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Depression of the Melting Temperature by Moisture for α -Form Crystallites in Human Hair Keratin

Abstract: DSC thermal analysis has been carried out for human hair samples with various moisture contents to investigate the melting temperature depression behavior of α -form crystallites in human hair. This is achieved by adopting a novel technique using silicon oil as the thermal medium, which permits hair samples to retain a range of moisture contents in between completely dry and fully saturated. The results show that the melting temperature of the α -form crystallites in human hair varies with moisture content from 205°C for dry hair to 155°C for the hair sample with moisture content of 23%. These experimental results are particularly useful for clarification of the conceptual ambiguities associated with the molecular properties of α -helices and α -form crystallites. Furthermore, the Flory–Huggins theory was employed to determine the water–helix interaction parameter ($\chi = 4.5$) and the α -form crystallinity of human hair (22%), a figure consistent with that obtained by the XRD method (21%). © 2004 Wiley Periodicals, Inc. *Biopolymers* 77: 38–43, 2005

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INTRODUCTION

It is straightforward in concept to use DSC to measure the melting temperature of α -form crystallites in human hair keratin. However, it has not been easy to obtain consistent DSC thermograms despite taking extra care during the experiments. This is because human hairs are biological polymers consisting of a range of histological

components. Thermal events relating to physiochemical changes in the histological components or the interaction of the histological components with the environment in which the measurements take place could interfere with the melting of α -form crystallites. This will result in complicated thermograms with low reproducibility. Indeed, the curve shapes and peak sizes reported in the literature vary significantly.^{1–8}

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Recent research has shown that the quality of DSC thermograms can be significantly improved by introducing silicon oil as the thermal medium for keratin materials. Silicon oil is chosen because it provides faultless thermal contact as well as protects the keratin samples from oxidation. In addition, the unique capability of silicon oil to retain moisture in samples has paved the way to investigate the melting temperature depression behavior of α -form crystallites in human hair keratin.

We are thus interested in obtaining experimental data showing the melting temperature depression by moisture of the α -form crystallites in human hair keratin. According to thermodynamics

$$T_m = \Delta H / \Delta S \quad (1)$$

while the melting enthalpy, ΔH , is constant irrespective of the measuring environment, the melting entropy, ΔS , increases with increasing the content of water molecules, resulting in a lower melting temperature, T_m . The α -form crystallites in human hair keratin have a melting temperature over 200°C when dry, which is depressed to around 150°C when measured wet (vide infra). In previous publications, only samples with a fixed moisture content between completely dry and fully saturated were investigated.^{9–12} However, the capability of the DSC technique for retaining moisture should permit measurements of hair samples with the full range of moisture contents.

Experimental verification of the above mentioned relationship is important to clarify a couple of conceptual ambiguities regarding the physiochemical nature of the α -form crystallites in human hair keratin. One is the concept of whether there is crystalline keratin or just well-oriented amorphous keratin in hair. Wide angle x-ray diffraction shows only a couple of diffraction spots with a broad intensity profile for hair fiber. It can be argued that the x-ray diffraction spots could have originated from well-oriented amorphous keratin rather than poorly ordered crystalline keratin.

The other concept is whether the endotherm observed on a DSC thermogram is associated with melting of the crystallites or denaturation of proteins. There is an argument that the DSC endotherm observed for hair keratin is due to denaturation rather than “melting” of keratin. Denaturation has a broad definition without precisely specifying the physiochemical nature. For most soft proteins, denaturation is thought to be a correct interpretation for the DSC endotherm. However, we can classify the endotherm for hair keratin as definitely melting, which should

permit the concepts and methodologies developed from classic thermodynamics and solid physics to be applied to human hair.

To clarify these arguments, one needs to seek additional evidence. Thermodynamics is ideal as it has a well-established knowledge base specifying the intrinsic characteristics of a crystal compared with amorphous material. There is a need to experimentally verify that melting temperature depression of α -form crystallites in human hair keratin follows the thermodynamics law.

The objective of this study was to experimentally demonstrate the above expectation by using the novel DSC technique.

EXPERIMENTAL

Sample Preparation

Hair samples were collected from a hairdresser. Golden hair from a 4-year-old Caucasian female and brown hair from an Asian adult male in his 30s were used in this study. Hair samples were first washed using a commercial shampoo and rinsed using ionized water.

