Characterization of proteins

GENERAL INTRODUCTION

Research and development is important in the pharmaceutical industry. To create a perfectly characterized and stable protein based drug, researchers use various techniques, including differential scanning calorimetry (DSC). When heated, a protein will unfold, or become denatured, which is characterized by an endothermic effect on a DSC curve. Once unfolded, proteins can also aggregate, which is typically an exothermic process. Differential scanning calorimetry is therefore a key tool.
Setaram has developed a microcalorimeter called the MICROCALVET ULTRA 4C for these applications with the following characteristics:
• Dual sample capacity
• Removable and cleanable cells so as to avoid problems due to gel formation or aggregate deposition.
• Improved detection limit
• A temperature range from -24°C up to 170°C

Examples of applications in the field of proteins characterization are detailed below.

1- RNAse Unfolding

INTRODUCTION

Thermally induced structural differences of proteins do not only depend on their origin and extraction and purification conditions, but also on their environmental conditions like pH, salt content, or solvent type.
In this example, Ribonuclease was tested under different conditions. RNase is a type of nuclease that catalyzes the degradation of RNA into smaller components.

EXPERIMENT

Bovine pancreatic RNase A is one of the classic model systems of protein science.
The samples being analyzed are 5mg/mL aqueous solutions of Ribonuclease A, 0.15M NaCl, pH7. Urea was added to one of the solutions with a concentration of 3M. About 750µL of each solution were placed separately into two batch cells. For compensation, corresponding buffer solutions were placed in reference cells. The four cells were then inserted into the four calorimetric chambers of MICROCALVET ULTRA 4C. They were then heated between 10°C and 90°C at 1 K/min.

RESULTS AND CONCLUSION

Figure 1 shows the results of this test. An endothermic peak correlating with the unfolding of the enzyme is recorded in each case at different temperatures. The peak temperature or Tm, which corresponds to the point at which half the protein in solution are unfolded, is compared. The 10.3°C downward shift of Tm in the sample containing urea corresponds to the formulated protein becoming unstable.

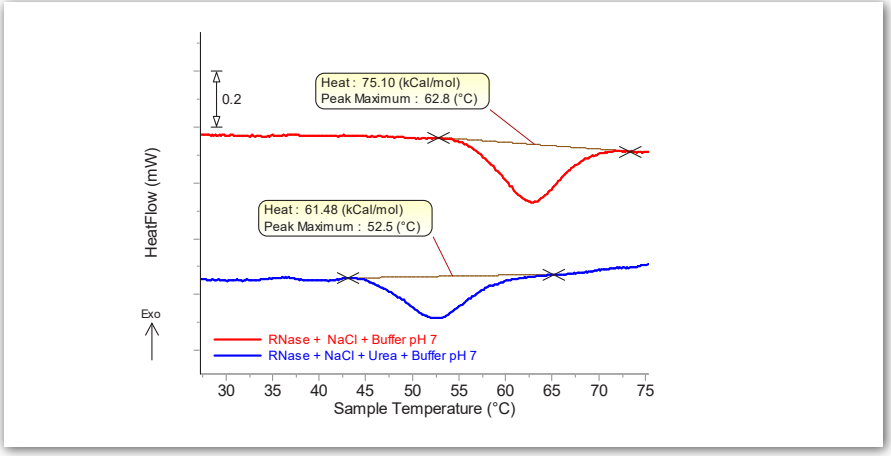


Figure 1 – Endothermic peaks corresponding to unfolding of the proteins in the two tested RNase solutions

2- Beta-lactoglobulin Unfolding, Aggregation and Decomposition

INTRODUCTION

Globular proteins are widely used in food and pharmaceutical industries because their unique folded structure leads to interesting active principle or flavor binding, water solubility, and gelation properties. β-Lactoglobulin (β-LG) is the major whey protein of cow and sheep's milk. β-LG is a well-characterized globular whey protein which exists mainly in its dimeric form between pH 3 and 7.

EXPERIMENT

Commercial β-Lactoglobulin from Sigma in aqueous buffer (pH 4.01 and 7.00), with concentrations of 7.5g/L and 25g/L, sodium chloride content of 0 or 0.5M. Heating profile: from 30°C to 160°C at 0.5K/min.

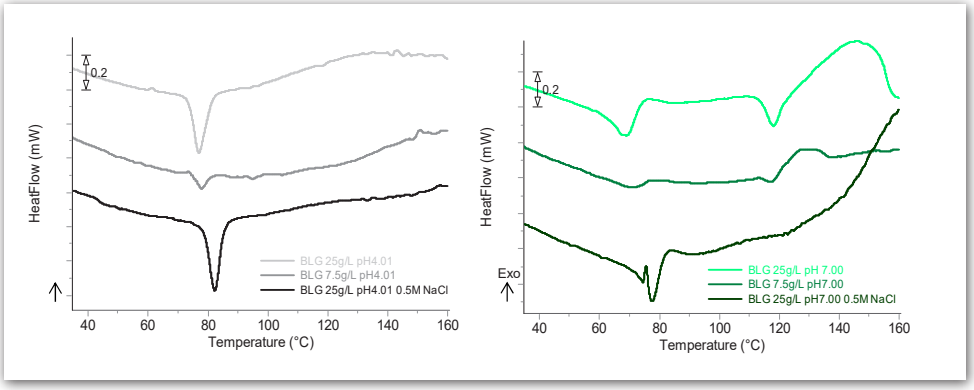


Figure 2 – HeatFlow vs. Temperature data of thermal unfolding and aggregation of β-LG under different concentration, pH and salt conditions

RESULTS AND CONCLUSION

A major endothermic peak corresponding to unfolding is observed at pH 4.01 and is not significantly affected by the β-LG concentration. In the presence of NaCl, the protein is stabilized with a peak shift of more than 5°C.
At pH 7.00, the mechanism is more complex and endothermic thermal effects are detected at high temperatures, which fit previous observations [1]. They correspond to the depolymerization of the unfolded protein followed by the formation of smaller peptides. The exotherms have been attributed to Maillard reaction between the peptides and polysaccharide impurities contained in the material. In the presence of NaCl, a thin exotherm can be seen during the unfolding endotherm. Raemy et al [2] showed that this peak corresponds to protein aggregation, in particular an increase in aggregate size.

