

Protein Denaturation Studies Using the Pyris 6 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 bullet, use "Normal".

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 T_g), melting temperatures, heats of melting, percent crystallinities, and recrystallization (temperatures and heats).

PerkinElmer offers the high performance Pyris 6 DSC for characterization of biotechnological materials, including proteins in solution.



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.

The level of sensitivity provided by the Pyris 6 DSC is such that the performance of the instrument is on a microwatt or microcalorimetric level, which is necessary for successfully measuring the weak protein denaturation transitions, especially of dilute solutions.

One of the important features necessary to obtain high quality protein denaturation data is exceptional baseline performance in terms of linearity and reproducibility. The Pyris 6 DSC provides unparalleled performance with regards to its baseline response. This is demonstrated in Figure 1 which displays 10 baseline experiments performed over a two day period on the Pyris 6 DSC instrument. The baseline performance is extremely stable and exhibits very low noise, both features of which are essential for getting excellent results on proteins.

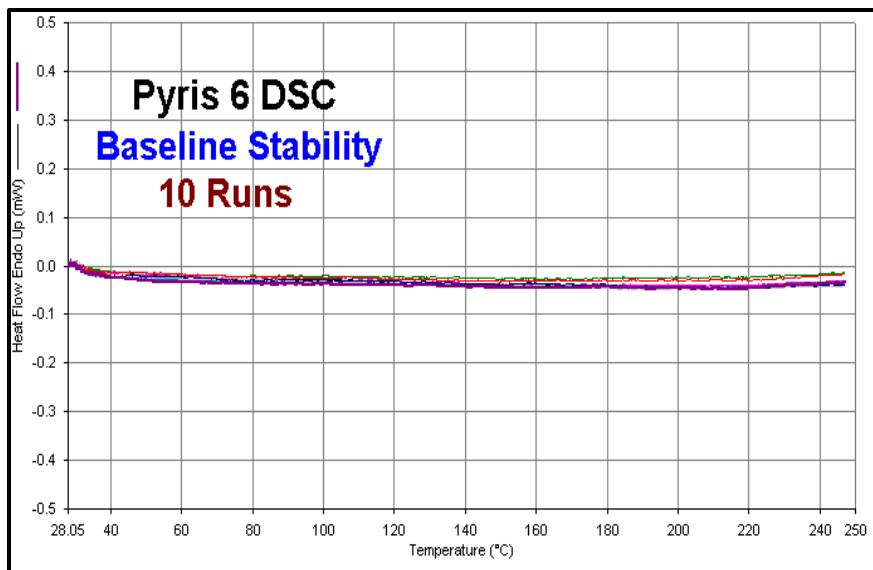


Figure 1. Baseline reproducibility of Pyris 6 DSC

The following experiment conditions were utilized to analyze the protein denaturation event.

Instrument	Pyris 6 DSC
Sample volume	60 μ L
Reference	water (58 μ L)
Containers	stainless steel
	Capsules with O-ring
Initial temp	25 °C
Final temp	110 °C
Heating rate	2 °C/min

The special stainless steel capsules are ideal for the analyses of proteins in solution for two main reasons:

- The capsules keep the water completely contained so that no evaporation interference is obtained
- The capsules hold a relatively large volume of solution (60 μ L) which provides an extra degree of sensitivity, especially for dilute solutions

DSC RESULTS ON PROTEINS

Displayed in Figure 2 are the DSC results generated on a protein solution of 'normal' concentration (5%) using the Pyris 6 DSC.