Two methods of sample preparation have been adopted to obtain samples with either high or low moisture contents. 1) Hair samples were immersed in ionized water for longer than 30 min and centrifuged at 5,000 or 11,000 rpm at 20°C for 5 min using the Eppendorf Centrifuge 5804 R. They were then promptly cut using sharp scissors to fine fragments approximately 0.5–1 mm long. This was achieved by cutting inside a 10-ml beaker. The fragment samples were left in an air-conditioned room for air drying for different time periods to obtain samples with different moisture contents. When a part of the sample was packed into a DSC cell, another part of the same sample was weighed, oven dried, and weighed again to calculate the moisture content. 2) On the other hand, we used a different method to prepare hair samples with low moisture contents. Hair samples were first chopped into fine fragments by cutting inside a 10-ml beaker with scissors. The fragmented hair was dried, then placed in an air-conditioned room for moisture reabsorption. Samples with various moisture contents were obtained by simply waiting for different time periods of reabsorption. It has been found that this dual approach represents an easy and reliable method of sample preparation. The water content was calculated according to

$$\text{water content(\%)} = \frac{\text{weight of hair sample} - \text{weight of dried hair sample}}{\text{weight of hair sample}} \times 100$$

DSC Measurement

A TA Instrument 2920 DSC equipped with a refrigerator cooling device was employed for this study. To carry out the

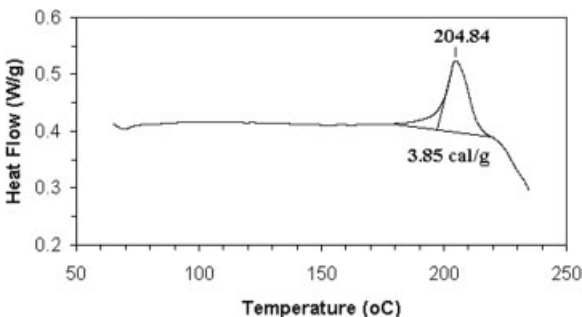


FIGURE 1 DSC thermograph for the hair sample with 1.1% moisture from a 4-year-old Caucasian female.

hair DSC measurements, a high volume pan accessory was required. The stainless steel pans provided by the TA Instrument were of 100 μl high volume, with pressure capability of 3.8 MPa, which is equivalent to a 250°C temperature limit for aqueous samples. One obtains the melting temperature corresponding to dry samples when an unsealed DSC pan is used as a result of moisture evaporation during heating.

The main technical specifications of the silicon oil used as the thermal medium were high temperature stability, flush point 315°C, density 1.050 g/cm^3 , refractive index = 1.4950 (as described in the *Aldrich Handbook of Fine Chemical and Laboratory Equipment*, Sigma, Aldrich, Sydney, 2002).

For the reference a stainless steel pan was sealed with a lid but no O-ring and no silicon oil were used. Over 20 mg of accurately weighed hair fragments were packed into the DSC pan, followed by an injection of silicon oil using a syringe to make up the total weight (hair + silicon oil) 80–90 mg. The pan was then sealed with an O-ring. The DSC runs were from around 30 to 245°C at 5°C/min. The nitrogen gas flow was 50 mL/min.

Due to the poor thermal conductivity and heavy mass of the high volume stainless steel pans compared with conventional aluminum hermetic and nonhermetic pans, a DSC instrument should be calibrated by using the same pan and lid under the same heating rate and gas flow rate. The DSC instrument was calibrated by placing 5 mg of indium in the stainless steel pan, followed by injection of 80 mg silicon oil and sealed with an O-ring, while the stainless steel pan sealed with a lid but no oil and no O-ring was used as the reference.

