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Analyzing protein denaturation using Fast Differential Scanning Calorimetry

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Abstract

This paper investigates the possibility to measure protein denaturation with Fast Differential Scanning Calorimetry (FDSC). Cancer can be diagnosed by measuring protein denaturation in blood plasma using Differential Scanning Calorimetry (DSC). FDSC can reduce diagnosis time from hours to minutes, requiring significantly smaller sample quantities. To show the feasibility of measuring protein denaturation with FDSC, protein denaturation in human hair is measured. We have been able to observe the phenomena of water evaporation and pyrolysis as they were measured in hair by DSC, however, the protein denaturation peaks are largely obscured by the water evaporation and pyrolysis phenomena, as the current set up only allows dry measurements.

Keywords: FDSC; DSC; Hair; Keratin; Protein denaturation; Blood plasma

1. Introduction

Protein denaturation in human blood plasma has been measured with DSC to detect early stage cancer [1-3]. In DSC, the plasma sample is heated at a specific temperature scan rate (in °C/min) to determine the heat capacity of the sample as a function of temperature. Plasma from individuals with cancer and healthy individuals show a significant difference in heat capacity for many cancer types. Table 1 reports the

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parameters used for measuring protein denaturation in plasma by DSC. Compared with DSC, FDSC can measure faster due to the smaller samples. It was suggested that the slow kinetics of the denaturation process require the slow temperature scan rate [4]. However, we believe that the thermal time constant of the large sample is instead the limiting factor. To demonstrate the working principle of the measurement method here proposed we used tiny samples of human hair to measure protein denaturation with FDSC. Hair was chosen to show the working principle, because with the current available FDSC chip it is not possible to measure protein denaturation in fluids, as the fluids would vaporize during the measurement. Human hair contains fibrous protein α-keratin, which shows a denaturation temperature at around 227 °C and has a denaturation enthalpy of the order of 9 J/g in dry open pan DSC measurements [5].

Table 1. Overview of DSC sample size, temperature range and temperature scan rate for cancer diagnosis using blood plasma.

Research work	Sample size	Temperature range	Temperature Scan rate	Run time
T. Fekecs [2]	850 µl	0 – 100 °C	0.3 °C/min	5.5 hrs.
C. Garbett[3]	100 µl	20 – 110 °C	1.0 °C/min	1.5 hrs.
Foreseen for FDSC	1 µl	30 – 90 °C	1-100 °C/s	<1 min

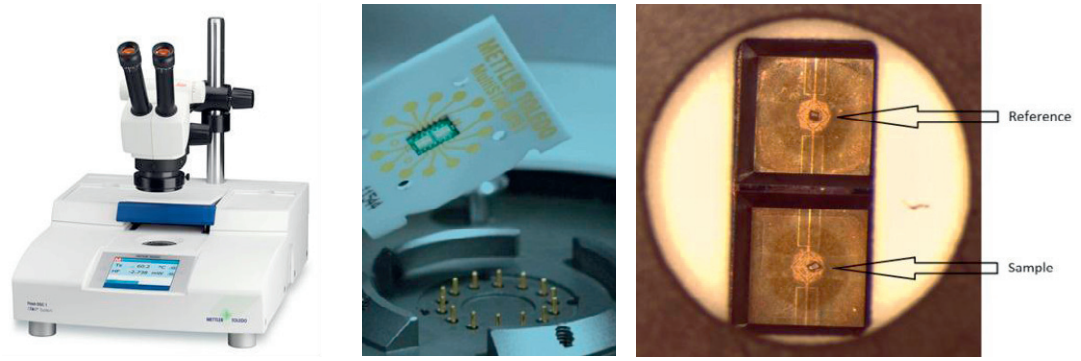

Fig. 1. (a) Flash DSC1 from Mettler Toledo; (b) FDSC chip (5 mm × 3.3 mm) on ceramic baseplate (24 mm × 24 mm); (c) Close up of the FDSC chip loaded with hair samples on the central sample areas (0.5 mm ø)

2. Experimental

Fig. 1 shows the Flash DSC1 and the FDSC chip from Mettler Toledo used for the experiments [6]. The FDSC chip consists of a sample and a reference cell. The sample cell was loaded with a hair sample with a length of about 0.2-0.4mm in length. To obtain a flat baseline the reference cell was loaded with a hair sample which was already heated up to 300 °C before the measurement (protein denaturation in hair is non-reversible) for the measurements up to 300 °C. The FDSC measurements were done in the temperature range from 30 °C to 300 °C with a temperature scan rate ranging from 1-1000 °C/s.

