangement of intermediate filaments (IFs) and the composition of the associated matrix of intermediate filaments associated proteins (IFAPs) (7). Para-type cells are located on the inside and ortho-cells on the outside of the crimp (3). The structural differences between cell types develop in the follicle (1, 8, 9, 10).

To induce curl there have to be similar fractions of the two major cell types (11) with a substantial tendency towards lateral segregation. How these conditions are met has been established microscopically for wool but is experimentally more challenging for hair (5, 12). Against this background, there is only limited evidence on whether the o/p-hypothesis applies in a similar way also for hair, or whether other hypotheses are more plausible (1, 13, s4, s5).
Differential Scanning Calorimetry (DSC) measures the heat flow differences between a sample and an inert reference under (usually) linear heating conditions (14). The method with keratin samples in water (DSC\textsubscript{w}) enables to monitor the denaturation process of $\alpha$-helical proteins in the IFs (15). DSC\textsubscript{w}-curves show a main peak marking the denaturation temperature $T_D$ (16). The peak area gives the denaturation enthalpy $\Delta H_D$ and is related to the amount of undamaged $\alpha$-helical material in the IFs. $T_D$ is controlled by the cross-link density, that is, the cystine content of the IFAPs, which kinetically hinders the unfolding of the helical material (15, 16).

**Question Addressed**

The occurrence of bi-modal DSC\textsubscript{w}-curves for wool (16) suggests that a separation of underlying peaks (deconvolution) may enable to estimate cortical cell fractions (17). Using this approach for a set of ethnic hairs, the objective was to investigate, whether differences in overall cell composition could be detected and whether they are related to hair form.

**Experimental Design & Curve Analysis**

Ethnic hair types were in the form of commercial hair tresses of mixed or individual origin and were analysed in various contexts (15, 16, s6). Reference material is highly curled, fine Merino wool, for which the validity of the o/p-hypothesis is well established. Figure 1 shows typical DSC\textsubscript{w}-curves. The curves could be fitted by a maximum of three Gaussian peaks of equal width (see Supplement). All fits show a dominant middle peak (II) with a minor peak on the low (I) and high temperature side (III), respectively (see Figure 1).
Figure 1:

DSC\textsubscript{w}-curves in water (▬) for Merino wool (A), African (B) and Asian (C) hair. The curves are deconvoluted (see Supplement) using three Gaussian distributions of equal width (standard deviation). For each of the peaks, labelled as I-III with increasing temperature, mean peak locations and fractional areas are given (see Table 1). The fine line following the experimental curve (─) is the fitted DSC\textsubscript{w}-curve. No curve is shown for European hair, since shape differences between hair types are comparatively small (15, 16). Insets are cross-sections (TEM) which highlight the contrast of cell types (or lack thereof) in the hair types (s7).
Results and Discussion

Table 1 summarizes the results of the deconvolution procedures.

**Table 1:** Denaturation temperatures $T_D$ and fractional enthalpies $f$ for the assumed three components of the DSC$_w$-curves in various keratin fibres, as determined through curve deconvolution. Superscripts $I$, $II$, and $III$ relate to the Gaussian peaks with increasing temperature. Data are arithmetic means ± standard error for five-fold determinations. Coefficients of determination are in all cases $R^2 > 0.98$. ortho = $f^I + f^II$. Analysis of Variance indicates homogeneity for $f_I$, $f_{II}$, $f_{III}$, and ortho across hair types ($p > 0.33$). Even a non-conservative post-hoc Comparison of Means (LSD) test (s8) failed to indicate any trends towards significant differences ($p > 0.16$).

<table>
<thead>
<tr>
<th>Hair Type</th>
<th>$T_D^I$</th>
<th>$T_D^{II}$</th>
<th>$T_D^{III}$</th>
<th>$f^I$, %</th>
<th>$f^{II}$, %</th>
<th>$f^{III}$, %</th>
<th>ortho, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merino wool</td>
<td>132.3 ± 0.69</td>
<td>138.0 ± 0.44</td>
<td>143.7 ± 0.22</td>
<td>12 %</td>
<td>56 ± 6.3</td>
<td>32 ± 8.2</td>
<td>68 ± 8.2</td>
</tr>
<tr>
<td>African</td>
<td>144.9 ± 0.97</td>
<td>150.1 ± 0.48</td>
<td>153.6 ± 0.87</td>
<td>11 %</td>
<td>68 ± 6.0</td>
<td>21 ± 5.6</td>
<td>79 ± 5.6</td>
</tr>
<tr>
<td>Asian</td>
<td>141 ± 2.6</td>
<td>145 ± 2.2</td>
<td>149 ± 2.4</td>
<td>9 %</td>
<td>60 ± 2.5</td>
<td>31 ± 2.9</td>
<td>69 ± 2.9</td>
</tr>
<tr>
<td>European</td>
<td>139.8 ± 0.41</td>
<td>144.6 ± 0.27</td>
<td>147.9 ± 0.70</td>
<td>10.3 %</td>
<td>64 ± 2.6</td>
<td>26 ± 2.4</td>
<td>74 ± 2.4</td>
</tr>
</tbody>
</table>

For wool Peaks II and III are assigned to the denaturation of helical material in ortho- and para-cells, respectively (17). While the temperature difference between the main cell types ($T_D^{III} - T_D^{II}$) is about 6 °C for wool it drops to 3-4 °C in hair. Since $T_D$ is related to the sulphur content of the IFAPs, this corresponds to the lack of contrast between cell types in hair cross-sections in the Transmission Electron Microscope (see Fig.1) (1, 12).
The smaller peak at low temperature (I) is attributed to a distinct type of lower-sulphur, ortho-cortical cells. The presence of meso-cells, as a low-sulphur version of para (18), could not be confirmed, supporting the view (19) that meso is not a distinct cell type.

The peak areas may plausibly be associated with cell-type fractions (17). Assigning Peaks I & II to ortho yields 68% - 79% with no significant differences between types (see Table 1). This range corresponds to the fraction of ortho-cells in coarse wools (5, s5).

Conclusions

Deconvolution of DSC\textsubscript{w} –curves of ethnic hairs is shown to be a viable method to estimate the fractions of the cortical cell types. On the basis of the o/p-hypothesis significant differences between cell fractions may have been expected in view of the shape differences of ethnic hairs. However, none were apparent. With similar cell fractions for ethnic hair types, curliness can thus be assumed to be primarily driven by the specific extent of cell type lateral segregation.
Authors Contributions

Authors contributed equally to the paper.

Conflict of Interests

The authors have declared no conflicting interests.

Supporting Information

Additional supporting information may be found online in the supporting information tab for this article:

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S.1.1 Materials & Methods

S.1.2 Deconvolution of DSC-curves

S.1.3 Concluding Supplementary Notes

S.2 Supplementary References
References