RESULTS AND DISCUSSION

Figures 1–5 show five DSC thermograms for the golden hair samples of the 4-year-old female with five different moisture contents. Clearly, an endotherm corresponding to the melting of the α -form crystallites can be identified. The endotherm shifts toward lower temperature with increasing the moisture content. Peak temperature of the endotherm is defined as the melting temperature in this study. After the endo-

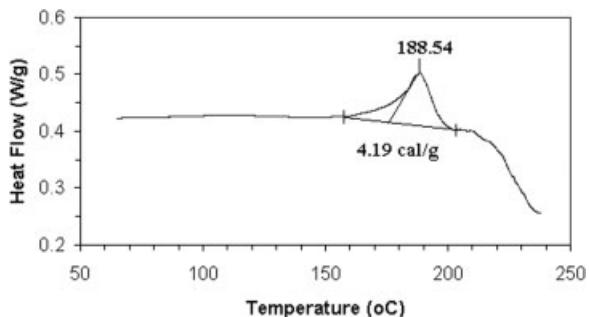


FIGURE 2 DSC thermograph for the hair sample with 2.7% moisture from a 4-year-old Caucasian female.

therm, the DSC curves are seen to return to the baseline, followed by further exo- and endotherms resulting from other thermal events such as oxidation and thermal degradation of various histological components in hair at higher temperatures. The existence of a baseline segment between the melting temperature of the α -form crystallites and the later exo- and endotherms resulting from other thermal events is critical for determination of the melting enthalpy. The reproducibility and overall quality of the DSC thermograms are considered satisfactory.

More DSC results are listed in Table I, where ΔH (cal/g) is the melting enthalpy as measured from the DSC thermograms. However, a parameter of interest is the melting enthalpy based on dry weight of hair, ΔH_{dry} (cal/g), which was calculated according to

$$\Delta H_{\text{dry}} = \frac{\Delta H}{1 - \text{moisture content}} \quad (2)$$

Both measured melting enthalpy and the melting enthalpy based on dry weight of hair are also shown in Table I. Unlike the melting temperature, the melting enthalpy remained constant within experimental error. It is common knowledge that the melting enthalpy measurement is less accurate than that for the melting

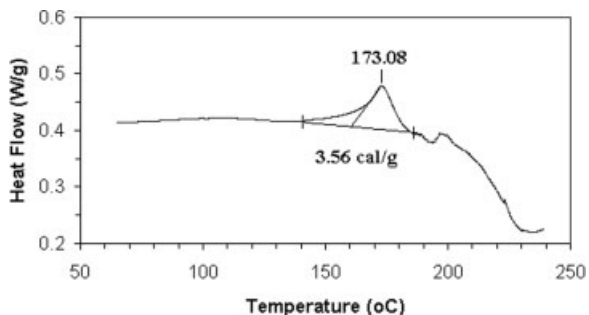


FIGURE 3 DSC thermograph for the hair sample with 10.9% moisture from a 4-year-old Caucasian female.

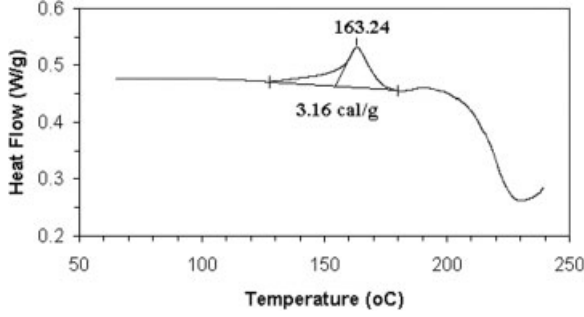


FIGURE 4 DSC thermograph for the hair sample with 14.3% moisture from a 4-year-old Caucasian female.

temperature by DSC. The difference between the two samples is not considered significant. The average melting enthalpy based on the dry weight of hair for all the samples listed in Table I is 3.94 cal/g. This is compared with a previous publication of one of the authors, which obtained a value of 3.47 cal/g (=14.5 (J/g)/4.18).¹² The difference could be a result of instrument factor.

A plot of the moisture content against the melting temperature is shown in Figure 6. The plot appears to be linear or slightly parabolic. The melting temperature is seen to increase as the moisture content decreases, varying from 155 to 205°C. It appears that the DSC results for both the 4-year-old Caucasian female and the Asian male in his 30s can be fitted with a single fitting curve. However, the hair of the 4-year-old female showed higher moisture content compared with that of the adult male under the same preparation procedure.

It can be concluded from these results that there are well-defined crystalline components in hair, clarifying the two long-standing conceptual ambiguities. The α -form crystallites in hair share all the characteristics of a crystal. We are observing melting rather than denaturation, allowing all the concepts and methodologies developed from classic crystal physics to be applied to hair.

