Application Note

CD-0024

Temperature control and CD/fluorescence spectrum of lysozyme using Temperature/wavelength scan measurement program

Introduction

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 bio-phamaceutical industry. An important requirement in the manufacturing and quality control of protein-based biopharmaceuticals is in the assessment of stability during storage and the affects 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.

The JASCO J-1500 Temperature/Wavelength Scan Measurement Program allows the measurement of a CD spectrum at a specific temperature, as well as temperature controlled measurement at a fixed wavelength. The optional FMO-522 Emission monochromator also allows simultaneous measurement of a fluorescence spectrum. In this technical note, we describe the temperature controlled simultaneous measurement at 222 nm of both CD and fluorescence signals for a lysozyme sample using the J-1500 CD spectrophotometer.

Keywords: Bio medicinal product, Thermal denaturation, Secondary structure, Tertiary structure

Sample preparation

An aqueous solution of 0.025 mg/mL lysozyme, derived from egg-white was prepared and measured using a rectangular quartz cell.

Measurement parameters

(Temperature control measurement)
Rising temp. condition: 1ºC/min (20 - 90ºC)
Data interval: 0.1ºC
Measurement wavelength: 222 nm
Spectral bandwidth (SBW): 1 nm
Response: 4 sec

(CD spectrum measurement)
Measurement temp.: 5ºC interval (20 – 90ºC)
Wavelength range: 190 – 260 nm
Data interval: 0.5 nm
Response: 2 sec
Spectral bandwidth (SBW): 1 nm
Scan speed: 100 nm/min

(Fluorescence measurement)
Measurement temp.: 5ºC interval (20 – 90ºC)
Excitation bandwidth: 1 nm
Excitation wavelength: 280 nm
Fluorescence bandwidth: 10 nm
Wavelength range: 300 – 420 nm
Data interval: 2 nm
Response: 1 sec

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Results

(CD spectrum measurement)

Fig. 1 shows the temperature control CD spectrum of the lysozyme solution. The CD intensity is decreased with rise in temperature and negative peak at 208 nm is shifted to 203 nm in response to temperature change from 20ºC to 90ºC. This result indicated that lysozyme helix structures transform to random structures.

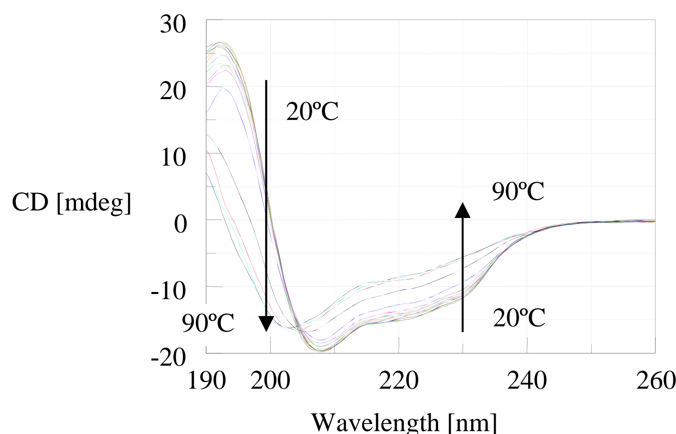

Fig. 1 Temperature control CD measurement of lysozyme from 20 ºC to 90 ºC

Fig. 2 shows the CD data at the 222 nm which reflects the helix structure against the temperature. In the region from 70ºC to 80ºC, the CD intensity is drastically decreased. Analysis using Denatured Protein Analysis Program provide the 74.38ºC of denaturation temperature (Tm).

After that, lysozyme solution with 90ºC was cooled to 20ºC and CD measurement was carried on. Fig. 3 shows the CD spectrum of cooling lysozyme solution, which indicates that the most part of lysozyme is re-folding by cooling.

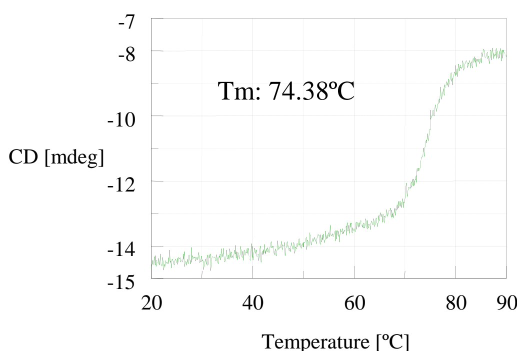

Fig. 2 Temperature control data of lysozyme (222 nm)

