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Estimation of α-Helix Content in Keratin Fibers by Differential Scanning Calorimetry

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In the DSC curves of α-keratin (intact wool keratin) an endothermic peak was observed at the temperature around 232 °C which had been interpreted in terms of helix melting point. When wool fiber was stretched the native α-keratin transformed to stretched β-keratin and the meantime, the endothermic peak decreased with stretching and finally disappeared. This DSC measurement was thought to be great advantage to confirm the existence of α-form in keratin fibers.

The molecular mechanism involved in the α-β transformation has always been a challenging aspect of keratin structure.

In a series of studies on keratin structure, Astbury and his co-workers[1)] have demonstrated that by stretching a native α-keratin fiber in an appropriate medium, transformation to the β-keratin form can be accomplished. The major evidence of the transformation was the accompanying change that occurred in wide angle X-ray diffraction patterns from the characteristics of the native α-form to the β-structure.

Recently, Arai,[2)] using Lincoln wool, showed that the intensity of 5.1 Å of α-reflection decreased lineary with fiber stretching, and the reflection disappeared at 55 % of extension. However, when we use a fine wool such as Merino wool, clear X-ray reflection patterns are not obtainable.

Moreover, when a keratin fiber is immersed in a concentrated aqueous solution of lithium bromide at 100 °C, the X-ray pattern is displayed as a halo indicating either the organized α-helixes or newly developed β-structure are no longer present as organized states in the fiber.[3)] In this haloneous X-ray pattern, we often encountered with a difficulty in confirmation of α-reflection (5.1 Å) because of overlapping with β-reflection (4.65 Å).

In differential scanning calorimetry (DSC) curves of α-keratin one or two endothermic peaks have been observed in the temperature range 230-255 °C which have been interpreted in terms of helix melting points and cystin decomposition points.[4)] Spei[5)] has performed DSC investigation of microfibrillar (helical component) and matrix (non-helical) proteins in the disulfide forms isolated from wool, and he has shwon that the first peak at 235 °C is a microfibrillar peak and the second peak at 254 °C a matrix peak. These assignments have confirmed also on intact keratin fibers such as human hair and Lincoln wool fibers stretched from 0 to 60 % extension.[6)]

Recently, we found that in DSC curves of stretched wool fibers, an endothermic peak area at the temperature around 232 °C decreased with increasing extension ratio and finally disappeared when the fibers were extended over 80 %.

In the present communication, then, we will deal with a possibility of the estimation of the relative helix content in keratin fibers from DSC curves of stretched and supercontracted Merino wool fibers, comparing with results obtained from X-ray studies.

All the DSC curves were obtained with a Perkin-Elmer DSC-4 differential scanning calorimeter controlled by a microcomputer. DSC measurements were performed for 4-6 mg of wool filaments in the sample pan with a heating rate of 8 °C/min, purging 30 cm^3/min of nitrogen gas.

Extended wool fibers were prepared by the following procedure: small amounts of solvent-scoured Merino wool fibers were arranged in the direction of the fiber axis and were set on a hand-stretching apparatus and subjected to stretching in steam. The speciments, stretched at various extension ratios, were dried with cold air on the stretching apparatus to maintain temporary set.

Supercontraction was produced by immersing wool fibers in 8.0 mol/L aqueous solution of lithium bromide at 100 °C for the desired time as the same method as described in the previous paper.[3)]

In Fig. 1, DSC curves of Merino wool fibers extended at various extension ratios are shown. As seen in the figure, the endothermic peak area decreases with an increase of extension ratio from 20 to 60 %. Beyond 80 % of extension, the peak disappeared completely.

According to the peak assignment by Spei[6)], the decrease of the peak area suggests the decrease of helix content in wool keratin by stretching.

Astbury and Woods[7)] found a change in the X-ray pattern of wool fibers that α-pattern began to disappear from a little extension ratio and the β-pattern was fully developed at 60 to 70 % extension.

As mentioned before, Arai has shown a linear decrease of 5.1 Å α-reflection during fiber stretching, and the reflection disappeared at about 55 % extension.

For the quantitative treatment of the DSC curves obtained in the present experiments, the endothermic heat was calculated for each sample by the integration of the peak area. The results calculated are plotted in Fig. 2 against the extension ratio. The fact that the decrease of the endothermic heat begins to decrease from about 20 % extension and reaches to zero at 70 % extension is in fair agreement with the X-ray data of Astbury and Woods.[7)] Unfortunately a linear relationship was not observed in Fig. 2 unlikely to the X-ray data of Arai.[2)] However one may estimate the relative helix content in wool keratin modified by a physical manner. At least, this DSC measurement is thought to be great advantage to finding of the existence of α-form in wool keratin, because we often encounter with a difficulty in confirmation of haloneous α-reflection pattern (5.1 Å) which is overlapped with β-reflection (4.65 Å) in X-ray measurements of modified wool fibers, especially for supercontracted wool fibers.

Therefore, as the next step of experiment, we performed DSC measurements of supercontracted wool fibers.