The depression of the melting temperature has been well explained by the Flory–Huggins theory:

$$\frac{1}{T_m} - \frac{1}{T_m^0} = \frac{R}{\Delta H_u} \left(\frac{V_u}{V_1} \right) (\phi_1 - \chi \phi_1^2) \quad (3)$$

where T_m is the melting temperature of hair with moisture; T_m^0 is the melting temperature of hair without moisture; R is the gas constant = 8.3143 [J K⁻¹ mol⁻¹]; ΔH_u is the melting enthalpy of the crystalline component in hair [J mol⁻¹]; V_u is the molar volume of crystalline component in hair; V_1 is the molar

volume of moisture component; ϕ_1 is the volume fraction of the moisture component; and χ is the moisture–helix interaction parameter.

The volume fraction of moisture deserves discussion. First, it is a volume fraction, so it should be calculated from the weight fraction by using the known density. Second, the Flory–Huggins theory requires that ϕ_1 be the fraction of water molecules that mix with α -helices of the α -form crystallites during melting. Human hair has a similar degree of crystallinity as that of Lincoln wool, which is 21% based on the dry weight.¹³ Water molecules reside in the amorphous region; only those in the adjacent area will participate the melting process of the α -form crystallites. For the first instance, it is assumed that ϕ_1 equals the volume water fraction of the hair sample times the crystallinity. To summarize in a formula, one writes:

$$\phi_1 = \text{measured moisture content } X_c \left(\frac{\rho_u}{\rho_1} \right) \quad (4)$$

where, ρ_u (1.3) and ρ_1 (1.0) are the density of α -form crystallites and water, and X_c is the crystallinity.

Clearly, Eq. (3) is a parabolic function of the reciprocal of the melting temperature, $1/T_m$, against the moisture volume fraction, ϕ_1 , with three adjustable parameters, A , B , and C being

$$A = \frac{1}{T_m^0} = 0.002108 \rightarrow T_m^0 = 201.2(^{\circ}\text{C}) \quad (5)$$

$$B = \frac{R}{\Delta H_u} \left(\frac{V_u}{V_1} \right) \rightarrow 0.00502 \quad (6)$$

$$C = - \frac{R}{\Delta H_u} \left(\frac{V_u}{V_1} \right) \chi \rightarrow - 0.0227 \quad (7)$$

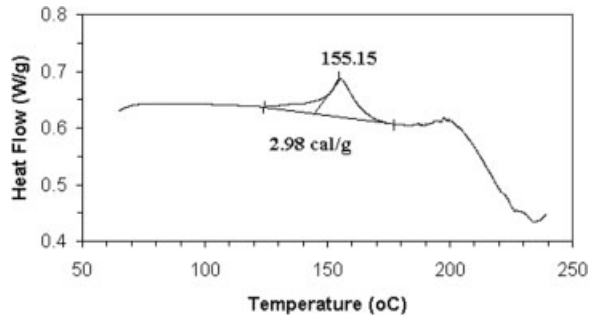


FIGURE 5 DSC thermograph for the hair sample with 22.5% moisture from a 4-year-old Caucasian female.

Table I Melting Temperature, Measured Melting Enthalpy, and the Melting Enthalpy Based on Dry Weight of Hair Determined from the DSC Thermograms for the Hair Samples

Hair Samples from a 4-year-old Caucasian Female				Hair Samples from an Asian Male in his 30s			
Water Content of Hair (wt %)	T_m (°C)	ΔH (cal/g)	ΔH_{dry} (cal/g)	Water Content of Hair (wt %)	T_m (°C)	ΔH (cal/g)	ΔH_{dry} (cal/g)
0	205	3.86	3.86	0	202	4.45	4.45
1.1	197	4.51	4.56	0.5	203	4.60	4.62
2.7	190	4.19	4.30	2.9	190	4.43	4.56
3.8	189	4.68	4.86	10.5	178	2.96	3.31
4.3	185	3.62	3.78	10.7	176	3.49	3.91
10.9	173	3.56	4.00	11.9	176	3.42	3.88
14.3	163	3.16	3.69	15.6	167	2.69	3.19
19.2	158	3.23	4.00	17.1	165	2.26	2.76
22.5	155	2.98	3.84				
22.9	156	2.74	3.55				