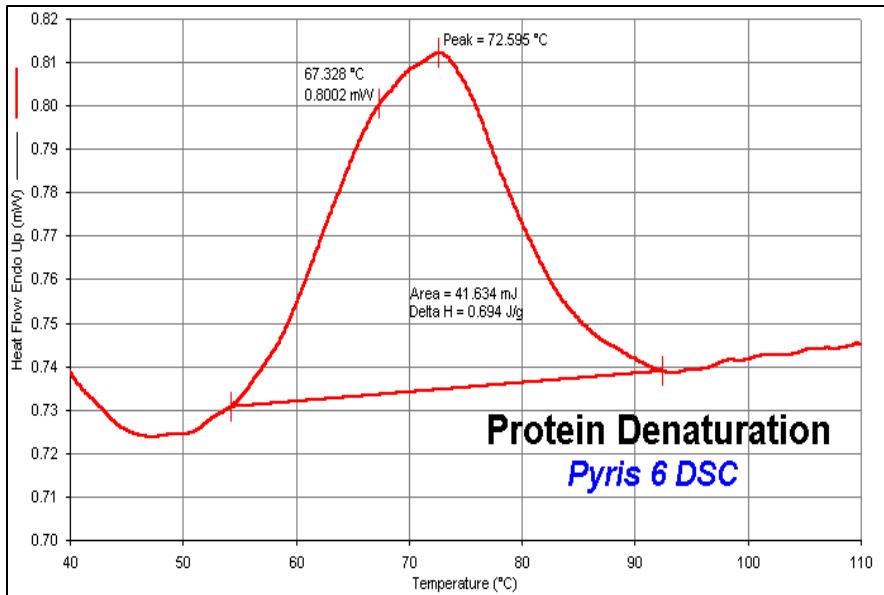


Figure 2. DSC results on 5% protein solution

The protein denaturation event is observed at 72.6 °C for this particular protein with a shoulder peak at 67.3 °C. This protein yields a broad, less intense denaturation peak as compared to some other proteins or the same concentration. The level of sensitivity obtained from the Pyris 6 DSC is outstanding, as the full Y-axis (heat flow) scaling is only 120 μ W.

Additionally, the Pyris 6 DSC had the resolution and sensitivity to detect the fine structure or two peak temperatures associated with this protein solution.

As the protein becomes more dilute, it becomes increasingly more difficult to detect the weak protein denaturation transition. This particular protein solution was diluted five-fold (5X dilution) to generate a solution of only 1% concentration. This dilution is important for protein researchers as the more dilute solutions can prevent possible interference of neighboring proteins during the unfolding or denaturation. For some proteins, the properties of the denaturation can become significantly different at more dilute concentrations.

Displayed in Figure 2 are the DSC results obtained on the dilute 1% protein solution

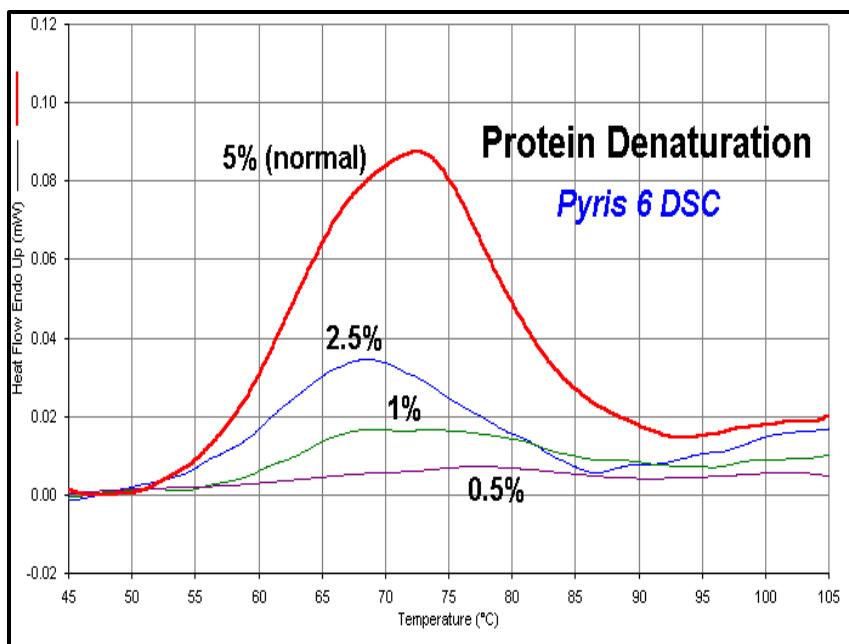
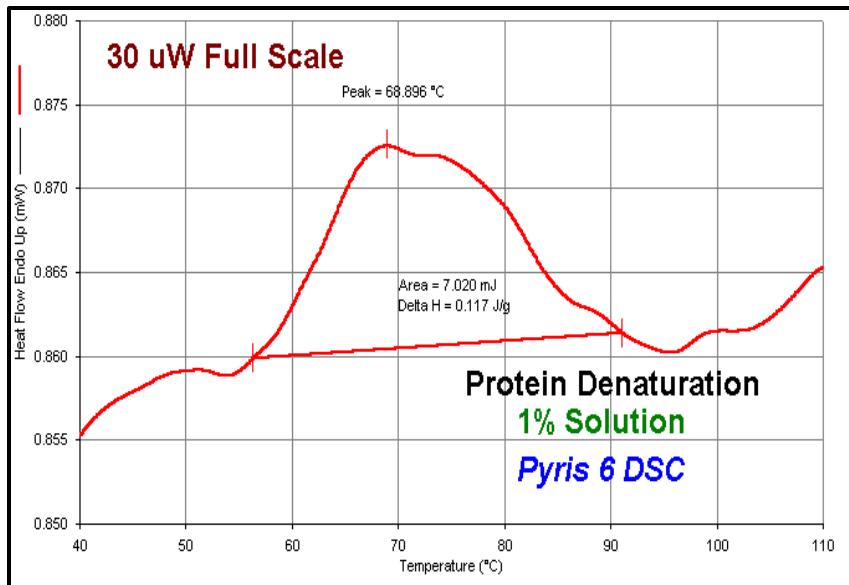
Full Y-axis scaling is an ultra sensitive 30 uW. Despite the very weak intensity of the dilute protein denaturation event, the Pyris 6 DSC provides the extremely high sensitivity to be able to successfully detect this transition.

