Advantages of Using a Nano DSC when Studying Proteins that Aggregate and Precipitate when Denatured

Introduction:

The Nano DSC instrument from TA Instruments can detect changes in heat down to the nanowatt range, generated by as little as 2 micrograms of protein. Differential Scanning Calorimetry (DSC) is a technique that is nondestructive when used on proteins that denature reversibly. However, when a protein unfolds, interior hydrophobic and hydrophilic regions of the protein are exposed to the aqueous buffer solution. When the exposed regions are hydrophobic, adjacent protein molecules will aggregate in order to cover up or shield these regions from the surrounding aqueous solution and if enough molecules aggregate together, then a precipitate will form. When a protein is aggregating, the heat signal on a DSC is the sum of both the endothermic unfolding of the protein and the exothermic aggregation of the protein. Aggregation/precipitation typically has a higher enthalpy than unfolding, so both processes occurring simultaneously usually results in unusable data. The main advantage of the TA Instruments Nano DSC capillary cell design is that it separates protein molecules with enough space that for many samples any aggregation is delayed until after the protein has unfolded. In other sample cell configurations, such as “coin” configurations, all of the molecules are located in a small confined space, allowing aggregation to proceed unchecked and thus have limited utility when analyzing some proteins.

In other published studies, the continuous capillary cell configuration has demonstrated an ability to successfully attenuate the aggregation of biomolecules, such as DNA and proteins[1,2].

Protein Sample:

The protein sample consisted of a purified human IgG_1 monoclonal antibody in a physiological buffer. For the DSC analysis, the antibody concentration was adjusted to 1.0 mg/ml.

DSC Protocol:

The DSC protocol was set from 25°C-110°C at 1°C/min.

Differential Scanning Instruments:

A sample of the IgG_1 antibody was run in two ultrasensitive DSC instruments. One instrument contained a coin shaped sample cell and the second instrument was the Nano DSC with a continuous capillary sample cell configuration.

Results:

Figure 1
Data obtained with a DSC with
"Coin" Shaped Sample Cell

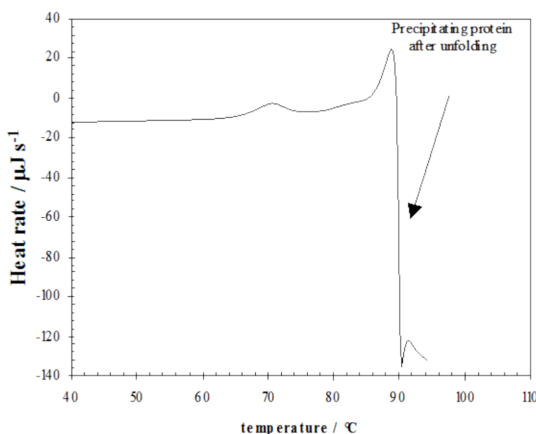
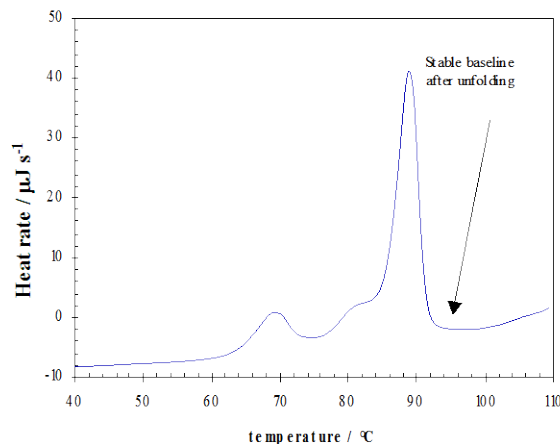


Figure 2
Data obtained with a Nano DSC with
a Continuous Capillary Sample Cell



Summary:
The Nano DSC with the continuous capillary sample cell demonstrated the ability to attenuate the protein aggregation/precipitation event following the final domain unfolding event exhibited by the DSC containing the coin shaped sample cell. Although there was some evidence of aggregation/precipitation in the sample when removed from the sample cell, the Nano DSC's ability to establish a stable baseline following the final domain unfolding event is essential to obtaining a relatively intact thermodynamic profile of the antibody scanned in this experiment.

Figure 1 shows the data collected by the DSC with the coin shaped cell. The large exothermic event following the T_m peak at approximately 89°C does not allow the determination of the ΔH or differential heat capacity (ΔCp) from the data collected.

Figure 2 shows the data collected by the Nano DSC with the continuous capillary cell. Although a visual inspection of the sample at the conclusion of the scan revealed some aggregation/precipitation, the continuous capillary cell attenuated the precipitation of the antibody enough to allow a reproducible post-transition baseline to be achieved above 95°C. Even though protein aggregation/precipitation may not be completely prevented in a continuous capillary cell DSC, the attenuation of these events until after the scan is completed results in a more complete thermodynamic data set. The final analysis of this data will, at minimum, give a good representation of the number of domains in the antibody structure, domain transition temperatures (T_m), and differential heat capacities (ΔCp).

References:

[1] Dragan, A.I., Russell, D.J. and P.L. Privalov. DNA hydration studied by pressure perturbation scanning microcalorimetry. Biopolymers 91(1): 95-101. 2009.

[2] Privalov, P. L. In Protein Structure, Stability and Interactions; Shriver, J. W., Ed.; Humana Press-Springer: US, 2008.