Curve-fitting computer software was employed to obtain the three adjustable parameters, and their values are shown on the above equations as well. The fitting curve is shown in Figure 7. From B and C , the water helix interaction parameter $\chi = 4.5$ is obtained; this parameter will be useful in deepening our understanding on the properties of α -helix revealed by Pauling and Corey^{14,15}

From B , one can also retrieve the α -form crystallinity by a rearrangement leading to

$$B = \frac{R}{\Delta H_{dry} M_u} \left(\frac{M_u / \rho_u}{M_1 / \rho_1} \right) = \frac{R X_c}{\Delta H_{dry}} \left(\frac{\rho_1}{\rho_u} \right) \frac{1}{M_1}$$

$$X_c = \frac{B \Delta H_{dry} M_1}{R} \left(\frac{\rho_u}{\rho_1} \right) \quad (8)$$

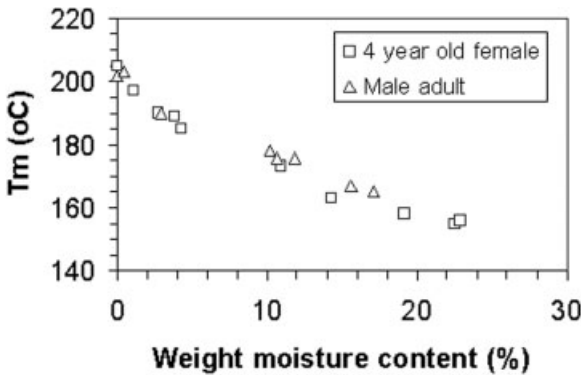


FIGURE 6 Plot of weight moisture content against the melting temperature for the hair of a 4-year-old female and the hair of an adult male.

where, ΔH_{dry} is the melting enthalpy of α -form crystallites in hair measured per gram that was mentioned in the previous section; M_u and M_1 are the molecular weights of α -helix (unknown) and water (18.016). The degree of crystallinity obtained in this method equals 23%, which is consistent with the value of 21% obtained using the XRD method, indicating that the Flory–Huggins theory is applicable in this case.

Eqs. (4)–(8) also offer a method to determine crystallinity of hair without reference to the value of crystallinity obtained using other methods. One first gives a tentative value for X_c in Eq. (4) to facilitate calculation of Eqs. (5)–(7). Eq. (8) then results in a new value for crystallinity, which may differ from the tentative value. By a process of iteration, we obtain the crystallinity of human hair as 22%, which is consistent with the value of 21% obtained using the XRD method.

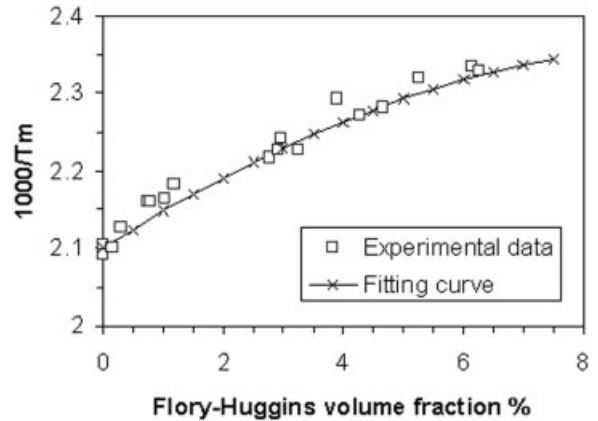


FIGURE 7 Plot of Flory–Huggins volume moisture content against the melting temperature for the hair samples.

CONCLUSION

By adopting a new technique of using silicon oil as the thermal medium, the DSC thermograms have been successfully obtained for human hair samples with a range of different moisture contents. The melting temperature of the α -form crystallites varied from 205°C for completely dried hair to 155°C for the hair samples with around 23% moisture, while the melting enthalpy based on dry weight of hair was maintained almost constant, leading to the conclusion that there are crystallites well defined by classical physics in human hair keratin. The depression of the melting temperature has allowed us to use the Flory–Huggins theory to calculate two molecular properties of α -helix, i.e., the water–helix interaction parameter $\chi = 4.5$ and the α -form crystallinity of 22%. These parameters will be useful in deepening our understanding of the molecular properties of human hair keratin.

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