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Application News

No.T150

Thermal Analysis

Heat Denaturation of Proteins by DSC

Higher-order protein structures play a role in the function of a protein. Protein geometry, and therefore function, is greatly influenced by many factors, including the surrounding solvent, temperature, pH, etc. Therefore, the evaluation of protein stability in the presence of solvents and with respect to the pH of the environment is very important. One common method for analyzing protein stability is differential scanning calorimetry (DSC).
DSC is used to measure heat energy released or absorbed by a sample compared to a reference, and is used to quantify the energy associated with chemical reactions, phase changes, and structural changes. When the higher-order structure of a protein is irreversibly changed, causing activity loss due to degeneration, this is referred to as thermal denaturation. The result is an endothermic peak in DSC analysis. Using the DSC-60 Plus differential scanning calorimeter, we measured the thermal denaturation temperatures of lysozyme and egg-derived protein, and observed the influence of pH on the thermal denaturation temperature of lysozyme.

Thermal Denaturation Temperatures of Egg White-Derived Protein and Lysozyme

The thermal denaturation temperature was measured for egg white-derived protein, as shown in Fig. 1, and lysozyme from chicken egg white, in Figs. 2 and 3. The protein and lysozyme were analyzed in pH 7.05 phosphate buffer in sealed aluminum cells. For lysozyme, measurements were performed at concentrations of 0.2 % and 2.5 %, whereas the egg white-derived protein had a concentration of 10.4 %.
In Fig. 1, an endothermic peak derived from albumin is seen at 83.5 °C. Fig. 2 and Fig. 3 show the results for lysozyme present at concentrations of 2.5 % and 0.2 %, respectively. Even with dilution to 0.2 % lysozyme, the endothermic peak due to thermal denaturation was clearly detected.

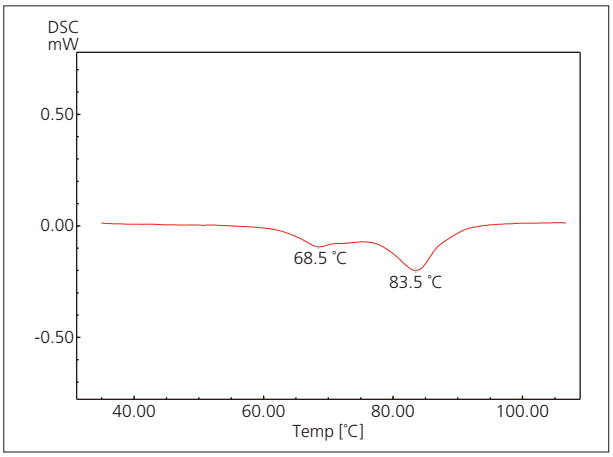

Fig. 1 Analysis of 10.4 % Egg White-Derived Protein in pH 7.05 Buffer

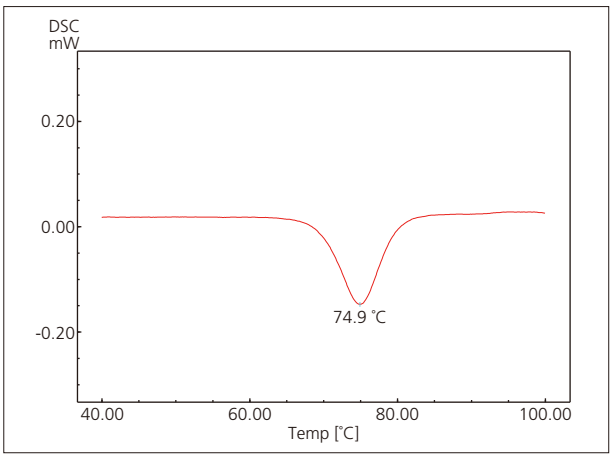

Fig. 2 Analysis of 2.5 % Lysozyme in pH 7.05 Buffer

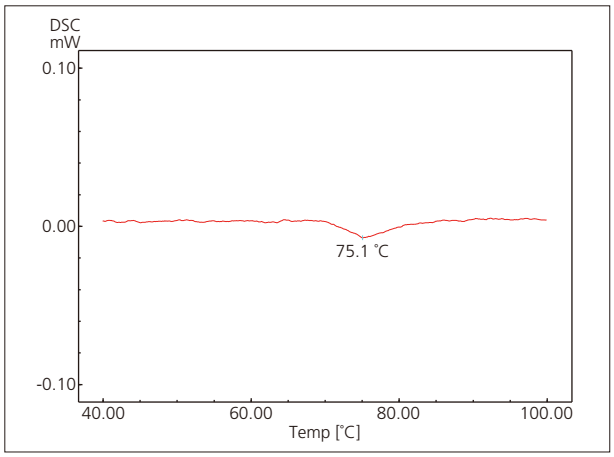

Fig. 3 Analysis of 0.2 % Lysozyme in pH 7.05 Buffer

pH Dependence of the Thermal Denaturation Temperature of Lysozyme

A 0.2 M sodium phosphate buffer solution was added to lysozyme produced from chicken eggs to create 10 % lysozyme samples with pHs of 4.2, 7.05, and 9.10. The thermal denaturation temperatures for these samples were measured by heating at 5 ˚C/min from 40 ˚C up to 100 ˚C. The result is that the thermal denaturation temperature clearly shifts to the higher temperatures as acidity increases (Fig. 7) from 72.0 ˚C at pH 9.10 to 77.4 ˚C at pH 4.20.