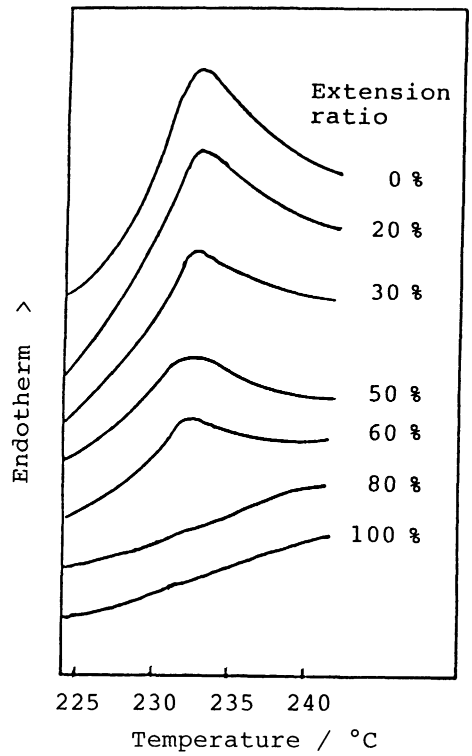

Fig. 1. DSC curves of extended Merino wool fibers.

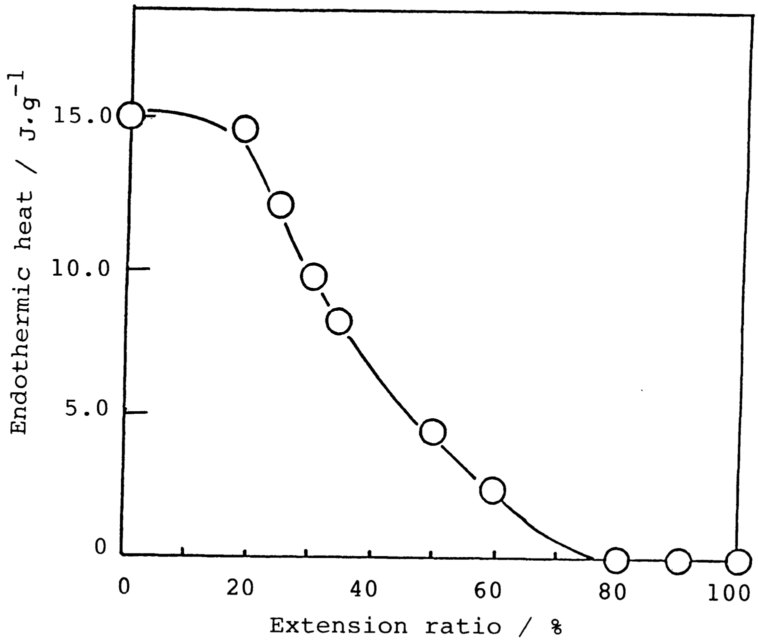

Fig. 2. Endothermic heat at helix melting point of extended Merino wool fibers.

When a keratin fiber was placed into aqueous lithium bromide of concentration greater than 6.4 mol/L and at 100 °C, X-ray measurements indicated that α-helical material was no longer present in the fiber and a normal wool fiber contracted irreversibly by 50 % of its native length with the treatment for more than 15 min.[3)] In this stage, the X-ray pattern is displayed as a single halo indicating either the originally aligned α-helixes or developed β-structure are no longer present as organized states in the fibers.

Figure 3 shows DSC curves of wool fibers supercontracted in 8.0 mol/L aqueous solution of lithium bromide at 100 °C for different time. As seen in the DSC curves in the figure, even in a short time treatment, the intensity of the endothermic peaks decreases remarkably in comparison with the original one, and the peak does not disappear even for 300 min treated fibers.

The endothermic heat calculated for contracted wool fibers is listed in Table 1. On the supposition that the endothermic heat correlates with the helix content, considerable amounts of helixes are remained in the fibers even after a prolonged treatment in contrast to our X-ray data. A similar observation has been obtained by Haly,[8)] who found that the X-ray diagram of supercontracted wool showed traces of the α-pattern.

It is worthy to note that the peak maxima shown in Fig. 3 appears near 224 °C, whereas those in Fig. 1 appears at 232 °C. This indicates the modification method affects strongly on the melting point of helix structure. This will be discussed in a subsequent paper.

In conclusion, as shown above, the DSC measurement is a very useful tool in investigation of helical component of keratin fibers.

Further DSC study on the stability of α-structure in keratin fibers modified by various methods are under progress.

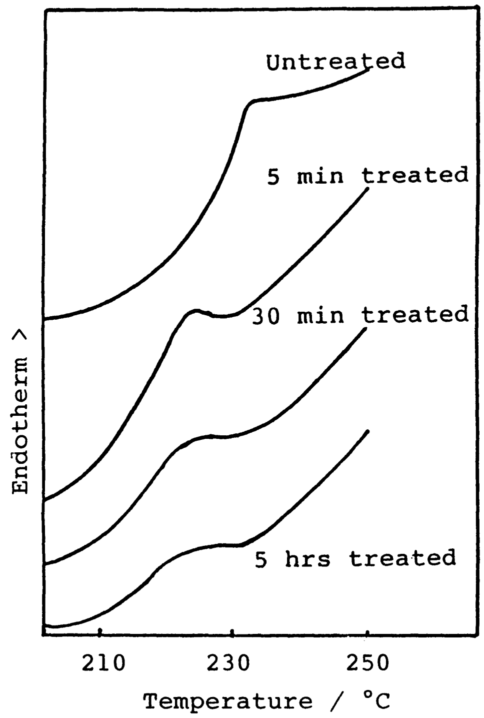

Fig. 3. DSC curves of 8.0 M LiBr treated Merino wool fibers.

Table 1. Endothermic heat of helix melt in LiBr treated Merino wool fiber

Time treated	Endothermic heat
min	$J(g\ fiber)^{-1}$
0	15.1
2	4.4
5	3.8
10	3.4
30	3.0
60	3.0
120	3.0
240	3.0
300	2.8

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