FP APPLICATION NOTE 1-09

Fluorescence Measurement of Heat-Denatured Lysozyme

Experiments for the denaturation of proteins are generally measured by Circular Dichroism. However, proteins contain aromatic amino acids (AAA) and will fluoresce when excited with UV light. During heat denaturation of proteins, the secondary structure of the protein will change and the aromatic amino acid residues will change slightly which can be detected by fluorescence.

Experimental

Emission (EM) spectra of lysozyme using an excitation (EX) of 280 nm are measured while controlling the temperature, to examine the relation between temperature and the fluorescence spectrum. The model FP-6500 spectrofluorometer and an ETC-272 Peltier thermostatted cell holder are used for the measurements in this experiment, using the instrument parameters outlined below.

Parameters:

Temperature range: 15 to 90°C
Temperature interval: 5°C
EM bandwidth: 5 nm EX bandwidth: 3 nm
EX wavelength: 280.0 nm
Response: 0.5 sec Gain: Medium
Wavelength range: 290 to 450 nm

A buffered aqueous solution of 0.1mg/mL of lysozyme was used as the sample, the measurement performed by stirring the sample with a magnetic stirrer to ensure even sample temperatures in the cell. A temperature ramping rate of 1°C/min was controlled by the Peltier cell holder. Spectra are measured within 60 seconds after reaching the individual set temperature points.

Results

The graph below (Figure 1) demonstrates the change of intensity vs. temperature at 340 nm. Lyzosyme is known to denature at a temperature of 70°C; however the graph of fluorescence vs. temperature shows only a decrease in intensity as the temperature increases. By contrast, the EM Spectra from 40 to 90 degrees plotted using a contour view demonstrates a transition of the spectra at 70 degrees (Figure 2).

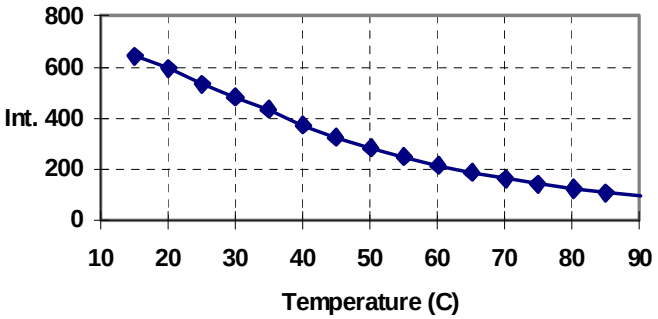

Figure 1: Temperature Dependence of Lysozyme aqueous solution at EX 280 nm, EM 340 nm

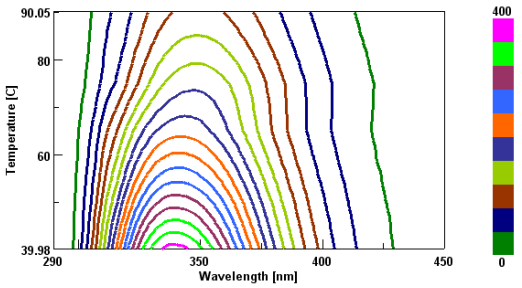

Figure 2: Contour view of EM spectra from 40 to 90 degrees.

The EM spectra at room temperature have an EM maximum at 340 nm with a corresponding maximum at 348 nm for 90 degrees. A plot of the intensity ratios for the two wavelengths versus temperature results in a graph that demonstrates a heat-denaturation at 70°C (Figure 3), in agreement with literature values.

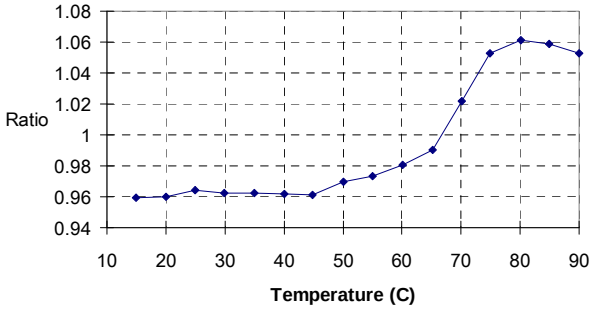

Figure 3: Intensity ratio of 340/348 nm vs. temperature

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