In conclusion, the high sensitivity and excellent baseline stability of the DSC-60 Plus enables easy measurement of thermal denaturation temperatures of proteins, even at low concentrations.

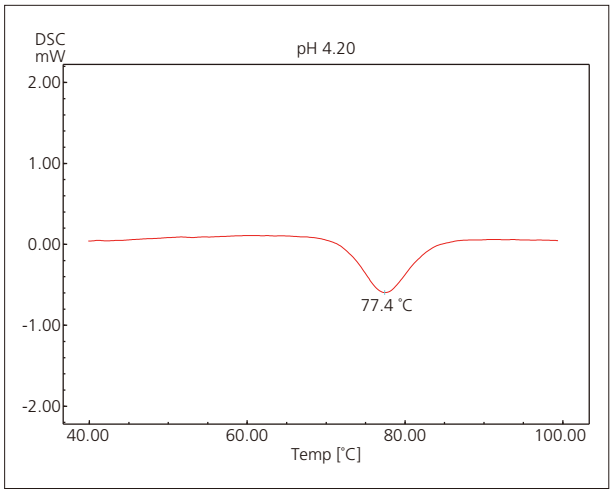

Fig. 4 Analysis of 10 % Lysozyme Solution at pH 4.20

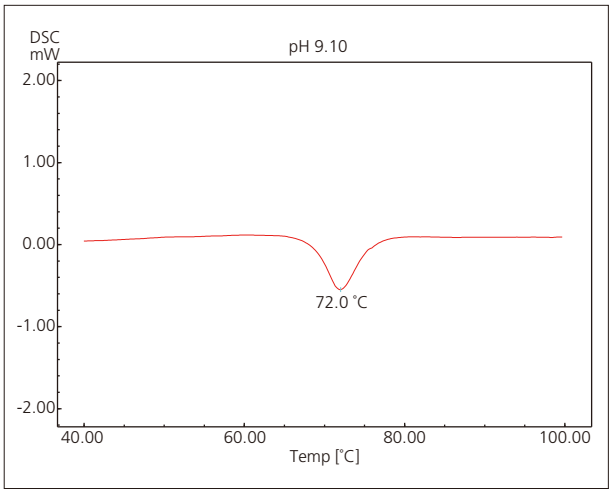

Fig. 6 Analysis of 10 % Lysozyme Solution at pH 9.10

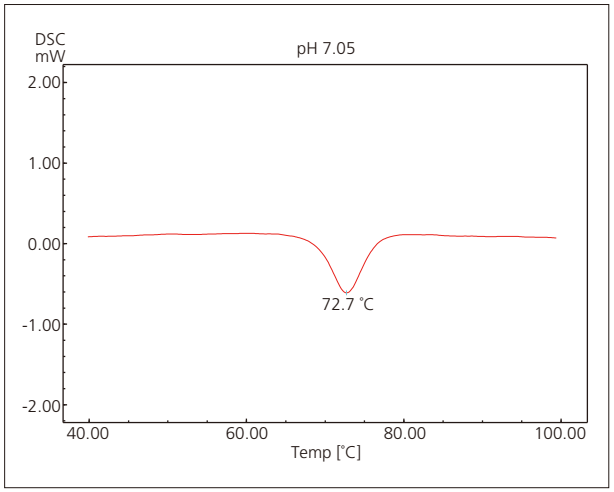

Fig. 5 Analysis of 10 % Lysozyme Solution at pH 7.05

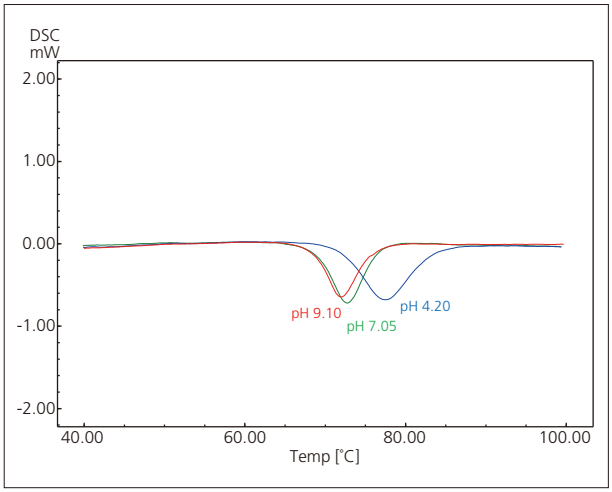

Fig. 7 Overlaid Analysis of 10 % Lysozyme Solution at pH 4.20, pH 7.05 and pH 9.10

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