3. Experiments results

To understand the FDSC thermograms we first looked at the amount of water inside human hair. This is around 10-15% at an ambient humidity of about 50% RH [7]. Fig. 2(a) shows an FDSC thermogram for a human hair sample at a temperature scan rate of 5 °C/s in the range of 30-210 °C. Curve 1 is the first heating curve and curve 2 and 3 are the second and third heating curves, curve 4 is the first cooling curve and curve 5 and 6 are the second and third cooling curves. As can be seen there is a decrease in power

between the first and the second measurement due to the evaporation of water. We can analyse this using the Eq. (1), where P is the power taken up by the sample, m is the mass of the sample, c_p the specific heat capacity of the sample and β is the temperature scan rate, while dH/dt (rate of enthalpy change) indicates the power taken up for phase changes, such as water evaporation or protein denaturation. The weight of the hair sample is calculated to be around 4 µg based on its volume.

$$P = \{m \times c_p + \mathrm{d}H/\mathrm{d}t\} \times \beta \qquad (1)$$

At room temperature the difference in endothermic power between curves 1 and 2 is about 13 µW. With a temperature scan rate of 5 °C/s this implies a difference of 2.6 µJ/K. As the specific heat capacity of water is about 4.2 kJ/kgK this is equivalent to a loss of water of about 0.6 µg of water. This is 15% of the mass of the hair, as expected. At temperatures slightly above room temperature the difference between the curves starts to increase due to the evaporation of water (dH/dt becomes non-zero).
The total energy difference between curves 1+4 and 2+5 (first and second measurement) is around 1.7mJ, which should include the total enthalpy of water evaporation, plus some heat capacity effect, of the not-yet evaporated water (this fraction decreases until it becomes zero in the first cooling curve 4 at around 140 °C). For the water evaporation the overall enthalpy is estimated [7] to be of the order of 1.6-3.3 kJ/g. With water content of 0.6 µg this implies 1-2 mJ. Assuming that the water is on average present for 100 °C, an additional energy is needed for the heat capacity of about 0.6 µg × 100 °C × 4.2 J/gK = 0.25 mJ, which is small compared to the total excess energy. For the phase change 1.45 mJ would remain, giving an average enthalpy of water evaporation of 2.4 kJ/g. The accuracy of this calculation is limited by the estimates of the sample weight and water content, which are inaccurate by perhaps 10-25%.