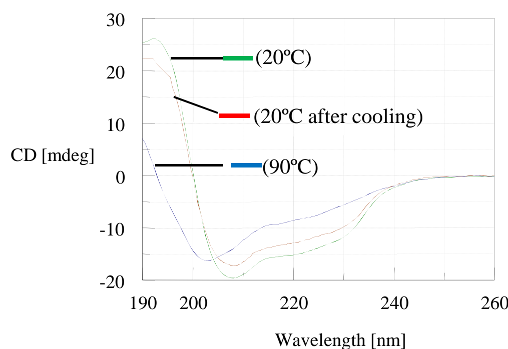

Fig. 3 CD spectrum of lysozyme (Green: 20ºC initial, Blue: 90ºC, Red: 20ºC final)

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Fig. 4 shows the temperature control fluorescence spectrum of lysozyme. The fluorescence peak of tryptophan residue has peak at 340 nm in 20ºC. The peak is shifted with rise of temperature and reached to 352 nm in 90ºC. This result probably means that the tryptophan residue which is inside of protein is appear on the surface of protein by the thermal denaturation.

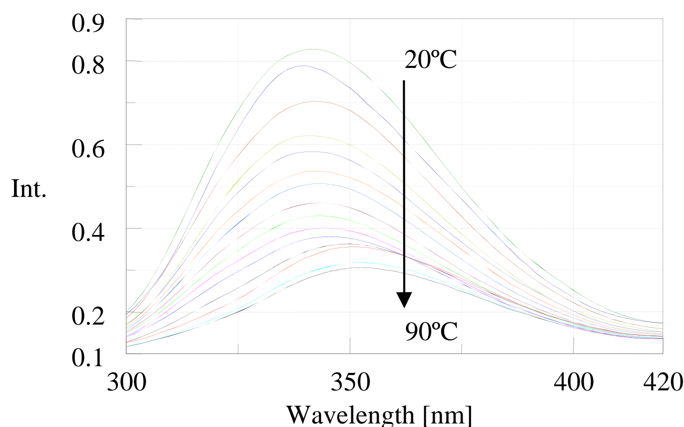

Fig. 4 Temperature control fluorescence spectrum of lysozyme from 20ºC to 90ºC

Fig. 5 shows the peak ratio plot of fluorescence intensity at 340 nm and 352 nm against the temperature. In the region from 70ºC to 75ºC, the peak ratio is drastically increased, which means that the surrounding environment of triptophan residue changes drastically at 74ºC like helix denaturation temperature.

After cooling lysozyme solution from 90ºC to 20ºC, fluorescence measurement is carried on again. Fig. 6 shows the fluorescence spectrum of lysozyme at each temperature. Cooling spectrum is similar to the spectrum before heating, which means that tryptophan residue is embedded in protein again.

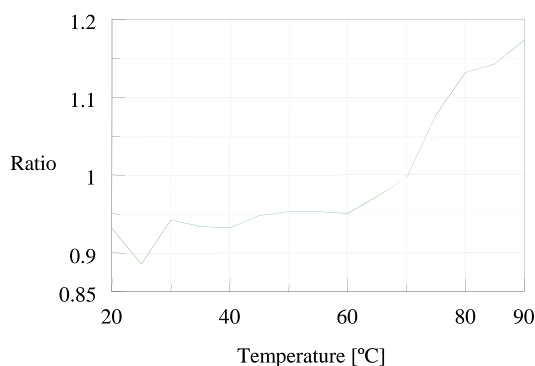

Fig. 5 Temperature control plot of fluorescence intensity ratio (352 nm/340 nm)

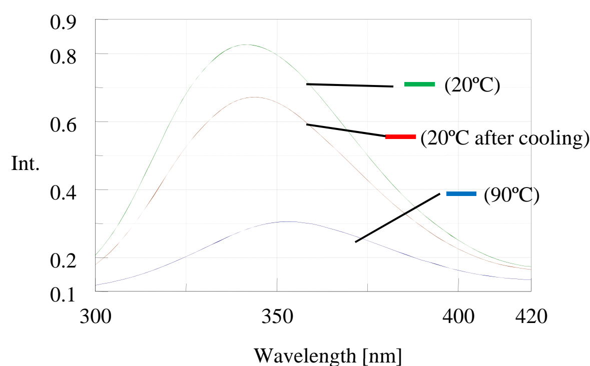

Fig. 6 Fluorescence spectrum of lysozyme (Green: 20ºC initial, Blue: 90ºC, Red: 20ºC final)

Reference

(1) S. V. Konev, *"Fluorescence and Phosphorescence of Proteins and Nucleic Acids", Plenum Press,* New York, p. 21 (1967)

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