References:

[1] S. Photchanachai et al, J Food Science—Vol. 66, No. 5, 2001
[2] A. Raemy et al, Food Hydrocolloids 20 (2006) 1006–1019

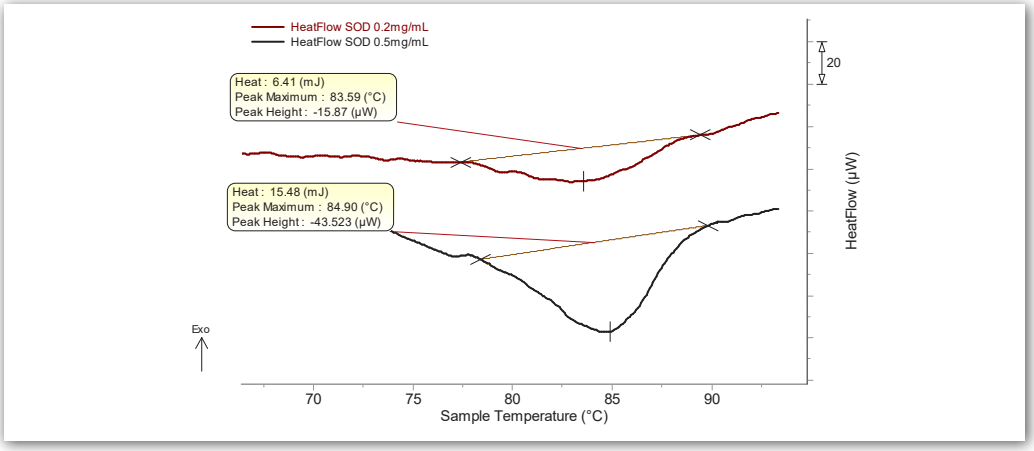


Figure 3 – Endothermic unfolding peaks of the two tested SOD solutions

3- Tests with small amounts of protein

INTRODUCTION

Samples of protein available after synthesis and purification are generally small and they need to then be divided into even smaller samples to be characterized using different analytical techniques. This means that the samples are usually highly diluted. In this example a sample of protein is tested using only 160µg in the cell.

EXPERIMENT

The analyzed samples are 0.2 and 0.5mg/mL aqueous solutions of Superoxide Dismutase Mutant G41D in 20mM HEPES, pH7.8 buffer. About 800µL of each solution were introduced in two different batch cells. For compensation, corresponding buffer solutions were placed into the reference cells. The four cells were placed into the four calorimetric chambers of the MICROCALVET ULTRA 4C. They were heated from 15°C to 100°C at 1 K/min.

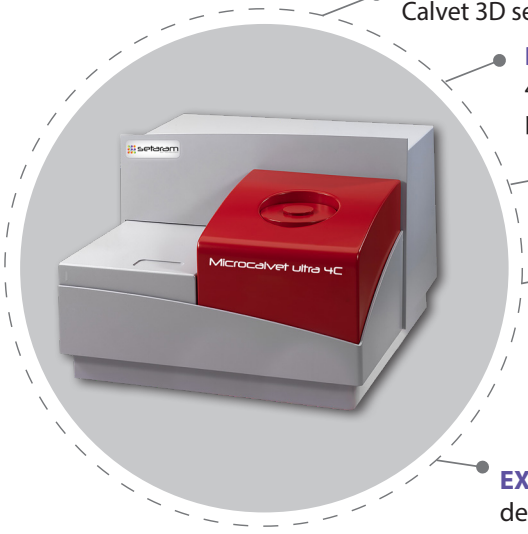
RESULTS AND CONCLUSION

The DSC curves depicted in Figure 3 show an endothermic effect in the 80-90°C range corresponding to the unfolding of the enzyme. Both lead to significant endothermic peaks with Tm in the 84°C range. For the 0.2mg/mL solution, a peak height as low as -15.9µW could be detected.

INSTRUMENT

MICROCALVET ULTRA

-20 to 170°C



HIGHEST HEAT MEASUREMENT ACCURACY
Calvet 3D sensor based on Peltier elements with Joule effect calibration

DUAL SAMPLE TESTING
4C means four cells, i.e. dual sample, for greater throughput and a preserved accuracy even with large heat effects

MODIFIABLE TEMPERATURE CONDITIONS
for increased flexibility and replication of real life conditions between -20 and 170°C

CONVENIENT INTERCHANGEABLE CRUCIBLES AND CELLS
to perform even the most demanding experiments using one instrument :
- high pressure (up to 400 bar) and high vacuum, pressure measurement and control
- mixing experiment

EXTERNAL COUPLING CAPABILITY
designed to increase your research options including manometry, BET instrumentation, gas analyzers, humidity controllers and gas panels