Measuring the Thermal Denaturation of Proteins Using the Power Compensated DSC

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Introduction

With the increasing focus upon biotechnological applications of thermal analysis, the use of high sensitivity DSC instruments to study the thermal properties of proteins in aqueous solution is becoming increasingly more important. In an aqueous solution, proteins have specialized three-dimensional structures that allows them to support specific biological functions. When heat is applied to the protein, this shape breaks down because of molecular thermal motions. This break down is referred to as thermal denaturation. The denaturation process is very low energy, but can be detected by very high sensitivity DSC instruments.

The use of DSC to characterization protein denaturation is important from drug discovery purposes as it can help in assessing the shape factor of the given protein and in the development of anti-viral drugs or treatments. The temperature of the denaturation event provides valuable data on the thermal stability of the given protein.

Experimental

DSC measures the heat flow into or from a sample as it is heated, cooled and/or held isothermally. The technique provides valuable information on softening temperatures (or Tg), melting temperatures, heats of melting, percent crystallinities, and recrystallization (temperatures and heats).

PerkinElmer offers the high performance, power compensated Pyris 1 DSC for characterization of biotechnological materials, including proteins in solution.

The Pyris 1 DSC offers the following features and benefits:

- Use of low mass (1 g) individual sample and reference furnaces for rapid response times
- Ability to heat and cool very quickly (500 C/min)
- Ability to achieve isothermal conditions rapidly
- Measurement of true heat flow rather than temperature differential for more accurate calorimetric determinations
- Use of PRT or platinum resistance thermometers, rather than thermocouples, for the most accurate and precise measurement of sample temperature
- Outstanding resolution
- Very high sensitivity for detection of weak or low energy transitions
- StepScan DSC for the separation of ‘fast’ and ‘slow’ thermal events (on the time scale of the DSC experiment). This provides for better data interpretation and for a clearer measurement of the glass transition event (Tg).

For the analyses of the thermal denaturation of protein, the need for a high performance DSC instrument becomes essential for the following reasons:

- The concentration of the protein in aqueous solution must be dilute because if the protein concentration is too high, interaction between the molecules can cause coagulation and prevent the detection of the denaturation event. The use of a dilute solution necessitates the use of a high sensitivity DSC instrument.
- Because of the slow kinetics of the protein denaturation process, a slow heating rate (2 or 1 C/min) to detect the transition. The effective sensitivity of a DSC becomes less as the heating rate is decreased and requires a high sensitivity DSC.

Experimental Conditions for Studying Protein Denaturation

Two proteins in aqueous solution were characterized using the high performance DSC. The samples were labeled as A and B and contained about 5 mg protein/mL of solution. The following experimental conditions were utilized to

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Figure 1. DSC results on protein solution A.

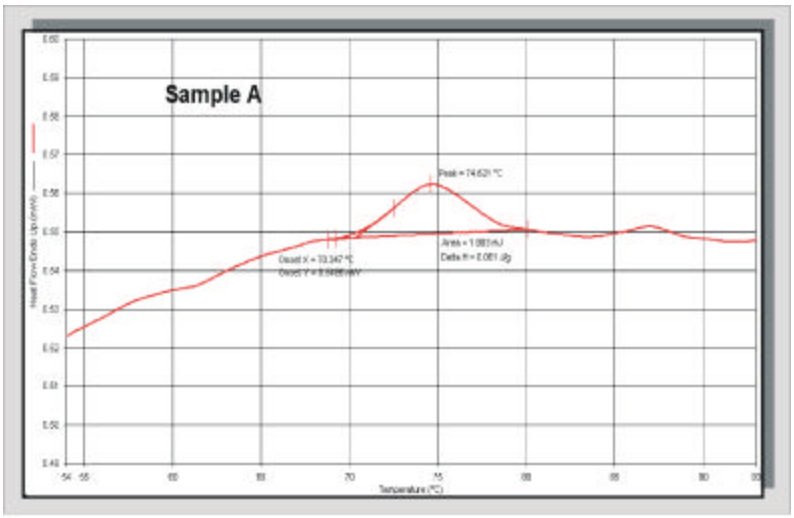
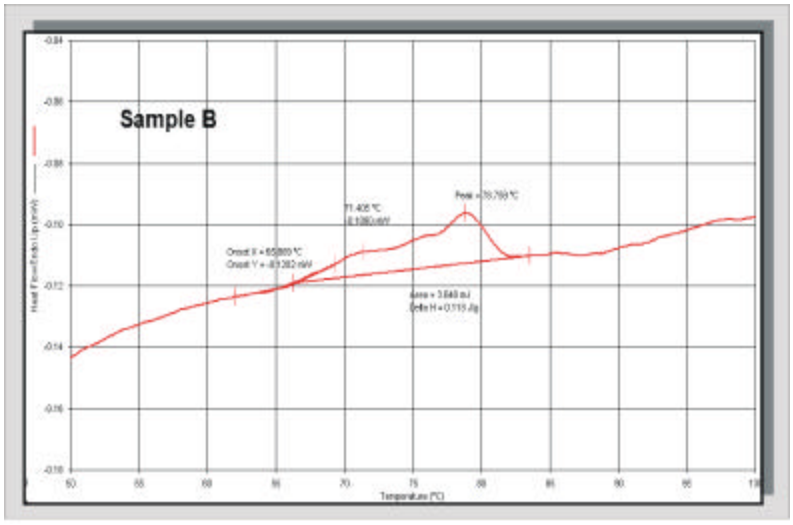


Figure 2. DSC results on protein solution B.



characterize the weight loss properties of the samples in the following table:

The use of water as a counterbalance on the reference side helps to make the DSC more sensitive to the properties of the dilute protein and allows the DSC to equilibrate more rapidly once the experiment is initiated.

Instrument:	Power compensated DSC
Sample container:	Stainless steel sealed containers with O-ring
Sample mass:	Approximately 30 mg of solution
Reference:	SS container with counterbalancing mass of water
Initial temperature:	20 C
Final temperature:	100 C
Heating rate:	2 C/min
Purge gas:	Nitrogen at a flow rate of 20 mL/min

The use of the special stainless steel sealed pans with O-ring contains the water and prevents leakage while heating the solution to elevated temperatures. These sealed pans are ideal for the characterization of the thermal denaturation of proteins in solution.

DSC Results

Displayed in Figure 1 are the DSC results obtained for protein solution A. The plot shows the DSC heat flow as a function of sample temperature with an endothermic response oriented upwards.

The protein denaturation transition is observed as a very small endothermic peak at 74.6 C with an onset temperature of 70.3 C. The heat of denaturation is only 0.061 J/g of solution.

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Displayed in Figure 2 are the DSC results generated on protein solution B.

This sample yields a broader protein denaturation transition between 65.9 and 82.5 C with peaks observed at 71.4 and 78.9 C. The heat of denaturation for sample B is 0.118 J/g, which is nearly double that of sample A (0.061 J/g).

Summary

The PerkinElmer power compensated Pyris 1 DSC yielded very good results on the two dilute protein aqueous solutions and was able to show the denaturation transition at about 75 C. The detection of the protein denaturation transition requires a high performance DSC instrument because of the inherently weak nature of the event, the dilute nature of the solution, and the requirement of slow heating rates. The thermal characteristics of the proteins are important for drug discovery purposes as it provides information on the shape factor and the thermal stability of proteins.

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