Application Note
CD-0024

Temperature-Dependent CD and Fluorescence Spectral Measurements of Lysozyme

Introduction

J-1500
Spectrometer

Recently, there has been a significant increase in the research and manufacturing of biomedicines derived from proteins, which are becoming more widely available in the biopharmaceutical industry. An important requirement in the manufacturing and quality control of protein-based biopharmaceuticals is in the assessment of stability during storage and the effects of storage conditions. The measurement of denaturation and thermal stability are of considerable importance in guaranteeing the efficacy of biopharmaceuticals. CD measurement offers significant advantage in the assessment of protein secondary structure due to its requirement for small amount of sample coupled with high sensitivity measurement. Therefore, CD measurement is becoming one of the most popular techniques used in the analysis of the thermal stability and changes in protein structure caused by ionic strength and pH. The use of fluorescence spectroscopy in the probing tryptophan residues also yields important information about the tertiary structure of proteins.

This application note illustrates temperature-dependent CD and fluorescence measurements of lysozyme obtained simultaneously using the J-1500 CD spectrometer, FMO-522 Emission Monochromator accessory, and Temperature/ Wavelength Scan Measurement program.

Keywords

J-1500, FMO-522 Emission monochromator, Circular dichroism, Fluorescence, Secondary structure, Tertiary structure, Thermal stability, Biochemistry

JASCO INC.
28600 Mary's Court, Easton, MD 21601 USA
Tel: (800) 333-5272, Fax: (410) 822-7526
Application Library: https://www.jascoinc.com/applications

Experimental

Measurement Conditions			
CD		**Fluorescence**	
Data Pitch	0.5 nm	Data Interval	2 nm
Bandwidth	1 nm	Excitation Bandwidth	1 nm
D.I.T.	2 sec	Excitation Wavelength	280 nm
Scan Speed	100 nm/min	Emission Bandwidth	10 nm
		D.I.T.	1 sec

Temperature Measurement			
Temperature Range	20-90°C	Gradient (Heating Rate)	0.1°C
Wavelength	222 nm	D.I.T.	4 sec
Bandwidth	1 nm		

An aqueous solution of 0.25 mg/mL of lysozyme, derived from egg white, was prepared and measured using a 5x5 mm rectangular quartz cell.

Results

Figure 1 shows the temperature-dependent CD spectra of lysozyme. The data illustrate that the CD intensity decreases with increasing temperature and the negative maxima at 208 nm is shifted to 203 nm with increasing temperatures. These results indicate that the helical structure of the protein at room temperature converts to a more random structure at higher temperatures.

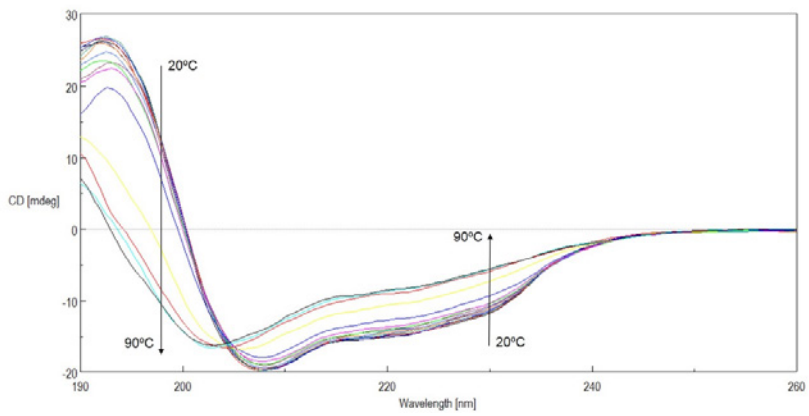

Figure 1. Temperature-Dependent CD spectra of lysozyme from 20 to 90°C.