The denaturation peak is observed at 68.9 C and the heat of denaturation is only 0.12 mJ/mg of solution.

The protein solution was analyzed using the Pyris 6 DSC at concentrations of 5% (original), 2.5%, 1.0% and a very dilute 0.5%. Displayed in Figure 4 is an overlay of the DSC results generated on the protein solutions at the various concentrations.

The Pyris 6 DSC has the ability to observed the protein denaturation events at concentrations ranging from the normal 5% down to the very dilute 0.5%. This is a high level of performance which is normally associated with microcalorimetric devices. The advantage of the Pyris 6 DSC over microcalorimeters, however, is that the DSC 6 permits the analyses of solids or frozen solutions as well as liquids. This is useful for studying the freeze-drying properties of aqueous formulations. The Pyris 6 DSC provides ease of use, flexibility, wide temperature and sample handling range, as well as very high sensitivity.

The Pyris 6 DSC can provide results on proteins for R&D and comparative purposes. Displayed in Figure 5 are the results generated on two different proteins (A and B) at the same solution concentration (2.5%).



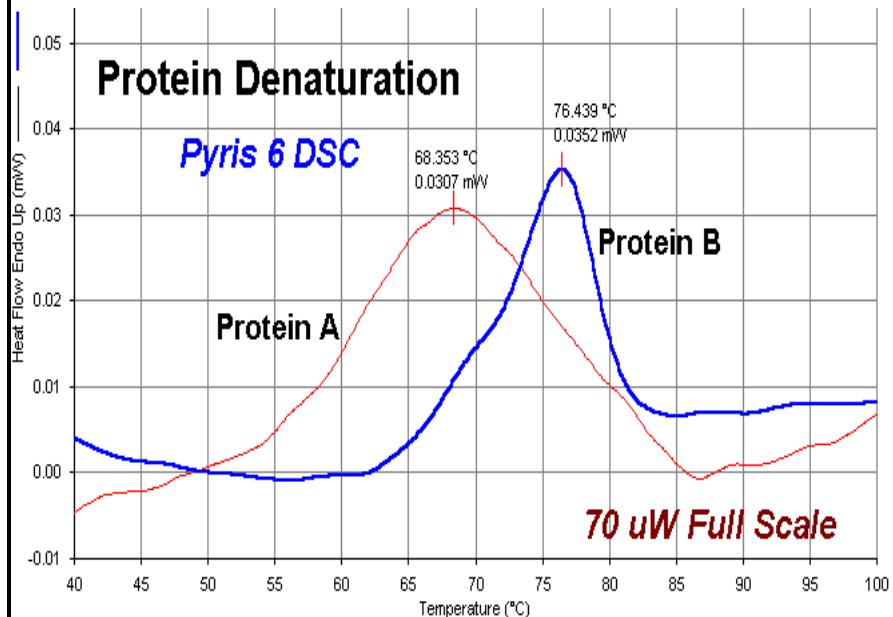
The DSC results reveal that protein B is more thermally stable than is protein A based on its significantly higher denaturation temperature (76.4 C versus 68.4 C). The shape of the denaturation endothermic peak for protein B is significantly narrower as compared to A. This is related to the particular shape factor differences of the two proteins and provides valuable information on the nature of the proteins.

SUMMARY

The PerkinElmer Pyris 6 DSC provides very high sensitivity and stable performance which are ideal for the demanding application of the study of protein denaturation. The sensitivity response of the Pyris 6 DSC is on a microwatt or microcalorimetric level, which is necessary when characterizing dilute protein solutions. The analysis of protein is enhanced with the use of the special, large volume stainless steel capsules.

The Pyris 6 DSC offers the pharmaceutical or drug discovery research scientist with the ability to handle both solids or frozen solutions, in addition to liquid solutions. This provides a high degree of applications flexibility which is not normally available with the microcalorimeters that have been traditionally used to study protein denaturation. With the Pyris 6 DSC, the research scientist can study protein denaturation of solutions as well as the subambient properties of frozen formulations for freeze-drying purposes.

Figure 5. DSC results on denaturation of 2.5% solutions of proteins A and B



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