Application Note
FP-0001

Fluorescence Measurement of Heat-Denatured Lysozyme

Introduction

Fluorescence spectroscopy can be used to monitor changes in the solvent environment and aromatic amino acid residues of a protein. Increasing the solution temperature can induce structural changes in a protein as it unfolds, which can be reflected in the emission spectrum. The thermodynamic properties can then be obtained to provide information regarding the protein's thermal stability.

FP-8500
Spectrofluorometer

This application notes illustrates the temperature-dependence of lysozyme by monitoring the protein's tertiary structure using a FP-8500 and thermostatted cell holder.

Keywords

FP-8500, Fluorescence, Temperature-dependence, ETC-272 Peltier thermostatted cell holder

Experimental

Measurement Conditions			
Excitation Wavelength	280 nm	Wavelength Range	290 - 450 nm
Excitation Bandwidth	3 nm	Emission Bandwidth	5 nm
Response Time	0.5 sec	Gain	Medium
Temperature Range	15-90°C	Temperature Interval	5°C

A buffered aqueous solution of 0.1 mg/mL of lysozyme was stirred 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 and spectra were measured within 60 seconds after reaching the individual set temperature points.

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Results

Figure 1 illustrates the change in the emission intensity at 340 nm as a function of temperature. As a protein is denatured and begins to unfold, the fluorescence intensity is expected to decrease. While lysozyme is known to denature around 70°C, Figure 1 shows a consistent decrease in the fluorescence intensity as the temperature increases. However, the contour plot of the emission spectrum (Figure 2) illustrates a slight shift in the spectra at 70°C.

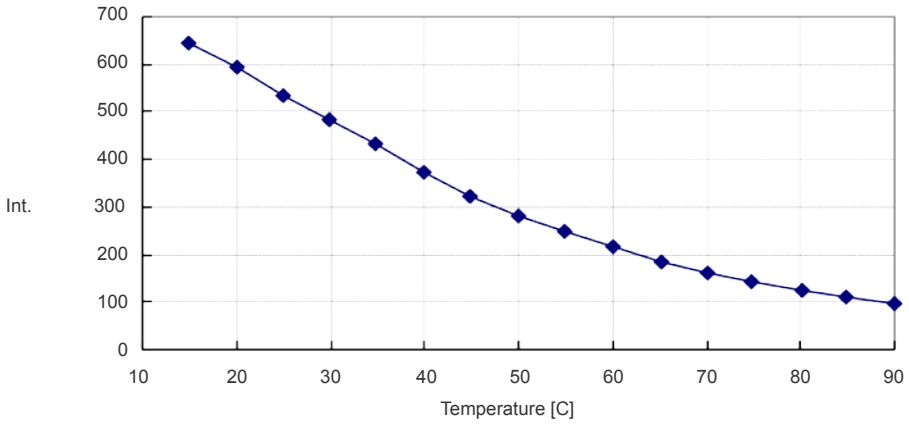

Figure 1. The temperature-dependent florescence intensity of lysozyme at 340 nm.

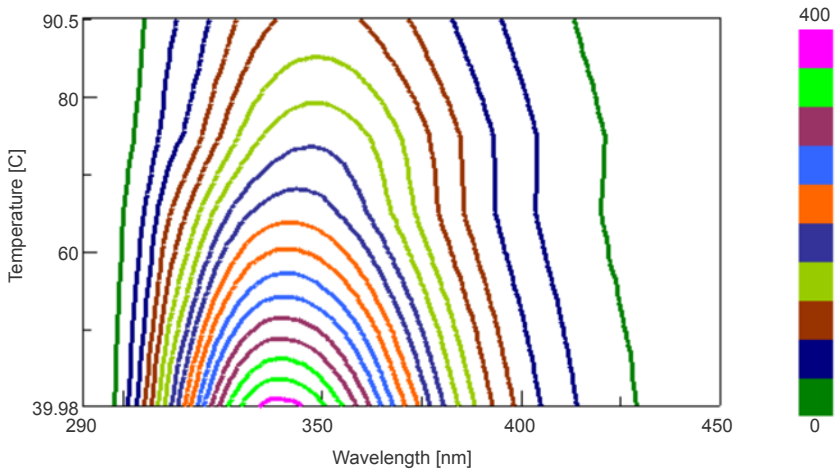

Figure 2. Countour plot of the emission spectra from 40 to 90°C.

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The emission spectra of lysozyme at room temperature has an emission maximum at 340 nm while at 90°C the maximum is at 348 nm. Figure 3 depicts a plot of the intensity ratios for the two wavelengths as a function of temperature. Figure 3 clearly shows a transition at 70°C, corresponding to the denaturation temperature of lysozyme.

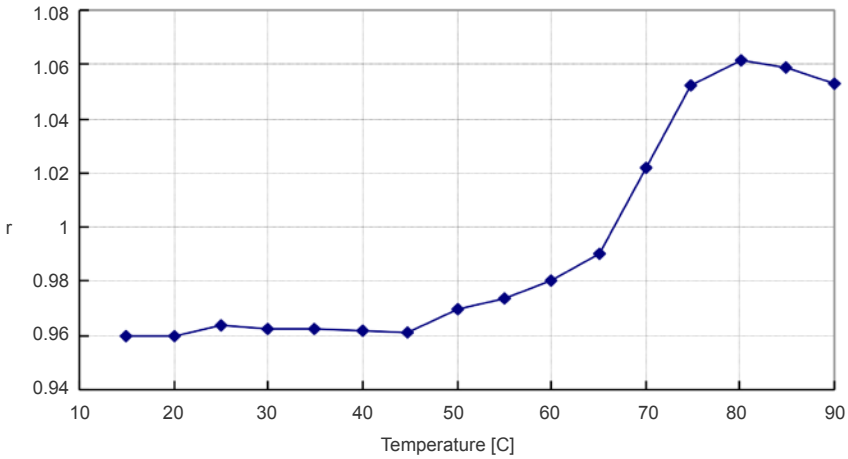

Figure 3. Maximum fluorescence intensity ratio (340/348 nm) as a function of temperature.

Conclusion

This application note demonstrates that the FP-8500 can be used to obtain thermal denaturation data for protein structure studies.

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