JASCO INC.
28600 Mary's Court, Easton, MD 21601 USA
Tel: (800) 333-5272, Fax: (410) 822-7526
Application Library: https://www.jascoinc.com/applications

To determine the transition temperature of the secondary structure conversion, the CD signal was monitored at 222 nm as a function of temperature. Figure 2 illustrates that from 70 to 80°C, the CD intensity drastically decreases and the melting temperature (Tm) was calculated to be 74.38°C.

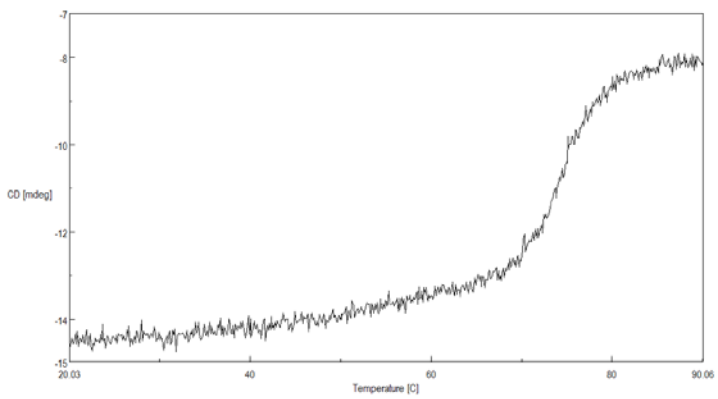

Figure 2. CD thermal melt data of lysozyme at 222 nm.

Once the initial melt was complete, the lysozyme solution was cooled back down to 20°C, to verify folding was reversible. Figure 3 shows the CD spectrum of the initial 20°C spectrum (black) compared with the spectrum measured at 90°C (red) and at 20°C after the melt (blue). The spectra before and after are very similar, indicating that the protein does refold once the temperature is reduced, however, the refolding process is not complete.

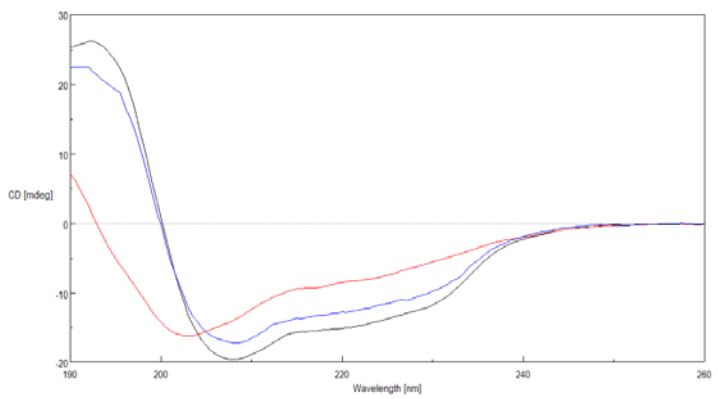

Figure 3. CD spectra of lysozyme measured at 20°C (black), heated to 90°C (red), and cooled back down to 20°C (blue).

JASCO INC.
28600 Mary's Court, Easton, MD 21601 USA
Tel: (800) 333-5272, Fax: (410) 822-7526
Application Library: https://www.jascoinc.com/applications

Tryptophan is commonly found in most proteins and has an emission maximum which is very sensitive to the polarity of its surrounding environment. In a nonpolar environment or when the residue is buried inside a protein, the fluorescence maximum is seen near 320 nm. Tryptophan in a polar environment or when it is solvent exposed, has an emission maximum near 350 nm. Figure 4 shows the temperature-dependent fluorescence spectra of lysozyme. Initially the emission maximum is at 340 nm but upon increasing temperatures, the peak is redshifted and at 90°C is at 352 nm. This result indicates that the tryptophan residue commonly tucked away inside the interior of the protein at 20°C moves to the periphery of the protein as it unfolds due to increasing temperatures.