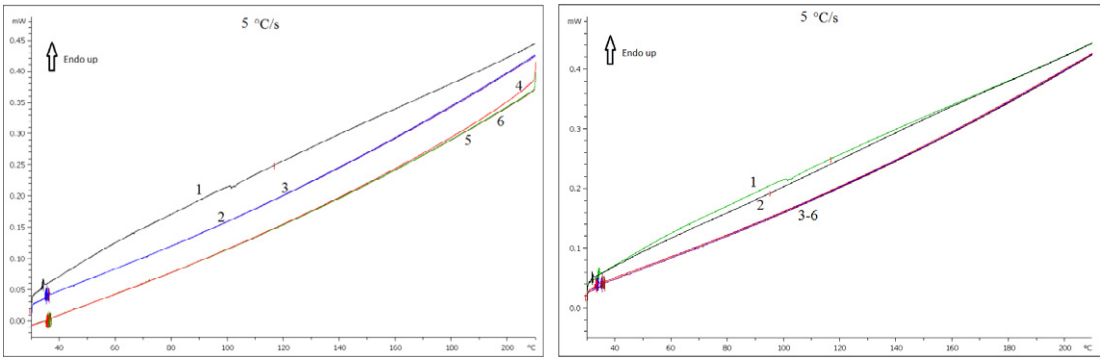

Fig. 2. (a) FDSC measurement at a temperature scan rate of 5 °C/s. Curves 1, 2 and 3 are the first, second and third heating measurements. Curves 4,5 and 6 are the first, second and third cooling measurements. The decrease in heat capacity between the first and the second and third measurement is due evaporated water content; (b) The same hair sample is measured again after 15 hours, curve 1 is the original curve, curve 2 the new curve measured after 15 hours, curve 3-6 represents the original curves and the new curves after evaporated water content (the four curves are on top of each other).

The same sample of hair was, after the measurement and all water was evaporated, left in the ambient atmosphere for 15 hours, and then measured again, see Fig.2b. Curve 1 is the first heating curve of Fig.2a, curve 2 is the first heating curve after 15 hr. recuperation, and the small difference between curves 1 and 2 shows that the hair has taken up again almost all the water that had been evaporated during the first measurement. This is in line with what is found by others [7], that water loss and gain of hair is a reversible process happening within 24 hours. Next we looked at the pyrolysis of hair, an irreversible destruction of the protein mass. In a dry-hair open-pan DSC measurement pyrolysis starts just after the denaturation peak, at around 240 °C. In FDSC measurements the start of the pyrolysis is dependent on the temperature scan rate. Fig. 3 shows three FDSC measurements at different temperature scan rates; curve 1

at 1 °C/s, curve 2 at 100 °C/s and curve 3 at 1000 °C/s. At a temperature scan rate of 1 °C/s the pyrolysis starts at 248 °C, at 100 °C/s around 257 °C and at 1000 °C/s at 281 °C, what implies a peak displacement between the temperature scan rates of 1°C/s and 1000 °C/s of 33 °C. At some measurements at a temperature scan rate of 1000 °C/s a small endothermic peak can be seen around 260 °C with an enthalpy of 0.6 J/g which cannot be seen at lower temperature scan rates. It can be speculated that this is due to protein denaturation, since at 1000 °C/s the displacement is 33 °C which means this peak actually occurs around 227 °C, the peak of protein denaturation in DSC. The measured enthalpy of the peak is, however, very low compared to DSC measurement results, which suggests that in FDSC the protein denaturation peak (≈ 25 µJ) is largely obscured by smeared out peaks of the water evaporation (1700 µJ).

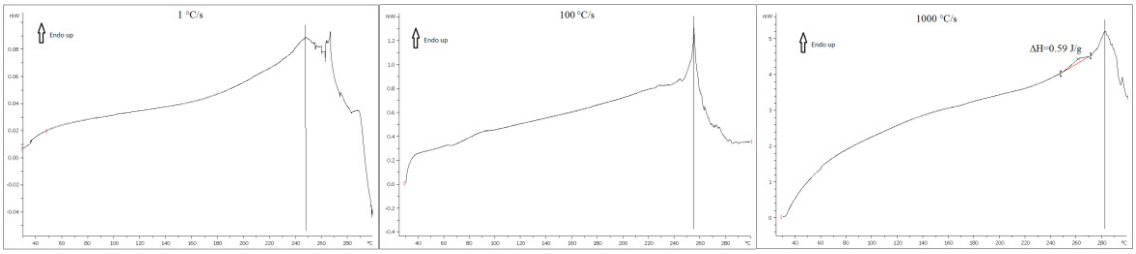

Fig. 3. Peak displacement at different temperature scan rates. The vertical line indicates the start of the pyrolysis of the hair sample.

4. Results analysis and discussion

The Fast DSC results agree with the DSC results regarding to water content and pyrolysis. However, it is difficult to measure the protein denaturation peak because it is largely obscured by the water evaporation and pyrolysis. Therefore we will make an adjustment to the FDSC chip to allow measurement in liquids, because with the open FDSC chips the evaporation of water prevents a proper analysis of hair, blood plasma and other liquid samples.

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