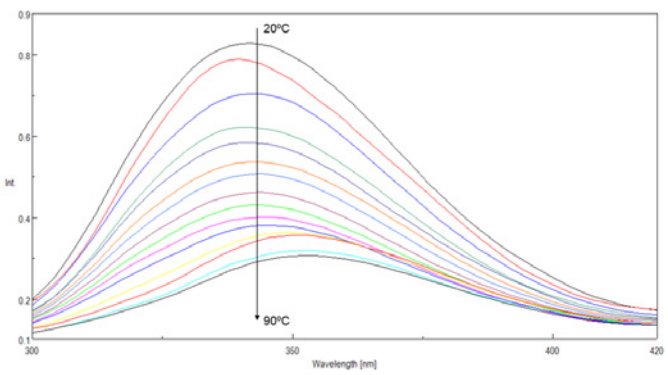

Figure 4. Temperature-Dependent fluorescence spectra of lysozyme.

Figure 5 shows the peak ratio plot of the fluorescence intensity at 340 nm and 352 nm as a function of temperature. From 70 to 75°C, the peak ratio significantly increases, indicating that the protein environment surrounding the tryptophan residues is altered and corroborating the melting temperature at 74°C calculated from the CD data.

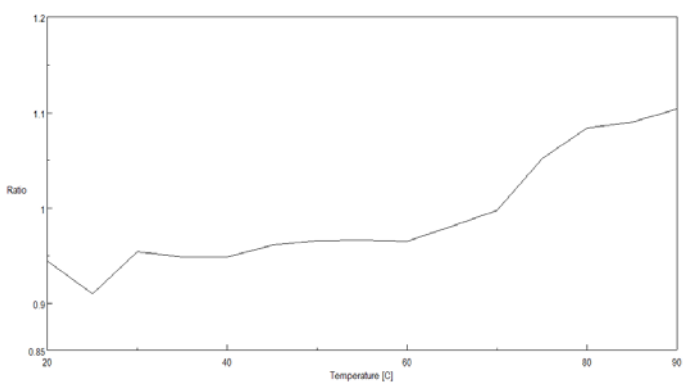

Figure 5. Fluorescence thermal melt data. The fluorescence intensity was plotted as the ratio of 352 and 340 nm.

JASCO INC.
28600 Mary's Court, Easton, MD 21601 USA
Tel: (800) 333-5272, Fax: (410) 822-7526
Application Library: https://www.jascoinc.com/applications

The ability of lysozyme to refold was also evaluated using fluorescence and the results are shown in Figure 6. They suggest that after cooling the protein, the lysozyme structure almost completely returns to its initial state, confirming the CD data which also indicate partial lysozyme refolding upon cooling.

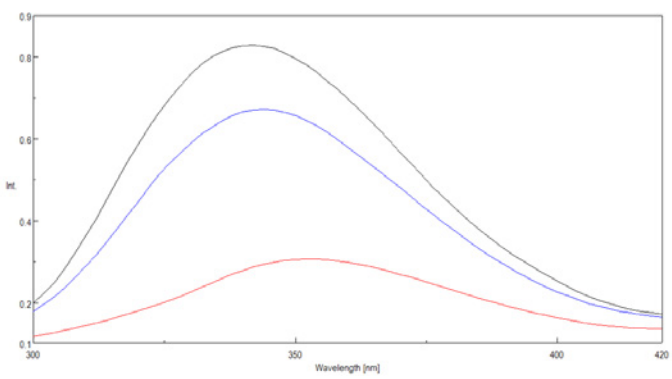

Figure 6. Fluorescence spectra of lysozyme measured at 20°C (black), heated to 90°C (red), and cooled back down to 20°C (blue).

References

1. S. V. Konev, "*Fluorescence and Phosphorescence of Proteins and Nucleic Acids*", Plenum Press, New York, 1967.

JASCO INC.
28600 Mary's Court, Easton, MD 21601 USA
Tel: (800) 333-5272, Fax: (410) 822-7526
Application Library: https://www.jascoinc.com/applications